Mitochondrial capacity in skeletal muscle is not stimulated by weight loss, despite increases in insulin action and decreases in intramyocellular lipid content.

Frederico G.S. Toledo*, Elizabeth V. Menshikova, Koichiro Azuma, Zofia Radiková, Carol A. Kelley, Vladimir B. Ritov and David E. Kelley

Division of Endocrinology and Metabolism, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Running Title: Weight loss effects on muscle mitochondria

Corresponding Author: Frederico G.S. Toledo
3459 Fifth Avenue – Montefiore Hospital 807N
Pittsburgh, PA 15213

Clinical trial registry number NCT00222924.

Received for publication 5 October 2007 and accepted in revised form 12 January 2008.
ABSTRACT

Purpose: In obesity and type-2 diabetes, exercise combined with weight loss increases skeletal muscle mitochondrial capacity. It remains unclear whether mitochondrial capacity increases because of weight loss, improvements in IR or physical training. In this study, we examined the effects of an intervention of weight loss induced by diet and compared to a similar intervention of weight loss by diet with exercise. Both are known to improve IR, and we tested the hypothesis that physical activity, rather than improved IR, is required to increase mitochondrial capacity of muscle.

Methods: 16 sedentary overweight/obese volunteers were randomized to a 16-week intervention of diet (D) (n=7) or diet+exercise (D+E) (n=9). Insulin sensitivity was measured using euglycemic clamps. Mitochondria were examined in muscle biopsies before and after intervention. We measured mitochondrial content and size by electron microscopy, electron transport chain (ETC) activity, cardiolipin content and mtDNA content. Intra-myocellular content of lipid (IMCL) and fiber-type distribution were determined by histology.

Results: D and D+E achieved similar weight loss (10.8% and 9.2%, respectively); only D+E improved aerobic capacity. Insulin sensitivity improved similarly in both groups. Mitochondrial content and ETC activity increased following D+E, but remained unchanged following D, and mitochondrial size decreased with weight loss despite improvement in IR. IMCL decreased in D, but not in the D+E intervention.

Conclusions: Despite similar effects to improve IR, these interventions had differential effects on mitochondria. Clinically-significant weight loss in the absence of increased physical activity ameliorates IR and IMCL, but does not increase muscle mitochondrial capacity in obesity.

KEYWORDS: mitochondria, insulin resistance, skeletal muscle, obesity
Mitochondrial oxidative capacity is decreased in skeletal muscle of obese individuals and correlated with insulin resistance (IR) (1). It remains unclear, however, whether reduced mitochondrial capacity in skeletal muscle is causative for IR or instead arises as a consequence of IR (2). Recent clinical investigations from our group have demonstrated that the reduced muscle mitochondrial content and functional capacity in obesity and type-2 DM are reversible with moderate weight loss combined with moderate-intensity regular physical activity (3-5), a lifestyle intervention that was modeled upon contemporary clinical recommendations (6). It was also observed that increases in mitochondrial parameters correlated with improved insulin sensitivity (4) and in type-2 DM with glucose lowering (3). One supposition is that physical activity may be the most important factor for improving mitochondrial capacity in IR since exercise training promotes mitochondrial biogenesis in skeletal muscle of healthy individuals (7-9). Nonetheless, other explanations should be considered. Calorie restriction evokes a panoply of changes such as reduced visceral adiposity and intramyocellular lipid (IMCL) content, and increased insulin sensitivity. The role of improved IR, in particular, merits consideration because insulin signaling has been postulated to affect mitochondrial capacity in muscle (2), and therefore it is possible that it is the improvement in IR that induces improvements in mitochondrial content and function in obesity and type-2 DM.

