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Predictors of Postabsorptive Ghrelin Secretion after Intake of Different Macronutrients

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Context: Release of ghrelin, a gastrointestinal hormone regulating feeding and energy balance, is blunted in obesity, a condition associated with insulin resistance.

Objective: The objective was to identify anthropometric and metabolic predictors of postabsorptive ghrelin secretion.

Design: We evaluated ghrelin, insulin, glucose, and leptin secretion overnight and after intake of different macronutrients.

Subjects: Ten obese subjects (age, 31.8 ± 2.5 yr; body mass index, 43.4 ± 0.8 kg/m^2) and six lean subjects (age, 33.5 ± 2.4 yr; body mass index, 21.8 ± 1.4 kg/m^2) participated in the study.

Main Outcome Measures: The main outcome measures were resting energy expenditure (REE); fat mass; nighttime approximate entropy (ApEn) and synchronicity (cross-ApEn) of ghrelin, insulin, and leptin; insulin sensitivity by homeostatic model approach insulin-sensitivity (HOMA-S%); postabsorptive area under the curve (AUC); and Δ of ghrelin, insulin, glucose, and leptin after carbohydrate-, lipid-, and protein-rich test meals.

Results: Nighttime ApEn scores were higher in obese than lean subjects ($P < 0.01$). Cross-ApEn revealed a synchronicity between ghrelin-insulin, ghrelin-leptin, and insulin-leptin in both groups. Compared with baseline, ghrelin decreased significantly ($P < 0.01$) in lean and obese subjects after carbohydrates (42.2 *vs.* 28.5%; $P < 0.05$), lipids (40.2 *vs.* 26.2%; $P < 0.01$), and proteins (42.2 *vs.* 26.3%; $P < 0.01$) devoid of between-meal ghrelin differences. Significant associations occurred between nocturnal ghrelin ApEn and insulin (r = 0.53; $P < 0.05$), postmeal ghrelin AUCs and REE (r = −0.57; $P < 0.05$), and HOMA-S% (r = 0.52; $P < 0.05$), postmeal ghrelin Δ and HOMA-S% (r = 0.60; $P < 0.05$). REE ($\beta = -0.57$; $P = 0.02$) and ghrelin ApEn ($\beta = -0.62$; $P = 0.01$) were predictors of postmeal ghrelin AUC and Δ, respectively.

Conclusions: Obesity determined a decreased orderliness of ghrelin secretion and a relative loss of ghrelin-insulin synchrony. Postabsorptive ghrelin secretion decreased significantly both in obese and lean subjects, was related to insulin sensitivity, and was predicted by energy expenditure and hormone pulsatility. ***(J Clin Endocrinol Metab* 91: 4124–4130, 2006)**

IT IS WELL ESTABLISHED that ghrelin, the putative ligand of the GH-secretagogue receptor (GHS-R), yields GHS-R-independent orexigenic effects mediated centrally by neuropeptide Y- and Agouti-related protein-containing hypothalamic neurons, and is most likely key to insulin and leptin-mediated short-term regulation of feeding and energy balance (1, 2). Evidence exists that ghrelin stimulates food intake when administered either centrally to rodents or peripherally to rodents and humans (3–5), with the role of ghrelin as appetite hormone confirmed by its meal-related variations encompassing a preprandial rise and a progressive decrease within 60–90 min after food or glucose intake (6, 7). Pre- and postabsorptive studies have further demonstrated a direct proportion between ingested calories and the resulting depth and duration of postprandial ghrelin suppression (6–9); in contrast, meal size and intermeal intervals did not appear to predict the magnitude of preprandial ghrelin rise (10, 11).

Superimposed to short-term fluctuations, ghrelin secretory rate greatly depends on anthropometric factors involved in the long-term regulation of energy balance, *i.e.* genetic setting and ethnicity, body composition, and energy efficiency (12). A number of evidences suggest that insulin homeostasis plays a pivotal role in the control of ghrelin secretion, because both peptides covary reciprocally after food or glucose ingestion (6, 7), and insulin infusion is able to blunt ghrelin secretion during clamp studies in healthy subjects as well as in type 1 or 2 diabetic patients (13–15). Ghrelin levels are remarkably decreased in states of insulin resistance (16–18) as well as in obesity, a disorder prone to develop hyperinsulinemia and insulin resistance, which is associated with lower fasting levels and diminished hormonal pulsatility of ghrelin compared with the lean state (6, 19, 20). An opposite relationship also links serum leptin to plasma ghrelin levels (6, 8, 19, 21), and rodent studies showed that these hormones exert counteracting effects on neuropeptide Y-positive hypothalamic neurons controlling appetite (2).

Although hypoghrelinemia of obesity could be viewed as a mechanism compensating for hyperphagia and weight accrual, there is controversial evidence that ghrelin is responsive to meal ingestion in obesity (8, 22). To further expand current knowledge on the control of postabsorptive ghrelin secretion, our study tested the hypotheses that ghrelin se-

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Abbreviations: ApEn, Approximate entropy; AUC, area under the curve; BMI, body mass index; GHS-R, GH-secretagogue receptor; HOMA-S%, homeostatic model approach insulin-sensitivity; REE, resting energy expenditure.

