

Deficiency of Subsarcolemmal Mitochondria in Obesity and Type 2 Diabetes

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The current study addresses a novel hypothesis of subcellular distribution of mitochondrial dysfunction in skeletal muscle in type 2 diabetes. Vastus lateralis muscle was obtained by percutaneous biopsy from 11 volunteers with type 2 diabetes; 12 age-, sex-, and weight-matched obese sedentary nondiabetic volunteers; and 8 lean volunteers. Subsarcolemmal and intermyofibrillar mitochondrial fractions were isolated by differential centrifugation and digestion techniques. Overall electron transport chain activity was similar in type 2 diabetic and obese subjects, but subsarcolemmal mitochondria electron transport chain activity was reduced in type 2 diabetic subjects (0.017 ± 0.003 vs. 0.034 ± 0.007 units/mU creatine kinase [CK], $P = 0.01$) and sevenfold reduced compared with lean subjects ($P < 0.01$). Electron transport chain activity in intermyofibrillar mitochondria was similar in type 2 diabetic and obese subjects, though reduced compared with lean subjects. A reduction in subsarcolemmal mitochondria was confirmed by transmission electron microscopy. Although mtDNA was lower in type 2 diabetic and obese subjects, the decrement in electron transport chain activity was proportionately greater, indicating functional impairment. Because of the potential importance of subsarcolemmal mitochondria for signal transduction and substrate transport, this deficit may contribute to the pathogenesis of muscle insulin resistance in type 2 diabetes. *Diabetes* 54:8–14, 2005

Skeletal muscle has a high reliance on oxidative phosphorylation. Distribution of energy within myocytes is served not only by the ATP-phosphocreatine shuttle but also by subcellular compartmentalization of mitochondria in skeletal muscle (1,2). Energy generated by oxidative phosphorylation in support of muscle contraction is largely generated by mitochondria located in an intermyofibrillar location, characteristically near the Z-line of muscle fibers (3). In contrast, subsarcolemmal mitochondria generate ATP for numerous

energy-requiring processes at the cell surface, including ion exchange, substrate transport, cell signaling, and protein synthesis (4). It has previously been postulated that the functional capacity of subsarcolemmal mitochondria may be crucial for fatty acid oxidation, glucose transport, and propagation of insulin signaling or other signaling cascades (4), each of importance to insulin action and thus potentially to the pathogenesis of insulin resistance in type 2 diabetes and obesity (5–7). However, the relation of subcellular distributions of mitochondria in human skeletal muscle to insulin resistance in type 2 diabetes and obesity has not been previously examined.

Prior studies have found that reduced oxidative enzyme capacity, measured in muscle homogenate, is correlated with severity of insulin resistance (8,9), a reduced capacity for lipid oxidation (10), and increased risk for obesity (11). Recently, we reported that activity of the electron transport chain is reduced and that intermyofibrillar mitochondria are smaller in type 2 diabetes (12). Both size of intermyofibrillar mitochondria and electron transport chain activity in muscle homogenate correlated with severity of insulin resistance (12). However, in that earlier study, the subpopulation of subsarcolemmal mitochondria was not specifically examined, either morphologically or with respect to electron transport chain activity. Given the importance of insulin signaling and glucose transport to insulin action, the current study was undertaken to test a novel hypothesis that a depletion of subsarcolemmal mitochondria is related to the pathogenesis of insulin resistance in muscle. To test this hypothesis, electron transport chain activity in subsarcolemmal mitochondria and intermyofibrillar mitochondrial fractions was assayed, and subsarcolemmal mitochondria were examined using a transmission electron microscope (TEM).

RESEARCH DESIGN AND METHODS

Research volunteers were recruited by advertisement from the general community, and each potential volunteer had a screening medical examination. Three groups of volunteers were recruited: type 2 diabetic, obese nondiabetic, and nondiabetic lean subjects. The obese nondiabetic and type 2 diabetic groups were recruited to match for BMI, and these groups were sedentary. The lean nondiabetic participants were recruited to match for age, and they reported more regular physical activity, although aerobic capacity was not quantified in study participants. With regard to those with type 2 diabetes, enrollment was limited to those treated with diet and exercise alone, sulfonylureas, or metformin; the latter medications were withdrawn under medical supervision at least 4 weeks before the study. Written informed consent was obtained, and this research project was approved by the University of Pittsburgh institutional review board.

