

Differential Association of Basal and Postprandial Plasma Ghrelin With Leptin, Insulin, and Type 2 Diabetes

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To gain further insight into the regulatory role of insulin and leptin on plasma ghrelin, 56 normal weight, 128 normoinsulinemic obese, 121 hyperinsulinemic obese, and 30 type 2 diabetic normoinsulinemic and 75 type 2 diabetic hyperinsulinemic obese patients were examined. In the obese subjects, basal hyperinsulinemia was associated with significantly lower ghrelin independent of BMI, age, and leptin. In normoinsulinemic (normal weight and normoinsulinemic obese) subjects, ghrelin was inversely related to stepwise increasing leptin. Multiple regression analysis and matching for insulin revealed a significant negative interaction of ghrelin with leptin but not insulin. In type 2 diabetic normoinsulinemic subjects, ghrelin was significantly lower compared with that in normoinsulinemic obese subjects. In type 2 diabetic hyperinsulinemic subjects, ghrelin was significantly lower than in normoinsulinemic subjects, whereas no further reduction was observed compared with hyperinsulinemic obese subjects. The postprandial decrease was significantly attenuated in normoinsulinemic obese and hyperinsulinemic obese subjects (-214.8 ± 247 pg/ml [normal weight], -137.6 ± 107 pg/ml [normoinsulinemic obese], -85.5 ± 69 pg/ml [hyperinsulinemic obese], $P < 0.001$; mean \pm SD), whereas type 2 diabetes had no independent postprandial effect. In conclusion, the present data support the concept that leptin could be of importance for suppression of basal ghrelin during moderate weight gain in normoinsulinemic subjects, whereas hyperinsulinemia but not leptin is responsible in more severe obesity. Postprandial suppression of ghrelin is attenuated by as yet unknown mechanisms that are related to body weight but not to insulin or type 2 diabetes. *Diabetes* 54:1371–1378, 2005

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HOMA-IR, homeostasis model assessment for insulin resistance.

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Meal ingestion activates satiety signals mainly via gastric distention supported by the nutrient content. This information is conveyed to hypothalamic areas of feeding regulation predominantly by afferent vagal pathways. Distention but not nutrient-induced satiety can be abolished by vagotomy, which suggests the regulation of feeding behavior by additional hormonal factors (rev. in 1–3).

Ghrelin, a recently discovered orexigenic hormone of gastric origin, (4,5) is a major candidate for this endocrine arm of gastric feeding regulation. Administration of ghrelin stimulates appetite and food intake in several species including humans (5–9). Plasma levels of ghrelin decrease in response to carbohydrate-rich meals, whereas carbohydrate-poor meals elicit a postprandial increase of circulating ghrelin concentrations (10–14). Because a ghrelin receptor antagonist reduces food intake and weight gain in mice (15), it is most likely that endogenously released ghrelin contributes to the acute regulation of feeding behavior and weight control.

The early postprandial reduction of circulating ghrelin would attenuate the hormonal gastric feeding drive, thereby supporting neurally activated satiety signals. The progressively increasing ghrelin levels during the later postprandial and interdigestive phase back to baseline could then contribute to the recurrence of appetite and hunger sensations (10). Thus, ghrelin is part of the neuroendocrine gastrohypothalamic axis that is important for short-term regulation of satiety and meal size.

The available evidence indicates that ghrelin secretion not only responds to acute food intake but also to the overall nutritional status of the organism. Basal plasma ghrelin levels are increased in states of malnutrition such as cachexia and anorexia nervosa, whereas obesity is associated with lower basal ghrelin. Changes of body weight are accompanied by the corresponding inverse changes of plasma ghrelin concentrations, respectively (16–23).

One possibility to explain lower ghrelin levels in obese subjects is the elevation of insulin, which is supported by some (24–26) but not all (27,28) insulin infusion studies. In vitro in the isolated stomach, insulin is a potent inhibitor of ghrelin secretion (29). This inverse relationship is supported further by increased ghrelin levels in insulin-deficient diabetic rats (30). Postprandial suppression of ghrelin is associated with meals containing a sufficient

TABLE 1
Demographic data of the study population

	NW subjects	NO subjects	HO subjects	DNO subjects	DHO subjects
<i>n</i> (men/women)	56 (36/20)	128 (26/102)	121 (37/84)	30 (12/18)	75 (29/46)
Age (years)	30 ± 10.5	45 ± 13.6*	44 ± 14.3*	55 ± 12.1	52 ± 13
BMI (kg/m ²)	22 ± 2.2	33.8 ± 5.66*	41.4 ± 8.8*†	34 ± 7.1	40 ± 9.5‡
Height (cm)	176 ± 0.08	169 ± 0.11*	172 ± 0.11*†	172 ± 0.11	170 ± 0.17‡
Weight (kg)	68 ± 10.5	96.6 ± 18.1*	122.5 ± 30.8*†	99.5 ± 18.6	116.5 ± 30.3‡
Systolic blood pressure (mmHg)	112.9 ± 13.5	122.8 ± 47.5	132.4 ± 51.7*	132.1 ± 22.5	138.3 ± 28.9
Diastolic blood pressure (mmHg)	70.4 ± 13.5	80.4 ± 39.6	84.2 ± 40.7*	77.9 ± 15.3	85.1 ± 15.6§
HbA _{1c} (%)	5.25 ± 0.67	5.57 ± 1.1	5.60 ± 1.1	7.42 ± 2.2	7.23 ± 2.3

