

**MECHANISM FOR IMPROVED INSULIN SENSITIVITY
AFTER GASTRIC BYPASS SURGERY**

Short Title:

Gastric Bypass and Insulin Sensitivity

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Context: Surgical treatments of obesity have been shown to induce rapid and prolonged improvements in insulin sensitivity.

Objective: To investigate the effects of gastric bypass surgery and the mechanisms that explain the improvement in insulin sensitivity.

Design: The study was a cross-sectional, non-randomized, controlled study.

Setting: This study was conducted jointly between the Departments of Exercise Science and Physiology at East Carolina University in Greenville, NC.

Subjects: Subjects were recruited into four groups: 1) lean (BMI<25; n=93), 2) weight-matched (BMI=25 to 35; n=310), 3) morbidly obese (BMI>35; n=43), and 4) post-surgery patients (BMI≈30 kg/m²; n=40). Post-surgery patients were weight stable one year post surgery.

Main Outcome Measures: Whole-body insulin sensitivity, muscle glucose transport, and muscle insulin signaling were assessed.

Results: Post-surgery subjects had insulin sensitivity index values that were similar to the lean and higher than morbidly obese and weight-matched control subjects. Glucose transport was higher in the post-surgery vs. morbidly obese and weight-matched groups. IRS1-pSer³¹² in the post-surgery group was lower than morbidly obese and weight-matched groups. IκBα was higher in the post-surgery vs. the morbidly obese and weight-matched controls, indicating reduced IKKβ activity.

Conclusions: Insulin sensitivity and glucose transport are greater in the post-surgery patients than predicted from the weight-matched group, suggesting improved insulin sensitivity after bypass is due to something other than, or in addition to, weight loss. Improved insulin sensitivity is related to reduced IKKβ activity and enhanced insulin signaling in muscle.

Gastric bypass surgery has been employed as a tool to assist in weight loss in morbidly obese individuals. For over 20 years, our group has been performing gastric bypass surgery, which consists of surgical reduction in the size of the stomach and bypassing a portion of the proximal small intestine. We were the first to report that gastric bypass surgery results in rapid and long term improvement in insulin sensitivity and an ultimate reversal of diabetes (1-3). More recently, Dixon et al (4) observed that laparoscopic adjustable gastric banding (LAGB) resulted in a higher remission rate of type 2 diabetes compared with conventional medical treatment. Additionally, the reversal of diabetes by various surgical treatments of obesity was confirmed by meta-analysis, wherein gastric bypass surgery and LAGB were found to resolve diabetes in approximately 84% and 48% of post-surgery patients, respectively (5).

Although an increase in whole-body insulin sensitivity after gastric bypass surgery is well established, the mechanisms involved are not fully understood. A potential signaling defect in the insulin signaling cascade in the muscle of obese and type 2 diabetics may be a cause for reduced insulin sensitivity. We have shown previously that insulin-stimulated muscle glucose transport is blunted in obese individuals and patients with type 2 diabetes and that insulin-receptor tyrosine kinase activity is diminished in obese and diabetic skeletal muscle (6, 7). Serine phosphorylation of the insulin receptor substrate (IRS)-1 has been implicated in inhibiting insulin signal transduction (8, 9) and while there are many potential serine phosphorylation sites on IRS-1 (10), the IRS1 Ser³¹²(Human)/Ser³⁰⁷(rodent) residue is of particular interest given its proximity to the phosphotyrosine-binding (PTB) domain in IRS-1. The inhibitor of κ B kinase β (IKK β), a serine kinase, has been suggested as a potential culprit that results in deficient insulin signal transduction given that it has been shown to phosphorylate IRS-1 on Ser^{312/307} (8, 9, 11, 12). Moreover, IKK β is activated by fatty acid metabolites, such as diacylglycerol (DAG) and ceramide (13, 14), which have been shown to be higher in insulin resistant states, such as obesity (15).