It is well established that IR improves in response to weight loss. The addition of exercise to a weight loss intervention may not improve IR more than weight loss alone (10). Accordingly, weight loss alone may be more pivotal than physical activity for amelioration of IR in obesity. Though the relative contribution of exercise and weight loss to improving IR has received much attention, there are few reports that specifically address the effects of weight loss upon muscle mitochondrial biogenesis, which is in contrast to an abundant literature describing the effects of physical activity. This issue of the comparative effects of weight loss and physical activity on muscle mitochondrial has potential clinical importance in view of the association between mitochondrial dysfunction and IR. Kern et al observed an increase in skeletal muscle oxidative capacity in obese women following moderate weight loss, but the study did not include a specific metric of aerobic fitness (11). Simoneau et al (12), in an intervention study in which moderate weight loss was induced without changes in physical activity or aerobic fitness, observed no improvement in muscle oxidative enzyme activity (12). More recently, this issue was studied in the CALERIE study, though these participants were not obese (13). Calorie restriction was associated with increased expression of oxidative phosphorylation genes, but the related enzyme activities were unchanged (13).

The current study was undertaken to examine how mitochondrial capacity responds to diet-induced weight loss in IR and concomitantly compared the results to an intervention of weight loss plus moderate exercise. We hypothesized that both interventions would reduce systemic and central adiposity and improve IR, but that mitochondrial capacity would increase only with the addition of exercise. A goal of this study was to achieve similar weight loss in the two intervention arms and thereby strengthen comparative examination of changes in insulin sensitivity and of skeletal muscle mitochondria. Muscle biopsies were obtained for determination of mitochondrial size and volume density, electron transport
Weight loss effects on muscle mitochondria

SUBJECTS AND METHODS

Research volunteers. The protocol was approved by the University of Pittsburgh Institutional Review Board. All participants had a screening medical history, physical examination, and screening laboratory tests. We sought to select a study population with a high likelihood of having insulin resistance: eligibility criteria included a BMI > 28 kg/m², waist circumference > 94 cm (men) or > 80 cm (women) (14), weight stability (< 3 kg change in the prior 2 months), a sedentary lifestyle (< 20 min of exercise activity/week), and age > 30 years. Enrolled participants were all White-Caucasian or African-American, reflecting the demographics of Pittsburgh. Participants were excluded if they had diabetes, anemia, cardiopulmonary, neuromuscular or renal disease, abnormal urine sediment, abnormal TSH, alkaline-phosphatase, or serum transaminases greater than 2.5 times the upper limit of the reference range.

Lifestyle Intervention. After screening and baseline metabolic assessments, participants were randomized to a diet (D) or a diet plus exercise (D+E) intervention (n=7 and n=9, respectively). The goals were to achieve ≥7% weight loss in both groups within 16-20 weeks of intervention, and for those in the D+E group to exercise 3-5 days/week at moderate intensity (60–70% of maximal heart rate). The mean duration of intervention was 19.2±0.4 weeks in the D+E group and 18.6±0.7 weeks in the D group (P=0.42). To achieve weight loss, a research dietitian met weekly with participants to give instructions to reduce portion size, lower consumption of fat, maintain daily food records and undertake related behavior change to reduce calorie intake by approximately 25%. In the D+E group, most participants chose walking, on a treadmill or otherwise, for exercise and were instructed to begin with 30 min sessions for the first month, and increase to 40 min for the next month at which time a sub-maximal treadmill stress test was performed to adjust the exercise prescription. At least one session weekly was supervised by an exercise physiologist, and heart rate was recorded at each session, and participants were requested to maintain an exercise log and record heart rate during unsupervised exercise. Exercise intensity was quantified by the average heart rate recorded by a wireless monitor (Polar, Kempele, Finland) for each exercise session in the participant’s personal exercise log. This provided an estimate of energy expenditure during each exercise bout based on the regression of heart rate and VO₂ determined at baseline.

Metabolic assessments. Prior to and after interventions, all participants underwent metabolic and physical fitness evaluations, which included an oral 75-g glucose tolerance test after an overnight fasting, a glucose clamp to measure insulin sensitivity, body composition studies, exercise tests and a muscle biopsy. Participants were asked not to exercise for 2 days preceding the metabolic assessments. A modified Bruce treadmill protocol was used to measure maximal aerobic capacity. Fat mass and fat-free mass were assessed by dual-energy x-ray absorptiometry. Cross-sectional computed tomography (CT) images were obtained, centered at L₃₋₄ and the mid-thigh to examine abdominal and thigh adipose tissue distribution.

