Weight Regain after a Diet-Induced Loss Is Predicted by Higher Baseline Leptin and Lower Ghrelin Plasma Levels

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Context: Appetite-related hormones may play an important role in weight regain after obesity therapy.

Objective: Our objective was to investigate the potential involvement of ghrelin, leptin, and insulin plasma levels in weight regain after a therapeutic hypocaloric diet.

Design: A group of obese/overweight volunteers (49 women and 55 men; 35 $\pm$ 7 yr; 30.7 $\pm$ 2.4 kg/m^2) followed an 8-wk hypocaloric diet ($-$30% energy expenditure) and were evaluated again 32 wk after treatment. Body weight as well as plasma fasting ghrelin, leptin, and insulin concentrations were measured at three points (wk 0, 8, and 32).

Results: After the 8-wk hypocaloric diet, the average weight loss was $-5.0 \pm 2.2\%$ ($P < 0.001$). Plasma leptin and insulin concentrations decreased significantly, whereas ghrelin levels did not markedly change. In the group regaining more than 10% of the weight loss, leptin levels were higher ($P < 0.01$), whereas ghrelin levels were lower ($P < 0.05$). No differences were observed in insulin levels. Weight regain at wk 32 was negatively correlated with ghrelin and positively associated with leptin levels at baseline (wk 0) and endpoint (wk 8). These outcomes showed a gender-specific influence, being statistically significant among men for ghrelin and between women for leptin. Moreover, a decrease in ghrelin after an 8-wk hypocaloric diet was related to an increased risk for weight regain (odds ratio = 3.109; $P = 0.008$) whereas a greater reduction in leptin (odds ratio = 0.141; $P = 0.001$) was related to weight-loss maintenance.

Conclusions: Subjects with higher plasma leptin and lower ghrelin levels at baseline could be more prone to regain lost weight, and hormones levels could be proposed as biomarkers for predicting obesity-treatment outcomes. **(*J Clin Endocrinol Metab* 95: 5037–5044, 2010)**

The prevalence of obesity continues to rise worldwide (1). This metabolic disorder is associated with several comorbidities such as diabetes and cardiovascular and neurodegenerative diseases as well as some types of cancer (2).

Diverse therapeutic strategies have been tried to decrease body adiposity. Nutritional interventions, such as caloric restriction diets, can be an efficient therapeutic approach to promote weight loss in obese subjects (3). Although most therapeutic trials involving drugs (4) or dietary treatments can show mean weight losses higher than 5% of body weight (5, 6), the long-term success of maintaining the lost weight is usually poor (7). Therefore, the

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Abbreviations: BMI, Body mass index; GHS-R, growth hormone secretagogue receptor; WM group, weight maintenance group; WR group, weight regain group.

identification of factors associated with weight regain can enhance our understanding of energy homeostasis regulation and would have positive implications for the personalization of therapeutic approaches.

Several biological and psychological factors may contribute to the weight regain after a treatment-induced weight loss (8). There is also evidence that some weight reduction therapies fail long term due to compensatory adaptations in metabolism, which promote rapid and efficient weight regain (9). Among the mechanisms implicated, orexigenic and anorexigenic hormones appear to have a clinically relevant role (10).

Ghrelin is a circulating orexigenic hormone (11), which is implicated in both the short-term control of food intake at single meals and in long-term body-weight regulation (12). Furthermore, insulin and leptin are two key endocrine adiposity factors providing signaling for the central nervous system to regulate food intake (13). Indeed, there is a complex interaction between insulin, leptin, and ghrelin; leptin regulates ghrelin levels and affects changes in body weight, whereas insulin and ghrelin are involved in the physiological response to food intake and are reciprocally regulated (14).

The aim of the present study was to evaluate whether plasma levels of ghrelin, leptin, and insulin can influence weight regain after an energy-restricted therapy and whether they may be used to identify those subjects more prone to regain the weight loss induced by a hypocaloric diet.

Subjects and Methods

Study subjects

The study protocol initially included 162 obese/overweight subjects [79 women and 83 men; 35 ± 6 yr; with a mean body mass index (BMI) of 31.1 ± 2.9 kg/m^2] recruited through local advertisements. All participants provided a medical history, physical examination, and routine laboratory tests. Exclusion criteria were history of diabetes mellitus, high blood pressure, or dyslipidemia or changes in body weight greater than ±3 kg during the 3 months before the study. Other exclusion criteria included pregnancy and previous surgically or drug-treated obesity as well as alcohol or drug abuse. Furthermore, consumption of vitamin or mineral supplements as well as regular prescription of medical drugs during this time was not allowed and were further reasons for exclusion. Written informed consent to participate in the trial was obtained before the start of the study. The protocol used for this study was approved by the Clínica Universitaria de Navarra Ethics Committee (Ref. 54/2006), in agreement with the Helsinki Declaration, and followed national and European Union guidelines.

