

Original article

Differential acute and chronic responses in insulin action in cultured myotubes following from nondiabetic severely obese humans following gastric bypass surgery

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Abstract

Background: Roux-en-Y gastric bypass (RYGB) surgery has been shown to induce positive metabolic adaptations for individuals with severe obesity (body mass index ≥ 40 kg/m²), including improved peripheral insulin action. Although a major site of insulin action, the time course changes in skeletal muscle glucose metabolism following RYGB is unclear.

Objectives: To investigate the acute and chronic effects of RYGB surgery on insulin-stimulated glucose metabolism in cultured human primary myotubes derived from nondiabetic severely obese humans.

Setting: East Carolina University Bariatric Surgery Center and East Carolina Diabetes and Obesity Institute.

Methods: Primary human skeletal muscle cells were isolated from biopsies obtained from 8 women with severe obesity before, 1 month, and 7 months following RYGB surgery. Glucose metabolism, glycogen content, and insulin signal transduction were determined in differentiated myotubes.

Results: Insulin-stimulated glycogen synthesis and glucose oxidation increased in human myotubes derived from patients with severe obesity at both 1 and 7 months post-RYGB. However, there were no alterations indicative of enhanced insulin signal transduction. At 1 month post-RYGB, muscle glycogen levels were lower (-23%) and phosphorylation of acetyl CoA carboxylase 2 (ACC2) was elevated (+16%); both returned to presurgery levels at 7 months after RYGB in myotubes derived from patients. At 7 months post-RYGB, there was an increase in peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1 α) protein content (+54%).

Conclusion: These data indicate that insulin action intrinsically improves in cultured human primary myotubes derived from nondiabetic severely obese patients following RYGB surgery; however, the cellular alterations involved appear to consist of distinct acute and chronic components. (Surg Obes Relat Dis 2017;13:1853–1862.) © 2017 American Society for Metabolic and Bariatric Surgery. All rights reserved.

Keywords:

Metabolic surgery; Skeletal muscle; Glycogen synthesis; Insulin signaling; PGC1 α

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Introduction

Skeletal muscle comprises ~40% of total body mass and accounts for 70%–90% of glucose disposal under insulin-stimulated conditions [1]. Unfortunately, individuals with severe obesity (body mass index ≥ 40 kg/m²) exhibit defects in skeletal muscle metabolism such as reduced rates of insulin-stimulated glucose uptake, glycogen synthesis, glucose oxidation, and impaired insulin signal transduction [2–4]. However, Roux-en-Y gastric bypass (RYGB) surgery leads to improved metabolic health, as indicated by enhanced insulin action and the reversion of type 2 diabetes [5]. Although the clinical efficacy of RYGB is well-documented, the sequencing of the resolution of the obesity-related deficiencies in glucose metabolism in skeletal muscle and the underlying cellular alterations involved are not clearly evident. For example, improved glycemic control and reversal of type 2 diabetes are evident almost immediately (~1 wk) after RYGB [6]. Conversely, peripheral insulin action only improves ~3 months or longer after RYGB, and seems to be connected with weight loss [3,7]. These findings suggest that distinct alterations are involved in the acute and chronic periods after RYGB, which ultimately contribute to enhanced glucose metabolism.

Primary human skeletal muscle cells have been used to study muscle-specific adaptations in substrate metabolism. Interestingly, despite proliferation and differentiation in an environment void of *in vivo* factors (i.e., hormones, blood lipids, neural input, etc.), our group has reported that defects in metabolism evident *in vivo* are retained in myotubes from individuals with severe obesity [8,9]. Recently, Nascimento et al. reported an increase in basal glycogen synthesis in primary human muscle cells at 6 months after gastric bypass surgery, suggesting that RYGB can alleviate intrinsic defects in carbohydrate metabolism evident with severe obesity [10]. However, such data do not provide a temporal indication of when after RYGB such changes occur (i.e., acutely or chronically). The purpose of the present study was to use the primary human skeletal muscle cell culture model to examine whether RYGB alters insulin action in skeletal muscle and, if so, the time course involved. Our findings reveal that RYGB remodels skeletal muscle in a manner that improves muscle-specific insulin action as early as 1 month postsurgery, which is retained at 7 months postsurgery, and that different cellular alterations seem to contribute to improving insulin action in the acute versus chronic periods after the surgery.

Methods

Subject recruitment

Female patients with severe obesity (body mass index [BMI] > 40 kg/m²) undergoing RYGB (details of surgery can be found in [3]) ($n = 8$) who were between the ages of 30 to 60 were recruited from the East Carolina University

bariatric surgery center. All patients were sedentary and were not involved in an exercise program either before or after the intervention. Participants were excluded if they had a diagnosis of diabetes, heart diseases, or a history of cancer. Participants were also excluded if they were being treated with medications that would alter glucose metabolism. All procedures were approved by the East Carolina University Institutional Review Board and informed consent was obtained.

Primary muscle cell cultures

Skeletal muscle biopsies were obtained from the vastus lateralis before and at 1 month and 7 months following RYGB using the percutaneous needle biopsy technique; we were not able to obtain samples in 2 of the patients at the 1-month timepoint because of medical issues with these patients after surgery. A fasting venous blood sample was obtained for analysis of plasma glucose and insulin. Satellite cells were isolated from the muscle biopsies and cultured into myoblasts as described previously [11]. Myoblasts were subcultured onto type I collagen-coated plates, and upon reaching 80%–90% confluence, were differentiated to myotubes by switching from growth to differentiation media (Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific, Waltham, MA) supplemented with 2% horse serum (Thermo Fisher Scientific), .3% bovine serum albumin (Sigma Aldrich, St. Louis, MO), .05% fetuin (Sigma Aldrich), and 100 mg/mL penicillin/streptomycin). Experiments were performed on day 7 of differentiation.

