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Plasma Ghrelin Concentrations Are Decreased in Insulin-Resistant Obese Adults Relative to Equally Obese Insulin-Sensitive Controls

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Ghrelin, an orexigenic hormone that may play a role in body weight regulation, is reduced in states of obesity. Because obesity is associated with insulin resistance and compensatory hyperinsulinemia, we determined whether these metabolic characteristics were independently associated with suppressed ghrelin concentrations. To investigate this hypothesis, using steady-state plasma glucose concentrations, we identified 20 insulin-resistant (IR) and 20 insulin-sensitive (IS) individuals who were equally obese. The mean body mass indexes were 32.5 ± 0.4 and 32.0 ± 0.4 kg/m^2 for the IR and IS groups, respectively. Fasting insulin concentrations were 19.5 and 7.4 μU/ml ($P < 0.001$), respectively. Ghrelin concentrations were suppressed in the IR group (252 ± 19 pg/ml) relative to the IS group (412 ± 35 pg/ml; $P < 0.001$). Ghrelin correlated inversely with both insulin resistance ($r = -0.64$; $P < 0.001$) and fasting insulin concentration ($r = -0.58$; $P < 0.001$). Multivariate analysis confirmed that both insulin resistance and hyperinsulinemia independently predicted low ghrelin concentrations. Our results demonstrate that in obese individuals, insulin resistance and hyperinsulinemia are inversely associated with ghrelin concentrations. Thus, insulin resistance or related metabolic abnormalities may constitute part of a feedback mechanism by which body weight is regulated in humans. (*J Clin Endocrinol Metab* 89: 1630–1635, 2004)

GHRELIN, A PEPTIDE hormone secreted primarily by the stomach and small intestine, is the only known circulating orexigen (1–4). Increasing evidence suggests that ghrelin plays a role in regulating premeal hunger and meal initiation as well as long-term energy balance (5, 6). For example, acute ghrelin injections potently stimulate appetite and food intake in multiple species, including humans (2, 3, 7, 8), and chronic administration increases body weight, not only by stimulating food intake, but also by decreasing energy expenditure and fat catabolism (2, 3, 7). In addition, blockade of ghrelin signaling using ghrelin receptor antagonists, antighrelin antibodies, or genetic techniques has been reported to decrease food intake and body weight (3, 9–12). Finally, low body mass and weight loss are associated with elevations in ghrelin, whereas high body mass and weight gain are associated with decreased ghrelin concentrations (6, 13–18). These observations suggest that ghrelin may serve as a signal to the brain of nutrient excess or depletion (6).

Despite the evidence favoring a role for ghrelin in long-term energy balance, it is unclear how a hormone secreted by the gastrointestinal tract can sense and respond to changes in body energy stores. This raises the possibility that a circulating adiposity signal, such as leptin or insulin, may regulate the production of ghrelin. Several lines of evidence suggest that leptin does not subserve this function (19). Insulin, a hormone that circulates in high concentrations in individuals who are insulin resistant, typically in the setting of obesity (20), is an attractive candidate for this signal. The changes in plasma insulin concentrations before and after meals are a virtual mirror image of fluctuations in ghrelin concentrations (5, 16). Additionally, short-term insulin infusions have been shown to decrease ghrelin concentrations in rodents (21) and humans (22–26), consistent with a possible causal role of insulin in suppressing ghrelin secretion.