Data are means ±SD unless otherwise indicated. DHO, type 2 diabetic hyperinsulinemic obese; DNO, type 2 diabetic normoinsulinemic obese; HO, hyperinsulinemic obese; NO, normoinsulinemic obese; NW, normal weight. **P* ≤ 0.01 vs. NW; †*P* ≤ 0.01 vs. NO; ‡*P* ≤ 0.01 vs. DNO; §*P* = 0.03 vs. DNO.

amount of carbohydrate to raise glucose and insulin levels substantially (10,11,13,14,20,31,32). In patients with type 1 diabetes, a replacement of basal insulin is mandatory for the postprandial suppression of ghrelin (33), suggesting a permissive effect of insulin for ghrelin cell responsiveness to potential inhibitory factors such as gastrointestinal hormones (29,34). Thus, increased insulin levels in obese subjects are one potential pathway for a cross talk between the energy reserve of the organism and the gastric neuroendocrine control system of short-term feeding regulation.

A second pathway could be generated by a direct effect of fat cell secretory products on ghrelin secretion. A major candidate in this context is leptin, which is elevated in the plasma of obese subjects (35,36). An inverse relationship between leptin and ghrelin has been reported (18,21), which would support a contribution of leptin to obesity-related low ghrelin levels. The experimental evidence, however, for such a negative feedback control is still a matter of debate. In mice, intraperitoneal leptin inhibits ghrelin release (37), and in rats, leptin can prevent the rise of ghrelin during moderate food restriction (38). In line with this observation are the inverse amplitude changes of leptin and ghrelin pulse discharge in rats (39). Furthermore, in the isolated rat stomach, leptin is a potent inhibitor of ghrelin secretion (40,41). In contrast to these findings in rodents are the observations of Chan et al. (42) in normal weight (NW) subjects, in which the administration of recombinant leptin had no effect on plasma ghrelin concentrations.

The aim of the present study was to evaluate the association between ghrelin, leptin, and insulin in greater detail to gain more insight into the differential and possibly independent control mechanisms of basal and postprandial ghrelin release.

RESEARCH DESIGN AND METHODS

A total of 410 patients who visited the outpatient clinic of the department of nutritional medicine were evaluated. The majority of patients were admitted for the treatment of obesity. The NW subgroup (BMI 18.5–24.9 kg/m²) consisted of patients who came for a routine checkup, exclusion of hyper- or dyslipidemia, celiac disease, and lactose or fructose intolerance. As part of the routine examinations, carbohydrate metabolism was evaluated by a standard test meal consisting of bread, butter, and marmalade (260 kcal, 62% carbohydrate, 32% fat, and 6% protein). In patients in whom the postprandial plasma glucose levels exceeded 150 mg/dl, an additional oral glucose tolerance test was performed to exclude impaired glucose tolerance or diabetes mellitus.

For a better discrimination between insulin and leptin effects on ghrelin,

the obese nondiabetic and diabetic subjects were divided according to their fasting basal insulin level. Fasting insulin <6 μU/ml (mean ± 2 SD of NW subjects) was considered as cutoff. Type 2 diabetes was either newly diagnosed in patients in the outpatient center (76%) or diagnosed up to 9 months before admission. The patients included in the study population had neither diabetes-related complications nor were being treated with metformin or any other antidiabetic drug.

Patients who were taking medication that is supposed to influence carbohydrate metabolism and related endocrine functions (e.g., β-blocker, steroids, diuretics, and antidiabetic drugs) were excluded from the analysis unless the respective substances were withdrawn at least for 2 weeks before the test meal. The demographic data of the patients are summarized in Table 1.

After informed consent was obtained, all examinations were performed according to the guidelines of the Ethical Committee of the Technical University of Munich and in accordance to the principles of the Declaration of Helsinki.

Laboratory analysis. Examinations started at 8.00 A.M., after a 12-h overnight fast. An indwelling catheter was inserted into a forearm vein for collection of blood samples at –15, 0, 15, 30, 60, 90, 120, 150, and 180 min. The test meal had to be consumed within 10 min. Samples were collected into plastic tubes containing 1.2 mg of EDTA and 500 kIU of Trasylol for hormone analysis and into NaF-containing tubes for the determination of glucose. They were kept chilled until centrifugation at 2000 rpm for 15 min at 4°C. The separated plasma was stored at –20°C until the time of assay. All samples of one subject were run in duplicate in the same assay.

