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PAPER

Muscle insulin receptor concentrations in obese patients post bariatric surgery: relationship to hyperinsulinemia

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OBJECTIVE: Obesity results in insulin resistance. Bariatric surgery for obese individuals induces weight loss, improves insulin sensitivity, and lowers insulin levels. We investigated the mechanisms of this improvement.
DESIGN: Insulin receptor (IR) content, IR signaling, and adiponectin levels were measured in nine morbidly obese subjects before and after bariatric surgery.
SUBJECTS: Seven female and two male, average age 44 ± 2y, BMI >40 kg/m^2 and/or at least 100 lbs over ideal body weight, undergoing elective bariatric surgery.
MEASUREMENTS: Before surgery BMI, fasting plasma glucose, adiponectin, and insulin levels were measured. A fasting muscle biopsy was obtained from the vastus lateralis for IR concentration and autophosphorylation activity measurements. These procedures were repeated 1 y after surgery.
RESULTS: At 1 y after surgery, the subjects had lost an average of 48.3 ± 5.6 kg ($P<0.001$), insulin sensitivity had significantly increased as determined by the minimal model (*SI* 0.72 ± 0.18 *vs* 3.86 ± 1.43, $P<0.05$), and IR content had increased two-fold in muscle (2.1 ± 0.4 *vs* 4.3 ± 0.7 ng/mg protein, $P<0.01$). The increase in IR content was related to fasting insulin levels. In the subjects with the lowest IR function, there was also an increase in IR function. Plasma adiponectin increased by 40% following weight loss (7.4 ± 1.6 pre *vs* 10.3 ± 1.3 mg/ml post, $P<0.05$). There was no significant change in muscle content of the IR inhibitor, PC-1.
CONCLUSION: Increased IR content, most likely regulated by insulin levels, may be one contributor to the increased insulin sensitivity that occurs when morbidly obese patients undergo bariatric surgery.
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Keywords: receptor, insulin; adiponectin; surgery; gastric; surgery; bariatric; weight loss; human; muscle, skeletal; glucose tolerance test

Introduction

Obesity is a major problem in the United States and other developed countries. In addition to the many adverse effects of being overweight, obesity is associated with hyperinsulinemia, insulin resistance, and an increased risk for type-II diabetes. Although this relationship has long been recognized, the mechanism(s) whereby increased fat mass negatively impacts insulin action in key insulin-sensitive tissues, most notably skeletal muscle, remains unknown.

Cellular insulin signaling is initiated by binding of insulin to the alpha subunits of the insulin receptor (IR). This binding results in a conformational change in the beta subunits of the IR, allowing ATP to bind with subsequent autophosphorylation of multiple tyrosine residues.[1–3] These events in the IR are followed by tyrosine phosphorylation and activation of downstream signaling molecules.[4,5] *In vitro* evidence from numerous studies in cultured cell systems[6,7] indicate that hyperinsulinemia downregulates IR content. *In vivo*, skeletal muscle from obese subjects demonstrates impaired insulin action at the earliest steps of IR signaling.

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For instance, muscle biopsies from obese subjects have reduced IR content and impaired insulin-induced activation of IR autophosphorylation.[8–10] We have demonstrated a strong correlation between increasing levels of obesity, hyperinsulinemia, and decreasing IR signaling capacity in muscle from Pima Indians.[11]

Weight loss is known to improve insulin sensitivity in obese subjects.[12–14] In morbidly obese individuals who are insulin resistant, the major weight loss induced by gastric surgery (bariatric surgery) is associated with a marked increase in insulin action in muscle.[15,16] However, the effects of this weight loss on IR signaling capacity in muscle have not been studied. Since this surgical procedure is known to lower the insulin levels, it is possible that upregulation of IR content and/or function is a contributing mechanism improving insulin action. In the present study, we examined the changes in insulin levels, insulin sensitivity, and muscle IR content and function, in morbidly obese subjects before and after surgically induced weight loss. We also measured the changes of circulating adiponectin, a hormone produced by adipocytes that appears to increase insulin sensitivity.[17]

