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Plasma Acylation-Stimulating Protein, Adiponectin, Leptin, and Ghrelin before and after Weight Loss Induced by Gastric Bypass Surgery in Morbidly Obese Subjects

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We examined fasting plasma insulin, acylation-stimulating protein (ASP), leptin, adiponectin, ghrelin, and metabolic/cardiovascular risk profile before and 15 ± 6 months after isolated Roux-en-Y gastric bypass surgery in 50 morbidly obese subjects. Average preoperative plasma lipids were mostly normal, whereas ASP, insulin, and leptin were elevated, and adiponectin and ghrelin were decreased. Postoperatively, body weight decreased significantly (−36.4 ± 9.6%) and was best predicted by preoperative adiponectin concentration in weight-stable subjects ($r = -0.59$; $P = 0.02$). Plasma lipids and insulin resistance improved, leptin and ASP decreased (−76.3 ± 14.6% and −35.9 ± 52.2%; $P < 0.001$), and adiponectin increased (50.1 ± 47.0%; $P < 0.001$). The decrease in apolipoprotein B was best predicted by the decrease in ASP ($r = 0.55$; $P = 0.009$), whereas the improved postoperative insulin sensitivity was best predicted by the increase in adiponectin ($r = 0.70$; $P = 0.01$). Despite bypassing 95% of the stomach and isolating the fundus from contact with ingested nutrients, circulating ghrelin did not decrease after surgery. In fact, plasma ghrelin increased postoperatively in the subset of subjects undergoing active weight loss (+60.5 ± 23.2%; $P < 0.001$); ghrelin, however, remained unchanged in weight-stable subjects. In summary, 1) preoperative adiponectin concentrations may be predictive of the extent of weight loss; 2) changes in ASP and adiponectin are predictive of decreased apolipoprotein B and improved insulin action, respectively; and 3) plasma ghrelin increases after gastric bypass surgery in patients experiencing active weight loss. (*J Clin Endocrinol Metab* 88: 1594–1602, 2003)

OBESITY HAS BECOME an epidemic in affluent societies. Cohort and cross-sectional studies have shown that obesity is associated with coronary artery disease, diabetes, and hypertension (1). Obesity is an inevitable consequence of chronic positive energy balance. The regulation of energy balance between intake and expenditure and the subsequent metabolic profile that evolves during positive energy balance are mediated by a complex network of signals originating from a number of endocrine tissues, including pancreas, adipose tissue (2), and, as recently discovered, stomach (3). These peripheral signals are integrated in the central nervous system (primarily in the hypothalamus) in the regulation of energy intake and expenditure (4, 5).

Adipose tissue synthesizes and secretes a number of cytokine hormones that are involved in the regulation of energy homeostasis, insulin action, and lipid metabolism (6). Acylation-stimulating protein (ASP), which is identical to C3adesarg, is a lipogenic adipocytokine whose precursors, complement C3, adipsin, and factor B, are synthesized and secreted by adipose tissue in a differentiation-dependent manner (7, 8). ASP is linked to the pathogenesis of obesity via its action to enhance triglyceride synthesis and storage in the adipocyte. ASP increases both glucose uptake as well as fatty acid esterification in a manner that is independent of but additive to insulin (9). Mice with a genetic knockout of C3, which are unable to produce ASP, are resistant to diet-induced obesity and insulin resistance (10). The concentration of ASP is elevated in obesity (11), type II diabetes mellitus (12), and coronary artery disease (13). Weight loss in obese subjects induced by fasting (14) or hypocaloric diets (15) decreases the concentration of plasma ASP.

Leptin, the product of the *ob* gene, is also an adipocytokine secreted by white adipose tissue (16). Originally leptin was proposed to act as a signal indicating abundant adipose stores to the hypothalamus to limit energy intake and increase energy expenditure (17). Subsequently, it has been suggested that the primary role of leptin is in adaptation to negative energy balance (18, 19). Accordingly, decreases in circulating leptin are associated with increased hunger (20), and leptin replacement prevents the compensatory decrease in metabolic rate and thyroid axis function after diet-induced weight loss in humans (21). Furthermore, there is some evidence linking leptin to a direct regulation of adipose tissue metabolism through inhibition of lipogenesis and stimulation of lipolysis (22, 23). Circulating leptin concentrations are elevated in obesity and decrease after weight loss (24).

Adiponectin, is one of the most abundant adipose tissue-specific factors (25, 26). Recent data suggest that adiponectin

Abbreviations: apoB, Apolipoprotein B; ASP, acylation-stimulating protein; BMI, body mass index; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; LDL, low density lipoprotein; NEFA, nonesterified fatty acids; TG, triglycerides.

is a mediator of insulin sensitivity and an enhancer of fatty acid oxidation (27). In contrast to ASP and leptin, plasma levels of adiponectin are lower in obese subjects, and the low levels are associated with risk factors for coronary artery disease (28, 29) and insulin resistance (30, 31). Low levels of adiponectin are also associated with the reduced ability of insulin to phosphorylate insulin receptor tyrosine residues and are predictive of the development of insulin resistance in humans (32). Administration of adiponectin to rodents increases insulin sensitivity, an action that appears to result from lowered hepatic glucose production and increased muscle fatty acid oxidation (33), and adiponectin knockout mice exhibit insulin resistance (33, 34). Circulating adiponectin concentrations have been reported to increase after weight loss (35).

Ghrelin is a recently described hormone predominantly produced by the stomach fundus that acts on GH secretagogue receptors to increase GH release from the pituitary gland (36). Ghrelin administration increases food intake, decreases fat oxidation, increases adiposity in rodents (37), and triggers hunger and increased food intake in humans (38). Circulating ghrelin levels are suppressed by meal ingestion or intragastric glucose administration, but not gastric distension, and rise during fasting (3, 39). Plasma ghrelin concentrations are reduced in obese subjects (39), and diet-induced weight loss increases circulating ghrelin concentrations (40, 41).