Insulin-stimulated glycogen synthesis

The rate of glycogen synthesis was determined as previously described [12]. Briefly, after 3 hours of serum starvation, myotubes were treated with media containing D-[U-¹⁴C] glucose (PerkinElmer, Waltham, MA) (1 μ Ci/mL, 5.0 mM glucose) in the presence or absence of 100 nM insulin for 2 hours at 37°C. After incubation, cells were washed with ice-cold phosphate-buffered saline and solubilized in .05% sodium dodecyl sulfate (SDS). An aliquot was transferred to a 2 mL tube containing carrier glycogen (2 mg) and heated for 1 hour at 100°C. The remaining lysate was used to assess protein concentration (bicinchoninic acid assay, Pierce Biotechnology, Rockford, IL). Glycogen was precipitated by the addition of 100% ethanol and overnight incubation at 4°C. Glycogen pellets were centrifuged (11,100 $\times g$ for 15 minutes at 4°C), washed once with 70% ethanol, and resuspended in dH₂O. Incorporation of radioactive glucose into glycogen was determined with liquid scintillation.

Insulin-stimulated glucose oxidation

Following 3 hours of serum starvation, cells were incubated in a sealed plate with reaction media containing D-[U-¹⁴C] glucose (American Radiolabeled Chemicals, St. Louis, MO) (1 μ Ci/mL, 5.0 mM glucose) in the presence

or absence of 100 nM insulin for 2 hours at 37°C. Following incubation, reaction media was transferred to a modified 48-well microtiter plate with fabricated grooves between 2 adjoining wells to allow for acid-driven ^{14}C CO_2 from media to be trapped by 1 M NaOH [13]. Incorporation of radioactive glucose into CO_2 was determined with liquid scintillation. Cells were washed with ice-cold phosphate-buffered saline and solubilized in .05% SDS to assess protein concentration.

Lipid oxidation

Basal rates of in vitro lipid oxidation in primary myotubes derived from RYGB patients was determined as previously [14]. Briefly, myotubes were incubated at 37°C in sealed 12-well collagen I-coated plates containing differentiation media supplemented with 12.5 mM HEPES, .5% BSA, 1 mM carnitine, 200 μM sodium oleate, and 1 $\mu\text{Ci}/\text{mL}$ [$1\text{-}^{14}\text{C}$] oleate (PerkinElmer) for 3 hours. After the incubation period, the medium was transferred to new plates and assayed for the collection of $^{14}\text{CO}_2$ production, which was quantified via liquid scintillation counting.

Immunoblot analyses

Myotubes were serum-starved for 3 hours, followed by treatment with 100 nM of insulin for 10 minutes. Cells were harvested in ice-cold lysis buffer containing 50 mM HEPES, 12 mM sodium pyrophosphate, 100 mM sodium fluoride, 100 mM ethylenediaminetetraacetic acid, 10 mM sodium orthovanate, 1% Triton X-100, and protease and phosphatase inhibitor cocktails (Sigma-Aldrich, St. Louis, MO). Lysates were sonicated for 5 seconds, rotated for ~ 1 hour at 4°C, and centrifuged at 12,000 revolutions per minute for 15 minutes at 4°C.

Equal amounts of protein were subjected to SDS-polyacrylamide gel electrophoresis, after which proteins were transferred to polyvinylidene difluoride membranes. Membranes were incubated with the following primary antibodies: phosphorylation of acetyl CoA carboxylase 2 (ACC2) (Ser79) (Cell Signaling, Beverly, MA), total ACC2 (Cell Signaling); phospho-Akt (Ser473 and Thr308) (Cell Signaling), total Akt (Cell Signaling); phospho-AMP-activated protein kinase alpha (AMPK α) (Thr172) (Cell Signaling), total AMPK α (Cell Signaling); phospho-Akt-substrate at 160 kDa (AS160) (Thr642) (Abcam, Cambridge, MA), total AS160 (Millipore, Billerica, MA), beta-actin (LI-COR Biosciences, Lincoln, NE), GLUT4 (Millipore), phosphoglycogen synthase kinase 3 α (GSK-3 α) (Ser21) (Cell Signaling), total GSK-3 α (Cell Signaling), hexokinase II (Santa Cruz Biotechnology, Dallas, TX), and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1 α) (Abcam). Membranes were probed with IRDye secondary antibodies (LI-COR Biosciences)

and band intensities quantified using Odyssey software (LI-COR Biosciences).

Muscle glycogen content

Myotubes were serum-starved for 24 hours and lysates were harvested as described in the section “Immunoblot analyses.” Muscle glycogen content in cell lysates was examined by methods previously described [15]. Briefly, 2N HCl was added to lysates and hydrolyzed for 2 hours at 95°C. The samples were neutralized with 2N NaOH and 1 M Tris-HCl (pH 7.4). Glycogen content was measured using a hexokinase reagent (Thermo Fisher Scientific), and data are expressed as glucose content per μg protein.

