

# Increases in Adiponectin Predict Improved Liver, but Not Peripheral, Insulin Sensitivity in Severely Obese Women During Weight Loss

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**Obesity-related glucose intolerance is a function of hepatic (homeostatic model assessment-insulin resistance [HOMA-IR]) and peripheral insulin resistance ( $S_i$ ) and  $\beta$ -cell dysfunction. We determined relationships between changes in these measures, visceral (VAT) and subcutaneous (SAT) adipose tissue, and systemic adipocytokine biomarkers 1 and 6 months after surgical weight loss. HOMA-IR decreased significantly ( $-50\%$ ) from baseline by 1 month and decreased further ( $-67\%$ ) by 6 months, and  $S_i$  was improved by 6 months (2.3-fold) weight loss. Plasma concentrations of leptin decreased and adiponectin increased significantly by 1 month, and decreases in interleukin-6, C-reactive protein (CRP), and tumor necrosis factor- $\alpha$  were observed at 6 months of weight loss. Longitudinal decreases in CRP ( $r = -0.53$ ,  $P < 0.05$ ) were associated with increases in  $S_i$ , and decreases in HOMA-IR were related to increases in adiponectin ( $r = -0.37$ ,  $P < 0.05$ ). Decreases in VAT were more strongly related to increases in adiponectin and decreases in CRP than were changes in general adiposity or SAT. Thus, in severely obese women, specific loss of VAT leads to acute improvements in hepatic insulin sensitivity mediated by increases in adiponectin and in peripheral insulin sensitivity mediated by decreases in CRP. *Diabetes* 56:735–742, 2007**

**I**nsulin resistance is a key feature of several obesity-related diseases, including glucose intolerance, type 2 diabetes, cardiovascular disease, stroke, cancer, and infertility (1). The links between excess adipose tissue and the development of insulin resistance are not well understood. Recently, however, evidence shows that

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BPD, bilio-pancreatic diversion; CRP, C-reactive protein; FSIGTT, frequently sampled intravenous glucose tolerance test; HOMA-IR, homeostatic model assessment-insulin resistance; IL-6, interleukin-6; SAT, subcutaneous adipose tissue; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; VAT, visceral adipose tissue.

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adipose tissue is an active endocrine organ, releasing several molecules with hormonal and pro-inflammatory action. These "adipocytokines," including interleukin-6 (IL-6), leptin, resistin, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and adiponectin, have been shown to influence insulin action (rev. in 2). However, the contribution of each of the adipocytokines to the development of glucose dysregulation in humans is not well understood, particularly when assessed serially during significant weight loss in women.

Insulin resistance induced by obesity reflects decreased sensitivity of the periphery (skeletal muscle and adipose tissue) and the liver to the effects of insulin, resulting in decreased glucose uptake by the muscles and glucose overproduction by the liver. Individuals with glucose intolerance have a secondary defect, inability of pancreatic  $\beta$ -cells to increase insulin secretion as needed to compensate for reduced insulin sensitivity, termed  $\beta$ -cell dysfunction. Methods that use fasting glucose and insulin concentrations to characterize insulin sensitivity, such as the homeostatic model assessment-insulin resistance (HOMA-IR), are thought largely to measure insulin resistance in the liver, whereas the euglycemic-hyperinsulinemic clamp and the intravenous glucose tolerance test may assess both peripheral insulin resistance and  $\beta$ -cell function (3).

Although the prevalence of severe obesity (BMI  $>40$  kg/m<sup>2</sup>) at 4.9% of the U.S. population has reached epidemic proportions (4), there is little information regarding the impact of  $>40$  kg of excess fat (5) on glucose and insulin metabolism. Also, although many studies have examined the relationships of total adipose tissue as well as visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) to insulin resistance (rev. in 6), there is limited information in the severely obese (7,8). Paradoxically, although obesity is a strong risk factor for type 2 diabetes, we and others have reported that only 20–30% of severely obese patients have type 2 diabetes (9,10). This suggests that severe obesity per se may not be a sufficient determinant of diabetes.

Individuals with severe obesity who undergo weight loss and improve their glucose tolerance are ideal subjects for studies that aim to clarify these issues. Accordingly, we have characterized the effects of generalized and abdominal adiposity on rigorous informative measures of glucose intolerance, including peripheral insulin sensitivity, hepatic insulin sensitivity, and  $\beta$ -cell function in severely obese women who display a wide variation in fat distribution. Using this clinical weight loss model, we had previously reported that weight loss-induced decreases in

systemic concentrations of the inflammatory mediator C-reactive protein (CRP) predicted improvements in insulin sensitivity (9). In the present study, we determined whether associations between weight loss-induced changes in circulating adipocytokines and changes in glucose tolerance could point to the potential importance of different factors in modulating insulin action. The aims of this study were to determine longitudinal relationships between changes in adiposity, changes in systemic concentrations of adipocytokines, and changes in measures of glucose tolerance at 1 and 6 months after surgical weight loss interventions in severely obese women.

## RESEARCH DESIGN AND METHODS

Subjects in the study were 28 severely obese female patients who had weight loss treatment at Emory Bariatrics by undergoing laparoscopic roux-en-y gastric bypass surgery (11). The study had a longitudinal design in which each patient served as his own control. Subjects were eligible for surgery after evaluation (clinical, psychological, and nutritional) when they were recruited for the study. Exclusion criteria were 1) male sex, 2) age <18 years or >65, 3) BMI <35 kg/m<sup>2</sup>, and 4) current smoking history. All medication use, including that used to treat metabolic syndrome, was monitored throughout the study. Recent diet history, using 3-day food records, was collected at each study visit. The Emory University institutional review board approved the study, and all patients gave informed consent before they were enrolled.

