Tissue-specific control of mitochondrial respiration in obesity-related insulin resistance and diabetes

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Holmström MH, Iglesias-Gutierrez E, Zierath JR, Garcia-Roves PM. Tissue-specific control of mitochondrial respiration in obesity-related insulin resistance and diabetes. Am J Physiol Endocrinol Metab 302: E731–E739, 2012. First published January 17, 2012; doi:10.1152/ajpendo.00159.2011.—The tissue-specific role of mitochondrial respiratory capacity in the development of insulin resistance and type 2 diabetes is unclear. We determined mitochondrial function in glycolytic and oxidative skeletal muscle and liver from lean (+/−) and obese diabetic (db/db) mice. In lean mice, the mitochondrial respiration pattern differed between tissues. Tissue-specific mitochondrial profiles were then compared between lean and db/db mice. In liver, mitochondrial respiratory capacity and protein expression, including peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), was decreased in db/db mice, consistent with increased mitochondrial fission. In glycolytic muscle, mitochondrial respiration, as well as protein and mRNA expression of mitochondrial markers, was increased in db/db mice, suggesting increased mitochondrial content and fatty acid oxidation capacity. In oxidative muscle, mitochondrial complex I function and PGC-1α and mitochondrial transcription factor A (TFAM) protein levels were decreased in db/db mice, along with increased level of proteins related to mitochondrial dynamics. In conclusion, mitochondrial respiratory performance is under the control of tissue-specific mechanisms and is not uniformly altered in response to obesity. Furthermore, insulin resistance in glycolytic skeletal muscle can be maintained by a mechanism independent of mitochondrial dysfunction. Conversely, insulin resistance in liver and oxidative skeletal muscle from db/db mice is coincident with mitochondrial dysfunction.

mitochondrial dysfunction; mitochondrial biogenesis; oxidative capacity; energy metabolism

Obesity is a major risk factor for development of insulin resistance and type 2 diabetes mellitus (T2DM) (49). Insulin resistance in skeletal muscle and liver, coupled with β-cell failure, represents underlying defects in T2DM. Thus, there is a growing appreciation that defects in insulin action in multiple tissues contribute to whole body insulin resistance, disturbances in energy balance, and T2DM.

Mitochondrial dysfunction has been implicated in the development of insulin resistance and the pathogenesis of T2DM (32, 37, 38). The vast majority of mitochondrial proteins, as well as factors controlling the expression and proliferation of the mitochondrial genome, are encoded in the nucleus. Studies of rodent tissues, including analysis of mitochondrial DNA copy number, cytochrome c oxidase activity, and mitochondrial mass in liver, cardiac muscle, and oxidative and glycolytic skeletal muscle (8, 9, 14, 50, 51) provide evidence for marked tissue-specific differences in mitochondrial properties, concurrent with varying metabolic characteristics and demands unique to each specific tissue. Furthermore, quantitative mass spectrometry and 2-D gel electrophoresis of the mitochondrial proteome reveal tissue-specific mitochondrial protein programs (13, 24, 34). Mitochondria are finely tuned to meet the specific needs of the tissue as well as the environmental or pathophysiological state that the specific tissue encounters (1). Thus, obesity and T2DM may be accompanied by tissue-specific differences in the mitochondrial profile.

Several lines of evidence suggest that mitochondrial function in liver or skeletal muscle is associated with insulin sensitivity and T2DM in animal models (2, 10, 12, 20, 40, 48) and humans (25, 27, 36–39). Yet differences in methodology, experimental design, age, genetic background, and sex of the animals/individuals under investigation may independently influence mitochondrial function. Therefore, the mechanism by which mitochondrial function contributes to the development of insulin resistance in T2DM remains to be clarified. Cross-sectional studies involving an integrative approach may provide a better understanding of the causative role of mitochondrial dysfunction in T2DM.

Here, we tested the hypothesis that obesity and insulin resistance are accompanied by tissue-specific alterations in mitochondrial function. We studied db/db mice on a C57BL/6J genetic background and characterized mitochondrial performance in key tissues for insulin action, including glycolytic and oxidative skeletal muscle and liver. Obese diabetic db/db mice are characterized by insulin resistance in glycolytic (22, 43, 52) and oxidative (6, 19, 35) skeletal muscle and liver (19, 28, 53). We provide evidence for tissue-specific changes in mitochondrial function in db/db mice, indicating that mitochondrial adaptations to an excessive nutrient supply and inactivity are influenced by the metabolic plasticity and function of each organ or cell type.