Plasma ghrelin levels were determined with a commercial radioimmunoassay that has been used in several previous studies (10,13,14,17,24,25) (Phoenix Pharmaceuticals, Belmont, CA). The assay uses ¹²⁵I-labeled bioactive ghrelin as a tracer molecule and a polyclonal antibody raised in rabbits against full-length octanoylated human ghrelin, which detects both active and inactive ghrelin. The interassay and intra-assay coefficients of variation were 10 and 4%, respectively. No cross-reactivity was observed with gastrin, somatostatin, gastric inhibitory polypeptide, glucagon-like peptide 1(7–36)amide, neuromedin C, cholecystokinin, and insulin, respectively.

Insulin was determined using a radioimmunoassay from DPC (Los Angeles, CA). Leptin was measured by a radioimmunoassay purchased from Linco Research (St. Charles, MO). Insulin sensitivity was determined by the homeostasis model assessment for insulin resistance (HOMA-IR) (43). The HOMA-IR was calculated as [fasting blood glucose (milligrams per deciliter) × fasting insulin (microunits per milliliter)]/405. Glucose was measured by the hexokinase method (Roche Diagnostics, Mannheim, Germany).

Statistical analysis. Normally distributed data are expressed as means ± SD, if not otherwise stated. In the case of non-Gaussian distribution (ghrelin, leptin, and insulin) mean, SD, and interquartile range are stated. Incremental levels were calculated for the 180-min postprandial period as the sum of differences between each time point and the mean baseline level. To compare means, the *t* test for independent samples or Welch test was used. Comparisons of means within groups were performed by *t* test for paired data considering multiple testing according to the procedure of Bonferroni. Multiple regression analysis using forward and backward variable selection was used to investigate the influence of independent variables including BMI, leptin, insulin, sex, age, and the interaction term of insulin and leptin on the dependent variable ghrelin. Ghrelin, leptin, and insulin levels were log-transformed to meet linear model assumptions. All *P* values given are two-sided and subject to a significance level of 0.05. Statistical examinations were performed using SPSS (version 11.5).

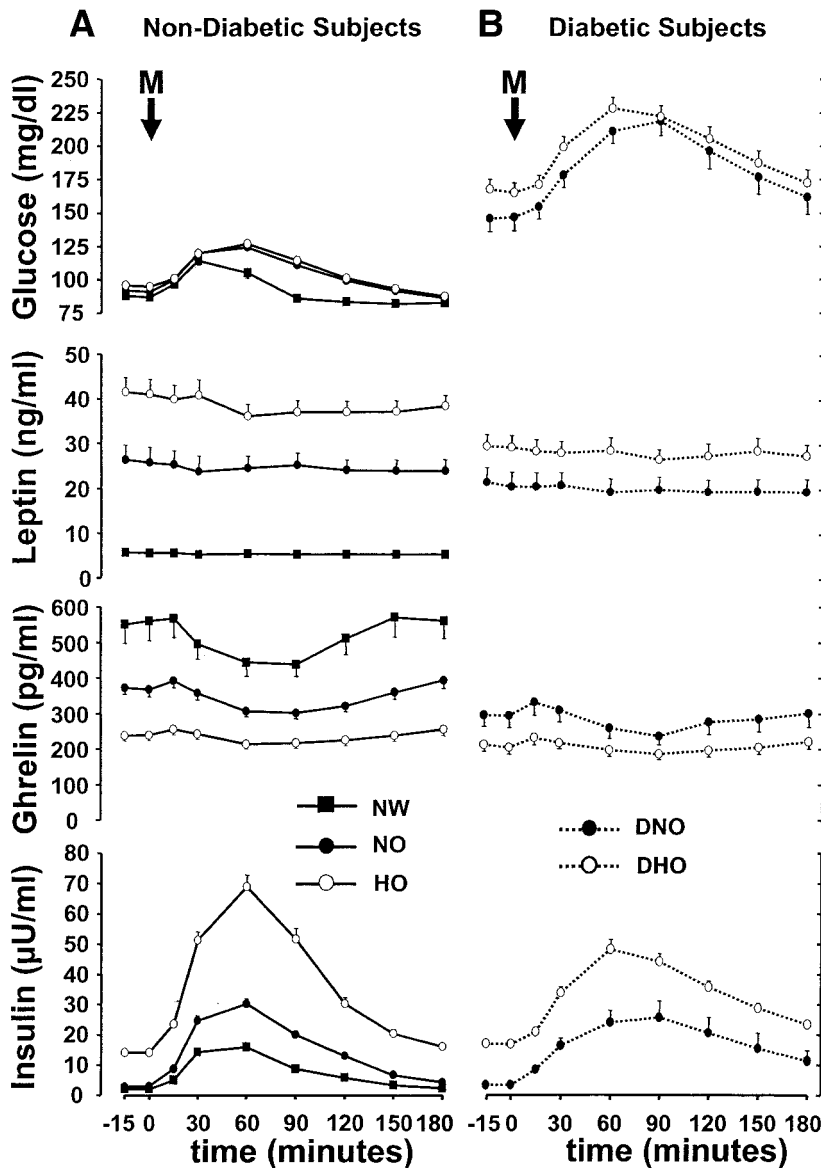


FIG. 1. Effect of a carbohydrate-rich test meal (M) on peripheral plasma insulin ($\mu\text{U}/\text{ml}$), ghrelin (pg/ml), leptin (ng/ml), and glucose (mg/dl) in 56 normal weight (NW), 128 normoinsulinemic obese (NO), and 121 hyperinsulinemic obese (HO) subjects (A) and 30 normoinsulinemic (DNO) and 75 hyperinsulinemic (DHO) patients (B) (means \pm SE).

