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Original article

Impaired skeletal muscle mitochondrial function in morbidly obese patients is normalized one year after bariatric surgery

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Abstract

Background: Obesity and type 2 diabetes are associated with impaired skeletal muscle mitochondrial metabolism. As an intrinsic characteristic of an individual, skeletal muscle mitochondrial dysfunction could be a risk factor for weight gain and obesity-associated co-morbidities, such as type 2 diabetes. On the other hand, impaired skeletal muscle metabolism could be a consequence of obesity. We hypothesize that marked weight loss after bariatric surgery recovers skeletal muscle mitochondrial function.

Methods: Skeletal muscle mitochondrial function as assessed by high-resolution respirometry was measured in 8 morbidly obese patients (body mass index [BMI], 41.3 ± 4.7 kg/m²; body fat, $48.3\% \pm 5.2\%$) before and 1 year after bariatric surgery (mean weight loss: 35.0 ± 8.6 kg). The results were compared with a lean (BMI 22.8 ± 1.1 kg/m²; body fat, $15.6\% \pm 4.7\%$) and obese (BMI 33.5 ± 4.2 kg/m²; body fat, $34.1\% \pm 6.3\%$) control group.

Results: Before surgery, adenosine diphosphate (ADP)-stimulated (state 3) respiration on glutamate/succinate was decreased compared with lean patients (9.5 ± 2.4 versus 15.6 ± 4.4 O₂ flux/mtDNA; $P < .05$). One year after surgery, mitochondrial function was comparable to that of lean controls (after weight loss, 12.3 ± 5.5 ; lean, 15.6 ± 4.4 O₂ flux/mtDNA). In addition, we observed an increased state 3 respiration on a lipid substrate after weight loss (10.0 ± 3.2 versus 14.0 ± 6.6 O₂ flux/mtDNA; $P < .05$).

Conclusion: We conclude that impaired skeletal muscle mitochondrial function is a consequence of obesity that recovers after marked weight loss. (Surg Obes Relat Dis 2013;9:936–941.) © 2013 American Society for Metabolic and Bariatric Surgery. All rights reserved.

Keywords:

Morbid obesity; Weight loss; Skeletal muscle mitochondrial function; Gastric banding

Obesity is one of the Western world's primary healthcare issues, and the increasing incidence of obesity is accompanied by an equal rise in co-morbidities such as type 2 diabetes, dyslipidemia, and hepatosteatosis [1]. The excess amount of adipose tissue in obesity is associated with a dysfunction in several tissues; hepatosteatosis, which causes

impaired liver function and increased lipid deposition in skeletal muscle, is suggested to induce insulin resistance [1,2]. In skeletal muscle from obese and obese type 2 diabetic patients, a decreased mitochondrial function compared with lean and nondiabetic patients has been reported. This could exacerbate the negative effect of intramyocellular lipid (IMCL) accumulation on insulin sensitivity [3–7]. However, although diet-induced weight loss decreased intramyocellular lipid content and has been shown to improve skeletal muscle insulin sensitivity in nondiabetic overweight patients [8,9], skeletal muscle

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mitochondrial function (as determined by nicotinamide adenine dinucleotide [NADH] oxidase enzyme levels) per se did not change [8,10]. This may suggest that reduced mitochondrial function in obesity is an intrinsic characteristic of obese patients. However, it should be noted that weight loss in the aforementioned studies was low (9–18 kg) [8–10] in comparison to that after bariatric surgery (37–42 kg) [11], and it cannot be excluded that further weight reduction does improve skeletal muscle mitochondrial metabolism. Indeed, it has been shown before that proteins associated with mitochondrial biogenesis in skeletal muscle were enhanced and related to improved insulin sensitivity after bariatric surgery, suggesting that skeletal muscle mitochondrial function may be suppressed by excessive weight [12,13]. However, to our knowledge no study has evaluated the effect of bariatric surgery on skeletal muscle mitochondrial function per se, and therefore these findings need further confirmation. To test our hypothesis that skeletal muscle mitochondrial function improves after bariatric surgery, we investigated ex vivo skeletal muscle mitochondrial function in morbidly obese patients before and 1 year after laparoscopic adjustable gastric banding (LAGB) surgery and compared these findings with those of an obese and lean control cohort.