Obesity is associated with an increased low-grade inflammatory tone, as evidenced by increased levels of proinflammatory cytokines as well as greater activity of the inflammatory factor, NF- κ B (16-19). NF- κ B is a nuclear transcription factor that is responsible for the transcription of a multitude of cytokines (including TNF- α and IL-1 β) (20, 21). NF- κ B is prevented from reaching the nucleus by an inhibitor, known as inhibitor κ B α (I κ B α). I κ B α is phosphorylated by IKK β , which results in the ubiquitination and degradation of I κ B α and NF- κ B is free to migrate into the nucleus. In addition to regulating NF- κ B activity, IKK β has been shown to directly inhibit insulin signaling (8, 9, 22). To highlight the role of NF- κ B pathway activity in inhibiting insulin signaling, thiazolidinediones, a known insulin-sensitizing drug, inhibit NF- κ B activity (22, 23). Similarly, Yin et al. (24) discovered that the mechanism of action for salicylate in improving insulin sensitivity was its inhibitory actions on IKK β .

We are interested in the causes of insulin resistance in muscle of obese individuals and the mechanisms that improve insulin resistance after patients have gastric bypass surgery. The purpose of this study was to investigate gastric bypass surgery-induced increases in insulin sensitivity at the whole body and muscle level. We also sought to determine a potential mechanism for the rapid and sustained improvement in insulin sensitivity as a result of gastric bypass surgery.

Materials and Methods

Subjects and study design. To investigate the reversal of insulin resistance after bypass surgery three experiments were performed. In the first experiment whole-body insulin sensitivity was measured in post-surgery patients and non-surgical controls by an intravenous glucose tolerance test (IVGTT) as outlined below. The second experiment involved glucose transport measured in rectus abdominis muscle strips from patients having elective surgery. These values were compared to previous data published for gastric bypass patients after weight loss (1). The activity of IKK β and IRS-1 serine³¹² phosphorylation was assayed in vastus lateralis muscle biopsies of post-surgery patients

and non-surgery controls in the third experiment. All subjects were female and considered to be in good health after filling out a medical history questionnaire. Subjects were informed of potential risks associated with the study and signed an informed consent document, which was approved by the East Carolina University Institutional Review Board.

Whole-body insulin sensitivity experiment. The first experiment consisted of 128 female subjects who were divided into four groups. One group consisted exclusively of post-gastric bypass surgery patients who were weight stable at least 12 mo after surgery (BMI \approx 30). The other three groups of non-surgery subjects were divided according to BMI: 1) lean (BMI < 25), 2) weight-matched to post-surgery patients (BMI = 25 to 35), and 3) morbidly obese (BMI > 35). As mentioned, this first cohort of subjects was used to obtain insulin sensitivity data, including fasting insulin and glucose, as well as insulin sensitivity with a 3-h intravenous glucose tolerance test (IVGTT) (outlined below).

Muscle glucose transport experiment. In the second experiment glucose transport was measured in incubated muscle strips obtained during elective abdominal surgery. Subjects were recruited into similar BMI groups, namely, lean (BMI < 25), weight-matched controls to post-surgery patients (BMI = 25-35), and morbidly obese (BMI >35). The data from these groups were then compared to data previously reported (1) for five post-surgery subjects in order to compare the given groups with post-surgery patients.

IKK β activity and IRS1-Ser³¹² experiment. The third experiment included 36 subjects who were evenly recruited into four groups, similar to the BMI groups mentioned above. This cohort of subjects had a muscle biopsy (vastus lateralis) that was used to obtain all muscle protein data.

Roux-en-Y gastric bypass surgery. The gastric-bypass surgery was performed as described previously (3). Briefly, Roux-en-Y gastric bypass surgery consists of surgical reduction in the size of the stomach and bypassing of a portion of the proximal small intestine (foregut).

Anthropometrics. Body mass was measured to the nearest 0.1 kg on a digital electronic scale. Height was measured to the nearest 0.5 cm with a stadiometer and BMI calculated [mass (kg)/height² (m²)].

IVGTT. Insulin sensitivity was determined by a minimal model analysis using a 3-h IVGTT (25), as described previously (26). After fasting samples were obtained, glucose (50%) was injected into a catheter placed in an antecubital vein at a dose of 0.3 g/kg body mass. Insulin, at a dose of 0.025 U/kg body mass was injected at minute 20. Blood samples were obtained at minutes 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 and the samples were then centrifuged, and plasma transferred and frozen at -80°C for the subsequent determination of insulin and glucose. Insulin was determined with immunoassay (Access Immunoassay System, Beckman Coulter, Fullerton, CA) and glucose was determined with an oxidation reaction (YSI model 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH). Insulin sensitivity index (S_I) was calculated by using the minimal model (25); a higher S_I indicates greater insulin sensitivity.

