Original Article

Excess Lipid Availability Increases Mitochondrial Fatty Acid Oxidative Capacity in Muscle

Evidence Against a Role for Reduced Fatty Acid Oxidation in Lipid-Induced Insulin Resistance in Rodents

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A reduced capacity for mitochondrial fatty acid oxidation in skeletal muscle has been proposed as a major factor leading to the accumulation of intramuscular lipids and their subsequent deleterious effects on insulin action. Here, we examine markers of mitochondrial fatty acid oxidative capacity in rodent models of insulin resistance associated with an oversupply of lipids. C57BL/6J mice were fed a high-fat diet for either 5 or 20 weeks. Several markers of muscle mitochondrial fatty acid oxidative capacity were measured, including ^{14}C-palmitate oxidation, palmitoyl-CoA oxidation in isolated mitochondria, oxidative enzyme activity (citrate synthase, β-hydroxyacyl CoA dehydrogenase, medium-chain acyl-CoA dehydrogenase, and carnitine palmitoyl-transferase 1), and expression of proteins involved in mitochondrial metabolism. Enzyme activity and mitochondrial protein expression were also examined in muscle from other rodent models of insulin resistance. Compared with standard diet–fed controls, muscle from fat-fed mice displayed elevated palmitate oxidation rate (5 weeks +23%, $P < 0.05$, and 20 weeks +29%, $P < 0.05$) and increased palmitoyl-CoA oxidation in isolated mitochondria (20 weeks +49%, $P < 0.01$). Furthermore, oxidative enzyme activity and protein expression of peroxisome proliferator–activated receptor γ coactivator (PGC)-1α, uncoupling protein (UCP) 3, and mitochondrial respiratory chain subunits were significantly elevated in fat-fed animals. A similar pattern was present in muscle of fat-fed rats, obese Zucker rats, and *db/db* mice, with increases observed for oxidative enzyme activity and expression of PGC-1α, UCP3, and subunits of the mitochondrial respiratory chain. These findings suggest that high lipid availability does not lead to intramuscular lipid accumulation and insulin resistance in rodents by decreasing muscle mitochondrial fatty acid oxidative capacity. *Diabetes* 56: 2085–2092, 2007

Insulin resistance, which represents an impaired ability for insulin to exert its effects on glucose and lipid homeostasis, is a key metabolic defect associated with obesity and type 2 diabetes. The factors underlying the development of insulin resistance are not fully elucidated; however, there is substantial literature linking lipid accumulation in skeletal muscle to reduced insulin sensitivity (1). Specifically, potent lipid metabolites, including fatty acyl CoAs, diacylglycerols, and ceramides, whose concentrations correlate with intramuscular triglyceride levels, have been shown to antagonize the metabolic actions of insulin in skeletal muscle (1).

Several mechanisms may be responsible for lipid accumulation in skeletal muscle. Both increased fatty acid uptake into muscle from circulation and/or diminished fatty acid oxidation by muscle mitochondria may augment lipid accumulation within skeletal muscle. In humans (2) and rodents (3,4), dysregulated insulin action has been linked with an increased uptake of fatty acids into muscle, suggesting that an increased availability of fatty acids contributes to excess muscle lipid. There is also a growing body of evidence suggesting that defective muscle mitochondrial metabolism, and a subsequent impaired ability to oxidize fatty acids, may be a causative factor in the accumulation of intramuscular lipid and the development of insulin resistance. In muscle from obese insulin-resistant humans, the activity of enzymes of oxidative metabolism and fatty acid utilization are reduced (5). Muscle mitochondria from subjects with insulin resistance or type 2 diabetes are reduced in size and number and have diminished activity of proteins in the respiratory chain (6–8). These defects appear to be particularly pronounced in the subsarcolemmal mitochondria, which are considered to be most the important site for muscle fatty acid oxidation (7). In vivo functional studies using magnetic resonance spectroscopy have also demonstrated reduced mitochondrial oxidative and phosphorylation capacities in skeletal muscle from insulin-resistant elderly subjects (9),

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Received for publication 22 January 2007 and accepted in revised form 18 May 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 29 May 2007. DOI: 10.2337/db07-0093

βHAD, β-hydroxyacyl CoA dehydrogenase; [^{3}H]-2-DOG, [^{3}H]-2-deoxyglucose; CPT, carnitine palmitoyl-transferase; MCAD, medium-chain acyl-CoA dehydrogenase; PGC, PPARγ coactivator; PPAR, peroxisome proliferator–activated receptor; UCP, uncoupling protein.

from patients with type 2 diabetes (10), and from insulin-resistant offspring of patients with type 2 diabetes (11).

Consistent with the observed defects in mitochondrial metabolism in insulin resistance and type 2 diabetes, some (12,13), but not all (6,14), studies have shown reduced expression of peroxisome proliferator–activated receptor (PPAR) γ coactivator (PGC)-1α, a transcriptional coactivator that is a key regulator of mitochondrial biogenesis and fatty acid oxidation in skeletal muscle (15). It must be noted, however, that the results of many of the abovementioned studies are correlative and it still remains to be determined whether reduced fatty acid metabolism in muscle mitochondria is an innate characteristic or whether it is secondary to other metabolic irregularities such as increased lipid availability.

