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Partitioning of Adipose Lipid Metabolism by Altered Expression and Function of PPAR Isoforms After Bariatric Surgery

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Abstract

Background—Bariatric surgery remains the most effective treatment for reducing adiposity and eliminating type 2 diabetes, however the mechanism(s) responsible have remained elusive. Peroxisome proliferator activated receptors (PPAR) encompass a family of nuclear hormone receptors that upon activation exert control of lipid metabolism, glucose regulation, and inflammation. Their role in adipose tissue following bariatric surgery remains undefined.

Materials and Methods—Subcutaneous adipose tissue biopsies and serum were obtained and evaluated from at time of surgery and on postoperative day 7 in patients randomized to Roux-en-Y gastric bypass (n=13) or matched caloric restriction (n=14), as well as patients undergoing vertical sleeve gastrectomy (n=33). Fat samples were evaluated for changes in gene expression, protein levels, β -oxidation, lipolysis, and cysteine oxidation.

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The corresponding authors have had full access to the data in the study and accept final responsibility for the decision to submit for publication.

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Author Contributions:

C.J., H.X., A.V.H., S. I. and D.A.B. performed all experiments and data interpretation, and conceived the study design. G.L., D.B.L., and S.I. performed all surgeries and participated in all facets of serum and tissue collection. K.E. conceived dietary plan and ensured subject compliance. N.K., F.J.S., S.K., A.X and C.J. collected and stored all specimens. N.K., F.J.S., S.K., and C.J. performed all qRT-PCR analysis. A.V.H. and H.X. performed adipose tissue fractionation, western blot, fatty acid oxidation, fatty acid and glycerol measurements. K.S. measured cysteine oxidation in SAT. R.F. and H.X. measured sera FABP4. C.J., H.X., A.V.H., S.K., S.I. and D.A.B. wrote the paper.

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All authors have nothing to disclose.

Results—Within 7 days, bariatric surgery acutely drives a change in the activity and expression of PPAR γ and PPAR δ in subcutaneous adipose tissue thereby attenuating lipid storage, increasing lipolysis and potentiating lipid oxidation. This unique metabolic alteration leads to changes in downstream PPAR γ/δ targets including decreased expression of FABP4 and SCD1 with increased expression of carnitine palmitoyl transferase 1 (CPT1) and uncoupling protein 2 (UCP2). Increased expression of UCP2 not only facilitated fatty acid oxidation (increased 15-fold following surgery) but also regulated the subcutaneous adipose tissue redoxome by attenuating protein cysteine oxidation and reducing oxidative stress. The expression of UCP1, a mitochondrial protein responsible for the regulation of fatty acid oxidation and thermogenesis in beige and brown fat, was unaltered following surgery.

Conclusions—These results suggest that bariatric surgery initiates a novel metabolic shift in subcutaneous adipose tissue to oxidize fatty acids independently from the beiging process through regulation of PPAR isoforms. Further studies are required to understand the contribution of this shift in expression of PPAR isoforms as a contributor to weight loss following bariatric surgery.

Introduction

By the year 2040, global estimates of the prevalence of obesity-linked type 2 diabetes mellitus (T2DM) are approximately 640 million people worldwide¹. The mainstay treatment remains weight loss achieved by lifestyle modifications and medications². Unfortunately, these strategies have not shown durable success^{3,4}. As an endocrine organ, adipose tissue plays an important role in the pathogenesis of obesity and T2DM, and hyperplastic adipocytes secrete a variety of fatty acids and adipokines to achieve homeostasis⁵. The most effective treatment for obesity and T2DM is bariatric surgery^{3,6,7}. The two most common procedures are vertical sleeve gastrectomy (VSG) and Roux-en-Y gastric bypass (RYGB)⁸. For both procedures, by one-week post-surgery, insulin sensitivity is significantly improved with only minimal weight loss⁹. However, the mechanism(s) responsible for such rapid metabolic changes after surgery has been incompletely characterized¹⁰.

The peroxisome proliferator-activated receptor γ (PPAR γ) nuclear receptor plays a major role in controlling adipocyte lipid storage and insulin sensitivity^{11,12}. PPAR δ is also abundantly expressed in adipose tissue and promotes lipid utilization by upregulating genes involved in fatty acid oxidation, such as carnitine palmitoyltransferase 1 (CPT1)^{13,14}. Herein we report on a novel surgically-driven pathway resulting in increased adipose fatty acid β -oxidation derived from down-regulation of PPAR γ and increased expression and activation of PPAR δ . The switch in PPAR expression regulates several key target genes such as FABP4, SCD1, CPT1 and UCP2. Moreover, increased expression of uncoupling protein 2 (UCP2) mediates a reduction in adipose tissue oxidative stress and changes the tissue redoxome profile.

Materials and Methods

Study design

The University of Minnesota and St. Cloud Hospital Institutional Review Boards approved all investigations and informed consent was obtained from each participant. Thirty-three

patients > 21 years of age and BMI ≥ 35.0 kg/m² undergoing VSG were recruited and informed consent was obtained. Abdominal subcutaneous adipose tissue (SAT) biopsies from 20 of the VSG patients were processed for tissue analysis. Based on a treatment effect of -0.5 after VSG and 0.18 after hypocaloric restriction for total effect of 0.68 , we estimated that 12 patients per group would allow us to detect at least a 40% difference of tissue protein level of FABP4 between groups with the power of 0.8 and alpha value of 0.05. The details of the study methods of the 27 patients recruited and randomized to RYGB ($n=13$; 3 males and 10 females) or diet ($n=14$; 4 males and 10 females) have been published¹⁵. Briefly, inclusion criteria included men and women older than 21 years of age with a BMI ≥ 35.0 kg/m², and meeting criteria by ADA standards for pre-diabetes or T2DM. We exclude patients with severe medical comorbidities, inability to undergo surgery, patients with unanticipated surgical complications, and subjects on thiazolidinediones (TZD) as these are known to affect the PPAR pathways.