Muscle strip incubations. Human rectus abdominis muscle strip preparation and incubation was performed according to the procedure previously described (6). Muscle strips were incubated in the presence (100 nM) or absence of insulin. 2-Deoxy-D-glucose was used at a concentration of 5 mM for 60 min and glucose transport was measured as described previously (6). 2-[1,2-³H(N)]deoxy-D-glucose (30.2 Ci/mmol) was obtained from Du Pont-NEN (Boston, MA).

Muscle IRS-1, pSer³¹², and I κ B α protein content. A muscle sample was obtained from the vastus lateralis with the percutaneous needle biopsy technique. Biopsy samples were immediately frozen in liquid nitrogen. Frozen muscle samples were homogenized in ice-cold lysis buffer [50 mM HEPES, 50 mM Na⁺ pyrophosphate, 100 mM Na⁺ fluoride, 10 mM EDTA, 10 mM Na⁺ orthovanadate, and protease

and phosphatase (1 and 2) inhibitor cocktails (Sigma, St. Louis, MO)], followed by addition of 1% triton and brief sonication. After centrifugation for 1 h at 45,000 *g* at 4°C, supernatants were extracted (cytosolic portion) protein content was detected using a BCA protein assay (Pierce, Rockford, IL) and individual homogenate volumes were adjusted so that precisely 50 µg of protein were loaded into each lane. For IRS1-Ser³¹² and total IRS-1 analysis, 200 µg of homogenates were subjected to 10 µl IRS-1 monoclonal IP antibody (Santa Cruz Biotech, Santa Cruz, CA) overnight then coupled to protein A sepharose beads and rotated for 2 hours at 4°C (Amersham Biosciences, Uppsala Sweden) and washed. After addition of sample buffer, samples were separated by SDS-PAGE using 7.5% or 10% Tris-HCl gels and then transferred to PVDF membranes for probing by the following antibodies: rabbit polyclonal IRS-1 and IRS-1 phospho serine³¹² purchased from Millipore (Billerica, MA) and IκBα antibodies were purchased from Cell Signaling (Beverly, MA). Following incubation with primary antibodies, blots were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies. Horseradish peroxidase activity was assessed with ECL solution (Thermo Scientific, Rockford, IL), and exposed to film. The image was scanned and band densitometry was assessed with Gel Pro Analyzer software, version 4.2 (Media Cybernetics, Silver Spring, MD). Content of phospho-proteins (using phospho-specific antibodies) was calculated from the density of the band of the phospho-protein divided by the density of the protein using the appropriate antibody.

Statistics. All data are shown as mean ± SE. Comparisons between the groups with insulin sensitivity, muscle protein levels, and muscle glucose transport rates were made using one-way ANOVA, followed by post hoc analyses where appropriate. Correlations between variables were determined by Dunnett T3 post hoc analysis.

Results

Subject characteristics are presented in Table 1. None were taking medications that would interfere with metabolism, and all were free from known cardiovascular disease and diabetes mellitus, and a few were glucose tolerance impaired (fasting glucose > 110 mg/dL; two in the lean group, two in the obese group, and five in the weight-matched group). The mean weight of all gastric bypass subjects decreased from 136.6±21.5 to 81.9±13.2 kg (mean±SD, $P<0.001$), resulting in a mean weight loss of 54.7±18.7 kg. Similarly, BMI fell from 48.6±5.7 to 28.8±4.6 kg/m² ($P=0.001$). The BMI of the post-surgery groups was not significantly different than the weight-matched control groups.

Fasting glucose, insulin and insulin resistance (HOMA). Average plasma glucose concentration for the post-surgery subjects (76±10.2 mg/dl) was lower when compared with all non-surgery control subjects (95.3±13.8 mg/dl) (Figure 1A; $P<0.001$). There was no significant change in fasting glucose with increasing BMI in either control or post-surgery groups. Fasting insulin concentration and BMI were positively correlated ($r=0.598$, $P<0.001$) for all non-surgery control subjects. In contrast, the same correlation in post-surgery subjects revealed no significant relationship ($r=0.304$, $P=0.169$; Figure 1B). Fasting glucose and insulin values were used to calculate homeostasis model assessment (HOMA) values, an index of insulin resistance. HOMA values were significantly related with BMI in the non-surgery subjects ($P<0.01$, $r=0.283$, Figure 2A), whereas the post-surgery patients revealed no significant correlation.