Materials and methods

Subjects

Morbidly obese (BMI $>40\,kg/m^2$ and/or at least 100 lbs over ideal body weight) subjects (seven female and two male, average age 44 ± 2y) were examined prior to and 1 y after elective gastric surgery to induce weight loss at the Department of Surgery, East Carolina University.[18] Descriptive data for the subjects are presented in Table 1. For seven patients, the Roux-en-Y gastric bypass procedure was performed, and the remaining two patients underwent banded gastroplasty.[19] Previous characterization of morbidly obese subjects undergoing gastric surgery demonstrated that, in the 12 months following surgery, body mass had stabilized and remained significantly depressed, and insulin action dramatically improved.[18] For data and sample collection prior to surgery and at 12-month follow-up, subjects reported to the laboratory at approximately 0800 h after a 12-h fast. At 3 days prior to the study, subjects were instructed to consume at least 250 g of carbohydrate per day. Body mass and stature were measured and a fasting blood sample was obtained for the measurement of plasma glucose, insulin, and adiponectin levels. A muscle sample was obtained from the vastus lateralis using the percutaneous needle biopsy technique. All procedures were approved by the East Carolina University Human Subjects Committee, and informed consent was obtained before performing any experimental procedures.

Insulin action

An insulin sensitivity (SI) was calculated on the basis of the minimal model, as described by Finegood *et al*.[20] SI is an index of the ability of insulin to promote the disposal of glucose during an *in vitro* glucose tolerance test (IVGTT). A higher SI indicates an enhanced insulin sensitivity. Glucose and insulin dosages were calculated on the basis of body surface area, because of the high body mass of morbidly obese subjects. Briefly, the IVGTT procedure consisted of obtaining four baseline samples before the intravenous injection of glucose ($12\,g/m^2$) at time zero and insulin ($1.5\,U/m^2$) 20 min later. In all, 25 samples were obtained between zero and 180 min and subsequently analyzed by spectrophotometry for glucose (procedure HK 16-UV, Sigma, St Louis, MO, USA) and by microparticle enzyme immunoassay for insulin (IMx, Abbott, Abbott Park, IL, USA). SI was calculated with the minimal model of insulin action (MINMOD, version 3.0).

Adiponectin

Plasma adiponectin levels were measured using a radioimmunoassay for human adiponectin (Linco Research, St Louis MO, USA). The assay utilizes ^{125}I-labeled adiponectin and an anti-adiponectin rabbit antiserum to determine adiponectin concentrations by the double antibody/PEG 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 inter-assay 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. Plasma adiponectin levels measured in 40 paired samples using this assay, and the enzyme-linked immunoabsorbent assay (ELISA) developed by Arita and colleagues[21] were similar (10.4 ± 6.6 *vs* 10.1 ± 5.5 μg/ml) and linearly

Table 1 Clinical characteristics of subjects before and after surgically induced weight loss

Variable	*Pre-Surgery*		*Post-Surgery*	
Weight (kg)	137.4 ± 6.2	(106.1–163.6)	$89.1\pm4.1^{*}$	(75.0–112.3)
BMI (kg/m^2)	47.0 ± 2.7	(36.0–56.5)	$30.4\pm1.4^{*}$	(25.1–37.6)
Fasting Insulin (μU/ml)	30.4 ± 8.1	(5.0–68.8)	$6.1\pm1.5^{\dagger}$	(2.5–15.1)
Fasting Glucose (mg/dl)	150 ± 27	(73–317)	$79\pm5^{\dagger}$	(55–92)
SI ($min^{-1}(\mu U/ml)^{-1}$)	0.72 ± 0.18	(0.18–1.39)	$3.86\pm1.43^{\dagger}$	(0.69–13.09)

Values are presented $\pm$ the standard error with the range in parentheses. Pre-and post-surgery data are significantly different; $^{*}P<0.001$, $^{\dagger}P<0.05$.

related ($r=0.896$, $P<0.0001$; RL Gingerich, unpublished observation).

Preparation of muscle extracts

Soluble extracts were prepared from frozen muscle tissue to measure the tissue content of IR, IR autophosphorylation capacity, and PC-1 content. Approximately 50 mg of frozen tissue was pulverized under liquid nitrogen. The resultant powder was homogenized in 0.5 ml buffer (50 mM HEPES HCl, 150 mM NaCl, 1 mM PMSF, 2 μM leupeptin, and 2 μM pepstatin, pH 7.4) at 4°C, using a polytron homogenizer (Kinematica, Lucerne, Switzerland) for 10 s at a setting of 9. Triton X-100 was added to a final concentration of 1%, and the homogenates were solubilized for 60 min at 4°C. The solubilized homogenate was then centrifuged at 100 000 *g* for 60 min at 4°C. The supernatants were collected and stored at −70°C. Protein content of the muscle extracts was determined by the Bradford method.[22]