Isolated longitudinal Roux-en-Y gastric bypass surgery is a procedure used to treat morbid obesity [body mass index (BMI), >40 kg/m^2] by restricting the volume of the stomach available for use to less than 15 ml and bypassing a subsection of the small intestine (42, 43). Weight loss after gastric bypass surgery is known to result from decreased energy intake rather than nutrient malabsorption (42). Although the mechanism(s) by which gastric bypass surgery induces long-term weight loss, often without increases in hunger, are not well understood, it has recently been hypothesized that reductions of circulating ghrelin may contribute to the success of weight loss after bypass surgery (41).

ASP, adiponectin, leptin, and ghrelin are all involved in the regulation of energy homeostasis and have been examined separately in relation to obesity and moderate weight loss. However, the complementary roles that these hormones may have in long-term regulation of energy balance have not been examined in the same population of morbidly obese subjects under weight-stable conditions and during dynamic weight loss. In addition, in studies examining ASP and ghrelin concentrations after gastric bypass surgery, only postgastric bypass values have been presented (41, 44). The aim of this study was therefore to examine plasma ASP, adiponectin, leptin, ghrelin, and metabolic/cardiovascular risk profile (*i.e.* glucose, insulin, and lipid parameters) before and after massive weight loss induced by gastric bypass surgery in morbidly obese subjects. As adipose tissue plays an active role in the regulation of energy balance and nutrient metabolism, we hypothesized that preoperative levels of the adipose tissue hormones ASP, leptin, and adiponectin would be predictive of the extent of weight loss, and that changes in these hormones would predict improved metabolic/cardiovascular profile after weight loss.

Subjects and Methods

Study subjects

Fifty morbidly obese subjects (39 women and 11 men) underwent standardized isolated longitudinal Roux-en-Y gastric bypass surgery for the treatment of obesity at the Royal Victoria Hospital by two surgeons. Detailed descriptions of the surgical procedures have been previously published (42, 43). In brief, a small longitudinal 4- to 8-cm long gastric pouch, 1.5 cm in diameter ($<$15 ml), is created along the lesser curvature of the stomach. The jejunum is divided 100 cm distal to the ligament of Treitz and advanced in a retrocolic/retrogastric position to create a 100-cm Roux-en-Y limb, which is anastomosed to the gastric pouch. The gastric pouch-jejunal anastomosis is 1–1.2 cm in diameter (around an 18-gauge naso-gastric tube).

Pre- and postoperative anthropometric measurements (weight and height) were made, and blood samples were collected at the Obesity Clinic where patients were followed at a decreasing frequency six times during the first year after the surgery and semiannually thereafter (an average of 6 ± 2 times/patient in this study). Postoperative weight and plasma values reported here correspond to those collected during the last visit, 15 ± 6 months after the surgery. Information regarding patients' anthropometric measurements, medical history, surgery details, and medication used were collected from the patients' hospital charts. Surgery details regarding gastric pouch size, length of the bypassed intestine, and gastrojejunostomy diameter were collected from the patient's operation report. Patients were excluded if they had reported symptomatic coronary artery disease, were taking lipid-lowering drugs, or were less than 6 months postsurgery at the time of data collection. Postoperatively, patients were classified as either weight stable (neutral energy balance) or weight reducing (negative energy balance). Weight stability was defined as weight loss of less than 10% over the 6-month period preceding the final measurement (average, $<$1.7% weight loss/month) (45). All subjects had signed a written consent to the study, which was approved by the research ethics board of the Royal Victoria Hospital.

Plasma lipids, apolipoprotein B (apoB), and glucose concentrations

Pre- and postoperative blood samples were collected for all 50 subjects for the measurement of 12 plasma parameters of interest. Due to the lack of sufficient sample volume for some subjects at the time of data analysis, some plasma parameters could not be measured for all subjects. The number of subjects for whom data were available for each parameter is indicated in Table 1 or Figs. 1 and 2.

Venous blood samples (10 ml) were collected after an overnight fast in tubes containing EDTA and were immediately centrifuged at 1500 rpm for 15 min at 4 C. Plasma samples were frozen at −80 C for later measurement of lipids, apoB, glucose, insulin, leptin, ASP, adiponectin, and ghrelin. Plasma triglycerides (TG) and nonesterified fatty acids (NEFA) were measured by commercial enzymatic colorimetric kits (Roche, Laval, Canada). High density lipoprotein (HDL) cholesterol was separated according to the method described by Gidez *et al.* (46) by heparin/manganese chloride precipitation (Sigma-Aldrich, St. Louis, MO). Total and HDL cholesterol were measured using a commercial enzymatic colorimetric kit (Roche). Low density lipoprotein (LDL) cholesterol was calculated according to Friedewald's equation (47): LDL cholesterol = total cholesterol – HDL cholesterol – (TG/2.2), where all values are in millimolar concentrations, except when plasma TG exceeded 4.6 mM. ApoB was measured by a commercial nephelometric assay using Immage analyzer (Beckman-Coulter, Brea, CA). Plasma glucose was measured using a 2000 STAT Plus analyzer (YSI, Inc., Yellow Springs, OH).

