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Exercise and Weight Loss Improve Muscle Mitochondrial Respiration, Lipid Partitioning, and Insulin Sensitivity After Gastric Bypass Surgery

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Both Roux-en-Y gastric bypass (RYGB) surgery and exercise can improve insulin sensitivity in individuals with severe obesity. However, the impact of RYGB with or without exercise on skeletal muscle mitochondria, intramyocellular lipids, and insulin sensitivity index (S_I) is unknown. We conducted a randomized exercise trial in patients (n = 101) who underwent RYGB surgery and completed either a 6-month moderate exercise (EX) or a health education control (CON) intervention. S_I was determined by intravenous glucose tolerance test. Mitochondrial respiration and intramyocellular triglyceride, sphingolipid, and diacylglycerol content were measured in vastus lateralis biopsy specimens. We found that EX provided additional improvements in S_I and that only EX improved cardiorespiratory fitness, mitochondrial respiration and enzyme activities, and cardiolipin profile with no change in mitochondrial content. Muscle triglycerides were reduced in type I fibers in CON, and sphingolipids decreased in both groups, with EX showing a further reduction in a number of ceramide species. In conclusion, exercise superimposed on bariatric surgery–induced weight loss enhances mitochondrial respiration, induces cardiolipin remodeling, reduces specific sphingolipids, and provides additional improvements in insulin sensitivity.

Roux-en-Y gastric bypass (RYGB) is the most commonly performed bariatric procedure in the U.S. and provides large and sustained weight loss, increases insulin sensitivity in multiple organs, and leads to diabetes remission in a significant percentage of patients (1). Improvements in glycemic control and tissue-specific insulin sensitivity after RYGB surgery occur over two discrete phases (2). The first phase is immediately (weeks to months) after surgery wherein glycemic control is normalized; in the second phase (up to ~18 months), peripheral tissue (principally muscle) insulin sensitivity slowly improves. However, even after substantial postoperative weight loss, peripheral tissue insulin sensitivity typically remains much lower than in metabolically healthy lean individuals (3). Exercise is another treatment option that we have recently demonstrated to be a feasible and an effective adjunct therapy to improve insulin sensitivity and glucose effectiveness in bariatric surgery patients with severe obesity (4).

Although a heavily studied area over the past decade, the precise mechanisms that mediate skeletal muscle insulin resistance in human obesity remain unclear. Impairments in mitochondrial oxidation have been implicated (5), and evidence points to a reduced capacity for mitochondria to oxidize fatty acids, which in turn leads to incomplete fatty acid oxidation (FAO) (6) and accumulation of long-chain acyl-CoA, sphingolipids, and diacylglycerol

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(DAG) (7). Experiments in cell culture and animal models suggest that intramyocellular sphingolipids and DAGs contribute to insulin resistance by inhibition of Akt/protein kinase B and insulin receptor substrate 1/2 signaling (8). Studies in human muscle are equivocal, with some showing that muscle ceramide content is elevated in insulin resistance and obesity (9,10) and others reporting no association (11,12). The extent to which muscle DAG content is related to insulin resistance is also unclear (12,13), with one report of elevated muscle DAG in insulin-sensitive athletes (14). A lack of clarity also exists regarding the nature of mitochondrial dysfunction contributing to muscle insulin resistance in obesity and whether calorie restriction interventions that improve insulin sensitivity also improve mitochondrial function (15). Weight loss by dietary caloric restriction alone has been reported to either induce mitochondrial biogenesis (16), reduce mitochondrial respiration (17), or have no effect on mitochondrial content and electron transport chain (ETC) activity (18). Weight loss by bariatric surgery induces greater changes in weight and insulin sensitivity than observed with diet-based weight loss. However, whether surgery-induced weight loss improves peripheral tissue insulin sensitivity through favorable changes in mitochondria and muscle lipids has not been extensively studied (19–21). Moreover, whether adding exercise training modulates the response in this setting is unknown.

Exercise has previously been demonstrated to improve muscle insulin sensitivity concomitant with improved mitochondrial function and reduced intramyocellular DAG and ceramide (22,23). To date, no studies have been done on the effects of exercise on muscle mitochondria, intramuscular lipids, and insulin sensitivity in the context of bariatric surgery–induced weight loss. To address this paucity in the literature, we conducted a 6-month prospective randomized exercise trial with percutaneous skeletal muscle biopsy specimens and intravenous glucose tolerance tests (IVGTTs) to examine the effects of a moderate exercise intervention on muscle mitochondria respiration, intramyocellular lipids (sphingolipids, DAGs, cardiolipin [CL]), and insulin sensitivity during bariatric surgery–induced weight loss. We hypothesized that RYGB surgery–induced weight loss with and without exercise would affect mitochondrial energetics and intramyocellular lipid partitioning and that this would be associated with improved insulin sensitivity. The exercise intervention was specifically focused on the period following surgery when significant weight loss improves peripheral tissue insulin sensitivity (~3–9 months postsurgery).

RESEARCH DESIGN AND METHODS

Patient Recruitment

The study volunteers were a subset of RYGB surgery patients enrolled in a larger randomized controlled exercise trial (4) who completed the study and who underwent muscle biopsy (n = 101). Participants were recruited from two academic bariatric surgery practices in Pittsburgh, Pennsylvania, and Greenville, North Carolina. The study protocol was approved by the human ethics committees of the University of Pittsburgh and East Carolina University. All participants provided written informed consent to participate in the study. Male and female patients were eligible if they were between the ages of 21 and 60 years, had a BMI <55 kg/m^2 and underwent RYGB surgery, were not diabetic, and volunteered to undergo muscle biopsy before and after the intervention. Other aspects of the study design, patient recruitment, and inclusion and exclusion criteria are described in detail elsewhere (4).

Intervention Groups

Between 1 and 3 months after bariatric surgery, participants were randomized to a 6-month intervention of health education control (n = 51) (CON) or an exercise program (n = 50) (EX) (4). Study measurements were made over separate clinic visits before and after the 6-month interventions. The exercise program started after completion of baseline metabolic and body composition assessments and the initial muscle biopsy. Participants in the EX group were required to participate in three to five exercise sessions per week with at least one directly supervised session per week to assure that the target exercise intensity (60–70% maximum heart rate) and duration were achieved and to document progress. Participants progressed over 3 months to a minimum of 120 min/week of exercise, which was maintained for the final 3 months of the program. The CON group was asked to attend six health education sessions. The sessions were held once a month and included lectures, discussions, and demonstrations providing up-to-date information on health topics such as medication use, nutrition, and communicating with health-care professionals. Physical activity habits were also reported and documented at the health education session.

Intravenous Glucose Tolerance Test

A 3-h insulin-modified IVGTT was performed in the morning hours after an overnight fast and at least 48 h removed from the last exercise session to measure insulin action based on the Bergman minimal model calculations (24), as previously described (4).

Body Composition and Cardiorespiratory Fitness

Fat and lean mass were determined by DXA using a GE Lunar bone densiometer (GE Healthcare). Cardiorespiratory fitness (VO_{2peak}) was measured by indirect calorimetry (Moxus Modular VO_2 System, AEI Technologies, Inc.) during a 5–12-min graded exercise test on a cycle ergometer (Lode), as previously described (4). Twelve-lead electrocardiogram recordings were monitored by the study physician and interpreted for contraindications to exercise. Body weight, waist circumference, blood pressure, and plasma lipid levels were measured by standard clinical protocols.