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cretion in response to calorie intake might be an expression of endogenous energy homeostasis; might be associated with the orderliness of nocturnal ghrelin secretion; might occur in the obese as well as in the lean state; and might be related to macronutrient type, insulin sensitivity, and leptin homeostasis. To address these issues, isovolumic isoenergetic test meals containing different amounts of carbohydrates, lipids, and proteins were administered to lean and obese individuals previously subjected to continuous nocturnal sampling.

Patients and Methods

Our study populations consisted of 10 nondiabetic, otherwise healthy obese individuals, admitted to our institution for work-up and treatment of obesity [five males and five females; age, 31.8 ± 2.5 yr; body mass index (BMI) 43.4 ± 0.8 kg/m^2], and six healthy lean subjects recruited from hospital staff as age/sex-matched controls (three males and three females; age, 33.5 ± 2.4 yr; BMI, 21.8 ± 1.4 kg/m^2). Subjects were enrolled after approval by the local ethical committee and once a written informed consent had been obtained. All individuals were nonsmokers, low alcohol consumers (≤125 ml of wine per day), and free from gastrointestinal, cardiovascular, or metabolic disorders. Diabetes mellitus was excluded by oral glucose tolerance test (2-h plasma glucose concentration <7.8 mmol/liter). Physical examination, electrocardiogram, and routine blood and urine analysis were carried out to exclude acute medical illness. All women reported a regular menstrual cycle and were studied during the early follicular phase.

Obese patients were tested 3 d after admission to the hospital while maintained with an isocaloric diet containing 30% lipids, 50% carbohydrates, and 20% proteins. Healthy controls underwent the same type of dieting and were admitted to the hospital on the morning of the examination day. All subjects underwent anthropometric measurements after voiding and dual-energy x-ray absorptiometry for measurement of fat body mass (GE-Lunar, Madison, WI). Resting energy expenditure (REE, kcal/24 h) was determined in a thermoregulated room (22–24 C) by computed open-circuit indirect calorimetry, measuring resting oxygen uptake and resting carbon dioxide production by a ventilated canopy (Sensormedics, Milan, Italy) at 1-min intervals for 30 min and expressed as 24-h value. The predicted REE was calculated by the Harris-Benedict formula (23). BMI was calculated as weight (kilograms)/height (meters)^2.

For the entire study duration, obese individuals were studied as inpatients whereas controls were studied as outpatients. As depicted in Fig. 1, the overnight study was carried out between 2000 and 0800 h (dinner ingested between 1900 and 1930 h) on bed-laying subjects in a thermoregulated room (lights out at 2230 h, wake up at 0700 h). During the night, blood samples were drawn half-hourly for determination of ghrelin, insulin, and leptin levels and collected separately through an indwelling cannula inserted at the antecubital vein, kept patent by continuous slow saline infusion. At 0800 h on the morning following the night study and for 2 subsequent days, each fasting participant underwent a meal test consisting of isovolumic isoenergetic liquid meal (500 kcal) rich in carbohydrates (Ensure; Abbott, Milan, Italy; nutritional composition: carbohydrates, 53%; lipids, 30%; proteins, 17%), lipids (Pulmocare, Abbott; carbohydrates, 28%; lipids, 55.5%; proteins, 16.5%), or proteins (Cubitan; Nutricia, Milan, Italy; carbohydrates, 45%; lipids, 25%; proteins, 30%). Each patient received the different meals in random order. For postmeal analyses, blood was drawn at 20-min intervals for 120 min for determination of ghrelin, insulin, leptin, and glucose levels.

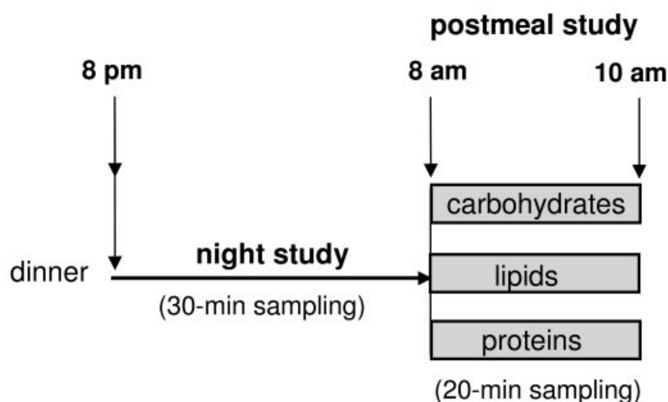

FIG. 1. Schematic illustration of timing and frequency of blood sampling. The meal tests were administered in random order on 3 consecutive days after the night study.