The normal concentration for NEFA was 0.100–0.800 mM for men and women (48). The normal cut-off points for plasma TG and total, HDL, and LDL cholesterol were set between the 25–75th percentiles of a healthy North American population distribution with the same average age (49). The normal concentration for plasma TG was between 0.75–1.31 mM for women and 1.00–1.96 mM for men. The normal concentrations for total, HDL, and LDL cholesterol were 4.45–5.69, 1.24–1.68, and 2.69–3.78 mM for women and 4.63–5.92, 0.93–1.32, and 2.97–4.06 mM for men, respectively. The normal range for plasma apoB was set between the 25–75th percentile in the Framingham Offspring Study, which was

TABLE 1. Pre- and postoperative characteristics in weight-stable and weight-reducing subjects

	Weight-stable (n = 25, 18 women)			Weight-reducing (n = 25, 21 women)		
	Preoperative	Postoperative	% Change	Preoperative	Postoperative	% Change
Age (yr)	42 ± 9	43 ± 10		39.4 ± 7	40 ± 7	
Duration (months)		17.5 ± 4.9[a]			12.3 ± 5.7	
Weight (kg)	134.6 ± 26.8	85.6 ± 17.6[b]	−35.9 ± 10.0	139.3 ± 31.9	87.4 ± 21.1[b]	−37.0 ± 9.3
BMI (kg/m^2)	48.5 ± 10.1	30.7 ± 6.1[b]	−35.9 ± 10.0	52.0 ± 9.3	32.6 ± 6.6[b]	−37.0 ± 9.3
% Weight loss/month			−0.5 ± 0.6[a]			−3.4 ± 1.4
TG (mM)	1.25 (1.66)	0.83 (1.12)[b]	−30.1 ± 25.	1.44 (1.93)	0.82 (1.03)[b]	−39.3 ± 21.8
NEFA (mM)	0.377 ± 0.155	0.321 ± 0.163	−3.6 ± 62.4	0.393 ± 0.159	0.321 ± 0.239	−8.9 ± 65.1
T-CHOL (mM)	4.79 ± 0.84	4.06 ± 0.64[b]	−14.2 ± 12.2	5.13 ± 0.92	3.91 ± 0.82[b]	−22.6 ± 17.5
HDLC (mM)	1.24 ± 0.38	1.13 ± 0.45[a]	−5.7 ± 30.8	1.04 ± 0.35	0.82 ± 0.30[c]	−11.9 ± 45.5
LDLC (mM)	3.43 ± 0.85	2.57 ± 0.66[b]	−22.2 ± 22.0	3.92 ± 0.92	2.68 ± 0.78[b]	−29.5 ± 22.7
apoB (mg/dl)	111.0 ± 47.6	70.8 ± 14.7[b]	−26.6 ± 23.2	117.5 ± 36.5	75.5 ± 20.7[b]	−30.1 ± 23.9
Glucose (mM)	5.64 (8.58)	4.36 (4.68)[c]	−23.0 ± 21.4	5.24 (6.00)	3.69 (4.01)[b]	−33.9 ± 23.5
Insulin (μU/ml)	27.2 (31.8)	11.7 (12.5)[b]	−33.4 ± 37.5	25.6 (36.2)	10.1 (12.7)[b]	−52.7 ± 18.6
HOMA-IR	7.76 (9.52)	1.92 (2.57)[b]	−55.3 ± 26.2	7.80 (10.23)	1.69 (2.05)[b]	−72.4 ± 13.3

T-CHOL, Total cholesterol; HDLC, HDL cholesterol; LDLC, LDL cholesterol. All data are presented as mean ± SD, except for nonparametric values; TG, glucose, insulin, and HOMA-IR are presented as median (75th percentile). For insulin, glucose, and HOMA-IR, n = 13 in weight-stable and n = 17 in weight-reducing subjects.

[a] $P < 0.05$ weight-reducing *vs.* weight-stable subjects; [b] $P < 0.001$ and [c] $P < 0.05$ post- *vs.* preoperative values.

93–111 mg/dl for women and 103–118 mg/dl for men (50). The normal range for plasma glucose for men and women was between 2.5–5.3 mM (48).

Plasma ASP, insulin, leptin, adiponectin, and ghrelin concentrations

Plasma ASP was assayed by an in-house ELISA using a monoclonal antibody as capture antibody and a polyclonal antibody as detecting antibody as described in detail previously (11, 51). A standard curve over the range of 0.08–3.13 nM was prepared using purified plasma human ASP. The plasma ASP level in obese subjects in this study was compared with that in a reference nonobese Canadian population, which was previously published by our group (23.5 ± 10.8 nM) (11).

Plasma insulin, leptin, adiponectin, and ghrelin were all assayed by standardized RIA kits. Insulin was measured with a human insulin-specific RIA kit (Linco Research, Inc., St. Charles, MO) that does not react with proinsulin. The normal range for plasma insulin was 4.9–24.3 μU/ml for both men and women (48). Leptin was measured with a standardized RIA kit (Linco Research, Inc.) using a ^{125}I-iodinated human leptin tracer as previously described (52). Standards over the range of 0.5–100 ng/ml were prepared using recombinant human leptin. Leptin concentrations in normal weight subjects are 16.2 ± 6.9 ng/ml for women and 3.7 ± 1.7 ng/ml for men (53). Plasma adiponectin levels were measured using a standardized RIA kit for human adiponectin (Linco Research, Inc.). The assay uses ^{125}I-labeled adiponectin and an antiadiponectin rabbit antiserum to determine adiponectin concentrations by the double-antibody/polyethylene glycol technique. Standards over the range of 1–200 ng/ml were prepared using recombinant human adiponectin. All plasma samples were diluted 1:200, yielding an effective range of 0.2–40 μg/ml. The intra- and interassay coefficients of variation at adiponectin concentrations in the range of 3–15 μg/ml are 1.8–6.2% and 6.9–9.3%, respectively. With this assay we measured plasma adiponectin concentrations of 8.3 ± 3.2 μg/ml in 22 normal weight healthy subjects (mean ± SD; unpublished observations). For ghrelin, a standardized RIA kit (Phoenix Pharmaceuticals, Inc., Belmont, CA) was used with an antibody directed against the central portion of the ghrelin molecule. The detection limits for the ghrelin RIA assay were 2.9–378.9 pM. The reference group value for plasma ghrelin concentrations in normal weight subjects is 132.4 ± 13.1 pM (39).