Rodent models, both genetic and dietary, are commonly used to examine the mechanisms underlying the development of insulin resistance in humans. In many of these animal models, such as high-fat–fed rodents and those with an absence of leptin signaling (e.g., obese Zucker rats), an oversupply of lipids has been identified as a key factor leading to insulin resistance (1). Whether defects in mitochondrial fatty acid metabolism are present in skeletal muscle of these animals is, however, not clear. Studies (3,16–20) have shown mitochondrial fatty acid oxidation and the activity of oxidative enzymes are elevated in muscle of fat-fed rats and obese Zucker rats. Equally, however, several recent studies (21–24) in rodent models of insulin resistance have reported deficits in a variety of markers of mitochondrial metabolism in muscle.

Therefore, the aim of the current study was to determine whether high lipid availability contributes to insulin resistance, in part, through a decreased mitochondrial fatty acid oxidative capacity in skeletal muscle. Specifically, we have examined fatty acid oxidative capacity and markers of mitochondrial function (oxidative enzyme activity and protein expression) in skeletal muscle from C57BL/6J mice fed a high-fat diet for either 5 or 20 weeks, as well as fat-fed Wistar rats, obese (*fa/fa*) Zucker rats, and *db/db* mice.

RESEARCH DESIGN AND METHODS

Eight-week-old male C57BL/6J mice and male Wistar rats were purchased from the animal resources center (Perth, Australia). The animals were kept in a temperature-controlled room (22 ± 1°C) on a 12-h light/dark cycle with free access to food and water. Mice were fed ad libitum for a period of 5 or 20 weeks with a standard lab diet (8% calories from fat, 21% calories from protein, and 71% calories from carbohydrate, 2.6 kcal/g; Gordon's Specialty Stock Feeds, Yanderra, Australia) or with a high-fat diet (45% of calories from fat, 20% calories from protein, and 35% calories from carbohydrates, 4.7 kcal/g, based on rodent diet no. D12451; Research Diets, New Brunswick, NJ). Rats were fed the standard or high-fat diet ad libitum for a period of 4 weeks. Quadriceps muscles were also obtained from 10- to 12-week-old *db/db* mice and obese Zucker rats and their respective lean controls. All experiments were carried out with the approval of the Garvan Institute/St. Vincent's Hospital Animal Experimentation Ethics Committee, following guidelines issued by the National Health and Medical Research Council of Australia.

Metabolic assays. Glucose tolerance tests (2g/kg glucose i.p.) were performed in overnight-fasted mice. Blood samples were obtained from the tail tip at the indicated times, and glucose levels were measured using a glucometer (AccuCheck II; Roche, New South Wales, Castle Hill, Australia). [^{3}H]-2-deoxyglucose ([^{3}H]-2-DOG) uptake in isolated soleus muscles was determined in sealed flasks containing pregassed (95% O_2/5% CO_2) Krebs-Henseleit buffer, pH 7.3, supplemented with 0.01% BSA, 25 mmol/l HEPES, 8 mmol/l mannitol, and 2 mmol/l pyruvate. Muscles were allowed to recover for 20 min and were incubated with or without insulin (1 mU/ml) for 40 min at 30°C. [^{3}H]-2-DOG uptake was then measured for 16 min using 0.25 μCi/ml [^{3}H]-2-DOG as described previously (25). Serum measurements were performed on blood collected from the chest cavity and centrifuged at 14,000*g* for 10 min to obtain the serum. Insulin concentrations were measured using a sensitive radioimmunoassay kit (Linco Research, St. Louis, MO). The concentration of nonesterified fatty acids was determined using a colorimetric kit (Wako Pure Chemical Industries, Osaka, Japan).

Indirect calorimetry studies. Oxygen consumption rate (V_{O_2}) was measured using an eight-chamber indirect calorimeter (Oxymax series; Columbus Instruments, Columbus, OH) with an airflow of 0.6 l/min. Studies were commenced after 2 h of acclimation to the metabolic chamber (20 × 10 × 12.5 cm). V_{O_2} was measured in individual mice at 27-min intervals over a 24-h period under a consistent environmental temperature (22°C). During the study, mice had ad libitum access to food and water.

Determination of body composition. Fat and lean body mass was measured using dual-energy X-ray absorptiometry (Lunar PIXImus2 mouse densitometer; GE Healthcare) in accordance with the manufacturer's instructions.