Clinical data

Demographic data on sex, age, and T2DM were collected for obese patients at the time of surgery and seven days following bariatric surgery. Weight and height were measured immediately prior to surgery (preop) and during the postoperative visit. Body mass index (BMI) was calculated as weight (kg) divided by height (m²).

Surgical Procedures

Details of the surgical procedures have been previously described¹⁵. With regards to the RYGB, a 20–30 mL vertically oriented gastric pouch was constructed using 3.5 mm staples. A 10–12 mm gastrojejunostomy was created in an antecolic, antegastric fashion, with the Roux limb measuring approximately 150 cm from the gastrojejunostomy. For the laparoscopic VSG, a 4.8 mm stapler load was used to divide the greater curvature of the stomach 5 cm from the pylorus and remaining 3 cm from the *angularis incisura*. 3.5 mm stapler loads were fired thereafter progressing up to the angle of His to complete the VSG.

Tissue sampling and blood collection

SAT samples and blood specimens were obtained from patients on the day of intervention and seven days post-intervention. Approximately 2–3 g of fat was obtained from each subject and either immediately frozen with liquid nitrogen and stored at -80°C or processed by collagenase digestion, or for measurements of β -oxidation and lipolysis.

Assessment of DM medication score and insulin resistance

Fasting plasma glucose and plasma insulin levels were obtained at the time of initiation of intervention and seven days post-intervention. Homeostasis Model of Assessment for Insulin Resistance (HOMA-IR) was calculated according to the method described by Matthews *et al*¹⁶. Hemoglobin A1c (HbA1c) was determined preoperatively on 20 patients (S. Table 1).

β -oxidation measurement in subcutaneous adipose tissue explants

Pre- and post-surgery SAT biopsies were minced and incubated with KRH buffer supplemented with ¹⁴C-palmitic acid for 1 h at 37°C . Minced tissue were transferred with

medium into a 20 ml-glass reaction vial, which has a 1 mL tube containing 400 μ L of 1 M NaOH. Perchloric acid was added to stop the reaction, and samples were incubated for 1 h shaking at 80 rpm. $^{14}\text{CO}_2$ was trapped into the central tube and measured by liquid scintillation counting.

Lipolysis measurement in human subcutaneous adipose tissue explants

0.3 g of adipose tissue was minced and incubated with 500 μ l assay buffer at 37°C for 1 hour. The tissue mixture was centrifuged at 500 \times g for 10 minutes and the liquid between the pellet and floating adipose tissue was moved to a new tube. Free fatty acids were measured using kits obtained from Wako Diagnostics (Mountain View, CA) and glycerol was measured using a Glycerol Colorimetric Assay Kit from Cayman Chemical Company (Ann Arbor, MI), each sample was measured in triplicates and presented as a composite mean with standard error of the mean.

Collagenase Digestion

After fat was obtained, it was immediately minced and digested with type I collagenase in Krebs-Ringers-HEPES (KRH) buffer supplemented with 10 mg/ml bovine serum albumin (BSA). After incubation at 37°C for one hour, the mixture was filtered with a cell strainer (100- μ m-pore-size nylon; Falcon) to remove undigested tissues. The stromal vascular fraction (SVF) was collected by centrifugation at 500 \times g for 10 minutes and both fractions, the floating primary adipocyte and the SVF were washed, and TRIzol © (Invitrogen, Carlsbad, CA) reagent was used for RNA isolation.

Real-time PCR

Total RNA was extracted from approximately 0.3 g of adipose tissue using TRIzol © (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. After DNase treatment, cDNA was synthesized using iScript cDNA synthesis kit (BioRad, Hercules, CA). Relative quantification of mRNA was performed by RT-PCR using iQ SYBR green Supermix and the MyiQ detection system (BioRad, Hercules, CA). Each sample was measured in duplicate. Human primers for target genes are listed in Supplemental Table 3. Gene expression data are expressed as arbitrary units normalized to the reference gene hTBP.

Immunoblotting

Immunoblotting was performed as previously described¹⁷. Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer supplemented with protease inhibitors. Equal amounts of protein were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane. After a blocking step, membranes were incubated with primary antibody overnight at 4°C. Membranes were washed and incubated with secondary antibody conjugated to Li-Cor IRDye for 1 h and visualized using Odyssey infrared imaging (Li-Cor Biosciences, Lincoln, NE). The antibodies used were anti-PPAR γ (Santa Cruz, Dallas, Texas, sc-7273), anti-FABP4 (laboratory stock), anti-CD36 (R&D Systems, Minneapolis, MN, AF2519), anti-Perilipin1 (American Research Products, Waltham, MA,

03-651156), anti-sulfenic acid modified cysteine (Milipore, Darmstadt, Germany, ABS30) and anti- β -actin (Sigma-Aldrich, St. Louis, MO, A3854).

Mitochondrial isolation

Mitochondrial isolation was carried out as previously described¹⁷. Adipose tissue was minced in ice-cold mitochondrial isolation buffer (20 mM Tris pH 7.4, 220 mM mannitol, 70 mM sucrose, 1 mM EDTA, 0.1 mM EGTA) and supplemented with protease inhibitors. The tissue was then further homogenized with 15 strokes of a Dounce homogenizer and centrifuged at $700 \times g$ for 10 minutes at 4° C. Avoiding the lipid layer, the supernatant was transferred to a fresh tube and centrifuged at $10,000 \times g$ for 15 min at 4° C.