Statistical analysis

Comparisons were made between basal and insulin-stimulated conditions from presurgery (pre-), 1 month, and 7 months postsurgery using 2-way repeated measures analysis of variance (condition \times time) followed by Tukey post hoc analyses where appropriate. Comparisons of insulin action (% or fold change), as well as body mass and blood chemistry measures between pre- and 1 month, as well as pre- and 7 months, were performed by one-way analysis of variance and Student's *t* test. Data are expressed as means \pm standard error of the mean. Significance was set as $P \leq .05$.

Results

Subject characteristics are presented in Table 1. Patients lost weight at 1 month (~ 20 kg, $P < .05$), with a further reduction of ~ 20 kg ($P < .05$) at 7 months following surgery. Fasting insulin and glucose levels were significantly lower ($P < .05$) at 7 months compared to 1 month pre- and postsurgery.

Insulin-stimulated glucose metabolism

No changes with RYGB were evident at any timepoint after surgery in basal glycogen synthesis and glucose oxidation (Fig. 1A and C). Although there was no insulin response in glycogen synthesis before surgery, insulin-stimulated glycogen synthesis rates were significantly higher than the basal condition in myotubes from patients

Table 1
Subject characteristics

	Presurgery	1 month	7 months
Body mass (kg)	139.3 \pm 6.7	120.3 \pm 9.1*	100.4 \pm 5.3*†
BMI (kg/m ²)	50.2 \pm 2.0	43.2 \pm 2.8*	35.7 \pm 2.2*†
Glucose (mmol/l)	5.1 \pm .1	4.8 \pm .2	4.4 \pm .2*†
Insulin (pmol/l)	95 \pm 8	76 \pm 21	51 \pm 10*

BMI = body mass index.

* $P < .05$ versus presurgery.

† $P < .05$ versus 1 month.

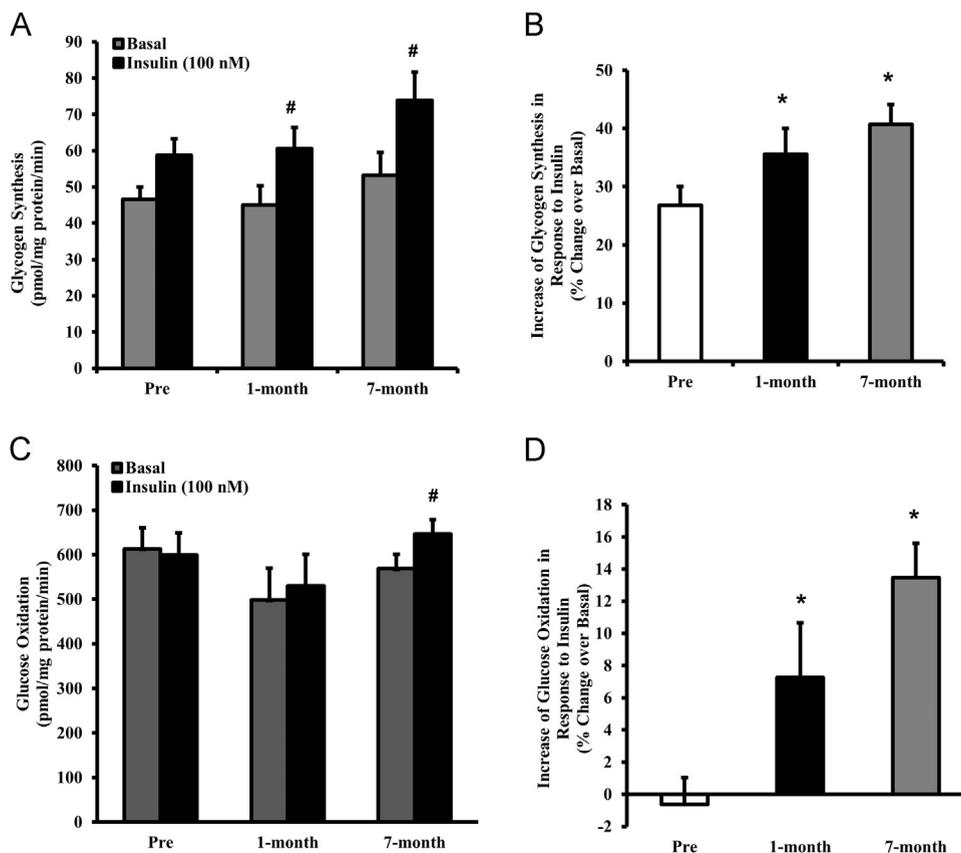


Fig. 1. Glucose metabolism in primary human skeletal muscle myotubes derived from patients presurgery (pre-), 1 month, and 7 months after Roux-en-Y gastric bypass (RYGB) surgery. (A) Glucose incorporation into glycogen under basal and insulin stimulated conditions. (B) Percentage increase in insulin-stimulated glycogen synthesis over the basal condition. (C) Glucose oxidation under basal and insulin-stimulated conditions. (D) Percentage increase in insulin-stimulated glucose oxidation over the basal condition. Data are presented as mean \pm standard error of the mean. n = 7, pre- and 7 months post-RYGB; n = 5, 1 month post-RYGB. # $P < .05$ versus basal at respective timepoint, * $P < .05$ versus pre-RYGB.

at 1 month and 7 months following RYGB surgery (Fig. 1A) ($P < .05$). To assess insulin action in myotubes, the relative increase of glycogen synthesis over basal after insulin stimulation (percentage change) was analyzed. The relative increase of insulin-stimulated glycogen synthesis over basal was significantly improved ($P < .05$) at both the 1-month ($\sim 36\%$) and 7-month ($\sim 41\%$) timepoints compared to before surgery (Fig. 1B). Insulin-stimulated glucose oxidation was significantly higher only at 7 months ($P < .05$), but not 1-month postsurgery compared with basal state (Fig. 1C). However, the relative increase in glucose oxidation in response to insulin stimulation over the basal condition (percentage change), which eliminates subject to subject variation in glucose oxidation rates, significantly improved at both 1 month ($\sim 7.3\%$) and 7 months (15.4%) following RYGB surgery compared with the presurgery state (Fig. 1D) ($P < .05$).