Sampling and analytical procedures

Plasma aliquots for determination of ghrelin concentrations were collected into plastic tubes containing EDTA and allowed to sit on ice for 10 min. After centrifugation, plasma was separated with aprotinin added and stored immediately at −80 C until assay. Frozen aliquots were assayed within 2 months after collection without additional freeze-thaw cycles; samples obtained from each subject were processed in single assays to minimize analytical variations. Serum samples for insulin and leptin were separated after centrifugation and subjected to analysis. Commercial kits were used for RIA measurement of plasma ghrelin (Phoenix Pharmaceuticals, Mountain View, CA; sensitivity, 1 pg/tube; intraassay coefficient of variation, 5.3%) and serum leptin levels (Linco Research, Inc., St. Charles, MO; sensitivity, 0.5 ng/ml; intraassay coefficient of variation, 8.3%). Insulin levels were measured by chemoluminescence (Immulite 2000 Analyzer; Diagnostic Products Corp., Los Angeles, CA). Plasma glucose was assessed by enzymatic methods (Roche Molecular Biochemicals, Mannheim, Germany). Factors for conversion from metric to SI units are: insulin, microunits per milliliter × 6 = picomoles per liter; glucose, milligrams per deciliter × 0.05551 = millimoles per liter. Insulin homeostasis was calculated by a homeostatic computed model approach insulin-sensitivity (HOMA-S%) available at www.OCDEM.ox.ac.uk, which can be used to determine insulin sensitivity and β-cell function from paired fasting plasma glucose and RIA insulin, specific insulin, across a range of 1–2200 pmol/liter for insulin and 1–25 mmol/liter for glucose (24).

To quantify the serial irregularity of nocturnal hormone secretion, we used approximate entropy (ApEn), a large-scale, translation-invariant and model-independent regularity statistic quantifying the orderliness of sequential measures, such as hormone time series, over a sufficiently prolonged period of time (25). ApEn is complementary to pulse detection algorithms widely employed to appraise hormone secretion time series. It evaluates both dominant and subordinated patterns in data and detects changes in underlying episodic behavior not reflected in peak occurrences or amplitudes. Profiles were analyzed with window length $m = 1$ and tolerance parameter $r = 20\%$ of the average SD of the individual subject time series. Cross-ApEn was evaluated to quantify asynchrony (conditional irregularity) and compare sequences from two distinct yet intertwined variables in the network of ghrelin with insulin or leptin concentrations (26). Cross-ApEn differs from cross-correlation analysis because it is independent of lags. Larger ApEn values correspond to greater randomness (irregularity).

Statistical analysis

Anthropometric and hormonal data are expressed as mean ± SEM. Hormonal levels were calculated during the study night as mean values, 4-h mean blocks, and area under the curve (AUC) of plasma concentrations *vs.* time by trapezoidal formula, as well as by ApEn and cross-ApEn as mentioned above. Postprandial hormonal responses were calculated as percent change over baseline values and AUCs. For comparative analyses, two-tailed paired and unpaired Student's t test were used when appropriate. Associations between hormonal variables and anthropometric parameters were analyzed by Pearson's analysis. Independent anthropometric and glucose-homeostatic variables potentially influencing ghrelin responses to meals were tested by multiple linear regression analysis from a variety of iterative model-building strategies. Statistical analyses were performed using SPSS 11.0 (SPSS, Inc., Chicago, IL) and Prism (GraphPad Software, Inc., San Diego, CA). Significance was set at $P < 0.05$.

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Results

Anthropometric characteristics of study participants are illustrated in Table 1. Study groups had similar mean age and sex distribution and dissimilar measures of adiposity, energy expenditure, and insulin homeostasis. A significant correlation was seen between BMI and fat mass (r = 0.62; $P < 0.01$) as well as REE (r = 0.84; $P < 0.001$).

Nocturnal profiles. Twelve-hour nocturnal profiles of ghrelin, insulin, and leptin plotted at 30-min intervals are summarized in Fig. 2 and Table 2. In line with literature data, lean controls showed declining ghrelin levels until 2400 h, followed by an increase until 0200 h, whereas obese patients showed no fluctuations of ghrelin, this being significantly lower ($P < 0.01$) than in controls. Peak-to-nadir variations were 33% and 17% in lean and obese subjects, respectively. Also, females had higher ghrelin levels than males both in the lean (275 ± 58.3 *vs.* 155.8 ± 27.5 pg/ml; $P < 0.001$) and obese groups (123.3 ± 1.5 *vs.* 102.2 ± 1.6 pg/ml; $P < 0.001$). Insulin levels decreased to reach their nadir at 0400 h in lean controls (59% lower than 12-h mean). Mean and AUC insulin levels were significantly higher in obese patients ($P < 0.01$), where nocturnal nadirs at 2400 and 0600 h were 29 and 36% lower, respectively, than 12-h mean values. Leptin levels were expectedly decreased in controls ($P < 0.01$ *vs.* obese) where peak and nadir values occurred at 2400 and 0700 h (19% and 20% variations over the 12-h mean, respectively); overall peak-to-nadir variation was 49%. Obese patients had more fluctuating leptin levels, with peaks 20 and 13% higher than nadir and 12-h mean concentrations, respectively.