Dimedone labeling of sulfenic acids

Mitochondria pellets were solubilized using sonication in radioimmunoprecipitation assay (RIPA) buffer containing protease inhibitors, 1 mM dimedone, 10 mM iodoacetamide, 10mM N-ethylmaleimide, and 200 units/mL catalase to metabolize intracellular hydrogen peroxide. The lysates were incubated for 1 hr on ice followed by the addition of Laemmli protein sample buffer and the proteins were separated on an SDS-PAGE gel for cysteine oxidation (sulfenic acid) detection¹⁸.

ELISA measurement of serum FABP4

Serum FABP4 was measured with human FABP4 Quantikine ELISA kit from R&D systems (#DFBP40) according to the manufacturer's instructions. Each sample was measured in duplicate.

Statistical analysis

The data are expressed as mean \pm standard error. Statistical analysis was performed using unpaired two-sided Student's t-test when comparing between groups and paired two-sided Student's t-test to compare pre- and post-surgical measures within groups. Unadjusted Pearson correlations were calculated to evaluate relations between HbA1c and target of interest, and changes in FABP4 and hormone of interest. Data with p-values less than 0.05 were considered statistically significant, unless otherwise stated.

Results

Expression of *PPAR* γ and downstream targets decrease after bariatric surgery

VSG was initially performed on 20 subjects (Supplemental Table 1). One-week post-VSG, subjects had minimal, although statistically significant weight loss and an improved HOMA-IR (Fig. 1A,B). Patients who were prediabetic or diabetic had higher initial HOMA-IR levels (5.3 ± 0.9) and exhibited a greater decrement in levels post-surgery (3.4 ± 0.5 ; $P = 0.02$).

SAT*PPAR* γ mRNA levels decreased significantly one-week after VSG, on average by 60% (Fig. 1C), along with several well-established *PPAR* γ targets including fatty acid binding protein 4 (*FABP4*), phosphodiesterase 3B (*PDE3B*), adipose triglyceride lipase (*ATGL*), cluster of differentiation 36 (*CD36*), stearoyl-CoA desaturase 1 (*SCD1*), uncoupling protein 1 (*UCPI*) and perilipin 1 (Fig. 1D). Assessment of *PPAR* γ protein levels indicated a similar

trend ($P = 0.09$), on average decreasing by nearly 60% (Fig. 1E). Furthermore, protein levels of CD36 also decreased significantly whereas Perilipin 1 trended downward (Fig. 1E).

To identify which cell population(s) was responsible for the decreased expression of *PPAR* γ and its gene targets, we performed collagenase digestion of SAT to obtain a primary adipocyte fraction (ADIP) and SVF. mRNA levels of *PPAR* γ and its downstream targets were consistently decreased in both fractions. One notable exception was *PDE3B* mRNA, although decreased in ADIP, was nearly tripled in SVF (Fig. 1F,G).

In addition to VSG, RYGB is a commonly performed bariatric procedure that adds a malabsorptive component to the treatment¹⁹. We previously performed a study that randomized obese diabetic subjects to RYGB versus a hypocaloric matched diet¹⁵. Weight loss after RYGB was similar to that observed following VSG (Fig. S1A). The RYGB group had a higher HbA1c and was on more anti-diabetic medications than the VSG cohort¹⁵. While there was no decrease in *PPAR* γ gene expression in post-RYGB patients, several *PPAR* γ gene targets including *PDE3B*, *ATGL*, *SCD1*, and *UCP1* decreased significantly or trended towards reduced levels (Fig. S1C). Protein levels of *PPAR* γ and its downstream targets, CD36 and Perilipin 1, decreased or trended downward after RYGB, similar to that seen post-VSG (Fig. S1D).

In an attempt to reconcile the differences in diabetic status among our patients undergoing VSG as compared to RYGB, we pooled all surgical patient data together and evaluated whether preoperative diabetic status, specifically HbA1c or HOMA-IR, was associated with acute post-surgical differences in *PPAR* γ gene expression and that of its downstream targets. HbA1c strongly correlated with changes in mRNA levels of *PPAR* γ (Fig. S2A). HbA1c also correlated with several downstream targets, specifically *FABP4*, *PDE3B*, and *CD36*, but not with *ATGL* or *SCD1* (Fig. S2B–F). These findings suggest that acutely after surgery, patients with higher preoperative HbA1c have smaller changes in *PPAR* γ and downstream targets. As such, both bariatric procedures result in decreased adipose *PPAR* γ activity, an effect previously suggested to improve insulin sensitivity^{20,21}.

Expression of *PPAR* δ and its targets increase after bariatric surgery

PPAR δ activity supports lipid metabolism in adipose and muscle via regulation of genes involved in fatty acid uptake, β -oxidation, and energy uncoupling. After VSG and RYGB, the expression of *PPAR* δ increased 2–3 fold in SAT (Fig. 2A, Fig. S4A). Furthermore, several downstream targets of *PPAR* δ , including *CPT1*, pyruvate dehydrogenase kinase 4 (*PDK4*), perilipin 2, and angiopoietin like 4 (*ANGPLT4*), increased significantly after VSG (Fig. 2B–E). After RYGB, *CPT1* increased while *PDK4* did not change (Fig. S4B,C). In contrast to *PPAR* γ , changes in *PPAR* δ and *CPT1* did not correlate with preoperative HbA1c (Fig. S2H,I). *PPAR* α , another participant in lipid metabolism, and its downstream target, *CPT2*, were unchanged after bariatric surgery or hypocaloric diet (Fig. 2F,G and Fig. S4D,E). Overall, bariatric surgery is associated with a dramatic change in the expression and activity *PPAR* γ and *PPAR* δ in SAT resulting in reduced gene expression of enzymes and proteins associated with lipid synthesis in favor of those linked to lipid oxidation.