Homogenate oxidations. Palmitate and glutamate oxidation were measured in muscle homogenates using a modified method of that described by Kim et al. (26). Briefly, muscles were homogenized in 19 volumes of ice-cold 250 mmol/l sucrose, 10 mmol/l Tris-HCl, and 1 mmol/l EDTA, pH 7.4. For assessment of substrate oxidation, 50 μl of muscle homogenate was incubated with 450 μl reaction mixture (pH 7.4). Final concentrations of the reaction mixture were (in mmol/l): 100 sucrose, 80 KCl, 10 Tris-HCl, 5 KH_2PO_4, 1 $MgCl_2$, 2 malate, 2 ATP, 1 dithiothreitol, 0.2 EDTA, and 0.3% fatty acid–free BSA. Substrates were 0.2 mmol/l [1-^{14}C]palmitate (0.5 μCi) plus 2 mmol/l L-carnitine and 0.05 mmol/l coenzyme A or 10 mmol/l [1-^{14}C]glutamate (0.1 μCi). After 90 min of incubation at 30°C, the reaction was stopped by the addition of 100 μl of ice-cold 1 mol/l perchloric acid. CO_2 produced during the incubation was collected in 100 μl of 1 mol/l sodium hydroxide. For palmitate incubations, ^{14}C counts present in the acid-soluble fraction were also measured and combined with the CO_2 values to give the total palmitate oxidation rate. Protein content in the homogenates were measured using the Bradford method (protein assay kit; Bio-Rad Laboratories, Regents Park, Australia).

Mitochondrial respiration measurements. Mitochondria were isolated from the quadriceps muscle as described by Bruce et al. (27). Oxygen consumption was measured at 30°C in a Clark-type oxygen electrode (Strathkelvin Instruments, Motherwell, Scotland). The respiration medium contained (in mmol/l): 225 mannitol, 75 sucrose, 10 Tris-HCl, 10 KH_2PO_4, 10 KCl, 0.8 $MgCl_2$, 0.1 EDTA, and 0.3% fatty acid–free BSA, pH 7.0. Substrates were 0.3 mmol/l palmitoyl-CoA, 2 mmol/l L-carnitine, and 2 mmol/l malate or 10 mmol/l glutamate and 2 mmol/l malate. For each assay 0.1–0.3 mg mitochondria were used and state III respiration was initiated by the addition of 0.2 mmol/l ADP. Protein content of the mitochondrial preparations were measured as described above.

Enzyme activity measurements. Powdered muscle samples were homogenized 1:19 (wt/vol) in 50 mmol/l Tris-HCl, 1 mmol/l EDTA, and 0.1% Triton X-100, pH 7.2, using a Polytron instrument (Kinematica, Littau-Lucerne, Switzerland) and were subjected to three freeze-thaw cycles. Citrate synthase, β-hydroxyacyl CoA dehydrogenase (βHAD), and medium-chain acyl-CoA dehydrogenase (MCAD) were determined at 30°C, as described previously (28,29), using a Spectra Max 250 microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). Carnitine palmitoyl-transferase (CPT)-1 activity was measured at 30°C in isolated mitochondria and corrected to muscle wet weight based on recovery rates of citrate synthase (27). Enzyme activities are presented as units per gram wet weight, where units are defined as micromoles per minute.

Immunoblotting. Powdered muscle samples were resuspended in radioimmunoprecipitation assay buffer (PBS, pH 7.5; 1% nonidet NP-40; 0.5% sodium deoxy-cholate; and 0.1% SDS), supplemented with protease and phosphatase inhibitors (10 μg/ml phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mmol/l Na_3VO_4, and 10 mmol/l NaF) and solubilized for 2 h at 4°C. Equal amounts of tissue lysates (10 μg protein) were resolved by SDS-PAGE and immunoblotted with appropriate antibodies against PGC-1α (Chemicon International, Temecula, CA), uncoupling protein (UCP) 3 (Affinity Bioreagents, Golden, CO), cytochrome oxidase (complex IV) subunit 1 (Invitrogen, Victoria, Australia), and an antibody cocktail that recognizes several subunits of the mitochondrial respiratory chain (MS601; Mitosciences, Eugene, OR). Immunolabeled bands were quantitated by densitometry.

Statistical analyses. Data are presented as means ± SE. An unpaired Student's *t* test was used for comparison of relevant groups. Differences at $P < 0.05$ were considered to be statistically significant.

RESULTS

Body composition and oxygen consumption. Table 1 shows the body mass, fat mass, and oxygen consumption measured in 5 and 20 weeks fat-fed mice along with their standard diet controls. Mice fed a high-fat diet for 5 weeks weighed the same as their standard diet–fed controls; how-

TABLE 1
Body mass, fat mass, and oxygen consumption in standard diet– and fat-fed mice

	5 weeks		20 weeks	
	Standard diet	Fat	Standard diet	Fat
Body mass (g)	28.7 ± 0.5	28.8 ± 0.5	31.7 ± 0.7	35.9 ± 0.8*
Fat mass (%)	14.5 ± 1.3	24.8 ± 1.9*	13.9 ± 0.6	27.8 ± 2.9*
Epididymal fat mass (g)	0.28 ± 0.02	0.63 ± 0.05*	0.46 ± 0.09	1.71 ± 0.21*
Brown fat mass (mg)	68 ± 3	76 ± 4	79 ± 8	130 ± 2*
Vo_2 (ml/g/h)	3.57 ± 0.05	3.72 ± 0.06	3.42 ± 0.03	3.43 ± 0.10
RER (Vco_2/Vo_2)	0.96 ± 0.01	0.86 ± 0.01*	0.95 ± 0.01	0.81 ± 0.01*