RESULTS

Basal state. Basal plasma insulin concentrations were 2.00 ± 1.97 (0.53–2.86) $\mu\text{U}/\text{ml}$ (mean \pm SD [interquartile range]) in NW subjects, 2.84 ± 1.58 (1.45–5.01) $\mu\text{U}/\text{ml}$ in normoinsulinemic obese (NO), and 14.1 ± 8.17 (8.0–18.0) $\mu\text{U}/\text{ml}$ in hyperinsulinemic obese (HO) subjects ($P < 0.001$ vs. NW and NO) (Fig. 1).

Basal ghrelin was significantly lower in the NO group (370.3 ± 215.5 [211.8–517.9] pg/ml , $P = 0.002$) compared with NW (555.7 ± 398 [324.8–659.3] pg/ml), and a further reduction to 237.9 ± 162.7 (135.6–303.6) pg/ml ($P = 0.001$ vs. NW and NO) was observed in HO subjects (Fig. 1). Corresponding leptin concentrations were 5.6 ± 5.1 (1.89–7.95) ng/ml (NW), 26.1 ± 19.6 (8.5–32.7) ng/ml (NO) ($P < 0.001$), and 41.3 ± 35.3 (21.9–47.5) ng/ml (HO) ($P < 0.001$ vs. NW and NO). The mean basal plasma glucose level was 87.6 ± 10.9 (81.5–92.5) mg/dl in NW, 91.4 ± 9.8 (84.5–97.5) mg/dl in the NO group ($P < 0.02$), and 95.3 ± 12.2 (86.2–103.5) mg/dl in the HO group ($P < 0.001$ vs. NW and $P = 0.006$ vs. NO). HOMA-IR was 0.49 ± 0.58 (0.1–0.74) in NW, 0.65 ± 0.38 (0.32–0.97) in NO ($P = 0.02$), and 3.34 ± 2.03 (1.83–4.14) in HO subjects ($P < 0.001$ vs. NW and NO).

Obese subjects matched for BMI, sex, age, and leptin.

Forward and backward variable selection resulted in the same covariate model. Multiple linear regression analysis demonstrated that BMI (standardized regression coefficient $\beta = -0.359$, $P < 0.001$), sex ($\beta = 0.229$, $P = 0.001$; with women having higher levels than men), and insulin ($\beta = -0.151$, $P = 0.025$) but not leptin ($\beta = 0.127$, $P = 0.152$) and age ($\beta = 0.116$, $P = 0.908$) had a significant association with ghrelin. When patients with normal and high insulin levels were matched for BMI, sex, age, and leptin, the normoinsulinemic group consisted of 48 subjects (5 men and 43 women; BMI 39.1 ± 4.2 kg/m^2 ; 43 ± 11.8 years), and the high-insulin group contained 48 subjects (6 men and 42 women; BMI 38.4 ± 2.95 kg/m^2 ; 45.1 ± 2.3 years). In this HO subgroup (insulin, 12.8 ± 6.9 $\mu\text{U}/\text{ml}$; leptin, 33.4 ± 13.1 ng/ml), ghrelin was significantly lower with 261.6 ± 171.1 pg/ml compared with the 334.6 ± 173.9 pg/ml ($P = 0.042$) of the NO subgroup (insulin, 2.8 ± 1.4 $\mu\text{U}/\text{ml}$; leptin, 32.2 ± 13.9 ng/ml). On the other hand, increasing leptin concentrations in the hyperinsulinemic subjects had no influence on basal ghrelin levels (Table 2).

TABLE 2
Basal ghrelin levels of HO subjects in relation to stepwise increasing leptin concentrations

	Leptin (ng/ml)				
	5.0–9.9	10.0–19.9	20.0–29.9	30.0–39.9	≥40
Insulin (μU/ml)	14.6 ± 10.3	12.3 ± 6.1	15.0 ± 9.8	13.8 ± 7.8	14.1 ± 8
Ghrelin (pg/ml)	191.1 ± 187.1	270.9 ± 200.9	202.5 ± 109.8	251.05 ± 187.6	251.63 ± 158.5
BMI (kg/m ²)	36 ± 9.8	36 ± 5.7	41 ± 5.2	41 ± 8.8	45 ± 9.3
n	6	19	24	24	44

Data are means ± SD.