Caloric restriction does not regulate PPAR γ or PPAR δ activity

It has been demonstrated that plasma glucose and insulin levels decrease as early as one-week after bariatric surgery prior to substantial weight loss and independent of diet⁹. To assess whether the observed post-surgical effects on PPAR γ or PPAR δ were dependent upon caloric restriction, we performed our analysis on a calorically restricted group from our previous randomized controlled trial¹⁵.

Although acute weight loss after hypocaloric restriction was similar to that observed in the surgical cohorts (Fig. S3A), there was no change in subcutaneous adipose *PPAR γ* mRNA levels (Fig. S3B). Evaluations of adipose expression of PPAR γ targets following hypocaloric restriction revealed increases in *PDE3B* and *FABP4*, decreases in *SCD1*, and no change in *UCPI* (Fig. S3C). In contrast to the surgical groups, PPAR γ protein levels increased five-fold after hypocaloric restriction, without changes to CD36 or Perilipin 1 (Fig. S3D). Similarly, neither expression of *PPAR δ* nor its downstream targets changed after hypocaloric diet (Fig. S4A–C). As such caloric restriction does not induce a similar change in the activity of PPAR γ or PPAR δ as does bariatric surgery.

Adipose tissue β -oxidation and lipolysis increase following VSG

To explore the functional consequences of increased PPAR δ activity, we measured β -oxidation in adipose tissue explants from six additional subjects undergoing VSG (S. Table 2) using ¹⁴C-palmitate oxidation. Consistent with the upregulation of PPAR δ activity and targets, β -oxidation was increased 15-fold following VSG (Fig. 3A). One potential contributor of β -oxidation is increased fatty acid availability via increased lipolysis²². Indeed, previous reports have shown via hyperinsulinemic-euglycemic clamps that lipolysis is increased after bariatric surgery^{9,23}. Similar to that shown by Gastaldelli et al. and Camastra et al. following RYGB, adipose tissue explants obtained from seven VSG subjects (S. Table 2) one week after VSG demonstrated a 2-fold increase in lipolysis (Fig. 3B–D)^{9,23}.

These results suggest that increased lipid mobilization following VSG leads to increased fatty acid oxidation within WAT. This increase in lipid oxidation does not appear to be due to beiging or browning since *UCPI* was not upregulated after bariatric surgery and indeed, shows a strong trend towards being downregulated (Fig. 1D, Fig. S1C).

Bariatric surgery downregulates FABP4 and upregulates FABP5

Fatty acid binding proteins, notably FABP4 and FABP5, play critical roles in lipid metabolism and are PPAR γ and PPAR δ targets, respectively²⁴. As shown in Fig. 4A and B, adipose FABP4 protein decreased nearly 40% per patient post-VSG. Although *FABP4* mRNA levels did not significantly change in the RYGB population, protein levels dropped by nearly 20% (Fig. 4A,B). FABP4 protein levels did not change in the diet cohort. FABP4 has also been identified as a circulating adipokine that could contribute to systemic insulin resistance through actions on liver and pancreatic β -cells^{25,26}. Therefore, we measured the serum levels of FABP4 in the three cohorts one-week after intervention. While serum levels of FABP4 did not change in either the VSG or RYGB cohorts, they were increased by about 30% in the diet cohort (Fig. S5). Protein levels of FABP5, another fatty acid carrier in

adipose tissue and a PPAR δ target, increased after both VSG and RYGB (Fig. S6A,B), but not in the diet group (Fig. S6C).

Thus, consistent with altered expression of PPAR isoforms, there is an acute switch in lipid carrier proteins after bariatric surgery that results in the downregulation of FABP4 and upregulation of FABP5 in SAT. While FABP4 expression decreases in adipose tissue, a compensatory upregulation of FABP5 was observed. Interestingly, FABP5 has been reported to deliver ligands to activate PPAR δ , resulting in increased expression of genes involved in β -oxidation²⁷.

Bariatric surgery increases UCP2 levels and decreases cysteine oxidation in SAT

We have previously demonstrated that reduced inflammation resulting from decreased FABP4 expression is dependent upon the upregulation of UCP2¹⁷ and UCP2 has also been identified as a downstream target of PPAR δ in a number of different tissues²⁸. As shown in Fig. 4C, *UCP2* expression increased approximately 7-fold following VSG and 3-fold following RYGB, but did not change in the diet cohort. Furthermore, *UCP2* was upregulated in both ADIP and SVF in VSG (Fig. 4D). The changes in gene expression of UCP2 following surgery correlated with preoperative HbA1c (Fig. S2G). UCP2 exerts its cellular function, in part, by reducing reactive oxygen species (ROS) which, if left unchecked, can alter a variety of cellular functions by oxidizing protein residues such as cysteines²⁹. Consistent with the upregulation of *UCP2*, we observed reduced cysteine oxidation in adipose tissue following VSG (Fig. 5A,B).

Discussion

Bariatric surgery is the most efficacious treatment of two of the most burdensome diseases in healthcare today: obesity and T2DM^{3,4}. Here we report a novel finding of altered expression of PPAR γ and PPAR δ occurring acutely in subcutaneous adipose tissue of human subjects after bariatric surgery, independent of caloric restriction. This alteration in the main regulators of adipose tissue metabolism has important implications in energy metabolism and insulin sensitivity given the active role of SAT. While visceral adipose tissue has been considered the primary metabolically active depot, evidence suggests that SAT is also critical to overall energy metabolism, even serving as the preferred storage depot in postprandial states³⁰. Furthermore, in a comparison of multiple weight loss interventions including bariatric surgery, SAT loss was consistently observed to be greater than visceral adipose tissue loss, and visceral fat loss was linked to subcutaneous fat loss³¹.