MATERIALS AND METHODS

Reagents. All reagents were purchased from Sigma-Aldrich (St. Louis, MO), unless stated otherwise.

Animal care. We studied 16- to 19-wk-old obese diabetic db/db mice and lean nondiabetic (+/−) littermates bred on a C57BL/6j background from the Jackson Laboratory. The db/db mouse is an obese model of T2DM that expresses a nonfunctional leptin receptor, Leprdb, which renders the animal insensitive to the hormone (3). Animals homozygous for the mutation are hyperphagic and are significantly heavier than control animals at a very young age; they are also hyperglycemic and hyperinsulinemic (17). The animals were kept in a standard temperature- and humidity-controlled environment with a 12:12-h light-dark cycle. Mice had access to nesting material and were provided with water and standard rodent chow ad libitum. Prior to tissue collection, mice were sedated with 0.02 ml/g body wt

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of Avertin (2.5% solution of 99% 2,2,2-tribromoethanol and tertiary amyl alcohol). Following dissection, tissues [liver and skeletal muscle: soleus, extensor digitorum longus (EDL), tibialis anterior, and gastrocnemius] were either processed immediately for mitochondrial respirometry or frozen with tongs cooled in liquid nitrogen and stored at −80°C until further use. All experiments were approved by the animal ethics committee for Northern Stockholm and were conducted in accordance with regulations for the protection of laboratory animals.

Mitochondrial respirometry. Mitochondrial function was assessed using high-resolution respirometry (Oroboros Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria), as described previously (16, 18, 29, 45). All respirometry experiments were performed on fresh tissues immediately following dissection. Liver was homogenized (mechanical permeabilization) in amino acid-depleted respirometry medium (0.5 mM EGTA, 3 mM MgCl2, 60 mM K-lactobionate, 10 mM KH2PO4, 20 mM HEPES, and 110 mM sucrose, pH 7.1) and the equivalent of 1–2 mg wet wt tissue was added to the experimental chamber. Oxidative soleus and glycolytic EDL skeletal muscle was placed in relaxing buffer (in mM: 2.8 Ca2K2EGTA, 7.2 K2 EGTA, 5.8 KCl, 0.5 dithiothreitol, and 50 MES, pH 7.1) immediately after dissection, and fibers were gently separated under a microscope using an iris forceps. Following sarcolemmal permeabilization in relaxing buffer supplemented with 0.005% (wt/vol) saponin, the tissue was equilibrated in respirometry medium [in mM: 0.5 EGTA, 3 MgCl2, 60 K-lactobionate, 20 taunine, 10 KH2PO4, 20 HEPES, 110 sucrose and 0.1% (wt/vol) bovine serum albumin, pH 7.1]. Thereafter, skeletal muscle fibers were blotted for 30 s and 1.0–2.5 mg of tissue was added to each chamber. All the tissues were assessed in respirometry medium. Oxygen flux (denoted as “Leak” in figures) was measured by adding malate (final concentration 2 mM) and pyruvate (10 mM) in the absence of ADP. Oxidative phosphorylation was quantified by the addition of ADP (5 mM, “OXPHOS”) followed by additions of glutamate (20 mM) for additional oxidation of reduced substrates through complex I (CI) and succinate (10 mM) for convergent electron flow through both complex I and II (CI+II). Subsequently, carbonylcyanide-4-(trifluoromethoxy)-phenyl-hydrazone (FCCP) was titrated to achieve maximum flux through the electron transfer system (ETSII). Mitochondrial respiration was inhibited by the sequential addition of rotenone (0.1 μM) and antimycin A (2.4 μM), respectively. The remaining O2 flux after inhibition with antimycin A (O2 flux independent of the electron transfer system) was subtracted from the values of each of the previous steps. Oxygen flux values are expressed relative to tissue wet weight per second (JO2, pmol O2·mg−1·s−1). Flux control ratios, the relative contribution of Leak, CI, CI+II, and ETSII to maximal electron flux through the electron transport system, were calculated by dividing each value by that of ETSII+II.