Muscle Biopsy and Specimen Collection Procedures

Percutaneous muscle biopsy samples were obtained after an overnight fast and at least 48 h removed from the last exercise session as described previously (13). Briefly, muscle biopsy specimens were obtained under local anesthesia (2% buffered lidocaine) from the medial vastus lateralis

15 cm above the patella using a 5-mm Bergström muscle biopsy cannula with suction (Stille Surgical Instruments, Eskilstuna, Sweden). Immediately after the biopsy procedure, the specimen was blotted dry and trimmed of visible adipose tissue using a standard dissecting microscope (Leica EZ4; Leica Microsystems, Heerbrugg, Switzerland). Three portions of the specimen (~30 mg each) were snap frozen in liquid nitrogen and stored at −80°C for lipidomics analysis, immunoblotting, and assays of mitochondrial enzyme activity. A separate portion (~10 mg) was placed in relaxing and biopsy-preserving solution (BIOPS media; OROBOROS Instruments, Innsbruck, Austria) for high-resolution respirometry. For immunohistochemistry, a portion (~30 mg) was mounted on a small piece of cork with mounting medium (Shandon Cryochrome; Thermo Scientific, Pittsburgh, PA), frozen in isopentane cooled with liquid nitrogen for 2–3 min (−160°C), and then placed into liquid nitrogen. All frozen samples were stored at −80°C until analysis.

Mitochondrial Biochemistry

A portion of frozen muscle (20–30 mg) was used to measure NADH oxidase, citrate synthase, and creatine kinase activities and CL as described in the Supplementary Data.

High-Resolution Respirometry

Immediately after the muscle biopsy procedure, muscle fiber bundles (~1–3 mg each) were prepared as described in the Supplementary Data. The fiber bundles were gently placed into the respirometer chambers (Oxygraph-2K; OROBOROS Instruments), and after a stable baseline was reached, two assay protocols were run in duplicate at 37°C and between 230–150 nmol/mL O_2 in buffer Z with blebbistatin (25 μmol/L) (Supplementary Fig. 1). In protocol 1, complex I–supported LEAK (CI_L or state 4) respiration was determined through the addition of glutamate (5 mmol/L) and malate (2 mmol/L). ADP (4 mmol/L) was added to elicit complex I–supported oxidative phosphorylation (OXPHOS) (CI_P or state 3) respiration. Succinate (10 mmol/L) was then added to elicit complex I and II–supported OXPHOS ($CI\&II_P$). Cytochrome c (10 μmol/L) was added to assess the integrity of the outer mitochondrial membrane. Finally, FCCP (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone) (2 μmol/L) was added to determine complex I and II–supported electron transfer system (ETS) capacity ($CI\&II_E$) or maximal uncoupled respiration. In protocol 2, FAO-supported LEAK (FAO_L) respiration was determined through the addition of palmitoylcarnitine (25 μmol/L) and malate (2 mmol/L). ADP (4 mmol/L) was added to elicit FAO-supported OXPHOS (FAO_P). Glutamate (5 mmol/L) was then added to elicit CI and FAO_P respiration. Succinate (10 mmol/L) was added to stimulate CI&II and FAO_P respiration. Finally, cytochrome c (10 μmol/L) was added to assess mitochondrial integrity. After the assay protocols, the fiber bundles were retrieved from the respirometry chambers, dried, and weighed on an analytical balance (XS105; Mettler Toledo, Columbus, OH). Oxygen flux was normalized to dry weight of the fiber bundle.

Analysis of Sphingolipid and DAG Species

Intramuscular sphingolipids and DAGs were quantified by high-performance liquid chromatography-tandem mass spectrometry as described previously (25) and in the Supplementary Data. Intramuscular DAG and ceramide content was normalized to tissue wet weight (pmol/mg tissue).

Fiber Type and Intramyocellular Triglyceride Content

Determination of intramyocellular triglyceride (IMTG) content was performed using a modified version of methods previously used in our laboratory (13) and as described in the Supplementary Data. Oil red O staining intensity and cross-sectional area was determined in type I and type II myocytes. Analysis is based on >200 fibers/section.

Protein Content by Immunoblot

A portion of frozen muscle (~30 mg) was prepared for immunoblot analysis for GLUT protein expression as described in the Supplementary Data. Gel-to-gel variation was controlled by using a standardized sample on each gel. Protein loading was controlled by normalizing bands of interest to α-tubulin.

Statistical Analysis

Group differences in baseline characteristics were determined using two-sample Student *t* test (two-tailed) or χ^2 or Fisher exact tests. Any variables with high skew were log- or square root–transformed to achieve a normal distribution. The general linear mixed model with repeated measures (PROC MIXED) was performed to detect group and time effects in the outcome variables. Group, time, and group * time were treated as fixed effects and subjects nested within each group as random effects. Age, sex, and race were covariates. *P* values for post hoc tests were adjusted by false discovery rate (FDR). $P < 0.05$ was considered significant. Analyses were performed using SAS 9.1 or JMP Pro 11 for Macintosh (SAS Institute Inc.).

RESULTS

Study Participants

The characteristics of the study groups before and after the interventions are shown in Table 1. There were no baseline differences in age, sex, race, mass, fat mass, or BMI between groups. The average time from the date of RYGB surgery to randomization into study groups was 77 ± 24 days. There was no difference between groups in average time from surgery to randomization. All muscle biopsy procedures and IVGTTs were conducted 1 day before randomization. No serious adverse events occurred in either study group, and no differences were found in reported adverse events between groups. By design, all participants completed the interventions, and participants in the EX group performed an average ± SEM of 154 ± 17 min/week of structured exercise. Supplementary Table 1 contains further exercise compliance data. Both groups reported similar medication use at baseline, and no difference was found in medication use after intervention (data not shown).