PPAR γ in particular is a major mediator of adipose tissue biology and increasing the activity of PPAR γ via TZDs has traditionally been considered insulin sensitizing. Paradoxically, humans with a less active PPAR γ (Pro12Ala), also have improved glucose homeostasis and are metabolically protected against T2DM^{32,33}. Furthermore, given its adipogenic function, increasing PPAR γ activity has the side effect of weight gain³⁴. Based on our findings of reduced PPAR γ activity following both RYGB and VSG surgeries, TZDs may be mechanistically counterproductive to the beneficial effects of bariatric surgery.

The decreased adipose *PPAR γ* expression and activity post-surgery indicates an acute metabolic switch that favors the use of adipose tissue for energy dissipation rather than for energy storage. Supporting this concept, increased *PPAR δ* activity favors lipid utilization via increased fatty acid oxidation and energy uncoupling¹³. Indeed, in several mouse models exhibiting increased lipolysis in WAT, there is a concurrent increase in white adipose tissue *PPAR δ* and *UCP2* expression that drives fatty acid oxidation and thermogenesis^{35–37}. Furthermore, these models do not exhibit elevated serum free fatty acids or hepatic/skeletal muscle fatty acid oxidation demonstrating the important role that adipocyte fatty acid oxidation can play in overall energy metabolism and adiposity^{35–37}. Consistent with this, we observed a 15-fold increase in WAT fatty acid oxidation, increased lipolysis, and decreased serum fatty acids post-surgery. Fatty acid oxidation in WAT has been underappreciated since most lipolysis-liberated fatty acids are re-esterified or released under normal physiological conditions¹⁸. Fasting has been demonstrated to increase fatty acid oxidation in adipocytes by 2-fold¹⁸. Considering the surplus of triglycerides stored in adipose tissue of obese patients, a slight increase of the oxidation pathway over time might influence whole body energy balance. Further studies are required to understand the contribution of the increase in *PPAR δ* activity as a contributor to weight loss following bariatric surgery. Our study demonstrates a dramatic increase in adipose tissue FFA disposal through an oxidative pathway post-bariatric surgery, independently from SAT browning via *UCP1* expression. This not only sheds light on potential mechanisms of the surgery in adipose tissue, but also provides support for therapeutic targets for the medical management of diabetes. Indeed, a *PPAR δ* agonist has shown promise in clinical trials with regards to lipid metabolism³⁸. In fact, a study that randomized patients to a *PPAR δ* agonist or placebo found improvements in metabolic syndrome in *PPAR δ* recipients³⁸. While this study focused on the effects of *PPAR δ* agonism on skeletal muscle, our findings suggest that adipose tissue may play a role in this improvement as well. Further evaluation is needed to clarify the effect of *PPAR δ* agonism in SAT.

Decreased levels of FABP4 have been associated with improved insulin sensitivity in both humans and mice^{39–41}. A polymorphism at the promoter region of FABP4, which leads to a decrease of about 60% in expression, has been associated with a decreased risk of type 2 diabetes and cardiovascular disease⁴². In this study, we observed an average reduction of 40% and 20% of FABP4 protein levels after VSG and RYGB, respectively, independent of caloric restriction. Interestingly, FABP4 expression has been shown to decrease WAT fatty acid oxidation⁴³. Therefore, decreased FABP4 post-surgery is consistent with increased β -oxidation. The increased fatty acid oxidation could generate mitochondrial reactive oxygen species⁴⁴. However, Xu et al. as well as Steen et al. previously identified an inverse relationship between FABP4 and *UCP2*^{17,45,46}. Indeed, the expression of *UCP2*, a downstream target of *PPAR δ* , was increased over 7-fold in WAT after bariatric surgery. Notably, a polymorphism in the promoter region of *UCP2* that increases its expression has been linked to a reduced risk of obesity and diabetes in humans^{47–49}. This dramatic increase of *UCP2* would be predicted to reduce ROS levels. Supportive of this, mitochondrial protein cysteine oxidation was decreased after bariatric surgery. As such, bariatric surgery results in a decrease in oxidative stress in adipose tissue. Although the current study focused on early changes after bariatric surgery, there is evidence of persistence of this pathway

longitudinally. Affonso et al. reported increased *UCP2* expression in SAT of patients 6 months following RYGB and correlated this change with increased resting metabolic rate⁵⁰. Moreover, Simón et al. as well as Terra et al. found decreased serum FABP4 levels at 6 and 12 months following RYGB^{51,52}. Surprisingly, Engl and colleagues observed no change in serum FABP4 levels despite 25 kg weight loss at one year after laparoscopic gastric band placement, a purely dietary restrictive bariatric procedure. These studies in conjunction with our observations support a reduction in FABP4 that may be uniquely associated with bariatric surgery and not necessarily with caloric restriction alone⁵³. As such, the downregulation of FABP4 may potentially serve as a biomarker to predict longitudinal outcomes.