Tissue homogenate preparation and Western blot analysis. Oxidative and glycolytic skeletal muscle (soleus and EDL, respectively) and liver were homogenized individually in ice-cold lysis buffer [135 mM NaCl, 2.7 mM KCl, 1 mM MgCl2, 1% (vol/vol) Triton X-100, 10% (vol/vol) glycerol, 20 mM Tris-HCl, pH 7.8, 10 mM NaF, 1 mM EDTA, 0.5 mM Na2VO4, 0.2 mM phenylmethylsulfonyl fluoride and 1:100 protease inhibitor cocktail]. Protease inhibitor cocktail was from VWR (West Chester, PA). Homogenates were rotated end over end for 1 h at 4°C and then subjected to three freeze-and-thaw cycles (−80°C to 4°C) to disrupt the mitochondria. The homogenates were subjected to centrifugation at 10,000 g for 15 min at 4°C, and the supernatant was collected. Protein concentration was measured using a Pierce BCA protein assay kit (Rockford, IL), and sample dilutions were adjusted to yield equal protein concentrations. Reagents for denaturing electrophoresis (SDS-PAGE) and Western blot analysis were purchased from Bio-Rad (Hercules, CA). Homogenates for Western blot analysis were mixed with Laemmli buffer, separated by SDS-PAGE, and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA). The membranes were blocked for 1 h at room temperature with 5% nonfat dry milk in Tris-buffered saline with 0.1% (vol/vol) Tween. Immunoblots were probed with the following primary antibodies. Mouse anti-human monoclonal antibodies against mitochondrial dynamin-like GTase-ptic atrophy 1 (OPA1; mitochondrial fusion) and mitochondrial transcription factor A (TFAM; Abnova, Taipei, TW); mouse anti-mouse monoclonal antibodies against NADH-dehydrogenase-1α subcomplex subunit 9 (NDUFA9, complex I), succinate dehydrogenase flavoprotein subunit 70 kDa (SDHA, complex II), cytochrome b-c1 complex subunit 1 and 2 (UQRC1/2, complex III), cytochrome c oxidase subunit 1 (COX1, complex IV), and ATP synthase subunit-α (ATPSA1), obtained from Invitrogen (Carlsbad, CA). Monoclonal mouse anti-mouse antibodies against mitochondrial superoxide dismutase (SOD2) were from Abcam (Cambridge, MA). Polyclonal rabbit anti-human mitofusin-2 (MFN2), glycerolaldehyde-3-phosphate dehydrogenase (GAPDH), and polyclonal rabbit anti-mouse antibodies against dynamin-1-like protein (DNM1L; mitochondrial fission) were from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal rabbit anti-mouse antibodies against pexisome proliferator activated receptor-γ coactivator 1α (PGC-1α) and polyclonal mouse anti-human antibodies against tubulin were obtained from Millipore. After overnight incubation at 4°C with the primary antibodies, membranes were washed extensively and incubated with appropriate horseradish peroxidase-conjugated goat anti-mouse and anti-rabbit IgG from Bio-Rad. Antibody-bound protein was detected using enhanced chemiluminescence from GE Healthcare (Little Chalfont, UK) and quantified by densitometry (GS-800 Calibrated Densitometer and Quantity One analytical software, Bio-Rad). Data are presented as fold over lean (+/+?) mice. Skeletal muscle data for OPA1 represents a ratio of long (a-b) to short (c-e) isoforms.

Quantitative PCR. Total mRNA from liver was extracted and purified using QiAshredder and an RNeasy Mini Kit (QiAGEN, Hilden, Germany), according to the manufacturer’s instructions. Total mRNA was extracted from glycolytic and oxidative skeletal muscle (tibialis anterior and soleus, respectively) according to the instructions provided by the manufacturer, using TRIzol (Invitrogen) followed by purification with the RNeasy Mini Kit. The mRNA concentration was measured using a Nanodrop 1000 (Thermo Scientific, Wilmington, MA). cDNA was performed using Klenow Fragment and III first-strand synthesis supermix (Invitrogen) with oligo(T)17 primers according to the protocol provided by the manufacturer. Quantification of mRNA expression was performed using real-time PCR as described previously (31). Real-time PCR reagents and predesigned TaqMan assays were purchased from Applied Biosystems (Foster City, CA). Custom mouse-specific primer/probe sets to detect gene expression by SYBR Green real-time PCR were selected by using Primer Express (Applied Biosystems), and sequences are available upon request. Reactions were performed in duplicate in a 96-well format using a Prism 7000 Sequence Detector (Applied Biosystems).