Insulin sensitivity. Research volunteers were admitted to the University of Pittsburgh General Clinical Research Center on the evening before these procedures, and a glucose clamp (13) was performed the next day after an

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CK, creatine kinase; TEM, transmission electron microscope.

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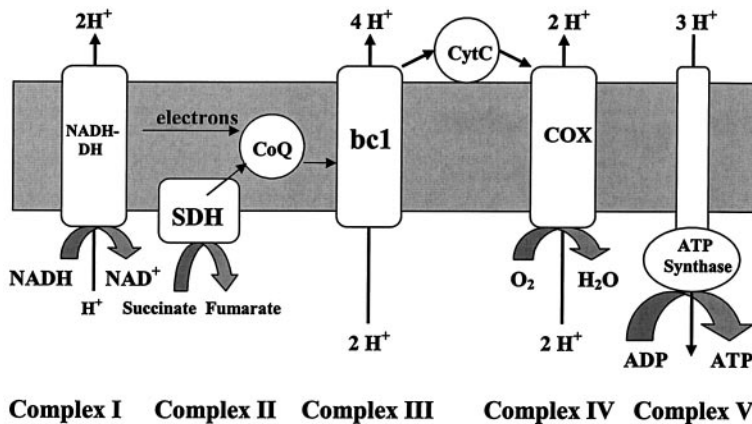


FIG. 1. The electron-transport chain of mitochondria is depicted in this schema showing the four respiratory complexes, consisting of NADH-dehydrogenase (NADH-DH), succinate dehydrogenase (SDH), cytochrome bc1 (bc1), and cytochrome oxidase (COX), and ATP synthase (Complex V), which couples the membrane potential to phosphorylation of ADP.

overnight fast. A percutaneous muscle biopsy of the vastus lateralis was performed (12) ~1 h preceding the start of the insulin infusion. To perform the glucose clamp, insulin was infused at $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ for 4 h, maintaining euglycemia by infusion of dextrose. A primed (200 mg) continuous infusion (2 mg/min) of the stable isotope 6,6- D_2 -glucose was used to measure glucose flux during the insulin infusions. The priming dose was adjusted for the severity of hyperglycemia in those with type 2 diabetes (14), with a baseline equilibration period of 3 h. Tracer was added to 20% dextrose infusions to maintain stable plasma enrichment during insulin infusions, as previously described (15). Systemic indirect calorimetry was performed during the last 30 min of insulin infusion using an open-circuit spirometry metabolic monitor system (Delta Trac, Anaheim, CA) to estimate glucose and lipid oxidation (16). A timed overnight urine collection was obtained to measure nitrogen in order to estimate protein oxidation.

Preparation of mitochondrial fractions. Muscle biopsy samples of vastus lateralis muscle (~10–15 mg wet wt) were homogenized in ice-cold basic medium (in mmol/l: 100 mannitol, 80 K-gluconate, 20 K-flouride, 1 MgCl_2 , 0.2 EGTA, 10 histidine, 10 glucose, and 10 tetraethylammonium-3-[N-morpholino]-2-hydroxypropanesulfonic acid, pH 7.6, at 21°C) containing 5.0 mg/ml BSA, 100 $\mu\text{mol/l}$ deferoxamine mesylate, and antiprotease cocktail III, using a Polytron homogenizer according to the procedures described by Krieger et al. (17). All procedures were performed at 4°C. Soluble and particulate fractions were prepared as previously described (18,19) by centrifugation (45,000g for 20 min) to pellet a particulate (subsarcolemmal mitochondrial + intermyofibrillar mitochondrial) fraction containing >95% of tissue mitochondria. Aliquots (250 μl) of the particulate fraction, corresponding to ~5 mg of tissue, were suspended by Pellet Pestle for 10 s and then centrifuged (600g for 5 min) to separate myofibrillar fragments. Subsarcolemmal mitochondria, which remain in the supernatant, were then obtained by centrifugation (50,400g for 60 min). To release intermyofibrillar mitochondria, the myofibrillar fraction was incubated in 0.6 mol/l KCl/20 mmol/l pyrophosphate- Na_4 at 4°C under nitrogen and in the presence of antiproteases. High KCl has been shown to release sarcoplasmic reticulum vesicles from myofibrils by partially dissolving myosin (20), but without a significant effect on electron transport chain activity (21). This incubation only partially frees intermyofibrillar mitochondria, and those remaining tightly bound to cellular matrix were obtained in a centrifugation pellet (600g for 5 min). The supernatant, containing freed intermyofibrillar mitochondrial fraction, was centrifuged (50,400g for 60 min). Subsarcolemmal and the two intermyofibrillar mitochondrial fractions (free and bound) were resuspended in medium I (100 mmol/l KCl, 0.5 mmol/l EGTA, 0.1 mg/ml BSA, and 25 mmol/l KH_2PO_4 , pH 7.1, at 21°C) and incubated with alamethicin (20 $\mu\text{g/ml}$) (12,22) to permeabilize the inner mitochondrial membrane. Alamethicin does not inhibit electron transport chain activity. Mitochondrial preparations were suspended in 500 μl of basic medium containing 0.5 mmol/l EGTA, 0.1 mg/ml BSA, and 25 mmol/l KH_2PO_4 , pH 7.0, at 21°C and kept at -80°C until assay. The yield of mitochondrial protein was determined as earlier described (23), based on determinations of succinate dehydrogenase (19).