IR ELISA

IR content of muscle extracts was determined by specific ELISA, as described previously.[23] Briefly, microtiter 96-well plates were coated with 2 μg/ml of a monoclonal antibody to the IR α-subunit (MA-20) for 18 h at 4°C. After washing and blocking the plate, solubilized cellular extract containing 10 μg protein of each sample was added to each well for triplicate determination and allowed to bind overnight at 4°C. Readout of bound IR was accomplished with the sequential addition of biotinylated monoclonal anti-IR antibody CT-1, peroxidase-conjugated streptavidin (Pierce, Rockford, IL, USA), ELAST ELISA Amplification System (NEN Research Products, Boston, MA, USA) for signal enhancement, and TMB peroxidase substrate System (Kirkegaard & Perry, Gaithersburg, MD, USA) for color development. The absorption at 450 nm of each well was measured in a microtiter plate reader (DuPont-NEN, Boston, MA, USA). IR content for each sample was calculated as an average of triplicate values.

IR autophosphorylation ELISA

The autophosphorylation capacity of muscle IR was determined in soluble extracts using an ELISA specific for IR tyrosine phosphorylation, as described previously.[11] In this assay, solubilized cellular extract containing 10 pg IR was added to each well of a 96-well microtiter plate coated with 2 μg/ml MA-20 and allowed to bind overnight. Immunocaptured IR was then incubated in 50 mM HEPES, 150 mM NaCl, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 0.1% Triton X-100, 0.05% BSA, 10 μM ATP, pH 7.6 ± 100 nM insulin for 1 h at 22°C. The extent of tyrosine phosphorylation was then determined by incubation with a biotinylated antiphosphotyrosine antibody (UBI, Lake Placid, NY, USA), followed by an identical procedure for color development, as employed in the IR content ELISA.

PC-1 content ELISA

Tissue content of membrane glycoprotein PC-1 was determined by specific ELISA, as described previously.[23] The protocol was similar to that described above for the IR ELISA with the capture antibody to PC-1 provided by Dr I Yamashina from Kyoto University, Kyoto, Japan. In addition, 2 μg muscle extract protein was added to each well, and the standard curve is constructed by multiple dilutions of 0–1.5 ng purified PC-1. The secondary antibody was a biotinylated anti-PC-1 monoclonal antibody.[23]

Statistics

Values are expressed as mean ± standard error (s.e.m.). Comparisons of variables before and after weight loss were performed with a paired *t*-test. Relationships between variables and changes in the variables with weight loss were determined with Pearson product correlations. Significance was denoted at $P<0.05$.

Results

Subject characteristics

Clinical and anthropometric data on nine subjects prior to and after surgery are shown (Table 1). Four of the subjects were classified as diabetic prior to surgery, having fasting glucose values > 127 mg/dl: all achieved normoglycemia after surgery. There was no difference between the diabetic and nondiabetic morbidly obese subjects on any clinical or biochemical measure of insulin action. Further, there was no difference between these groups in the effect of weight loss on any variable except fasting glucose. Thus, data from all the nine subjects were analyzed together.

Weight loss

All subjects had dramatic weight loss post surgery. They lost an average of 48.3 ± 5.6 kg over the 1 y period following surgery; individual weight loss varied from 27.7 to 72.7 kg (Table 1). BMI was reduced from 47.1 ± 2.4 to 30.4 ± 1.4 kg/m^2 (Figure 1).

Insulin sensitivity assessment

Insulin action in the subjects was assessed by FSIVGTT; minimal model analysis was employed to calculate *SI*(20). This value increased five-fold from 0.72 to 3.86 (min^{-1}/μU/ml) (Table 1). In accordance with this increase in insulin sensitivity, fasting plasma insulin levels fell five-fold from 30 to 6 μU/ml (Table 1).