Data are means ± SE of 6–11 animals per group. Fat mass (%) was determined by dual-energy X-ray absorptiometry scanning. Vo_2 and RER (respiratory exchange ratio) represents the average values recorded over a 24-h period. *$P < 0.01$ vs. standard diet–fed mice.

ever, they displayed a 2.3-fold increase ($P < 0.01$) in the mass of the epididymal fat pad and an increase ($P < 0.01$) in the percentage of whole-body adiposity by dual-energy X-ray absorptiometry scanning (Table 1). At the 20-week time point, fat-fed mice weighed on average 4.2 g more than standard diet–fed animals ($P < 0.01$), and this was associated with a 3.7-fold increase ($P < 0.01$) in epididymal fat pad mass and an increased ($P < 0.01$) percentage of whole-body adiposity by dual-energy X-ray absorptiometry scanning (Table 1). Brown adipose tissue mass was not significantly different between standard diet– and fat-fed mice at the 5-week time point; however, following 20 weeks of fat feeding there was a 65% ($P < 0.01$) increase in the size of the brown adipose depot in fat-fed mice.

We measured energy expenditure and food intake, as changes in either of these parameters may have contributed to the increased adiposity observed in the fat-fed animals. Whole-body oxygen consumption was measured over 24 h using indirect calorimetry, and we observed no difference in oxygen consumption (expressed per gram of body mass) between standard diet– and fat-fed mice (Table 1); however, when expressed per gram of lean mass there was a 20 and 19% higher V_{O_2} ($P < 0.01$) in animals fed the high-fat diet for 5 and 20 weeks, respectively. As expected, the respiratory exchange ratio was significantly lower in the fat-fed animals, reflecting the difference in diet between the two groups (Table 1). We also measured total cage food intake and calculated the average energy intake per animal and found that at the 5-week time point fat-fed animals were consuming 12% more calories than standard diet–fed controls (11.2 ± 0.2 vs. 12.6 ± 0.5 kcal/day, $n = 21$, $P < 0.02$), while at the 20-week time point fat-fed mice consumed 30% more calories than standard diet controls (10.1 ± 0.1 vs. 13.1 ± 0.3 kcal/day, $n = 12$, $P < 0.001$).

Glucose tolerance, glucose uptake, and serum parameters. To determine whether the increased adiposity in the fat-fed mice was associated with reduced insulin action, we examined whole-body glucose clearance during an intraperitoneal glucose tolerance test (Fig. 1). At both the 5- and 20-week time point, high-fat feeding resulted in a significant impairment in glucose clearance (Fig. 1*C*). In a group of 5 weeks fat-fed mice, we also observed impaired insulin action at the level of skeletal muscle, with an approximate 40% reduction in insulin-stimulated [^{3}H]-2-DOG uptake compared with standard diet–fed controls (Fig. 1*D*). Fat-fed mice displayed higher circulating insulin levels after both 5 weeks (0.48 ± 0.07 vs. 0.74 ± 0.08 ng/ml, $n = 6$, $P < 0.05$) and 20 weeks (0.65 ± 0.11 vs. 1.86 ± 0.36 ng/ml, $n = 5$, $P < 0.05$) of the high-fat diet. High-fat feeding also resulted in a significant increase in circulating nonesterified fatty acid levels compared with standard diet–fed controls at both the 5-week (0.68 ± 0.05 vs. 1.12 ± 0.16 mmol/l, $n = 6$, $P < 0.05$) and 20-week (0.71 ± 0.04 vs. 0.92 ± 0.04 mmol/l, $n = 4$–5, $P < 0.01$) time points.

Muscle fatty acid oxidative capacity and enzyme activity. To determine the effect of the high-fat regime on muscle fatty acid oxidative capacity, we measured the palmitate oxidation rate in tissue homogenates. Skeletal muscle from both 5 and 20 weeks fat-fed mice displayed a significantly increased capacity for fatty acid oxidation ($P < 0.05$) compared with muscle from standard diet controls (Fig. 2*A*). Recently, it has been suggested that a reduced ratio of complete (measured as CO_2 production) to incomplete (measured as acid-soluble metabolites) fatty acid oxidation may be important in high-fat diet–induced insulin resistance (23); however, we observed no difference in this ratio between standard diet– and fat-fed mice in our assays (5 weeks: 2.2 ± 0.3 vs. 2.2 ± 0.2; 20 weeks: 2.6 ± 0.3 vs. 2.4 ± 0.2). We also measured glutamate oxidation and found no significant difference between standard diet– and fat-fed animals at either the 5-week (96 ± 8 vs. 104 ± 10 nmol/h/mg protein; standard diet versus fat) or 20-week (102 ± 13 vs. 125 ± 11 nmol/h/mg protein; standard diet versus fat) time point.