Materials and methods

Study protocol

Approval was obtained from the Maastricht University Medical Centre institutional review board. Written informed consent was received before the first muscle biopsy from 2 male and 6 female morbidly obese patients who were awaiting laparoscopic adjustable gastric banding (LAGB), with a mean body mass index (BMI) of $41.3 \pm 4.7 \text{ kg/m}^2$ (Table 1). One female subject took levothyroxine for hypothyroidism and was euthyroid for several years. Diabetes mellitus was an exclusion criterion. Skeletal muscle mitochondrial function of the 8 morbidly obese patients before and after weight loss was compared with

that of 10 healthy lean male and 10 healthy obese male control patients (Table 1). These reference patients were studied by our group before; however, current results for skeletal muscle mitochondrial function were not reported previously [14].

Body composition

Body composition (fat free mass, fat mass) was determined under fasted conditions by dual x-ray absorptiometry (DXA, type Discovery A, Hologic, Bedford, MA).

Muscle biopsies

Several weeks before surgery, a muscle biopsy according to Bergström et al. [15] was taken under fasted conditions at about 8:30 AM. Part of the muscle was directly used for the fresh preparation of permeabilized muscle fibers as described [3]. The remaining part of the muscle was directly frozen in liquid nitrogen and stored at -80°C until further analysis.

To determine skeletal muscle mitochondrial capacity, high-resolution respirometry was performed in permeabilized muscle fibers at 37°C in a 2-chamber-Oxygraph (OROBOROS Instruments, Innsbruck, Austria).

High-resolution respirometry protocol

Coupled (state 3) respiration, initiated by addition of 2 mM ADP, was measured for complex I-linked substrate combination malate + glutamate, both in the presence and absence of the lipid substrate octanoyl-carnitine. Coupled respiration was then maximized with convergent electron input through complex I and complex II, by adding saturating concentrations of succinate (10 mM). Subsequently, mitochondrial respiration uncoupled from adenosine triphosphate (ATP) synthesis (state 4) was determined via addition of the ATP-synthase inhibitor oligomycin. In addition, the chemical uncoupler FCCP [carbonyl-cyanide-4-(trifluoromethoxy)-phenylhydrazone] was used to

Table 1

Subject characteristics for 2 male and 6 female morbidly obese patients before (MO-before) and after (MO-after) weight loss compared with 10 male obese (obese) and 10 male lean (lean) control patients.

	MO-before	MO-after	Obese	Lean	P			
						MO-before/obese	MO-before/obese	MO-before/obese
Number	8	8	10	10				
Age (yr)	40 ± 9	41 ± 9	29 ± 10.2	23 ± 2.7	<.001*	.025 [†]	<.001 [†]	
Body mass (kg)	126.7 ± 20.0	91.7 ± 18.8	107.7 ± 14.8	76.2 ± 10.5	<.001*	.034 [†]	.032 [†]	
BMI (kg/m ²)	41.3 ± 4.7	29.8 ± 4.8	33.5 ± 4.2	22.8 ± 1.1	<.001*	.002 [†]	<.001*	
Body fat % (BF%)	48.3 ± 5.2	34.1 ± 8.5	34.1 ± 6.3	15.6 ± 4.7	<.001*	<.001*	<.001*	
Fat mass (kg)	61.4 ± 11.5	32.1 ± 11.8	37.4 ± 11.1	11.8 ± 4.1	<.001*	<.001*	<.001*	
Fat-free mass (kg)	63.8 ± 13.0	59.0 ± 13.2	70.3 ± 5.9	64.4 ± 9.2	<.001*	.176	.235	

BMI = body mass index; MO = morbidly obese.

*P < .001.

[†]P < .05.

maximize oxygen flux to assess maximal mitochondrial capacity (state u respiration).

Normalization for mitochondrial DNA content

To determine mitochondrial density, the mitochondrial DNA (mtDNA) copy numbers were determined according to Phielix et al. [3], and all muscle biopsy samples were run in one analysis. For comparison, oxygen fluxes per wet-weight muscle mass were normalized for mtDNA. The mitochondrial DNA copy numbers did not differ significantly before and after weight loss (mtDNA copy number, 960.6 ± 425.1 before, 868.8 ± 386.0 after weight loss; $P = .371$). All results showed similar trends and significance when oxygen fluxes were not corrected for mtDNA (results not shown). Oxygen fluxes (O_2 flux) shown are expressed as picomoles $O_2 \times \text{seconds}^{-1} \times \text{milligrams}$ of tissue $^{-1} \times \text{mtDNA} \times 10^6$.