In summary, we have identified a novel alteration in the activity of PPAR γ and PPAR δ that leads to the downregulation of FABP4 and upregulation of UCP2 in subcutaneous adipose tissue after bariatric surgery. The altered expression of these molecular regulators leads to a metabolic shift in adipose tissue involving an increase of white adipose tissue lipolysis, lipid utilization through adipose fatty acid oxidation, and the control of ROS via UCP2 activity that in sum may contribute to the benefits of bariatric surgery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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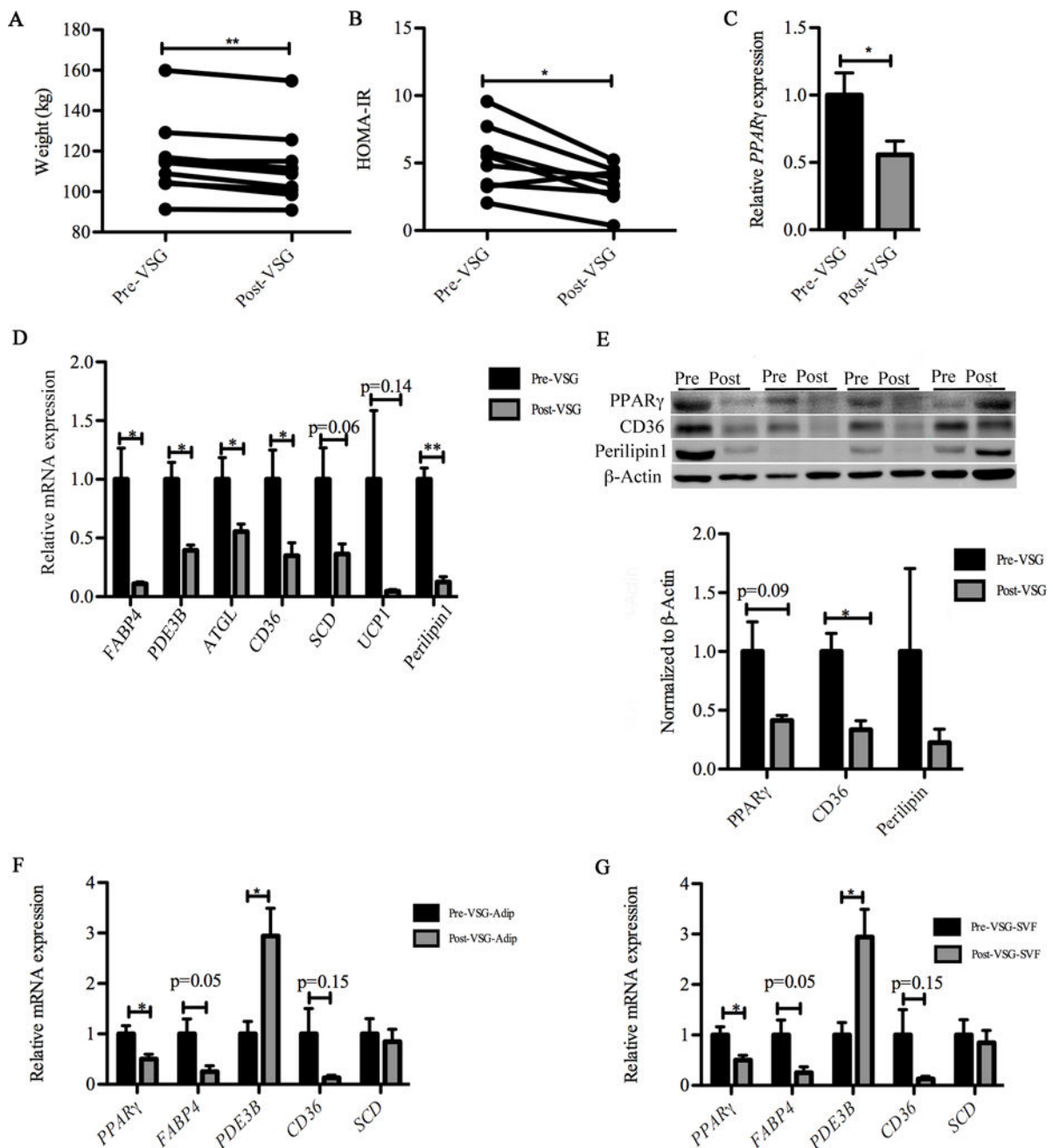


Figure 1. VSG downregulates *PPARγ* and *PPARγ* target expression in adipose tissue. **(A)** Body weight of patients pre- and one-week post-VSG ($n=20$); **(B)** HOMA-IR of patients pre- and one-week post-VSG; **(C)** Relative *PPARγ* mRNA levels in SAT pre- and one-week post-VSG ($n=12$); **(D)** Relative mRNA levels of *FABP4* (fatty acid binding protein 4), *PDE3B* (phosphodiesterase 3B), *ATGL* (adipose triglyceride lipase), *CD36* (cluster of differentiation 36), *SCD* (stearoyl-CoA desaturase 1), *UCP1* (uncoupling protein 1) in SAT pre- and one-week post-VSG ($n=12$); **(E)** Protein levels of *PPARγ*, *CD36*, and *Perilipin 1* measured by western blot in adipose tissue of patients pre- and one-week post-VSG ($n=4$); **(F)** mRNA

levels of *PPAR* γ , *FABP4*, *PDE3B*, *CD36*, and *SCD* in the primary adipocyte fraction of SAT of patients pre- and one-week post-VSG ($n=8$); (G) mRNA levels of *PPAR* γ , *FABP4*, *PDE3B*, *CD36*, and *SCD1* in stromal vascular fraction of SAT of patients pre- and one-week post-VSG ($n=8$). * denotes $p<0.05$; ** denotes $p<0.01$.

