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Improved Insulin Sensitivity 3 Months After RYGB Surgery Is Associated With Increased Subcutaneous Adipose Tissue AMPK Activity and Decreased Oxidative Stress

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Morbidly obese individuals are predisposed to a wide range of disorders, including type 2 diabetes, atherosclerotic cardiovascular disease, fatty liver disease, and certain cancers. Remarkably, all of these disorders can be improved or prevented by Roux-en-Y gastric bypass (RYGB) surgery. We have reported that decreased AMPK activity, together with increased oxidative stress and inflammation in adipose tissue, is associated with insulin resistance in morbidly obese bariatric surgery patients. In the current study, we assessed how these parameters are affected by RYGB surgery. Eleven patients (average age of 46 ± 4 years) were studied immediately prior to surgery and 3 months postoperatively. We measured subcutaneous adipose tissue AMPK phosphorylation (threonine 172, an index of its activation), malonyl-CoA content, protein carbonylation (a marker of oxidative stress), plasma adiponectin, and mRNA expression of several inflammatory cytokines. After surgery, AMPK activity increased 3.5-fold and oxidative stress decreased by 50% in subcutaneous adipose tissue. In addition, malonyl-CoA levels were reduced by 80%. Furthermore, patients had improvements in their BMI and insulin sensitivity (HOMA) and had increased circulating high–molecular weight adiponectin and decreased fasting plasma insulin levels. In contrast, the expression of inflammatory markers in subcutaneous adipose tissue was unchanged postoperatively, although plasma CRP was diminished by 50%.

Roux-en-Y gastric bypass (RYGB) is recognized as one of the most effective clinical interventions to achieve significant and sustainable weight loss in morbidly obese individuals. It has been reported to either cause remission or significantly improve type 2 diabetes and fatty liver disease, and it diminishes mortality from cardiovascular disease and the incidence of certain cancers (1–3). Despite this, the molecular mechanisms underlying its effects are incompletely understood. Factors such as gut hormones, alterations in gut microbiota, and decreased food intake are thought to be at least partially responsible (4–6).

Initially known as a fuel-sensing enzyme, mounting evidence has demonstrated that AMPK plays a much greater role in regulating cellular function. Studies in rodents and cultured cells indicate that its activation attenuates inflammation and oxidative stress and increases mitochondrial biogenesis (7,8). Likewise, decreased AMPK has been observed in tissues of obese and insulin-resistant rodents (7), and therapy with AMPK activators has been shown to reverse the insulin resistance in these rodents (8). We have previously demonstrated that decreased subcutaneous and visceral adipose tissue AMPK activity is associated with insulin resistance in morbidly obese bariatric surgery patients (9,10). In contrast, increases in inflammatory genes were predominantly observed in visceral fat (9).

In this study, we explored the link between adipose tissue AMPK activity, insulin sensitivity, and other

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parameters in 11 morbidly obese individuals before and 3 months after RYGB. Our data showed that postoperatively subcutaneous adipose tissue AMPK activity was increased as were insulin sensitivity and plasma adiponectin, whereas adipose tissue oxidative stress and the concentration of malonyl-CoA were diminished. In contrast, the mRNA expression of the inflammatory genes interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), and IL-10 in adipose tissue was unchanged despite a decrease in plasma CRP level.

RESEARCH DESIGN AND METHODS

Patients

Eleven patients (eight women and three men) with a mean ± SE age of 46.6 ± 4.3 years were studied. Five of the 11 patients had type 2 diabetes at baseline. Pre- and postoperative clinical characteristics are listed in Table 1. Insulin sensitivity was assessed by HOMA with a value of >2.3 considered insulin resistant and a value of <2.3 considered insulin sensitive (11). The study was approved by the Boston University Medical Center institutional review board. All participants were candidates for laparoscopic RYGB and had signed informed consent forms prior to their enrollment.

Blood measurements were carried out after an overnight fast. With the exception of adiponectin, all analyses were performed by the Boston Medical Center clinical chemistry laboratory. Plasma adiponectin was measured using a commercial human high–molecular weight (HMW) ELISA kit (R&D Systems, Minneapolis, MN). In humans, HMW adiponectin levels are known to reflect insulin sensitivity more accurately than levels of low– or medium–molecular weight adiponectin (12).

Biopsies of abdominal subcutaneous adipose tissue (~0.5 g) were obtained either at the time of surgery (baseline group) or under local anesthesia during a study visit 3 months after the RYGB surgery (postoperative group). The tissues were immediately frozen in liquid nitrogen and stored at −80°C until further processing.