Plasma values

Plasma thyroid-stimulating hormone (TSH) and free thyroxine (FT_4) were measured using an electrochemiluminescence immunoassay (Roche, Basel, Switzerland) and a fluorimmunoassay (PerkinElmer, Waltham, MA). Plasma insulin, glucose, free fatty acids (FFA), and the homeostasis model of assessment–insulin resistance (HOMA-IR) were calculated as described [14].

Follow-up

All 8 patients underwent LAGB without any perioperative or postoperative complications. One year after LAGB a second muscle biopsy was taken.

Statistical analysis

Reported data were expressed as means \pm SD. Statistical analyses were performed with PASW Statistics 18.0 for Mac OS 10.6.4. Repeated measurements before and 1 year after bariatric surgery were compared using paired Student's *t* tests. Comparisons between groups (morbidly obese patients before and after bariatric surgery, obese and lean control patients) were made using a one-way ANOVA with a post hoc Bonferroni correction. A *P* value $< .05$ was considered significant.

Results

Body composition was significantly altered 1 year after surgery (Table 1), with a mean weight loss of $27.9\% \pm 6.6\%$. After a mean follow-up of 18 months, there was no difference in body composition compared with the 1-year time point (body mass 91.7 ± 18.8 versus 91.5 ± 18.3 kg; BMI 29.8 ± 4.8 versus 29.7 ± 4.7 kg/m 2 ; $P = .844$), which indicates patients

were weight stable at the moment of the second muscle biopsy.

ADP-stimulated (state 3) respiration increased after weight loss upon complex I-linked substrates (for malate + glutamate 4.2 ± 1.4 versus 6.8 ± 2.0 O_2 flux; $P = .029$; for malate + octanoyl-carnitine + glutamate [MOG] 4.2 ± 2.1 versus 8.9 ± 4.2 O_2 flux; $P = .028$) as well as upon parallel electron input into both complex I and complex II (for malate + octanoyl-carnitine + glutamate + succinate [MOGS] 10.0 ± 3.2 versus 14.0 ± 6.6 O_2 flux; $P = .031$), although did this not reach statistical significance for malate + glutamate + succinate (MGS; 9.5 ± 2.4 versus 12.3 ± 5.5 O_2 flux; $P = .086$; Fig. 1). Leak respiration upon the ATP-synthase inhibitor oligomycin (i.e., respiration not related to ATP synthesis; state 4 respiration) was determined as a marker for mitochondrial uncoupling but remained unaffected by weight loss (6.1 ± 2.7 versus 5.4 ± 2.4 O_2 flux; $P = .093$; Fig. 1). The relative contribution of state 4 to state 3 respiration (uncoupling ratio [UCR]) decreased significantly after weight loss (0.6 ± 0.2 versus $.4 \pm 0.1$; $P = .049$; Fig. 1).

Maximally uncoupled mitochondrial respiration, as analyzed after addition of FCCP in the presence of octanoyl-carnitine, did not increase significantly after weight loss (15.0 ± 4.4 versus 18.0 ± 8.2 O_2 flux; $P = .212$; Fig. 1).

State 3 respiration upon complex I and II substrates MGS in muscle fibers was significantly lower in morbidly obese patients before bariatric surgery compared with lean patients (9.5 ± 2.4 versus 15.6 ± 4.4 O_2 flux; $P = .028$) and tended to be lower compared with obese controls (9.5 ± 2.4 versus 13.7 ± 5.8 O_2 flux; $P = .076$; Fig. 2). State 4 leak respiration was not different for morbidly obese compared with lean and obese controls (MO-before 6.1 ± 2.7 ; lean 4.7 ± 1 ; obese 3.9 ± 1.5 O_2 flux; $P = .127$; Fig. 2). After weight loss, there were no statistically significant differences for both state 3 and state 4 respiration for morbidly obese patients (MO-after) compared with lean and obese controls (state 3: MO-after 12.3 ± 5.5 ; lean 15.6 ± 4.4 ; obese 13.7 ± 5.8 O_2 flux; $P = .411$; state 4: MO-after 5.4 ± 2.4 ; lean 4.7 ± 1.6 ; obese 3.9 ± 1.5 O_2 flux; $P = .210$; Fig. 2). Although there were significant age differences between the morbidly obese and lean and obese control groups (Table 1), there was no relation between state 3 or state 4 respiration and age (state 3: $P = .910$; state 4: $P = .610$).

