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ORIGINAL RESEARCH PAPER

Oral ingestion of a hydrolyzed gelatin meal in subjects with normal weight and in obese patients: Postprandial effect on circulating gut peptides, glucose and insulin

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ABSTRACT. *Gut hormones [ghrelin, peptide YY (PYY) and glucagon-like peptide-1 (GLP-1)] are an important group of hormones that target appetite control. They are released from endocrine L cells of the small bowel in proportion to the volume, components and calories in a meal. In the current study, 20 g of gelatin (flavored and sweetened) were given to obese patients (n=12) and lean subjects (n=10). Subsequently, plasma samples were collected at-30-minute intervals up to 180 minutes and glucose, insulin, PYY, GLP-1 and ghrelin were assayed using specific and sensitive immunofluorometric and radioimmunoassays. As expected, obese patients had normal serum glucose levels, higher serum insulin, and lower plasma concentration of ghrelin at all times compared to lean subjects. GLP-1 plasma levels were significantly elevated at 60 minutes, peaking at 120 minutes in obese patients and lean subjects. As a consequence, there was a significant rise in serum insulin levels with a significantly higher peak level at 60 min (obese) and 30 min (lean). There were no significant changes in PYY plasma concentrations and no correlation was found between body mass index and concentrations of ghrelin, PYY and GLP-1 in the group of obese patients. In conclusion, a single gelatin meal induces a rise in plasma GLP-1 followed by an increase in serum levels of insulin. These findings may be applied to maximize satiety in obese patients as a means of improving adherence to calorie-controlled diets as well as provide better control of diabetic patients.*
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INTRODUCTION

The alarming rise of obesity all over the world, in both industrialized and emergent countries, has contributed to an explosion in type 2 diabetes mellitus, predicted to afflict 210 million people worldwide by 2010 (1). Obesity is also associated with an increased risk of cardiovascular disease, hypertension, hyperlipemic disorders and osteoarthritis (2). Current pharmacologic treatments for obesity lead to temporary weight loss (with repeated recurrences) and may sometimes not be well tolerated or even suitable for many obese patients.

Recent developments toward unraveling the intricate physiology of appetite and energy expenditure have paved the way for new pharmacological treatments. Gut hormones are an important group of hormones that target appetite control and are released from the gastrointestinal tract in response to the type of meal (3). These hormones, which include insulin, leptin, ghrelin, cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), provide short-term information on hunger and satiety and influence hypothalamic, limbic and brainstem circuits that control appetite and energy expenditure. They also modulate short-term signals that determine meal initiation and termination (4, 5).

Batterham et al. (6) conducted a study on PYY, a peptide secreted postprandially by endocrine L cells that line the distal small bowel and colon in proportion to the volume of the meal and the calories ingested. A single infusion of PYY two hours before the meal reduced appetite and food consumption (by about 30 percent) at a buffet lunch. The PYY response was diminished in obese subjects compared with the response observed in lean subjects. Other recent reports have indicated that GLP-1 and PYY

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Ghrelin, GLP-1, PYY, insulin, glucose, gelatin, obesity.

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are co-secreted by endocrine L cells of the small bowel, and that levels of both hormones are low in the fasting state, increasing within 30 to 60 min after meals (7). A recent meta-analysis reported that infusion of GLP-1 was associated with dose-dependent reduction in energy intake (food) in both lean and obese subjects (8).

Consumption of dietary protein generally produces greater satiety compared with carbohydrate (9). Moreover, differences in gastrointestinal hormones response to liquid preloads containing protein or glucose were also observed (10). The result of a recent study suggested that satiety signals from protein, but not from carbohydrate, are diminished in obese boys; however the metabolic explanation for this observation needs further investigation (11).

The aim of our study was to investigate whether the ingestion of a gelatin based meal was effective in inducing a rise in PYY and GLP-1, and in affecting the concentration of ghrelin in normal lean subjects and in obese patients. Moreover, a rise in GLP-1 would subsequently increase plasma insulin levels, an effect that has a potential therapeutic application in patients with diabetes.