Electron transport chain activity. The electron transport chain is shown in Fig. 1. Activity of succinate oxidase (succinate: O_2 oxidoreductase) was measured in a reaction starting from succinate dehydrogenase and was based on assay of the accumulation of fumarate, the end product of succinate oxidation. This procedure is a modification of a previously developed assay (24). Briefly, the assay couples fumarase and malic dehydrogenase reactions to oxidize fumarate and reduce NAD, with high-performance liquid chromatography and fluorescence detection used to measure NADH (18). Succinate dehydrogenase was preactivated, as previously described, to remove inhibi-

tion by tightly bound oxalacetate (23). Activity of CK was measured as an index of muscle fiber content in biopsy samples, as previously described (12,18), and electron transport chain activity was expressed normalized to CK activity. CK activity was measured at 30°C by monitoring the generation of NADPH in a coupled enzymatic reaction (hexokinase/glucose-6P dehydrogenase) (25).

mtDNA determinations. mtDNA content was measured using real-time PCR, as described earlier (26). Detection of a 69-bp fragment of mtDNA (nucleotides 14918–14986) and a 77-bp fragment of β -globin, both based on markers published by Miller et al. (27), were used to determine the relative copy number of mtDNA per diploid nuclear genome. Primers and FAM-labeled Taqman TAMRA probes (no. 450025; Applied Biosystems, Foster City, CA) were designed using Primer Express software, version 1.5 (Applied Biosystems), and are listed in Table 1. DNA (mitochondrial and nuclear) was extracted from tissue samples using a QIAamp DNA mini-kit (Qiagen, Chatham, CA). The concentration of each sample was determined using a GeneQuant spectrophotometer (Pharmacia Biotech).

PCR conditions. Detection of mtDNA and β -globin was performed as two separate reactions but within the same run for each sample. All samples were run in duplicate for each gene. Reactions were carried out in the presence of $1 \times$ Taqman Universal PCR Master Mix (4304437; Applied Biosystems), 1 $\mu\text{mol/l}$ each forward and reverse primer, 0.25 $\mu\text{mol/l}$ FAM-labeled Taqman/TAMRA probe, and 20 ng sample DNA to a final volume of 25 μl . Amplification reactions were performed in a Prism 7700 spectrofluorometric thermal cycler (Applied Biosystems) with the following cycle conditions: 50°C for 2 min uracil-DNA N-glycosylase incubation, 95°C denaturation and enzyme activation step for 10 min followed by 40 cycles of 95°C denaturation for 15 s, and 60°C annealing and elongation for 60 s. Fluorescence spectra were recorded during the annealing phase of each PCR cycle. Sequence Detection System software (version 1.7) for the Prism 7700 was used to generate the FAM fluorescence.