Table 1—Clinical changes in 11 morbidly obese patients before and 3 months after RYGB surgery

Parameter	Baseline	Post-RYGB
HOMA	5.8 ± 1.4	2.6 ± 0.6**
Weight (kg)	117.9 ± 6.0	96.1 ± 3.8***
BMI (kg/m^2)	41.7 ± 1.4	33.9 ± 1.1***
Waist circumference (cm)	125.7 ± 3.9	107.5 ± 3.4**
Hip circumference (cm)	130.7 ± 4.3	115.3 ± 2.3**
Plasma insulin (μIU/mL)	20.1 ± 4.5	9.6 ± 1.6**
Glucose (mg/dL)	118.9 ± 15	99.9 ± 8.2
HbA_{1c} (%)	6.2 ± 0.5	5.8 ± 0.4
HbA_{1c} (mmol/mol)	44 ± 5.5	40 ± 4.4
hsCRP (mg/L)	5.4 ± 1.7	2.7 ± 0.7*
HMW adiponectin (ng/mL)	5,605 ± 1,592	7,538 ± 2,036**
Type 2 diabetes	5	2
Metformin user	5	2

Data are means ± SE. No significant changes were observed in plasma total LDL, HDL, cholesterol, or triglycerides, although triglycerides were decreased from 127 + 26 to 101 + 11 mg/dL (data not shown). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, compared with the baseline group.

Western Blot Analyses

Total proteins were isolated from subcutaneous fat, and their concentrations were determined using the bicinchoninic acid assay (Thermo Scientific, Rockford, IL). Fifteen micrograms of protein were separated by gel electrophoresis, transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA), and then incubated with primary antibodies against phosphorylated AMPK (threonine^{172} [Thr^{172}]) and total AMPK (Cell Signaling Technology), using HSP90 (Santa Cruz Biotechnology, Santa Cruz, CA) as a loading control. Proteins were visualized by enhanced chemiluminescence (Thermo Scientific) and quantified with Scion Image software (National Institutes of Health).

RNA Isolation and Real-Time Quantitative PCR

Adipose tissue was homogenized in TRIzol (Invitrogen, Frederick, MD). Total RNA was extracted using the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA) and reverse transcribed into cDNA (Invitrogen). Real-time quantitative PCR was performed as described previously (9).

Protein Carbonylation Assay

Protein carbonylation was determined with an OxyBlot Protein Oxidation Detection Kit (Millipore) to provide a measure of oxidative stress, as described previously (9). In brief, 10 μg of protein lysate was derivatized with 4-dinitrophenylhydrazine according to the manufacturer's instructions. Total carbonylation was visualized by enhanced chemiluminescence (Thermo Scientific) and the bands quantified with Scion Image software.

Malonyl-CoA Assay

Human subcutaneous adipose tissue was homogenized in 6% perchloric acid and then centrifuged at 14,000*g* for 10 min at 4°C. After that, the supernatant was neutralized with 2 mol/L KOH and 0.4 mol/L KCl, to pH 7.0, and centrifuged at 14,000*g* for 10 min at 4°C. The resultant supernatant was subjected to malonyl-CoA assay, using the method by McGarry et al. (13).

Statistical Analysis

Data are expressed as means ± SE. All baseline and postoperative values were compared using the Wilcoxon matched pairs test. Spearman correlation analysis was used when appropriate. Minimal level of significance was set at $P < 0.05$. GraphPad Prism software (La Jolla, CA) was used for all analyses.

RESULTS

Patient Characterization

The clinical characteristics of the patients at baseline and 3 months after RYGB surgery are shown in Table 1. Postoperatively, there were significant reductions in body

weight, BMI, waist circumference, hip circumference, and fasting plasma insulin. Moreover, patients uniformly showed an improvement in insulin sensitivity based on HOMA evaluation and a decrease in CRP. Fasting plasma glucose and glycosylated hemoglobin A_{1c} (HbA_{1c}) levels showed downward trends postoperatively, but the differences did not reach statistical significance. Circulating HMW adiponectin was increased postoperatively. Of the five patients with type 2 diabetes, three were free of diabetes 3 months postoperatively. The two patients whose diabetes was not resolved by RYGB had higher HbA_{1c} levels preoperatively and a longer duration of type 2 diabetes. Also, preoperatively they were on diabetic medications other than metformin. Sex did not appear to be a factor that predicted outcome.

Subcutaneous Adipose Tissue AMPK Phosphorylation Is Uniformly Improved Postoperatively

Western blot analysis was performed to assess AMPK phosphorylation at Thr^{172}, an indicator of AMPK activity (14). As shown in Fig. 1*A*, the abundance of phosphorylated AMPK postoperatively was ~3.5-fold higher than preoperatively. As shown in Supplementary Table 1, AMPK phosphorylation was increased in every subject, regardless of baseline insulin sensitivity. Since AMPK can be activated by adiponectin (15), we performed correlation analysis to determine whether the improvement in AMPK and in adiponectin (Table 1) were related to each other. However, such correlation was not statistically significant (Fig. 1*B*).