As a further measure of fatty acid oxidative capacity, we measured oxygen consumption in isolated mitochondria, with palmitoyl-CoA as the substrate. In mitochondria from 20 weeks fat-fed mice, there was a significant increase (49%; $P < 0.01$) in ADP-stimulated respiration rate compared with standard diet controls, while for 5 weeks fat-fed mice respiration rate was 20% higher than standard diet controls; however, this difference did not reach statistical significance (Fig. 2*B*). Similar to the homogenate oxidations, there was no significant difference in mitochondrial respiration when glutamate was used as an alternative substrate (data not shown).

We also measured the activity of a range of enzymes associated with fatty acid utilization and oxidative capacity, including βHAD, MCAD, CPT-1, and citrate synthase. Consistent with the results observed in the homogenate oxidations and mitochondrial respiration measurements, there was a significant increase in the activity of all of these enzymes in muscle from 5 and 20 weeks fat-fed mice compared with standard diet controls (Table 2).

Expression of proteins involved in mitochondrial metabolism. Our data suggested an increase in mitochondrial content in skeletal muscle of mice fed a high-fat diet. Accordingly, we examined the protein expression of several subunits of the respiratory chain, both nuclear (30-

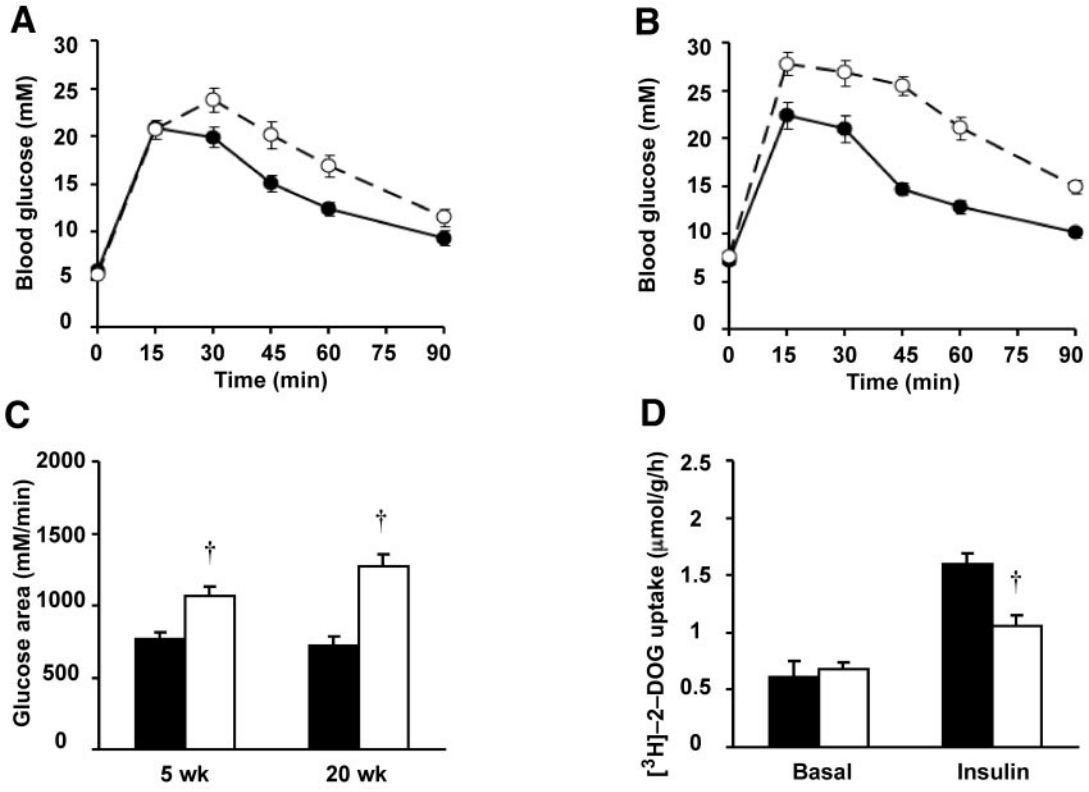

FIG. 1. Glucose tolerance test in overnight-fasted standard diet–fed (●) and fat-fed (○) mice. Blood glucose levels after an intraperitoneal glucose load (2 g/kg) in 5 (*A*) or 20 (*B*) weeks standard diet– and fat-fed mice. *C*: Incremental areas under the curve as an indicator of glucose clearance in the standard diet– and fat-fed animals. *D*: [^{3}H]-2-DOG uptake in isolated soleus muscles from standard diet–fed and 5 weeks fat-fed mice, incubated with or without 1 mU/ml insulin for 40 min at 30°C. ■, standard diet; □, high-fat diet. Data represent the means ± SE of 10–11 mice for panels *A–C* and 3–5 mice for panel *D*. †$P < 0.01$ vs. standard diet–fed controls.

kDa subunit of complex II, core protein 2 subunit of complex III, and the α subunit of complex V) and mitochondrial (ND6 subunit of complex I and subunit 1 of complex IV) encoded, as well as the expression of PGC-1α, given its important role in the regulation of mitochondrial biogenesis and fatty acid oxidation (15). In muscle from mice fed the high-fat diet for 5 or 20 weeks, there was increased protein expression of all subunits from the respiratory chain compared with standard diet–fed controls (Fig. 3). Consistent with the increased level of mitochondrial proteins, we found increased PGC-1α protein content in both 5 weeks (64%, $P < 0.01$, $n = 7$) and 20 weeks (42%, $P < 0.01$, $n = 8$) fat-fed animals compared with standard diet controls (Fig. *3*).