Threshold cycle calculations. The threshold cycle number was calculated using SDS software version 1.7 (Applied Biosystems) and an automatic setting of the baseline. The baseline value was the average fluorescence of PCR cycles 3–15 plus 10 times its standard deviation. These values were used for the calculations of relative copy number calculations by expressing threshold cycle number differences of the β -globin and mtDNA PCR, as described earlier (26): $R_c = 2^{\Delta\text{Ct}}$ and $\Delta\text{Ct} = \text{Ct}_{\beta\text{-globin}} - \text{Ct}_{\text{mtDNA}}$, where Ct is the threshold cycle number and Rc is the relative copy number.

Transmission electron microscopy. To complement the assessment of mitochondrial distributions based on subcellular separation methods, as described above, a TEM was used to examine the thickness of the layer of

TABLE 1
Sequences of amplification primers and probes

Primer	Sequence
mtDNA-forward	5'-GAC GCC TCA ACC GCC TTT-3'
mtDNA-reverse	5'-GCG GAT GAT TCA GCC ATA ATT TA-3'
β -Globin-forward	5'-TGA AGG CTC ATG GCA AGA AA-3'
β -Globin-reverse	5'-AAA GGT GCC CTT GAG GTT GTC-3'
Probe	Sequence
mtDNA probe	5'-6FAM-CAT CAA TCG CCC ACA TCA CTC GAG AC-TAMRA-3'
β -Globin probe	5'-6FAM-CCA GGC CAT CAC TAA AGG CAC CGA-TAMRA-3'

TABLE 2
Clinical characteristics of research volunteers

	Lean	Obese	Type 2 diabetes
<i>n</i> (F/M)	4/4	3/9	2/9
Age (years)	43.2 ± 2.1*	50.5 ± 3.4	52.6 ± 2.2
Weight (kg)	66.3 ± 5.4*	90.9 ± 4.1	95.2 ± 4.8
BMI (kg/m ²)	22.5 ± 0.9*	30.3 ± 1.1	29.4 ± 1.1
Fasting glucose (mg/dl)	82.0 ± 1.2*	89.2 ± 2.0*	163.8 ± 13.4
HbA _{1c} (%)	5.0 ± 0.1*	5.0 ± 0.1*	8.0 ± 0.4
Fasting insulin (μU/ml)	5.0 ± 0.3*	11.1 ± 0.7	11.4 ± 1.5
<i>R</i> _d (mg · min ⁻¹ · kg FFM ⁻¹)	12.2 ± 0.7*	6.4 ± 0.4	4.9 ± 0.8

**P* < 0.05, lean or obese vs. type 2 diabetes. FFM, fat-free mass.

subsarcolemmal mitochondria, using a previously published method (28). Muscle samples used for the TEM were cut into small pieces (1 × 1 × 2 mm), fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon at either a longitudinal or transverse orientation. After an initial low-power screening of semithin (300 nm) sections stained with toluidine blue to optimize the plane of sectioning (29), ultrathin (60 nm) longitudinal sections were cut and mounted on copper grids and stained with lead citrate (30) and uranyl acetate (31). Sections were examined using a TEM (JEOL 100CXII) at an accelerating voltage of 80 kV. The thickness (μm) of subsarcolemmal mitochondria in the longitudinal orientations was measured using an image analysis system (National Institutes of Health image 1.61).

Statistics. Data are presented as the means ± SE, unless otherwise indicated. ANOVA was used to compare groups and subfractions of mitochondria, and ANCOVA was performed to adjust for the effects of age and sex. Linear regression and stepwise multiple regression was used to examine the relationships of muscle electron transport chain activity and insulin sensitivity.

RESULTS

Research volunteers. The clinical characteristics of research volunteers are shown in Table 2. The obese nondiabetic and obese type 2 diabetic groups were matched for age and BMI. Insulin sensitivity tended to be lower in those with type 2 diabetes, but it was not significantly different from obese control subjects (*P* = 0.17). Fasting plasma glucose and HbA_{1c} were higher in type 2 diabetic than in obese control subjects.