Malonyl-CoA and Oxidative Stress

Malonyl-CoA is both an intermediate in the de novo synthesis of long-chain fatty acids and an inhibitor of fatty acid oxidation (16). AMPK suppresses malonyl-CoA production by phosphorylating acetyl-CoA carboxylase (ACC) (16). In keeping with the postoperative increase in AMPK phosphorylation, we found a significantly decreased malonyl-CoA level in the postoperative group compared with the baseline group (Fig. 1*C*). Protein carbonylation was measured as an index of oxidative stress. As shown in Fig. 2, it was significantly diminished postoperatively.

Expression of Inflammatory Genes Did Not Change in Subcutaneous Adipose Tissue After RYGB Surgery

Results from real-time PCR indicate that the mRNA levels of proinflammatory cytokines TNF-α and IL-1β did not differ between the baseline and postoperative groups (data not shown). The mRNA level of the anti-inflammatory cytokine IL-10 was moderately higher postoperatively (data not shown); however, the difference was not statistically significant.

DISCUSSION

The overall objective of this study was to investigate whether there is a link between subcutaneous adipose tissue AMPK phosphorylation/activity and insulin sensitivity

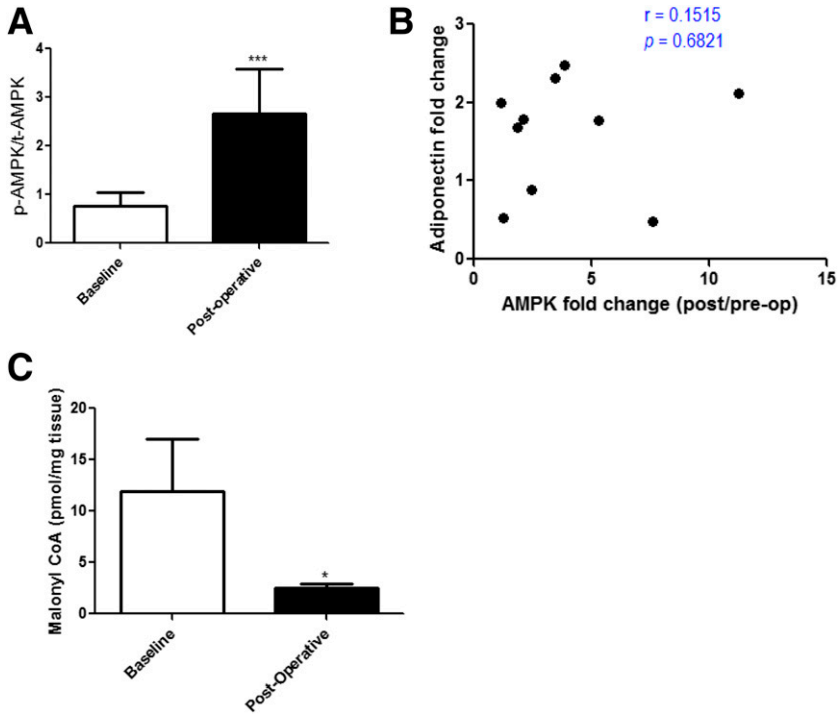

Figure 1—*A*: Comparison of AMPK phosphorylation at Thr^{172}, a marker of AMPK activation, in the subcutaneous fat of 11 pair-matched pre- and postbariatric surgery patients (***$P < 0.001$ compared with baseline group). *B*: Spearman correlation analysis of changes in adipose tissue AMPK phosphorylation and circulating adiponectin before and after RYGB. *C*: Comparison of malonyl-CoA levels in the subcutaneous adipose tissue in baseline and postoperative groups.

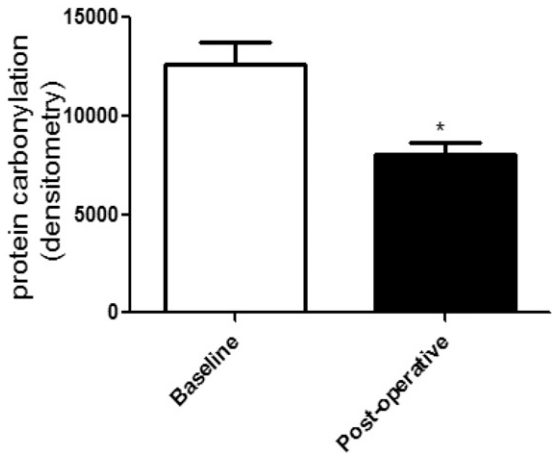

Figure 2—Comparison of the protein carbonylation (a measure of oxidative stress) in the subcutaneous fat of 11 pair-matched pre- and postbariatric surgery patients (*$P < 0.05$ compared with baseline group).