**Glucose tolerance testing.** Measurement of glucose tolerance, anthropometry, adipose tissue distribution, and plasma inflammatory biomarkers was obtained at baseline (before treatment) and at 1 and 6 months after surgery. During the week before the baseline and the 6 months after weight loss, measures were obtained, and subjects were weight stable ( $\pm 1$  kg) (12) and placed on a diet containing sufficient carbohydrate ( $\geq 150$  g) to allow for optimal glucose tolerance testing. These steady-state conditions not were feasible at the 1-month weight loss studies when patients were undergoing  $\sim 3$  kg/week weight loss. Patients did not take insulin sensitizer medications the night before or the morning of the glucose tolerance test.

Insulin action was assessed via the frequently sampled intravenous glucose tolerance test (FSIGTT) and by HOMA-IR. Patients were admitted into the Emory General Clinical Research Center on the night before FSIGTT testing and fasted overnight (12 h). An intravenous catheter was inserted into an antecubital vein for blood sampling. Baseline samples were obtained at  $-15$  and  $-5$  min. Glucose (0.3 g/kg body wt, as dextrose 50 g/dl) was then administered within 2 min, and subsequent samples were obtained at 2, 4, 6, 8, 10, 14, 19, 22, 24, 27, 30, 40, 50, 70, 90, 120, 150, 180, 210, and 240 min, relative to the start of glucose infusion. At 20 min, subjects received an intravenous bolus of human insulin (0.02 units/kg body wt). Minimal modeling analysis of glucose and insulin levels (13) was used to quantify insulin sensitivity ( $S_i$ ), insulin secretion (acute insulin response to glucose), and the disposition index (MinMod Millennium, Los Angeles, CA). HOMA-IR was calculated using fasting insulin ( $\mu\text{U/ml}$ )  $\times$  fasting glucose (mmol/l)/22.5.

**Anthropometry, body composition, and fat distribution.** Body fat composition was measured by air plethysmography (BOD-POD; Life Measurement Instruments, Concord, CA). Abdominal fat distribution was measured by computed tomography, using a GE High Speed Advantage CT scanner (General Electric Medical Systems, Milwaukee, WI) as described previously (5). Volumes of VAT and SAT were determined from scans taken from the L1 to the L5 vertebral region (140 kV, 240–340 mA/s, and 10 mm slice thickness). Adipose tissue within an attenuation range of  $-190$  to  $-30$  Hounsfield units was highlighted and computed using software (GE Medical Systems). In our scanner, we are able to analyze patients with body weight <182 kg (400 pounds). We have found that all patients fit in the scan tube, however, in a few cases, depending on the shape of the patient, small portions of periphery of subcutaneous fat are outside the field of view of the scanner. Because the present study only includes women, with an average weight of 127 kg (range 108–146 kg), we rarely experienced problems measuring VAT and SAT volumes. If these measures were not adequately obtained, the patient's data were not included in the analysis. Sagittal abdominal diameter was obtained at the L4-L5 intervertebral space using an abdominal caliper (14). Body height was measured without shoes. Body weight was measured with subjects in light clothing, in the fasting state, and immediately after voiding in the morning. Waist circumference was obtained by tape measure at 2.54 cm above the iliac crest, whereas hip circumference was determined as the maximum value over the buttocks.

**Metabolic measures.** Serum insulin and glucose were quantified at the Emory University Hospital Laboratory using the Beckman Coulter DX1 and Beckman Coulter Alex 20 automated systems, respectively (Beckman Coulter, Brea, CA). The limit of the assay for insulin was 1  $\mu\text{U/ml}$ , and that for glucose was 0.17 mmol/l. Plasma leptin was measured using a commercial human enzyme-linked immunosorbent assay (ELISA) kit, (Diagnostic Systems Laboratory, Webster, TX). The inter- and intra-assay variability is 4.4 and 4.0%, respectively. Plasma resistin, adiponectin, IL-6 (high sensitivity), and TNF- $\alpha$  (high sensitivity) were measured using commercial human ELISA kits (R&D Systems, Minneapolis, MN). We found the sensitivity of the assays and the inter- and inter-assay precision to be similar to those stated by the manufacturers. High-sensitivity CRP was measured using the SYNCHRON LX20 high-sensitivity immunoassay (Beckman Coulter). The sensitivity of the assay is 0.07 mg/dl. Free fatty acids were measured by ARUP Laboratories (Salt Lake City, UT).

**Statistical analysis.** The statistical software STATISTICA (StatSoft, Tulsa, OK) was used for analysis. The differences between baseline and 1- and 6-month after surgery measures were analyzed using Student's *t* tests. Because many outcome measures were not normally distributed, the relationships between changes in  $S_i$ , HOMA-IR, and various metabolic and anthropometrical parameters were examined as linear correlations using the Spearman rank order test. Multiple regression analysis was done to determine whether the correlations between adiponectin and HOMA-IR were confounded by VAT. The significance level was set at  $P < 0.05$ . Results are expressed as means  $\pm$  SE.

## RESULTS

**Patient characteristics.** Twenty-eight patients completed the full longitudinal analysis. Their average age was  $36.1 \pm 1.7$  years (range 24–55), and 25% of women were postmenopausal. Nine women were self-described as African American in descent, 16 were Caucasian, and 3 were Hispanic. The mean BMI was  $48.2 \pm 0.7$  kg/m<sup>2</sup> (range 39.7–55.3). All women had abdominal obesity (waist circumference  $>88$  cm), and 61% of patients had two other criteria of Adult Treatment Panel III–defined metabolic syndrome (dyslipidemia, hypertension, and glucose intolerance) (15). Six patients (21% of the population) had diabetes at baseline and were taking medication to improve insulin sensitivity. Diabetes was resolved in four patients acutely after surgery, and these patients were not taking medication at their 1-month evaluations. Two patients remained on medication for diabetes but were taking lower doses by their 6-month evaluations.