Insulin signal transduction

Insulin-stimulated phosphorylation of Akt (Ser473 and Thr308) (Fig. 2A and B), GSK3 α (Ser21) (Fig. 2C), and

AS160 (Thr642) (Fig. 2D) were significantly elevated in myotubes derived from patients compared with the basal state at all timepoints ($P < .05$, main effect of insulin). However, insulin signal transduction was not potentiated after the surgery. There were no changes in total Akt, GSK3 α , or AS160 protein content with RYGB.

Glycogen content, ACC2, and AMPK α

Because of possible interactions with insulin action, glycogen content and the phosphorylation status of AMPK α and ACC2 were determined. At 1 month, there was a $\sim 23\%$ decrease in glycogen content ($P < .05$); however, there was no difference in glycogen content in cultured myotubes derived from human skeletal muscle cells obtained from patients 7 months postsurgery in comparison to presurgery (Fig. 3). Although basal phospho-AMPK α (Thr172) was not significantly altered following surgery (Fig. 4A), the basal phosphorylation state of ACC2 (Ser79) increased in myotubes derived from patients at 1 month following RYGB (Fig. 4B) ($P < .05$).

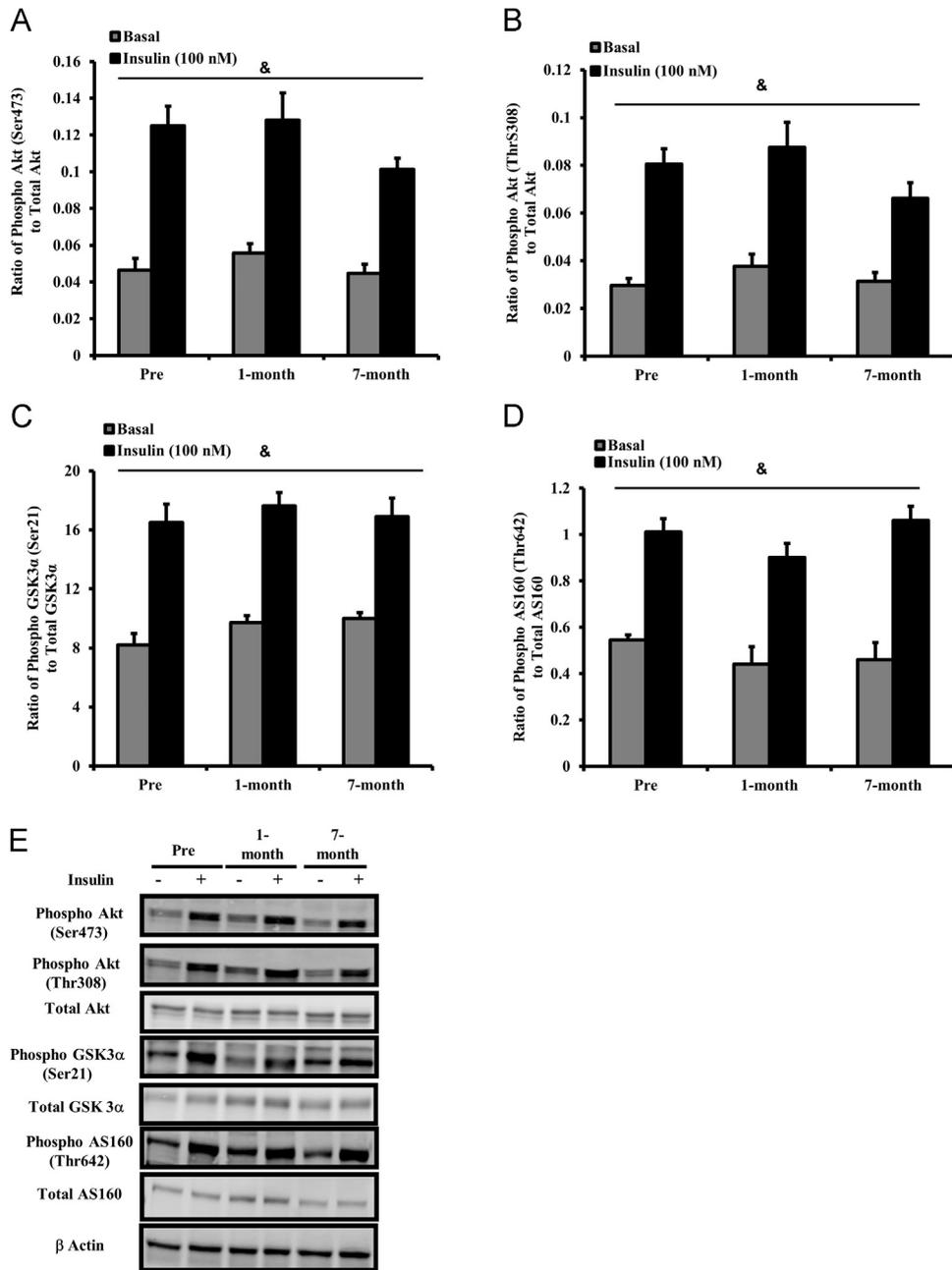


Fig. 2. Insulin signaling in primary human skeletal muscle myotubes derived from patients presurgery (pre-), 1 month, and 7 months after Roux-en-Y gastric bypass (RYGB) surgery. Ratios of phosphorylated to total expression of the protein are presented. Quantification of (A) Akt phosphorylation on site Ser473, (B) Akt phosphorylation on site Thr308, (C) glycogen synthase kinase 3 alpha phosphorylation on site Ser21, (D) AS160 phosphorylation on site Thr642, and (E) representative immunoblots for the quantifications. Beta-actin was used to verify equal loading. Data are presented as mean \pm standard error of the mean. n = 8, pre- and 7 months post-RYGB; n = 6, 1 month post-RYGB. &P < .05 main effect of insulin. GSK3 α = glycogen synthase kinase 3 alpha; AS160 = Akt substrate of 160 kDa.

PGC1 α and downstream targets

Although glycogen content appeared to be linked with changes in insulin action 1 month postsurgery, this did not explain the improvements in insulin action at 7 months after the intervention. We thus examined whether PGC1 α , a major transcriptional coactivator involved in substrate utilization, was altered following surgery. As shown in

