

Dysfunction of Mitochondria in Human Skeletal Muscle in Type 2 Diabetes

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Skeletal muscle is strongly dependent on oxidative phosphorylation for energy production. Because the insulin resistance of skeletal muscle in type 2 diabetes and obesity entails dysregulation of the oxidation of both carbohydrate and lipid fuels, the current study was undertaken to examine the potential contribution of perturbation of mitochondrial function. Vastus lateralis muscle was obtained by percutaneous biopsy during fasting conditions from lean ($n = 10$) and obese ($n = 10$) nondiabetic volunteers and from volunteers with type 2 diabetes ($n = 10$). The activity of rotenone-sensitive NADH:O₂ oxidoreductase, reflecting the overall activity of the respiratory chain, was measured in a mitochondrial fraction by a novel method based on providing access for NADH to intact mitochondria via alamethicin, a channel-forming antibiotic. Creatine kinase and citrate synthase activities were measured as markers of myocyte and mitochondria content, respectively. Activity of rotenone-sensitive NADH:O₂ oxidoreductase was normalized to creatine kinase activity, as was citrate synthase activity. NADH:O₂ oxidoreductase activity was lowest in type 2 diabetic subjects and highest in the lean volunteers (lean 0.95 ± 0.17 , obese 0.76 ± 0.30 , type 2 diabetes 0.56 ± 0.14 units/mU creatine kinase; $P < 0.005$). Also, citrate synthase activity was reduced in type 2 diabetic patients (lean 3.10 ± 0.74 , obese 3.24 ± 0.82 , type 2 diabetes 2.48 ± 0.47 units/mU creatine kinase; $P < 0.005$). As measured by electron microscopy, skeletal muscle mitochondria were smaller in type 2 diabetic and obese subjects than in muscle from lean volunteers ($P < 0.01$). We conclude that there is an impaired bioenergetic capacity of skeletal muscle mitochondria in type 2 diabetes, with some impairment also present in obesity. *Diabetes* 51: 2944–2950, 2002

Metabolism of both glucose and fatty acids by skeletal muscle is impaired in type 2 diabetes (1,2). The manifestations of insulin-resistant glucose metabolism include reduced glucose transport and phosphorylation and reduced rates of glycogen synthesis (3), whereas abnormal fatty acid metabolism entails increased accumulation of triglyceride and other lipid moieties as well as dysregulation of lipid oxidation during fasting and insulin-stimulated conditions (4,5). There are likely multiple factors that contribute to perturbations of both glucose and fatty acid metabolism. An impaired functional capacity of mitochondria is one factor that might contribute to perturbations in the metabolism of both substrates. A reduction in the activity of marker enzymes of oxidative pathways has been observed in skeletal muscle obtained from individuals with obesity and type 2 diabetes and correlates with the severity of insulin-resistant glucose metabolism (6). These findings raise the possibility of impaired mitochondrial function as an additional aspect of the pathogenesis of insulin resistance. An impaired mitochondrial capacity for fat oxidation during fasting conditions, as noted in obesity and type 2 diabetes (7), could lead to insulin-resistant glucose metabolism through the accumulation of lipid intermediates (8). It has also been postulated that impaired mitochondria function could directly contribute to insulin-resistant glucose metabolism due to inefficient provision of ATP for hexokinase as well as other reactions requiring phosphorylation (9).

Therefore, the current study was undertaken to test the hypothesis that skeletal muscle in obesity and type 2 diabetes has an impaired functional capacity of mitochondria. To test this hypothesis, we have assessed activity of the mitochondrial electron transport chain in human skeletal muscle and performed quantitative studies of the morphology of mitochondria in these samples. The specific activity of rotenone-sensitive NADH:O₂ oxidoreductase, representing the overall activity of the mitochondrial electron-transport chain, was measured. To permit assessment of the electron transport activity despite impermeability of the inner mitochondrial membrane for NADH, the antibiotic alamethicin was used. Alamethicin is a channel-forming antibiotic known to increase the permeability of biological membranes through the creation of transmembrane pores up to 20 Å in diameter, providing ready access for NADH to enter intact mitochondria, as previously demonstrated with rat skeletal muscle homogenate (10) and rat heart mitochondria (11). Creatine kinase and citrate synthase were measured in these samples as markers for muscle fiber content in tissue samples

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mtDNA, mitochondria DNA; FPG, fasting plasma glucose.