**Changes in adiposity after weight loss surgery.** Significant decreases in adiposity were observed after weight loss as shown in Table 1. Weight loss compared with baseline was  $11.9 \pm 0.8$  kg at 1 month and  $34.3 \pm 1.4$  kg at 6 months. The majority of weight loss was fat mass, which decreased by  $8.9 \pm 0.7$  and  $29.6 \pm 1.2$  kg, as compared with lean mass, which decreased by  $3.3 \pm 0.6$  and  $4.6 \pm 0.5$  kg at 1 and 6 months, respectively. Greater percentages of VAT were lost compared with SAT at 1 and 6 months (VAT,  $-12$  and  $-44\%$ ; SAT,  $-3$  and  $-33\%$ , respectively,  $P < 0.05$ ); changes in SAT volumes were not significant at 1 month. Other anthropometrical measures of abdominal adiposity, including waist circumference and sagittal abdominal diameter, were also decreased from baseline at 1 and 6 months of weight loss.

**Changes in metabolic variables after surgical weight loss.** Metabolic variables at baseline, 1 month, and 6 months are shown in Table 2. At 1 and 6 months, improvements were observed in plasma concentrations of LDL ( $-16 \pm 6$  and  $-22 \pm 5$  mg/dl, respectively), triglycerides ( $-26 \pm 12$  and  $-26 \pm 8$  mg/dl), and CRP ( $-0.19 \pm 0.23$  and  $-0.79 \pm 0.17$  mg/dl). Systolic blood pressure was decreased at 1 and 6 months, and diastolic blood pressure was decreased at 6 months. Transient increases were

TABLE 1  
Anthropometrical characteristics of 28 severely obese patients undergoing roux-en-y gastric bypass surgery

	Baseline	1 month	$\Delta$ 1 month	6 months	$\Delta$ 6 months
Weight (kg)	126.7 $\pm$ 1.9 (107.5–146.2)	115.2 $\pm$ 1.9 (95.7–133.7)	–11.9 $\ddagger$	92.4 $\pm$ 2.2 (73.0–127.7)	–34.3 $\ddagger$
Fat mass (kg)	71.3 $\pm$ 1.6 (51.5–88.3)	62.9 $\pm$ 1.5 (44.5–76.3)	–8.9 $\ddagger$	41.7 $\pm$ 1.8 (30.0–72.7)	–29.6 $\ddagger$
Lean mass (kg)	55.0 $\pm$ 1.0 (43.4–64.6)	51.7 $\pm$ 0.9 (41.1–60.7)	–3.3 $\ddagger$	50.5 $\pm$ 0.9 (38.2–56.4)	–4.6 $\ddagger$
BMI (kg/m <sup>2</sup> )	48.2 $\pm$ 0.7 (39.7–55.3)	43.4 $\pm$ 0.8 (34.7–52.2)	–4.8 $\ddagger$	35.5 $\pm$ 0.9 (27.2–48.0)	–12.7 $\ddagger$
VAT (cm <sup>3</sup> )	4,029 $\pm$ 330 (1,310–8,430)	3,580 $\pm$ 330 (1,410–7,490)	–520 $\ddagger$	2,240 $\pm$ 230 (490–5,470)	–1,790 $\ddagger$
SAT (cm <sup>3</sup> )	12,990 $\pm$ 320 (9,930–17,660)	12,530 $\pm$ 360 (8,919–17,810)	–500	8,510 $\pm$ 390 (3,780–12,730)	–4,480 $\ddagger$
WC (cm)	135.6 $\pm$ 2.5 (112.5–165.0)	130.0 $\pm$ 2.5 (106.5–163.0)	–5.0 $\ddagger$	112.6 $\pm$ 2.7 (89.0–140.5)	–23.3 $\ddagger$
SAD (cm)	30.3 $\pm$ 0.4 (26.0–34.0)	28.2 $\pm$ 0.5 (24.0–33.5)	–2.2 $\ddagger$	23.8 $\pm$ 0.5 (19.5–29.2)	–6.6 $\ddagger$

Data are means  $\pm$  SE (range). WC, waist circumference; SAD, sagittal abdominal diameter.  $\Delta$ , change in measures from baseline. \* $P < 0.05$ ,  $\ddagger P < 0.01$ ,  $\ddagger\ddagger P < 0.001$ .

observed in free fatty acids ( $0.33 \pm 0.06$  mmol/l) and aspartate aminotransferase ( $4.4 \pm 3.9$  units/l), and a transient decrease was observed in alanine aminotransferase ( $-4.9 \pm 5.2$  units/l) at 1 month after weight loss, but values became comparable with baseline at 6 months. HDL concentrations were reduced compared with baseline at 1 month ( $-13 \pm 2$  mg/dl) and at 6 months ( $-4 \pm 1$  mg/dl), but the HDL-to-LDL ratio was not changed by weight loss intervention.

**Changes in adipocytokines after weight loss intervention.** Systemic plasma concentrations of adiponectin, leptin, resistin, and IL-6 and changes during weight loss are reported in Table 3. In response to weight loss, adiponectin increased at 1 month and was further increased by 6 months after weight loss intervention. In contrast, leptin decreased at 1 month and decreased further by 6 months. IL-6 concentrations were not changed at 1 month but decreased at 6 months. We did not observe any changes in resistin over the 6-month period.