Fig. 5A, although there was no change at the 1-month timepoint, PGC α protein content increased by \sim 54% in cultured myotubes derived from surgery patients at 7 months following RYGB ($P < .05$). To examine potential downstream targets of PGC1 α , we examined GLUT4 and hexokinase protein content. Despite an increase in PGC1 α , total GLUT4 was unaltered following RYGB surgery (Fig. 5B). However, hexokinase, which has been

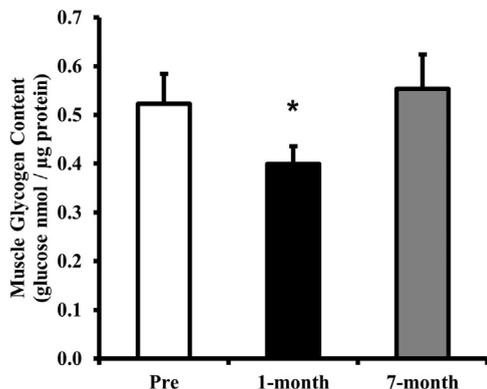


Fig. 3. Muscle glycogen content in myotubes derived from patients presurgery (pre-), 1 month, and 7 months after surgery. Data are presented as mean ± standard error of the mean. n = 8, pre- and 7 months post-Roux-en-Y gastric bypass; n = 6, 1 month post-Roux-en-Y gastric bypass. *P < .05 versus pre. RYGB: Roux-en-Y gastric bypass.

shown by previous groups to be altered with PGC1α overexpression [16], tended to increase in myotubes derived from surgery patients at 7 months following RYGB surgery (Fig. 5C) (P = .09). Finally, lipid oxidation (Fig. 5E) was unaltered following RYGB surgery.

Discussion

RYGB surgery induces numerous metabolic benefits in individuals with severe obesity, including improved whole-body insulin action at 3–12 months postintervention [3,5,7]. Despite the role of skeletal muscle in glycemic control, there are few studies that have examined glucose metabolism in skeletal muscle following RYGB. Studies from Friedman et al. [17] and Bikman [3] have observed improved glucose transport in muscles derived from RYGB patients ~1 year postsurgery. However, the acute alterations in skeletal muscle metabolism following RYGB (weeks/months) are unclear. Using human primary muscle cells derived from RYGB patients at different timepoints postsurgery, we observed that insulin-stimulated glucose metabolism (i.e., glycogen synthesis and glucose oxidation) improved at 1 month and remained elevated at 7 months following surgery (Fig. 1). These novel data suggest that relatively early (i.e., 1 month) after RYGB, insulin-resistant skeletal muscle exhibits a transition to a more insulin-sensitive tissue.

Recently, Albers et al. examined key signaling proteins associated with skeletal muscle glucose metabolism under insulin-stimulated conditions (hyperinsulinemic-euglycemic clamp) [7]. In concordance with whole-body insulin