Leptin-ghrelin relationship in normoinsulinemic subjects. Multiple regression analysis of the normoinsulinemic subjects demonstrated a significant association of ghrelin with leptin ($\beta = -0.477, P < 0.0001$) and sex ($\beta = 0.408, P < 0.0001$; with women having higher levels than men) but not with insulin ($\beta = -0.084, P = 0.578$) and age ($\beta = -0.052, P = 0.516$). The highest ghrelin concentration of 658.6 ± 507.5 pg/ml was observed in the men with leptin levels <2.0 ng/ml (Fig. 2), and ghrelin concentration significantly decreased to 444.6 ± 392.6 pg/ml ($P < 0.05$) at leptin levels of 2–4.9 ng/ml and further to 302.4 ± 276.9 pg/ml at leptin levels of 5–9.9 ng/ml ($P < 0.01$). Thereafter, ghrelin values were at a plateau around 400 pg/ml despite substantially higher leptin concentrations. In the women, a similar tendency was observed, but because of the small number of subjects in the low leptin group, no statistics were calculated.

Interestingly, the decrease of ghrelin levels was not only associated with an increase of leptin but also with a small but significant rise of insulin (Fig. 2). To exclude an additional effect of insulin, men with low (<5 ng/ml) or high leptin were matched for insulin. Ghrelin levels were significantly lower in the high leptin group (Fig. 3). They remained unchanged in a third group matched for high leptin and BMI that had higher insulin levels, indicating that in normoinsulinemia, the inverse leptin-ghrelin relation is independent of small but significant alterations of insulin.

Diabetic subjects. Similar to the nondiabetic obese subjects, patients with type 2 diabetes were divided into two

groups according to basal insulin levels below (normoinsulinemic subjects with type 2 diabetes [DNO]) or above (hyperinsulinemic subjects with type 2 diabetes [DHO]) $6 \mu\text{U/ml}$, respectively. Insulin concentrations were 3.25 ± 1.73 (2.2–4.7) $\mu\text{U/ml}$ (DNO) and 16.8 ± 7.9 (10–21.6) $\mu\text{U/ml}$ (DHO) ($P < 0.001$), respectively. The corresponding glucose levels were 148.0 ± 54.2 (106.8–175.5) mg/dl and 168.4 ± 63.8 (122.7–200.4) mg/dl ($P = 0.13, \text{NS}$; Fig. 1). Basal ghrelin levels were significantly lower in the DNO group compared with the DHO group (DNO 300.9 ± 172.1 [161.7–395.9] pg/ml vs. DHO 216.5 ± 150.6 [110.2–275.7] pg/ml, $P = 0.015$). Leptin in DHO patients (29.9 ± 22.5 [15.7–395.9] ng/ml) was significantly higher compared with DNO patients ($21.4 \pm 18.18.2$ [9.5–27.1] ng/ml, $P = 0.04$).

In comparison with the nondiabetic NO subjects, ghrelin was significantly lower in the DNO patients (DNO 300.9 ± 172.1 [161.7–395.9] pg/ml vs. NO 370.3 ± 215.5 [211.8–518] pg/ml, $P < 0.001$). No significant difference was observed between DHO and HO patients (237.9 ± 162.7 [135.6–303.6] pg/ml, NS). Plasma leptin concentration in DNO was lower but not significantly different from the 26.1 ± 9.6 (8.5–32.7) ng/ml of the NO group ($P = 0.13, \text{NS}$), whereas DHO leptin was significantly lower than HO (41.3 ± 35.3 [21.9–47.5] ng/ml, $P < 0.001$; Fig. 1).

Postprandial changes. After meal ingestion, insulin levels were significantly elevated above baseline between 15 and 150 min with a maximum at 60 min in all three groups (Fig. 1)

In NW subjects, postprandial ghrelin remained unchanged during the first 15 min decreasing thereafter

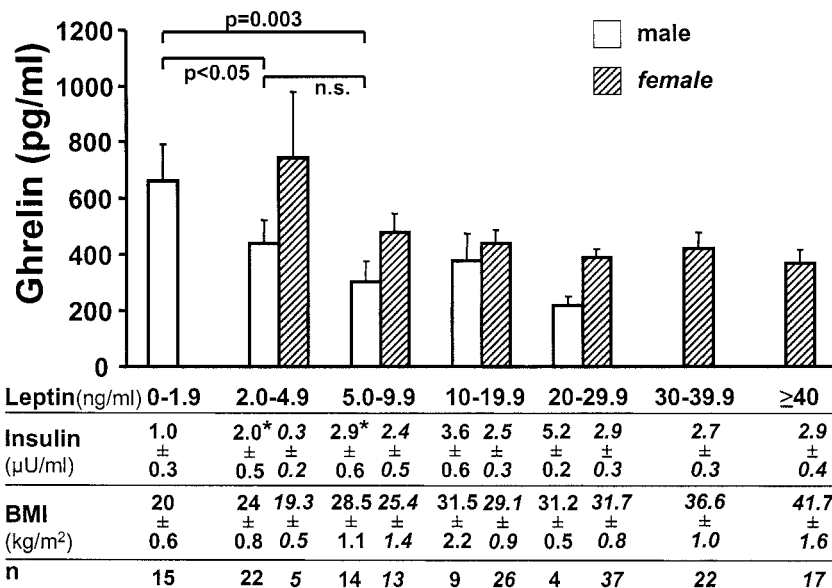


FIG. 2. Basal plasma ghrelin levels in subgroups of male and female normoinsulinemic nondiabetic subjects in relation to stepwise increasing basal leptin concentrations (means ± SE). *Significant difference versus the leptin group 0–1.9.