The data implicating chronic hyperinsulinemia and/or insulin resistance in the negative regulation of ghrelin are suggestive, but inconsistent. For example, in a manner opposite that of ghrelin, insulin concentrations increase with increasing adiposity (20) and decrease with fasting (27) and weight loss (16). These findings raise the possibility that insulin is the mediator between body mass and ghrelin concentrations, and that hyperinsulinemia and/or insulin resistance, as signs of nutrient excess, suppress orexigenic ghrelin concentrations. Five published studies have evaluated this relationship (28–32). Results are conflicting, with some reports showing an inverse relationship between ghrelin and insulin concentrations or surrogate measures of insulin resistance, and others showing no association. It is important to note that conclusions from these investigations are limited by the fact that none included a physiological or precise measure of insulin resistance, relying instead on homeostatic model assessment (HOMA), which has been shown to be no more highly associated with insulin resistance than the fasting insulin concentration (33). Furthermore, the majority of published studies did not control for body mass, a major

Abbreviations: BMI, Body mass index; HDL-C, high density lipoprotein cholesterol; HOMA, homeostatic model assessment; IR, insulin resistant; PCOS, polycystic ovarian syndrome; PWS, Prader-Willi syndrome; SSPG, steady state plasma glucose.

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potential confounder between any observed association between ghrelin and insulin concentrations.

We clarified the relationships among insulin resistance, hyperinsulinemia, and ghrelin by evaluating 40 obese adults without concurrent medical diagnoses, who were matched for body mass index (BMI) and who were classified as either insulin resistant or insulin sensitive using a highly validated and precise physiological measure of insulin resistance, the steady-state plasma glucose test.

Subjects and Methods

Subjects

The study population consisted of 40 obese individuals who had responded to newspaper advertisements seeking healthy volunteers for a weight loss study. Potential participants were screened at Stanford University General Clinical Research Center, after giving written informed consent (protocol approved by the Stanford human subjects committee). To be eligible for this study, volunteers were required to be free of major medical illnesses, such as renal or hepatic disease, congestive heart failure, malignancy, or coronary artery disease; without history of eating disorders or change in weight within the past 4 wk; free of medications known to alter body weight or insulin resistance; and have a BMI between 29–35 kg/m^2.

Protocol

Volunteers meeting the above criteria and willing to be considered for further evaluation returned after an overnight fast for measurement of insulin-mediated glucose disposal (*i.e.* insulin sensitivity) by a modification (34) of the insulin suppression test as originally described (35). This test consists of a 180-min infusion with octreotide (0.27 μg/m^2·min), insulin (25 mU/m^2·min), and glucose (240 mg/m^2·min). Blood is drawn at 10-min intervals from 150–180 min of the infusion to measure plasma glucose and insulin concentrations, and the mean of these four values is defined as the steady state plasma insulin or glucose (SSPG) concentration, respectively, for each individual. As steady state plasma insulin concentrations are similar for all subjects, the SSPG value provides a direct measure of the ability of insulin to mediate disposal of an infused glucose load; the higher the SSPG, the more insulin resistant the individual. Subjects were classified as insulin resistant if their SSPG value was 180 mg/dl or higher and as insulin sensitive if their SSPG was 120 mg/dl or less. These values represent the upper and lower 40th percentiles of SSPG concentrations, as measured in 490 healthy nondiabetic individuals (33). Of 100 screened individuals, 20 qualified as insulin sensitive. We then selected 20 insulin-resistant individuals matched to the insulin-sensitive subjects with respect to age, gender, and BMI. The characteristics of the two experimental groups are shown in Table 1. BMI was nearly identical in both groups: 32.0 ± 0.4 and 32.5 ± 0.4 (range, 29–35) kg/m^2 for the insulin-sensitive and -resistant groups, respectively. The only difference between the groups was the higher SSPG (by definition) concentration in the insulin-resistant group.

Hormone assays

Glucose was measured as previously described (36). Samples were assayed for total immunoreactive ghrelin concentration with a modification of a commercial RIA (Phoenix Pharmaceuticals, Belmont, CA). This assay uses an ^{125}I-labeled ghrelin tracer and a rabbit polyclonal antibody against full-length, octanoylated human ghrelin that recognizes the acylated and des-acyl forms. Although only acylated ghrelin is bioactive (1), total ghrelin appears to be a reasonable surrogate for the acylated form because the ratio of the two levels is constant under a wide variety of conditions that affect ghrelin (10, 37). The lower and upper detection limits were 80 and 2500 pg/ml, and the intra- and interassay coefficients of variations were 5.4% and 9.2%, respectively. Insulin was measured via RIA as previously described (38). Although the study was not designed to evaluate the relationship between ghrelin and high density lipoprotein cholesterol (HDL-C), we included HDL-C (39) in our multivariate analyses because of a recent publication showing that HDL-C can bind to ghrelin (40) and therefore might influence measured ghrelin concentrations.