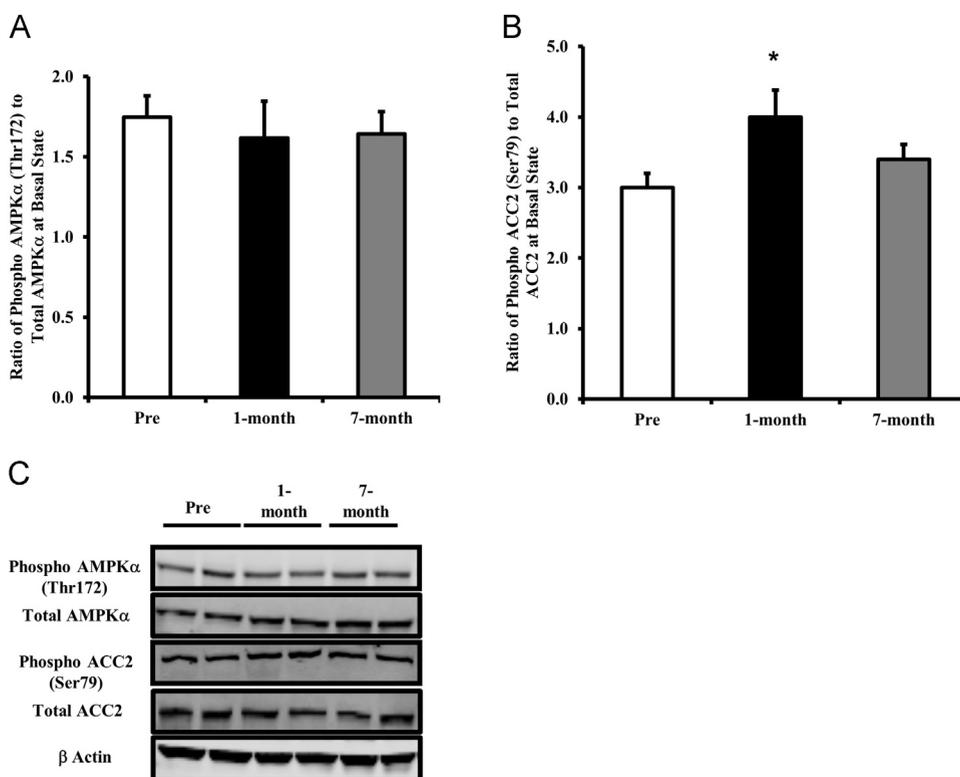


Fig. 4. Basal phosphorylation of AMP-activated protein kinase alpha on site Thr172 (A) and acetyl CoA carboxylase 2 on site Ser79 (B) in primary human skeletal muscle myotubes derived from patients presurgery (pre), 1 month, and 7 months after surgery. Ratios of phosphorylation to total expression of the protein are presented. (C) Representative immunoblots for the quantifications. Beta-actin was used to verify equal loading. Data are presented as mean ± standard error of the mean. n = 8, pre- and 7 months post-Roux-en-Y gastric bypass; n = 6, 1 month post-Roux-en-Y gastric bypass. *P < .05 versus pre. RYGB: Roux-en-Y gastric bypass; AMPKα = AMP-activated protein kinase alpha; ACC2: acetyl-CoA carboxylase 2.