For metabolic studies and biopsies, volunteers were admitted to the University of Pittsburgh General Clinical Research Center. Following a standardized dinner (7 Kcal/kg; 50% carbohydrates, 20% protein and 30% fat), they fasted for at least 12 hours overnight. The next morning a primed (200
mg/m²), continuous (2 mg·min⁻¹·m⁻²) infusion of [6,6-²H₂]-glucose was started 150 min prior to initiating the euglycemic hyperinsulinemic clamp to measure rates of glucose utilization (Rd) and endogenous glucose production (EGP). These were calculated using non-steady-state equations based on plasma [6,6-²H₂]-glucose enrichment determined by GC/MS. The clamp was started with an insulin infusion (40 mU·m⁻²·min⁻¹) and plasma glucose maintained at 90±5 mg/dL (~5 mMol/L) for 4 hours with a variable dextrose infusion. Plasma and serum were sampled before and during the steady-state phase of the clamp for determinations of FFA, glucose and insulin concentrations.

Muscle biopsies. samples of vastus lateralis skeletal muscle were obtained by percutaneous needle biopsy after local lidocaine anesthesia. Approximately 50-70 mg of muscle tissue was obtained each time and immediately dissected of any adipose and connective tissue under low-magnification microscopy. A portion was saved for light and electron microscopy experiments and the remainder immediately stored in liquid nitrogen for biochemical determinations. Histological and biochemical assays were then conducted in parallel in paired samples when available.

Light Microscopy. samples were mounted in Cryomatrix (Shandon, Pittsburgh, PA), then frozen directly in isopentane cooled to its freezing point with liquid nitrogen. From each tissue block, serial transverse sections (8 µm) were cut using a cryostat at −20°C and then mounted on Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA). Immunohistochemistry with an anti-myosin monoclonal antibody was performed to identify fiber types (I, IIa, and IIb). Lipid content was determined with Oil-red-O staining as in previous studies (15). Using digital imaging software, regions of interest were outlined in the cytoplasm of each muscle fiber and average staining density determined. All values were subtracted from background density. Images were acquired with an optical microscope (Nikon Microphot-FXL, Tokyo, Japan) connected to a digital video camera (Sony, Tokyo, Japan) and analyzed using digital image software (MVIA, Monaca, PA).

Transmission Electron Microscopy. 10mg of muscle was cut into small pieces (1x1x2 mm), fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon for TEM. After tissue fixation, randomly-sampled transverse sections of muscle fibers were obtained followed by 10-12 random micrographs acquired with an electron microscope (JEM-1210, JEOL-Ltd., Tokyo, Japan) on a final magnification of 36,000x. Mitochondrial volume density. i.e., the fraction of the cell occupied by mitochondria, was determined in images of the inter-myofibrillar compartment as in our previous studies (3; 4), using a digitally overlapped grid of 144 intersection points and stereological analysis methodology. Mitochondrial cross-sectional area was determined by digital imaging software (Metamorph 6.3; Molecular Devices Corp., Sunnyvale, CA). Measurements were conducted in a blinded fashion.

Mitochondrial biochemistry. the remainder 20-30 mg of muscle available was homogenized as in our previous studies (16-18). To prepare soluble and particulate fractions, homogenates were centrifuged at 45,000g x 20min (17). The soluble fraction was mixed (1:1) with buffer containing 50% glycerol (17) and saved for analysis of creatine kinase. The pellet (particulate fraction) containing mitochondria was suspended in storage medium containing 0.1 mg/ml BSA, 20 µmol/l leupeptin, and 25% glycerol (17). To release mitochondria trapped by the myofibrillar matrix, the particulate fraction was treated with the KCl/pyrophosphate method (16; 18; 19). Samples were stored at -80°C before analyses.
NADH-oxidase activity and cardiolipin content in the particulate fraction were measured by HPLC (1; 20) and expressed relative to creatine kinase (CK) activity to normalize for minor variations in muscle content (17). As in our previous studies, CK activity (U/g wet weight tissue) was not affected by the interventions (diet+exercise: 5951±411 vs. 6352±576, diet-only: 5644±511 vs. 5520±439, means not statistically different).