TABLE 1. Demographic characteristics of insulin-sensitive compared with insulin-resistant individuals

Characteristic	Insulin sensitive (n = 20)	Insulin resistant (n = 20)	*P* value
Age (yr)	53 ± 2	48 ± 2	NS
Gender (F/M)	12/8	12/8	NS
Weight (kg)	93.5 ± 3.4	93.4 ± 3.3	NS
BMI (kg/m^2)	32 ± 0.4	32.5 ± 0.4	NS
SSPG (mg/dl)	91 ± 4	252 ± 10	<0.001

Statistical analyses

Data are expressed as the mean ± SEM. An unpaired *t* test was used to compare clinical and laboratory characteristics between insulin-sensitive and insulin-resistant individuals. χ^2 analysis was used to compare categorical variables between the two groups. Multiple linear regression was performed to ascertain the independent effect of insulin resistance (as a categorical variable) on ghrelin after adjustment for BMI, age, and HDL-C. Because of colinearity, fasting plasma insulin was not included in the same regression model; it was analyzed in a separate model, with BMI, age, and HDL-C, to predict ghrelin concentrations. Due to unequal variances in the two groups, log-transformed values for insulin, SSPG, and ghrelin were used for correlations, and log-transformed ghrelin was used as the dependent variable in multivariate regression analysis. $P < 0.05$ was considered statistically significant. All analyses were performed using SAS 8.0 (SAS Institute, Inc., Cary, NC).

Results

The mean (±SEM) plasma ghrelin concentration was lower in the insulin-resistant group (252 ± 19 pg/ml) than in the insulin-sensitive group (412 ± 35 pg/ml; $P < 0.001$) despite equivalent body weight and BMI values in the two groups (Fig. 1A). The fasting insulin concentration was more than twice as high in the insulin-resistant group (19.5 ± 2.7 μU/ml)

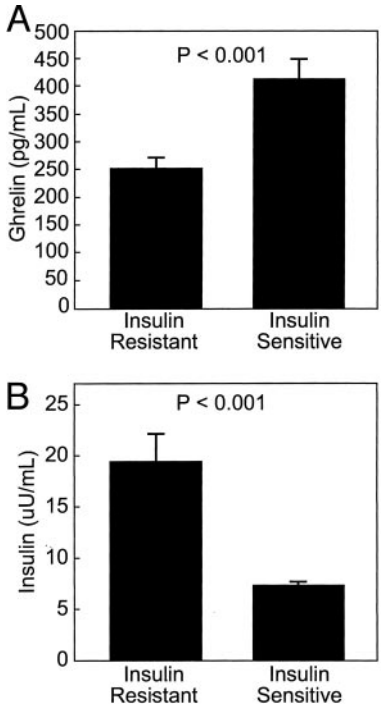

FIG. 1. Plasma ghrelin (A) and insulin (B) concentrations in insulin-resistant and insulin-sensitive obese individuals.

as in the insulin-sensitive group (7.4 ± 0.4 μU/ml; $P < 0.001$; Fig. 1B). Figure 2 displays the inverse relationship between log plasma ghrelin and log SSPG concentrations (r = −0.64; $P < 0.001$) as well as between log plasma ghrelin and log fasting plasma insulin concentrations (r = −0.58; $P < 0.001$). For ease of interpretation, the actual values are presented, but the correlation coefficients are for log-transformed SSPG, insulin, and ghrelin concentrations.

