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# The nutritional control of ghrelin secretion in humans

## The effects of enteral vs. parenteral nutrition

■ **Abstract** *Background* The nutritional control of ghrelin has not been fully clarified yet. Particularly, the influence of aminoacids and lipids is controversial and, moreover, whether the intraluminal gastric contact with

nutrients is required or if the modulatory action of nutrients on ghrelin secretion is mediated by insulin is still matter of debate. *Aim of the study* To clarify the role of nutrients in the control of ghrelin secretion evaluating the effects of intravenous and oral lipids and aminoacids compared with glucose and fructose load in healthy subjects. *Methods* A total of 6 healthy overnight-fasted volunteers underwent the following testing sessions: (a) iv arginine (ARG, 0.5 g/kg); (b) oral protein load (PRO, 50 g); (c) iv lipid-heparin infusion (Li He, Intralipid 10% 250 ml); (d) oral fat load (OIL, soy oil 40 g); (e) oral glucose load (OGL, 100 g); (f) oral fructose load (OFL, 100 g); (g) iv saline (SAL, 3 ml); (h) oral water load (WL, 200 ml). Total ghrelin, insulin, and glucose were assayed every 15 min from 0 up to +180 min. *Results* WL and SAL did not modify insulin, glucose and ghrelin. ARG induced a prompt but transient increase ( $P < 0.05$ ) of insulin and glucose ( $P < 0.01$ ), without modifying ghrelin secretion. PRO induced a

mild but sustained increase of insulin secretion ( $P < 0.05$ ) without affecting glucose and ghrelin. Li-He progressively increased circulating glucose ( $P < 0.01$ ) without modifying insulin and ghrelin secretion. No significant variations in circulating glucose, insulin, and ghrelin occurred after OIL. OGL significantly ( $P < 0.01$ ) increased insulin and glucose levels and progressively decreased ( $P < 0.05$ ) ghrelin levels. OFL induced a mild ( $P < 0.05$ ) increase of insulin without modifying glucose levels. Similarly, OFL was followed by a milder decrease ( $P < 0.05$ ) of ghrelin levels. *Conclusions* Differently from carbohydrates and independently from their modulatory effect on insulin secretion and glucose levels, both lipids and aminoacids play a negligible role in the acute control of ghrelin secretion either after acute enteral and parenteral administration.

■ **Key words** ghrelin – aminoacids – lipids – enteral nutrition – parenteral nutrition

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## Introduction

Ghrelin is a 28-amino acid peptide predominantly produced by the stomach although a remarkable expression has also been detected in several other central and peripheral endocrine and non-endocrine tissues [1, 2].

Both in animals and in humans, ghrelin circulates in the bloodstream in two different forms endowed with different biological properties [1, 2]. When acylated in serine 3, ghrelin binds with high affinity the GH secretagogues receptor type 1a (GHS-R1a), the activation of which mediates ghrelin's well-known GH-releasing effect but also other endocrine and non-endocrine actions among which a profound influence on energy metabolism via the hypothalamic modulation of appetite but also at the peripheral level by influencing the endocrine pancreatic function as well as glucose and lipid metabolism [1, 2].

In adulthood, circulating total ghrelin levels mostly reflect gastric secretion [3]. In rats, ghrelin secretion occurs following a pulsatile pattern that is not strictly correlated with GH levels but is markedly influenced by food intake episodes [4]. Similarly, in humans, ghrelin secretion undergoes circadian variations with superimposed remarkable transient decreases after meals [5, 6].

The physiological mechanisms accounting for the meal-induced inhibition of ghrelin secretion have been extensively investigated, but not fully clarified yet. Specifically, the involvement of several factors has been described, including the central activation of the cephalic phase of appetite [7, 8], the caloric content of the ingested meals [9, 10], the glycemic content of nutrients as well as the insulin responses to food intake [5, 11–13], and even several neuro-hormonal intestinal signals [14, 15].

Among all these factors, the direct role of nutrients and of their intraluminal contact with ghrelin-secreting gastric mucosa have obviously been investigated though without definitive results [16–18].