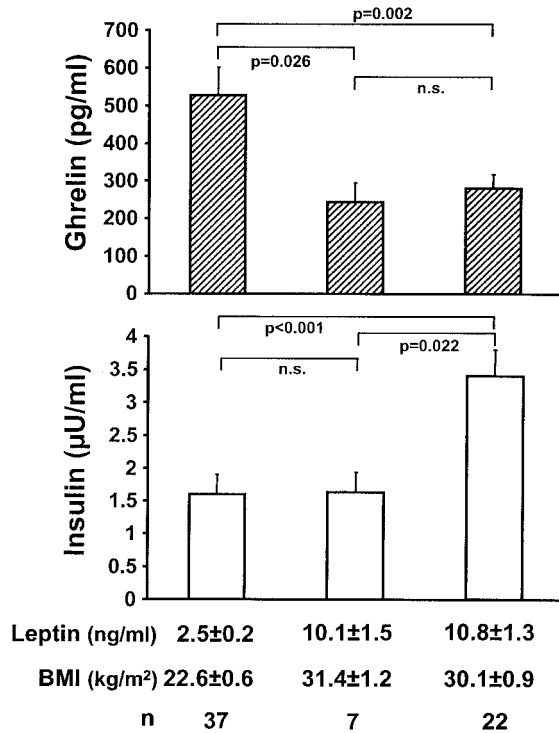


FIG. 3. Basal ghrelin levels in male normoinsulinemic subjects matched for insulin (left and middle bars) or elevated leptin concentrations (middle and right bars), respectively, indicating that significant changes of insulin within the range below 6 µU/ml have no effect on ghrelin (means ± SE).

significantly between 30 and 120 min ($P < 0.01$). In the NO group, ghrelin rose significantly by 22 pg/ml during the first 15 min ($P = 0.007$), decreasing thereafter significantly between 60 and 120 min ($P < 0.001$). At 180 min, ghrelin levels were again significantly above the mean basal value by 23 pg/ml ($P = 0.049$). In the HO group, there was a small but significant increase by 17 pg/ml ($P = 0.007$) at 15 min, decreasing thereafter to significantly lower values at 60 and 90 min ($P < 0.001$).

The maximal decrease of ghrelin levels below baseline was significantly attenuated in NO compared with NW patients and was significantly postponed by 12 min (Table 3). A further attenuation of ghrelin suppression was observed in HO subjects, whereas the maximal rise of insulin showed the inverse pattern and occurred 10–12 min earlier than trough levels of ghrelin. The patients of the NO and HO subgroup, respectively, matched for age, sex, BMI, and leptin had comparable Δ -insulin and Δ -ghrelin levels with similar significant differences.

Corresponding to the different trough values of ghrelin

was the steepness of the slope during the fall and rise of postprandial ghrelin concentrations in the respective groups (Table 4). The initial decrease of ghrelin was significantly steeper than the subsequent rise back to baseline in all three groups. Similar to the maximal changes of insulin and ghrelin, the steepness of the respective slopes was also inversely related.

Plasma glucose rose by 27 mg/dl ($P < 0.001$) within 30 min in NW subjects, returning to baseline levels at 90 min. In NO and HO subjects, the maximal increase of glucose was 33 and 31 mg/dl within 60 min, respectively ($P < 0.001$; Fig. 1). In comparison with the NW group, glucose levels of both obese groups were significantly higher at 60, 90, and 120 min ($P < 0.01$). Plasma leptin concentrations remained unchanged during the entire postprandial period in all three groups (Fig. 1).

In both diabetic groups, postprandial insulin was significantly elevated between 15 and 180 min ($P = 0.01$; Fig. 1). In comparison with the nondiabetic obese subjects, the initial rise was attenuated. In the DNO group, the maximal rise of insulin was similar to NO subjects but occurred nearly 30 min later (Table 3). In the DHO group, the maximal rise of insulin was significantly less in comparison with HO, but the occurrence was similarly delayed.

In DNO patients, ghrelin levels rose significantly by 36 pg/ml at 15 min ($P < 0.001$), decreasing thereafter below baseline levels between 60 and 90 min ($P = 0.02$). Ghrelin in the DHO group showed a similar pattern with a significant rise at 15 min by 24 pg/ml ($P = 0.007$). Thereafter, ghrelin decreased but was significantly below baseline only at 90 min ($P < 0.001$; Fig. 1).

The maximal decrease of ghrelin in the DNO group (-131 ± 95.3 pg/ml) was not different from the NO group. In the DHO group, the ghrelin decrease was significantly attenuated compared with the DNO subjects (-82.9 ± 81.4 pg/ml, $P = 0.01$), but it was identical to the HO group (Table 3).