Multiple linear regression analysis was used to evaluate the roles of insulin resistance (SSPG concentration) and plasma insulin concentration as independent predictors of ghrelin concentration. As SSPG concentrations were not continuous (the middle 20th percentile was excluded from the study population), in the first model insulin resistance was entered as a categorical variable, along with BMI and age. The results, shown in Table 2, demonstrate that only insulin resistance was a significant independent predictor of ghrelin, and that the presence of insulin resistance was associated with an average 145 pg/ml reduction in the plasma ghrelin concentration. As the results in Fig. 2 indicated that fasting plasma insulin concentrations were not dichotomous, they were entered as a continuous variable, along with BMI and age, in a second model, shown in Table 3. These results demonstrate that the fasting plasma insulin concentration was also an independent predictor of the ghrelin concentration, with each increase (microunits per milliliter) in insulin contributing a mean 6.6 pg/ml reduction in ghrelin concentration.

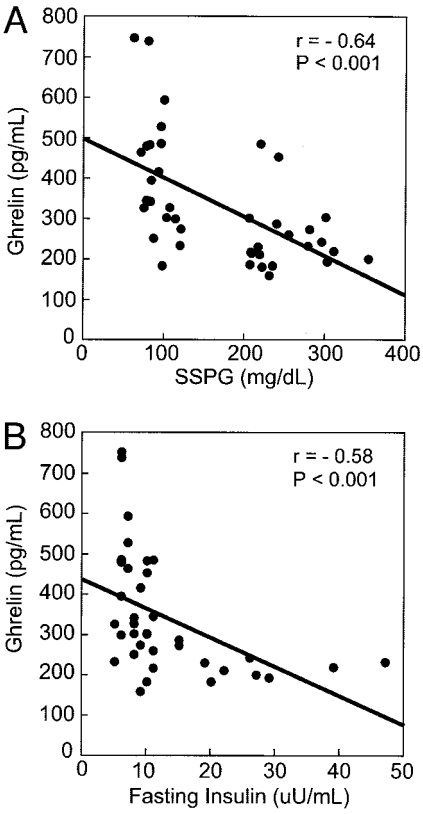

FIG. 2. Relationship between insulin resistance and ghrelin concentrations (A), and between fasting insulin and ghrelin concentrations (B). Insulin resistance was quantified via SSPG.

TABLE 2. Multivariate analysis with insulin resistance as a predictor of ghrelin concentration

Variable	Regression coefficient	SE	*P* value
Constant	536	397	0.19
Age (yr)	−1.1	2.2	0.63
BMI (kg/m^2)	−2.33	11.9	0.85
Insulin resistance[a]	−145	39	0.001

[a] Defined as SSPG concentration in the top 40th percentile of the healthy nondiabetic population.

TABLE 3. Multivariate analysis with fasting plasma insulin concentration as a predictor of ghrelin concentration

Variable	Regression coefficient	SE	*P* value
Constant	335	431	0.44
Age (yr)	−0.4	2.3	0.86
BMI (kg/m^2)	3.3	13.2	0.81
Insulin (μU/ml)	−6.6	2.2	0.005

Finally, as HDL-C has been reported to bind to ghrelin (40) and may thus affect its levels, the models in Tables 2 and 3 were rerun, including HDL-C along with the variables previously entered. In neither instance was there a statistically significant association between HDL-C concentration and ghrelin, nor did the inclusion of HDL-C change any of the estimates.

Discussion

The results of our study demonstrate that among obese, otherwise healthy adults with equivalent BMI values, plasma ghrelin concentrations are lower in those who are insulin resistant *vs.* those who are insulin sensitive. Because BMI was virtually identical in the two groups, our results help to clarify mixed observations in prior studies examining the relationships between ghrelin, obesity, and insulin resistance. Based on our findings, it appears that insulin resistance is independently and inversely associated with ghrelin concentration, with 41% of the variability in ghrelin attributable to insulin resistance. Adjustment, via multivariate analysis, for small degrees of variation among individual BMIs, confirmed that the inverse association between insulin resistance and ghrelin was significant and independent of BMI. Furthermore, multivariate analysis did not show any independent contribution of BMI to ghrelin concentrations.