Table 1—Participant characteristics

	CON (n = 51)		EX (n = 50)		P value		
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	Group	Time	Group * Time
Age (years)	42.1 ± 9.9	—	41.6 ± 9.3	—	0.830	—	—
Race ratio	5AA/46C	—	8AA/42C	—	0.350	—	—
Sex ratio	8M/43F	—	6M/44F	—	0.590	—	—
Weight (kg)	106.8 ± 25.4^{B}	84.7 ± 21.9^{A}	108.2 ± 20.2^{B}	84.6 ± 17.0^{A}	0.910	<0.001	0.710
BMI (kg/m^2)	38.1 ± 6.9^{B}	30.3 ± 5.8^{A}	38.8 ± 5.6^{B}	30.4 ± 5.4^{A}	0.970	<0.001	0.690
Waist (cm)	112.2 ± 15.0^{B}	94.7 ± 12.8^{A}	113.2 ± 16.3^{B}	94.3 ± 13.0^{A}	0.850	<0.001	0.620
Fat mass (kg)	49.4 ± 14.5^{A}	30.3 ± 10.9^{B}	51.6 ± 10.4^{A}	30.8 ± 10.5^{B}	0.940	<0.001	0.480
Lean mass (kg)	51.0 ± 10.3^{A}	50.1 ± 10.7^{A}	50.3 ± 7.5^{A}	49.6 ± 7.3^{A}	0.880	0.380	0.930
VO_{2peak} (mL/min)	1,935.3 ± 468.1^{A,B}	1,859.8 ± 552.2^{B}	1,955.3 ± 429.3^{A,B}	2,068.7 ± 609.2^{A}	0.030	0.940	0.120
VO_{2peak} (mL/min/kg FFM)	18.4 ± 3.5^{A}	22.2 ± 5.0^{B}	18.2 ± 3.4^{A}	24.6 ± 6.6^{C}	0.012	<0.001	0.027
IVGTT							
S_I ($min^{-1} \cdot \mu U/mL$)	2.29 ± 1.43^{A}	3.76 ± 2.19^{B}	2.27 ± 1.14^{A}	4.52 ± 2.56^{C}	0.110	<0.001	0.052
AIRg (pmol/L)	392.9 ± 350.1^{A,B}	295.6 ± 238.6^{B}	449.5 ± 364.6^{A}	365.3 ± 342.9^{A,B}	0.170	0.030	0.760
D_I ($\times 10^{-5} \cdot min^{-1}$)	726.2 ± 593.4^{A}	1,103.3 ± 1044.2^{A,B}	871.4 ± 626.7^{A}	1,396.7 ± 951.7^{B}	0.058	0.002	0.730
Fasting insulin (μIU/mL)	6.4 ± 4.3^{A}	4.1 ± 2.4^{B,C}	5.3 ± 2.1^{A,B}	3.8 ± 1.7^{C}	0.140	<0.001	0.300
Fasting glucose (mg/dL)	88.2 ± 12.8^{A}	88.6 ± 11.3^{A}	83.4 ± 7.7^{B}	82.4 ± 7.7^{B}	<0.001	0.820	0.630
HOMA-IR (mg/dL × μIU/mL)	1.4 ± 1.1^{A}	0.9 ± 0.6^{B}	1.1 ± 0.5^{B}	0.7 ± 0.3^{B}	0.054	<0.001	0.390

Data are mean ± SD unless otherwise indicated. SI conversion factors: To convert glucose values from mg/dL to mmol/L, multiply by 0.0555, and to convert insulin values from μIU/mL to pmol/L, multiply by 6.945. AA, African American; AIRg, acute insulin response to glucose; C, Caucasian; D_I, disposition index; F, female; FFM, fat-free mass; HOMA-IR, HOMA for insulin resistance [(fasting glucose × fasting insulin)/22.5]; M, male. ^{A,B,C}Significant differences between group/time points with FDR, $P < 0.05$.

Weight, Body Composition, and Cardiorespiratory Fitness

Both groups experienced similar reductions in mass, fat mass, BMI, and waist circumference after the interventions (Table 1). Changes in blood pressure (systolic and diastolic) and blood lipids (total, LDL, and HDL cholesterol and triglycerides) were reduced to a similar degree in the EX and CON groups similar to that reported in the parent trial (4). EX but not CON had a significantly improved VO_{2peak} (Table 1), an index of cardiorespiratory fitness and an effective predictor of future morbidity and mortality.

Intravenous Glucose Tolerance Test

The baseline insulin sensitivity index (S_I) for both groups was similar (Table 1). Baseline fasting insulin, glucose, and HOMA for insulin resistance were slightly higher in the CON than in the EX group. After the interventions, S_I was improved in both groups. The EX group, however, had a significantly greater improvement in S_I than the CON group. The intervention effects on weight, body composition, fitness, and S_I for these groups were the same or similar as those reported in the parent trial (4).

Muscle CL and OXPHOS Content

CL and OXPHOS protein expression were measured as surrogates of mitochondrial content. Total CL did not change in either intervention group, indicating no change in inner mitochondrial membrane content (Fig. 1*A*). However, exercise altered individual molecular CL species (based on fatty acyl carbon chain length and degree of saturation). Exercise increased the CL-$(C18:2)_4$ species and decreased the CL-$(C18:2)_3(C18:1)_1$, CL-$(C18:2)_2(C18:1)_2$, and CL-$(C18:2)_3(C18:0)_1$ species. CL-$(C18:2)_3(C18:3)_1$ was not altered by exercise (Fig. 1*B*). These data indicate that the exercise intervention remodeled the CL profile in mitochondria independent of changes in total content. We also found that neither the total OXPHOS content nor each of the five ETS subunit proteins were significantly changed by either the CON or the EX group (Fig. 1*C*–*E*). Relative change of each ETS subunit did not change for either intervention (Fig. 1*F*).

Mitochondrial Respiration

Measuring mitochondrial respiration is a widely used approach to assess mitochondrial ETS function. In the first assay protocol, which used carbohydrate-derived substrates, we found that exercise improved CI_P and $CI\&II_P$ respiration. ETS capacity was also improved with exercise. In contrast, the CON group had no change in steady-state respiration (Fig. 2*A*). The second assay protocol used fatty acid (palmitoylcarnitine) and carbohydrate-derived substrates. Exercise improved FAO_L respiration and CI&II & FAO_P respiration. There was also an increase for both groups (time effect) for FAO_P respiration and CI&II and FAO_P respiration (Fig. 2*B*), suggesting an independent effect of RYGB surgery–induced weight loss. A benefit of the mitochondrial respiration protocol is that ratios of oxygen flux in various respiratory states can be examined. Flux control ratios provide an internal normalization and qualitative assessment of coupling and substrate control independent of mitochondrial content, quality of the fiber bundle preparation, and inadvertent variation in assay conditions. We found an increase in both groups (time effect) for the CI phosphorylation system control ratio (Fig. 2*C*). This ratio is an expression of the limitation of CI ETS capacity by the phosphorylation system that increases with increasing capacity of the phosphorylation system. Similarly, there was an increase in both groups for the CI&II phosphorylation system control ratio (Fig. 2*D*). On examining the ratio of $CI_P/CI\&II_P$ (CI control ratio), we found an increase for both groups (Fig. 2*E*). This observation indicates that the relative contribution of CI to maximal OXPHOS respiration increases after RYGB surgery–induced weight loss, with or without exercise. There was no change in the LEAK control ratio with either intervention (Fig. 2*F*), indicating no change in inner mitochondrial membrane uncoupling. Finally, the cytochrome *C* response for both groups was $<10\%$, indicating maintenance of mitochondrial membrane integrity.

Mitochondrial Enzyme Assays

Activities of both succinate dehydrogenase and citrate synthase, enzyme complexes of the tricarboxylic acid cycle, tended to increase in the EX but not in the CON group (Table 2). NADH oxidase, representing the activity of the entire ETC, also tended to be higher in the EX group. Moreover, the ratio of NADH oxidase to CL significantly increased in the EX group, reflecting an exercise-induced increase in the ETC activity per unit of mitochondrial mass. Creatine kinase activity did not change with the interventions (Table 2).

IMTG and DAG Content

There was no change in fiber type proportion or fiber cross-sectional area (Table 3). IMTG content in type I myofibers was decreased in the CON group but not in the EX group, indicating an exercise-induced preservation of higher IMTG during weight loss (Fig. 3*B*). The exercise effect was not seen in type IIA fibers for which both groups showed decreased (time effect) IMTG content (Fig. 3*C*). We found no significant change in any species or total content of DAG in either group.