Homeostasis model assessment for insulin resistance (HOMA-IR)

HOMA-IR was calculated from fasting plasma insulin and glucose levels as (insulin × glucose)/22.5, where the insulin concentration is reported as milliunits per liter and glucose as millimolar concentrations (54). Thus, the normal range for HOMA-IR calculated from normal ranges of insulin and glucose is 0.54–5.72 mU/liter·mM.

Statistical analysis

Parametric data were expressed as the mean ± SD. Nonparametric data were expressed as the median and 75th percentile. Data were compared using paired *t* test (pre- *vs.* postoperative) and two-sample *t* test (women *vs.* men, weight-stable *vs.* weight-reducing subjects) for normal data or signed rank test and Mann-Whitney rank-sum test for nonparametric data. Correlation was analyzed by the Pearson product-moment correlation for parametric and Spearman rank test correlation for nonparametric variables, and regression was analyzed by forward stepwise regression. Correlation and regression analyses were conducted for weight-stable subjects only. Statistical analysis was performed using SigmaStat (Jandel, San Rafael, CA), with significance set at $P < 0.05$ and a power of more than 80%.

Results

Body weight, plasma lipids, and apoB

As many of the metabolic parameters studied may be regulated by nutritional status, subjects were separated into two groups based on postoperative weight stability (Table 1). The gender differences in the pre- or postoperative states were not significant; thus, data from women and men were pooled (gender differences are reported for each parameter where they are significant).

The average preoperative BMI was 50.2 ± 8.1 kg/m^2 in women (n = 39) and 50.7 ± 14.9 kg/m^2 in men (n = 11). BMI was dramatically reduced after surgery (16–55% weight lost), and seven subjects in the weight-stable and four in the weight-reducing groups attained ideal body weight (BMI, 20–25 kg/m^2). However, despite massive weight loss (~50 kg on the average in both groups), most subjects were still categorized as obese, and to a comparable degree in both weight-stable and weight-reducing groups (52% and 64% above BMI >30 kg/m^2, respectively).

Preoperative elevated plasma TG was the most frequent lipid abnormality occurring in 49% of women (>1.31 mM) and 36% of men (>1.96 mM), followed by low plasma HDL cholesterol, occurring in 44% of women (<1.24 mM) and 36%

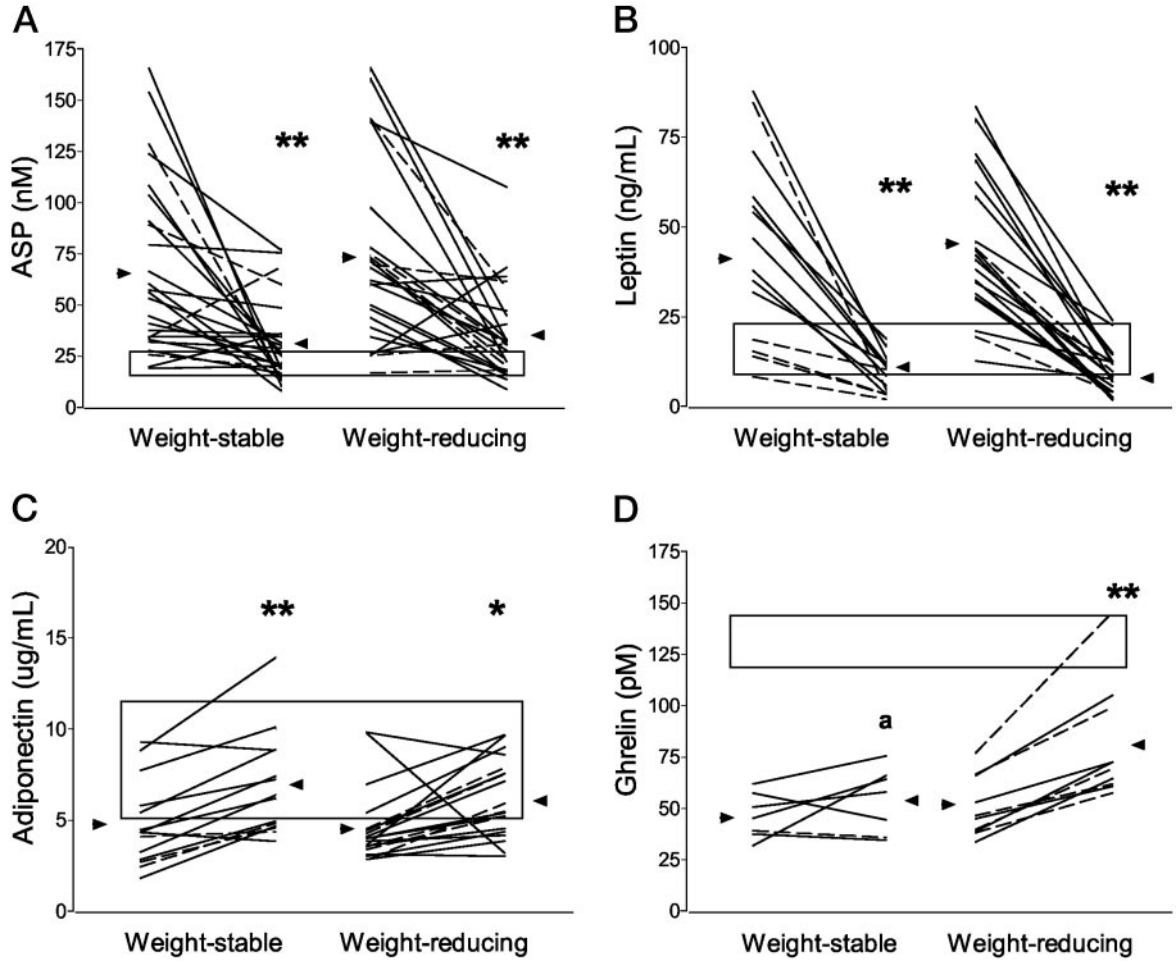

FIG. 1. Pre- to postoperative changes in plasma ASP, leptin, adiponectin, and ghrelin after weight loss in weight-stable and reducing subjects. The *box* represents the average ± 1 SD for a healthy lean reference group for each hormone (see *Subjects and Methods*; except for leptin where the box represents average ± SD for women only). *Arrows* indicate the average value for each hormone in the pre- and postoperative states in this study. Women are shown by *solid lines*, and men are indicated by *dotted lines*. *, $P = 0.01$; **, $P < 0.001$ (post- *vs.* preoperative values). a, $P = 0.04$ (weight-stable *vs.* weight-reducing postoperative ghrelin concentrations).