These findings clarify and extend those of three prior studies (28–30) that demonstrated a significant inverse association between insulin resistance and ghrelin. As adiposity is correlated with both insulin resistance and ghrelin levels, it is important to eliminate confounding by obesity in the evaluation of the relationship between insulin resistance and ghrelin. Two of the prior studies (29, 30) did not control for adiposity, which, as expected, differed substantially according to the degree of insulin resistance. Thus, it is not possible in these studies to distinguish contributions of obesity *vs.* insulin resistance toward lowering of the ghrelin concentration. The third study (28) showed that among children with obesity resulting from melanocortin-4 receptor mutations, leptin deficiency, or Prader-Willi syndrome (PWS), insulin concentrations in the first two obese groups were signifi-

cantly higher, and ghrelin concentrations were significantly lower than those in lean controls. The children with PWS, who were equally obese, had insulin concentrations less than half those in the other obese groups, and their ghrelin concentrations were more than twice as high, suggesting that insulin may play an independent role in modulating ghrelin concentrations. Indeed, multivariate analysis showed independent associations between both BMI and insulin with ghrelin in all but the PWS group.

In our study, BMI, which ranged from 29–35 kg/m^2, was not independently associated with ghrelin. This may indicate that the relationship between obesity and ghrelin is mediated via insulin resistance and/or compensatory hyperinsulinemia, and that adiposity *per se* does not affect ghrelin concentrations. Alternatively, our BMI range may have been too narrow and our sample size too small to identify a relationship between BMI and ghrelin. Thus, although our study cannot rule out a contribution of obesity to the regulation of ghrelin concentration, it demonstrates that ghrelin is not invariably suppressed in obese individuals, but, rather, is much more consistently suppressed among those obese individuals who are also insulin resistant.

It is important to note that virtually all prior studies on this topic used surrogate markers of insulin resistance that rely on fasting insulin concentrations, either alone or as a major component of indexes such as HOMA. The largest study (n = 490) validating HOMA as a measure of insulin resistance (measured by SSPG) showed that the correlation coefficient was 0.62, compared with a correlation coefficient of 0.61 for fasting insulin and insulin resistance, and a correlation coefficient of 0.98 for fasting insulin and HOMA (33). Thus, HOMA mainly reflects the hyperinsulinemia that accompanies insulin resistance and does not offer substantially more information regarding insulin resistance than does fasting insulin alone. This is particularly important in the study of obese individuals, in whom insulin concentrations may be elevated not only as a compensatory response to insulin resistance, but also due to decreased insulin clearance (41), rendering estimates of insulin resistance based on fasting insulin particularly subject to inaccuracies.

This may partially explain why two published studies found no association between insulin resistance, as measured by HOMA, and ghrelin. One, by Orio and colleagues (31), showed that although obese women with polycystic ovarian syndrome (PCOS) were more insulin resistant than obese non-PCOS controls, their ghrelin concentrations did not differ. Another, by Pagotto and colleagues (32), showed that although ghrelin concentrations were lower in obese PCOS subjects relative to obese non-PCOS controls, HOMA and fasting insulin were similar. Of note, the fasting insulin concentrations reported were similar (between groups) and relatively low in the Orio study (10 *vs.* 7 μU/ml) and were similar (between groups) and extremely high in the Pagotto study (21.3 *vs.* 21.5 μU/ml). As the two comparison groups in each of these studies had relatively similar insulin concentrations, it is possible that a more precise measure of insulin resistance might have demonstrated an association with ghrelin. Alternatively, Schofl *et al.* (30) found that in the "continuous infusion of glucose with model assessment" (CIGMA), which uses a 2-h glucose/insulin ratio in response to glucose infusion as an estimate of insulin sensitivity, increasing insulin sensitivity was independently predictive of ghrelin. Thus, the current analysis adds to prior findings by using a physiological and precise measure of insulin-mediated glucose disposal (insulin resistance) to identify two equally obese groups that are clearly different with respect to insulin resistance.