Intramyocellular Sphingolipid

We quantified a profile of sphingolipid species, purported lipotoxic mediators of insulin resistance. We found a significant decrease for the EX group and a time effect for total sphingolipid, total ceramide, and total unsaturated ceramide. There was also a group effect for total sphingolipid, ceramide, and saturated and unsaturated ceramide largely driven by the higher content in the EX group preintervention (Fig. 4*A*). Similar observations were made for many other ceramide species (Fig. 4*B* and *C*). Finally, there were no intervention effects on sphingosine species (Fig. 4*D*).

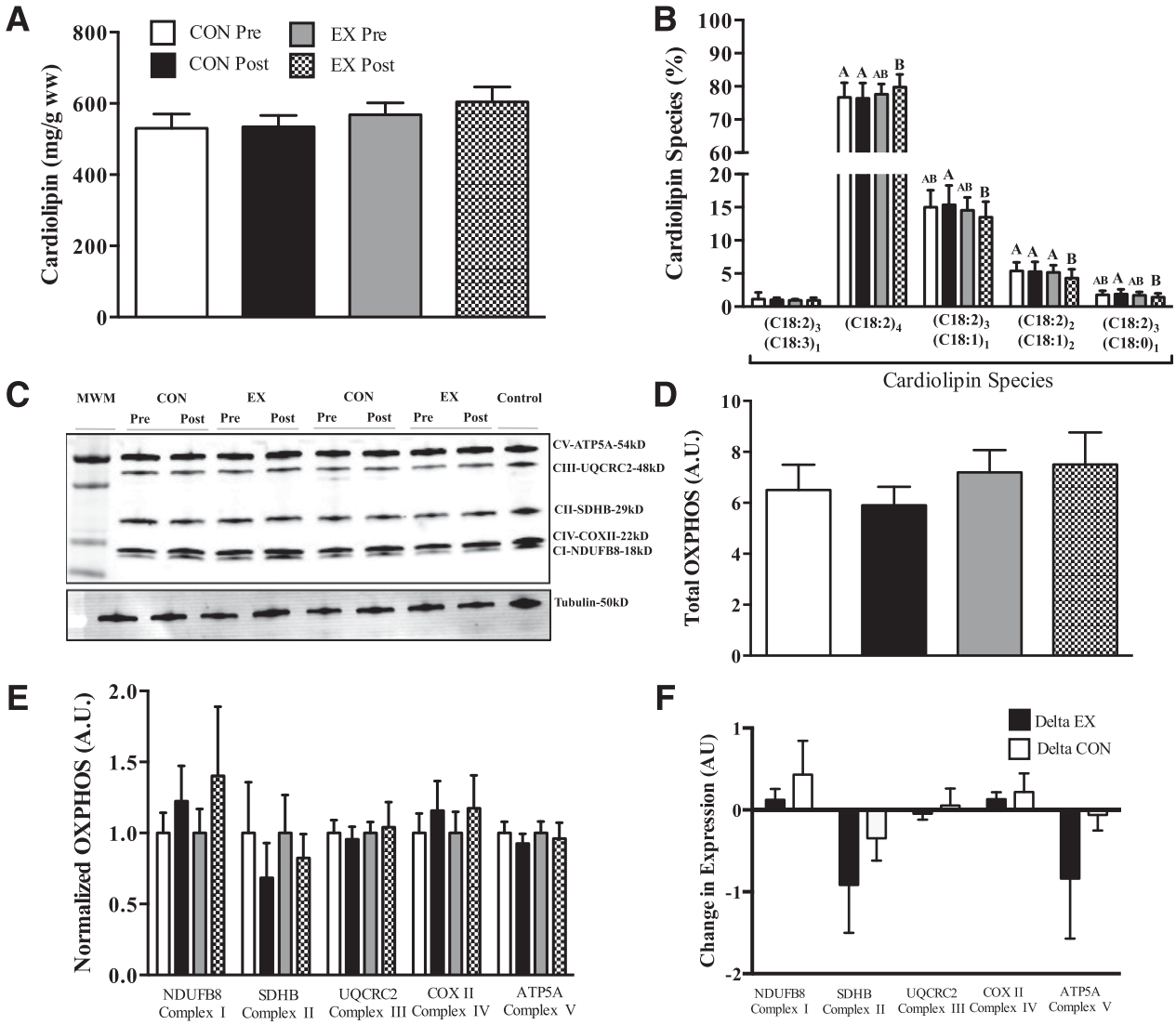

Figure 1—Exercise remodels CL profile without altering total CL or OXPHOS content in vastus lateralis after RYGB surgery. *A*: Total CL is not altered with RYGB surgery–induced weight loss with or without exercise (n = 21 EX, n = 20, CON). *B*: Exercise increases the relative proportion of CL-$(C18:2)_4$, whereas CL-$(C18:2)_3(C18:1)_1$, CL-$(C18:2)_2(C18:1)_2$, and CL-$(C18:2)_3(C18:0)_1$ all decreased. *C*: Representative Western blot for five OXPHOS proteins and α-tubulin. *D* and *E*: OXPHOS content is not altered with RYGB surgery–induced weight loss with or without exercise. *F*: There was no difference between groups in the change in expression of OXPHOS proteins (n = 19 EX, n = 17 CON). The letters A and B denote significant differences between group/time points ($P < 0.05$, ANOVA). Data are mean ± SEM. AU, arbitrary unit; MWM, molecular weight marker; ww, wet weight.

GLUT Expression

We measured protein expression of insulin dependent (GLUT4) and independent (GLUT1 and 12) GLUTs as potential mediators of improved S_I after the interventions. No change in expression of GLUT was found in either the EX or the CON group (Fig. 5).

DISCUSSION

Despite being recognized as a key element in the etiology of type 2 diabetes, skeletal muscle insulin resistance lacks a unifying mechanism that can be consistently translated to human disease or pathobiology. Moreover, whether improvements in insulin sensitivity with weight loss and exercise share common underpinnings is unclear. In this context, bariatric surgery–induced weight loss, with or without concomitant exercise training, provides a conceptual and practical framework to examine important questions about treatment for insulin resistance. We recently reported that insulin resistance is not generally normalized in the weeks to months after RYGB surgery and that regular exercise can further improve peripheral insulin sensitivity (4). In the present study, we examined whether alterations in mitochondrial energetics or intramyocellular lipids correspond with improved insulin sensitivity following RYGB surgery with or without regular exercise.

A key finding was that surgery-induced weight loss, particularly with adjunct exercise, imparted distinct effects