**Mitochondrial DNA.** (mtDNA): mtDNA copy number was measured by quantitative PCR (TaqMan, Applied Biosystems) and expressed relative to nuclear DNA copy number (5). DNA was extracted from biopsy samples (QIAamp DNA Mini Kit; Qiagen, Chatsworth, CA). 20 ng was used as a template against cytochrome b for mtDNA genome and against β-globin for nuclear DNA.

**Statistics.** data are presented as mean±SEM unless otherwise indicated. Statistical significance was accepted with p-values ≤0.05. Baseline characteristics in Table 1 were statistically examined by two-tailed unpaired T-tests, after confirming variables followed a normal distribution. Gender distribution was examined with Fisher’s test. To examine the factors of group and intervention, we employed two-way ANOVA with repeated measures. For body weight, fat mass and insulin sensitivity we further examined whether the mean percent change with intervention was similar between groups using two-tailed t-tests.

**RESULTS**

**Baseline and Post-Intervention Clinical Characteristics.** as shown in Table 1, the baseline (pre-intervention) characteristics of volunteers in both groups were similar and IR was apparent in both groups. In both groups, mean glycemia after an oral glucose tolerance test was greater than the diagnostic threshold of impaired glucose tolerance.

Significant weight loss was achieved in both the D and the D+E interventions (Table 2): the mean individual weight loss was -10.8±1.6% in the D group and -9.2±1.2% in the D+E group, values that were not statistically different from each other. There was a nearly identical decrease in fat mass in both groups (19±3% and 18±3%; in D and D+E, respectively). Comparable reductions in adiposity within various regional depots occurred in both groups.

Though loss of fat mass was similar in the D and D+E intervention groups, there was a difference in effect on aerobic fitness (table 2). At baseline, maximal aerobic capacity (VO₂max; normalized to lean body mass) was similar in both groups. Participants in the D group were instructed not to alter previous patterns of physical activity and at the end of intervention these participants had not significantly changed VO₂max (mean change of 1.6±4.0% from baseline). In contrast, there was a significant increase in VO₂max in the D+E group (10.7±3.9% from baseline). Intervention logs indicated that D+E participants participated in the prescribed 4 sessions weekly; 1.5±0.2 sessions weekly under supervision at the research facility and 2.5±0.4 sessions weekly without supervision. Thus the intent of the study was attained, which was to have both groups achieve similar weight loss but differ in the change in fitness and with little change in the D intervention, allowing us to examine the effects of exercise beyond those attributable to weight loss alone.

**Effects on Insulin Sensitivity:** as shown in table 3, both groups had elevated fasting insulin concentrations, which decreased following intervention. Systemic insulin sensitivity was quantified using euglycemic clamps. Pre- and post-intervention steady-state plasma glucose concentrations were similar between groups. Steady-state plasma insulin during clamp studies was slightly lower after interventions, consistent with an
Weight loss effects on muscle mitochondria

increase in insulin clearance after weight loss. As shown in Figure 1, insulin-stimulated glucose disposal (M), increased following intervention in both groups. The mean percent change in response to intervention was +38±9% in the D+E group and +29±7% in the D group. These increases were statistically similar and remained similar when M was normalized for insulin concentrations during steady-state conditions (M/I). Therefore, the addition of moderate-intensity exercise to the weight loss intervention, despite attainment of improved aerobic fitness, did not further improve IR.

Effects on Muscle Fiber Type, IMCL and Mitochondria: skeletal muscle fiber-type distribution was not altered by either intervention, as shown in Figure 2. However, differing responses upon IMCL were observed, as shown in Figure 3. IMCL did not change from baseline in response to D+E, a finding consistent with prior observations (15; 21). In contrast, there was a significant decrease in IMCL following the D intervention. As shown in Fig 4, D+E increased mitochondrial density, reflecting an increase in mitochondrial content. The mean increase was 49±16% from baseline. In contrast, mitochondrial density remained unchanged in the D group. Also, distinct patterns of mitochondrial ultrastructural adaptation occurred between the two interventions (Fig. 4): mean mitochondrial size decreased in the D group, on average by -17±4% from baseline (P<0.05), but remained unchanged in the D+E group. Biochemical findings corroborated the microscopy data (Fig. 5). Cardiolipin content, a marker for the amount of the inner mitochondrial membrane, increased in the D+E group, but not in the D group. Likewise, ETC activity increased in response to the D+E intervention but remained unchanged following the D intervention. Mitochondrial DNA (mtDNA) encodes important components required for mitochondrial respiration, so we examined whether the mtDNA content (expressed as mtDNA copies relative to nuclear DNA copies) was affected by interventions. There were no significant changes in mtDNA following the D intervention (2297±439 vs. 2508±414; pre- and post-intervention, respectively) or the D+E intervention (2049±296 vs. 2185±338).