Study design

The trial was based on a nutritional intervention controlled by trained dieticians from the Department of Nutrition and Food Sciences, Physiology, and Toxicology of the University of Navarra. Weight loss was induced by means of a hypocaloric diet devised to produce a 30% energy restriction (500–600 kcal/d) of the subject's total energy expenditure. This estimation was calculated by applying Harris-Benedict equations and a correction factor based on the overweight status of the subjects, which was adjusted for the level of physical activity (15). The pattern of physical activity remained unchanged in all stages and was assessed and controlled by a trained specialist, based on a validated physical activity questionnaire for overweight individuals (16). The nutritional intervention program consisted of 8 wk of a balanced hypocaloric diet containing 55% of the energy supply as carbohydrates, 15% as proteins, and 30% as fat, which was implemented by a food exchange system (17). Dietary compliance was assessed through 3-d weighed food records (5, 18). Calculations of the energy and nutrient intake were performed using appropriate software (Sanocare Human System L.S., Madrid, Spain) based on recognized Spanish food composition tables. Food questionnaires were completed during the week before the beginning of the intervention and 1 wk before the completion of the hypocaloric diet. These reports provided information on the baseline intake and the adherence to the prescribed diets (17). Before and after the nutritional treatment, selected anthropometric measurements were taken.

Upon completion of the dietary intervention (wk 8), volunteers were given general dietary guidelines to maintain the weight loss, but without calorie restrictions or specific follow-up instructions. Subjects were instructed on healthy habits, such as dietary intake and physical activity, following the Spanish Society for the Study of Obesity (SEEDO) guidelines and the Nutrition and Physical Activity for Obesity Prevention Strategy (NAOS) guidelines from the Health Ministry of the Spanish Government. Six months later (wk 32), 104 subjects returned to the clinical research unit for further assessment. Their dietary patterns were estimated from food questionnaires, and their changes in body weight were measured.

Venous blood samples were drawn after a 12-h overnight fast and were obtained at the beginning of the restriction diet (baseline, wk 0), at the end of the diet intervention (endpoint, wk 8), and 6 months after ending the treatment (follow-up, wk 32). The EDTA-plasma and serum of specimens were separated from whole blood and immediately frozen at −80 C until assay.

Anthropometric and body composition measurements were performed according to standardized procedures described elsewhere (15).

Hormone assays

Overnight fasting plasma levels of ghrelin were measured by RIA using a commercially available kit (Linco Research Inc., St. Charles, MO). This assay is able to detect both acylated and deacylated ghrelin. Serum concentrations of insulin and leptin were assessed using a RIA-based method (Diagnostic Products Corp., Los Angeles, CA).

Statistical analysis

The sample size of the current interventional trial was estimated taking the weight loss after treatment (main variable) into account and calculated according to the equation reported by Mera *et al.* (19). Thus, to detect differences, the sample size was established at a minimum of 85 obese/overweight subjects who finished the nutritional intervention. The original sample size recruited was 162 obese/overweight volunteers who completed

TABLE 1. Phenotypical characteristics and changes in anthropometrical and biochemical variables before and after the caloric restriction (CR) treatment in 49 women and 55 men (35.6 ± 6.6 yr old)

	Before CR (wk 0)	After CR (wk 8)	*P* value
Body weight (kg)	89.5 ± 12.3	84.7 ± 11.3	<0.001
BMI (kg/m^2)	30.7 ± 2.4	29.0 ± 2.2	<0.001
Fat-free mass (kg)	61.1 ± 12.3	58.9 ± 11.9	<0.001
Fat mass (%)	32.9 ± 7.7	31.3 ± 8.1	<0.001
Waist circumference (cm)	96.2 ± 8.2	92.1 ± 7.9	<0.001
Leptin (ng/ml)	22.5 ± 14.7	14.9 ± 14.5	<0.001
Ghrelin (pg/ml)	952 ± 326	964 ± 343	0.461
Insulin (mU/liter)	9.28 ± 4.68	7.36 ± 3.63	<0.001

the hypocaloric diet. Six months later, 104 subjects returned to the study site for further assessment. To reinforce the results, statistical analysis was performed using only the 104 subjects that completed the three points (wk 0, 8, and 32) of this study, and the median criterion was applied to avoid dropout impact.

Diet-induced changes in body weight and hormone values were calculated (in percent) as the difference between endpoint (wk 8) and baseline (wk 0) measurements and related to the baseline. To analyze the high or low changes of hormone levels during the intervention, the median (above and below the 50th percentile) cutoff values of the changes in these variables were considered in the analyzed population as previously applied (15) and based on a validated method to assign the studied population to two groups of disease risk (16). Body weight loss was also dichotomized according to the 5% of weight loss criterion. Weight regain (percent) was calculated as the difference between the follow-up period (wk 32) and endpoint (wk 8) with respect to the endpoint values. Successful weight-loss maintenance was considered by using the criterion of the 10% weight regain, according to previously published reports (15).

The normal distribution of variables was explored using the Kolmogorov-Smirnov and the Shapiro Wilk tests. Accordingly, the parametric Student's *t* or nonparametric Wilcoxon paired test was applied to detect differences before and after the treatment. Moreover, differences between the groups at all points of the nutritional trial were assessed by the parametric Student's *t* or nonparametric Mann-Whitney *U* test. A repeated-measures ANOVA was used to study the effects of time and groupings on body weight and hormone levels. The Pearson coefficient was used to evaluate the potential association between body weight regain and hormone levels before and after dieting. In addition, a logistic regression analysis was applied to assess the potential predictive factors concerning body weight regain. Thus, as independent variables, median cutoff values were used to classify the hormone diet-induced changes. Success in weight-loss maintenance was used as the dependent variable.