Subcellular distribution of mitochondria. Overall electron transport chain activity and that within the subsarcolemmal and intermyofibrillar mitochondrial fractions are shown in Table 3. In comparison with electron transport chain activity in muscle from lean volunteers, there were significant differences compared with obese and type 2 diabetic subjects in all subfractions of the electron transport chain, as well as in total electron transport chain activity (all *P* < 0.001 for group differences). With respect to electron transport chain activity in the subsarcolemmal mitochondrial fraction, there was an approximately sevenfold reduction in type 2 diabetes and a three- to fourfold reduction in obesity compared with lean subjects. These differences were highly significant for both type 2 diabetes

and obesity (*P* < 0.001). As well, electron transport chain activity in free and bound intermyofibrillar mitochondrial fractions was reduced in type 2 diabetic and obese compared with lean subjects (*P* < 0.01), although the gradient was less than that for subsarcolemmal mitochondria, being two to threefold reduced in the intermyofibrillar mitochondrial fractions. In comparing type 2 diabetes and obesity, there was similar total electron transport chain activity in skeletal muscle. Nonetheless, in type 2 diabetes, there was a significant depression of electron transport chain activity in the subsarcolemmal mitochondrial fraction in comparison with obesity. Electron transport chain activities in the free and bound intermyofibrillar mitochondrial fractions were similar in type 2 diabetes and obesity, with a tendency toward higher electron transport chain activity in type 2 diabetes within the bound intermyofibrillar mitochondrial fraction. ANCOVA based on sex and age did not affect the significance of the comparisons.

The mitochondrial protein yield was similar across the three fractions in lean volunteers (2.1 ± 0.3, 2.1 ± 0.2, and 2.0 ± 0.2 mg/g wet wt for subsarcolemmal and free and bound intermyofibrillar mitochondrial fractions, respectively). As shown in Fig. 2, electron transport chain activity in skeletal muscle of lean volunteers was distributed evenly across the fractions, with approximately one-third of overall activity in the subsarcolemmal mitochondrial fraction, which is consistent with prior studies (32). In type 2 diabetic subjects, mitochondrial protein yield was significantly lower than in muscle from lean volunteers (0.8 ± 0.1, 1.1 ± 0.2, and 1.3 ± 0.4 mg/g wet wt; *P* < 0.001). Also, expressed as a fraction of overall electron transport chain activity, the subsarcolemmal mitochondrial fraction was smaller in type 2 diabetic subjects than in lean (*P* < 0.001) and obese (*P* < 0.01) volunteers. The distribution of muscle electron transport chain activity in obesity also revealed a deficit in the subsarcolemmal mitochondrial fraction, although this was not as severe as in type 2 diabetes.

TABLE 3
Electron transport chain activity in subsarcolemmal and intermyofibrillar mitochondria fractions of human skeletal muscle

	Lean	Obese	Type 2 diabetes
<i>n</i>	8	12	11
Subsarcolemmal mitochondria fraction	0.121 ± 0.022	0.034 ± 0.007†	0.017 ± 0.003*†
Intermyofibrillar mitochondria-1 fraction	0.130 ± 0.018	0.065 ± 0.014†	0.040 ± 0.008†
Intermyofibrillar mitochondria-2 fraction	0.138 ± 0.016	0.051 ± 0.008†	0.068 ± 0.008†
Total electron transport chain activity	0.389 ± 0.050	0.150 ± 0.024†	0.125 ± 0.013†

Succinate oxidase (units/mU CK). **P* < 0.01, type 2 diabetes vs. obese; †*P* < 0.01, type 2 diabetes or obese vs. lean.

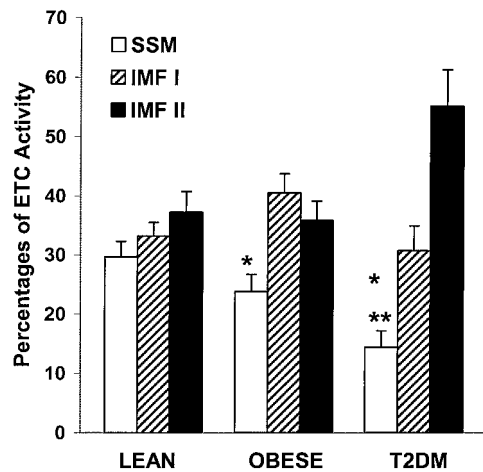


FIG. 2. The distribution of overall succinate oxidase activity (complex II-IV) among subsarcolemmal and intermyofibrillar mitochondrial (IFM) fractions, as a percentage of overall succinate oxidase activity, is shown for skeletal muscle from lean, obese, and type 2 diabetic (T2DM) research volunteers. *Lean vs. obese or type 2 diabetic subjects, $P < 0.01$; **type 2 diabetic vs. obese subjects, $P < 0.05$. ETC, electron transport chain; SSM, subsarcolemmal mitochondria.