METHODS

Study subjects

Healthy obese patients (n=12) and lean subjects (n=10) were recruited from the outpatient clinic and from the staff of Hospital das Clinicas, respectively. Clinical and laboratory data are shown in Table 1. The body mass index (BMI) for both genders in the obese group ranged from 34 to 50 kg/m^2. All obese patients had stable weight over the 3 months preceding the study. Lean subjects of both genders had a BMI between 18.4 and 23.8 kg/m^2 (Table 1). All subjects were between the ages of 19 and 48 years; the mean age for the obese group was 36.8 years and for lean subjects was 27.1 years. Criteria for exclusion were smoking, pregnancy, use of anorectic agents (phentermine, fenproporex, diethylpropion, mazindol, sibutramine), and use of medications that would alter gastric secretion (omeprazole and similar agents) or bowel movements (laxatives, orlistat, acarbose).

All subjects signed an informed consent for the study and approval was obtained from the Research Ethics Committee of the Hospital

TABLE 1
Obese patients and lean subjects: clinical and laboratory data.

Initials	Gender	Age (years)	BMI (kg/m^2)	Glucose (mg/dl)	Insulin (mU/l)
				Basal levels	
OBESE PATIENTS					
RSR	F	28	50.0	87.00	21.12
AMAC	F	46	34.0	102.00	11.63
MAB	F	48	34.0	75.00	30.58
MPRF	F	43	34.5	94.00	6.89
ABEP	F	47	50.0	85.00	9.53
MDL	F	25	43.5	82.00	20.67
FAO	M	31	38.1	79.00	30.76
CEAA	M	24	44.0	94.00	53.17
JDMP	M	42	34.5	87.00	14.87
DPLL	M	26	45.1	127.00	62.01
WSQ	M	46	37.8	108.00	21.47
JB	M	36	43.8	112.00	27.73
Mean		36.83	40.78	94.33	25.87
SD		9.11	5.79	14.66	16.13
LEAN PATIENTS					
VLVP	F	29	18.5	72.00	6.02
MFG	F	24	18.4	77.00	2.51
JBF	F	24	20.9	70.00	4.23
JAB	F	24	21.1	77.00	5.78
MNR	M	19	23.8	87.00	10.82
RVA	M	26	21.3	92.00	8.78
JCC	M	23	21.1	100.00	12.33
FOR	M	47	20.6	87.00	10.82
GMG	M	19	23.4	112.00	8.66
DG	M	36	22.5	80.00	8.89
Mean		27.10	21.16	85.40	7.88
SD		8.10	1.70	12.48	2.99

das Clinicas, University of São Paulo Medical School. The study was carried out in accordance with the Declaration of Helsinki.

Study protocol

Patients were asked to eat a light meal and refrain from drinking alcoholic beverages the night before the study. After this evening meal (8:00 pm), they were only allowed to drink water. The following day, at 7:30 am, they arrived at the Central Laboratory of the Hospital das Clinicas and an intravenous line was established. Before the first blood collection (time -30 minutes), patients were allowed to relax on a comfortable couch for 30 minutes. Blood was also collected at time 0 and subsequently at 30, 60, 90, 120, 150 and 180 minutes in serum-separating tubes and heparin-coated tubes containing 5,000 kallikrein inhibitor units (0.2 ml) of aprotinin (Bayer Chemical). All blood tubes were immediately placed on ice before centrifugation at 4ºC for 10 minutes at 1550 g to separate the serum at the end of the test. Samples were stored at -70°C until analysis. At time 0, all subjects were instructed to eat a meal with 20 grams of hydrolyzed gelatin (86% of protein), that contained gelatin 92.6%, fumaric acid 4.14%, sodium citrate 1.4%, sodium chloride 0.8%, and flavored with artificial sweeteners (acesulfame K 0.31% and aspartame 0.75%).