Insulin receptor content in muscle biopsies

Weight loss was associated with a doubling of the IR content in skeletal muscle biopsies from 2.1 ± 0.4 to 4.3 ± 0.7 ng/mg

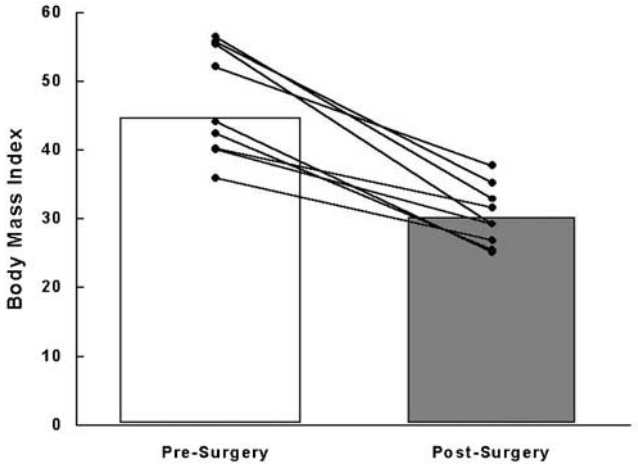

Figure 1 Change in body mass indices of obese patients before and after gastric surgery. Postsurgery values were significantly different from presurgery values, $P<0.001$.

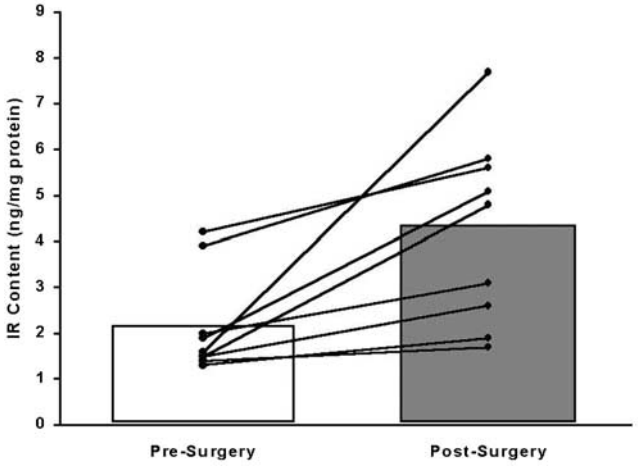

Figure 2 Skeletal muscle IR content in obese patients before and after gastric surgery. Vastus lateralis biopsies were obtained from patients in the fasted state before and after gastric surgery. IR content was measured by IR ELISA. Postsurgery values were significantly different from presurgery values, $P<0.01$.

protein **(**Figure 2**)**. When IR content was plotted as a function of fasting insulin concentration, it was observed that IR content fell markedly over the insulin range of 2–20 (Figure 3a). Within this range, IR content correlated with the log of fasting insulin ($r=-0.75$, $P<0.005$). Once IR content reached a minimum value ($\sim$1.5 ng IR/mg protein), higher insulin levels no longer corresponded to lower IR content. The highest increases in IR were observed in those patients with the greatest decrease in fasting insulin **(**Figure 3b**)**. A linear relationship was observed between these two parameters for decreases in fasting insulin greater than 70% ($r=-0.92$, $P<0.01$).

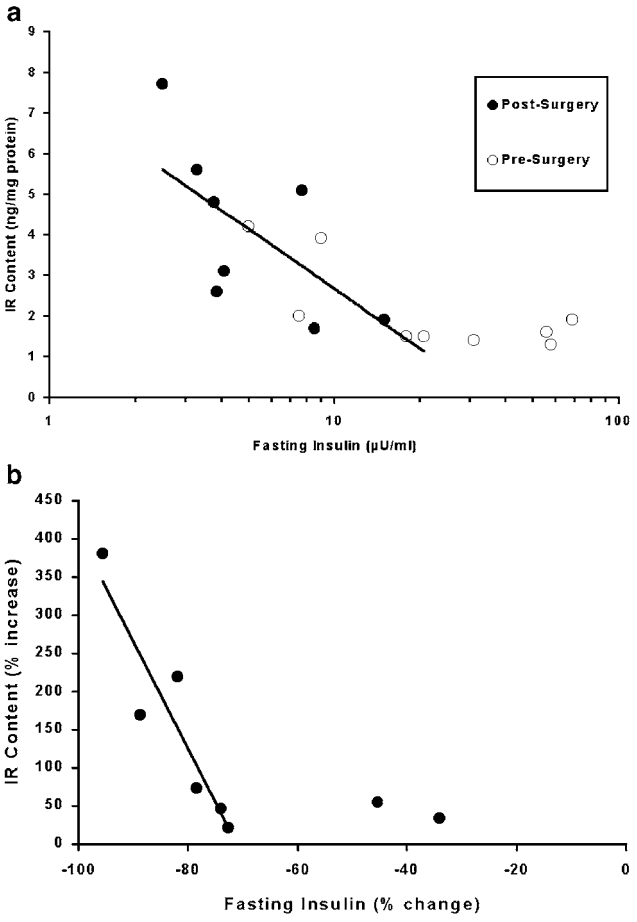

Figure 3 (a) Plot of skeletal muscle IR content *vs* fasting plasma insulin levels. ($r=-0.75$, $P<0.005$). (b) Change in skeletal muscle IR content induced by gastric surgery *vs* change in fasting plasma insulin levels ($r=-0.92$, linear-logarithmic plot, $P<0.01$).