TABLE 1
Clinical characteristics of research volunteers

	Lean	Obese	Type 2 diabetes
<i>n</i> (F/M)	6/4	2/8	6/4
Age (years)	39 ± 2	41 ± 2	47 ± 2
BMI (kg/m ²)	23.1 ± 0.5	30.0 ± 1.2*	34.9 ± 1.3*
Fasting plasma glucose (mg/dl)	86 ± 2	94 ± 3	140 ± 14†
Fasting plasma insulin (pmol/l)	33 ± 21	56 ± 26	155 ± 25†
HbA _{1c}	5.2 ± 0.1	5.4 ± 0.1	7.5 ± 0.4†

Data are means ± SE. **P* < 0.05 vs. lean; †*P* < 0.05 vs. nondiabetic.

and as a marker of mitochondria content, respectively. Because it has long been recognized that functional capacity of mitochondria is closely related to its structural organization, in parallel with measurements of activity of the electron transport chain, transmission electron microscopy was done to examine the size and morphology of skeletal muscle mitochondria.

RESEARCH DESIGN AND METHODS

Research volunteers. The research volunteers who participated in these studies included 10 healthy normal weight individuals (6 women and 4 men), 10 obese nondiabetic and otherwise healthy individuals (2 women and 8 men), and 10 individuals (6 women and 4 men) with type 2 diabetes and obesity. The clinical characteristics are shown in Table 1. In addition to a lower BMI, lean subjects had lower fasting insulin than obese or type 2 diabetic participants. Lean and obese volunteers were ~10 years younger than the group with type 2 diabetes.

The research volunteers underwent a screening medical examination before participation and were free of known cardiovascular disease. Volunteers with type 2 diabetes enrolled in this study were treated with diet and exercise alone, a sulfonylurea, or metformin, and these agents were withdrawn at least 4 weeks before these studies. Written informed consent was obtained, and the research project was approved by the University of Pittsburgh Institutional Review Board.

Insulin sensitivity. Research volunteers underwent measurement of insulin sensitivity using the glucose clamp method (12) in conjunction with estimation by indirect calorimetry of systemic rates of carbohydrate and lipid oxidation (13). A percutaneous muscle biopsy to obtain tissue for *in vitro* studies was performed, as previously described (14).

On the evening before these procedures, research subjects were admitted to the University of Pittsburgh General Clinical Research Center and prepared for determinations of insulin sensitivity as previously described (15). To determine rates of overall glucose appearance in obese and type 2 diabetic subjects, a primed (20 μCi, increased in proportion to fasting plasma glucose [FPG] by the factor FPG/100)-continuous (0.20 μCi/min) infusion of [³H]glucose was started 2 h before insulin infusion so that steady-state conditions were attained during the final 30 min of the 4-h insulin infusion. During the insulin infusion, an adjustable infusion of 20% dextrose was given to maintain euglycemia and [³H]glucose was added to the dextrose infusion to maintain a stable specific activity (16). Isotope was not given to lean subjects, as it was assumed that endogenous glucose production was fully suppressed during the insulin infusion (40 mU · m⁻² · min⁻¹) in these subjects (17). Systemic indirect calorimetry was performed during the last 30 min of insulin infusion using an open-circuit spirometry metabolic monitor system (DeltaTrac, Anaheim, CA) to estimate glucose and lipid oxidation (13).

Glucose specific activity was determined with liquid scintillation spectrometry after deproteinization of plasma with barium sulfate and zinc hydroxide. Serum insulin was determined using a commercially available radioimmunoassay kit (Pharmacia, Uppsala, Sweden). Rates of plasma glucose appearance and utilization were calculated using the Steele equation, modified for variable rate glucose infusions that contain isotope (16). Nonoxidative glucose disposal was calculated as the difference between glucose utilization and glucose oxidation.

Electron microscopy. For muscle tissue obtained at biopsy, ~50 mg was immediately frozen in liquid nitrogen and stored at -70°C until analyses. The portion of the sample to be used for electron microscopy was cut into small pieces (1 × 1 × 2 mm) and fixed in 2.5% glutaraldehyde, postfixed in 1%

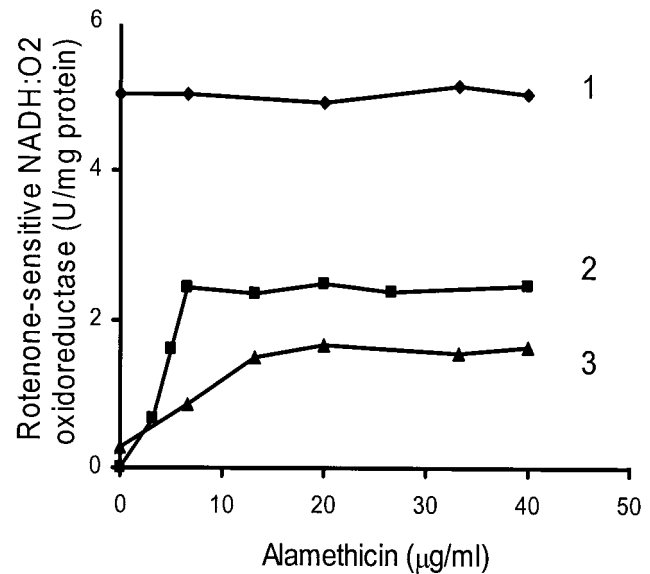


FIG. 1. Effect of alamethicin on rotenone-sensitive NADH:O₂ oxidoreductase activity in bovine heart submitochondrial particles (1), rat liver mitochondria (2), and rat heart homogenate (3).