Hormone assays

Serum glucose and serum insulin were assayed by colorimetric and immunofluorometric standardized assays, respectively, by the Central Laboratory of Hospital das Clinicas, University of São Paulo Medical School. Plasma PYY, GLP-1 and total ghrelin were assayed in duplicate using commercially available specific and sensitive radioimmunoassays (PYY-67HK; GLP1T-36HK; GHRT-89HK; Linco Research, Missouri, USA) following the manufacturers' protocols. All samples from a given subject were analyzed using the same kit to avoid inter-assay variation.

Statistical analysis

Plasma concentrations of ghrelin, GLP-1 and PYY were compared between groups (obese versus lean) using analysis of variance for repeated measures (ANOVA), as well as the moment/time of observation (repetition factor), using a correlation matrix of symmetrical components. The Kolmogorov-Smirnov test indicated that all gut hormones data were normally distributed. For significance analysis, two by two analysis using Tukey multiple comparison procedure was employed.

RESULTS

All the twenty-two subjects (obese n=12, lean n=10) included in the study were evaluated for their respective glucose and insulin curves as well as secretion of gut peptides (ghrelin, GLP-1 and PYY) before and after the gelatin meal.

Serum glucose and insulin levels

The results are shown in Table 1 and Figure 1. Serum glucose concentrations were similar in both groups at baseline and remained unchanged after the meal. At all times, serum insulin levels in obese patients were significantly higher as compared to lean subjects ($p<0.05$). After the gelatin meal, both obese patients and lean subjects had a significantly rise in serum insulin, peaking at 30 min (lean subjects) and at 60 min (obese patients) ($p<0.01$ as compared to basal). In both groups, insulin values returned to basal levels after 180 min.

Plasma values of ghrelin

Mean plasma ghrelin levels were consistently higher at all times in lean subjects (values

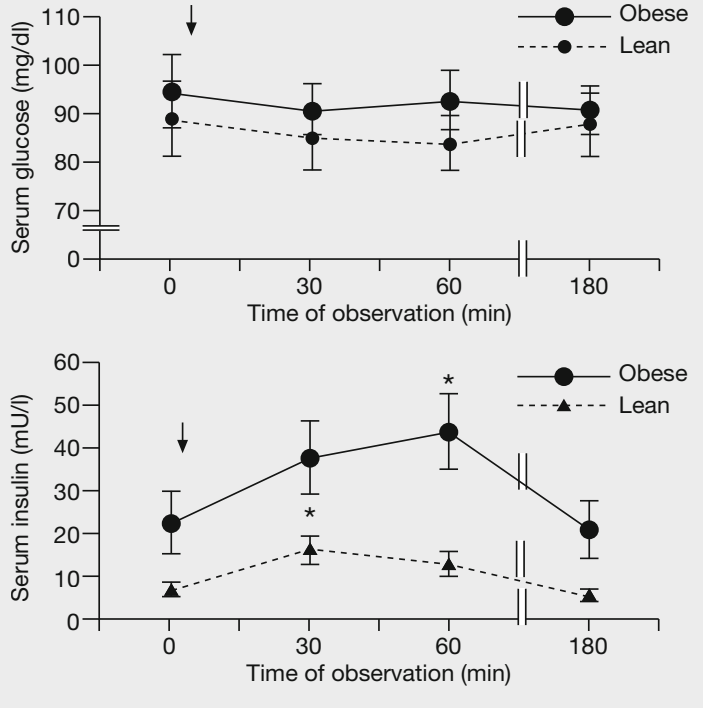

FIGURE 1

Mean and SD of serum glucose and insulin concentrations before and after the gelatin meal (↓) in both obese patients and lean subjects. Obese patients had a significantly higher concentration of serum insulin at all times ($p<0.05$). Both obese patients and lean subjects had a significant rise in insulin concentrations that peaked at 60 min (obese) and 30 min (lean) ($p<0.01$) as compared to basal levels. Glucose concentrations were similar in both groups at baseline and remained unchanged after the meal.

between 1,080.2±241 pg/ml and 1,271.66± 385pg/ml) compared to values in obese patients (values between 925.89±238.63 and 1,155.74±596 pg/ml); however, it did not attain statistical significance (p=0.249) (Fig. 2). Mean ghrelin values did not differ over time for either group (p=0.052).