In particular, it is at present unknown whether the signals mediating the meal-related inhibition of ghrelin secretion originate from the gastrointestinal tract or from postabsorptive sites [16–18]. Although parenteral nutrients and/or insulin infusion can suppress ghrelin levels when administered for prolonged periods or at supraphysiological doses both in animals and in humans [13, 19–22], physiological doses that mimic postprandial fluctuations do not modify ghrelin in humans [23, 24]. In contrast, enteral nutrients consistently suppress ghrelin levels, even at low doses [25].

Both after oral and intravenous administration, carbohydrates, mainly glucose, represent one of the most potent inhibitory inputs on ghrelin secretion

[21, 22, 26]. In fact, in experimental conditions of prolonged glucose- or fructose-enriched diets, glucose has been shown to inhibit ghrelin secretion more potently than fructose, that on its turn exerts a significantly milder impact also on insulin and glucose levels [26]. On the other hand, discordant results have been reported on the role of lipids and proteins [12, 20, 27–33].

Based on these data, aim of our study was to further investigate the role of lipids and proteins in the regulation of ghrelin secretion in humans, in particular evaluating the potential role of the direct intraluminal contact of nutrients with gastrointestinal mucosa. In particular the effects of intravenous arginine, known as the most potent aminoacid insulin secretagogue [34, 35], administered at a dose commonly used to explore insulin and GH secretion in clinical practice [35] have been compared with those of oral mixed aminoacid load, to mimic aminoacid meal content. Moreover, the effects of intravenous lipid-heparin emulsion and of oral soy oil, characterized by similar free fatty acids content, have been also evaluated. The effects of glucose and fructose loads have been studied as positive controls.

## Subjects and methods

A total of 6 healthy young male volunteers (age [mean  $\pm$  SEM]: 28.3  $\pm$  3.1 year; body mass index: 21.9  $\pm$  0.9 kg/m<sup>2</sup>) were studied. All the subjects gave their written informed consent to participate to the study, which had been approved by an independent Ethical Committee.

All the subjects underwent the following testing sessions in random order at least 7 days apart:

- iv arginine (ARG, 0.5 g/kg from 0 min to +30 min; 150 kcal) (Table 1);
- oral amino acids load [Protifar, Nutricia, The Netherlands (PRO), 50 g at 0 min; 180 kcal] (Table 1);
- iv lipid-heparin infusion (Li He, Intralipid 10% 250 ml from 0 min to +120 min; 275 kcal) (Table 1);
- oral lipid load (OIL, soy oil 40 g at 0 min; 360 kcal) (Table 1);
- oral glucose load (OGL, 100 g at 0 min; 400 kcal)
- oral fructose load (OFL, 100 g at 0 min; 400 kcal)
- saline (SAL, 3 ml iv at 0 min as a bolus); and
- oral water load (WL, 200 ml at 0 min).

All the tests began at 08:30–09:00 h after an overnight fasting. An indwelling catheter has been placed into a forearm antecubital vein, kept patent by the slow infusion of isotonic saline. Blood samples were

**Table 1** Chemical composition of the protein and lipid loads

Testing sessions	Chemical composition
iv arginine load	arginine: 100%
oral amino acids load	95.6% proteins, 0.5% carbohydrates, 3.9% lipids; aminoacid composition/100 g proteins: L-alanine 3.5 g; L-arginine: 3.6 g; aspartic acid: 8 g; L-cysteine: 0.9 g; glutamic acid: 22.7 g; glycine: 2 g; istidine: 3.0 g; L-isoleucine: 5.4 g; L-leucine: 10.2 g; L-lysine: 8.8 g; methionine: 2.7 g; L-phenylalanine: 5.1 g; L-proline: 10 g; L-serine: 6.1 g; L-threonine: 4.7 g; L-tryptophan: 1.4 g; L-tyrosine: 4.9 g; L-valine: 6.5 g
iv lipid-heparin	1000 ml contain: purified soybean oil 100 g, purified egg phospholipids 12 g, glycerol anhydrous 22 g, water for injection q.s. ad 1000 ml
oral lipid load	soybean oil: 100%

taken every 15 min from 0 up to +180 min in order to assay total ghrelin, insulin, and glucose levels.