**Longitudinal relationships between changes in abdominal adiposity and systemic adipocytokines.** We determined correlations between longitudinal changes between VAT and SAT volumes and concentrations of adipocytokines over 6 months of weight loss (Table 4). There were stronger correlations between visceral fat ( $r = 0.35$ ,  $P = 0.07$ ; Fig. 1A) and CRP compared with those between subcutaneous fat ( $r = 0.27$ ,  $P = 0.17$ ). There were equally strong positive correlations between both visceral and subcutaneous fat volumes and leptin concentrations ( $r = 0.40$ ,  $P = 0.04$  and  $r = 0.40$ ,  $P = 0.04$ , respectively). However, there were significant correlations between increases in adiponectin and decreases in visceral (Fig. 1B) but not subcutaneous adiposity ( $r = -0.37$ ,  $P < 0.05$  vs.  $r = -0.35$ ,  $P > 0.05$ , respectively). Thus, in most cases, correlations were stronger with visceral adiposity than with subcutaneous adiposity. As might be expected, multiple regression analysis found that relationships between VAT and adiponectin were partially explained by total body fat. No correlations were found with changes in IL-6 or resistin and adipose tissue volumes.

**Changes in glucose regulation after weight loss intervention.** Changes in peripheral insulin sensitivity ( $S_i$ ), hepatic insulin resistance (HOMA-IR), glucose levels, insulin secretion (acute insulin response to glucose), and disposition index are given in Table 2. At baseline, 68% of patients had peripheral insulin resistance as defined by Saad et al. (16) [ $S_i < 2.0 \times 10^4$  (mU/l)<sup>-1</sup> · min<sup>-1</sup>], 25% had hepatic insulin resistance as defined by Radikova et al. (17) (HOMA  $> 4$  mmol/l per  $\mu$ U/ml), and 36% had impaired fasting glucose (glucose  $> 5.6$  mmol/l)

(18). At 1 and 6 months after the start of weight loss, 64 and 21%, respectively, of subjects continued to exhibit peripheral insulin resistance, but only one patient continued to have hepatic insulin resistance and impaired fasting glucose. The HOMA index decreased dramatically at 1 month ( $-50\%$ ) and 6 months ( $-67\%$ ), and peripheral insulin sensitivity was not changed at 1 month but increased markedly (2.3-fold) at 6 months. No changes in first-phase insulin secretion were observed over the 6-month period, but disposition index rose 7.2-fold at 6 months ( $P < 0.01$ ).

**Longitudinal relationships between changes in glucose regulation and metabolic variables.** As shown in Table 5 and Figs. 2 and 3, changes in adiposity during the 6-month weight loss period revealed strong correlations among metabolic variables. Decreases in VAT were significantly correlated to improvements in peripheral insulin sensitivity ( $r = -0.38$ ,  $P = 0.05$ ). Also, CRP concentrations decreased concomitantly with the improvement in  $S_i$  ( $r = -0.53$ ,  $P = 0.01$ ). When patients with diabetes were excluded from the analysis, decreases in CRP were correlated with increases in  $S_i$  ( $r = -0.38$ ,  $P = 0.05$ ). There were no correlations between changes in  $S_i$  and changes in other measures of adiposity, including SAT and fat mass, or changes in other metabolic variables, including free fatty acids, adiponectin, leptin, IL-6, and liver enzymes. In contrast, for both the 1- and 6-month weight loss periods, significant correlations were observed between changes in the HOMA index and changes in adiponectin ( $r = -0.41$ ,  $P = 0.03$  [Fig. 2A] and  $r = -0.37$ ,  $P = 0.04$  [Fig. 2B]). Decreases in HOMA-IR were weakly correlated to decrease in VAT over 6 months of weight loss ( $r = 0.33$ ,  $P = 0.09$ ). However, there were no correlations between changes in HOMA and any changes in adiposity over 1 month. There was also a significant correlation between changes in VAT and adiponectin over 6 months ( $r = -0.37$ ,  $P < 0.04$ ). When patients with diabetes were excluded from the analysis, the longitudinal relationship between HOMA-IR and adiponectin was not significant ( $r = -0.27$ ,  $P = 0.18$ ). Multivariable regression revealed that when change in adiponectin was added as a variable, the Pearson correlation coefficient between change in HOMA and change in VAT over 6 months was no longer significant, suggesting that the relationship between HOMA and VAT can be partially explained by adiponectin.

Although there was an improvement in disposition index at 6 months, there were no significant correlations between changes in disposition index and changes in measures of adiposity or changes in IL-6, resistin, adiponectin, or leptin (data not shown).

TABLE 2  
Clinical and metabolic characteristics of 28 severely obese patients undergoing surgical weight loss