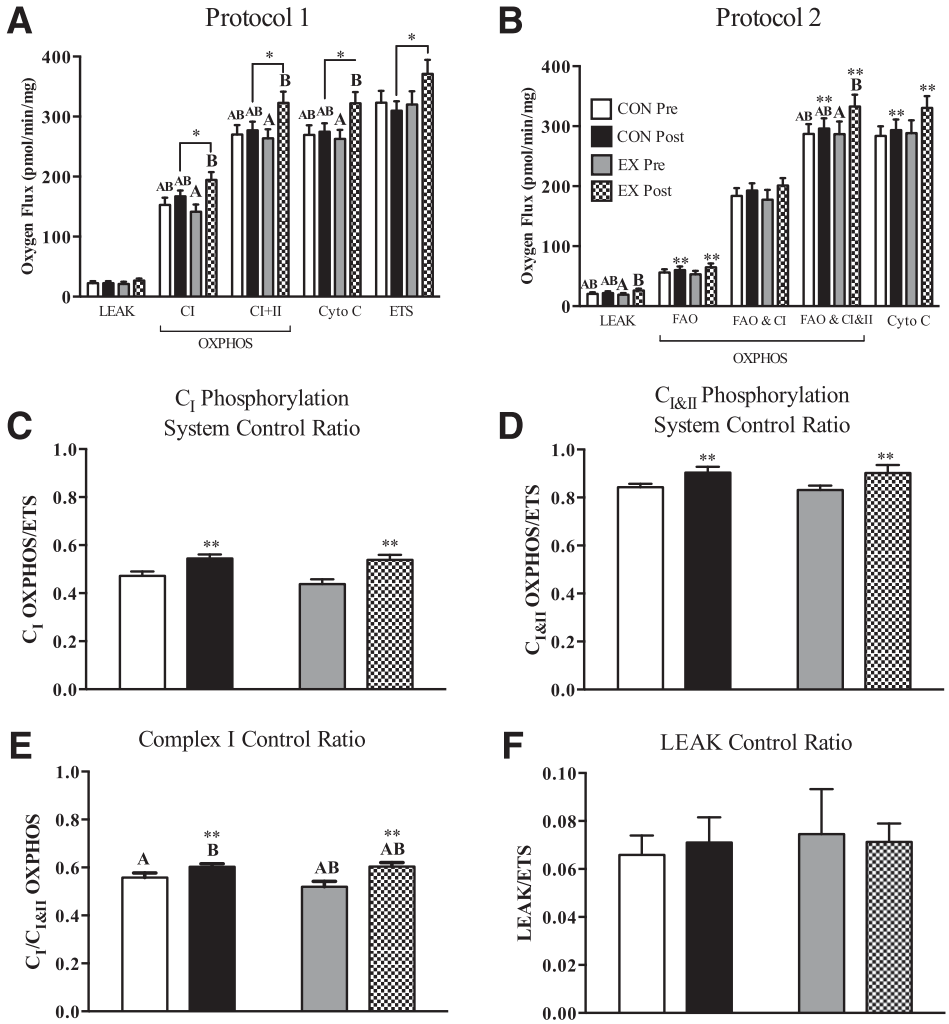

Figure 2—Exercise improves mitochondrial respiratory capacity, whereas RYGB surgery–induced weight loss improves the phosphorylation system control ratio. *A*: Exercise improves ADP (4 mmol/L)-stimulated respiration supported by CI (5 mmol/L glutamate and 2 mmol/L malate), CI&II (10 mmol/L succinate), and ETS capacity (2 μmol/L FCCP). *B*: Exercise improves LEAK respiration and ADP (4 mmol/L)-stimulated respiration supported by FAO and CI&II (25 μmol/L palmitoylcarnitine, 2 mmol/L malate, 5 mmol/L glutamate, and 10 mmol/L succinate). There is also a time effect for ADP (4 mmol/L)-stimulated respiration supported by FAO (25 μmol/L palmitoylcarnitine, 2 mmol/L malate) and FAO and CI&II (25 μmol/L palmitoylcarnitine, 2 mmol/L malate, 5 mmol/L glutamate, and 10 mmol/L succinate). *C*: There was an increase in both groups (time effect) for the CI phosphorylation system control ratio. *D*: There was an increase in both groups (time effect) for the CI&II phosphorylation system control ratio. *E*: There was an increase in both groups (time effect) for the CI control ratio. *F*: No change was seen in the LEAK control ratio (n = 30 EX, n = 41 CON). The letters A and B denote significant differences between groups ($P < 0.05$, ANOVA). Data are mean ± SEM. *$P < 0.05$, group × time interaction; **$P < 0.05$, time effect. Cyto C, cytochrome c.

on mitochondrial energetics in the absence of any notable alterations in mitochondrial content. Much controversy exists regarding the role of mitochondria in muscle insulin resistance and how weight loss affects this relationship (15). Calorie restriction–induced weight loss in humans, an intervention that improves peripheral insulin resistance (15), has been reported to elicit muscle mitochondrial biogenesis (16), reduce mitochondrial respiration (17), or have no effect on mitochondrial content and ETC activity (18). In the context of bariatric surgery–induced weight loss, only a handful of studies have examined skeletal muscle. These studies show changes in gene expression related to glucose metabolism and mitochondrial function in muscle biopsy samples and myotubes derived from biopsy specimens (26), changes that may be mediated by an altered epigenome (27). Others have shown that the expression of genes associated with mitochondrial biogenesis in skeletal muscle are enhanced and related to improved insulin sensitivity after bariatric surgery (28). The few studies that examined mitochondrial respiratory performance after bariatric surgery produced equivocal results (19,20).

We used a number of analytical approaches to assess mitochondrial function more comprehensively. Our observation that surgery-induced weight loss did not alter markers of mitochondrial content is in line with a growing consensus

Table 2—Muscle mitochondria enzyme activity

	CON (n = 20)		EX (n = 21)		P value		
	Preintervention	Postintervention	Preintervention	Postintervention	Group	Time	Group * Time
SDH (AU)	33,217 ± 7,242	32,787 ± 8,293	33,311 ± 8,019	35,385 ± 7,791	0.7142	0.2468	0.0804
Citrate synthase (U/mU CK)	3.39 ± 0.88[A,B]	3.67 ± 0.94[A,B]	3.39 ± 0.69[A]	4.49 ± 1.30[B]	0.3101	0.0042	0.0716
NADH oxidase (U/g ww)	1.39 ± 0.49[A,B]	1.67 ± 0.63[A,B]	1.55 ± 0.72[A]	2.27 ± 1.14[B]	0.0821	0.0003	0.093
NADH oxidase (U/mg CL)	2.76 ± 1.03[A,B]	3.20 ± 1.06[A,B]	2.76 ± 0.99[A]	3.72 ± 1.13[B]	0.1353	<0.0001	0.0183
Total CK (U/g ww)	5,509 ± 897	5,634 ± 850	5,930 ± 971	5,617 ± 1,058	0.472	0.6004	0.2265

Data are mean ± SD. AU, arbitrary unit; CK, creatine kinase; SDH, succinate dehydrogenase; ww, wet weight. [A,B]Significant differences between group/time points with FDR, $P < 0.05$.

that weight loss alone does not induce mitochondrial biogenesis (15,18). Surgery-induced weight loss, however, did induce a robust increase in $CI_P/CI\&II_P$ control ratios, indicating increased capacity for OXPHOS without increased ETS capacity. Weight loss in the CON group also increased the $CI_P/CI\&II_P$ control ratio, indicating a relative increase in the contribution of electron flow from CI to maximal OXPHOS respiration. Taken together, these data suggest a unique remodeling of mitochondrial respiratory parameters with surgery-induced weight loss that occur along with improved insulin sensitivity.

The exercise program further improved S_I compared with surgery-induced weight loss alone, concomitant with distinct improvements in mitochondrial respiration. Abundant evidence indicates that aerobic exercise induces muscle mitochondrial biogenesis and improves oxidative capacity (23,29), but few exercise studies with a focus on mitochondria have been conducted in obese patients (29,30), and none have been conducted in patients after bariatric surgery. We found no increase in OXPHOS protein or total CL content after exercise (a surprising observation that may be due to massive weight loss modulating the effect of exercise on mitochondria), whereas mitochondrial enzymes, including citrate synthase and NADH oxidase activities, increased robustly.