Whole-body insulin sensitivity. The post-surgery group had insulin sensitivity index (S_I) values that were 361% higher than the morbidly obese and 47% higher than weight-matched groups ($P<0.005$ for both; Figure 2B). The post-surgery group's S_I levels were not significantly different from the lean group, despite having a greater BMI. The S_I value of the morbidly obese was 68% lower than the weight-matched controls, suggesting an inverse relationship between whole-body insulin sensitivity and BMI ($P<0.05$). This is consistent with the positive

correlation between BMI and HOMA from all non-surgery subjects (Figure 2A; $P < 0.01$, $r = 0.283$).

Muscle glucose transport. We previously reported that muscle glucose transport was significantly lower in morbidly obese patients than in lean controls (1, 6). Additionally, in five gastric bypass patients who subsequently had elective abdominal surgery after weight loss, muscle glucose transport was not different than that of lean subjects, despite the post surgery group having an average BMI of 31 (1). To determine how post-surgery muscle glucose transport compared to a weight-matched control group, we retrieved muscle glucose transport measurements from a database of our previous experiments (Figure 3A). Previously published post-surgery glucose transport values (1) are shown in figure 3A for comparison. Insulin-stimulated glucose transport values were lower in the weight-matched group and morbidly obese groups compared to the lean control groups ($P < 0.05$). There were no differences in insulin-stimulated glucose transport between the morbidly obese and weight-matched groups. This is in agreement with our previous report that muscle glucose transport is related to BMI up to a BMI of 30, after which there is no further change (27); i.e., muscle insulin resistance is maximal at a BMI of 30. Post surgery glucose transport values were similar to the lean group and greater than morbidly obese and weight-matched subjects.

We previously reported that insulin-stimulated glucose transport was depressed in muscle of morbidly obese patients, compared to lean controls (6). With the larger number of observations in the present data set we extend this observation by reporting that basal glucose transport (in the absence of insulin) is also lower in muscle of morbidly obese patients. Also, a significant positive relationship was found between the rates of basal and insulin-stimulated glucose transport ($P < 0.001$, $r = 0.726$, Figure 3B). This suggests that the factors that regulate basal glucose might also be those that regulate the rate in the presence of insulin. This was unexpected as we had predicted that insulin resistance might only have an effect on insulin-stimulated glucose transport.

Insulin sensitivity and IRS1-Ser³¹². Inasmuch as serine³¹² phosphorylation of the IRS-1 protein has been implicated in inhibiting insulin signaling, this parameter was investigated in vastus lateralis muscle biopsies of lean, weight-matched, morbidly obese, and post-surgery subjects (group 3 in Table 1). Ser³¹² phosphorylation of IRS-1 was significantly greater in the morbidly obese and weight-matched subjects versus the lean and post-surgery subjects ($P < 0.05$). IRS1-Ser³¹² phosphorylation followed a similar pattern to the insulin-stimulated glucose transport data. In particular, IRS1-pSer³¹² did not differ between the morbidly obese and weight-matched groups (Figure 4A). Moreover, IRS1-Ser³¹² phosphorylation in post-surgery subjects was significantly reduced by 47% compared with morbidly obese ($P = 0.04$) and tended to be lower when compared with the weight-matched subjects ($P = 0.057$).

IκBα and IRS1-Ser³¹². Due to the implicated role of IKKβ as a serine kinase of IRS1-Ser³¹², muscle levels of IκBα, a substrate of IKKβ that is rapidly ubiquitinated and degraded upon phosphorylation, were measured in an effort to determine IKKβ activity (28). Greater IKKβ activity would be evident in reduced levels of IκBα. Similar to our observations with IRS1-phospho-Ser³¹² and insulin-stimulated glucose uptake in muscle, IκBα levels in the post-surgery subjects were similar to lean, and were significantly elevated by 44% compared with the morbidly obese ($P < 0.05$) and 47% compared with the weight-matched controls ($P < 0.05$), which demonstrated increased IKKβ activity in the weight-matched and morbidly obese compared to the lean and post-bypass subjects (Figure 4B). With correlational analysis, we discovered a highly significant inverse relationship between IκBα and phospho-Ser³¹² levels ($P = 0.003$, $r = -0.47$, Figure 4C).