	Baseline	1 month	$\Delta$ 1 month	6 months	$\Delta$ 6 months
Glucose (mmol/l)	5.62 $\pm$ 0.31 (3.67–10.67)	4.42 $\pm$ 0.19 (3.39 $\pm$ 7.72)	–1.10 $\ddagger$	4.31 $\pm$ 0.11 (3.39–6.61)	–1.32 $\ddagger$
Insulin ( $\mu$ U/ml)	13.04 $\pm$ 1.20 (2.00–32.04)	7.33 $\pm$ 0.80 (2.00–16.46)	–6.10 $\ddagger$	4.76 $\pm$ 0.70 (1.00–18.40)	–8.30 $\ddagger$
HOMA (mmol/l per $\mu$ U/ml)	3.32 $\pm$ 0.37 (0.58–8.06)	1.50 $\pm$ 0.23 (0.32–4.98)	–1.81 $\ddagger$	0.97 $\pm$ 0.19 (0.17–5.41)	–2.35 $\ddagger$
$S_i$ ( $10^4$ mU/l) $^{-1} \cdot \text{min}^{-1}$	1.93 $\pm$ 0.23 (0.20–6.39)	1.99 $\pm$ 0.26 (0.06–4.47)	0.32	3.16 $\pm$ 0.30 (1.09–8.84)	1.24 $\ddagger$
AIRg ( $\mu$ U $\cdot$ ml $^{-1} \cdot \text{min}^{-1}$ )	432 $\pm$ 85 (–18 to 1605)	555 $\pm$ 149 (16–3,200)	77	330 $\pm$ 38 (26–706)	–103
Disposition index ( $\text{min}^{-1}$ )	619 $\pm$ 104 (–34.3 to 1610)	716 $\pm$ 107 (14.6–1,550)	89	881 $\pm$ 87 (110–1,890)	263 $\ddagger$
hsCRP (mg/dl)	1.31 $\pm$ 0.23 (0.37–4.5)	1.15 $\pm$ 0.22 (0.25–4.82)	–0.19*	0.52 $\pm$ 0.10 (0.52–1.90)	–0.79 $\ddagger$
Systolic blood pressure (mmHg)	136 $\pm$ 4 (104–193)	127 $\pm$ 3 (102–166)	–9 $\ddagger$	123 $\pm$ 3 (93–158)	–14 $\ddagger$
FFA (mmol/l)	0.71 $\pm$ 0.04 (0.21–1.11)	1.04 $\pm$ 0.05 (0.67–1.68)	0.33 $\ddagger$	0.74 $\pm$ 0.04 (0.38–1.33)	0.02
HDL (mg/dl)	43 $\pm$ 1.7 (27–63)	33 $\pm$ 2 (17–52)	–13 $\ddagger$	39 $\pm$ 1 (28–58)	–4*
LDL (mg/dl)	103 $\pm$ 7 (53–177)	83 $\pm$ 6 (22–172)	–16 $\ddagger$	80 $\pm$ 4 (29–123)	–22 $\ddagger$
Triglycerides (mg/dl)	120 $\pm$ 12 (43–300)	97 $\pm$ 6.3 (52–203)	–26 $\ddagger$	92 $\pm$ 8.9 (38–230)	–26 $\ddagger$
AST (units/l)	28.0 $\pm$ 5.7 (14.0–135)	32.2 $\pm$ 4.7 (16.0–80.0)	4.39*	21.2 $\pm$ 1.35 (14.0–38.0)	–1.53
ALT (units/l)	29.6 $\pm$ 5.6 (11.0–125)	31.1 $\pm$ 5.3 (14.0–84.0)	–4.94*	21.3 $\pm$ 2.4 (10.0–51.0)	–5.68

Data are means  $\pm$  SE (range). AIRg, acute insulin response to glucose; hsCRP, high-sensitivity CRP; FFA, free fatty acids; AST, aspartate aminotransferase; ALT, alanine aminotransferase.  $\Delta$ , change in measures from baseline. \* $P$  < 0.05,  $\ddagger P$  < 0.01,  $\ddagger P$  < 0.001.

## DISCUSSION

This study found that in the 6 months after surgically induced weight loss, severely obese women experienced considerable decreases in whole-body and abdominal adiposity along with improvements in important aspects of glucose regulation and insulin action: peripheral insulin sensitivity, hepatic insulin resistance, and  $\beta$ -cell dysfunction. Improvements were also observed in other clinically important biomarkers of the metabolic syndrome, including triglycerides and blood pressure, and in the inflammatory mediator CRP. Most importantly, early 1- and 6-month improvements in hepatic insulin resistance were best predicted by increases in systemic concentrations of adiponectin. Strong correlations were also observed between changes in peripheral insulin sensitivity, CRP, and VAT but not other measures of adiposity or systemic adipocytokines.

Although it has been well documented in the severely obese that weight loss induces improvements in glucose tolerance, few studies have explored the detailed dynamics of insulin and glucose regulation using robust tools such as the hyperinsulinemic-euglycemic clamp or the intravenous glucose tolerance test with minimal model analysis (19–25). Thus, little is known about whole-body insulin sensitivity and  $\beta$ -cell function and changes after weight loss in the severely obese, who now represent 4.9% of the U.S. population and a significant percentage (13.5%) of African-American females (4).

Our finding that peripheral insulin sensitivity was improved by 6 months of weight loss (a 25% reduction in

weight) is comparable with those of other studies (19,21,23–25). Even more rapid improvements in insulin sensitivity were reported in severely obese patients who have undergone bilio-pancreatic diversion (BPD) for weight loss, a surgery that induces nutrient malabsorption (26). Unlike for patients after BPD, for roux-en-y gastric bypass surgery patients, we found that a similar and modest amount of weight loss (–10% reduction at 1 month) did not change peripheral insulin resistance but normalized hepatic insulin resistance. Interestingly, although 68% of patients had peripheral insulin resistance at baseline, only 25% exhibited hepatic insulin resistance as defined (17), only 21% had type 2 diabetes, similar to previous reports (10), and only a third exhibited impaired fasting glucose. Such a low prevalence of glucose intolerance may reflect the primary importance of hepatic insulin resistance and/or compensatory insulin secretion (21,27) in this patient population.