Despite the limitations of fasting insulin as a marker of insulin resistance, in our study the insulin-resistant group (as defined by SSPG concentration) was markedly hyperinsulinemic compared with the insulin-sensitive group. Thus, the group with low ghrelin concentrations had both insulin resistance and hyperinsulinemia compared to the group with higher ghrelin concentrations. In multivariate analysis, the insulin concentration was inversely associated with ghrelin, as was the SSPG concentration. As, in the nondiabetic population, insulin rises in response to insulin resistance, it is difficult in an observational study to separate the effects of these two variables with respect to ghrelin. Thus, our study cannot distinguish whether it is resistance to insulin, hyperinsulinemia, or an unmeasured associated factor that contributes to decreased ghrelin concentrations. We speculate that the control systems that regulate ghrelin levels are not subject to the mechanisms causing insulin resistance in major glucose-disposing tissues, such as muscle. Consequently, even in insulin-resistant individuals, these control systems may respond to excessive insulin signaling driven by compensatory hyperinsulinemia, thereby suppressing ghrelin concentrations. We hypothesize that the compensatory hyperinsulinemia associated with insulin resistance contributes directly to decreased ghrelin concentrations, and that the pathway by which insulin suppresses ghrelin is not resistant to insulin's effect to the same degree as are muscle and liver. This hypothesis is consistent with studies showing that acute insulin infusions in animals or humans decrease ghrelin (21–26), and that chemically induced insulin deficiency raises ghrelin concentrations (42). In a manner opposite to that of ghrelin, insulin concentrations rise both acutely in the postprandial state and chronically in the setting of insulin resistance, which is generally a manifestation of obesity. It is, therefore, reasonable to consider that insulin suppresses an orexigenic hormone as part of a complex feedback system that regulates appetite and body weight. In support of this hypothesis, it has been shown in four prospective studies that individuals with insulin concentrations in the highest quartile, compared with those with insulin concentrations in the lowest quartile, gain less weight over 5–14 yr (43–46).

As our understanding of the endocrine functions of adipose tissue has expanded, various circulating hormones have been considered to play a role in body weight regulation and have thus been investigated with respect to ghrelin concentrations. Of these, leptin has received the most attention. Overall, however, extant evidence suggests that leptin is not an important regulator of ghrelin because 1) it correlates (inversely) weakly with ghrelin compared with other measures of adiposity; 2) it does not affect ghrelin when infused iv; and 3) in leptin-deficient or -resistant mice and humans who are obese, ghrelin concentrations are low, consistent with obesity, but inconsistent with the hypothetical inverse relationship between ghrelin and leptin (19). The same is true

in the converse situation, in which lean animals with leptin overexpression (transgenics, central nervous system adenoviruses, or chronic leptin infusions) have high ghrelin concentrations, the opposite of that expected in the setting of high leptin concentrations, if leptin were to regulate ghrelin negatively (19). Although insulin appears to be a more likely candidate for ghrelin regulation, it is possible that there are unidentified circulating factors that are associated with both high insulin and low ghrelin concentrations; in which case, insulin is merely a marker of another regulatory factor.

Given the growing epidemic of obesity, it has become increasingly important to understand the complex physiological processes that regulate body weight. For this reason, much attention has recently centered on ghrelin, the only known circulating orexigen. Our finding that insulin resistance and compensatory hyperinsulinemia are independently associated with suppression of ghrelin furthers our understanding of the variable expression of ghrelin in humans. With continued research, it should be possible to elucidate exactly how the associations among insulin resistance, hyperinsulinemia, and ghrelin participate in the more intricate web of factors that regulate body weight.

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