ApEn scores displayed statistical structures significantly different from random for ghrelin and leptin levels. Mean ApEn values differed between lean and obese subjects only for ghrelin values (0.81 ± 0.05 *vs.* 0.95 ± 0.02; $P < 0.01$), whereas similar ApEn scores were obtained for insulin (0.29 ± 0.02 *vs.* 0.32 ± 0.05) and leptin values (0.96 ± 0.04 *vs.* 0.94 ± 0.02). When stratified by gender, significant differences were documented in ApEn insulin values between obese females and males (0.47 ± 0.04 *vs.* 0.17 ± 0.03; $P < 0.001$). Assessment by cross-ApEn of the synchronicity of secretory pulses among ghrelin, insulin, and leptin time series revealed a temporally patterned synchronicity for both the lean and obese groups between ghrelin-insulin (0.96 ± 0.04 *vs.* 1.15 ± 0.04; $P < 0.01$), ghrelin-leptin [1.26 ± 0.06 *vs.* 1.29 ± 0.01; P = not significant (ns)], and insulin-leptin levels (1.15 ± 0.05 *vs.* 1.06 ± 0.06; P = ns).

Test meal studies. Before meal administration, ghrelin levels were on average 59 and 33% higher than nocturnal nadir in lean and obese subjects, respectively. After test meals, both lean and obese subjects experienced a significant decrease of ghrelin (Table 3 and Fig. 3) under carbohydrate ($P < 0.001$ and $P < 0.0001$, respectively), lipid ($P < 0.01$ and $P < 0.001$) and protein test meals ($P < 0.01$ and $P < 0.001$). Ghrelin nadir occurred within 60–90 min after ingestion to levels (lean *vs.* obese) 42.2 ± 7.7% *vs.* 28.5 ± 3.5% ($P < 0.05$), 40.2 ± 2.9% *vs.* 26.2 ± 3% ($P < 0.01$), and 42.2 ± 5.3% *vs.* 26.3 ± 3.1% ($P < 0.01$) lower than baseline. Postmeal ghrelin profiles were lower in obese than lean subjects under either meal ($P < 0.01$ for all). Within each study group, ghrelin nadir and AUC values were comparable under the three test meals (Table 3). Postmeal insulin response was significantly higher in obese than lean subjects under each test meal ($P < 0.01$ for all) and

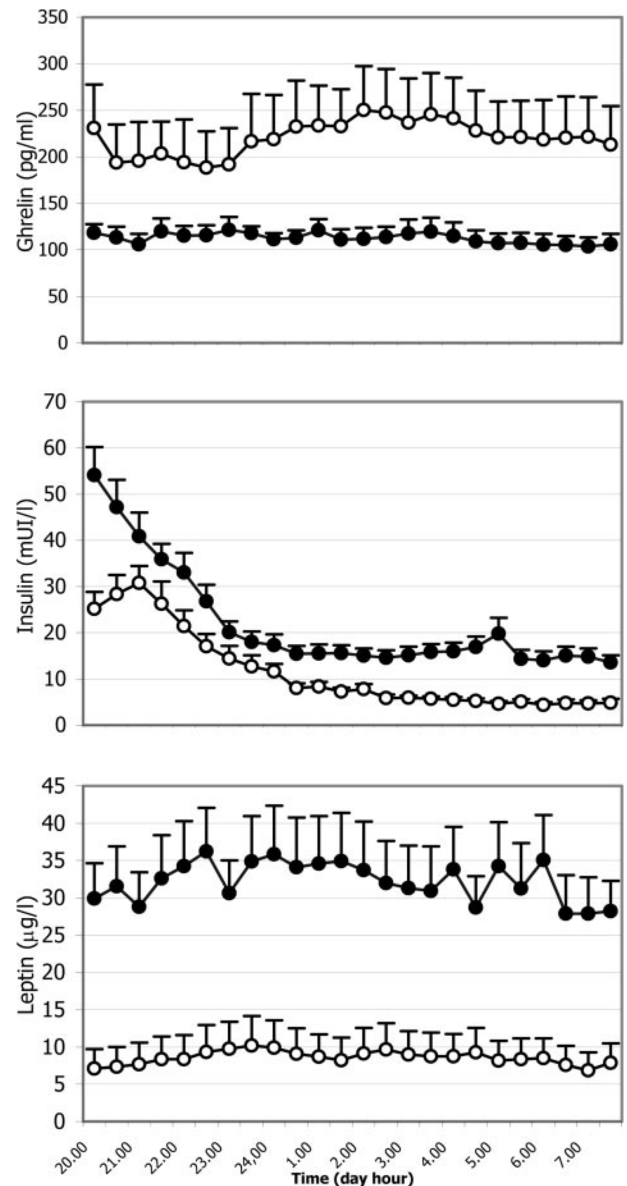

FIG. 2. Nighttime profiles of ghrelin (*upper panel*), insulin (*middle panel*), and leptin (*lower panel*) plotted at 30-min intervals from 2000 to 0730 h in lean (*open circles*) and obese subjects (*closed circles*). For significance, differences between the two groups were significant at all time points of the night study ($P < 0.05$).