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

Plasma total ghrelin levels (pg/ml) were assayed, after extraction in reverse phase C18 columns, by an immunoradiometric assay (Phoenix Pharmaceuticals, Inc., Belmont, CA) using <sup>125</sup>I-labeled bioactive ghrelin as a tracer and a rabbit polyclonal antibody vs. octanoylated and des-octanoylated h-ghrelin. The sensitivity of the assay was 30 pg/tube and the intra-assay coefficient of variation (CV) range was 0.3–10.7%.

Serum insulin levels (mU/l; 1 mU/l = 7.175 pmol/l) were measured in duplicate by radioimmunoassay (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.

Plasma FFA levels (mmol/l) were measured by enzymatic analysis using the “NEFA C ACS-ACOD Method” kit provided by Wako Chemicals GmbH (Neuss, Germany).

Plasma glucose levels (mg/dl; 1 mg/dl = 0.05551 mmol/l) were measured by gluco-oxidase colorimetric assay (GLUCOFIX, by Menarini Diagnostici, Firenze, Italy).

All samples from an individual subject were analyzed together.

The hormonal responses are expressed as delta absolute variations.

The statistical analysis was carried out using a non-parametric ANOVA (Friedman test) and then Wilcoxon test, as appropriate.

The results are expressed as mean ± SEM.

## Results

Baseline glucose, insulin, and total ghrelin levels were similar in all the testing sessions (Table 2).

Either WL or SAL did not modify fasting insulin (mean Δ variation [mean ± SEM]: −0.4 ± 1.2 and

−0.3 ± 1.3 mU/l), glucose (mean Δ variation: −2.0 ± 1.4 and −1.3 ± 1.4 mg/dl), FFA (mean Δ variation: −0.1 ± 0.06 and 0.0 ± 0.05 mmol/l), and ghrelin (mean Δ variation: 20.3 ± 13.2 and 8.1 ± 6.2 pg/ml) levels (Figs. 1, 2).

ARG induced a prompt but transient increase ( $P < 0.05$ ) of insulin levels peaking at 30 min ( $\Delta$  peak: 33.6 ± 13.5 mU/l) coupled with a transient increase in glucose levels ( $\Delta$  peak at 30 min: 7.9 ± 4.8 mg/dl;  $P < 0.01$ ) (Fig. 1). On the other hand, ARG did not induce any significant variation of ghrelin levels (mean Δ variation: 9.7 ± 7.1 pg/ml) (Fig. 1).

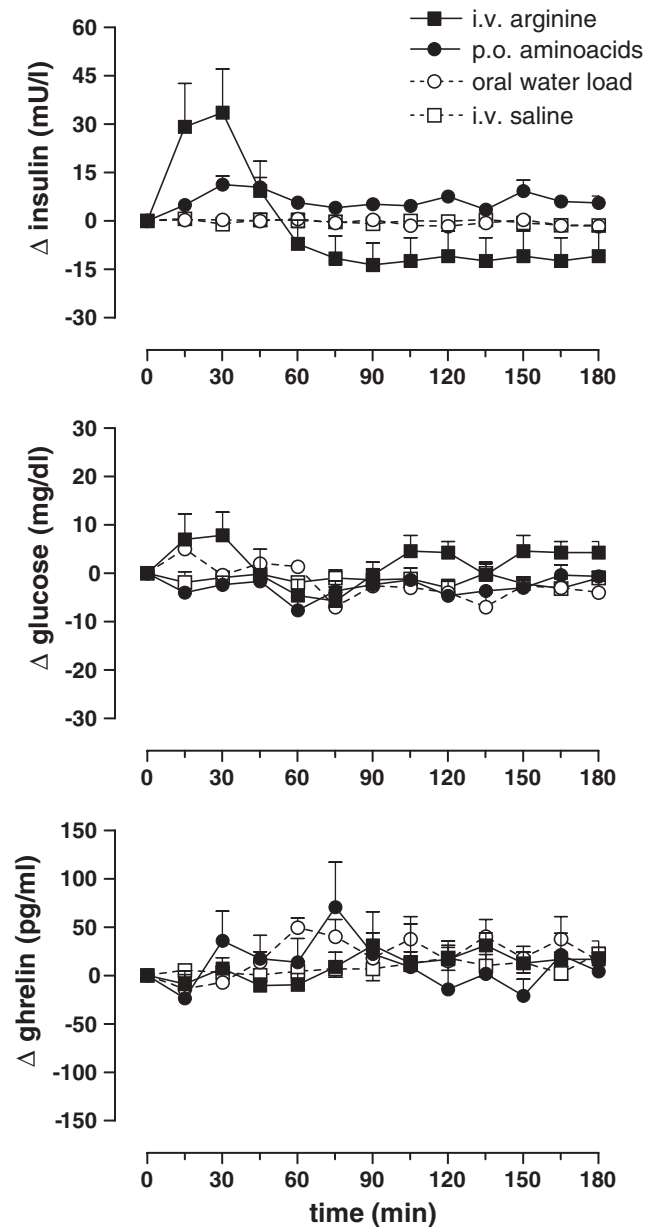
PRO induced a mild but sustained increase ( $P < 0.05$ ) in insulin levels (mean Δ variation: 6.1 ± 0.7 mU/l) (Fig. 1). On the other hand, PRO administration did not modify either glucose (mean Δ variation: −2.7 ± 1.0 mg/dl) or ghrelin (mean Δ variation: 10.6 ± 23.0 pg/ml) levels (Fig. 1).