of men (<0.93 mM). Yet despite those lipid abnormalities in some subjects, preoperative median TG and average total and LDL cholesterol were all within the normal range for both women and men. Preoperative mean HDL cholesterol in women (1.14 ± 0.39 mM) was, however, lower than the normal range. None of the patients studied had abnormal preoperative plasma NEFA; however, women had higher preoperative NEFA than men (mean, 0.417 ± 0.154 mM in women *vs.* 0.272 ± 0.107 mM in men; $P < 0.05$). Postoperatively, median TG and average total, LDL, and HDL (in men) cholesterol were all within the normal range. However, plasma HDL cholesterol remained low in both weight-reducing and weight-stable women (Table 1).

Preoperatively, plasma apoB was above the 75th percentile in 38% of patients (16 women and 3 men), 16 of whom had other lipid abnormalities, yet, on the average, it was normal in both genders. Abnormal plasma apoB was the only lipid parameter that was fully corrected after weight loss in all subjects with elevated preoperative values despite their remaining obese on average.

Plasma glucose and insulin

Mean plasma glucose levels were slightly increased before the bypass surgery. Preoperatively, 57% of the patients had HOMA-IR levels above the upper limit for the calculated normal range. Postoperatively, both glucose and insulin levels decreased significantly with weight loss, and all patients had HOMA-IR values within the normal calculated range (Table 1). Subjects who were receiving medical treatment for diabetes before the surgery (six women and four men) had discontinued all hypoglycemic agents.

Plasma ASP, leptin, adiponectin, and ghrelin

Most obese subjects (86%) had elevated preoperative plasma ASP concentrations (>23.5 ± 10.8 nM), with an average of 75.0 ± 44.2 nM in women and 55.4 ± 34.5 nM in men (gender difference not significant; Fig. 1A). Postoperatively, plasma ASP decreased in most subjects. However, the average ASP concentrations in both weight-stable (31.4 ± 19.8 nM) and weight-reducing (35.5 ± 22.5 nM) subjects were still higher than normal ($P < 0.001$ for both groups), consis-