Insulin receptor function

When the group was studied as a whole, maximal insulin stimulation of IR autophosphorylation was not significantly increased after weight loss (0.47 ± 0.05 *vs* 0.53 ± 0.04 OD units/ng IR, pre- *vs* post-weight loss, respectively) **(**Figure 4**)**. However, in the four subjects with lowest IR autophosphorylation values before surgery, this parameter increased significantly after surgery ($P<0.01$). Two patients were initially diabetic and two were not.

PC-1 is an IR inhibitor.[24] However, there was no change in muscle PC-1 content after weight loss (16.8 ± 1.2 *vs* 18.4 ± 3.9 ng/mg, pre- *vs* post-weight loss, respectively).

Plasma adiponectin levels

Adiponectin, a cytokine released from adipose cells and associated with increased insulin action,[17] was measured in the plasma **(**Figure 5**)**. The mean adiponectin level for all subjects increased by approximately 40% from

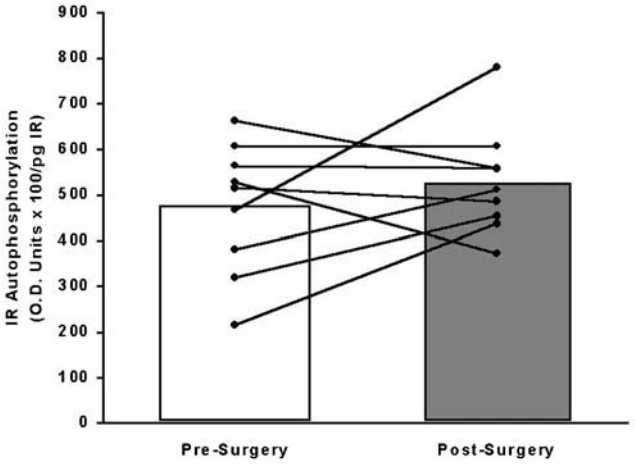

Figure 4 IR autophosphorylation in muscle samples before and after gastric surgery. Skeletal muscle biopsies were obtained in the fasted state. Tissue was solubilized, and insulin stimulation (100 nM) of IR autophosphorylation was measured by specific ELISA, as described in Materials and methods. For each sample, the data were normalized per 10 pg IR.

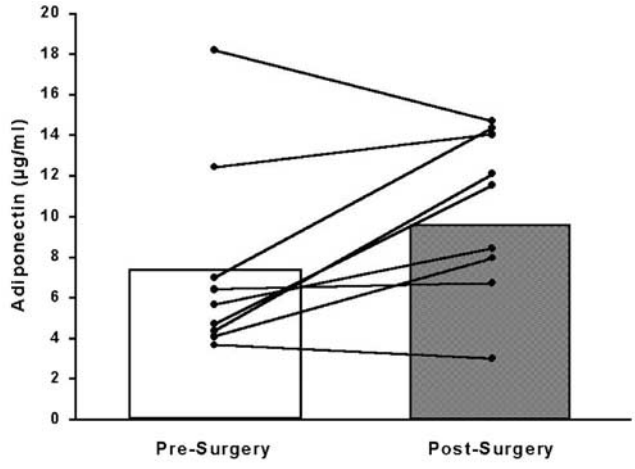

Figure 5 Fasting plasma adiponectin levels in obese patients before and after gastric surgery. The differences between the two groups were significant, $P<0.05$.

7.4 ± 1.6 to $10.3\pm1.4\,\mu g/ml$ following gastric surgery ($P<0.05$).