Plasma concentrations of GLP-1

GLP-1 concentrations are shown in Figure 3. Analysis of variance indicated that both groups (lean and obese) exhibited the same behavior over time (p=0.119). A significant rise of GLP-1 after the gelatin meal was observed (p=0.012) in both groups at 60 and 120 minutes compared to the basal values (p=0.028 and p=0.02 in lean and obese group, respectively). The highest GLP-1 plasma concentrations were observed at 120 minutes after the test meal.

Plasma concentrations of PYY

Mean PYY values were statistically higher in lean subjects than in obese patients at all times (p<0.04) (range values for lean subjects 146.04±42 pg/ml and 162.26±50 pg/ml; for obese subjects 96.53±29 pg/ml and 105.21±18 pg/ml). Analysis over time showed that obese patients exhibited a significant attenuation of plasma PYY secretion 30 minutes after the ingestion of gelatin compared to values at time 0 (p=0.034). Lean subjects also exhibited a significant reduction of PYY levels at 150 and 180 min compared to the levels before the gelatin meal (time -30, p=0.015; and time 0, p=0.009 respectively).

Correlation between BMI and basal mean levels of ghrelin, GLP-1 and PYY

Spearman's analysis showed no significant correlation between BMI (kg/m^2) and plasma levels of the three gut peptides at 0 and 30 minutes.

DISCUSSION

A growing number of gut-brain peptides have been shown to affect short-term food intake when administered acutely to experimental animals and humans (12). It is known that PYY and GLP-1 are released from the gut in proportion to the volume, calories and composition of nutrients in an ingested meal (3). The 28 amino acid peptide ghrelin stimulates food intake, whereas other peptides including GLP-1, PYY, CCK, and amylin inhibit food intake (13). Furthermore, PYY infusion reduces cumulative 24 hours food intake both in lean subjects and obese patients (6). In experimental animals, PYY and GLP-1 inhibit food intake additively, being co-secreted after a meal (7).

Several studies (10,11,14-17) have indicated that consumption of dietary protein seems to