osmium tetroxide, dehydrated, and embedded in Epon at either a longitudinal or transverse orientation. A smaller piece (0.5 × 0.5 × 1 mm) was prepared for immunoelectron microscopy by fixation in 2% paraformaldehyde and 0.0128% glutaraldehyde for 1 h. After an initial low-power screening of semithin (300 nm) sections stained with toluidine blue to optimize the plane of sectioning (18), ultrathin (60 nm) longitudinal and transverse sections were cut for each sample. The sections were mounted on copper grids and stained with lead citrate (19) and uranyl acetate (20). For each biopsy, at least 10 longitudinal and transverse sections were examined by transmission electron microscopy (JEOL 100CXII) at an accelerating voltage of 80 kV. A minimum of 10 micrographs were taken at 19,000× magnification. The micrographs were scanned by a computer-linked scanner using Fotolook PS 2.09.1 software (AGFA-GEVAERT, Ridgefield Park, NJ). The area of mitochondria in the transverse and longitudinal orientations was measured using an image analysis system (National Institutes of Health image 1.61). The muscle fiber type was identified by measuring the Z line width. For immunoelectron microscopy, a monoclonal mouse anti-human 65-kDa mitochondria protein (Chemicon International, Temecula, CA) was used.

Measurement of NADH:O₂ oxidoreductase. Muscle homogenate and particulate fractions from tissue samples (~10 mg) were prepared as previously described (21). Standard procedures for preparation of mitochondria from skeletal muscle have a yield of ~50% (22), probably because a significant proportion of muscle mitochondria remain embedded within the dense myofibrillar matrix. To unmask mitochondria, the particulate fraction was treated with 0.6 mol/l KCl (23,24) in the presence of 15 mmol/l sodium pyrophosphate (25). To verify that alamethicin, which was used to render the inner mitochondrial membrane permeable, did not have a direct effect on NADH oxidation by the respiratory chain, submitochondrial particles were prepared from beef heart by the Keilin-Hartree method (26). These submitochondrial particles are fragments of the inner mitochondrial membrane with an "inside-out" orientation or "open" membranes and therefore have unrestricted access to exogenous NADH (26).

In Fig. 1, submitochondrial particles from beef heart are shown to oxidize NADH at a high rate (5 μmol · min⁻¹ · mg protein⁻¹ at 37°C), and this was not affected by the addition of alamethicin in concentrations up to 40 μg/ml. In contrast, as shown in Fig. 1, fresh (intact) rat liver mitochondria do not reveal rotenone-sensitive NADH:O₂ oxidoreductase activity (due to impermeability of NADH), but after incubation with alamethicin, oxidation of exogenous NADH was robust and concentration dependent. Maximal rates of oxidation were observed at an alamethicin concentration of ~7 μg/ml, and increases of alamethicin up to 40 μg/ml did not affect NADH:O₂ oxidoreductase activity. Thus, alamethicin, although not having direct effects on the respiratory chain, renders the pathway accessible to assay in intact mitochondria.

We also tested the effect of alamethicin on the activity of mitochondrial rotenone-sensitive NADH:O₂ oxidoreductase activity in homogenate prepared from rat myocardium (Fig. 1). We found that the optimal activity of rotenone-sensitive NADH:O₂ oxidoreductase in myocardium homogenate can only be observed in the presence of added cytochrome c, which is explained by the