Discussion

The primary effect of gastric-bypass surgery in the obese is significant weight loss, but an important side effect of the procedure is the dramatic and rapid rescuing of insulin

sensitivity. We observed lower fasting glucose and insulin values from the post-surgery group compared with all other subjects. Further, we found that whole-body insulin sensitivity, as measured by IVGTT, is significantly greater in the post-surgery subjects compared with BMI-matched control subjects. Additionally, despite having a significantly higher BMI than the lean subjects, post-surgery subjects enjoy comparable S_I values.

We have previously shown that muscle insulin resistance in obese individuals is related to depressed insulin signal transduction (7). Since phosphorylation of the IRS1-Ser^{312(human)/307(rodent)} residue has been implicated in attenuating insulin signal transduction, we measured IRS1-pSer³¹² and I κ B α protein levels, as a surrogate for IKK β activity, and found that both I κ B α and IRS1-phospho-Ser³¹² protein levels in skeletal muscle from post-surgery patients were similar to that seen in muscle from lean subjects despite the post-surgery group having a significantly higher BMI (Figure 4A and 4B). The NF- κ B pathway has garnished a great deal of attention recently in regards to its novel effects on metabolic function and substrate utilization. In particular, IKK β has been studied in a variety of cells, including endothelial, hepatic, and skeletal muscle. Research into the role of the NF- κ B pathway in endothelial cells has been conducted due, in part, to the pathological consequences of inflammation in the vascular system (29-32). Recent work by Wu et al. (33) determined that activation of IKK β by TNF- α is suppressed by adiponectin. This is particularly interesting due to adiponectin's ability to improve insulin sensitivity (34). Further, studying the role of NF- κ B pathway proteins in the liver, Cai et al. (8) revealed that diet-induced obesity in mice lead to increased NF- κ B pathway signaling in the liver. Mice with a liver-specific constitutively active IKK β transgene suffered from systemic insulin resistance and further analysis revealed a dose-dependent relationship between NF- κ B activity in the liver and the onset of system insulin resistance. Moreover, in human hepatocytes, Gao et al (35) treated cells with TNF- α and found increased IRS1 phosphorylation at Ser³¹². This IRS1-Ser³¹² phosphorylation correlated with the

disappearance of I κ B α , which represents increased IKK β activity.

Since skeletal muscle is the main depot for insulin-stimulated glucose uptake, studying the effects of IKK β on insulin signaling in this tissue is worthwhile. A study by de Alvaro et al. (28) reported that IKK β activation in rat skeletal muscle cell culture resulted in a significant increase in IRS1-Ser³⁰⁷ phosphorylation. They also explored the role of a pharmacological IKK β inhibitor, salicylate, in preventing IKK β -induced insulin resistance. Targeted disruption of the *Ikk β* locus in mice results lower fasting glucose and insulin concentrations, as well as preventing insulin resistance from a high-fat diet. Similar results have been reported with salicylate treatment (9). Additionally, Kim et al. (12) employed lipid infusions in rodents to cause acute insulin resistance. They observed that this effect was attenuated with pretreatment with salicylates. Further, lipid infusions performed in *Ikk β* heterozygous knockout mice revealed improvements in insulin sensitivity during hyperinsulinemic-euglycemic clamps when compared to controls.

IKK β activation, and subsequent IRS1-Ser³¹² phosphorylation, has been shown to be a result of the accumulation of excess fatty acids within the muscle that are subsequently partitioned toward storage or the formation of fatty acid metabolites, resulting in insulin resistance (13-15, 23, 36). We have previously shown that intramyocellular lipid (IMCL) levels are significantly reduced following gastric bypass surgery (37). Moreover, Greco et al. (38) determined that surgery-induced weight loss results in a greater-than-expected reduction in IMCL when compared with diet-induced weight loss alone. IMCL depletion is certainly a likely explanation for the improvements we observed in post-surgery patients in regards to levels of I κ B α and IRS1-pSer³¹² due to their role in mediating lipid-induced insulin resistance and it may partly explain the differences in whole-body insulin sensitivity.