TABLE 1. Anthropometric characteristics of the study groups

Parameters	Lean	Obese
Age (yr)	33.5 ± 2.4	31.8 ± 2.5
Males/females	3/3	5/5
BMI (kg/m^2)	21.8 ± 1.4	43 ± 0.9[a]
Body fat mass (%)[c]	20 ± 3.3	46.8 ± 2.1[a]
REE (kcal/24 h)[d]	1659 ± 109	2112 ± 140[b]
HOMA-S%	168.7 ± 25.3	58.8 ± 10.5[a]

For significance, lean *vs.* obese: [a] $P < 0.01$; [b] $P < 0.05$.
[c] As determined by total-body dual-energy x-ray absorptiometry.
[d] As determined by indirect calorimetry.

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TABLE 2. Nocturnal AUC, mean and time block series (2000 to 2400 h; 0030 to 0400 h; 0430 to 0800 h) of ghrelin, insulin, and leptin levels in lean and obese subjects

	Ghrelin (pg/ml)		Insulin (mU/liter)		Leptin (μg/liter)	
	Lean	Obese	Lean	Obese	Lean	Obese
AUCs	152,258 ± 29,066	77,823 ± 6,931[a]	7,807 ± 862	14,710 ± 1,365[a]	5,944 ± 2,106	22,327 ± 2,668[a]
Mean levels	220.7 ± 58	112.8 ± 18[a]	11.5 ± 2.7	21.9 ± 3.7[a]	8.6 ± 0.3	32.2 ± 0.8[a]
Time blocks						
2000–2400 h	203.7 ± 45	115.5 ± 30[a]	20.8 ± 0.8	32.6 ± 1.7[a]	8.6 ± 0.4	32.7 ± 0.8[b]
0030–0400 h	240 ± 26	115.4 ± 27[a]	6.8 ± 0.2	15.4 ± 0.2[a]	8.9 ± 0.2	33.2 ± 0.5[a]
0430–0800 h	223.8 ± 18	107.2 ± 10[a]	5.1 ± 0.9	15.7 ± 0.7[a]	7.9 ± 0.2	30.2 ± 1.2[a]

For significance, lean *vs.* obese: [a] $P < 0.01$; [b] $P < 0.05$.

increased significantly after carbohydrates, lipids, and proteins ($P < 0.01$ for all) to proportions dissimilar between lean and obese subjects (Fig. 3). Leptin concentrations were significantly higher in obese than lean subjects during each test ($P < 0.001$ for each test), with a mild decrease after carbohydrates, lipids, and proteins in lean and obese subjects (Table 3).

Correlation analyses. Analysis of nocturnal datasets showed that ghrelin ApEn scores were positively correlated with fasting insulin (r = 0.53; $P < 0.05$). The association between nocturnal ghrelin ApEn and BMI (r = 0.64; $P < 0.01$) or fat mass (r = 0.60; $P < 0.05$) was opposite to that seen between nocturnal ghrelin AUC and BMI (r = −0.49; $P < 0.05$). Insulin and leptin AUCs were reciprocally correlated (r = 0.58; P = 0.02), and each was, in turn, positively associated both with fat mass (r = 0.56, P = 0.02; and r = 0.84, $P < 0.001$, respectively) and BMI (r = 0.67, P = 0.004; and r = 0.58, P = 0.02, respectively). Opposite associations related insulin AUC and ApEn scores to REE (r = 0.60, P = 0.02; and r = −0.65, $P <$ 0.01, respectively). Nocturnal and postabsorptive ghrelin AUCs were correlated following carbohydrates (r = 0.61; $P <$ 0.05), lipids (r = 0.67; $P < 0.01$), and proteins (r = 0.61; $P <$ 0.05).

As summarized in Table 4, analysis of postmeal datasets showed that ghrelin was unrelated to insulin levels, whereas it was positively associated with HOMA-S%, when measured as either AUC or percent variation. A relationship also became evident between ghrelin ApEn and ghrelin suppression after meals (r = −0.63; $P < 0.01$). Based on significant associations, group datasets from each meal test were pooled and tested by multivariate regression analysis after exclusion of dichotomized variables potentially interrelated by multicolinearity (*i.e.* fat mass, BMI, and weight). The fraction of ghrelin variance calculated as postmeal AUC and percent suppression was explained for 79 and 94%, respectively, by a model that included REE, ghrelin ApEn scores, HOMA-S%, as well as postmeal insulin and glucose AUCs and their percent variations. Stepwise analysis indicated that REE (β = −0.57; P = 0.02) and ghrelin ApEn values (β = −0.62; P = 0.01) independently predicted postmeal ghrelin AUC and percent variation, respectively. Group-stratified stepwise analysis confirmed that REE was a significant predictor of ghrelin AUC in normal-weight individuals (β = −1.03; $P <$ 0.0001), followed by fat mass (β = −0.83; $P < 0.0001$). Only in this state, insulin Δ was an independent predictor of percent ghrelin suppression (β = 0.64; P = 0.005). In the obese group, blood glucose AUC significantly predicted ghrelin AUC (β = −0.45; P = 0.013).