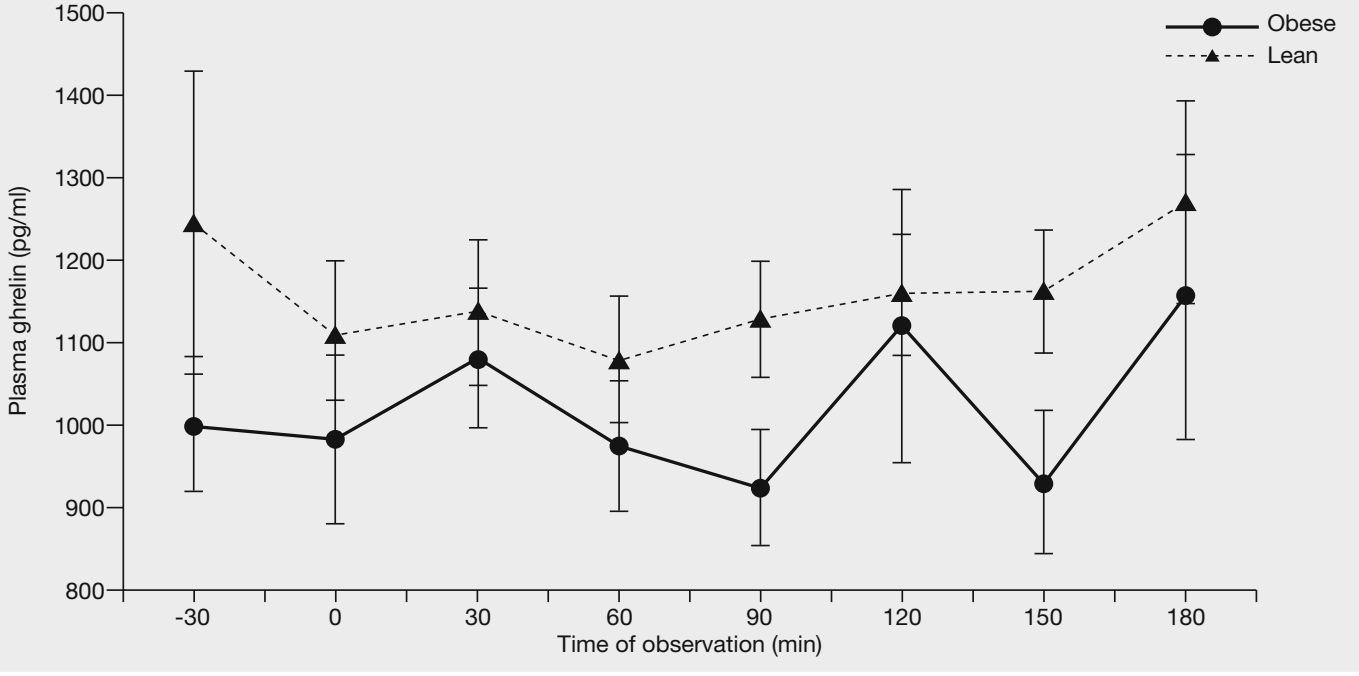

FIGURE 2
Mean and SD of plasma ghrelin levels before and after the gelatin meal (↓). Lean subjects had consistently more elevated plasma ghrelin levels at all time as compared to obese patients.