Additionally, we determined the protein expression of UCP3 because although its precise function has yet to be determined, it has been suggested to be a potentially important protein for the regulation of fatty acid transport, and metabolism and its expression is increased during periods of elevated fatty acid oxidation (30,31). There was a 1.8-fold ($P < 0.01$, $n = 6$) and 2.2-fold ($P < 0.01$, $n = 6$) increase in the expression of UCP3 in the 5 and 20 weeks

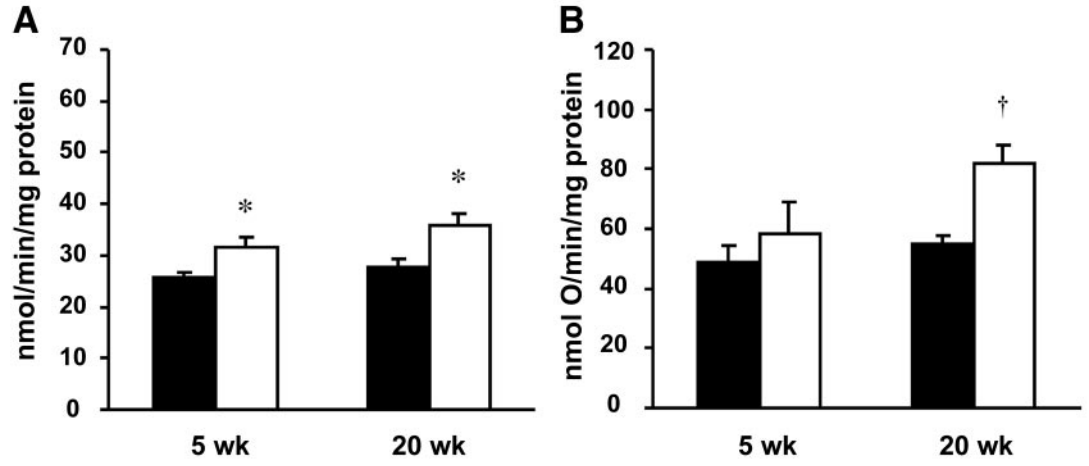

FIG. 2. Indicators of fatty acid oxidative capacity in 5 and 20 weeks standard diet–fed (■) and fat-fed (□) mice. *A*: Palmitate oxidation rate in muscle homogenates. *B*: ADP-stimulated respiration rate in isolated muscle mitochondria, with palmitoyl-CoA as substrate. Data represent the means ± SE of 5–6 mice. *$P < 0.05$; †$P < 0.01$ vs. standard diet–fed controls.

TABLE 2
Activity of enzymes of fatty acid utilization and oxidative capacity in standard diet– and fat-fed mice

	5 weeks		20 weeks	
	Standard diet	Fat	Standard diet	Fat
Citrate synthase	20.0 ± 1.6	25.0 ± 0.6*	16.9 ± 1.9	26.5 ± 1.9†
βHAD	2.40 ± 0.18	3.19 ± 0.11†	2.27 ± 0.28	3.82 ± 0.31†
MCAD	0.75 ± 0.05	1.12 ± 0.04†	0.63 ± 0.10	1.40 ± 0.14†
CPT-1	0.36 ± 0.05	0.49 ± 0.04*	0.30 ± 0.02	0.47 ± 0.07*

Data are means ± SE of 5–6 animals per group. Activities are expressed as units per gram wet weight. Citrate synthase, βHAD, and MCAD activities were determined directly in muscle homogenates, while CPT-1 activity was determined in isolated mitochondria and is expressed per gram wet weight based on recovery rates of citrate synthase (27). *$P < 0.05$; †$P < 0.01$ vs. standard diet–fed mice.

fat-fed mice, respectively, compared with standard diet–fed controls (Fig. *3*).

Fat-fed rats, obese Zucker rats, and *db/db mice*. To determine whether similar patterns may also be present in other rodent models of insulin resistance, we examined enzyme activity (βHAD, MCAD, and citrate synthase) and PGC-1α and mitochondrial protein expression in skeletal muscle from fat-fed Wistar rats, obese Zucker rats, and *db/db* mice. Compared with control animals, enzyme activity was significantly increased in muscle from fat-fed rats (15–27%), obese Zucker rats (33–41%), and *db/db* mice (17–46%) (Table 3). We also examined protein expression of PGC-1α, UCP3, and respiratory chain subunits (complex I and complex III) and found elevated protein levels in muscle from the insulin-resistant animals (Fig. 4).

DISCUSSION

Inappropriate lipid deposition in skeletal muscle is recognized as an important factor associated with insulin resistance (1). Recent studies (7–9,12,13) in humans have suggested that aberrant mitochondrial fatty acid metabolism may be associated with intramuscular lipid accumulation in conditions of reduced insulin action. Many of these studies, however, have been conducted in subjects with well-established insulin resistance, and whether defects in muscle mitochondrial metabolism are a cause or correlate of insulin resistance remains to be clarified.