Determination of mitochondrial vs. nuclear DNA ratio. Total DNA was extracted from liver and oxidative and glycolytic skeletal muscle (soleus and EDL, respectively) using a DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer’s instructions. Mitochondrial and nuclear DNA (COX1 and β-actin, respectively) were measured by quantitative PCR using SYBR Green.

Measurement of glycogen and triglyceride content. Glycogen content was measured using a standardized biochemical method in which glucose is phosphorylated in vitro by hexokinase, followed by conversion of glucose 6-phosphate to gluconic acid by glucose-6-phosphate dehydrogenase. This reaction also produces NADP, which is measured by fluorometry. Liver and oxidative and glycolytic skeletal muscle (soleus and gastrocnemius, respectively) were lysed in 1 N HCl at 100°C for 2 h. Following centrifugation at 2,000 g, liver samples were further diluted 1:4 with 1 N HCl. Samples were mixed 1:200 with fluorometry assay buffer [50 mM Tris buffer, pH 8.1, 300 μM ATP, 2 mM MgCl2, 0.02% (wt/vol) fatty acid-free BSA, 40 μM

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NADP, and 1 μg/ml glucose-6-phosphate dehydrogenase and supplemented with 1 mM imidazole and 0.02% (w/vol) fatty-acid free BSA) followed by fluorometry. Glycogen content was then calculated using a standard curve.

Triglyceride content was measured by an enzymatic colorimetric method in which free glycerol is eliminated independently of lipase and 4-aminophenazone, followed by enzymatic hydrolysis of triglycerides in the presence of lipase and 4-aminophenazone, and, finally, liberated glycerol is determined spectrophotometrically by means of an enzymatic colorimetric reaction. Liver and oxidative and glycolytic skeletal muscle (soleus and gastrocnemius, respectively) samples were homogenized in a 3:2 heptane-isopropanol solution with 1% (vol/vol) Tween. Thereafter, the Trig/GB kit (triglycerides/glycerol blanked; Roche Diagnostics Scandinavia, Bromma, Sweden) was used according to the manufacturer’s instructions along with Precinorm L standard (Roche Diagnostics Scandinavia).

Statistical analysis. Values are reported as means ± SE. Differences were determined using one-way ANOVA for comparison between tissues (Fig. 1) and unpaired Student’s t-test for comparison between lean (+/−) and db/db mice. Significance was established at $P < 0.05$.

RESULTS

Tissue-specific differences in mitochondrial respiration in lean mice. To determine whether mitochondrial respiratory capacity (per tissue weight) is tissue specific, we studied freshly dissected liver and glycolytic (EDL) and oxidative (soleus) skeletal muscle from lean nondiabetic mice using high-resolution respirometry (Fig. 1A). Leak respiration (oxygen flux achieved after addition of malate and pyruvate with no ADP present) was similar in liver and glycolytic EDL muscle, whereas respiration in oxidative muscle was higher ($P < 0.05$). The oxidative phosphorylation state (OXPHOS, following addition of ADP) was lower in liver and higher in soleus muscle compared with EDL muscle ($P < 0.05$). Maximal respiration in the coupled state, with electron input through both complex I and II (CI+II, following addition of glutamate and succinate) was similar between liver and EDL muscle but higher in the oxidative soleus muscle ($P < 0.05$). Maximum electron transport system capacity (ETSI+II, following addition of the exogenous uncoupler FCCP) was also similar between EDL muscle and liver and highest in soleus muscle ($P < 0.05$). Finally, ETSII, following complex I inhibition, was highest in liver ($P < 0.05$), followed by soleus and EDL. The difference in metabolic programming between tissues was further highlighted by the flux control ratio (i.e., the relative contribution of each respiratory state to the maximal electron transport system capacity ETSI+II) of complex I and II (Fig. 1B). In liver, the maximal electron capacity was primarily carried out by complex II rather than complex I. In skeletal muscle, the flux control ratio at each respiratory state was similar between EDL and soleus muscle except for higher CI efficiency in EDL muscle ($P < 0.05$; Fig. 1B). While oxygen flux was increased 35% in liver after addition of FCCP (Fig. 1A), it was essentially unaltered by exogenous uncoupling in EDL and soleus muscle, reflecting the efficiency of the phosphorylation system (adenine nucleotide translocase, phosphate transporter, and ATP synthase) in matching the potential of the electron transfer system in mouse skeletal muscle.