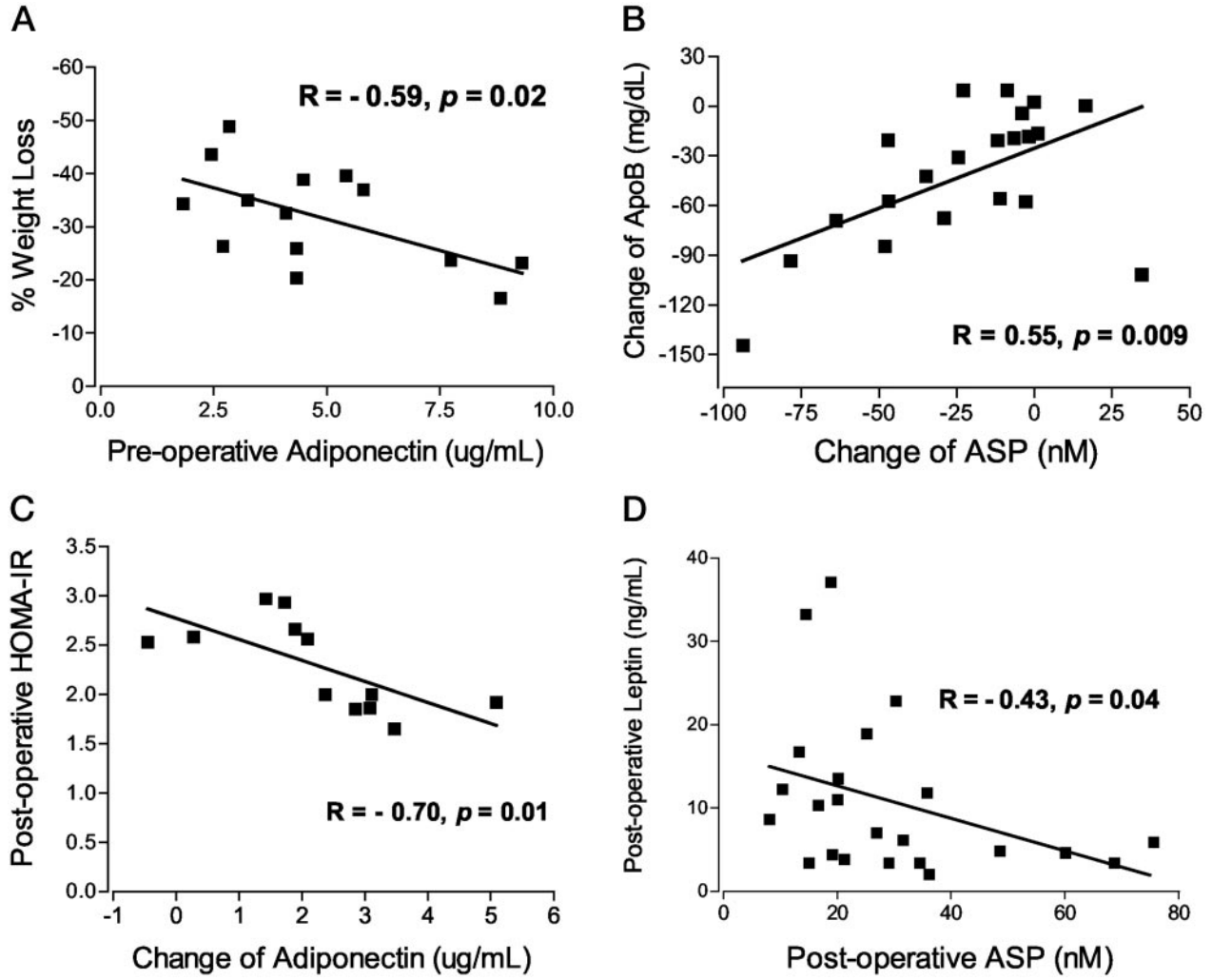

FIG. 2. Regression and correlation analyses in weight-stable subjects. The regression analysis is between preoperative plasma adiponectin concentration and percent drop in body weight (A), changes in plasma ASP and apoB concentrations (B), and changes in adiponectin and postoperative HOMA-IR (C). D, Correlation analysis between postoperative ASP and leptin concentrations in weight-stable subjects.

tent with the observation that most subjects in both groups were still obese (BMI, >30 kg/m^2).

Gender differences in plasma hormones were observed for leptin concentrations only. Women had significantly higher leptin in both preoperative (48.9 ± 19.3 ng/ml in women *vs.* 27.6 ± 24.4 ng/ml in men) and postoperative (11.1 ± 8.3 ng/ml in women *vs.* 5.1 ± 2.9 ng/ml in men) states. Leptin decreased postoperatively in almost all subjects (Fig. 1B), and in weight-reducing women fell below normal reference values despite the fact that the subjects remained obese (9.9 ± 6.4 ng/ml in postobese *vs.* 16.2 ± 6.9 ng/ml in reference group; $P = 0.01$). These low leptin levels, despite increased fat mass, are consistent with the known adiposity-independent effects of negative energy balance on leptin production (2).

Mean preoperative adiponectin concentrations were lower than those in the lean reference group (<8.3 ± 3.2 μg/ml) in both women (4.85 ± 2.18 μg/ml; $P < 0.001$) and men (4.09 ± 2.21 μg/ml; $P < 0.02$), and there were no significant gender differences. In contrast to the other adipose tissue hormones (ASP and leptin), adiponectin increased in response to weight loss after gastric bypass surgery in almost all subjects (Fig. 1C). Mean adiponectin was comparable to normal reference values in weight-stable and reducing subjects at 6.9 ± 2.18 and 6.2 ± 2.2 μg/ml, respectively.

Before surgery, all obese subjects had low plasma ghrelin concentrations compared with reference values in normal weight subjects (<119.3 pM), and there was no significant gender difference (48.4 ± 13.6 pM in women and 40.1 ± 16.9 pM in men; P = NS; Fig. 1D). Despite bypassing most of the stomach (~95%) and completely isolating the fundus from contact with nutrients, ghrelin concentrations did not decrease after surgery. Plasma ghrelin levels remained unchanged in the weight-stable subjects, but increased by approximately 60% in weight-reducing subjects (*i.e.* those in negative energy balance). Thus, the postoperative average ghrelin level in the weight-reducing group was higher than that in weight-stable subjects (80.8 ± 27.7 *vs.* 54.2 ± 15.8 pM; $P = 0.04$) despite the fact that their average postoperative BMI and gastric pouch size were similar to those in weight-stable subjects. Only one man in the weight-reducing group (who achieved a postsurgery body weight/BMI within the normal range) attained a plasma ghrelin concentration

within normal limits for nonobese subjects (119.3–145.5 pM). It should be noted, however, that all pre- and postoperative ghrelin levels measured were well within the detection limits of the ghrelin assay (2.9–378.9 pM).

Correlation analysis

Before surgery, the plasma parameters that correlated with BMI were leptin ($r = 0.60$; $P < 0.0005$), HOMA-IR ($r = 0.50$; $P = 0.003$), and plasma insulin ($r = 0.38$; $P = 0.03$). There was a positive correlation between plasma insulin and leptin ($r = 0.35$; $P = 0.04$) and a negative correlation between HOMA-IR and plasma ghrelin concentrations ($r = -0.47$; $P = 0.05$). The age of the subjects was correlated positively with plasma adiponectin ($r = 0.39$; $P = 0.03$) and glucose ($r = 0.38$; $P = 0.04$) and negatively with insulin concentrations ($r = -0.35$; $P = 0.04$). There was no association between any of the preoperative hormone levels and the metabolic parameters examined other than that between HOMA-IR and TG ($r = 0.37$; $P = 0.03$).

Postoperatively, there was a positive correlation between the preoperative value and the postoperative change in every plasma parameter measured; the higher the preoperative concentration, the greater the postoperative decrease (data not shown), except for adiponectin and ghrelin. The preoperative adiponectin level was negatively correlated with its percent increase after surgery ($r = -0.59$; $P = 0.02$), whereas no such correlation was observed for changes in plasma ghrelin levels.

As leptin, ASP, and adiponectin are all adipose tissue secreted factors, we examined the hypothesis that the preoperative concentrations of these hormones are predictive of the extent of weight loss in weight-stable subjects. Neither preoperative plasma leptin nor ASP concentrations were predictive of the extent of postsurgical weight loss. However, although adiponectin did not correlate with weight in the pre- or postoperative states, by forward stepwise regression analysis, proportional weight loss was best predicted by preoperative adiponectin concentrations ($r = -0.59$; $P = 0.02$). Thus, the lower the preoperative plasma adiponectin level, the greater the percent reduction in body weight (Fig. 2A).