Numerous cross-sectional and prospective investigations have demonstrated that visceral adiposity is independently associated with insulin resistance (rev. in 6). However, none of these studies were done in severely obese women, who display a wide variation in fat distribution and are known to bear an extreme burden of >40 kg of excess fat (5). Moreover, we examined associations between regional adiposity and mechanisms of glucose intolerance using measures of both peripheral and hepatic insulin resistance and  $\beta$ -cell function and rigorous multiple-slice technology to quantitate visceral and subcutaneous abdominal depots, whereas most other studies used

TABLE 3  
Changes in adipocytokines after weight loss intervention

	Baseline	1 month	$\Delta$ 1 month	6 months	$\Delta$ 6 months
Adiponectin (ng/ml)	5,820 $\pm$ 510 (1,780–14,460)	6,720 $\pm$ 660 (1,880–18,070)	860*	8,040 $\pm$ 650 (2,860–15,980)	2,170 $\ddagger$
Leptin (ng/ml)	159.3 $\pm$ 11.1 (69.2–357)	86.1 $\pm$ 6.0 (28.1–170)	–72.9 $\ddagger$	53.5 $\pm$ 6.6 (17.7–197)	–103 $\ddagger$
IL-6 (pg/ml)	3.93 $\pm$ 0.42 (0.79–9.92)	5.07 $\pm$ 0.72 (0.44–19.5)	0.75	2.96 $\pm$ 0.31 (0.73–7.71)	–1.2 $\ddagger$
Resistin (ng/ml)	19.3 $\pm$ 1.8 (9.5–43.7)	21.1 $\pm$ 2.7 (9.7–56.1)	1.2	19.9 $\pm$ 2.2 (11.3–52.7)	0.59

Data are means  $\pm$  SE (range).  $\Delta$ , change in measures from baseline. \* $P$  < 0.05,  $\ddagger P$  < 0.001.

TABLE 4  
Longitudinal correlations between changes in abdominal adiposity and changes in systemic cytokines

	$\Delta$ VAT	<i>P</i>	$\Delta$ SAT	<i>P</i>
$\Delta$ Adiponectin	<b>-0.37</b>	<b>0.04</b>	-0.35	0.06
$\Delta$ Leptin	<b>0.40</b>	<b>0.04</b>	<b>0.40</b>	<b>0.04</b>
$\Delta$ IL-6	0.15	0.44	0.07	0.74
$\Delta$ CRP	0.35	0.07	0.27	0.17

Data are %. Spearman analysis was used to determine correlation coefficients.  $\Delta$ , change at 6 months from baseline measures. Significant correlations are highlighted in bold.  $P < 0.05$ .

only single slices. We found wide ranges of abdominal adipose tissue volumes, visceral (from 1 to 8 l) and subcutaneous (from 7 to 18 l), demonstrating remarkable heterogeneity of storage of fat in the different depots. Also, insulin sensitivity and regional fat distribution have been shown to differ with patients of different race/ethnicities, and although we had a fairly heterogeneous population, we believe that larger numbers of subgroups are needed to determine whether relationships between glucose tolerance and regional adiposity are altered differentially.

Another study aim was to examine the relationships between changes in visceral and subcutaneous adiposity and changes in adipocytokines to explore the contributory role of each abdominal depot. We found that changes in adiponectin, leptin, and CRP were more highly correlated with changes in visceral than with subcutaneous adiposity. In most cases, comparable relationships between SAT and total body fat and adipokines were observed in this study, and relationships between SAT and adipokines may reflect those of adipokines and total body fat. In fact, we did find a strong correlation between changes in abdominal SAT and total body fat in this study ( $r = 0.7$ ,  $P < 0.000$ ). The finding that adiponectin was uniquely negatively related to visceral adiposity is in agreement with other cross-sectional studies (28–30), but several studies have reported that only subcutaneous fat is associated with leptin (28,29,31). Although most previous studies have not been longitudinal, some did report correlations between leptin and SAT but not VAT (32–34). More studies using robust

TABLE 5  
Correlations between changes in adiposity and metabolic variables and changes in peripheral and hepatic insulin sensitivity

	$\Delta S_i$	<i>P</i>	$\Delta$ HOMA-IR	<i>P</i>
$\Delta$ Fat mass	-0.13	0.64	0.08	0.68
$\Delta$ VAT	<b>-0.38</b>	<b>0.05</b>	0.33	0.09
$\Delta$ SAT	-0.20	0.31	0.08	0.68
$\Delta$ FFA	-0.16	0.43	-0.07	0.75
$\Delta$ CRP	<b>-0.53</b>	<b>0.01</b>	0.17	0.40
$\Delta$ Adiponectin	0.00	0.99	<b>-0.37</b>	<b>0.04</b>
$\Delta$ Leptin	0.06	0.75	0.01	0.97
$\Delta$ IL-6	-0.09	0.64	-0.06	0.77
$\Delta$ AST	0.04	0.88	-0.11	0.68
$\Delta$ ALT	0.07	0.80	0.074	0.78

Data are %. Spearman analysis was used to determine correlation coefficients.  $\Delta$ , change at 6 months from baseline measures. AIRg, acute insulin response to glucose; FFA, free fatty acids; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Significant correlations are highlighted in bold.  $P < 0.05$ .

methods to measure depot volumes are needed to resolve these discrepancies.

Consistent with a previous report from our group (9), we found that decreases in plasma CRP, a marker of systemic inflammation, were negatively correlated with improvements in peripheral insulin sensitivity during weight loss. Cross-sectional studies have also found associations between CRP concentrations and measures of insulin resistance (35–37). One other longitudinal study (25), which determined  $S_i$  after weight loss via caloric restriction, also reported strong negative correlations between CRP and insulin sensitivity, whereas one longitudinal study reported no relationship between change in CRP and improvement in glucose disposal after a 4-week physical training program (37). In the present study, we also did not find significant correlations between change in CRP and insulin sensitivity at 1 month of weight loss. At this time point, patients were losing weight rapidly, and the lack of steady-state conditions may have confounded measurements of insulin sensitivity. Thus the discrepant results may be explained by differences in timing of measurements and in weight loss therapy (i.e., physical training versus reduced food intake). One other study found no changes in either CRP or insulin sensitivity despite 12% weight loss over 3 months after abdominal liposuction (38). In combination, such findings and the present study support the relationship between CRP, peripheral insulin sensitivity, and visceral rather than generalized or subcutaneous abdominal obesity, which may explain why even severe obesity may not be a sufficient or necessary determinant of insulin resistance.