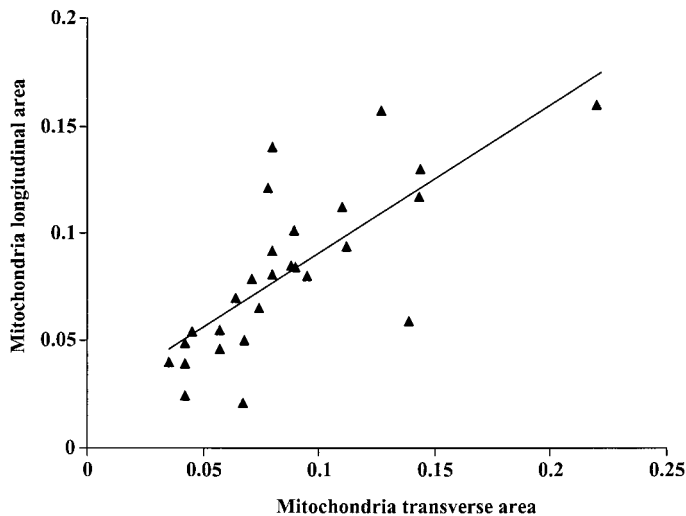


FIG. 2. Correlation ($r = 0.76$, $P < 0.001$) between mitochondria area measured in the longitudinal axis and the area measured in the transverse axis on electron micrographs of skeletal muscle.

release of endogenous cytochrome c from mitochondria through pores created by alamethicin. In studies of human skeletal muscle, the incubation medium for NADH:O₂ oxidoreductase contained 10 $\mu\text{mol/l}$ cytochrome c. To prepare the sample for assay for activity of NADH:O₂ oxidoreductase, an aliquot of the particulate fraction (200 μl) was added to 200 μl of concentrated extraction medium containing 30 mmol/l Na₄-pyrophosphate (pH 7.3 at 21°C), 1.2 mol/l KCl, and 2 mmol/l EGTA. The mixture was vortexed, incubated for 60 min on ice, and then homogenized by a Pellet Pestle.

An aliquot of the KCl pyrophosphate-treated particulate fraction (50 μl) was diluted by 450 μl diluent mixture containing 5 mmol/l MgCl₂, 0.2 mmol/l EGTA, 0.5 mg/ml BSA, 40 $\mu\text{g/ml}$ alamethicin, 0.1 mmol/l deferoxamine, 10 $\mu\text{mol/l}$ cytochrome c, 0.3 mg/ml phosphatidylcholine from soybean, 10 mmol/l HEPES, and 10 mmol/l histidine, at pH 7.5 and 21°C, and then homogenized by Pellet Pestle and kept on ice before assay. The reaction was started by mixing 50 μl diluted sample with 50 μl diluent mixture that contained 0.3 mmol/l NADH with or without 4 $\mu\text{mol/l}$ rotenone, and the mixture was incubated at 30°C for 10 min. As a control, samples were incubated in the presence of 2 $\mu\text{mol/l}$ rotenone, which blocks activity of NADH:O₂ oxidoreductase. Reactions were terminated by the addition of 12 μl of 1 mol/l HCl to destroy remaining NADH, neutralized, incubated with alcohol dehydrogenase and ethanol, and assayed as previously described (21). Activity of citrate synthase was determined by high-performance liquid chromatography monitoring of the generation of CoA-SH after conversion to a fluorescent adduct in a reaction with ThioGlo-1 (27). Activity of creatine kinase was measured at 30°C by monitoring of the generation of NADPH in a coupled enzymatic reaction (hexokinase/glucose-6P dehydrogenase) (28).

Statistics. Data are presented as means \pm SE, unless otherwise indicated. ANOVA was used to compare groups. Linear regression and stepwise multiple regression was used to examine the relationships of muscle enzyme activity or mitochondria size and insulin sensitivity.

RESULTS

Insulin sensitivity. There were significant group differences in insulin sensitivity ($P < 0.001$), rates of insulin-stimulated systemic glucose oxidation ($P < 0.001$), and nonoxidative glucose metabolism ($P < 0.001$) during euglycemic clamp conditions. The group values of lean, obese, and type 2 diabetic subjects for insulin-stimulated glucose utilization were 6.38 ± 0.74 , 4.66 ± 0.12 , and 1.65 ± 0.85 $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, respectively. For insulin-stimulated systemic glucose oxidation, the respective values were 3.16 ± 0.68 , 2.37 ± 0.81 , and 1.70 ± 0.41 $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. For insulin-stimulated systemic nonoxidative glucose metabolism, the respective values were 3.22 ± 0.35 , 2.28 ± 0.48 , and -0.01 ± 0.38 $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$.

TABLE 2

Electron microscopy measurements of mitochondria size in vastus lateralis skeletal muscle from lean and obese nondiabetic volunteers and type 2 diabetic subjects

	Z line width (nm)	Mitochondria area (μm^2)	
		Longitudinal	Transverse
Lean	0.09 ± 0.002	0.114 ± 0.02	0.116 ± 0.01
Obese	0.08 ± 0.004	$0.076 \pm 0.01^*$	$0.063 \pm 0.01^*$
Type 2 diabetes	0.08 ± 0.004	$0.063 \pm 0.01^*$	$0.063 \pm 0.01^*$

Data are means \pm SE. * $P < 0.01$ vs. lean.