Discussion

The current study confirms that nocturnal ghrelin secretion is less oscillatory and overall decreased in obese compared with lean adults, with a quantifiable level of orderliness significantly different from random in both groups, but

TABLE 3. Baseline, nadir or peak concentrations, maximum percent change (mean Δ, %), and mean AUC of ghrelin, insulin, plasma glucose, and leptin levels during 2-h sampling after the ingestion of isovolumic isocaloric (500 kcal) carbohydrate-, lipid-, and protein-rich test meals in lean and obese subjects

Meal type	Ghrelin (pg/ml)		Insulin (mIU/liter)		Glucose (mg/dl)		Leptin (μg/liter)	
	Lean	Obese	Lean	Obese	Lean	Obese	Lean	Obese
Carbohydrates								
Baseline	247 ± 39	117 ± 10[a]	5.9 ± 0.9	14.5 ± 1.3[b]	91.3 ± 2.2	91 ± 3.5	6.6 ± 2.4	20.3 ± 3.4[a]
Nadir or peak	144 ± 26[c]	90 ± 8[b,c]	51.3 ± 10.4[c]	128.5 ± 21.7[b,c]	128.3 ± 9.8[c]	135.7 ± 8.1[c]	5.2 ± 2.1	20 ± 3.1[a]
Mean Δ (%)	−42.2 ± 7.7	−28.5 ± 3.5[b]	939 ± 163	792 ± 137	41 ± 8	61 ± 7	−18 ± 7	−2.5 ± 11
AUC	21,111 ± 3,463	11,578 ± 1,036[a]	4,361 ± 1,027	10,831 ± 1,485[a]	12,797 ± 1,170	14,450 ± 950	681 ± 193	2,515 ± 384[a]
Lipids								
Baseline	233 ± 46	113 ± 12[a]	4.4 ± 2.7	17.1 ± 2.1[a]	88.3 ± 1.7	93.1 ± 3.9	6.6 ± 2.4	28 ± 7.1[b]
Nadir or peak	143 ± 24[c]	91 ± 8[b,c]	24.2 ± 3.5[c]	107 ± 21[b,c]	101.3 ± 4.1[c]	120.1 ± 5.5[a,c]	5.2 ± 2.1	21.6 ± 4.1[b]
Mean Δ (%)	−40.2 ± 2.9	−26.2 ± 3[a]	625 ± 75	536 ± 119	20 ± 3	35 ± 5	−12.4 ± 5	−19 ± 8
AUC	19,913 ± 3,486	11,674 ± 1,114[b]	2,398 ± 253	8,278 ± 1,496[a]	11,371 ± 324	13,245 ± 489[b]	746 ± 261	2,893 ± 594[b]
Proteins								
Baseline	255 ± 36	111 ± 10[a]	5.4 ± 1.1	14.7 ± 1.6[a]	87.8 ± 3.1	91.9 ± 3.1	7.1 ± 2.6	27.3 ± 4.5[a]
Nadir or peak	142 ± 18[c]	92 ± 8[a,c]	56.9 ± 10.6[c]	96.7 ± 11.1[b,c]	103 ± 6.8[c]	118.9 ± 6.3[b,c]	6.5 ± 2.4	26.4 ± 4.2[a]
Mean Δ (%)	−42 ± 5	−26 ± 3[b]	1,129 ± 226	744 ± 86	24 ± 4	37 ± 4	−16 ± 7	−5.3 ± 12.8
AUC	22,586 ± 3,412	11,635 ± 1,134[b]	4,586 ± 952	9,353 ± 1,335[b]	11,411 ± 717	13,237 ± 622	806 ± 295	3,329 ± 474[a]

For significance, lean *vs.* obese subjects: [a] $P < 0.01$, [b] $P < 0.05$; within-group significance: baseline *vs.* nadir or peak, [c] $P < 0.01$.

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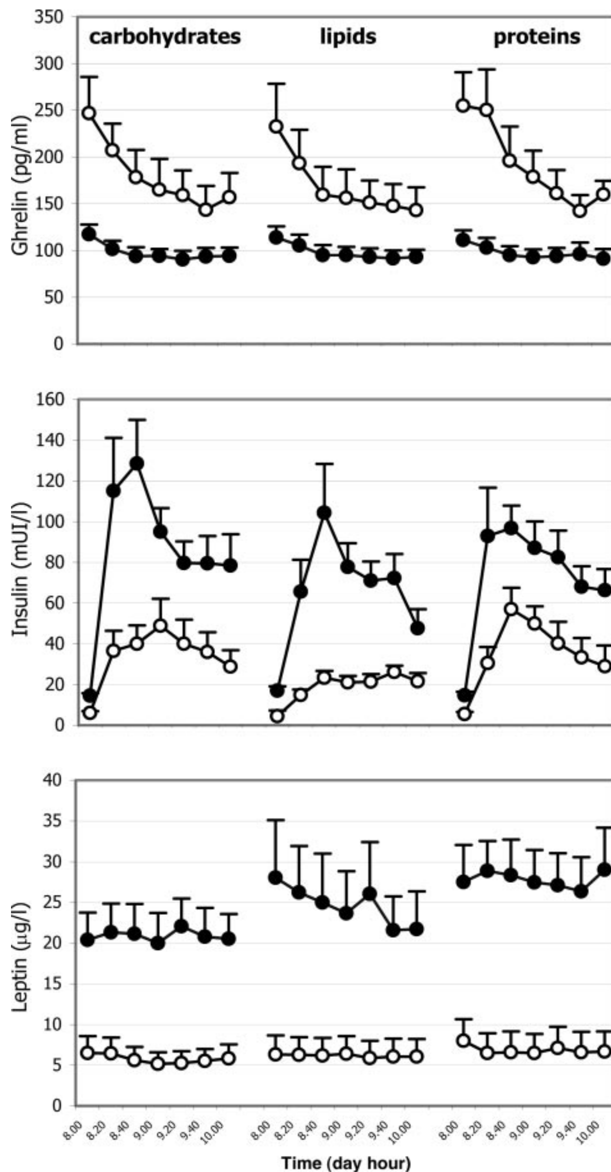