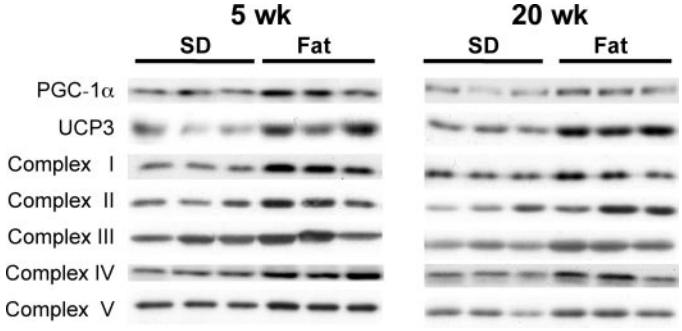

FIG. 3. Immunoblots for markers of mitochondrial metabolism and biogenesis in skeletal muscle from 5 and 20 weeks standard diet (SD)– and fat-fed mice. Equal amounts of muscle lysates (10 μg protein) were resolved by SDS-PAGE and immunoblotted with specific antibodies for PGC-1α, UCP3, and mitochondrial respiratory chain subunits.

In the current study, we examined markers of mitochondrial fatty acid metabolism in skeletal muscle from rodents, in which insulin resistance is associated with an oversupply of lipids. In C57BL/6J mice fed a high-fat diet, we observed a significant impairment in glucose tolerance and a 40% reduction in insulin-stimulated glucose uptake in skeletal muscle. Despite this, we observed increased fatty acid oxidative capacity; higher activity of βHAD, MCAD, CPT1, and citrate synthase; and elevated protein expression of PGC-1α, UCP3, and mitochondrial respiratory chain subunits in skeletal muscle from these animals. Similar findings were also observed in muscle from fat-fed Wistar rats, obese Zucker rats, and *db/db* mice, in which there was increased βHAD, MCAD, and citrate synthase activity and elevated expression of PGC-1α, UCP3, and subunits of the mitochondrial respiratory chain compared with respective control animals. Collectively, our findings suggest that mitochondrial fatty acid oxidative capacity is increased in skeletal muscle from insulin-resistant rodents.

Insulin resistance is associated with elevated fatty acid levels in the circulation. The increased capacity for fatty acid oxidation observed in skeletal muscle of insulin-resistant rodents in the current study is potentially a compensatory response to elevated fatty acid substrate availability. Mice with muscle-specific overexpression of lipoprotein lipase, which increases fatty acid influx into skeletal muscle, display extensive mitochondrial proliferation (32). Fatty acids have also been shown to increase PGC-1α expression in muscle cells (33) and β-cells (34), and this is associated with increased mitochondrial metabolism (33,35). The fatty acid subtype appears to be important (33), as palmitate alone reduces PGC-1α expression (36); however, this may be related to its activation of inflammatory pathways that are known to impact on PGC-1α expression (37).

TABLE 3
Activity of enzymes of fatty acid utilization and oxidative capacity in rodent models of insulin resistance

	Wistar rats		Zucker rats		*db/db* mice	
	Standard diet	Fat	+/+	*fa/fa*	*db/+*	*db/db*
Citrate synthase	23.4 ± 1.1	26.7 ± 1.2*	27.5 ± 3.1	37.3 ± 1.4*	25.4 ± 0.6	29.6 ± 1.3*
βHAD	2.16 ± 0.10	2.74 ± 0.24*	4.41 ± 0.35	6.18 ± 0.15†	3.61 ± 0.19	4.54 ± 0.29†
MCAD	1.34 ± 0.07	1.64 ± 0.08*	0.55 ± 0.06	0.73 ± 0.02*	0.28 ± 0.02	0.41 ± 0.02†

Data are means ± SE of 5–7 animals per group. Activities are expressed as units per gram wet weight. *$P < 0.05$; †$P < 0.01$ vs. control group.

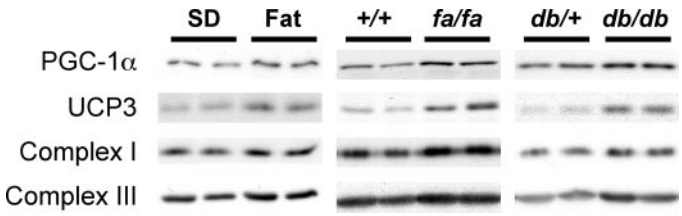

FIG. 4. Immunoblots for markers of mitochondrial metabolism and biogenesis in skeletal muscle from fat-fed Wistar rats, obese (*fa/fa*) Zucker rats, and *db/db* mice with respective control animals. Equal amounts of muscle lysates (10 μg protein) were resolved by SDS-PAGE and immunoblotted with specific antibodies for PGC-1α, UCP3, and mitochondrial respiratory chain subunits. SD, standard diet.