Exercise induced a remodeling of CL species, a phospholipid that is primarily localized to the inner mitochondrial membrane and plays a key role in maintaining OXPHOS complex integrity and function (31). We observed a distinct pattern of remodeling, with species containing the polyunsaturated linoleoyl-CoA [CL-$(C18:2)_4$] becoming more abundant and CL-$(C18:2)_3(C18:1)_1$, CL-$(C18:2)_2(C18:1)_2$, and CL-$(C18:2)_3(C18:0)_1$ decreasing with exercise. The increase in tetralinoleic CL may reflect lower exposure of CL to oxidative stress because polyunsaturated acyl chains in CL are particularly vulnerable to oxidative modification by reactive oxygen species (32). This would be in line with the increase in mitochondrial antioxidant capacity that has been reported with regular exercise (33). The increase in tetralinoleic CL also reflects a more mature CL pool, meaning that the immature form of CL is characterized by a random assortment of attached acyl chains that are saturated and variable in length. A mature CL pool has structural uniformity and contains predominantly tetralinoleoyl acyl chain (31) features that are essential to its function to maintain the integrity of the OXPHOS capacity. The importance of CL to mitochondrial function is highlighted by studies of patients with mutations in the tafazzin gene (Barth syndrome) (34) and studies in rodents where relatively small changes in CL profile reduce mitochondrial respiratory capacity in cardiomyocytes (35). Further investigations are needed to interrogate the role of CL in improved insulin resistance.

The differential response of various indices of mitochondrial content and CL remodeling to exercise may relate to the fact that obese individuals may not adapt to

contractile activity in a manner consistent with the plasticity of mitochondria (36,37). The impact of varying intensity and volume of exercise on mitochondrial remodeling, particularly in obese individuals, may also play a role (38). Overall, these data suggest that exercise-induced remodeling of the various components of the mitochondria may occur to different degrees in obese individuals (39). Congruent with a more mature CL pool, we observed an increase CI_P, $CI\&II_P$, and CI&II and FAO_P respiration after the exercise program. Although these effects have been previously reported for exercise (29,33), the present report is the first in obese patients following bariatric surgery. For the first time to our knowledge, the data link exercise-induced CL remodeling with improved mitochondrial OXPHOS and insulin sensitivity during RYGB surgery–induced weight loss.

Surgery-induced weight loss reduced IMTG in type I and II fibers, a finding in line with that of Gray et al. (21) and others who have shown that calorie restriction–induced weight loss also reduces IMTG (18,40). The present study is the first to comprehensively compare skeletal muscle sphingolipid and DAG species after bariatric surgery. The sphingolipid ceramide has been touted as a key mediator of insulin resistance through inhibition of Akt/protein kinase B signaling and mediation of inflammation (tumor necrosis factor-α). Associations between muscle ceramide content and insulin resistance in obesity have been reported in some (9,10,13) but not all studies (11). A limitation in the current literature is that many previous studies only measure total ceramide (41) or a small number of molecular species (9). However, the identity of sphingolipid species that is associated with improvements in insulin sensitivity with weight loss has not been adequately examined. In this study, we used state-of-the-art lipidomics to quantify individual molecular species of sphingolipid (17 total, including ceramide) in the muscle of RYGB surgery patients during weight loss and provide strong evidence that ceramide is reduced concomitant with improved insulin sensitivity. Of note, the reduction in ceramide may also be involved in the observed alterations in mitochondria respiration because ceramide can suppress the ETS at CI and CIII (42), leading to oxidative stress (43), and modulate mitochondrial permeability transition pore opening (44). However, in two previous studies, diet-induced calorie restriction (45) and RYGB- and laproscopic gastric banding–induced weight loss (20%) (46) did not alter intramyocellular ceramide. Although reconciling the present data to these findings is difficult, the data underscore the need for further studies to evaluate the importance of intramyocellular ceramide in improved insulin sensitivity with weight loss.

Exercise also induced distinct intramyocellular lipid partitioning and prevented the RYGB surgery–induced decrease in IMTG in type I myofibers, an observation that echoes the athlete's paradox (47) and is consistent with observations made during calorie-induced weight loss with exercise (18). Exercise also resulted in greater selective

Table 3—Muscle fiber type distribution and cross-sectional area in vastus lateralis

	CON (n = 33)		EX (n = 34)		P value		
	Preintervention	Postintervention	Preintervention	Postintervention	Group	Time	Group * Time
Type I CSA (μm^2)	3,268 ± 940	3,357 ± 1,605	3,499 ± 1,435	3,363 ± 1,460	0.7533	0.2756	0.7363
Type IIA CSA (μm^2)	2,790 ± 1,073	2,640 ± 1,848	3,007 ± 1,317	2,759 ± 1,522	0.9872	0.2181	0.7013
Type IIX CSA (μm^2)	3,562 ± 920	3,871 ± 1,767	3,657 ± 893	3,960 ± 1,815	0.78	0.8883	0.8028
Type I content (%)	44 ± 13	47 ± 12	49 ± 11	48 ± 14	0.2408	0.665	0.5501
Type IIA content (%)	45 ± 11	43 ± 11	44 ± 10	44 ± 12	0.6866	0.4226	0.6147
Type IIX content (%)	13 ± 11	20 ± 33	10 ± 6	13 ± 10	0.1102	0.1153	0.779

Data are mean ± SD. CSA, cross-sectional area.