Activity of NADH:O₂ oxidoreductase and citrate synthase in skeletal muscle. Skeletal muscle NADH:O₂ oxidoreductase activity was lower in type 2 diabetic subjects (lean 0.95 ± 0.17 , obese 0.76 ± 0.30 , type 2 diabetes 0.56 ± 0.14 units/mU creatine kinase; $P < 0.005$). Also, citrate synthase activity was reduced in those with type 2 diabetes (lean 3.23 ± 0.20 , obese 3.13 ± 0.31 , type 2 diabetes 2.44 ± 0.16 units/mU creatine kinase; $P < 0.005$). The activity of skeletal muscle NADH:O₂ oxidoreductase was reduced in obese compared with lean volunteers, whereas values for citrate synthase activity were not significantly different in lean and obese volunteers. The ratio of activities of skeletal muscle NADH:O₂ oxidoreductase and citrate synthase was examined to assess whether there was any effect of group on this proportionality. These ratios (lean 0.30 ± 0.03 , obese 0.25 ± 0.04 , type 2 diabetes 0.23 ± 0.02) did not differ significantly across groups ($P = 0.17$). There was no significant effect of gender on the activities of citrate synthase or NADH:O₂ oxidoreductase.

These enzyme activities, measured in the particulate fractions of skeletal muscle, are expressed relative to the activity of creatine kinase rather than wet weight of samples because potential inclusion of connective tissue, fat, or blood could significantly affect the specific activity of muscle enzymes (29). Creatine kinase activity was similar in skeletal muscle from lean ($5,033 \pm 1,139$ units/g wet wt), obese ($4,601 \pm 970$), and type 2 diabetic subjects ($4,987 \pm 652$) (means \pm SD).

Skeletal muscle activity of NADH:O₂ oxidoreductase correlated with insulin sensitivity measured by the glucose clamp technique ($r = 0.56$, $P < 0.01$). Similarly, insulin sensitivity was greater in those individuals with higher values for activity of skeletal muscle citrate synthase ($r = 0.52$, $P < 0.01$). The activity of NADH:O₂ oxidoreductase was negatively related to obesity ($r = -0.50$, $P < 0.01$).

Mitochondria morphology. The mitochondria examined by electron microscopy in this study were central mitochondria, mostly located near the Z line in longitudinal sections and between myofilaments in transverse sections. To control for the potential effect of fiber type on mitochondria size, the Z line width was also measured in the longitudinal sections, and the mean value was very close within the three groups, ranging from 0.08 to 0.09 nm (Table 2).

Mitochondria were smaller in skeletal muscle from obese volunteers and type 2 diabetic patients than in muscle from lean volunteers (Table 2). The mitochondria area was reduced by $\sim 35\%$ in type 2 diabetes and obesity, regardless of whether this was measured in longitudinal or transverse sections. There was a strong correlation be-

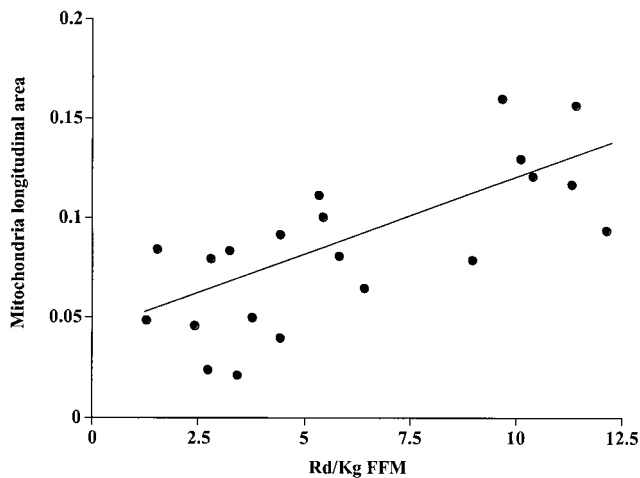


FIG. 3. Insulin sensitivity measured by the euglycemic clamp method plotted against skeletal muscle mitochondrial size measured by electron microscopy. There was a positive correlation between these variables ($r = 0.72$, $P < 0.01$). FFM, fat-free mass; Rd, glucose disposal rate.

tween measurements of mitochondria area taken in the longitudinal and transverse sections ($r = 0.76$, $P < 0.001$) (Fig. 2).

The size of mitochondria was also correlated with glucose disposal rate, the clamp-determined measurement of insulin sensitivity ($r = 0.72$, $P < 0.01$) (Fig. 3). A significant correlation did not exist between age or glycemic control (FPG or HbA_{1c}) and size of mitochondria.