Discussion

In this study, we investigated the effect of pronounced weight loss induced by bariatric surgery on skeletal muscle mitochondrial function in morbidly obese patients. ADP-stimulated (state 3) respiration increased significantly 1 year after bariatric surgery. Our data suggest that impaired skeletal muscle mitochondrial function in morbidly obese

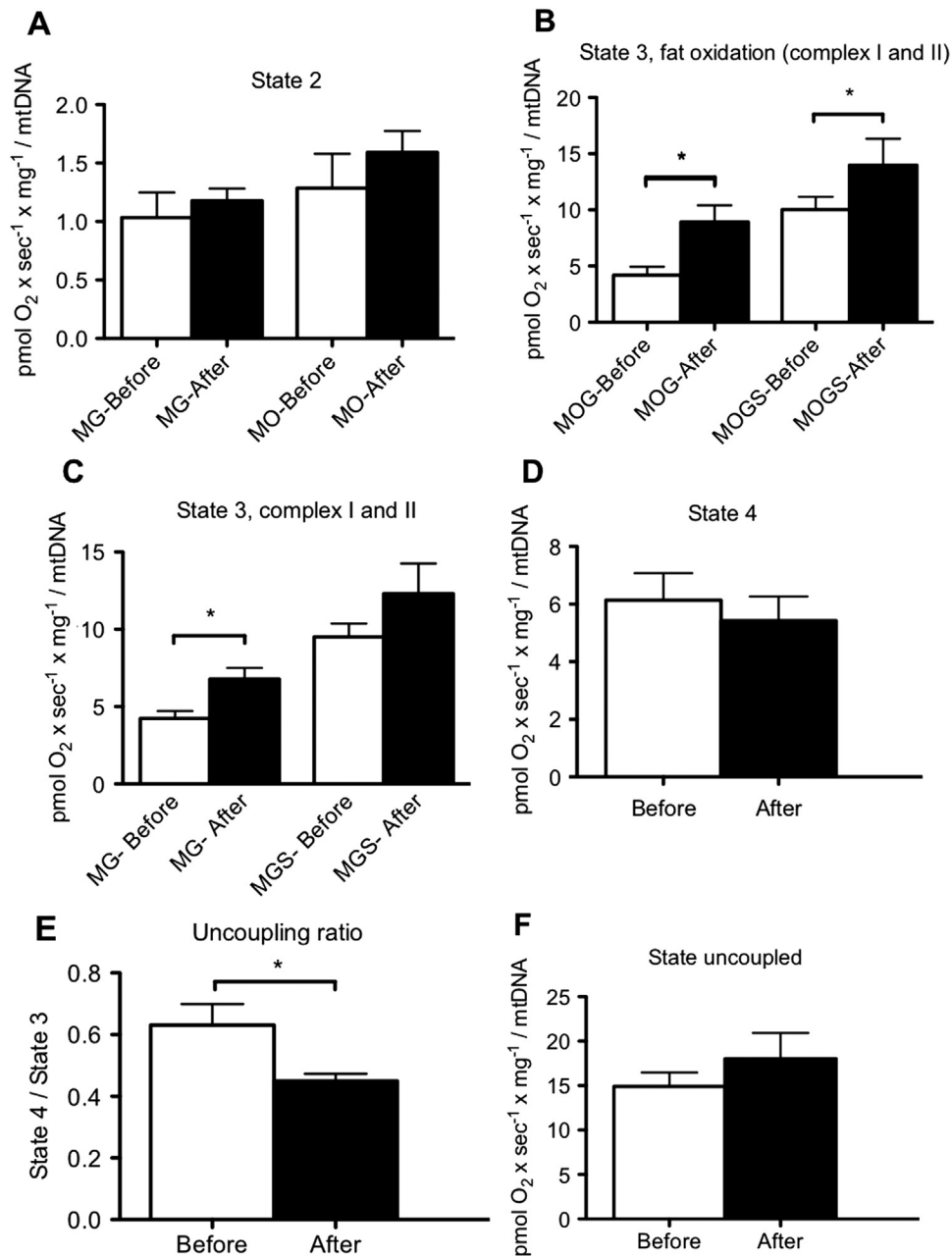


Fig. 1. Skeletal muscle fiber respirometry before (white bars) and after (black bars) weight loss. (A) Oxygen consumption in the presence of endogenous substrates (state 2 respiration) was similar before and after weight loss ($P = .310$). (B) Adenosine diphosphate (ADP)-stimulated state 3 respiration upon a lipid substrate for complex I (malate + octanoyl-carnitine + glutamate [MOG]) and complex I and II substrates (malate + octanoyl-carnitine + glutamate + succinate [MOGS]). (C) State 3 respiration upon complex I substrates (malate + glutamate [MG]) and complex I and II substrates (malate + glutamate + succinate [MGS]). (D) Leak (state 4) respiration obtained by addition of the ATP-synthase inhibitor oligomycin. (E) The relative contribution of state 4 to state 3 respiration (uncoupling ratio [UCR]). (F) Maximal respiration upon carbonyl-cyanide-4-(trifluoromethoxy)-phenylhydrazone (FCCP) (state uncoupled). O_2 flux shown: picomoles O_2 consumption \times seconds $^{-1}$ \times milligrams of tissue $^{-1}$ \times mtDNA $\times 10^6$. Values shown are mean + SEM, $n = 8$. * $P < .05$.

patients is reversible by bariatric surgery-induced weight loss.

Skeletal muscle fat oxidation is negatively related to BMI [16], resulting in an increased muscle fat accumulation in obesity [9,17]. This impaired lipid oxidation is associated with skeletal muscle insulin resistance and thus suggests a pathophysiologic basis for obesity-related type 2 diabetes