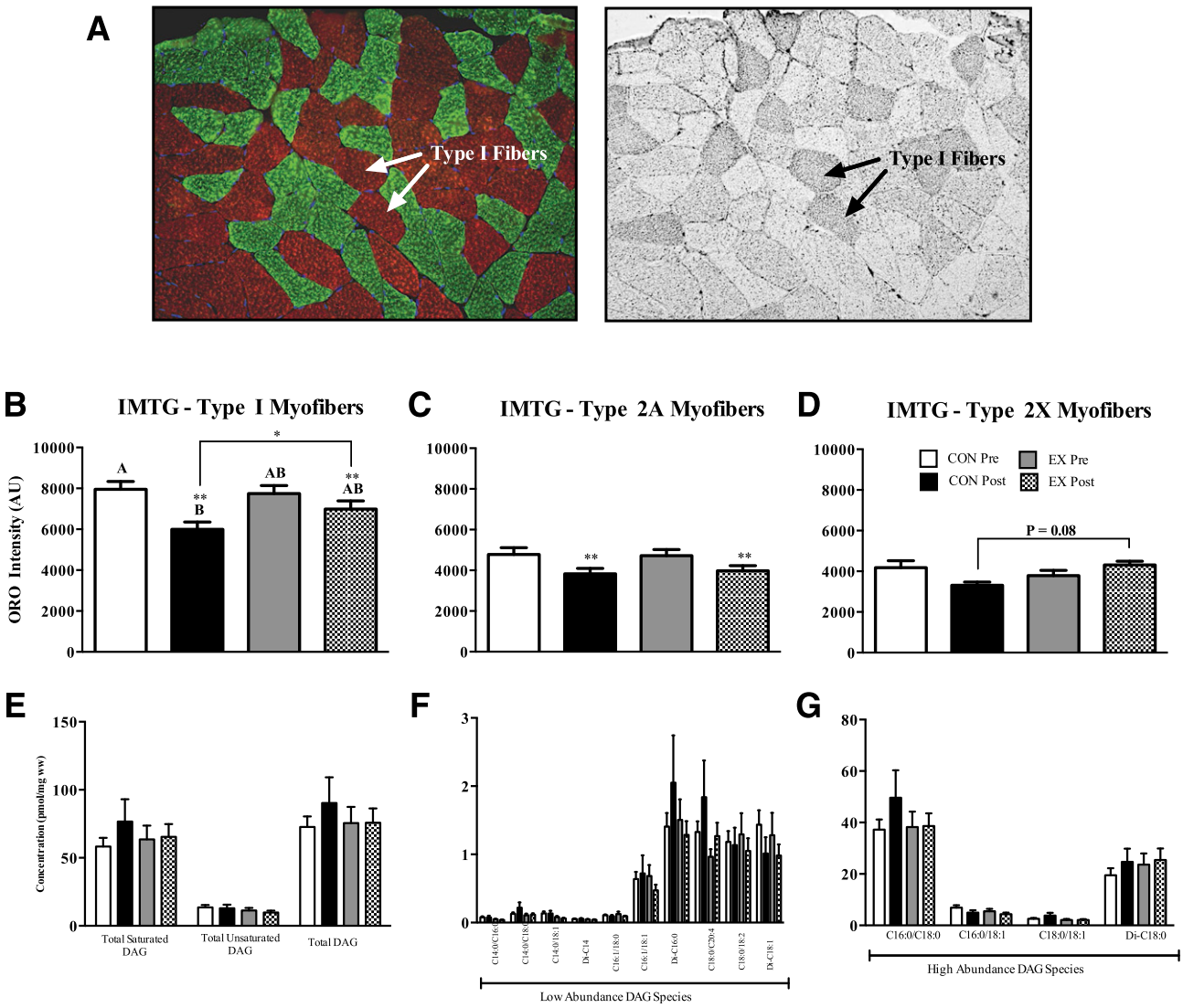

Figure 3—RYGB surgery reduces IMTG content in type I and type II myofibers without altering whole-muscle DAG content. *A*: Representative images of immunofiber typing and oil red O staining generated from a muscle biopsy specimen. *B*: The CON group but not the EX group had reduced IMTG in type I myofibers. *C*: IMTG content was significantly reduced in type IIA myofibers for both groups (time effect). *D*: There was no change in IMTG content in type IIX myofibers (n = 34 EX, n = 33 CON). *E–G*: There were no EX or CON group effects on any DAG species quantified (n = 20 EX, n = 16 CON). The letters A and B denote significant differences between group/time points ($P < 0.05$, ANOVA). Data are mean ± SEM. *$P < 0.05$, group × time interaction; **$P < 0.05$, time effect. AU, arbitrary unit; ORO, oil red O; ww, wet weight.

reductions in a number of individual ceramide species with long acyl chains (C18, C18:1, C24:1, C22:1) and total sphingolipid and ceramide content. This is consistent with our previous reports of individual ceramide species being elevated in obesity (10) and insulin resistance (13,14) and being reduced with exercise (22), further supporting a role for ceramide in human muscle insulin resistance.

A significant body of research in animal models supports a role for DAG as a mediator of muscle insulin resistance (48). However, evidence from studies of human muscle remain equivocal. Here, we did not observe changes in intramyocellular DAG during RYGB surgery–induced weight loss with or without exercise, indicating no relationship with improved insulin sensitivity. This result is in line with our previous reports that whole-muscle DAG does not play a role in insulin sensitivity in human muscle (10,13,14) and a report that intramyocellular DAG does not change after surgery-induced weight loss (46). Of note, Jocken et al. (49) reported paradoxically lower levels of DAG in obese compared with lean subjects. Although the consensus in human studies thus far suggests that whole-muscle levels of DAG are not associated with insulin resistance, this does not preclude the possibility that organelle/lipid membrane–specific accumulation of DAG species plays an important role in mediating insulin resistance in human obesity (50).

Finally, the expression of GLUT proteins did not appear to explain the exercise-induced improvements in S_I. GLUT4