Using regression analysis, we examined the potential relation of electron transport chain activity to insulin sensitivity. Consistent with our prior study (12), there was significant correlation between overall electron transport chain activity and insulin-stimulated glucose metabolism ($r = 0.67$, $P < 0.001$). The association between systemic insulin sensitivity and electron transport chain activity in subsarcolemmal and free and bound intermyofibrillar mitochondrial fractions ($r = 0.63$, 0.60 , and 0.65 , respectively) was similar, and each was statistically significant ($P < 0.001$).

TEM. A TEM was used to further and independently examine the hypothesis that there is a deficit of subsarcolemmal mitochondria in type 2 diabetes. Representative photographs of skeletal muscle from a lean volunteer and one with type 2 diabetes are shown in Fig. 3. In the muscle of lean volunteers, abundant subsarcolemmal mitochondria can be identified in the characteristic location adjacent to muscle nuclei. In contrast, in type 2 diabetes, there is a striking depletion of subsarcolemmal mitochondria. We performed quantitative measurement of the thickness of the layer of subsarcolemmal mitochondria. In type 2 diabetic subjects, the thickness of the subsarcolemmal mitochondria was reduced approximately threefold com-

TABLE 4
Mitochondrial DNA content in skeletal muscle in lean, obese, and type 2 diabetic research volunteers

	Lean	Obese	Type 2 diabetes
<i>n</i>	6	12	5
mtDNA	$2,514 \pm 505$	$1,665 \pm 213^*$	$1,850 \pm 220^*$
Succinate oxidase (units/mU CK)	0.39 ± 0.07	$0.14 \pm 0.02^*$	$0.11 \pm 0.01^*$

* $P < 0.05$ vs. values in lean.

pared with that of lean volunteers (1.16 ± 0.47 vs. $2.93 \pm 0.47 \mu\text{m}$, $P < 0.01$, $n = 6$ samples from each group). Although TEM assessments support the conclusion of depleted subsarcolemmal mitochondria in type 2 diabetes, the reduction in electron transport chain activity in the subsarcolemmal mitochondrial fraction was nearly two-fold greater than the reduction in mitochondria observed using a TEM.

Mitochondrial DNA content. To assess whether the reduction in muscle electron transport chain activity might be accounted for by reduced content of mitochondria, reduced functional capacity, or a combination of these factors, mtDNA content was determined as an index of mitochondrial content. This analysis included all volunteers from whom a sufficient sample of muscle was available, and these data are shown in Table 4. The key finding is that mtDNA content in skeletal muscle was reduced similarly in obese and type 2 diabetic subjects relative to lean volunteers. We next examined the ratio between total electron transport chain activity and mtDNA. This ratio was significantly reduced in obese and type 2 diabetic subjects relative to that found in lean volunteers. This indicates that reduced mitochondrial content, as revealed by lower mtDNA, does not fully account for the overall reduction in electron transport chain activity and that there is a reduced functional capacity of mitochondria in type 2 diabetes and obesity even after adjusting for mitochondrial content.

DISCUSSION

Skeletal muscle is a major site for insulin-stimulated glucose utilization. Insulin resistance of this tissue is characteristically severe in type 2 diabetes (33). The pathogenesis of muscle insulin resistance in type 2 diabetes and obesity is known to involve defects of insulin