In addition to having a smaller mean size, there were other morphological differences noted in the mitochondria in skeletal muscle from volunteers with type 2 diabetes and obesity. As shown in representative photographs in Fig. 4, mitochondria from muscle in lean volunteers have a more clearly defined internal membrane structure, including wider cristae, than those from obese and type 2 diabetic subjects.

In some of the muscle samples obtained from obese volunteers (3 of 10 subjects) and type 2 diabetic volunteers (4 of 10 subjects), a number of very large vacuoles were evidenced in the muscle fibers (Fig. 5).

These vacuoles were not seen in any of the samples from lean volunteers. The vacuole structures have a thick wall, segments of which have several layers of a double membrane structure, whereas the internal area is of a low density. Although the low density of the interior of the vacuoles resembled that of lipid droplets, the fragments of the membrane structure suggested the possibility of degenerated mitochondria. This was confirmed using immunoelectron microscopy, in which a mouse monoclonal anti-human 65-kDa mitochondria protein antibody was found to bind to these membrane structures.

DISCUSSION

Perturbations in the regulation of glucose and lipid metabolism are both involved in the insulin resistance in skeletal muscle in obesity and type 2 diabetes (2,3). Previously, our laboratory (30) as well as others (31) have observed that the severity of skeletal muscle insulin resistance in type 2 diabetes and obesity is related to diminished activity of oxidative enzymes. In addition, accumulation of triglycerides in skeletal muscle is also correlated with the severity

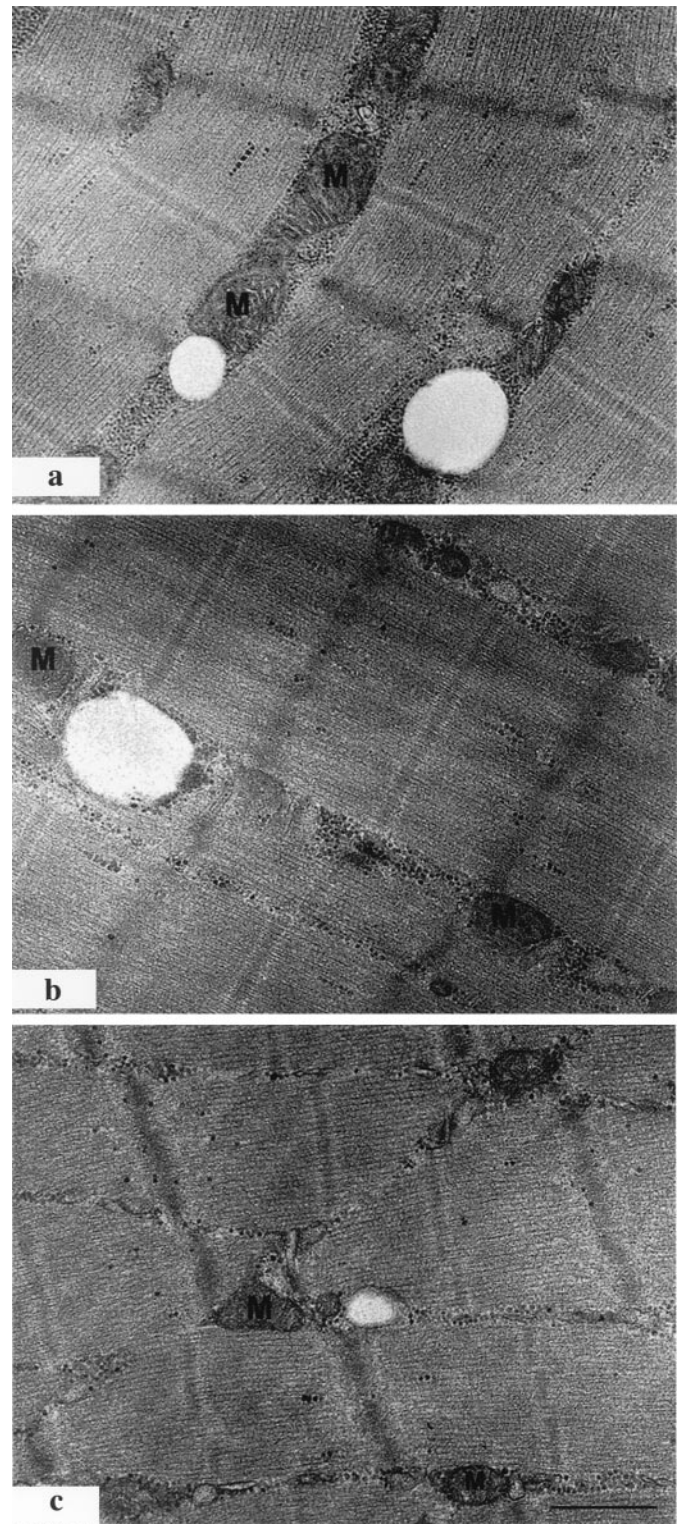


FIG. 4. Representative electron micrographs of skeletal muscle from a lean volunteer (a), an obese volunteer (b), and a volunteer with type 2 diabetes (c). The bar in the bottom right corner of c is 0.5 μ m. Several mitochondria are labeled with the letter M.