Statistical analysis was performed by SPSS version 13.0 software (SPSS Inc., Chicago, IL) for Windows XP (Microsoft, Redmond, WA). $P \leq 0.05$ was considered statistically significant.

Results

Changes in anthropometric parameters as well as in ghrelin, leptin, and insulin levels after nutritional intervention

After the 8-wk energy restriction period, patients lost an average of 5.0 ± 2.2% body weight (−4.5 ± 1.9% for women *vs.* −5.9 ± 2.2% for men; $P = 0.001$) accompanied by a statistically significant reduction in BMI and body fat mass, fat-free mass, and waist circumference (Table 1). The hypocaloric diet also induced a significant decrease in leptin and insulin levels, whereas ghrelin concentrations remained unaltered (Table 1). These changes were also statistically different when the subjects were sorted according to the 5% of weight loss criteria. Thus, decreases in plasma leptin and insulin were greater in subjects who lost at least 5% of their body weight, and no statistical differences in ghrelin levels were found between the two groups (Table 2).

After the follow-up period (wk 32), as a group, subjects maintained the body weight as compared with the endpoint (84.42 ± 13.22 *vs.* 84.74 ± 11.36 kg; $P > 0.1$). When patients were categorized according to the 10% of weight regain classification criterion, a total of 55 subjects (25 females and 30 males) maintained the weight loss [weight-maintenance group (WM group), −5.39 ± 4.09%] and 49 subjects (24 females and 25 males) regained at least 10% of the lost weight [weight-regain group (WR group), 5.17 ± 5.02%]. As expected, after the follow-up period, the WR group presented a statistically ($P < 0.001$) higher body weight (84.74 ± 14.41 kg) than the WM group (79.68 ± 10.01 kg).

Overall, gender-specific differences were found in body weight and hormone levels. Thus, it was observed that women presented lower body weight than men in all points of the study ($P < 0.001$). Moreover, leptin and ghrelin levels were statistically higher in females than

TABLE 2. Diet-induced changes of leptin, insulin, and ghrelin according to 5% of weight loss grouping criteria between baseline and wk 8

	≥5% body weight-loss group	<5% body weight-loss group	*P* value
Weight loss (% change)	−6.98 ± 1.87	−3.65 ± 0.95	<0.001
Leptin (% change)	−51 ± 17	−28 ± 26	<0.001
Insulin (% change)	−7.0 ± 1.9	−3.6 ± 0.9	<0.001
Ghrelin (% change)	5.3 ± 22.8	−0.5 ± 16.1	0.140