DISCUSSION

The effects of weight loss to improve obesity-related IR are well established, but there is less information concerning an impact on muscle mitochondria, an organelle that has begun to emerge as an independent target of intervention for improving IR. In the current study, both a D and a D+E intervention achieved approximately 10% weight loss and nearly identical loss of fat mass. This degree of weight loss is generally regarded as clinically significant, and consistent with this there was improvement in insulin-stimulated glucose disposal, indicating improved insulin sensitivity in muscle tissue. Notably, the improvement in IR was quite similar between D and D+E. However, there were differences between these interventions for the effect on muscle lipid content and muscle mitochondria. IMCL was reduced by D, yet this intervention did not improve mitochondrial capacity. In contrast, D+E led to an unambiguous increase in mitochondrial capacity, but did not lower IMCL; the latter finding being consistent with the notion that exercise training favors storage of triglyceride in muscle. These observations provide a valuable intervention-based perspective to examine the inter-actions of IR, obesity and skeletal muscle mitochondria.

The etiology of reduced mitochondrial capacity in skeletal muscle in IR is not yet fully established. Acute insulin administration increases mitochondrial ATP production (2; 22) and chronic treatment of diabetic individuals with subcutaneous insulin improves gene transcription for mitochondrial
Weight loss effects on muscle mitochondria

These studies give indirect support to the hypothesis that decreased mitochondrial capacity in IR might arise as a consequence of impaired insulin action. The alternative hypothesis is that mitochondrial dysfunction occurs as a primary abnormality and leads to IR (24). Studies from our laboratory and from other investigations have clearly shown that reduced mitochondrial capacity associated with obesity and type-2 DM is not a fixed defect. Rather, mitochondrial capacity can be considerably stimulated by an intervention combining moderate weight loss with moderate-intensity exercise (3; 4; 25). This intervention evokes a variety of physiological changes including decreased IR, and therefore the direction of causality between mitochondrial dysfunction and IR, if one does exist, can not be determined. Accordingly, the impetus for the current study was to achieve weight loss with and without concomitant exercise and compare effects on IR and on muscle mitochondria. In obese individuals with IR, improvement in IR occurs quickly upon initiating reduced caloric intake. Therefore, we postulated that if mitochondrial dysfunction in muscle arises as a consequence of IR, improvement in mitochondrial parameters should follow a period of sustained improvement in IR induced by weight loss. However, we did not observe this to occur, despite unequivocally improved IR and reduced IMCL. This result leads us to draw two conclusions: a) amelioration of IR is not contingent on a concomitant increase in mitochondrial capacity in muscle; and b) reduced mitochondrial content and functional capacity in obesity is not solely a consequence of IR.

Our data do not negate that insulin may promote effects on skeletal muscle mitochondria, as has been well documented under experimental conditions (23). However, our data do not support the notion that IR is a prime determinant of skeletal muscle mitochondrial dysfunction in obesity. The obese participants with IR experienced a robust improvement in insulin action, chronically compounded over 16 weeks. Yet, increases in mitochondrial content and oxidative function did not occur. In contrast, the D+E intervention resulted in a clear increase in mitochondrial content and oxidative capacity, confirming prior results in other cohorts of overweight/obese and type-2 diabetic subjects (3; 4). Such increases in mitochondrial content were observed in the setting of similar changes in body adiposity and IR as occurred with the D intervention, but with a differential effect on aerobic fitness. This observation indicates that physical activity is a chief factor controlling mitochondrial capacity in IR, calling into question whether it is sedentary behavior that is responsible for reduced mitochondrial capacity in obesity-related IR.