Discussion

In the present study, we evaluated insulin receptor content and function in morbidly obese subjects following bariatric surgery. Fasting insulin levels decreased and insulin sensitivity markedly improved after weight loss. A key finding was that, in skeletal muscle, a major target tissue for insulin action, insulin receptor content increased two- fold. The increase in IR content was related to the decrease in fasting plasma insulin (Figure 3). In addition, for all patients, both before and after surgery, muscle IR content was related to fasting plasma insulin. It is likely therefore that the IR content is regulated by plasma insulin levels.

Downregulation of IR number by insulin has been demonstrated *in vitro* with several cell types.[6,7,25] The downregulation can be attributed to both the accelerated degradation of IR-bound insulin and reduction of IR mRNA levels.[6] Analysis of the correlation between IR number and plasma insulin levels in this study revealed a biphasic relationship. When our patients' fasting plasma insulin levels were less than $20\,\mu U/ml$, we observed a strong correlation between IR number and fasting plasma insulin. When the patient's fasting plasma insulin level exceeded $20\,\mu U/ml$, downregulation of IR content appeared to reach its maximum. In contrast, *in vitro,* the downregulation of IR has been demonstrated across several orders of magnitude of insulin concentration.[26] This observation therefore raises the possibility that the mechanisms of IR downregulation *in vivo* are more complex than those in tissue culture.

In obese subjects, employing diazoxide to suppress insulin secretion, Wigand and Blackard[27] demonstrated that hyperinsulinemia was at least partly responsible for downregulating cellular IR content; however, the direct effects of the agent on this process could not be ruled out. IR content was also decreased in studies of several hyperinsulinemic states, including insulinoma patients[28] and obesity.[29] IR mRNA is not reported to be diminished in obese, insulin-resistant subjects.[30]

IR function is also modified by changing physiological conditions. We have previously demonstrated, employing the IR ELISA technique used herein, that IR autophosphorylation is decreased with increasing obesity.[11] Others have reported that IR function is impaired in muscle biopsies from obese subjects.[8–10] In earlier studies, we have demonstrated that insulin-stimulated IR autophosphorylation is improved in muscle from subjects following a 7-day exercise training program.[31] Conversely, we have shown that high-fat feeding in rats can induce impairments in IR autophosphorylation within 2 weeks, which is before the acquisition of increased fat stores occurs.[32] In this study, we found increases in IR autophosphorylation following bariatric surgery in the four subjects with the lowest pre-surgery IR autophosphorylation. However, the increase in this parameter for all patients did not reach statistical significance. Since analyses were performed using high insulin levels to stimulate the IR, it is possible that, at lower physiological levels, a significant difference may have been observed. It is also possible that regulation of IR function in obese patients is heterogeneous and that IR autophosphorylation, as analyzed herein, is improved by bariatric surgery and subsequent weight loss in some, but not all, patients.

The hormonal or metabolic pathways that couple increased whole body fat stores with impaired insulin signaling are unclear. It is known that elevated free fatty acids and triglycerides have detrimental effects on insulin signaling and that adipose tissue also plays a regulatory role.[33–36]

Adiponectin is an adipocyte-secreted peptide that may effect obesity-associated insulin resistance.[17,37] Adiponectin levels are reduced in obesity and increase following weight loss. We have recently demonstrated that plasma adiponectin is significantly correlated with IR signaling capacity.[38] Moreover, administration of adiponectin improves insulin sensitivity in animal models of lipoatrophic insulin resistance and obesity,[39] perhaps by either lowering hepatic glucose production, or by increasing muscle fatty acid oxidation via its effects to activate AMP kinase.[39,40] In the present study, adiponectin levels increased following weight loss. It is possible, therefore, that increases in adiponectin may contribute to improvements in insulin sensitivity resulting from weight loss. In support of this hypothesis, it has recently been demonstrated that the increase of adiponectin in morbidly obese subjects after weight loss induced by gastric bypass surgery was predictive of improved insulin sensitivity, as assessed by the HOMA-IR calculation.[41] The present studies extend this observation with a more specific measurement of insulin sensitivity, the SI.

In summary, we found that surgically induced weight loss in morbidly obese subjects resulted in a dramatic improvement in whole body insulin sensitivity, concomitant with a fall in plasma insulin levels. In all subjects, these changes were associated with an increase in the skeletal muscle IR content, and in subjects with the lowest IR function, there was an increase in IR function. In addition, we observed an increase in plasma adiponectin levels. It is likely, therefore, that changes in IR content and function, along with changes in other parameters, such as increased adiponectin production, and decreased tissue fat deposition, contribute to the regulation of insulin sensitivity.

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