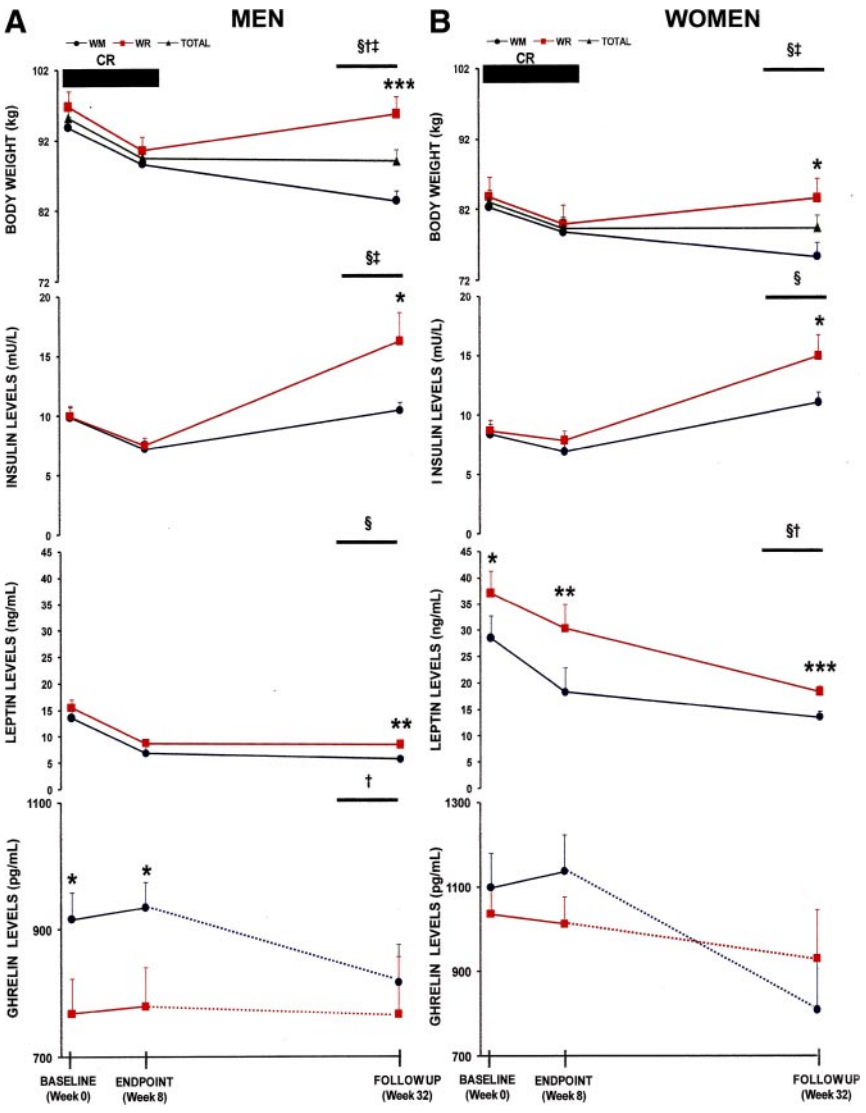

FIG. 1. Men's (A) and women's (B) body weight and plasma insulin, leptin, and ghrelin levels during the nutritional treatment (wk 0–8) as well as follow-up period, 6 months later (wk 32), depending on the weight maintenance group (n = 104). The follow-up data for ghrelin levels are shown, but they were not included in the statistical analysis due to insufficient blood samples (n = 31). Data show mean ± SE. WM, Subjects with successful weight maintenance; WR, subjects with weight regain. *Asterisks* denote statistical changes (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) between WM groups at each time point of the study evaluated by Student's *t* test according to the normal distribution of the variables. §, Statistically significant ($P < 0.05$) changes across time; †, changes concerning the group; ‡, statistical significance for the time-group interaction evaluated by means of a repeated-measures ANOVA. CR, Calorie restriction.

males ($P \leq 0.001$) but with no statistical differences in the insulin concentration between either gender categories.

Based on this gender dimorphism, the differences between WR and WM groups were evaluated separately in both gender categories. Thus, body weight and insulin changes between WR and WM were similar between men and women. Furthermore, leptin levels were significantly higher at all three points in WR compared with WM women (Fig. 1B). In contrast, in men, no differences between groups were observed (Fig. 1A). Ghrelin levels were significantly higher at baseline and wk 8 in WR compared with WM men (Fig. 1A), whereas women showed no statistical differences (Fig. 1B).

A correlation analysis showed that lower baseline (wk 0) and lower endpoint (wk 8) plasma levels of ghrelin (Fig. 2, A and B), especially in men, as well as higher leptin (Fig. 2, C and D) concentrations, especially in women, were significantly associated with higher weight regain. No association was found between the baseline ($r = -0.013$; $P = 0.894$) or endpoint ($r = 0.047$; $P = 0.639$) insulin levels and body weight regain. Moreover, weight regain was positively associated with the diet-induced changes of leptin (Fig. 2F) and tended to correlate inversely with the changes in ghrelin after dieting (Fig. 2E).

Association between ghrelin, leptin, and insulin hypocaloric diet-induced changes and the body weight evolution

To further investigate the influence of the assayed hormone levels after the energy restriction treatment on body weight maintenance, the subjects were categorized into two groups according to the median of the diet-induced (wk 0–8) changes in ghrelin (2.1%), leptin (−44.9%) and insulin (−21.9%) levels. Patients exhibiting changes from wk 0 to wk 8 in ghrelin below the median ($P = 0.023$) and changes in insulin ($P = 0.049$) and leptin ($P = 0.022$) above the median showed greater weight regain (Fig. 3).

To further assess the effect of the evaluated hormones on weight regain, a logistic regression analysis was performed to investigate potential predictors of weight regain. Thus, after adjusting for the age, gender, and amount of weight loss, the changes in ghrelin and leptin (as dichotomous variables) from wk 0 to wk 8 appeared as potential predictors of weight regain (Table 3). A diet-induced (wk 0–8) decrease in ghrelin was related to an increased risk for weight regain (odds ratio = 3.109; $P = 0.008$) whereas a diet-induced (wk 0–8) decrease in leptin (odds ratio = 0.141; $P = 0.001$) was related to weight-loss maintenance.