There are sparse prior data concerning the effects of weight loss on muscle oxidative capacity. Kern and colleagues reported an increase in muscle oxidative capacity among obese women following weight loss, but the contribution of physical activity in that intervention is uncertain, and aerobic capacity was not assessed (11). Simoneau reported that weight loss in obese IR adults did not increase oxidative enzyme activities in vastus lateralis, despite improvement in insulin sensitivity (12). In the present study, more detailed mitochondrial assessments were undertaken. In response to D, as noted above, we did not observe a change from baseline mitochondrial capacity, but we did find subtle ultrastructural mitochondrial changes. Mitochondrial size decreased by approximately 17%, an observation not previously reported. Interestingly, mitochondrial size has been correlated with insulin sensitivity (1; 4). In the present investigation, we show that weight loss induced even smaller mitochondria; thus dissociating this phenotype from IR. It is
potentially conceivable that the decrease in mitochondrial size is related to reduced energy metabolism as occurs with weight loss (26). Conversely, mitochondrial size has been shown to be increased in exercise-trained humans (27; 28) and to increase after exercise training in healthy, young subjects (29). Increased mitochondrial size has been postulated to support the high energy demands of exercise activity (30), reducing the diffusion distances to energy-demanding compartments like the sarcoplasmic reticulum and contractile apparatus (30). We have previously shown that some degree of mitochondrial enlargement can occur in obese non-diabetic and diabetic subjects following an intervention of moderate-intensity exercise with weight loss (3; 4). However, in the present study no mitochondrial enlargement was observed in the D+E group. The reason behind this incongruity among studies is unclear. A novel finding of our study is that weight loss may decrease mitochondrial size and thus one conceivable possibility is that the opposing effects of exercise training and weight loss upon mitochondrial size offset each other. Consistent with this notion, the magnitude of weight loss in the D+E group (9.2±1.2%) was more marked than in our former studies employing a similarly structured D+E intervention in sedentary obese individuals (8.4±2.0%) (4) and sedentary individuals with type-2 diabetes (7.1±0.9%) (3). On the other hand, other explanations should be considered. It remains uncertain whether differences in gender distribution among studies might have played a role as well, since the present study had more women (n=6) than men (n=3), while our former studies had a balanced male/female distribution. Despite these uncertainties, it is unlikely that the age of subjects was an influencing factor, because the mean age in the present study (42 years-old) is quite similar to that of our former studies with non-diabetics (39 years-old) and diabetics (44 years-old) (3; 4).

In the present study, there was no significant change in skeletal muscle mtDNA content after weight loss, with or without the addition of exercise. Other studies have also found that mitochondrial biogenesis can be induced by an intervention of weight loss and moderate intensity exercise in the absence of increased mtDNA (3; 5). Recently, however, the CALERIE study showed that calorie restriction in healthy, non-obese individuals increases skeletal muscle mtDNA (13). We believe our data are not necessarily incongruent with the latter observation due to the clear differences in characteristics of the research volunteers in the two studies. Those in the CALERIE study were lean or overweight and may not have had IR. Additionally, mtDNA content may not be the most accurate marker of mitochondrial mass in skeletal muscle because of the existence of a reticulated network of mitochondria in skeletal muscle (31).

Acute lipid loading of skeletal muscle achieved by infusion of a lipid emulsion is associated with decreased expression of PGC-1 and nuclear encoded mitochondrial genes (32), an observation that raises the suggestion that lipotoxicity may contribute to the pathogenesis of decreased oxidative enzyme activity in IR. On the other hand, recent studies in rodents indicate that a high fat diet is accompanied by increased oxidative capacity skeletal muscle (33). These observations implicate a possible role of intramyocellular lipid content upon mitochondria. In the current study, weight loss was associated with decreased IMCL content but this was not accompanied by changes in mitochondrial capacity. The most straightforward interpretation is that the chief determinant of oxidative capacity in skeletal muscle is physical activity, or more specifically, the energy demand created
during frequent bouts of physical activity rather than the level of IMCL or IR per se.