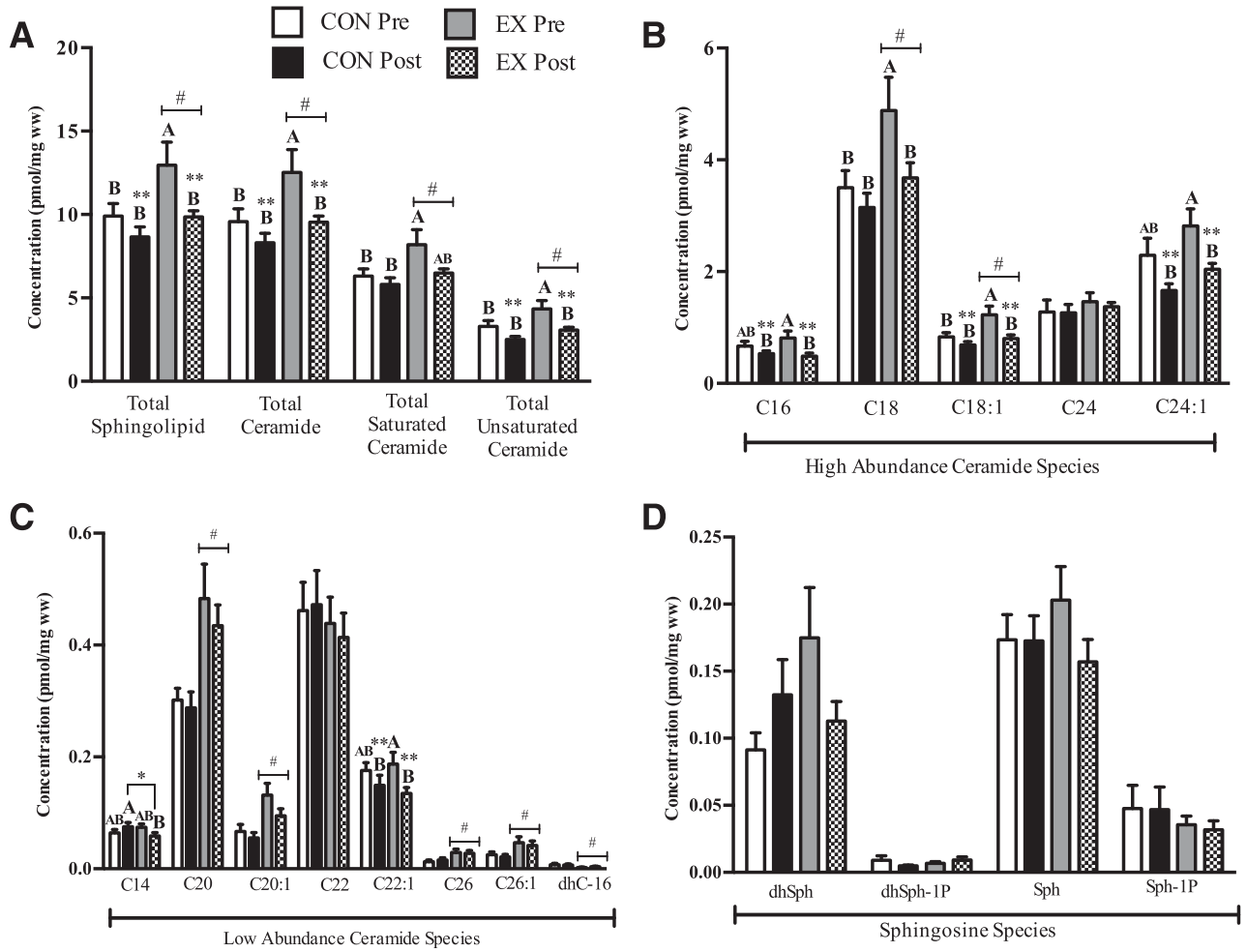

Figure 4—Ceramide content in muscle decreases with RYGB surgery–induced weight loss. Exercise may further reduce content of certain molecular species of ceramide. *A*: Total sphingolipid, ceramide, and unsaturated ceramide all decreased in both groups (time effect), with the decreases being more pronounced in the EX group. Ceramide levels before intervention were higher in the EX than in the CON group. *B*: C16, C18:1, and C24:1 ceramides were decreased in both groups (time effect), with the decreases being more pronounced in the EX group. There is also a group effect for C18 and C18:1 influenced mainly by higher levels of ceramide at EX preintervention. *C*: There was a number of group effects (higher in EX) for many of the low-abundance ceramides. *D*: There were no EX or CON effects on any sphingosine species quantified (n = 20 EX, n = 16 CON). The letters A and B denote significant differences between group/time points ($P < 0.05$, ANOVA). Data are mean ± SEM. *$P < 0.05$, group × time interaction; **$P < 0.05$, time effect; #$P < 0.05$, group effect. ww, wet weight.

is the key GLUT responsible for insulin- and contraction-stimulated glucose transport in skeletal muscle (51). Expression of GLUT4 is not different between healthy individuals with insulin resistance and those with type 2 diabetes (52), and caloric restriction has no effect on GLUT expression in muscle (53). However, many studies have demonstrated that increased GLUT4 expression is a key adaptation to long-term exercise (54,55) and that GLUT4 expression increases in muscle of obese patients with type 2 diabetes after exercise (56). Fewer studies have examined the effect of exercise on GLUT1 and 12 isoforms, which are expressed at lower levels than GLUT4. It is possible that similar to the lack of effect on mitochondria biogenesis or content in the present study, the dose of exercise was insufficient to elicit changes in GLUT proteins [exercise-induced expression of GLUT4 is regulated in parallel with mitochondrial biogenesis through the same signaling pathways (57)]. It is also possible that these patients are resistant to these exercise improvements for reasons not explored in this study.

A distinguishing feature of the present study was that we used a randomized exercise trial to determine the additional effects of exercise postsurgery, so we did not capture changes that could have initially occurred after surgery. Presurgery data would have allowed us to examine potentially important changes in myocellular metabolism during the initial period after surgery. Another limitation was that nutritional intake was not controlled or monitored and may represent an important factor that affects outcome measures, including intramyocellular lipids, mitochondrial function, and S_I. Moreover, future studies should examine other potential mediators of insulin resistance and their response

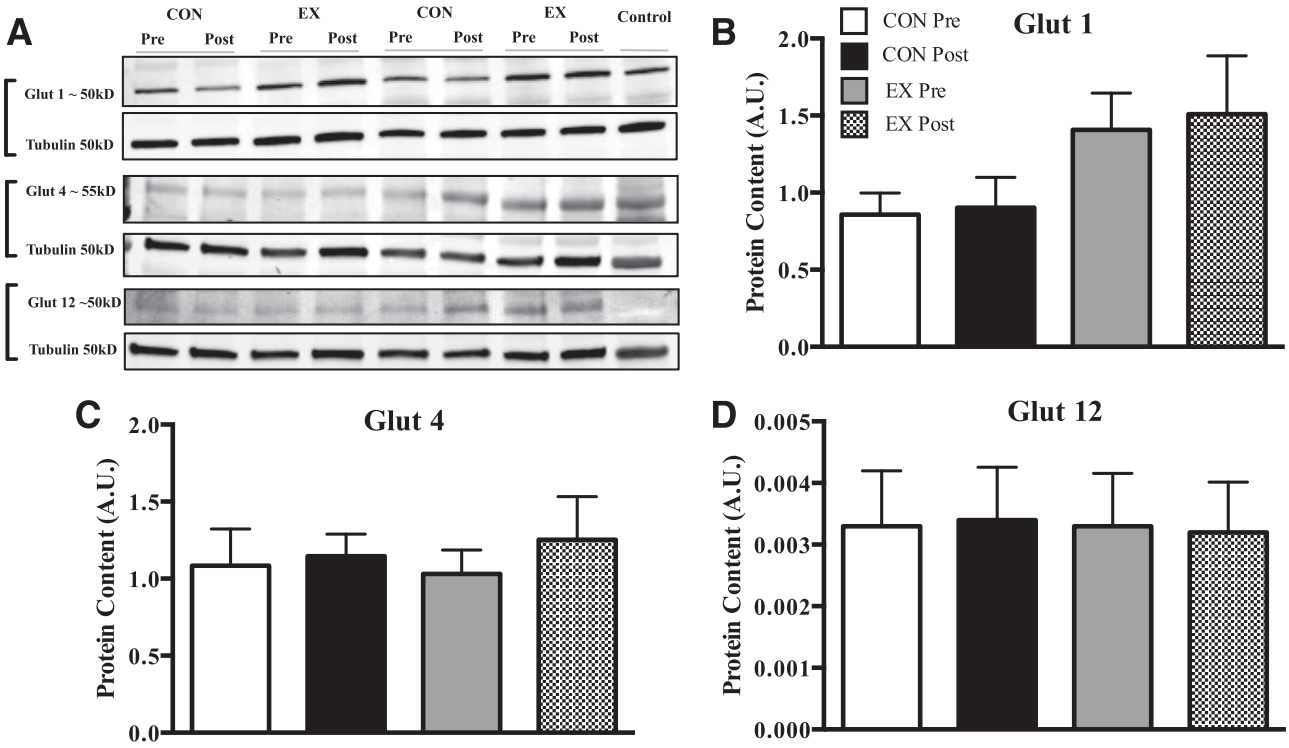

Figure 5—GLUT protein expression did not change with the EX or CON groups. *A*: Representative Western blots for GLUT1, GLUT4, GLUT12, and the housekeeping protein α-tubulin. GLUT1 (*B*), GLUT4 (*C*), and GLUT12 (*D*) protein expression in muscle did not change with either the CON or the EX interventions (n = 19 EX, n = 17 CON). Data are mean ± SEM. AU, arbitrary unit.

to surgery-induced weight loss and exercise (e.g., acylcarntines, amino acids, gut microbiome).

In summary, this randomized exercise intervention study provides the first evidence in RYGB surgery patients to show distinct weight loss and exercise effects on mitochondrial respiration and CL remodeling independent of mitochondrial content and reductions in IMTG and intramyocellular ceramide but not DAG. RYGB surgery–induced weight loss enhanced specific aspects of mitochondrial OXPHOS, a novel observation that may prove to be a valuable target for therapy. Exercise further improved S_I along with improved respiratory capacity, remodeling of the CL profile, and further reductions in specific ceramide species. Thus, several facets of myocellular energetics are not rectified with substantial weight loss from bariatric surgery alone and further support the implementation of an adjunct exercise program after bariatric surgery. These data provide valuable mechanistic insight into how both surgery-induced weight loss and exercise are complementary therapies to improve the metabolic profile in severe obesity.

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