Not surprisingly, in control subjects fasting insulin levels and insulin resistance (HOMA) have positive relationships with BMI. However, fasting insulin and insulin resistance are not associated with BMI in the post-surgery subjects. BMI was used as a global assessment

of body fat and weight loss in the current study. Whereas BMI is not an accurate indicator of body fat in certain populations with relatively greater muscle mass, given that all subjects in the current study were sedentary, we feel it is a legitimate measurement. Moreover, in regards to the weight loss and reduction in BMI associated with bypass surgery, it has been shown that the tremendous fat loss associated with gastric bypass surgery is mostly a result of a reduction in truncal fat (39).

A possible concern may be the possible confounding effect of a change in food intake as a result of gastric bypass surgery, which our group has previously explored (40). While patients experience an acute and meaningful decrease in caloric intake following surgery that results in significant weight loss, the outcomes we explored in the current study were measured approximately 12 mos following surgery in patients who were weight stable on a eucaloric diet, which would seemingly negate any dietary effect.

We interpret our data to suggest that the improvement in insulin sensitivity after gastric bypass is not exclusively due to weight loss and may implicate an effect of the surgery itself (2). A comparison of various surgical interventions lends support for this hypothesis. Namely, gastric banding surgery restricts food intake but does not change the channel of food flow. The remission rate for gastric banding is 48% while that for gastric bypass is 84% (5). In addition, Dixon et al. (4) reported that the remission of diabetes was correlated with the degree of weight loss, which is in contrast with the data we present here for gastric bypass. Moreover, Lafèrriere et al. (41) compared subjects who experienced comparable levels of weight loss due to hypocaloric diet and surgery. Whereas both interventions result in improved diabetes control, they found that patients who underwent bypass surgery enjoyed a better clinical outcome and no longer required diabetes medication.

If the improvement in insulin sensitivity after gastric bypass is not related to weight loss, an attractive alternative hypothesis is that increased insulin sensitivity and remission of diabetes after gastric bypass is due to restructuring of the gastrointestinal tract (2). According to this hypothesis, bypassing a

portion of the duodenum and jejunum causes either: 1) decreased secretion of a diabetes-inducing peptide (anti-incretin) from the foregut or 2) increased secretion of an anti-diabetes peptide (incretin) from the hindgut. This “foregut bypass hypothesis” was first suggested Pories (2) but has received recent attention by Rubino (42), who, with his colleagues, has extensively studied this hypothesis in a non-obese animal model of diabetes (GK rat). They found that a duodenal-jejunal bypass, which does not result in food restriction or weight loss, leads to improved glucose tolerance in the diabetic GK rat.

In summary, whole-body insulin sensitivity, as measured by IVGTT, and muscle glucose transport following gastric bypass are greater than predicted from the weight-matched control group. We interpret these findings to suggest that the improvement in insulin sensitivity is not a result of weight loss, but is more likely related to bypass of the foregut. Based on our results, we believe that IRS1-Ser³¹² phosphorylation is a potential cause for the insulin resistance seen in the weight-matched and morbidly obese compared to the lean group, and that gastric bypass appears to result in reduced IRS1-Ser³¹² phosphorylation to levels similar to lean controls. Finally, we have implicated IKK β , an NF- κ B pathway serine kinase, as a potential culprit responsible for phosphorylating IRS1-Ser³¹², and that bypass surgery may indirectly result in reduced IKK β activity in human skeletal muscle.

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FIGURE LEGENDS

Table 1. Characteristics of subjects/patients in the three groups.

Figure 1. The correlations between BMI and glucose (A) and insulin (B) for non-surgery control subjects (black circles) and post-surgery patients (white triangles). The data are from experiment 1 patients (see Table 1). **A.** The comparison of fasting glucose values between non-surgery control subjects (n=97) and post-surgery subjects (n=31), and **B.** Fasting insulin values between non-surgery subjects and post-surgery subjects. A positive and significant correlation was found ($P<0.001$, $r = 0.598$) for the non-surgery subjects. The same correlation in post-surgery subjects revealed no significant relationship ($P=0.169$, $r = 0.304$). Data are presented as mean \pm SD.