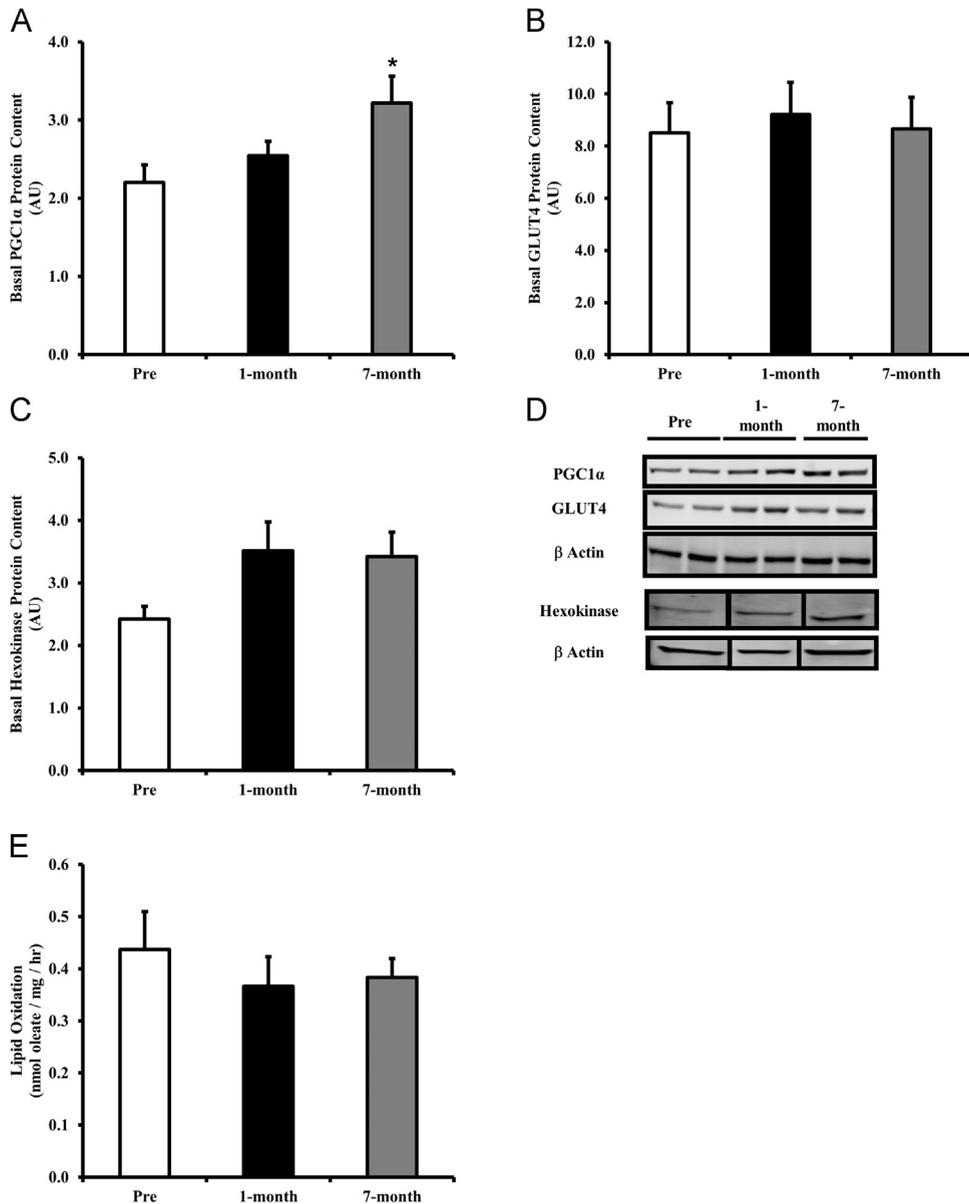


Fig. 5. Peroxisome proliferator-activated receptor gamma coactivator 1 alpha protein content (A) and downstream targets of peroxisome proliferator-activated receptor gamma coactivator 1 alpha, GLUT4 (B), and hexokinase (C) at basal state in primary human skeletal muscle myotubes derived from patients presurgery (pre-), 1 month, and 7 months after surgery. (D) Representative immunoblots for the quantifications. Beta-actin was used to verify equal loading. (E) In vitro rates of lipid oxidation following Roux-en-Y gastric bypass surgery. Data are presented as mean \pm standard error of the mean. n = 8, pre- and 7 months post-RYGB; n = 6, 1 month post-RYGB. * $P < .05$ versus pre. Hexokinase (C) approached being significantly different from pre ($P = 0.09$). RYGB = Roux-en-Y gastric bypass; PGC1 α = peroxisome proliferator-activated receptor gamma coactivator 1 alpha.

sensitivity, they reported no changes in the insulin-signaling cascade either 1 week or 3 months following RYGB. These results are in contrast to the observations in the present study; however, the discrepancy may be a result of systemic factors that may alter skeletal muscle insulin sensitivity. Specifically, de Weijer et al. [18] observed that 2 weeks following RYGB surgery, there was a significant increase in lipolysis resulting in an increase in plasma free fatty acids, which may have masked muscle-specific changes in the acute period after the intervention, as a rise in plasma free fatty acids has been shown to induce insulin resistance [19].

To eliminate the influence of these potentially confounding factors and specifically focus on metabolic adaptations in skeletal muscle, we used human primary muscle cells derived from patients pre-, 1 month, and 7 months after RYGB. The results of the present study suggest that skeletal muscle-specific changes in insulin sensitivity may occur rapidly following RYGB surgery, but are masked by whole-body measures because of systemic factors. Thus, future examination should explore the systemic factors that impede whole-body insulin sensitivity improvements immediately following RYGB surgery.

Although the metabolic phenotype of primary skeletal muscle myotubes has been examined in relation to obesity and type 2 diabetes, few studies have examined whether acute interventions that improve whole-body metabolism in patients with metabolic disease translate to the level of the muscle cell raised in culture. Bourlier et al. observed that 8 weeks of exercise training improved *in vitro* glucose metabolism in primary muscle cell myotubes derived from individuals with severe obesity [20]. In addition, Nascimento et al. have shown improvements in basal glycogen synthesis in skeletal muscle cells derived from RYGB patients 6 months following surgery [10]. The present study, which showed improved insulin-stimulated glucose metabolism, expands on the growing knowledge that acute interventions (i.e., exercise training and RYGB surgery/weight loss) can remodel the metabolic characteristics of human skeletal muscle. More interestingly, glucose metabolism (both glycogen synthesis and glucose oxidation) were elevated to a similar extent in response to insulin stimulation and virtually no difference in myotubes derived from severely obese individuals 7 months following surgery compared with lean controls (data not shown), suggesting RYGB surgery may correct the defective metabolism of skeletal muscle from severely obese individuals at the muscle cell level. This is consistent with the whole-body correction of insulin-stimulated glucose metabolism at a similar timepoint following RYGB surgery [21,22]. Although further mechanistic experiments are needed to address the causation, such a finding provides evidence for future interventions to potentially target skeletal muscle cells to improve insulin action.

Insulin stimulates glucose metabolism by binding to its respective receptor, which initiates a signaling cascade involving an increase in Akt activity. Akt activation provides an upstream signal resulting in increased glucose entry into the cell, as well as enhanced activity of glycogen synthase, the major regulator of glycogen synthesis [23]. In the present study, we did not observe changes in insulin-induced phosphorylation of Akt (Fig. 2A and B) or its downstream target associated with glycogen synthesis, GSK3 α (Fig. 2C). This lack of change is supportive of the *in vivo* findings of Albers et al., who reported that insulin-stimulated Akt activity was not potentiated in skeletal muscle at either 1 week or 3 months following gastric bypass surgery [7]. Furthermore, the role of insulin-stimulated phosphorylation of GSK3 α on skeletal muscle glycogen synthesis has been questioned, as Bouskila et al. have shown that mice with mutated GSK3, which could not be phosphorylated following insulin stimulation, have normal insulin-stimulated glycogen synthesis [24].

As insulin signaling appeared to be unchanged following surgery, the cellular alterations linked with enhanced insulin action were unclear. Along with covalent modifications (i.e., phosphorylation of GSK3 α), intracellular glucose metabolism is also altered allosterically by substrate levels.

In particular, glycogen synthesis is controlled by either an increase in glucose-6-phosphate and/or reduction in glycogen levels. At 1-month post-RYGB, in conjunction with enhanced insulin action (Fig. 1), muscle glycogen levels were significantly reduced in the primary myotubes (Fig. 3). Similarly, glycogen levels also greatly diminish during intense exercise [25], and a relationship between reduced muscle glycogen levels and enhanced insulin action post-exercise has been suggested [26]. The present data suggests that, at 1-month RYGB, the muscle cells may have reduced muscle glycogen concentrations, which in-turn aids in facilitating glucose entry and storage under insulin-stimulated conditions.