As expected Li-He infusion increased FFA levels (mean Δ variation: 2.9 ± 0.6 mg/dl;  $\Delta$  peak at 150': 5.4 ± 0.6 mmol/l;  $P < 0.01$ ) and induced a progressive increase in circulating glucose levels (mean Δ variation: 14.4 ± 4.4 mg/dl;  $\Delta$  peak at 150': 20.5 ± 7.6 mU/l;  $P < 0.01$ ) without significantly modifying insulin levels (mean Δ variation: −2.1 ± 2.9 mU/l) (Fig. 2). Similarly, no significant variations in term of ghrelin levels occurred after Li-He infusion (mean Δ variation: 6.8 ± 11.4 pg/ml).

On the other hand, despite a still significant ( $P < 0.05$ ) although less pronounced ( $P < 0.01$  vs. Li He) increase of FFA levels (mean Δ variation: 0.5 ± 0.09 mmol/l;  $\Delta$  peak at 150 min:

**Table 2** Mean (±SEM) baseline insulin, glucose and ghrelin levels in the testing sessions

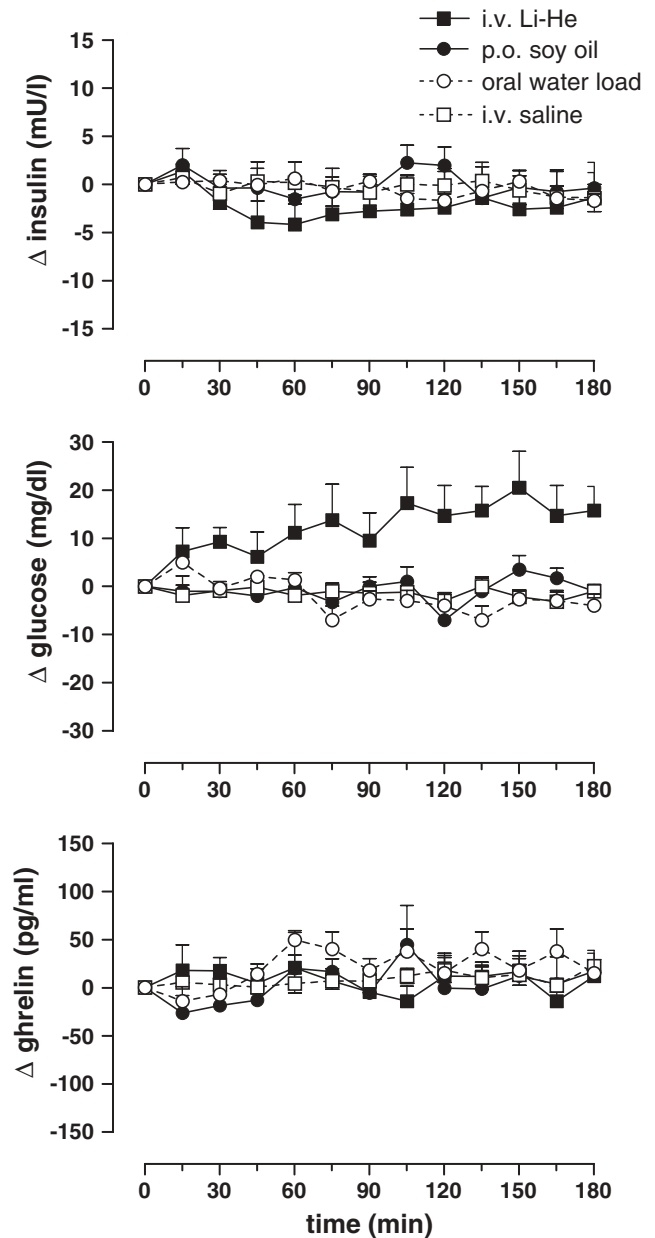
	Insulin (mU/l)	Glucose (mg/dl)	Ghrelin (pg/ml)
ARG	16.5 ± 7.6	76.4 ± 2.9	432.0 ± 23.3
PRO	15.1 ± 0.4	80.7 ± 0.9	378.3 ± 39.61
LiHe	17.9 ± 4.2	77.0 ± 3.3	423.3 ± 29.3
OIL	15.9 ± 2.5	75.5 ± 5.3	441.0 ± 44.6
OGL	20.2 ± 6.2	80.0 ± 3.6	416.0 ± 88.8
OFL	14.3 ± 1.0	77.0 ± 3.0	400.0 ± 74.3
SAL	14.0 ± 1.4	74.5 ± 2.0	374.6 ± 79.3
WL	17.4 ± 1.2	82.3 ± 3.2	374.3 ± 28.5



**Fig. 1** Mean ( $\pm$  SEM) delta insulin, glucose, and ghrelin concentration after iv arginine (ARG, 0.5 g/kg from 0 min to +30 min), oral amino acids load (PRO, 50 g at 0 min), saline (SAL, 3 ml iv at 0 min) or oral water load (WL, 200 ml at 0 min) in six healthy overnight-fasted volunteers

$1.3 \pm 0.06$  mmol/l), no significant variations in circulating glucose (mean  $\Delta$  variation:  $-0.03 \pm 1.3$  mg/dl), insulin (mean  $\Delta$  variation:  $-0.8 \pm 1.6$  mU/l), and ghrelin (mean  $\Delta$  variation:  $3.8 \pm 21.1$  pg/ml) levels were observed after OIL (Fig. 2).