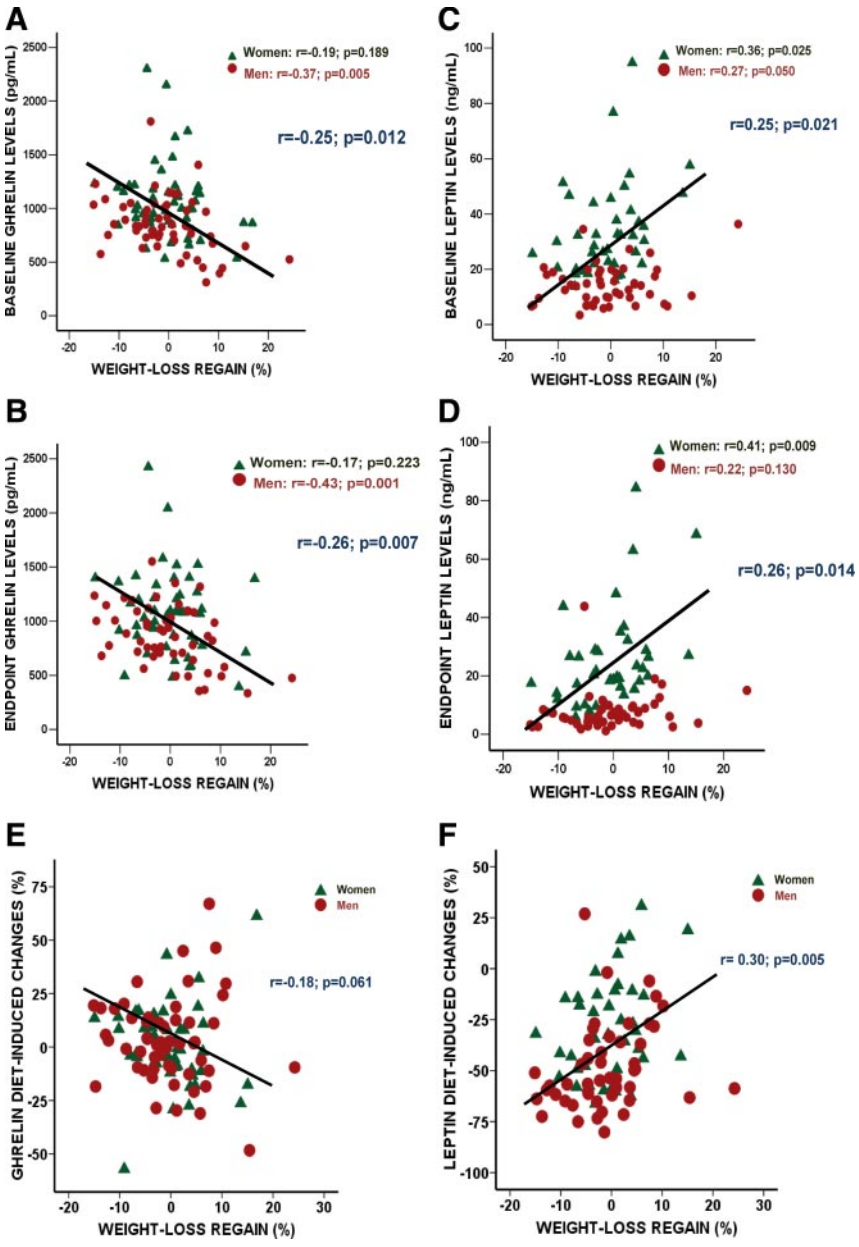

FIG. 2. Association between plasma ghrelin and leptin levels at baseline (A and B, respectively) and after 8 wk caloric restriction treatment (C and D, respectively), as well as the diet-induced changes in ghrelin (E) and leptin (F) with body weight regain 6 months after finishing dietary intervention. Those subjects who presented lower ghrelin and higher leptin fasting plasma levels after energy restriction treatment regained the lost body weight in the 6 months of follow-up.

Discussion

After a nutritional intervention to induce weight loss, the prevention of weight regain is the most clinically relevant issue in the efforts to reduce the epidemic of obesity. The novel findings from this study are that after a weight reduction induced by an 8-wk balanced hypocaloric diet, subjects who regained at least 10% of the lost weight 6 months later appeared to have higher fasting leptin levels and lower fasting ghrelin levels at all times during the study than those that maintained body weight. In contrast, no significant differences were found in the concentrations of fasting insulin. These responses had a gender-specific dependence. In addition, the energy-restricted diet-induced changes in fasting leptin and ghrelin appear as important risk predictors for regaining the lost weight.

Differential outcomes are often found when classifying patients as responders and nonresponders to chemotherapy (20) or antidiabetic (21) treatments. Concerning obesity management, important weight loss variability among individuals is encountered due to factors such as metabolic adaptations (9) or genetic predisposition (15, 22). Therefore, the identification of predictive factors concerning the treatment response is crucial to individualize therapies and to avoid the prescription of therapies in patients with potential resistance.

Several studies have shown reductions in leptin and insulin levels (5), together with an increase in ghrelin levels (23, 24) induced by different weight loss therapies. In agreement with such previous reports, the balanced hypocaloric diet applied to lose weight in the current work also induced a significant decrease in insulin and leptin levels after 8 wk. At the same time, ghrelin levels showed a small although not statistically significant increase after dieting.

Despite the demonstrated beneficial effect of an energy-restricted diet on obesity comorbidity risk factors (18, 25, 26), changes in appetite-regulating hormones have been hypothesized to occur with weight loss and may facilitate rapid and efficient weight regain after treatment (9). It has been demonstrated that subjects without a significant weight loss (<5% of initial weight) under dieting showed a compensatory increase of ghrelin levels that could explain the lack of relevant weight loss (23). In the present study, lower decreases in leptin and insulin levels were found in subjects who lost less than 5% of the initial weight. In accordance with other authors (27), ghrelin levels were no different depending on weight loss, although subjects without significant weight loss showed a lower increase in ghrelin. These discrepancies with previous reports could be due to technical

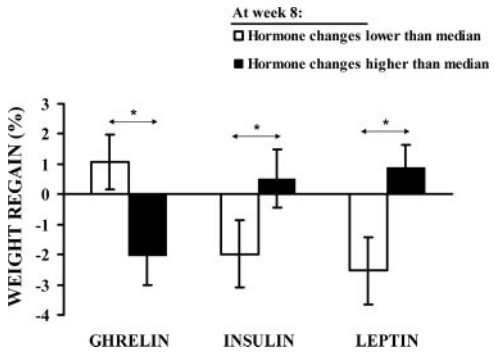

FIG. 3. Differences in body weight regain of subjects categorized according to the median of the diet-induced (wk 0–8) changes in insulin, leptin, and ghrelin. Data show mean ± SE. *, Statistically significant differences between both groups.

and experimental approaches (27). However, when the subjects included in this study were categorized into two groups according to success in body weight maintenance, those subjects who regained at least 10% of the lost weight showed higher levels of leptin and lower levels of ghrelin. Interestingly, these hormonal differences were found at all times including baseline, suggesting the existence of two different populations according to the leptin and ghrelin levels influencing the response outcomes.