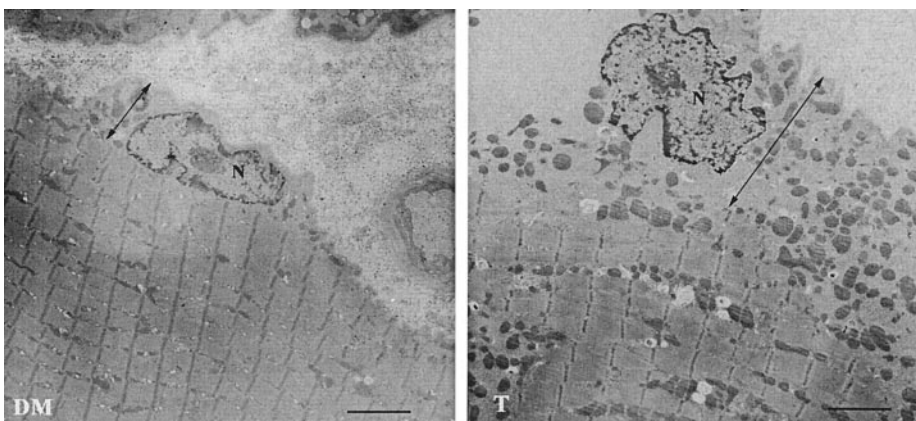


FIG. 3. Representative transmission electron microscopy of longitudinal sections of human skeletal muscle from a lean (T) and a type 2 diabetic (DM) research volunteer are shown (bar = $2.5 \mu\text{m}$). The thickness of the perinuclear distribution of subsarcolemmal mitochondria was measured using image analysis (National Institutes of Health image 1.61) and can be observed to be substantially depleted in type 2 diabetes.

signaling and glucose transport (7,34–36), but the underlying pathogenesis remains uncertain. Several recent studies, including one from our laboratory, suggest that mitochondrial dysfunction might be involved in the pathogenesis of insulin resistance (12,37–39). The mechanism by which mitochondrial dysfunction causes insulin resistance remains unclear, although one attractive hypothesis is a disposition to lipid accumulation caused by impaired capacity for lipid oxidation (10,40).

The current study was undertaken to examine the subpopulation of subsarcolemmal mitochondria. Although it is recognized that mitochondria in skeletal muscle exist as a reticulum, there is compartmentalization into subsarcolemmal and intermyofibrillar mitochondrial populations (41). Subsarcolemmal mitochondria provide energy for membrane-related processes, including signal transduction, ion exchange, substrate transport, and substrate activation—steps clearly relevant to insulin action—(4), whereas the intermyofibrillar mitochondria more directly support muscle contraction. In this study, we have made the novel observation that there is a disproportionate deficiency of electron transport chain activity within the subsarcolemmal mitochondrial fraction in type 2 diabetic compared with lean active adults. This deficit of subsarcolemmal mitochondria is also evident in sedentary obese individuals, though not as severe as in type 2 diabetic individuals. This deficiency of electron transport chain activity near the cell surface in skeletal muscle in type 2 diabetes may be of importance in understanding the cell biology of insulin resistance.

In a prior study, we reported an overall reduction in electron transport chain activity in muscle in type 2 diabetes and obesity (12), but we did not address mitochondrial distribution. Also, the previous TEM examination of mitochondrial morphology, which revealed smaller mitochondria in type 2 diabetic and obese than in lean volunteers, examined only intermyofibrillar mitochondria (12). In the current study, using differential centrifugation and cell digestion procedures to separate mitochondrial fractions, a striking deficiency of subsarcolemmal mitochondria was observed in type 2 diabetes and confirmed using a TEM. In skeletal muscle of lean healthy volunteers, the distribution of electron transport chain activity across the subsarcolemmal and free and bound intermyofibrillar mitochondrial fractions was symmetrical, with approximately one-third of overall electron transport chain activity in each fraction. This observation is strongly consistent with previous findings (32). In obese and type 2 diabetic subjects, a greater proportion of overall electron transport chain activity was found in the intermyofibrillar mitochondrial fractions, although the absolute activities were reduced compared with those of lean volunteers. No significant difference between obese and type 2 diabetic subjects was observed for absolute electron transport chain activity in intermyofibrillar mitochondrial fractions. However, it was found that absolute electron transport chain activity in the subsarcolemmal mitochondrial fraction and the relative proportion of overall electron transport chain activity accounted for by this fraction were reduced in type 2 diabetic compared with both lean and obese subjects.