FIG. 3. Postmeal profiles of ghrelin (*upper panel*), insulin (*middle panel*), and leptin levels (*lower panel*) plotted at 20-min intervals between 0800 and 1000 h after test meals rich in carbohydrates (*left*), lipids (*center*), and proteins (*right*) in lean (*open circles*) and obese subjects (*closed circles*). For significance, differences between the two groups were significant at all postmeal time points ($P < 0.05$).

remarkably greater in lean than obese subjects. Cross-ApEn for ghrelin and insulin profiles was significantly higher in obese than lean subjects, which signifies relative loss of ghrelin-insulin synchrony in obese when compared with lean subjects. In an analysis controlled for gender, females of both study groups showed significantly higher and more fluctuating nocturnal ghrelin concentrations than males. So far, the endogenous pattern of ghrelin secretion has been largely characterized in relation to food intake, nutritional disorders, and treatments for altered body weight, and daily oscillations of ghrelin are known to be blunted in obesity (6–8, 12, 19, 20, 22). Ghrelin secretion could be expected to be less organized in obesity, and the correlation herein observed between ApEn and a cluster of anthropometric/metabolic variables (*i.e.* fat mass, BMI, and fasting insulin) appeared to reflect this hypothesis. However, a prior study failed to document differences in ghrelin randomness and ghrelin-insulin synchrony between lean and obese individuals subjected to frequent sampling (20). Discrepancies between this (20) and our fasting evaluation might thus result from differences in sampling frequency or time frames analyzed, because 24-hr ApEn scores included up to four post-feeding conditions (20). In line with our findings, a study on nocturnal ghrelin ApEn obtained values (0.93 ± 0.09) comparable to ours using the same commercial immunoassay (27). Discrepancies may also originate, however, from different samples collection, preanalytical treatment (28), immunoassay standards (29) and immunoreactivity for ghrelin fragments (30). Noteworthy, total ghrelin levels measured by commercial kits used here and elsewhere (20) may differ by a factor of 10 (29). Also, assays measuring total ghrelin capture all ghrelin-like immunoreactive proteins. Hence, variations in ghrelin levels can be attributed not only to acylated ghrelin, which regulates appetite and energy balance centrally, but also to fragments derived from degradation, alternative splicing, or mutations of the ghrelin gene, which presumably would be inactive (31).

Analysis of postmeal ghrelin responsiveness to carbohydrate-, lipid-, and protein-rich test meals revealed that each preload caused a significant decrease of ghrelin secretion not only in the lean state, as already known (8–11, 22, 32, 33), but also in obese individuals, by an order of magnitude that was on average half in obese to that obtained in controls and similar among the different preloads. Like in other investigations (8, 9, 32), the preloads used in our study consisted of a combination of nutrients reflecting those of standard meals. An expected association related ghrelin secretion, under any study condition, with BMI and fat mass, which confirmed the tight dependence of ghrelin secretion on anthropometric determinants in fasting as well as postabsorptive conditions. A key finding was the independent predictive role of REE on postmeal ghrelin secretion. The evidence that this relationship was preserved in the lean state, where ghrelin is higher and more responsive to calorie ingestion, strengthens the relevance of this observation. These observations confirm previous data showing that ghrelin secretion was decreased in obesity as a compensatory mechanism in cases of impaired energy expenditure (34) and agree with the significant correlation obtained between ghrelin and either resting or postprandial energy expenditure in a study on young healthy women, independent of their variations in body composition, insulin levels, and daily energy intake (35). Indeed, caveats in our findings are the small study population, which does not allow us to expand current observations to other states of altered ghrelin homeostasis (*i.e.* childhood obesity, elderly status, and diabetes mellitus), as well as the lack of discrimination of the assay between acylated and des-acylated ghrelin. In turn, this does not allow us to attribute specific effects of the two variants on energy expenditure (36).