Insulin presented a profile in accordance with the body weight course changes during the trial, showing differences between WM and WR only at wk 32. Thereafter, the insulin data support the suitability of the classification of subjects applied in this trial to evaluate the differential weight regain status. On the other hand, whereas insulin levels did decrease from baseline to wk 8 in both WR and WM men and women, by wk 32, insulin levels were higher than baseline in both men and women in the WR group and returned to baseline levels in the WM group. In contrast, body weight at follow-up was not higher than at baseline in the WR group, and it remained lower than baseline in the WM group. Therefore, the findings in the WR group suggest that metabolically, this group was less insulin sensitive at the same body weight after cycling through weight loss/weight regain. The findings in the WM group suggest that insulin sensitivity improved with energy restriction rather than with weight loss.

TABLE 3. Logistic regression analysis to determine the risk of regaining more than 10% of weight loss after a diet-induced weight loss

	OR	95% CI	*P* value
Model 1: ghrelin change	3.109	1.346–7.181	0.008
Model 2: leptin change	0.141	0.044–0.454	0.001
Model 3: insulin change	0.541	0.233–1.256	0.153

The ghrelin, leptin, or insulin diet-induced changes classified as dichotomous variables according to the median cutoff value (reference is values below the median) were considered as independent variables. The model was adjusted by gender, age, and weight loss (percent). The weight regain (dependent variable) was encoded as 0 for weight maintenance (<10% of weight regain) and 1 for weight regain (≥10% of weight regain). Changes in ghrelin, leptin, and insulin were encoded as 0 for diet-induced increase or values above the median and 1 for diet-induced decrease or values below the median. CI, Confidence interval; OR, odds ratio.

Because ghrelin is a potent orexigenic signal and leptin is a satiety signal, a coordinated change in these hormone levels may elicit strong compensatory increases in appetite and weight gain (28). In agreement with this statement, it may be expected that those subjects who regain more easily the lost weight showed lower levels of leptin and higher levels of ghrelin. However, that was not the case, and the present results are consistent with a disruption in the sensitivity to these hormone signals, probably in the central nervous system of those subjects with a higher predisposition to regain body weight.

Previous reports and also the present study (data not shown) found a negative association between circulating ghrelin levels and BMI (29), postulating an increase in ghrelin secretion during fasting, malnutrition, cachexia, and anorexia nervosa and reduced ghrelin secretion in obesity. Ghrelin administration does not affect appetite in anorexia nervosa patients, suggesting that they are less sensitive to ghrelin in terms of food intake (30). These findings suggest altered ghrelin signaling in obesity and anorexia nervosa, the mechanisms of which are not presently understood.

The regulation of ghrelin is complex and is under genetic and other patient-related influences. Several polymorphisms or distinct mutations have been identified in the preproghrelin locus and in its receptor [growth hormone secretagogue receptor (GHS-R)] as well as additional GHS-R subtypes or other receptor families (31) or ligands other than ghrelin that may act on the GHS-R (32), which may influence a wide range of biological roles.

In the same line, obesity in humans is commonly associated with increased leptin levels, but despite the anorexigenic function of this hormone, appetite is not effectively suppressed. This phenomenon, called leptin resistance, could be the result of impairments at a number of levels in the signaling pathway from reduced access of the hormone to its receptor through changes in receptor expression or changes in postreceptor signal transduction (33).

Reinforcing the present results, it was found that baseline and endpoint levels of ghrelin and leptin could explain the variability in the body weight regain after the energy restriction treatment. Thus, higher levels of ghrelin predicted no body weight regain, especially in men. At the same time, higher leptin levels were associated with body weight regain more specifically in women. Moreover, ad-

justed by gender, age, and weight loss, the diet-induced changes in leptin and ghrelin were found to be protective factors of regaining the lost weight. These reports are in contrast to the hypothesis concerning the role of the weight loss-induced changes in these appetite hormones as metabolic adaptations to weight reduction that promote efficient weight regain. This statement usually comes from trials that do not evaluate body weight maintenance after treatment (23, 24) or from experiments with genetic and pharmacological manipulations of key components of the appetite hormone in animal models (34). On the contrary, the present results were obtained from humans on a moderate and balanced hypocaloric diet where subjects were given dietary guidelines to maintain weight loss, but without caloric restrictions or specific follow-up instructions. In this regard, it has been previously observed in obese humans that the appetite evaluated by a validated visual analog scale is inversely associated with ghrelin concentrations (35). Another report has found higher ghrelin concentrations in obese subjects fed a hypocaloric diet enriched in n-3 polyunsaturated fatty acids which have positive effects on weight loss (36) and on decreases in the sensation of hunger (35). Moreover, higher decreases in leptin levels induced by a fish-based energy-restricted diet were associated with better metabolic improvements, suggesting that weight maintenance control could be enhanced (5).