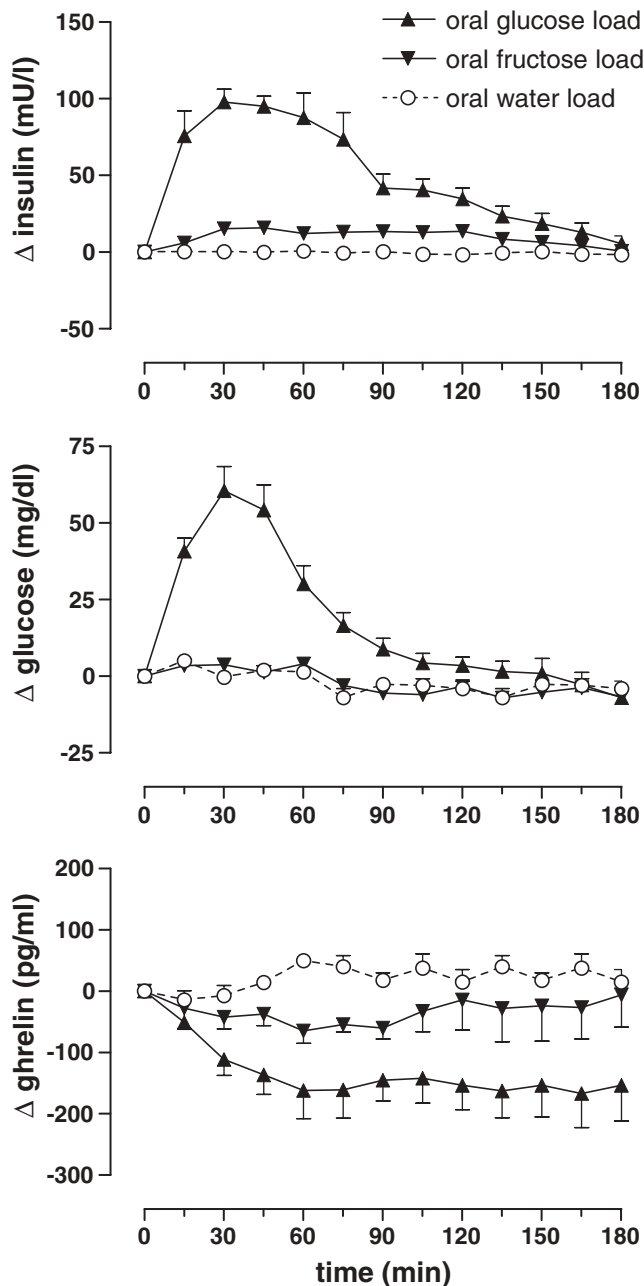
Oral glucose load induced a significant ( $P < 0.01$ ) increase in insulin (mean  $\Delta$  variation:  $46.7 \pm 5.8$  mU/l;  $\Delta$  peak at 30 min:  $97.9 \pm 8.4$  mU/l) and glucose (mean  $\Delta$  variation:  $16.3 \pm 2.2$  mg/dl;  $\Delta$  peak at 30 min:  $60.5 \pm 7.9$  mg/dl) levels. After OGL a pro-



**Fig. 2** Mean ( $\pm$  SEM) delta insulin, glucose, and ghrelin concentration after lipid-heparin infusion (Li He, Intralipid 10% 250 ml), oral fat load (OIL, soy oil 40 g), iv saline (SAL, 3 ml) or oral water load (WL, 200 ml) in six healthy overnight-fasted volunteers

gressive decrease ( $P < 0.05$ ) of ghrelin levels was also observed (mean  $\Delta$  variation:  $-84.5 \pm 51.7$  pg/ml) (Fig. 3).

Oral fructose load induced a mild but significant ( $P < 0.05$ ) increase of insulin levels (mean  $\Delta$  variation:  $9.3 \pm 1.3$  mU/l) without modifying glucose (mean  $\Delta$  variation:  $-2.2 \pm 0.6$  mg/dl). After OFL a significant ( $P < 0.05$ ) transient decrease of ghrelin levels was observed (mean  $\Delta$  variation:  $-32.1 \pm 23.6$  pg/ml) (Fig. 3).



**Fig. 3** Mean ( $\pm$  SEM) delta insulin, glucose, and ghrelin concentration after oral glucose load (OGL, 100 g), oral fructose load (OFL, 100 g), or oral water load (WL, 200 ml) in 6 healthy overnight-fasted volunteers

The insulin response to OGL was remarkably higher ( $P < 0.05$ ) than that to ARG, that, in turn was higher ( $P < 0.05$ ) than that induced by OFL. Similarly the inhibitory effect of OGL on circulating ghrelin levels were significantly more potent than that induced by OFL.

Notably, however, no correlation was found between insulin or glucose and total ghrelin levels after each stimulus.

## Discussion

Ghrelin, in its acylated form, is an endogenous ligand of the GHS-R1a that is under intensive investigation due to its tight relationship with feeding behavior and energy metabolism [1, 2]. Consistently with the tight link between nutritional status and ghrelin, its circulating levels are decreased by food intake and over-feeding [5, 6].

Notably, however, despite metabolic and nutritional signals clearly turned out to be the most consistent modulators of ghrelin secretion in humans [1, 2], the exact mechanisms mediating the nutritional regulation of ghrelin secretion are still largely unknown [13, 23, 36, 37].