[9]. In line with this notion, skeletal muscle mitochondria from obese type 2 diabetic patients displayed a reduced activity of the mitochondrial oxidative enzyme NADH-reductase, suggesting an impairment of mitochondrial function [5], which was confirmed by in vivo measurements [18]. Furthermore, a detailed ex vivo analysis of mitochondrial function in permeabilized skeletal muscle fibers

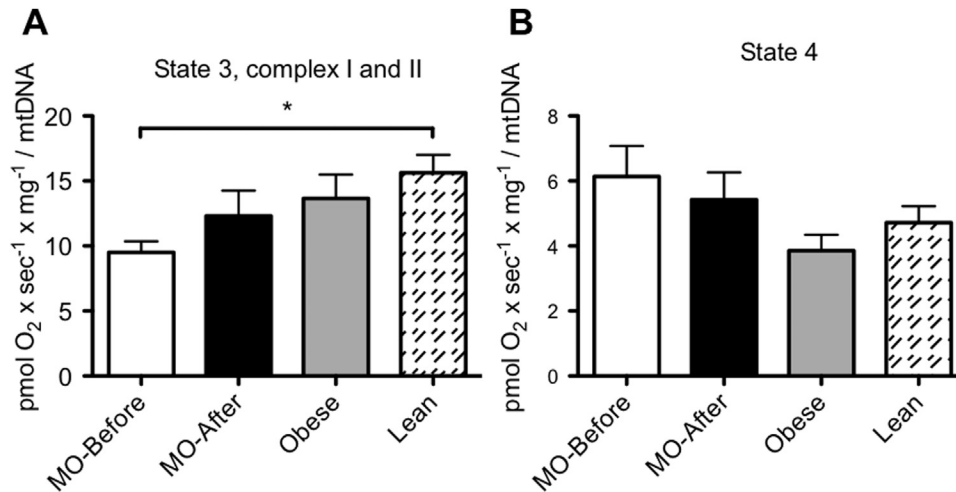


Fig. 2. Comparison between skeletal muscle mitochondrial function for morbidly obese patients before (MO-before) and after (MO-after) weight loss and lean and obese controls. (A) Adenosine diphosphate (ADP)-stimulated state 3 respiration upon complex I and II substrates malate, glutamate, and succinate. (B) Mitochondrial state 4 respiration after addition of oligomycin. O₂ flux shown: picomoles O₂ consumption × seconds⁻¹ × milligrams of tissue⁻¹ × mtDNA × 10⁶. Values shown are mean + SEM. **P* < .05.

showed that the observed in vivo mitochondrial dysfunction in overweight type 2 diabetes (mean BMI 28.9 kg/m²) was associated with a decreased intrinsic mitochondrial capacity, that is, ADP-stimulated (state 3) mitochondrial respiration corrected for mitochondrial density [3]. Skeletal muscle mitochondrial state 3 respiration was also reported to be reduced in nondiabetic morbidly obese patients eligible for bariatric surgery (mean BMI 40 kg/m²) compared with that for lean controls [6]. To observe whether obesity is causative for a decreased mitochondrial function, several reports have studied the effect of weight loss on skeletal muscle mitochondrial function, in both type 2 diabetic and nondiabetic patients. A diet-induced weight loss of 10.6 kg did not induce changes in mitochondrial NADH-reductase activity in nondiabetic obese patients (mean BMI before weight loss 33.4 kg/m²) [8], and 1 report even observed a decrease in mitochondrial cytochrome c oxidase activity after weight loss (12–17 kg weight loss; initial BMI 33–34 kg/m²) [10]. In contrast, after a more pronounced diet-induced weight loss in

nondiabetic patients (13–32 kg weight loss; initial BMI 33.8 kg/m²), an improved oxidative enzyme (succinate dehydrogenase) activity was observed [19].