We did not find significant correlations between the adipocytokines IL-6, leptin, resistin, and adiponectin and peripheral insulin sensitivity. Some, but not all, longitudinal studies have reported associations between insulin resistance and adipocytokines, including IL-6, leptin, resistin, and adiponectin (rev. in 2). However, many of these studies did not obtain direct measures of peripheral insulin sensitivity.

To our knowledge, this is the first weight loss intervention study to demonstrate that change in plasma adiponectin concentrations is associated with changes in hepatic and not peripheral insulin sensitivity. Although both hepatic and peripheral insulin resistance contribute to glucose intolerance, recent data suggests that they may be

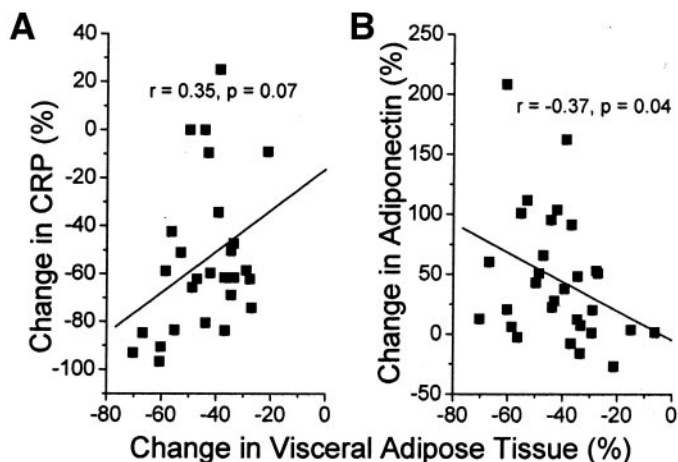
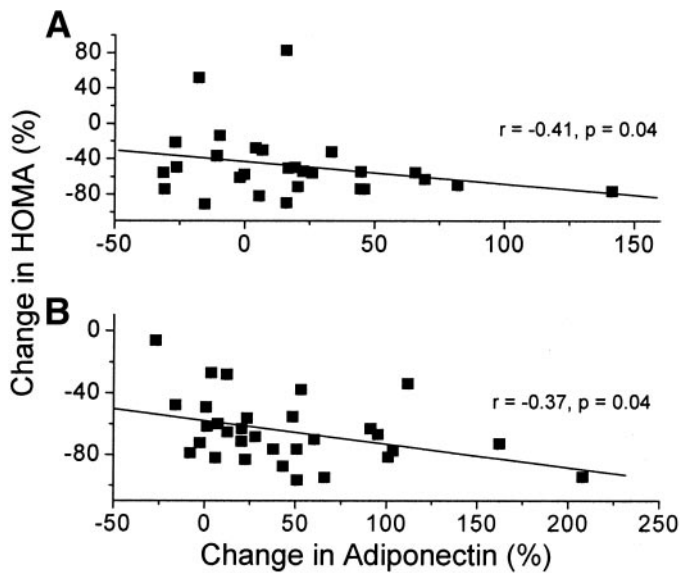


FIG. 1. Relationship between VAT and plasma cytokines. VAT volumes were measured by computed tomography, and CRP (A) and adiponectin (B) were measured using enzyme-linked immunosorbent assay in 28 severely obese women at baseline and 6 months post-weight loss. Percent changes from baseline in VAT were related to percent changes in CRP ( $r = 0.35$ ,  $P = 0.07$ ) and negatively related to percent changes in adiponectin ( $r = -0.37$ ,  $P < 0.05$ ).



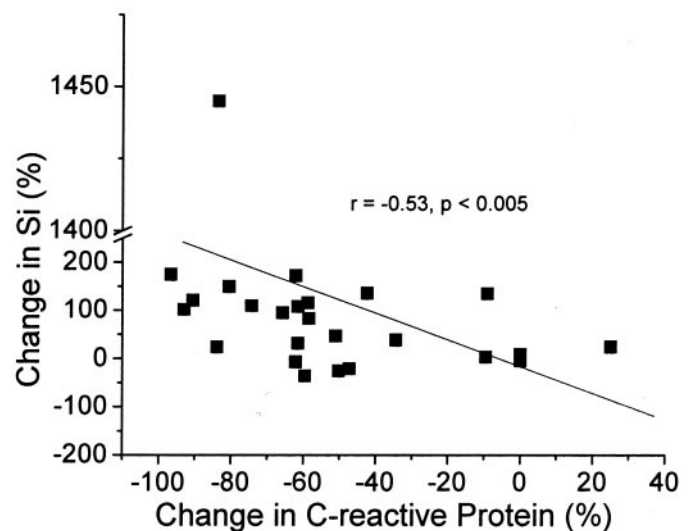
**FIG. 2.** Relationships between hepatic insulin resistance and plasma adiponectin. Hepatic insulin resistance was determined by HOMA, and plasma adiponectin was determined using enzyme-linked immunosorbent assay in 28 severely obese patients undergoing 1 month (A) and 6 months (B) of weight loss. Negative correlations were observed between HOMA and adiponectin at 1 month ( $r = -0.41$ ,  $P < 0.05$ ) and 6 months ( $r = -0.37$ ,  $P < 0.05$ ).