We also examined whether changes in adipose tissue hormone levels would be predictive of improved lipid profiles and insulin sensitivity after weight loss in weight-stable subjects. The decrease in apoB was best predicted by the decrease in ASP, whereas changes in adiponectin and leptin did not add any further predictive value to the changes in plasma apoB. By regression analysis, 67% of the decrease in apoB was predicted by the changes in ASP and TG, with ASP being the primary predictor (55%; $P = 0.009$; Fig. 2B). As for improved insulin sensitivity postoperatively, 70% of postoperative HOMA-IR values were predicted by the increase in adiponectin ($P = 0.01$), whereas in this model ASP and leptin did not improve the prediction of postoperative HOMA-IR. Thus, subjects who exhibited the greatest increase in adiponectin concentrations were also the most insulin sensitive in the postoperative state (*i.e.* lowest postoperative HOMA-IR value; Fig. 2C). The extent of weight loss also correlated with time since surgery ($r = 0.49$; $P = 0.01$) and the changes in leptin ($r = 0.69$; $P = 0.005$), insulin ($r = 0.62$; $P = 0.02$), and HOMA-IR ($r = 0.71$; $P = 0.01$).

In the postoperative state, leptin was the only hormone that correlated with BMI after weight loss in weight-stable subjects ($r = 0.62$; $P = 0.001$). An unexpected finding was the negative correlation between postoperative plasma concentrations of ASP and leptin in weight-stable subjects ($r = -0.43$; $P = 0.04$; Fig. 2D).

Discussion

Obesity is a complex metabolic state. In the present study, despite the subjects' pathological obesity, on the average, preoperative lipid levels were surprisingly normal. The regulation of lipid metabolism and energy homeostasis in extreme obesity is not well understood. However, along with insulin, the four more recently described hormones examined in this study are believed to have major roles in substrate metabolism and energy balance. Thus, we examined for the first time all four hormones and their association with cardiovascular risk factors in a population of morbidly obese subjects before and after massive weight loss induced by gastric bypass surgery.

The response of plasma lipids after surgery-induced weight loss has been reported to be influenced by the type of surgery performed, such as jejuno-ileal, biliopancreatic, or gastric bypass (55–58). Therefore, only subjects who underwent the same type of standardized surgery, isolated longitudinal Roux-en-Y gastric bypass, were evaluated in the present study. Isolated Roux-en-Y gastric bypass reduces the volume of the stomach by about 95% and is associated with reduced food intake (42). Although the mechanism(s) by which this surgery produces substantial long-term weight loss with decreased hunger and energy intake is not well understood, persistent diarrhea, vomiting, and protein malnutrition do not occur in these patients (43) nor is there any evidence of fat malabsorption (44). The beneficial effects of gastric bypass-induced weight loss on plasma lipids, insulin, and glucose and the correction of diabetes have been demonstrated by a number of studies (56, 59–63) as well as the present report.

One major cardiovascular risk factor that few studies have examined after gastric bypass-induced weight loss or in a large sample size is plasma apoB. An elevated plasma apoB concentration is an independent risk factor for ischemic heart disease. Controlling for plasma TG, HDL cholesterol, and total/HDL cholesterol ratios does not eliminate its significance (64). In contrast to a previous study (63), abnormal preoperative apoB concentrations were normalized after weight loss in all subjects studied. Of note, the reductions in plasma total and LDL cholesterol were relatively small compared with that in apoB. Thus, assessment of changes in plasma total and LDL cholesterol alone without examining changes in apoB could minimize the true benefit of surgical treatment of obesity in the reduction of cardiovascular disease risk factors.

We examined the hypothesis that the change in adipose tissue hormones, ASP, leptin, and adiponectin, may predict the amelioration of the metabolic/cardiovascular risk profile after weight loss. The decrease in plasma ASP was the vari-

able most predictive of the reduction of plasma apoB levels after weight loss (55%). The close association between these two variables may reflect the influence that fatty acid flux has on both parameters. In a previous study fibroblasts obtained from subjects with high plasma apoB and ASP concentrations exhibited reduced glucose transport and triglyceride synthesis capacity in response to stimulation with ASP (65). This is consistent with ineffective adipose tissue fatty acid trapping as TG and a net flux of fatty acids to the liver resulting in high apoB. In the present study increased fatty acid flux to the liver in obesity may result in hepatic apoB overproduction (66), whereas increased fatty acid flux to adipose tissue results in fat storage and increased ASP secretion. Consequently, with caloric restriction, the reduced ASP and apoB levels may reflect diversion of fatty acids for energy demands, reducing both adipose storage and excess hepatic lipoprotein secretion.

With respect to weight loss, preoperative plasma adiponectin concentrations were predictive of the extent of weight loss after bypass surgery. Lower preoperative adiponectin concentrations predicted a greater percent reduction in body weight and were associated with greater increases in plasma adiponectin concentrations after surgery. In rodents, adiponectin administration decreases hepatic glucose production and increases muscle fatty acid oxidation (33, 67). Therefore, it is possible that those subjects with the lowest preoperative adiponectin concentrations and who had the greatest increase in adiponectin after weight loss may have benefited from larger increases in muscle fatty acid oxidation. The increase in adiponectin after weight loss was also predictive of the improvement of insulin sensitivity as estimated by HOMA-IR values. Interestingly, thiazolidinedione (peroxisomal proliferator-activated receptor γ agonists) that improve insulin sensitivity in patients with type 2 diabetes also increase adiponectin gene expression and plasma adiponectin levels (68), suggesting a mechanism by which this class of drugs enhances insulin sensitivity. In addition to improving insulin sensitivity, one of the demonstrated effects of adiponectin is the inhibition of TNFα production and TNFα-induced monocyte adhesion to aortic endothelial cells, an early stage in the atherosclerotic vascular change (69). Obesity is associated with increased risk of both type II diabetes and cardiovascular disease, thus increased adiponectin production after weight loss in obese subjects may represent an important link between weight loss and improved insulin sensitivity and cardiovascular risk profiles.