Based on these data, aim of our study has been to compare for the first time in humans the acute effects of either intravenous and oral lipids and aminoacids on circulating ghrelin levels in the same subjects. To this aim, in separate sessions we tested the effects of oral mixed pure aminoacid load, mimicking aminoacid meal content, compared with intravenous arginine, known as the most potent aminoacid insulin secretagogue [34, 35], administered at a dose commonly used to explore insulin and GH secretion in clinical practice [35] and, then, the effects of soy oil, as commonly used nutritional component, with intravenous lipid-heparin emulsion, characterized by similar free fatty acids content. The effects of glucose and fructose loads have been also studied as positive controls.

The results we obtained show that, at least after acute administration, differently from carbohydrates, both lipids and amino acids do not modify total ghrelin levels and no significant differences occur after enteral or parenteral administration.

Our findings are apparently in contrast with some (but not all [20, 33]) other studies reporting that in humans oral fat administration is able to mildly decrease [27, 28, 30, 32], or to increase [12] circulating ghrelin levels. Similarly, oral protein intake has been variably reported either to increase [12, 27] and not to affect [29, 31] circulating ghrelin levels.

The explanations for these differences are not obvious and several hypothesis could be considered.

First, in contrast with our study in which we tested the effect of pure lipid or protein load, some of the studies in the literature have been performed using mixed protein-enriched or lipid-enriched meals in which the carbohydrates content was not negligible (>5%) [28, 32].

Since a dose-response curve of carbohydrates on ghrelin secretion has never been performed so far, it is theoretically possible that even low carbohydrates doses in mixed meals could play a significant inhibitory role on ghrelin secretion [25].

On the other hand, other studies reported a late stimulatory effect of oral proteins on ghrelin secretion occurring 210 min [29] or 300 min [31] after the ingestion. This time course would suggest that such stimulatory action on ghrelin secretion could be expression of an indirect metabolic response to amino acid absorption rather than a direct effect.

As expected, both aminoacids and lipid loads induced a remarkable metabolic impact. In fact, both oral amino acids load and even more intravenous arginine strongly stimulated insulin secretion and increased circulating glucose levels likely as the results of the well-known concomitant direct stimulatory effect of arginine on glucagon secretion [38]. On the other hand, free fatty acids infusion induced a remarkable progressive increase of glucose levels, due to the effects of free fatty acids on the inhibition of insulin-stimulated glucose uptake and glycogen synthesis as well as of insulin suppression of endogenous glucose production [39, 40].

Interestingly, however, despite this remarkable impact on insulin and glucose levels, neither aminoacids nor free fatty acids administrations were coupled with significant variations of circulating ghrelin levels. This finding is of particular interest also taking into account that no effect was recorded both after oral and also after intravenous administration despite obvious differences in administration profile and bioavailability. In particular both intravenous lipid-

heparin emulsion and arginine administration given as continuous infusion instead of acute bolus for safety reasons, were devoid of any modulatory effect on ghrelin secretion as the oral loads, despite an even stronger impact on insulin and glucose levels, in particular after arginine, known as the most potent aminoacid insulin secretagogue [34, 35]

On the other hand, as previously reported in experimental conditions of prolonged high fructose diet, also in our acute experimental conditions, fructose exerts a mild but still significant inhibitory effect on ghrelin secretion, despite its insulin-secreting effects is lower than that of arginine, that, in turn, as reported above does not modify ghrelin secretion.

Therefore, these data further question the existence of a univocal relationship between insulin or glucose and ghrelin and suggest that the acute nutritional control of ghrelin secretion is regulated by a multifactorial system not simply directly related to glucose metabolism.

In conclusions, the results of the present study show that independently from their modulatory effect on insulin secretion and glucose levels, both lipids and amino acids play a negligible role in the acute control of ghrelin secretion either after acute enteral or parenteral administration. Consistently with the milder impact on insulin and glucose levels, among carbohydrates, fructose exerts a less potent inhibitory effect on ghrelin secretion than glucose.

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