A limitation of our study is that it only examined vastus lateralis mitochondria. Therefore, it is uncertain whether our findings can be generalized to mitochondria of other muscles. Although this is a limitation, it allows for a direct comparison with findings from other human studies on IR and muscle mitochondria, since the majority of these published studies have also employed vastus lateralis muscle biopsies (1-5; 13; 22; 23; 25; 34).

In summary, moderate weight loss even when accompanied by reductions in IMCL and a significant improvement in IR does not augment mitochondrial content or function in obese adults with IR. These findings indicate that the reduced mitochondrial content of IR is unlikely a primary consequence of IR, and that improvement in skeletal muscle IR can occur independently of changes in mitochondrial capacity.

ACKNOWLEDGEMENTS

This study was supported by the University of Pittsburgh General Clinical Research Center (5 M01RR00056) and the Obesity and Nutrition Research Center (P30DK462) and was principally funded by National Institutes of Health-National Institute of Diabetes and Digestive and Kidney Diseases Grant DK49200-08. K.A. was supported by a mentor-based fellowship grant from the American Diabetes Association. Z.R. was a visiting postdoctoral fellow from the Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia. We express our appreciation to the research volunteers who participated in these studies, to Carol Kelley for her efforts as the research coordinator, and to the research nutritionists and exercise physiologists of the Obesity and Nutrition Research Center who implemented the intervention.
REFERENCES


33. Turner N, Bruce CR, Beale SM, Hoehn KL, So T, Rolph MS, Cooney GJ: Excess lipid availability increases mitochondrial fatty acid oxidative capacity in muscle: evidence against a
role for reduced fatty acid oxidation in lipid-induced insulin resistance in rodents. *Diabetes* 56:2085-2092, 2007

### TABLE 1
Basic characteristics of subjects:

<table>
<thead>
<tr>
<th></th>
<th>Diet and exercise group (n=9)</th>
<th>Diet-only group (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male / Female</td>
<td>3 / 6</td>
<td>3 / 4</td>
</tr>
<tr>
<td>Age (years)</td>
<td><strong>42.4 ±2.7</strong></td>
<td><strong>46.1 ±2.0</strong></td>
</tr>
<tr>
<td></td>
<td><em>Males:</em> 43.3 ±1.9</td>
<td>43.3 ±2.9</td>
</tr>
<tr>
<td></td>
<td><em>Females:</em> 42.0 ±4.0</td>
<td>48.2 ±2.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td><strong>34.8 ±1.1</strong></td>
<td><strong>33.4 ±1.2</strong></td>
</tr>
<tr>
<td></td>
<td><em>Males:</em> 34.6 ±2.1</td>
<td>33.6 ±2.0</td>
</tr>
<tr>
<td></td>
<td><em>Females:</em> 34.9 ±1.4</td>
<td>33.3 ±1.7</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td><strong>107.2 ±3.8</strong></td>
<td><strong>111.8 ±5.3</strong></td>
</tr>
<tr>
<td></td>
<td><em>Males:</em> 114.8 ±0.25</td>
<td>114.0 ±9.0</td>
</tr>
<tr>
<td></td>
<td><em>Females:</em> 104.7 ±4.7</td>
<td>110.1 ±7.4</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td><strong>5.5 ±0.1</strong></td>
<td><strong>5.5 ±0.1</strong></td>
</tr>
<tr>
<td></td>
<td><em>Males:</em> 5.4 ±0.1</td>
<td>5.2 ±0.3</td>
</tr>
<tr>
<td></td>
<td><em>Females:</em> 5.5 ±0.15</td>
<td>5.6 ±0.1</td>
</tr>
<tr>
<td>Glucose, fasting (mmol/l)</td>
<td><strong>5.35 ±0.15</strong></td>
<td><strong>5.48 ±0.10</strong></td>
</tr>
<tr>
<td></td>
<td><em>Males:</em> 5.69 ±0.36</td>
<td>5.46 ±0.24</td>
</tr>
<tr>
<td></td>
<td><em>Females:</em> 5.18 ±0.10</td>
<td>5.49 ±0.08</td>
</tr>
<tr>
<td>Glucose, 2h post challenge (mmol/l)</td>
<td><strong>7.83 ±0.50</strong></td>
<td><strong>9.05 ±0.44</strong></td>
</tr>
<tr>
<td></td>
<td><em>Males:</em> 7.42 ±1.18</td>
<td>9.46 ±1.02</td>
</tr>
<tr>
<td></td>
<td><em>Females:</em> 8.04 ±0.54</td>
<td>8.75 ±0.35</td>
</tr>
</tbody>
</table>