On the other hand, the findings of this trial are consistent with an advanced proinflammatory state in those subjects more prone to regain the diet-induced weight loss (15) because leptin has been proposed as a proinflammatory adipokine (37), whereas ghrelin appears to have antiinflammatory and antioxidant properties (38). Thus, leptin and ghrelin levels found in those subjects who are able to maintain the weight loss after treatment could be in accordance with a lower susceptibility to develop obesity-related morbidities. Finally, beside the widely demonstrated gender-specific differences in leptin levels (39), some studies have also reported gender differences in plasma ghrelin levels in normal subjects (40). However, as far as we know, this work presents for the first time that higher plasma ghrelin concentrations were found in obese women than in men.

A limitation of this study could be the single fasting hormone levels employed because insulin, leptin, and ghrelin concentrations can be dramatically influenced by food intake and can be modified by several metabolic conditions. However, fasting hormone level is a representative sample of baseline conditions, without influence of preprandial and postprandial factors. In fact, the evaluation of fasting hormone level is usually a reference point to compare two or more populations and under different conditions. On the other hand, leptin concentration at fasting is representative of the 24-h levels. Moreover, the assay of the area under curve values is a nuisance to the patients, and it has a high economic cost. Because of these reasons, we decided to evaluate the hormone levels at fasting at all points of the study.

In conclusion, these results suggest that those obese subjects who regain more weight after a weight-lowering program appear to present a different central response to leptin and ghrelin signaling than those that maintain the body weight loss. Although the mechanisms remain unclear, this altered regulation is in the same line as was found in obesity where leptin levels are higher and ghrelin levels are lower than in normal-weight subjects. The counterintuitive findings of ghrelin and leptin suggest that weight regain may be associated with some central or peripheral resistance to both hormones. On the other hand, these data concur with a putative proinflammatory state that could be implicated in the weight rebound.

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References

1. **Casanueva FF, Moreno B, Rodriguez-Azeredo R, Massien C, Conthe P, Formiguera X, Barrios V, Balkau B** 2009 Relationship of abdominal obesity with cardiovascular disease, diabetes and hyperlipidaemia in Spain. Clin Endocrinol (Oxf) 73:35–40
2. **Bray GA, Bellanger T** 2006 Epidemiology, trends, and morbidities of obesity and the metabolic syndrome. Endocrine 29:109–117
3. **Abete I, Parra MD, Zulet MA, Martínez JA** 2006 Different dietary strategies for weight loss in obesity: role of energy and macronutrient content. Nutr Res Rev 19:5–17
4. **Astrup A, Rössner S, Van Gaal L, Rissanen A, Niskanen L, Al Hakim**