ASP and leptin are also secreted by adipose tissue, but in contrast to adiponectin, circulating levels of these hormones tend to reflect the size of their tissue of origin, increasing with obesity (2, 11). Before surgery, nearly all obese subjects had elevated plasma ASP and leptin concentrations, and both hormones decreased with postsurgical weight loss, although the decreases in leptin were greater in magnitude. Despite weight stability, there was no correlation between changes in plasma ASP and weight loss. Similarly, although most weight-stable subjects remained obese, all had normal or below normal leptin concentrations compared with published reference values, suggesting that factors other than body adiposity regulate ASP and leptin production. Although circulating leptin concentrations are highly correlated with indexes of body adiposity, body fat mass is not the sole determinant of plasma leptin levels (20, 53, 70–72), and other nutritional factors, such as recent energy intake (53) and dietary macronutrient composition (73), are involved in the regulation of leptin production.

Two major determinants of circulating concentrations of the novel gastroenteric hormone ghrelin have been identified. The first is body weight, as plasma ghrelin concentrations are negatively correlated with BMI over a wide range (74, 75). In this study plasma ghrelin levels were indeed low in morbidly obese subjects, although we did not observe a significant correlation with BMI within this very obese population. The second factor suggested to regulate ghrelin secretion is food intake, as plasma ghrelin levels decrease shortly after food ingestion, a response that may involve increases in circulating insulin and glucose (39, 76) and/or contact of nutrients with the gastroenteric lumen (37). Cummings *et al.* (41) recently reported that plasma ghrelin concentrations over a 24-h period were markedly lower in five patients studied after gastric bypass surgery than in either normal weight or comparably obese control subjects. Whether plasma ghrelin was changed compared with its concentration before surgery in that study is unknown, as presurgical ghrelin levels were not measured in those patients. The researchers hypothesized that ghrelin levels were low in these patients because ingested nutrients bypassed most of the stomach and upper intestine. They suggested that the paradoxical absence of postprandial change in ghrelin, which is normally reduced by food ingestion, was due to override inhibition from a continuous empty stomach and duodenum. In agreement with these results, in the present study plasma ghrelin concentrations after surgery in weight-stable subjects remained low, comparably to those reported by Cummings *et al.* (41).

However, plasma ghrelin levels increased by approximately 60% in the subset of subjects experiencing active weight loss at the time the postsurgical samples were collected. In contrast, plasma ghrelin did not increase after surgery in weight-stable subjects (in neutral energy balance) despite a similar degree of weight loss, postoperative BMI, and size of the gastric pouch as in weight-reducing subjects (in negative energy balance). Thus, energy balance may be a more important determinant of postsurgical ghrelin levels after gastric bypass than body weight *per se*. The postsurgical increase in ghrelin in weight-reducing subjects suggests that ghrelin-secreting cells can increase the production of ghrelin in response to negative energy balance, even if the gastric fundus and upper small intestine are continuously prevented from exposure to incoming nutrients. Alternatively, the increase in ghrelin may reflect increased ghrelin secretion from extragastric sources, as a number of other organs, including much of the lower gastrointestinal tract, contain ghrelin and could therefore contribute to circulating ghrelin levels (77).

Nonetheless, plasma ghrelin concentrations after surgery in nearly all subjects, whether weight-stable or weight-reducing, remained lower than the levels reported in either normal weight or comparably obese subjects (41, 75). The finding that plasma ghrelin is not increased after weight loss in weight-stable patients contrasts with numerous reports that ghrelin concentrations are substantially increased even after far lesser degrees of weight loss produced by methods other than gastric bypass surgery, *i.e.* calorie-restricted diets (40, 41). Thus, it is possible that low circulating levels of this orexigenic hormone

could contribute to initial weight loss and/or maintenance of weight loss after this type of gastric bypass surgery.

The hormones examined (insulin, leptin, ghrelin, and ASP) may be reciprocal indicators/regulators of acute and chronic energy balance. Insulin and leptin have opposing actions to ghrelin on food intake; leptin and insulin inhibit feeding, whereas ghrelin is orexigenic. They are also opposing indicators of chronic energy balance; circulating leptin and insulin levels are increased in obesity and decrease with weight loss, whereas the ghrelin concentration has opposite changes (2, 3). Like leptin, plasma ASP is also elevated in obese subjects and decreases after weight loss (11, 15), and although there is little evidence for ASP action in the hypothalamus, administration of ASP has been reported to acutely increase short-term food intake in rodents (78). Improved insulin sensitivity and sustained low ghrelin concentrations after gastric bypass surgery would favor maintenance of weight loss in these patients. In contrast, a combination of reduced postoperative leptin and elevated ASP concentrations would favor increased food intake, decreased energy expenditure, and increased adipose tissue fat storage and eventually promote weight regain in postobese subjects. This subclass of patients with very low leptin levels and high ASP concentrations might therefore be at greater risk for postoperative weight regain.

In summary, weight loss is associated with amelioration and often total correction of a number of the metabolic abnormalities associated with extreme obesity. Our results underscore the coordinated roles of insulin, leptin, ASP, adiponectin, and ghrelin as signals in the long-term regulation of energy balance and carbohydrate and lipid metabolism. Furthermore, in this study of isolated Roux-en-Y gastric bypass patients we report novel data suggesting that 1) preoperative fasting adiponectin concentrations are predictive of the extent of weight loss; 2) changes in ASP and adiponectin predict decreases in apoB and improved insulin action, respectively; 3) plasma ghrelin concentrations increase after bypass surgery in patients experiencing active weight loss despite isolation of the fundus from incoming nutrients, but remain low compared with those in normal weight subjects; and 4) low ghrelin concentrations could contribute to decreased hunger and food intake, weight loss, and maintenance of weight loss after gastric bypass surgery.

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