The slope of decreasing and increasing ghrelin levels in both diabetic groups was similar to the nondiabetic subjects (Table 4), whereas the corresponding slopes of rising insulin were significantly attenuated in diabetic patients. The subsequent fall of insulin was identical in DNO but attenuated in DHO subjects.

The rise of plasma glucose was similar in both groups with significantly elevated levels over the entire 3-h experimental period (Fig. 1). Postprandial leptin concentrations did not change significantly from baseline in both diabetic groups.

DISCUSSION

The present data demonstrate that obesity (BMI >25 kg/m²) is associated with reduced basal plasma ghrelin

TABLE 3
Maximal postprandial rise of insulin and maximal decrease of ghrelin within the indicated time period after meal ingestion

	Δ -Insulin (µU/ml)	Time after meal (min)	Δ -Ghrelin (pg/ml)	Time after meal (min)
NW subjects	17.6 ± 11.2	49.8 ± 21	-214.8 ± 246.9	60.1 ± 23.2
NO subjects	31.6 ± 19.2*	56.0 ± 22.6	-137.6 ± 107.5*	72.6 ± 32.8*
HO subjects	62.2 ± 38.5*†	57.6 ± 23.1	-85.5 ± 69.3*†	69.2 ± 34.1*
DNO subjects	27.5 ± 30.7	86.1 ± 41.6¶	-131.1 ± 95.3§	74.5 ± 31.8*
DHO subjects	34.5 ± 25.1‡	81.4 ± 35.5¶	-82.9 ± 81.4	72.4 ± 34.6*

Data are means ± SD. DHO, type 2 diabetic hyperinsulinemic obese; DNO, type 2 diabetic normoinsulinemic obese; HO, hyperinsulinemic obese; NO, normoinsulinemic obese; NW, normal weight. * $P < 0.001$ vs. NW; † $P < 0.001$ vs. NO; ‡ $P < 0.001$ vs. HO; § $P = 0.024$ vs. DHO; ¶ $P = 0.001$ vs. NO and HO.

TABLE 4
Ratios of hormonal changes over time after meal ingestion

	Postprandial period			
	Early		Late	
	Insulin	Ghrelin	Insulin	Ghrelin
NW subjects	0.38 ± 0.22	-4.18 ± 0.53	-0.17 ± 0.15§	2.85 ± 4.27§
NO subjects	0.67 ± 0.57*	-2.29 ± 2.26*	-0.28 ± 0.23*§	1.85 ± 1.81*§
HO subjects	1.22 ± 0.77*†	-1.59 ± 1.5*†	-0.59 ± 0.44*†§	1.17 ± 1.42*†§
DNO subjects	0.40 ± 0.38†	-1.98 ± 1.48*	-0.23 ± 0.27§	1.55 ± 1.42*
DHO subjects	0.51 ± 0.43‡	-1.49 ± 2.5*	-0.33 ± 0.26‡§	1.03 ± 1.73*

Data are means ± SD. The early postprandial period comprises the phase to maximal insulin and trough ghrelin levels; the late period is the time phase thereafter until 180 min. Positive values indicate a rise and negative values indicate a decrease of the respective slope; a higher value represents a steeper slope. DHO, type 2 diabetic hyperinsulinemic obese; DNO, type 2 diabetic normoinsulinemic obese; HO, hyperinsulinemic obese; NO, normoinsulinemic obese; NW, normal weight. **P* < 0.01 or less vs. NW; †*P* < 0.01 or less vs. NO; ‡*P* < 0.01 vs. HO; §*P* < 0.05 or less vs. respective change during the early postprandial period.

levels and an attenuated postprandial decrease, which confirms several previously published observations (19–23,44). Some but not all studies support the concept that insulin is an inhibitor of ghrelin secretion (24–30,33). Thus, obesity-related hyperinsulinemia could at least in part be responsible for ghrelin suppression. On the other hand, leptin has to be considered as a tonic regulator of the gastrohypothalamic axis involved in short-term feeding regulation (45,46). Leptin provides information about the energy reserve stored in adipocytes, because plasma leptin concentrations correlate well with the body fat cell mass (35,36). From experiments in the isolated stomach and from animal studies, there is substantial evidence that leptin can exert an inhibitory effect on gastric ghrelin release (38–40), and in some but not in all studies in obese humans, an inverse relation between leptin and ghrelin levels has been described (21,44,47). This raises the possibility that not only insulin but also leptin contributes to ghrelin suppression in obese subjects. On the other hand, Chan et al. (42) recently presented evidence that leptin does not suppress ghrelin levels in NW subjects.

The reduction of basal ghrelin levels is not only associated with elevated insulin and leptin but also with higher BMI levels. This as well as age and sex are potential confounding factors (22,42,47). When patients were matched for age, sex, BMI, and also leptin, the difference in insulin was still associated with a significant reduction of ghrelin, whereas subgroups with comparable high insulin levels but progressively increasing BMI and leptin concentrations did not show any alteration of basal ghrelin. This supports the concept that in obese subjects with associated hyperinsulinemia, ghrelin suppression is due to insulin, whereas leptin is of only minor or no importance.