of insulin resistance and with diminished oxidative enzyme activity in these disorders (23). These observations led therefore to the hypothesis of the current investigation, which is that a functional impairment of mitochondria might contribute to the pathogenesis of insulin resistance in skeletal muscle.

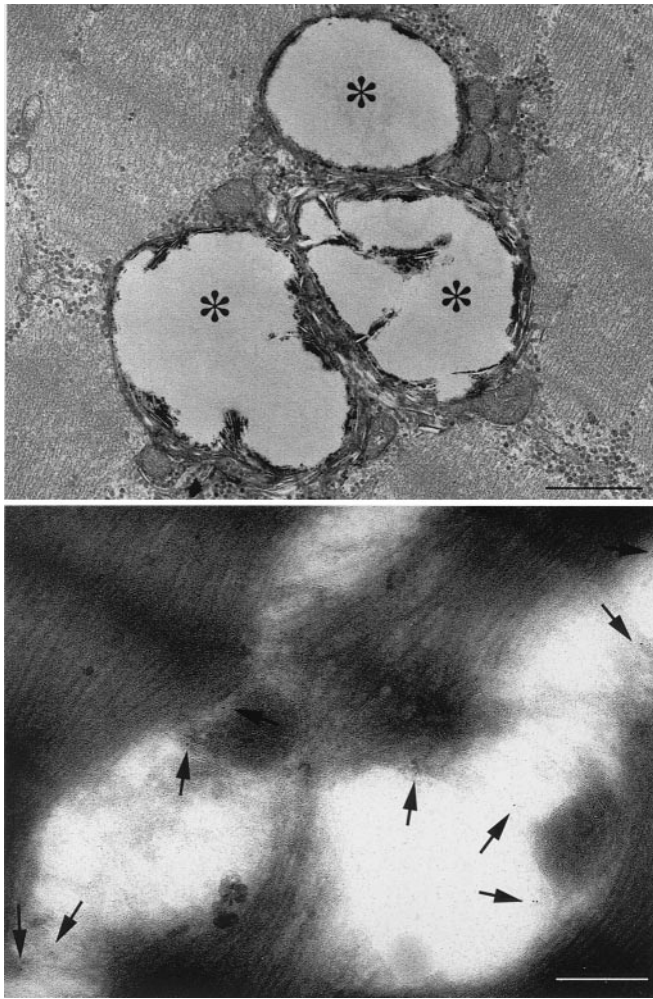


FIG. 5. Upper panel: immunogold particle staining of the membrane after incubation with an antibody against the human 65-kDa mitochondrial membrane protein. The magnification bar is 0.5 μm . Bottom panel: higher magnification view of the vacuoles observed in skeletal muscle from some individuals with type 2 diabetes or obesity, and noted to have a double-membrane structure consistent with mitochondria. The magnification bar is 0.28 μm .

Skeletal muscle is a tissue richly endowed with mitochondria and strongly reliant on oxidative phosphorylation for energy production. To test our hypothesis, we assessed the size and morphology of skeletal muscle mitochondria using electron microscopy and measured the activity of the electron transport chain. Both the parameters of mitochondria structure and functional capacity demonstrated perturbations in type 2 diabetes and, to a lesser degree, in obesity. Activity of rotenone-sensitive NADH: O_2 oxidoreductase was found to be reduced by $\sim 40\%$ in skeletal muscle from patients with type 2 diabetes. Skeletal muscle mitochondria were smaller in obesity and type 2 diabetes, and in some instances, particularly in type 2 diabetes, there was evidence of severely damaged mitochondria. Both findings correlated with the degree of insulin resistance.

The hypothesis that impaired functional capacity of mitochondria might contribute to insulin resistance in skeletal muscle is novel but not without important precedents (24,30). Others had earlier reported reduced activity of mitochondria tricarboxylic acid cycle enzymes in skeletal muscle in type 2 diabetic subjects (32–34). Distur-

bances of mitochondrial function in muscle and other tissue can lead to lipid accumulation (35), which in turn can cause or aggravate insulin resistance.