M, Madsen J, Rasmussen MF, Lean ME 2009 Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. Lancet 374:1606–1616
5. **Abete I, Parra D, Crujeiras AB, Goyenechea E, Martinez JA** 2008 Specific insulin sensitivity and leptin responses to a nutritional treatment of obesity via a combination of energy restriction and fatty fish intake. J Hum Nutr Diet 21:591–600
6. **Abete I, Parra D, Martinez JA** 2009 Legume-, fish-, or high-protein-based hypocaloric diets: effects on weight loss and mitochondrial oxidation in obese men. J Med Food 12:100–108
7. **Aronne LJ, Nelinson DS, Lillo JL** 2009 Obesity as a disease state: a new paradigm for diagnosis and treatment. Clin Cornerstone 9:9–25
8. **Schur EA, Cummings DE, Callahan HS, Foster-Schubert KE** 2008 Association of cognitive restraint with ghrelin, leptin, and insulin levels in subjects who are not weight-reduced. Physiol Behav 93: 706–712
9. **MacLean PS, Higgins JA, Jackman MR, Johnson GC, Fleming-Elder BK, Wyatt HR, Melanson EL, Hill JO** 2006 Peripheral metabolic responses to prolonged weight reduction that promote rapid, efficient regain in obesity-prone rats. Am J Physiol Regul Integr Comp Physiol 290:R1577–R1588
10. **Levin BE** 2004 The drive to regain is mainly in the brain. Am J Physiol Regul Integr Comp Physiol 287:R1297–R1300
11. **Seoane LM, Al-Massadi O, Caminos JE, Tovar SA, Dieguez C, Casanueva FF** 2007 Sensory stimuli directly acting at the central nervous system regulate gastric ghrelin secretion: an *ex vivo* organ culture study. Endocrinology 148:3998–4006
12. **Cummings DE** 2006 Ghrelin and the short- and long-term regulation of appetite and body weight. Physiol Behav 89:71–84
13. **Davis JF, Choi DL, Benoit SC** 2010 Insulin, leptin and reward. Trends Endocrinol Metab 21:68–74
14. **Gil-Campos M, Aguilera CM, Cañete R, Gil A** 2006 Ghrelin: a hormone regulating food intake and energy homeostasis. Br J Nutr 96:201–226
15. **Goyenechea E, Parra D, Crujeiras AB, Abete I, Martínez JA** 2009 A nutrigenomic inflammation-related PBMC-based approach to predict the weight-loss regain in obese subjects. Ann Nutr Metab 54: 43–51
16. **Martínez-González MA, López-Fontana C, Varo JJ, Sánchez-Villegas A, Martinez JA** 2005 Validation of the Spanish version of the physical activity questionnaire used in the Nurses' Health Study and the Health Profesionals' Follow-up Study. Public Health Nutr 8:920–927
17. **Tremblay A, Côté J, LeBlanc J** 1983 Diminished dietary thermogenesis in exercise-trained human subjects. Eur J Appl Physiol Occup Physiol 52:1–4
18. **Crujeiras AB, Parra D, Goyenechea E, Abete I, Martínez JA** 2009 Tachyphylaxis effects on postprandial oxidative stress and mitochondrial-related gene expression in overweight subjects after a period of energy restriction. Eur J Nutr 48:341–347
19. **Mera R, Thompson H, Prasad C** 1998 How to calculate sample size for an experiment: a case-based description. Nutr Neurosci 1:87–91
20. **Osterberg L, Levan K, Partheen K, Delle U, Olsson B, Sundfeldt K, Horvath G** 2009 Potential predictive markers of chemotherapy resistance in stage III ovarian serous carcinomas. BMC Cancer 9:368
21. **Wolford JK, Yeatts KA, Dhanjal SK, Black MH, Xiang AH, Buchanan TA, Watanabe RM** 2005 Sequence variation in PPARγ may underlie differential response to troglitazone. Diabetes 54:3319–3325
22. **Campion J, Milagro FI, Goyenechea E, Martinez JA** 2009 TNF-alpha promoter methylation as a predictive biomarker for weight-loss response. Obesity (Silver Spring) 17:1293–1297
23. **de Luis DA, Sagrado MG, Conde R, Aller R, Izaola O** 2008 Changes of ghrelin and leptin in response to hypocaloric diet in obese patients. Nutrition 24:162–166
24. **Romon M, Gomila S, Hincker P, Soudan B, Dallongeville J** 2006 Influence of weight loss on plasma ghrelin responses to high-fat and high-carbohydrate test meals in obese women. J Clin Endocrinol Metab 91:1034–1041
25. **Abete I, Parra D, De Morentin BM, Alfredo Martinez J** 2009 Effects of two energy-restricted diets differing in the carbohydrate/protein ratio on weight loss and oxidative changes of obese men. Int J Food Sci Nutr 60(Suppl 3):1–13
26. **Crujeiras AB, Parra D, Goyenechea E, Martínez JA** 2008 Sirtuin gene expression in human mononuclear cells is modulated by caloric restriction. Eur J Clin Invest 38:672–678
27. **Mager U, Kolehmainen M, de Mello VD, Schwab U, Laaksonen DE, Rauramaa R, Gylling H, Atalay M, Pulkkinen L, Uusitupa M** 2008 Expression of ghrelin gene in peripheral blood mononuclear cells and plasma ghrelin concentrations in patients with metabolic syndrome. Eur J Endocrinol 158:499–510
28. **Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG** 2000 Central nervous system control of food intake. Nature 404:661–671
29. **Ybarra J, Bobbioni-Harsch E, Chassot G, Huber O, Morel P, Assimacopoulos-Jeannet F, Golay A** 2009 Persistent correlation of ghrelin plasma levels with body mass index both in stable weight conditions and during gastric-bypass-induced weight loss. Obes Surg 19:327–331
30. **Miljic D, Pekic S, Djurovic M, Doknic M, Milic N, Casanueva FF, Ghatei M, Popovic V** 2006 Ghrelin has partial or no effect on appetite, growth hormone, prolactin, and cortisol release in patients with anorexia nervosa. J Clin Endocrinol Metab 91:1491–1495
31. **Camiña JP** 2006 Cell biology of the ghrelin receptor. J Neuroendocrinol 18:65–76
32. **Casanueva FF, Camiña JP, Carreira MC, Pazos Y, Varga JL, Schally AV** 2008 Growth hormone-releasing hormone as an agonist of the ghrelin receptor GHS-R1a. Proc Natl Acad Sci USA 105:20452–20457
33. **Tups A** 2009 Physiological models of leptin resistance. J Neuroendocrinol 21:961–971
34. **Nogueiras R, Tschöp MH, Zigman JM** 2008 Central nervous system regulation of energy metabolism: ghrelin versus leptin. Ann NY Acad Sci 1126:14–19
35. **Parra D, Ramel A, Bandarra N, Kiely M, Martínez JA, Thorsdottir I** 2008 A diet rich in long chain omega-3 fatty acids modulates satiety in overweight and obese volunteers during weight loss. Appetite 51:676–680
36. **Ramel A, Martínez JA, Kiely M, Morais G, Bandarra NM, Thorsdottir I** 2008 Beneficial effects of long-chain n-3 fatty acids included in an energy-restricted diet on insulin resistance in overweight and obese European young adults. Diabetologia 51:1261–1268
37. **Procaccini C, Lourenco EV, Matarese G, La Cava A** 2009 Leptin signaling: a key pathway in immune responses. Curr Signal Transduct Ther 4:22–30
38. **De Vriese C, Delporte C** 2008 Ghrelin: a new peptide regulating growth hormone release and food intake. Int J Biochem Cell Biol 40:1420–1424
39. **Considine RV, Premkumar A, Reynolds JC, Sebring NG, Ricks M, Sumner AE** 2008 Adiponectin and leptin in African Americans. Obesity (Silver Spring) 16:428–434
40. **Makovey J, Naganathan V, Seibel M, Sambrook P** 2007 Gender differences in plasma ghrelin and its relations to body composition and bone: an opposite-sex twin study. Clin Endocrinol (Oxf) 66: 530–537