The multiple regression analysis of the normoinsulinemic subjects demonstrates that ghrelin is negatively associated with leptin but not insulin. It must be noted, though, that the decrease of basal ghrelin is paralleled not only by an increase of leptin but also by a significant rise of insulin. The subsequent analysis of subgroups matched for insulin or leptin, respectively, suggests that such small, although significant, changes of insulin have no effect on the suppression of ghrelin levels, which supports the concept that leptin is important for the negative feedback regulation of ghrelin in states of moderately increasing body weight. The previously reported inefficacy of recombinant leptin

on plasma ghrelin in NW subjects (42) is in contrast to this concept and is as yet unexplained.

The inverse relation between leptin and ghrelin reaches its maximum at a leptin level of 10 ng/ml, which corresponds to a BMI of 28 kg/m². Despite substantially higher leptin and BMI, no further decrease of ghrelin was observed. If this effect of leptin is a mirror image of the action of leptin on the other regulatory pathways of feeding behavior, then the direct feedback control by the energy reserve would be limited to a rather small range of body weight. On the other hand, the limitation of the regulatory role of leptin to BMI 25–30 kg/m² could be indicative for the upper weight range that occurred during human evolution. At higher body weight and more severe metabolic deterioration, high insulin could in part compensate for the loss of leptin regulation.

In patients with type 2 diabetes, higher basal insulin was also associated with reduced ghrelin levels. Interestingly in normoinsulinemic subjects with type 2 diabetes, ghrelin was significantly lower compared with the nondiabetic group. Because insulin concentrations were comparable and leptin was even lower, other as yet unidentified factors related to the diabetic state must be responsible. The elevated glucose levels are most likely of no major importance considering previous *in vivo* studies (33). Furthermore, in the isolated rat stomach changes of perfusate glucose over a wide range between 30 and 300 mg/dl had no effect on ghrelin secretion (F.L, J.E., V.S., unpublished observations).

In contrast to the normoinsulinemic diabetic patients, those with high basal insulin had no further reduction of ghrelin compared with the nondiabetic group. This could at least in part be due to the previously reported attenuated suppressive action of insulin on ghrelin in diabetic patients (48).

The postprandial decrease of plasma ghrelin levels in NW subjects is in accordance with previously reported data. The reduction of ghrelin depends on an adequate basal and postprandial insulin secretion because ghrelin levels increase after test meals that elicit only a small rise of plasma insulin (10,11,13,14,20,31,32). In addition, intestinal hormones contribute to the suppression of ghrelin secretion (29,34). The present data show that in obese subjects, the postprandial decrease is attenuated, which is in line with previous observations (20,44,49,50). It extends

these data by demonstrating an inverse relation between postprandial ghrelin and insulin. Because the postprandial insulin response is substantially greater in both obese groups, one would expect the opposite effect on postprandial ghrelin, considering the inverse relationship between postprandial insulin and ghrelin in NW subjects (10,11,13,14,20). The mechanisms responsible for this attenuated postprandial suppression of ghrelin with increasing obesity are as yet unknown.

In contrast to NW subjects, there was an early rise of postprandial plasma ghrelin levels in obese subjects with and without diabetes before its subsequent suppression. A tendency toward an increase of ghrelin immediately after carbohydrate-rich test meals has also been observed in previous studies in NW subjects, although statistical significance has never been demonstrated (10,13,14). In the diabetic subjects, the rapid increase of ghrelin could at least in part be explained by the attenuated early-phase insulin response.

The reduction of basal ghrelin secretion in response to an increasing energy reserve of the body seems to be a reasonable negative feedback loop that helps to turn down further energy intake. Therefore, it is somewhat surprising that the postprandial decrease of ghrelin is attenuated in obese subjects. The disadvantage of this phenomenon could be a delayed and reduced support of neurally mediated gastric satiety signals. It should be noted, however, that hunger/satiety sensations and the early ghrelin response correlate poorly and that trough levels of ghrelin occur ~1 h after maximal satiety has been reached (14). Thus, the return of ghrelin back to baseline might be more important (10) as shown by a better correlation between these levels and subsequent meal size (14).

In the event that the ameliorated steepness of returning ghrelin levels in conjunction with lower absolute preprandial concentrations is important for feeding behavior, then obesity-related attenuation of postprandial ghrelin suppression could be a meaningful important regulatory feature. The available infusion studies with ghrelin do not permit an adequate answer to these questions (9), and more sophisticated dose-response studies are required.

In conclusion, the present data suggest that during moderate increases of body weight, leptin can be important for reduction of ghrelin release, whereas in more severe obesity, hyperinsulinemia without leptin is most likely responsible for the cross talk between the energy reserve and short-term feeding regulation. The greater postprandial rise of insulin in obesity is associated with an attenuated suppression of postprandial ghrelin levels, suggesting that in obese and type 2 diabetic subjects, a certain insulin resistance exists at the ghrelin cell that is different from the impairment of glucose disposal.

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