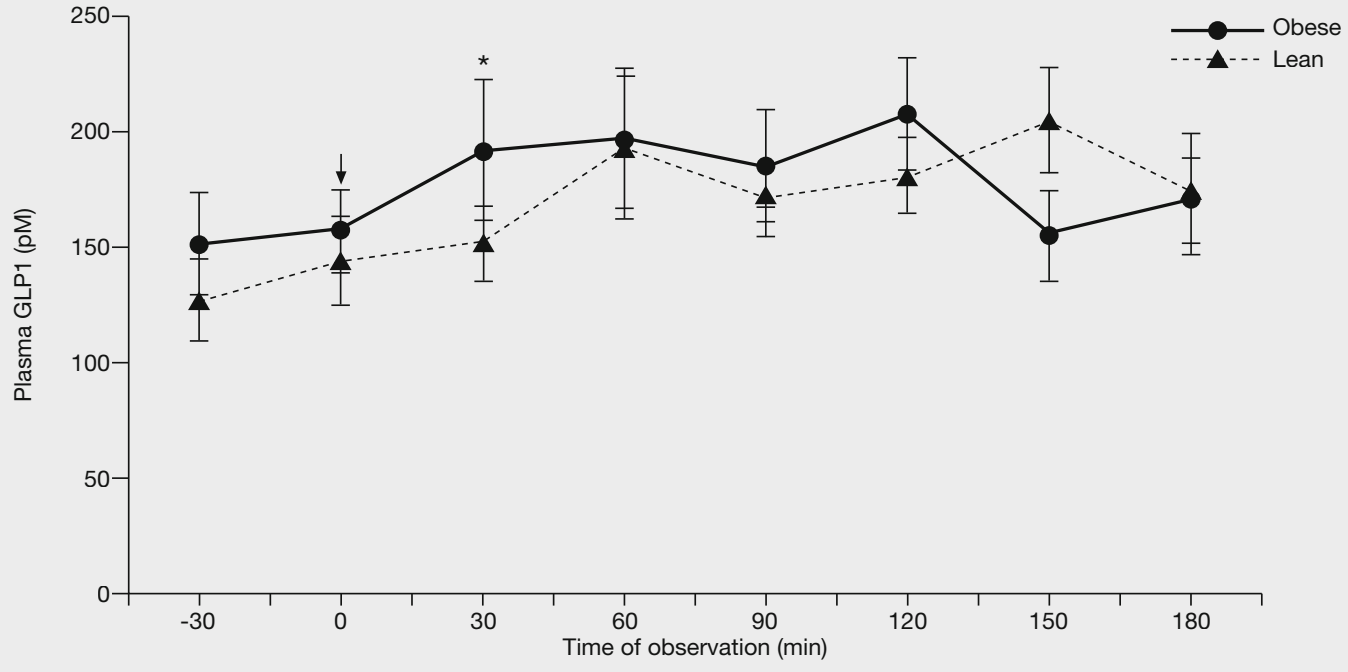

FIGURE 3
Mean and SD of plasma GLP-1 levels before and after the gelatin meal (↓) in both groups.

decrease postprandial appetite and subsequent food intake more intensively than carbohydrate and fat meals. Moreover these reports emphasize the importance of considering the impact of protein type on the appetite response to a meal. For instance, whey protein produces a prolonged suppression of ghrelin and elevation of GLP-1 and CCK that is reduced when combined with fructose (16). A high (30%) protein diet induces a higher GLP-1 concentration as compared to adequate (10%) protein diet. This is accompanied by increase satiety (15).

Our findings indicate that a simple protein meal consisting of 20 g of gelatin may induce an elevation of GLP-1 as early as 30 minutes after ingestion of the meal. The mean plasma level of GLP-1 was significantly higher at 60 and 120 minutes compared to basal levels, with a peak at 120 minutes, decreasing to basal values at 180 minutes.

Protein ingestion (gelatin meal) requires a longer time than carbohydrate (glucose, fructose) to be digested and absorbed, presumably reaching more distal regions in the small intestine. Gelatin protein is considered to be "rapidly" digested and absorbed relatively to other more complex proteins and similarly to whey (18). Therefore we may assume that gelatin, like whey, is a potent insulinotropic dietary protein through its stimulation of incretins such as GLP-1. This effect was nicely demonstrated in both obese patients and lean subjects in our study that presented a rise in serum insulin levels after the gelatin meal due to incretin (GLP-1) release. This finding may be useful for type 2 diabetic patients that are in need for incretins effect on insulin release to obtain a better control of glycemic levels.

Although plasma ghrelin levels were consistently lower in obese patients in comparison with lean subjects, the variance analysis of the data did not indicate statistical differences (p= 0.052). Low ghrelin levels in obese subjects have been shown in previous studies (19). Plasma levels of PYY were also consistently lower in obese patients compared to lean subjects (p=NS) but did not show significant changes over time. BMI (kg/m^2) did not correlate with any of the three peptides studied at 0 and 30 minutes.

Dietary manipulations that maximize satiety have obvious applications for the overweight population as a means of improving compliance with calorie-controlled diets. Therefore, a simple gelatin based meal containing around 20 g of gelatin ingested one hour before the main meals could be useful in reducing appetite and consequently, energy intake of the obese patient. Thus, it is possible that the increase of GLP-1 observed in obese patients could mediate a satiety signal in the complex hypothalamic centers, inducing a small but useful decrease in appetite. Moreover GLP-1 has insulinotropic properties (incretin) that would favor the release of insulin.

In conclusion, we have observed that a gelatin meal offered to both obese and lean subjects induces a significant rise in GLP-1 levels after 30 minutes, with a peak at 120 minutes. There was no effect on plasma concentrations of ghrelin and PYY. A gelatin meal preprandially could therefore be useful in maximizing satiety in the obese population as well as to providing a surge of insulin that may be helpful for a better control of diabetic patients.

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