Legend: There were no statistically significant differences between groups at baseline.
TABLE 2
Effect of each intervention on aerobic capacity and adiposity

<table>
<thead>
<tr>
<th></th>
<th>Diet and exercise group</th>
<th>Diet only group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Whole-body adiposity:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>94.8 ±4.4</td>
<td>86.3 ±4.6</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>40.2 ±1.7</td>
<td>33.1 ±1.9</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>52.6 ±3.8</td>
<td>51.3 ±3.5</td>
</tr>
<tr>
<td>Regional adiposity:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-thigh SAT (cm²)</td>
<td>147.8 ±13.3</td>
<td>119.5 ±12.9</td>
</tr>
<tr>
<td>Abdominal SAT (cm²)</td>
<td>468.3 ±29.8</td>
<td>388.3 ±31.4</td>
</tr>
<tr>
<td>VAT (cm²)</td>
<td>202.5 ±27.8</td>
<td>150.9 ±23.1</td>
</tr>
<tr>
<td>Maximal Aerobic capacity:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂max (ml O₂ . min⁻¹ . kg LBM⁻¹)</td>
<td>47.6 ±2.5</td>
<td>52.4 ±2.2</td>
</tr>
</tbody>
</table>

- There were no statistical differences between groups. P values for the effect of the intervention are shown. Non-significant P values are indicated as “ns”
- Abbreviations: SAT=subcutaneous adipose tissue; VAT=visceral adipose tissue; LBM=lean body mass
TABLE 3
Hyperinsulinemic euglycemic clamps before and after the intervention

<table>
<thead>
<tr>
<th></th>
<th>Diet and exercise group</th>
<th>Diet only group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td><strong>Fasting:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma FFA (mmol/L)</td>
<td>0.54 ±0.04</td>
<td>0.53 ±0.04</td>
<td>ns</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>5.47 ±0.15</td>
<td>5.23 ±0.12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma insulin (µU/ml)</td>
<td>19.2 ±2.0</td>
<td>12.4 ±1.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>During hyperinsulinemic clamp:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>4.98 ±0.03</td>
<td>5.06 ±0.07</td>
<td>ns</td>
</tr>
<tr>
<td>Plasma insulin (µU/ml)</td>
<td>89.7 ±6.6</td>
<td>80.7 ±5.9</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Legend:

- P values for the effect of intervention are shown. “ns” stands for non-significant P values.
- Asterisk (*) indicates P<0.05 between groups before intervention.
**FIGURE 1**

Legend: Insulin sensitivity measured by euglycemic clamps performed before (white bars) and after intervention (black bars). Glucose disposal (M) adjusted for lean body mass (LBM) improved after intervention in both groups. (* indicates P<0.05 and # indicates P<0.01 after intervention). M/I indicates glucose disposal normalized for insulin concentrations during steady state. There were no differences of statistical significance in insulin sensitivity between groups.
FIGURE 2

Legend: % proportion of skeletal muscle fiber-types before and after intervention. Fiber type distribution did not change after intervention in both groups.
FIGURE 3

Legend: Content of intramyocellular lipid (IMCL) before (white bars) and after intervention (black bars). Only the diet-only intervention induced changes in IMCL content.
FIGURE 4

Legend:
Mitochondrial density and size assessed by transmission electron microscopy and morphometry before (white bars) and after intervention (black bars). Left panel: mitochondrial density (%). Right panel: mean mitochondrial size (µm^2). * indicates P<0.05 after intervention.
Mitochondrial capacity assessed by biochemistry: mitochondrial cardiolipin content (U/mU/CK) and mitochondrial NADH-oxidase activity (U/mU CK) were measured before (white bars) and after intervention (black bars). # indicates P<0.01 after intervention.