in a well-characterized yet small (n = 11) group of RYGB patients. We demonstrated that 3 months postoperatively, there is a substantial improvement in the patient metabolic profile as assessed by changes in body weight, BMI, circulating HMW adiponectin, insulin sensitivity, and increased adipose tissue AMPK phosphorylation/activation. In addition, we found decreases in oxidative stress and malonyl-CoA level in subcutaneous adipose tissue of the postoperative group. In contrast, the mRNA levels of several inflammatory genes did not change, despite a decrease in circulating CRP levels.

AMPK is an energy sensor that restores cellular energy homeostasis. Recent developments have shown that AMPK is a probable target of major antidiabetic drugs such as metformin and thiazolidinediones (7,8). At the molecular level, evidence accumulated from cell culture and animal studies has demonstrated a role for AMPK in reversing adverse events such as oxidative stress, inflammation, and insulin resistance (8,17,18). We previously showed that diminished subcutaneous and visceral adipose tissue AMPK activity is associated with insulin resistance in RYGB patients (9,10). In addition, Kola et al. (19) reported decreased AMPK activity in the visceral adipose tissue of patients with Cushing syndrome, many of whom are insulin resistant. Findings from the current study indicate an association between improved AMPK activity and insulin sensitivity in morbidly obese individuals after RYGB weight-loss surgery. To the best of our knowledge, this is the first human study that links increased adipose tissue AMPK to improvement in insulin sensitivity after RYGB surgery. Intriguingly, increased AMPK phosphorylation was observed in every patient studied, including three individuals who were identified as insulin sensitive at baseline (Supplementary Table 1). Studies with a larger number of patients will be needed to determine whether the responses to RYGB surgery are qualitatively different in patients classified as insulin sensitive or resistant.

The decrease in malonyl-CoA content in adipose tissue postoperatively is most likely caused by the activation of AMPK upstream, as AMPK phosphorylates and inhibits ACC, a rate-limiting enzyme for malonyl-CoA synthesis. To the best of our knowledge, this is the first study in which malonyl-CoA has been measured in human fat.

As for the mechanism responsible for the elevated AMPK activity postoperatively, adiponectin is known to activate AMPK (15) and its plasma level increased significantly after RYGB (Table 1). Since adiponectin is produced by adipocytes exclusively, its increase could reflect a general improvement in fat cell function postoperatively. However, we did not find a significant correlation between changes in AMPK and adiponectin; the sample-to-sample variation was quite large (Fig. 1*B*). Future studies with more participants are necessary. Other possible candidates include substantial weight loss, as AMPK is activated by energy deficit (7). Although not measured in this study, GLP-1 is known to increase significantly after RYGB surgery (20), and it can activate AMPK at least in endothelial cells (21).

Bariatric surgery has been demonstrated to attenuate markers of oxidative stress in liver and plasma (22,23). The results of the current study indicate that it has a similar effect in subcutaneous adipose tissue. Whether such changes occur in visceral adipose tissue warrants exploration. Although AMPK activation can diminish oxidative stress, oxidative stress can also suppress AMPK activity (18). Thus, our data cannot discern whether the decrease in oxidative stress is a consequence or cause of the increased AMPK activity observed postoperatively.

Although we did not find any change in a small number of inflammatory genes, the circulating level of CRP decreased postoperatively. It is conceivable that the disappearance of tissue inflammation is a slower process. However, a more likely explanation is that inflammation is more prominent in visceral than subcutaneous fat (18), and it is a change in the latter, and possibly the liver, that led to the decreased CRP.

Finally, although bariatric surgery is associated with a durable remission of type 2 diabetes, about one-third of the patients experience a relapse within 5 years (24). Thus, measurements of AMPK in postoperative adipose tissue biopsies might provide insights as to why such remissions and relapses occur and what can be done to prevent the latter. For instance, if decreased AMPK activity is found to reoccur in adipose tissue, agents that could activate AMPK, such as metformin, thiazolidinediones, and GLP-1 analogs alone or in combination, could prove useful.

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Author Contributions. X.J.X. wrote the manuscript and designed and conducted all the experiments as well as data analyses. C.A. reviewed and edited the manuscript and performed the adipose tissue biopsies. D.H. and B.C. performed the adipose tissue biopsies. A.S. measured and analyzed malonyl-CoA data and edited the manuscript. N.R. reviewed and edited the manuscript. X.J.X. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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