Two recently published studies using gene array expres-

sion analyses have reported a significant decrease in the nuclear expression of genes encoding proteins of oxidative phosphorylation and other mitochondrial proteins in type 2 diabetes (42,43). These are striking observations because this pattern of impaired gene expression was the most consistent finding in the analyses of multiple pathways. Also, Stump et al. (39) recently reported that an 8-h insulin infusion stimulates increased muscle ATP production and mitochondria protein synthesis in lean volunteers, but not in type 2 diabetic subjects. More recently, noninvasive assessments of oxidative phosphorylation of soleus muscle, performed with magnetic resonance spectroscopy, indicate mitochondrial dysfunction in insulin resistance (37,38). Taken together, the various studies support an emerging concept that insulin resistance has a crucial component related to disordered functional capacity of mitochondria. Earlier, reduced oxidative enzyme activity in skeletal muscle was identified as a risk factor for development of obesity (11,44), an association that might relate to decreased capacity for fat oxidation in skeletal muscle. Our laboratory has observed that there is impaired fat oxidation within skeletal muscle during fasting conditions in type 2 diabetes (45) and in obesity (46), and results implicated reductions in oxidative enzymes as a causative factor (10). Ex vivo studies of human muscle support this hypothesis (40). In this regard, it is important to note that one of the physiological functions postulated for subsarcolemmal mitochondria is fat oxidation (4). One mechanism by which a deficiency in subsarcolemmal mitochondria may contribute to insulin resistance might be the accumulation of intramyocellular lipids (7).

The reduction in electron transport chain activity in muscle in type 2 diabetes and obesity could be caused by reduced mitochondrial content or reduced functional capacity of mitochondria. To address this issue, we measured mtDNA content in muscle. Assessment of mtDNA is regarded as a quantitative index of mitochondrial content in tissues, including skeletal muscle (26,27). Compared with the findings in muscle from lean healthy volunteers, we did find a significant decrease in mtDNA in muscle in obesity and type 2 diabetes. This indicates that decreased mitochondrial content at least partly explains reduced electron transport chain activity. However, in type 2 diabetes, the decrement in electron transport chain activity was greater than the corresponding decrement in mtDNA content. A similar, albeit less severe, pattern was seen in obesity. These findings suggest that functional impairments in electron transport chain activity in type 2 diabetes are not fully accounted for by reduced mitochondrial content. In further support of this concept, it is noteworthy that the decrement in electron transport chain activity within subsarcolemmal mitochondria in type 2 diabetes appears to be approximately twofold greater than the decrease in the thickness of the subsarcolemmal mitochondria layer, as assessed by the TEM.

The factors that account for a disproportionate depletion of electron transport chain activity in subsarcolemmal mitochondria in type 2 diabetes are unclear and warrant further investigation. Perhaps the explanation for lower subsarcolemmal mitochondria electron transport chain activity is simply decreased physical activity. In the current study, volunteers with type 2 diabetes and those who

were obese but nondiabetic were closely matched for age, sedentary lifestyle, sex, and weight. However, a formal measure of fitness and physical activity was not performed, and potential differences might have existed. Both groups manifested a disproportionate reduction in electron transport chain activity in the subsarcolemmal mitochondrial fraction compared with lean individuals, who were more physically active. These sharp differences in subsarcolemmal mitochondria electron transport chain activity—both absolute and as a percentage of overall muscle electron transport chain activity—between sedentary type 2 diabetic and obese subjects on the one hand and the lean active volunteers on the other suggest that factors of obesity and sedentary lifestyle are potentially important determinants of the distribution of muscle electron transport chain activity. Intervention-based studies should be helpful in revealing the impact of physical activity because exercise is considered to have particular effects on the subsarcolemmal mitochondrial fraction (4).

In summary, in this study we find a disproportionately large reduction of electron transport chain activity in the subsarcolemmal mitochondrial fraction in type 2 diabetic and obese subjects compared with lean volunteers. Furthermore, despite similar overall reductions in muscle electron transport chain activity in obesity and type 2 diabetes, there is a greater deficit within the subsarcolemmal mitochondrial fraction in type 2 diabetes. The reduction in electron transport chain activity in obesity and type 2 diabetes does not appear to be fully accounted for by reduced mitochondrial content. Given the important role that subsarcolemmal mitochondria have for bioenergetic support of signal transduction, fat oxidation, and substrate transport, an impairment of electron transport chain activity in this subcellular location may have particular relevance to the pathogenesis of insulin resistance in type 2 diabetes.

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