Measurement of rotenone-sensitive NADH: O_2 oxidoreductase activity provides an appropriate *in vitro* index of the overall capacity of the electron transport chain (36). However, assay of the activity of NADH: O_2 oxidoreductase activity in intact mitochondria isolated from frozen biopsy samples presents a technical challenge. The inner mitochondrial membrane is impermeable to NADH. In the current study, to assay the functional capacity of the electron transport chain in mitochondria isolated from frozen human skeletal muscle, the channel-forming antibiotic alamethicin was used (37).

The second major novel finding of the current study, one that is wholly consistent with the data on functional impairment of mitochondria, is that the morphology of mitochondria is altered in skeletal muscle in obesity and type 2 diabetes. Using transmission electron microscopy to measure the area of mitochondria, we found that it is significantly smaller in obesity and type 2 diabetes. To our knowledge, this is the first report of this finding. Altered morphology of mitochondria is a hallmark of a number of different myopathies known to result in disturbances of biochemical function of mitochondria, and the morphological examination of mitochondria has traditionally been regarded as a key complement to functional studies (35,38). Age is one factor known to affect the size of mitochondria in skeletal muscle, as mitochondria size is larger in infancy and gradually becomes smaller with aging (39); however, the small differences in age across the groups in this study would not account for the $\sim 30\%$ reduction in size in obesity and type 2 diabetes. Mitochondria can also vary in relation to muscle fiber type (40). It can be difficult to assess muscle fiber type at the magnification level of transmission electron microscope, but one criterion is to examine Z line width (40). Z line width was similar across groups for the muscle fibers used for analysis of mitochondria size in the current study.

Altered mitochondrial morphology in diabetes has been reported for tissues other than muscle, including neurons, and, for the most part, in animal models of diabetes (41,42). In some reports, swelling and disruption of mitochondria have been observed (43). In the current study, and only in muscle from obese individuals or those with type 2 diabetes, enlarged, fractured mitochondria were observed. Destruction of mitochondria is a key part of apoptosis, and whether the disrupted mitochondria observed in the current study signify increased apoptosis in skeletal muscle in type 2 diabetes and obesity will require further investigation.

There are at least several potential mechanisms by which impaired mitochondria function might contribute to insulin resistance of skeletal muscle. One such mechanism could be lipid accumulation within myocytes. Previous studies from our group, as well as work of others, have found that increased lipid accumulation in muscle is associated with insulin resistance and that, in turn, lipid accumulation in skeletal muscle in obesity and type 2 diabetes is related to reduced oxidative enzyme capacity (23). Insulin resistance might therefore develop as a consequence of lipid accumulation in skeletal muscle (8),

and defects in muscle lipid oxidation due to impaired mitochondria number or function could contribute fundamentally to this process. Gerbitz et al. (9) have postulated that disturbed oxidative phosphorylation capacity could be a direct cause of insulin resistance. In mitochondria, hexokinase is bound on the outer mitochondria membrane at contact sites in the proximity of adenine nucleotide translocator (44).

Equally or perhaps even more important than understanding how impaired mitochondrial function contributes to skeletal muscle insulin resistance is to understand why mitochondria are smaller and less efficient at electron transport in obesity and type 2 diabetes. One simple explanation could be that the individuals we studied were generally sedentary; it is well known that exercise increases mitochondria biogenesis, and the lack of exercise has an opposite effect (45). In this regard, exercise intervention studies would be very useful. However, a number of other mechanisms could also be considered. Altered phospholipid composition, especially of the inner mitochondria membrane, which is enriched in cardiolipin, could contribute to reduced mitochondrial function (46). An excess of long-chain fatty acid CoA, which can occur with caloric excess and obesity in type 2 diabetes, could damage mitochondria either directly or by channeling of palmitoyl-CoA to increased ceramide synthesis (47). Mitochondria DNA (mtDNA) is more susceptible than nuclear DNA to damage and has a less efficient repair mechanism; therefore, it is more likely to develop acquired mutations, as has been reported for skeletal muscle in aging (48,49). Increased mtDNA mutations have been reported in type 2 diabetic patients (50). One source of injury to mtDNA could be reactive oxygen species, and increased free radical damage to mtDNA has been reported for other tissues in type 2 diabetic patients (51).

In summary, in the current study we examined a novel hypothesis of impaired functional capacity of mitochondria in the pathogenesis of skeletal muscle insulin resistance in type 2 diabetic patients. The findings support this hypothesis, since mitochondria were found to be smaller and to have reduced activity of complex I of the electron transport chain. Further studies are needed to assess the inherited and acquired aspects of damage to mitochondria in type 2 diabetic subjects and to determine whether functional impairments are remedial.

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