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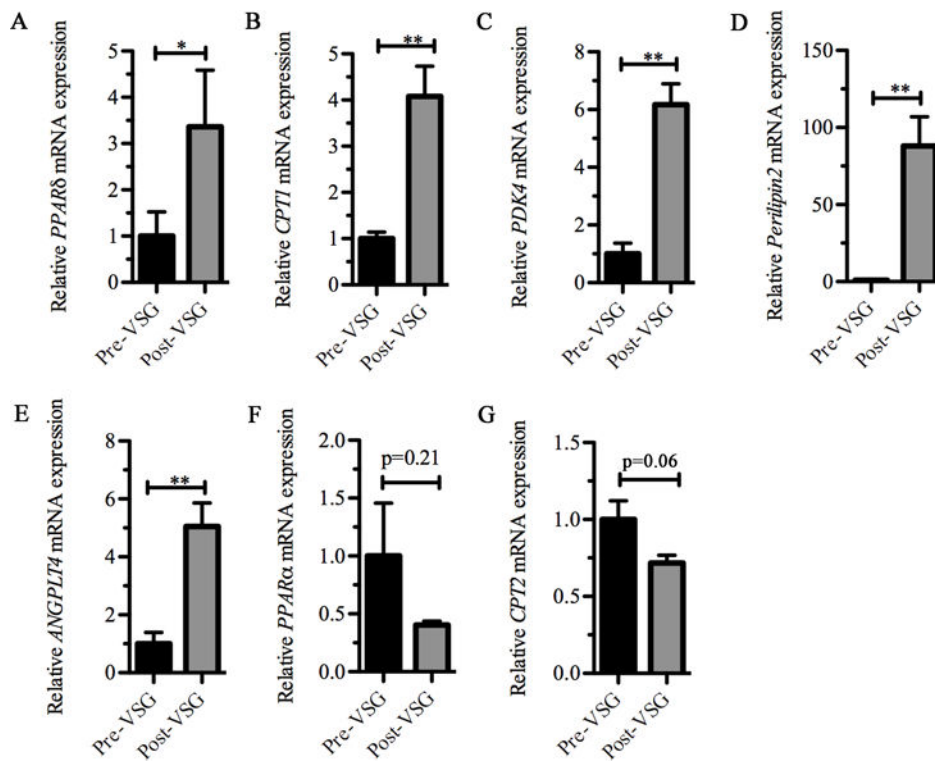


Figure 2. VSG upregulates *PPARδ* and *PPARδ* target expression in adipose tissue. Relative mRNA levels in SAT of subjects pre- and one week post-VSG ($n=12$) of (A) *PPARδ*; (B) *CPT1*; (C) *PDK4*; (D) *Perilipin2*; (E) *ANGPLT4*; (F) *PPARα*; (G) *CPT2*. * denotes $p < 0.05$; ** denotes $p < 0.01$.

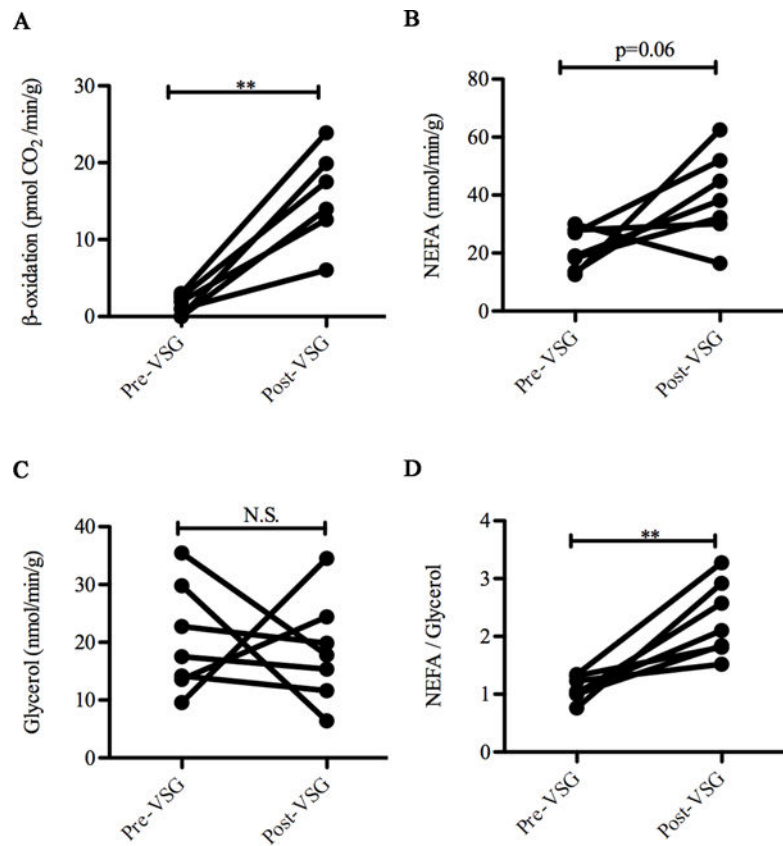


Figure 3. VSG increases adipose tissue fatty acid oxidation and lipolysis. **(A)** β -oxidation measured in SAT pre- and post-VSG ($n=6$); **(B)** Secreted fatty acids measured in SAT pre- and post-VSG ($n=7$); **(C)** Secreted glycerol measured in SAT pre- and post-VSG ($n=7$); **(D)** Ratio of secreted fatty acids to glycerol from SAT pre- and post-VSG ($n=7$). ** denotes $p<0.01$.