Bariatric surgery results in pronounced weight loss, and after Roux-en-Y gastric bypass and biliopancreatic diversion surgery (42–63 kg weight loss; mean initial BMI 45.9–53.5 kg/m²), proteins associated with mitochondrial biogenesis (PGC1 α and MFN2) were enhanced in skeletal muscle and related to improved insulin sensitivity as assessed by euglycemic-hyperinsulinemic clamp [12,13]. Finally, in patients with an approximate 50-kg weight loss after Roux-en-Y gastric bypass, skeletal muscle fatty acid oxidation (as assessed ex vivo with ¹⁴C-palmitate) did not change and was decreased compared with lean controls [20]. However, the postoperative BMI was relatively high (36.5 kg/m²) compared with that of the present study (30 kg/m²).

In contrast to the aforementioned weight loss studies using biochemical assessments of mitochondrial enzyme activities to assess skeletal muscle mitochondrial function,

Table 2
Plasma values before and after weight loss.

	MO-before	MO-after	Obese	Lean	<i>P</i>			
						MO-before/MO-after	MO-before/obese	MO-after/lean
TSH (mU/mL)	2.0 ± .7	1.9 ± .4	—	—	.341	—	—	—
FT ₄ (pmol/L)	13.5 ± 2.0	14.9 ± 1.4	—	—	.009*	—	—	—
Glucose (mM)	4.9 ± .4	5.2 ± 1.0	5.4 ± .4	5.0 ± .2	.199	.016*	.436	
Insulin (μU/mL)	25.4 ± 8.5	16.6 ± 5.2	17.3 ± 5.4	10.0 ± 2.3	.064	.028*	.009*	
HOMA-IR	4.9 ± 2.9	3.8 ± 1.1	4.2 ± 1.5	2.2 ± .5	.324	.134	.005*	
FFA (μM)	588.3 ± 234.3	567.0 ± 283.4	280.1 ± 128.1	259.0 ± 84.4	.750	.003*	.005*	

FFA = free fatty acids; FT₄ = free thyroxine; HOMA-IR = homeostasis model assessment–insulin resistance; TSH = thyroid-stimulating hormone.

Values shown for 2 male and 6 female morbidly obese patients before (MO-before) and after (MO-after) weight loss compared with 10 male obese (obese) and 10 male lean (lean) control patients.

**P* < .05.

we performed a detailed characterization of mitochondrial function via ex vivo mitochondrial respiration in permeabilized muscle fibers. In this study, we show ADP-stimulated state 3 respiration increased after a mean 35-kg weight loss and restored to normal levels. The mitochondrial density (as assessed by mtDNA copy numbers) did not change after weight loss and therefore the observed improvement in mitochondrial respiration is an intrinsic mitochondrial characteristic. The improvement in muscle mitochondrial metabolism up to levels comparable to those of lean control patients suggests that the observed weight loss could “restore” obesity-induced impairments in skeletal muscle mitochondrial function. Further studies should elucidate the molecular and clinical changes associated with the increase skeletal muscle mitochondrial function. All included morbidly obese patients were nondiabetic, and plasma values (Table 2) for plasma insulin showed a nearly significant decrease ($P = .064$). However, the HOMA-IR did not change after weight loss ($P = .324$). Although fasting insulin levels tended to be lower after weight loss (mean reduction 28.6%) further studies in morbidly obese diabetic populations are necessary to determine the effect of weight loss on skeletal muscle mitochondrial function and its relation to obesity-induced insulin resistance.

Conclusion

This study shows an increase in ex vivo skeletal muscle mitochondrial function in morbidly obese patients 1 year after LAGB-induced weight loss. The level of weight loss in this study was higher than previous reports that did not observe differences in mitochondrial function after weight loss. Possibly, the very pronounced weight loss after bariatric surgery is necessary to recover mitochondrial function in morbid obesity.

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Disclosures

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

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