The coordinated increase in the activity of β-oxidation and trichloroacetic cycle enzymes, along with the increased expression of respiratory chain subunits observed in the current study, suggest that PGC-1α is in part mediating the increase in fatty acid oxidative capacity and mitochondrial content by coactivating its known binding partners estrogen-related receptor α, PPARα, PPARδ, and nuclear respiratory factor-1 (15). Mechanistically elevated fatty acid could stimulate PGC-1α expression and increase fatty acid oxidative capacity via a number of pathways. Fatty acids are known ligands for the PPAR family of nuclear hormone receptors, and part of the increase in fatty acid oxidative capacity in the current study may be related to direct activation of PPARα or PPARδ by fatty acids. In skeletal muscle, activation of PPARδ has been shown to increase expression of PGC-1α (38). PGC-1α is also known to coactivate PPARδ (39), resulting in a feed-forward loop that stabilizes PGC-1α protein expression and drives the transcription of genes associated with fatty acid metabolism (38).

Other studies in insulin-resistant rodents have reported reduced expression of PGC-1α and other markers of mitochondrial metabolism in muscle (21–24). The reason for the disparity in results is unclear but may be related to methodological factors such as diet composition, the length of high-fat feeding, or the particular muscle groups examined (i.e., quadriceps versus gastrocnemius bundle). Furthermore, it must also be noted that many studies in rodents and humans have only examined mRNA expression for PGC-1α, and as PGC-1α is known to be posttranslationally modified, its gene expression may not always correlate with protein levels (40).

Despite our findings of elevated fatty acid oxidative capacity in insulin-resistant rodents, increased intramuscular lipid is a characteristic feature of these animal models (1,4). Excess lipid storage may be in part related to a greater increase in the efficiency of fatty acid uptake, as it has been observed in insulin-resistant rodents that there is increased clearance of fatty acid into muscle (3,4). We did not directly measure fatty acid uptake in our animals; however, adipose mass and circulating nonesterified fatty acids levels were elevated in fat-fed mice (Table 1), and the increased UCP3 protein in all of our rodent models is consistent with an increased influx of fatty acid into muscle (30,31,41). Skeletal muscle is quantitatively an important tissue for whole-body fat oxidation, and lipid overload in muscle may be linked to the reduction in muscle mass observed in insulin resistance (Table 1; [42]). Another factor to be considered is that our ex vivo measurements represent the capacity of enzymes and fatty acid oxidation pathways under favorable conditions of substrate availability, and in vivo, regulatory factors such as elevated levels of malonyl-CoA (43) or reduced activity of adiponectin and leptin signaling pathways (44) might contribute to ectopic deposition of lipids in muscle.

The proposed causative role for mitochondrial dysfunction in the development of insulin resistance is yet to be definitively demonstrated. Several studies (7–9,12,13) have reported defects in various markers of mitochondrial metabolism and biogenesis in skeletal muscle from subjects with obesity, insulin resistance, and type 2 diabetes. These results may be confounded by various disease factors; however, investigations demonstrating mitochondrial defects in first-degree relatives of patients with type 2 diabetes (6,11,12) suggest that mitochondrial dysfunction may be among the earliest defects that predisposes these subjects to lipid accumulation and insulin resistance, as opposed to increased lipid availability leading to decreased mitochondrial function and then insulin resistance. Studies in humans in which lipid availability has been experimentally altered have provided inconclusive results in this respect. Acute oversupply of lipids, via lipid infusion, has been shown to reduce gene expression for PGC-1α and mitochondrial respiratory chain components in muscle (45,46). However, 1 week of pharmacological reduction of plasma free fatty acids (and subsequently intramuscular acyl-CoA concentrations) in insulin-resistant subjects also reduced gene expression of PGC-1α and other mitochondrial markers in muscle (47). Dietary studies in humans are also equivocal with high-fat feeding studies reporting increases (48), decreases (24), or no change (49) in various markers of muscle mitochondrial metabolism. Thus, the effect of elevated lipid availability on muscle mitochondrial oxidative capacity in humans remains to be clarified.

In summary, our study demonstrates that fatty acid oxidative capacity and protein expression of PGC-1α and mitochondrial respiratory chain subunits are upregulated in skeletal muscle of a variety of rodent models of insulin resistance. We suggest that these changes likely represent a homeostatic response to attempt to compensate for elevated availability of lipids in these animals. We therefore conclude that increased lipid availability is unlikely to lead to lipid accumulation and insulin resistance via a specific effect to diminish mitochondrial fatty acid oxidative capacity.

ACKNOWLEDGMENTS

This work was supported by the National Health and Medical Research Council of Australia (NHMRC), the Diabetes Australia Research Trust (DART), and the Rebecca L. Cooper Medical Research Foundation. N.T. and C.R.B. are supported by Peter Doherty Fellowships and G.J.C. by a Research Fellowship from the NHMRC.

The contribution of Dr. Bronwyn Hegarty, Andrew Hoy, Donna Wilks, and Ron Enriquez to specific methodological and technical aspects of the study is gratefully acknowledged. We thank the Biological Testing Facility at the Garvan Institute for their help with animal care.

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