fundamentally different conditions. Resistance to suppression of glucose production in the liver has been shown to contribute to impaired fasting glucose concentrations, whereas reduced whole-body glucose uptake resulted in impaired glucose tolerance (39). Also, HOMA-IR was found to correlate with endogenous glucose production but not peripheral insulin sensitivity in patients with glucose intolerance (40). Adiponectin has been correlated with both HOMA-IR (rev. in 41) and whole-body insulin sensitivity (42,43) in cross-sectional human studies. In accordance with these reports, we observed significant cross-sectional correlations at baseline between adiponectin and peripheral insulin sensitivity ( $r = 0.39$ ,  $P < 0.05$ ); correlations at baseline between adiponectin and the HOMA index were not significant ( $r = -0.28$ ,  $P = 0.1$ ). However, there are few longitudinal previously published observations of relationships between changes in adiponectin and changes in HOMA-IR (44–46) or more direct measures of insulin sensitivity (37,43,47–49), and prior longitudinal studies have not examined both hepatic and peripheral insulin sensitivity. Two previous studies found inverse correlations between HOMA-IR and adiponectin (44,45), whereas a third study found decreases in HOMA-IR without changes in adiponectin concentrations after 6 weeks of weight loss (46). Discrepancies among these studies may be explained by differences in sex and severity of the metabolic models, and it also is possible that changes in HOMA-IR may occur via adiponectin-dependent and -independent mechanisms. Consistent with the present study, two other studies of whole-body insulin sensitivity also did not find longitudinal relationships with adiponectin (43,48).

In contrast to our data, previous studies described significant correlations between changes in peripheral insulin sensitivity and adiponectin (37,47,49). The findings in one study (37), described previously, may be different because of the intervention, which was 4 weeks of physical training. Another study (47) measured insulin sensitiv-

ity with an insulin suppression test, a different approach. The third longitudinal study (49) was done in a small number of subjects ( $n = 7$ ) who underwent BPD for weight loss, a surgery associated with nutrient malabsorption. It is also possible that adiponectin may contribute to both peripheral and hepatic insulin sensitivity but with different kinetics, because adiponectin and its receptors are present in different forms in human muscle and liver (50). The relationship that we found between adiponectin and hepatic glucose production has been suggested both in animal studies (51,52) and in one other human study (53). Stefan et al. (53) elegantly demonstrated that fasting adiponectin was negatively correlated with basal and insulin-stimulated endogenous glucose production (determined using clamp studies), both before and after adjusting for variables, including percent body fat and peripheral insulin sensitivity. These data, taken together with our present study, constitute strong evidence of an association between adiponectin and hepatic insulin sensitivity.

Severely obese individuals exhibit impaired glucose metabolism due to variable combinations of hepatic or peripheral insulin resistance and defective insulin secretion. This study and others (10,24,27,54) have demonstrated that there are differential kinetics of resolution of these processes after weight loss. We found acute (1-month) decreases in the HOMA-IR measure but slower improvements (by 6 months) in peripheral insulin sensitivity and  $\beta$ -cell function. Changes in CRP and in adiponectin were more strongly associated with improvements in peripheral and hepatic insulin sensitivity than were changes in general adiposity. Other studies have shown that changes in circulating inflammatory mediators and adipokines contribute independently of weight loss to improved insulin sensitivity (21,37,47,48). These studies highlight the role of cytokines of adipose origin in the etiology of insulin resistance. However, compared with subcutaneous abdominal fat, visceral adiposity was more associated with CRP and adiponectin, suggesting that the visceral depot plays a larger role in the regulation of these factors.



**FIG. 3.** Relationship between CRP and peripheral insulin sensitivity. Peripheral insulin sensitivity ( $S_i$ ) was determined via the FSIGTT, and high-sensitivity CRP was measured in enzyme-linked immunosorbent assay in 28 patients undergoing 6 months of weight loss. Percent changes in CRP were negatively correlated with changes in  $S_i$  ( $r = -0.53$ ,  $P < 0.005$ ).

Weaknesses of our study include the lack of data in men, the relatively small number of subjects, and the heterogeneity of the subjects with regard to glucose tolerance as well as race and ethnicity. When patients with diabetes were removed from the analysis, the relationship between CRP and  $S_i$  remained significant. However, although the trend persisted between HOMA-IR and adiponectin, the relationship was not significant; we expect to test this relationship in subjects with varying glucose tolerance as we obtain larger sample sizes. Also, future studies will determine whether the data observed in roux-en-y gastric bypass surgery patients may be generalized to patients undergoing other methods of weight loss.

In summary, we have shown that in severely obese subjects with substantial weight loss, there are strong longitudinal relationships between abdominal adiposity, inflammatory mediators, adipocytokines, and direct measures of in vivo insulin action, including peripheral and hepatic insulin sensitivity. However, there appears to be specificity of both cytokine involvement and loci of insulin sensitivity. Compared with other cytokines, including IL-6, leptin, and TNF- $\alpha$ , CRP was much more strongly associated with improvements in peripheral insulin sensitivity. Furthermore, we found longitudinal associations between changes in adiponectin and changes in hepatic but not peripheral insulin sensitivity. Our findings indicate that in severely obese women, visceral adiposity is positively associated with CRP and negatively associated with adiponectin. Because changes in insulin action were associated with changes in CRP and adiponectin, and because visceral adiposity appears to regulate these cytokines, our findings suggest that the diabetogenic effects of visceral adiposity may be mediated in part by these factors. Most significantly, the effects of adiponectin and CRP on hepatic and peripheral insulin sensitivity appear to be independent of adiposity, suggesting that these molecules represent potential pharmaceutical targets for the treatment of insulin resistance.

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