Liver mitochondrial function is decreased in db/db mice. In liver, Leak and OXPHOS mitochondrial respiration states, normalized to tissue wet weight, were similar between lean (+/−) and db/db mice (Fig. 2A). However, the maximal electron transport capacity was lower in the db/db mice following membrane uncoupling by FCCP (ETSI+II for lean control and db/db mice, respectively, $P < 0.01$). Due to the reduction in the electron transport system capacity in db/db mice, the flux control ratio for CI+II was higher in db/db mice than in lean littermates ($P < 0.01$; Fig. 2B). However, the flux control ratio for Leak, CI, and ETSII was unchanged between genotypes. The observed oxygen flux differences (Fig. 2A) could be due to alterations in nutrient availability, since hepatic triglyceride level was increased in db/db mice ($P < 0.05$; Table 1), whereas glycogen content was unchanged. These functional data are supported by protein expression studies (Fig. 2, C and D), since the abundance of components of complex I and IV of the oxidative phosphorylation system, NDUF9 ($P < 0.001$) and COX1 ($P < 0.01$), were decreased in liver from db/db mice. Conversely, protein expression of markers of the other electron
transfer complexes, SDHA and UQRC1, and ATP synthase were unaltered. Furthermore, DNM1L, a protein mediating mitochondrial fission, was increased in db/db mice (P/H11021 0.001), whereas MFN2, involved in mitochondrial fusion, was decreased to a similar degree in liver from db/db mice (P/H11021 0.01). OPA1, a protein that participates in mitochondrial fusion as well as levels of OPA1 processing products were analyzed (Fig. 2F). Immunoreactive OPA1 bands labeled a and b correspond to the two long isoforms and c–e to the short isoforms. A shift from long to short isoforms indicates proteolytic cleavage of the protein, which is linked to mitochondrial fragmentation (7). The levels of the short isoforms c and e were reduced in liver from db/db mice, whereas the level of the d isofrm was increased (P < 0.05; Fig. 2F). Nevertheless, a clear long-to-short isoform shift was not evident. PGC-1α was reduced at the protein level (Fig. 2C), providing further evidence for decreased mitochondrial function in liver from db/db mice. The mRNA expression of transcription factors regulating mitochondrial biogenesis and fuel selection, such as PPARα (P/H11021 0.05; Fig. 2E), as well as cytochrome b,
for fatty acid oxidation in glycolytic skeletal muscle from db/db mice.

Mitochondrial function in oxidative skeletal muscle from db/db mice. In oxidative soleus skeletal muscle, the OXPHOS state was reduced in db/db vs. control mice (P < 0.01; Fig. 4A). However, the flux control ratio at all states was unaltered between genotypes (Fig. 4B). Protein content and mRNA expression of the electron transfer system (NDUFA9, SDHA, UQCR1, and COX1) and ATP synthase complexes were unaltered in oxidative soleus muscle from db/db vs. lean mice (Fig. 4, C–E). However, PGC-1α, TFAM, and mitochondrial matrix SOD2 protein were reduced in db/db mice vs. lean mice (Fig. 4, C–D). Similarly, mRNA expression of MKN2 and DNM1L (P < 0.05) and OPA1 (ratio long/short isoforms; P < 0.01) was increased in db/db mice (Fig. 4, C and D). Collectively, these results indicate that mitochondrial function and biogenesis is altered in oxidative soleus muscle from db/db mice.

**DISCUSSION**

Mitochondrial dysfunction has been proposed as one of several mechanisms contributing to the development of insulin resistance and T2DM (32). Transcriptional or posttranslational events finely adjust the mitochondria to adapt to the specific environment and energy demands of the tissue (1, 13, 24, 34). Since multiple organs contribute to the progressive impairment in whole body insulin sensitivity, we determined mitochondrial respiration in several tissues important for glucose homeostasis in lean and obese diabetic db/db mice. We provide evidence for tissue-specific mitochondrial respiration patterns under physiological and pathological conditions. In obese, insulin-resistant, diabetic db/db mice, mitochondrial respiration is impaired in different respiratory states in liver and oxidative skeletal muscle and enhanced in glycolytic skeletal muscle. These tissue-specific adaptations in mitochondrial function are a consequence of transcriptional regulation, as observed by alterations in the mRNA expression and protein profiles, due to energy substrate oversupply and derangements in whole body glucose and energy homeostasis in obesity and diabetes. Using the db/db mouse as a model of obesity and insulin resistance, we cannot exclude the possibility that lack of leptin action also regulates mitochondrial function. Conversely, we have previously reported that enhanced leptin action in LepRb-Tyr985 mutant mice improves insulin sensitivity in the liver without alterations in hepatic mitochondrial function (45).