Beyond the allosteric effects on glycogen synthesis, lower muscle glycogen levels are also implicated in activating key metabolic-sensitive signaling pathways that aid in improved substrate metabolism. Wojtaszewski et al. reported that lowered glycogen levels enhance the phosphorylation status of ACC2 [27]. Although mostly known as a key regulator of lipid metabolism [28], recent evidence suggests that ACC2 may also be involved in glucose metabolism. In transgenic mice with a mutation in the phosphorylation site of ACC2, O'Neill et al. observed impaired whole-body insulin sensitivity, which was attributed to reduced skeletal muscle glucose uptake [29]. In conjunction with these studies, we observed an increase in the phosphorylation status of ACC2 (Ser79) in the basal state 1 month following RYGB surgery (Fig. 4). Collectively, these data suggest that, at 1 month post-RYGB, the improvements in insulin action in skeletal muscle are potentially linked with lower muscle glycogen levels, which, in turn, increase ACC2 phosphorylation.

Interestingly, at 7 months postsurgery, glycogen content returned to presurgery levels (Fig. 3), which suggests that another factor was involved with enhanced insulin-stimulated glucose metabolism in the more chronic period after RYGB. PGC1 α , a transcriptional coactivator, is a key regulator of substrate utilization and mitochondrial function in skeletal muscle [30]. A physiologic increase in PGC1 α protein content (20%–150%), similar to what we observed at 7 months after RYGB (Fig. 5A), is associated with enhanced glucose utilization. As PGC1 α is primarily associated with mitochondrial biogenesis and oxidative capacity and increased expression of PGC1 α has been shown to enhance mitochondrial oxidative capacity [31] and insulin sensitivity [32], we measured lipid oxidation and mitochondrial content (citrate synthase protein content, data not shown) in myotubes derived from RYGB patients. However, we did not find any significant changes in myotubes derived from severely obese patients following RYGB surgery, which is in line with previous research from our group [33]. We next investigated other downstream targets of PGC1 α that are associated with glucose metabolism, including GLUT4 and hexokinase. Although we did not observe an increase in GLUT4 protein content

postsurgery (Fig. 5B), hexokinase tended to increase 7 months following surgery (Fig. 5C). Benton et al. reported that modest overexpression of PGC1 α (~25%) in rat skeletal muscle improved insulin-stimulated phosphorylation of AS160 [34]. However, we did not observe a robust increase in insulin-stimulated AS160 phosphorylation at 7 months postsurgery (Fig. 2D). Thus, our findings suggest that the improvement in insulin action at 7 months post-RYGB may be the result of a yet-unidentified alteration that is linked with an increase in PGC1 α protein content.

In primary human skeletal muscle cell cultures, the expressed phenotype is likely the product of genetic and/or epigenetic influences. Epigenetic modifications are responsive to environmental cues, including conditions of nutrient excess or deprivation, and can occur rapidly following a change in the nutrient state. For example, Jacobsen et al. indicated that high fat feeding for only 5 days altered the epigenetic profile of human skeletal muscle [35]. At 1 month after RYGB, patients are essentially in a catabolic state because of the reduction in energy intake. It is possible that this change in the nutrient environment elicits metabolic changes at the cellular level, such as a reduction in glycogen content in muscle cells, possibly via epigenetic mechanisms. Similarly, changes in PGC1 α protein content 7 months following RYGB surgery may be associated with epigenetic modifications, as Barres et al. observed that promoter methylation of PGC1 α in skeletal muscle biopsies was reduced 6 months following gastric bypass surgery, which led to an increase in PGC1 α messenger RNA expression [36].

Whereas our results provide novel insight into the metabolic adaptations in myotubes derived from RYGB patients, we acknowledge the limitations of the study. Notably, we did not measure insulin sensitivity at the whole-body level (e.g., oral glucose tolerance tests or intravenous glucose tolerance tests) in these patients as this was not the primary interest of this study; therefore, understanding the relationship between whole-body and in vitro improvements in insulin action cannot be extrapolated. However, the novel findings from the present study that insulin action is improved in cultured myotubes at both acute and chronic stages after surgery will certainly lead to future investigations on the time course change of whole-body insulin sensitivity in RYGB surgery patients. Furthermore, improvements in insulin action in myotubes at 1 month and 7 months following RYGB surgery appear to be related to lower muscle glycogen levels and enhanced PGC1 α content, respectively; however, the precise mechanism(s) behind these metabolic adaptations is still unclear and needs to be further investigated.

Conclusions

By using human primary skeletal muscle cells, we were able to examine muscle-specific changes in insulin action

following RYGB surgery. Our findings suggest that improvements in insulin-stimulated glucose metabolism in primary human muscle cells occur as early as 1 month following surgery because of a lowered glycogen content. Furthermore, although glycogen content returned to levels observed before RYGB surgery, further improvements in insulin-stimulated glucose metabolism appear to be linked to an increase in PGC1 α protein content 7 months following surgery. These data indicate that insulin action intrinsically improves in cultured human myotubes derived from non-diabetic severely obese patients following RYGB surgery; however, the cellular alterations involved appear to consist of distinct acute and chronic components.

Disclosures

The authors have no commercial associations that might be a conflict of interest in relation to this article.

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