Figure 2. Whole-body insulin sensitivity determined by HOMA correlated with BMI (A) and IVGTT for four BMI groups (B). The data for the HOMA and insulin sensitivity analysis were taken from experiment 1 (see Table 1). **A.** The comparison of HOMA values for non-surgery control subjects (black circles) and post-surgery patients (white triangles). A positive and significant correlation between HOMA and BMI was found ($P<0.01$, $r = 0.283$) for the non-surgery subjects. The same correlation in post-surgery subjects revealed no significant relationship ($P=0.083$, $r = 0.446$). **B.** The difference in whole-body insulin sensitivity between BMI groups. *The post-surgery and lean groups are statistically higher ($P<0.005$) than the morbidly obese and weight-matched groups. † $P<0.05$ for the comparison between the morbidly obese and all other groups. Data are presented as mean \pm SD.

Figure 3. 2-Deoxy-D-glucose uptake in incubated muscle strips in the presence (100 nM) or absence of insulin (A) and the correlation between basal and insulin-stimulated glucose transport (B). The data were obtained from subjects in experiment 2 (see Table 1). **A.** A comparison of basal (empty bars) and insulin-stimulated (filled bars) glucose transport. *Insulin-stimulated glucose transport in the morbidly obese and weight-matched groups was statistically lower ($P<0.05$) than the lean and post-surgery groups. †Basal glucose transport in the morbidly obese and weight-matched groups was statistically lower ($P<0.05$) than the lean and post-surgery groups. Basal and insulin-stimulated glucose transport in muscle from morbidly obese subjects did not differ from the weight-matched subjects. **B.** The correlation between basal and insulin-stimulated glucose transport revealed a highly significant relationship ($P<0.001$; $r = 0.726$). Data are presented as mean \pm SD.

Figure 4. Muscle levels of IRS1-phospho-Ser³¹² (A) and I κ B α (B), and the correlation between IRS1-pSer³¹² and I κ B α (C). Non-surgery control subjects and post-surgery patients were from experiment 3 (see Table 1). **A.** Western blot analysis of serine³¹² phosphorylation of IRS1. * $P<0.05$ for the comparison between the lean group and the morbidly obese and weight-matched. † $P<0.05$ for the comparison between the post-surgery and the morbidly obese groups. The post-surgery group tended to have a lower level compared with the weight-matched group ($P=0.057$). **B.** Western blot analysis of I κ B α in skeletal muscle between groups. * $P<0.05$ for the observation that the lean and post-surgery groups were statistically higher than the morbidly obese and weight-matched groups. I κ B α levels were similar between post-surgery subjects and lean subjects. **C.** The correlation between I κ B α and IRS1-phospho-Ser³¹² levels between non-surgery and post-surgery subjects ($P=0.003$, $R=-0.47$). Data are presented as mean \pm SD.

Table 1. Characteristics of patients/subjects in the three experiments.

Subject characteristics			
Experiment	Age (y)	BMI (kg/m²)	Number of observations (n)
Experiment 1: Whole-body insulin sensitivity, fasting glucose and insulin			
Lean BMI<25 (non-surgery)	38.9 ± 15.0	23.4 ± 1.5	33
Weight-matched BMI 25-35 (non-surgery)	41.8 ± 14.1	28.5 ± 1.7	50
Morbidly obese BMI>35 (non-surgery)	40.3 ± 16.1	47.7 ± 9.5	14
Post-surgery	43.5 ± 9.8	29.4 ± 4.9	31
Experiment 2: Muscle glucose transport from rectus abdominis muscle taken during surgery			
Lean BMI<25	39.3 ± 6.4	22.4 ± 2.1	51
Weight-matched BMI 25-35	42.6 ± 19.0	28.9 ± 2.8	251
Morbidly obese BMI>35	41.6 ± 4.2	46.2 ± 4.0	20
Experiment 3: Vastus lateralis muscle biopsy for IRS1-Ser³¹² and IκBα			
Lean BMI<25 (non-surgery)	35.1 ± 14.4	21.7 ± 1.9	9
Weight-matched BMI 25-35 (non-surgery)	35.5 ± 10.1	30.9 ± 2.7	9
Morbidly obese BMI>35 (non-surgery)	32.8 ± 13.3	44.6 ± 4.2	9
Post-surgery	40.8 ± 8.1	29.9 ± 4.1	9

Mean ± SD. There is no statistical difference in BMI between the weight-matched and post-surgery groups.

