Liver plays an integral role in lipid metabolism and is a key organ for the maintenance of systemic glucose homeostasis. In rodents, high-fat diet (23, 33, 40) leads to obesity, insulin resistance, and hepatic steatosis with concomitant reductions in respiratory capacity and increased oxidative stress in liver
mitochondria. Here, we provide evidence for mitochondrial dysfunction in liver from db/db mice. Nevertheless, db/db mice have a higher overall oxidative capacity in liver compared with lean littermates due to the 2.8-fold increased liver mass. In contrast to our results in db/db mice, liver mitochondrial function in the insulin-resistant obese Zucker rat (i.e., the genetic equivalent of the db/db mouse) is unaltered (12), and oxidative phosphorylation efficiency is increased (11). These discrepancies between liver mitochondrial adaptive responses to obesity and insulin resistance could be due to several factors, including experimental design, age, genetic background, and species differences (10). However, using two-dimensional differential electrophoresis, dysregulated proteins in liver from db/db mice were localized in mitochondria, corresponding to decreased activity of mitochondrial complex I-V enzymes (48), in agreement with our present observations. Moreover, our findings are also consistent with earlier studies of white adipose tissue from db/db and ob/ob mice showing reduced mitochondrial respiration and fatty acid oxidation. Although some alterations in ultrastructure could be seen, functional decreases were largely attributed to reduced mitochondrial mass (4).

Several lines of evidence link mitochondrial dysfunction in skeletal muscle with both the emergence of insulin resistance and the transition between normal glucose tolerance and T2DM (27, 32, 37, 38, 41). In humans, skeletal muscle insulin resistance correlates with reduced expression of genes involved in mitochondrial oxidative phosphorylation (42) and alterations in ultrastructure and reduced mitochondrial size (27, 44). Yet, in vivo evidence both supports (26, 41) and challenges (5) the notion that mitochondrial dysfunction plays a role in the development of skeletal muscle insulin resistance in T2DM. Here, we provide evidence for an enhanced respiratory capacity in glycolytic skeletal muscle from insulin-resistant diabetic db/db mice due to an increase in mitochondrial biogenesis. This finding contrasts with the striking observation of mitochondrial dysfunction in liver from these same mice. Although this seems paradoxical, acute elevations in circulating free fatty acid levels are correlated with increased mitochondrial biogenesis in glycolytic skeletal muscle, possibly due to increased lipid availability (15, 21). Our results are consistent with earlier studies showing enhanced mitochondrial function (30, 46) and density (47) in insulin-resistant monoge-
necic or diet-induced obese rodents. Thus, skeletal muscle insulin resistance in db/db mice may develop by a mechanism independently of any alteration in mitochondrial function. However, additional studies are warranted to establish whether mitochondrial dysfunction causes skeletal muscle insulin resistance in this model, since diabetic rather than prediabetic db/db mice were studied.

Oxidative capacity can differ greatly between skeletal muscle types due to locomotor, structural, and metabolic demands (39). In contrast to glycolytic skeletal muscle, we found that, in oxidative skeletal muscle from db/db mice, mitochondrial respiratory capacity through complex I was reduced, consistent with reductions in key regulators of mitochondrial biogenesis and mitochondrial DNA content. These fiber type-specific responses may reflect the intrinsic increase in mitochondrial content and fatty acid oxidation in oxidative skeletal muscle. Indeed, intramyocellular lipid levels in soleus, but not in glycolytic tibialis anterior muscle, predict the degree of insulin resistance in first-degree relatives of T2DM patients (42). Our results provide evidence for fiber type-specific changes in mitochondrial function in db/db mice. Mitochondrial respiratory properties demonstrate a tissue-specific response that is likely dependent on the specific environment and specialized cell function of each organ.

In conclusion, tissue-specific mechanisms control mitochondrial performance under physiological and pathophysiological conditions. Moreover, in obese diabetic db/db mice, insulin resistance may develop in some tissues such as glycolytic skeletal muscle independently of mitochondrial dysfunction. Conversely, insulin resistance in liver and oxidative skeletal muscle from db/db mice is coincident with mitochondrial dysfunction. Thus, mitochondrial adaptations to obesity-induced changes in the environmental set point may occur in a tissue-specific manner and thereby influence cellular performance and whole body energy metabolism.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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