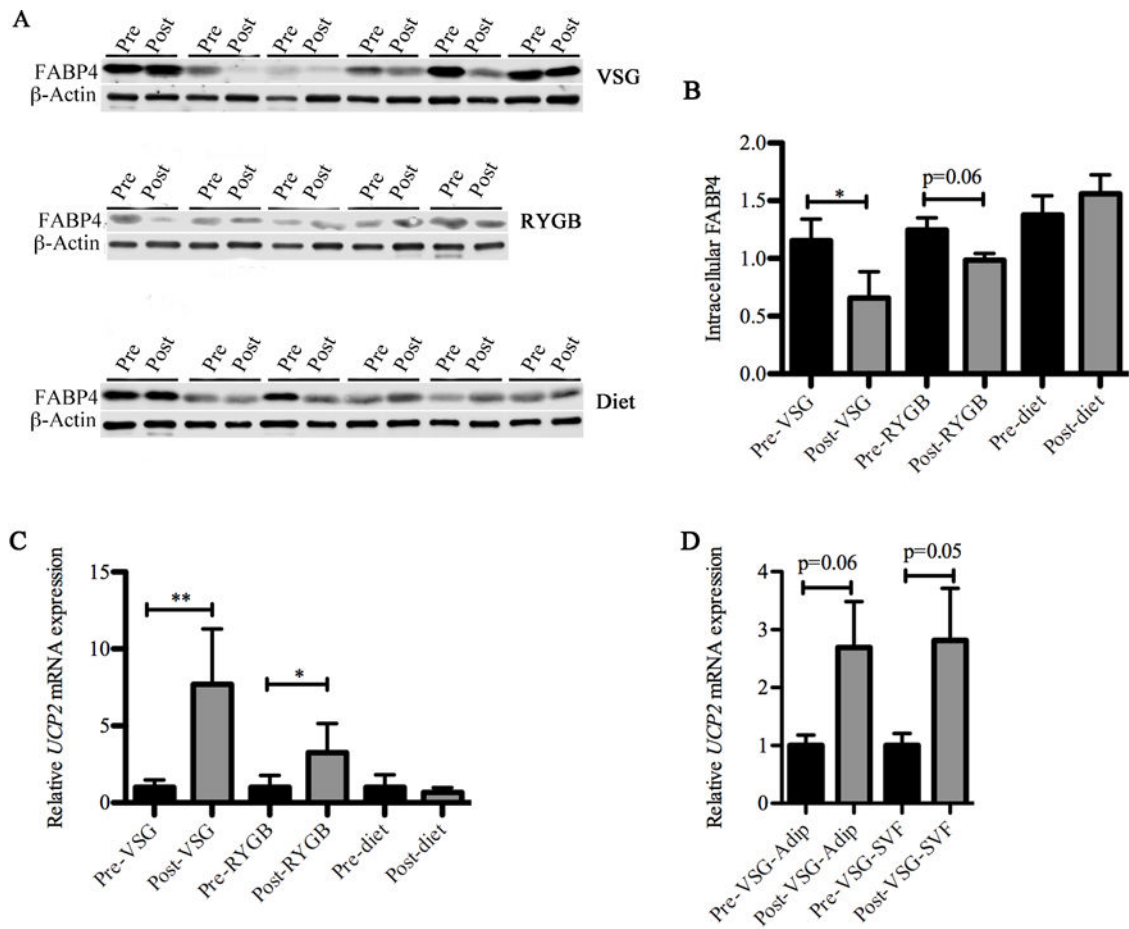


Figure 4.

The downregulation of FABP4 and upregulation of *UCP2* occurs in SAT of patients following bariatric surgery, but not after caloric restriction. (A–B) SAT FABP4 protein levels as determined by western blot in patients pre- and post-VSG ($n=6$), RYGB ($n=9$), and caloric restriction ($n=10$). (C) SAT expression of *UCP2* mRNA in patients pre- and post-VSG ($n=12$), RYGB ($n=13$), and caloric restriction ($n=14$), as determined by qRT-PCR; (D) mRNA levels of *UCP2* in the primary adipocyte fraction (ADIP) and stromal vascular fraction (SVF) in SAT of patients pre- and 7 days post-VSG ($n=8$). * denotes $p<0.05$; ** denotes $p<0.01$.

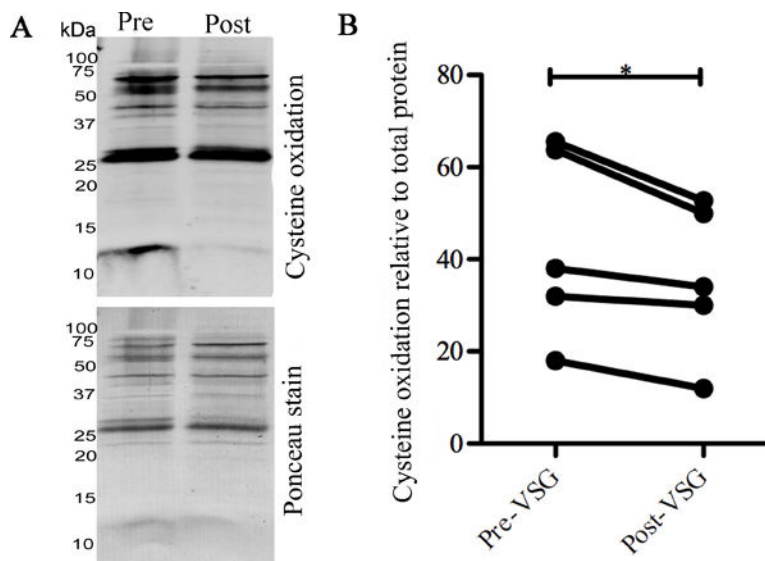


Figure 5. Cysteine oxidation in SAT was decreased 7 days following VSG; **(A)** SAT cysteine oxidation levels in patients pre- and post-VSG ($n=5$); **(B)** Quantification of cysteine oxidation levels in SAT of patients pre- and post-VSG. Total intensity of cysteine oxidation was normalized to total protein for each sample using Ponceau S staining solution. * denotes $p<0.05$.