Unlike body fat (12), the role of insulin homeostasis on ghrelin responsiveness to feeding is controversial. It is known that insulin administration blunts ghrelin secretion in normal as well as type 1 and 2 diabetic individuals undergoing euglycemic or hypoglycemic clamp studies (13–15). Insulin resistance negatively regulates fasting ghrelin levels

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TABLE 4. Bivariate regression analysis between postmeal ghrelin AUC or percent change (mean Δ, %) and anthropometric measures, postmeal hormonal changes, and insulin sensitivity index

Postmeal ghrelin	Anthropometric variables		Premeal variables			Postmeal Δ		
	BMI	%FM	REE	ApEn	HOMA-S%	Insulin	Glucose	Leptin
Carbohydrates								
AUC	-0.75^b	-0.64^b	-0.54^a	−0.07	0.29	−0.22	−0.34	0.17
% Δ	−0.31	-0.63^b	−0.09	-0.57^a	0.29	0.33	−0.09	0.11
Lipids								
AUC	-0.69^b	-0.56^a	-0.59^a	−0.21	0.52^a	−0.26	-0.52^a	0.07
% Δ	-0.63^a	-0.67^b	−0.08	−0.43	0.39	0.08	−0.08	−0.33
Proteins								
AUC	-0.80^b	-0.69^b	-0.57^a	−0.35	0.61^b	0.24	-0.57^a	−0.07
% Δ	-0.63^b	-0.73^b	−0.36	-0.54^a	0.73^b	0.41	−0.25	0.33
All meals								
AUC	-0.76^b	-0.65^b	-0.57^a	−0.25	0.52^a	−0.02	-0.55^a	0.06
% Δ	-0.72^b	-0.82^b	−0.14	-0.62^b	0.60^a	0.36	−0.38	−0.04

%FM, Percent free fat mass.
For significance of regression coefficients: ^{a} $P < 0.05$; ^{b} $P < 0.01$.

in obesity (16, 37), as well as in nonalcoholic fatty liver disease independent of BMI (17). A negative association has also been recorded between insulin or insulin resistance and ghrelin in a middle-aged hypertensive population (18). This relationship becomes questionable in postabsorptive conditions. Previous studies either found a negative association between postmeal insulin increment (nadir to peak) and ghrelin decrement (peak to nadir) in a population with a wide variation in BMI and age (16) or obtained a negative relationship between these hormones only in fasting conditions, in individuals bearing different nutritional disorders (22).

Our data do not imply direct postabsorptive associations between ghrelin and insulin levels. However, our observations of nocturnal ghrelin-insulin synchrony and postmeal associations either between ghrelin AUC and insulin sensitivity or between ghrelin and insulin Δ values are suggestive of an interaction between the two systems. Remarkably, insulin sensitivity was the only metabolic variable associated with both ghrelin AUC and the postmeal variation. Although our study cannot address whether reciprocal control mechanisms are involved, previous studies showed that ghrelin blunts insulin secretion in healthy subjects (4), and, at supraphysiological doses, it negatively regulates glucose-mediated insulin secretion in rodent pancreatic insulae (38). It may be interesting to note that reelaboration of our postmeal insulin-glucose datasets as a function of the Matsuda-DeFronzo equation, which is only validated for oral glucose tolerance test (39), simulated an index of insulin sensitivity that independently predicted postmeal ghrelin secretion (data not shown). We speculate that more adequate assessment may help to discriminate the effects of postabsorptive insulin sensitivity on ghrelin control *vs.* nutrient characteristics (10, 11, 22, 32, 40). Our results should neglect the potential effects of the gut insulinotropic hormone incretins, *i.e.* gastric inhibitory-peptide, glucagon-like peptide-1 and peptide-YY. Incretins are responsible for as much as half of the glucose-dependent insulin release after food ingestion, and debated evidence exists that the enteroinsular axis is impaired in obesity and in conditions of insulin resistance (41). Although it is possible that postmeal insulin increments occurring in insulin-resistant subjects are due to the elevation of plasma glucose and nonesterified fatty acids, clinical studies on obese and anorexic adolescents have been inconclusive so far with regard to the role played by incretins on the postmeal ghrelin secretion (22).

One potential factor involved in the control of ghrelin is leptin, an adipocytokine that signals the amount of energy accumulation to the hypothalamus and is implicated in long-term control of energy balance. *In vitro* studies have previously shown that leptin inhibits ghrelin production from the gastric mucosa (42), and leptin levels in humans are inversely related to ghrelin concentrations (6, 16). Additionally, a significant negative interaction between ghrelin and leptin levels has been found by multivariate analysis in normoinsulinemic subjects (43), whereas a 3-d leptin administration to fasting healthy subjects did not significantly alter ghrelin secretion (21). All experimental conditions of our study showed higher leptin levels in obese than normal subjects without reciprocal postmeal interactions between leptin and ghrelin. Associations were significant between postmeal leptin and insulin, but not with insulin sensitivity indices. This result does not seem to support a role for ghrelin or pancreatic β-cell function in the postmeal regulation of leptin.

In summary, we found that nocturnal ghrelin secretion was blunted in the obese state, where a relative loss of ghrelin-insulin synchrony occurred compared with lean subjects. Postprandial ghrelin suppression was independent of macronutrients contained in test meals and occurred in both lean and obese subjects, being negatively regulated however by adiposity. The overall secretion of ghrelin was related to insulin homeostasis and independently predicted by REE and the spontaneous hormone pulsatility. This may imply that ghrelin secretion, in addition to controlling satiety and substrate oxidation, may potentially reflect energy efficiency through peripheral signals involving insulin sensitivity.

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