Overexpression of Carnitine Palmitoyltransferase-1 in Skeletal Muscle Is Sufficient to Enhance Fatty Acid Oxidation and Improve High-Fat Diet–Induced Insulin Resistance

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OBJECTIVE—Skeletal muscle insulin resistance is associated with lipid accumulation, but whether insulin resistance is due to reduced or enhanced flux of long-chain fatty acids into the mitochondria is both controversial and unclear. We hypothesized that skeletal muscle-specific overexpression of the muscle isoform of carnitine palmitoyltransferase 1 (CPT1), the enzyme that controls the entry of long-chain fatty acyl CoA into mitochondria, would enhance rates of fatty acid oxidation and improve insulin action in muscle in high-fat diet insulin-resistant rats.

RESEARCH DESIGN AND METHODS—Rats were fed a standard (chow) or high-fat diet for 4 weeks. After 3 weeks, in vivo electrotransfer was used to overexpress the muscle isoform of CPT1 in the distal hindlimb muscles (tibialis anterior and extensor digitorum longus [EDL]). Skeletal muscle insulin action was examined in vivo during a hyperinsulinenic-euglycemic clamp.

RESULTS—In vivo electrotransfer produced a physiologically relevant increase of ~20% in enzyme activity; and although the high-fat diet produced insulin resistance in the sham-treated muscle, insulin action was improved in the CPT1-overexpressing muscle. This improvement was associated with a reduction in triacylglycerol content, the membrane-to-cytosolic ratio of diacylglycerol, and protein kinase C δ activity. Importantly, overexpression of CPT1 did not affect markers of mitochondrial capacity or function, nor did it alter skeletal muscle acylcarnitine profiles irrespective of diet.

CONCLUSIONS—Our data provide clear evidence that a physiological increase in the capacity of long-chain fatty acyl CoA entry into mitochondria is sufficient to ameliorate lipid-induced insulin resistance in muscle. Diabetes 58:550–558, 2009

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lin resistance when animals are placed on a high-fat diet (20), whereas overexpression of CPT1 protects myotubes against lipid-induced insulin resistance (21,22), arguing that alterations in fatty acid flux into the mitochondria are critical in regulating lipid effects on insulin sensitivity. Thus, to test whether increasing the capacity for fatty acid flux into the mitochondria is, in itself, sufficient to increase fat oxidation and alter insulin action, we used an approach in which we selectively overexpressed the muscle isoform of CPT1 in skeletal muscle in vivo. The results show that a physiological increase in CPT1 activity is sufficient to improve insulin resistance caused by a high-fat diet, suggesting that entry of long-chain fatty acids into the mitochondria is more critical in the regulation of fatty acid oxidation than the downstream capacity of the β-oxidation and TCA cycle.

RESEARCH DESIGN AND METHODS

Vector construction. The human CPT1B CDNA was a gift from Dr. Feike van der Leij (van Hall University of Applied Sciences, Leeuwarden, the Netherlands) and was constructed as previously described (23).

Animal maintenance and surgery. Male Wistar rats (~200 g; Animal Resources Centre, Perth, Australia) were acclimatized at 22 ± 0.5°C under a 12-h light/12-h dark cycle for 1 week on a standard chow diet (8% calories from fat, 21% calories from protein, and 71% calories from carbohydrates; Gordon’s Specialty Stocks Feed, Yanderra, New South Wales, Australia) with ad libitum water. Thereafter, one-half of the animals were switched to a high-fat diet (45% of calories from fat [lard]), 20% calories from protein, and 35% calories from carbohydrates; 4.7 kcal/g, based on Rodent Diet Diet D12451; Research Diets, New Brunswick, NJ for 4 weeks. One week before study, the right and left jugular veins of rats designated to undergo euglycemic-hyperinsulinemic clamp studies were cannulated as previously described (24). Rats were single housed and handled daily to minimize stress. Body weight was recorded daily, and only those rats that had fully recovered their presurgery weight were subsequently studied. All experimental procedures were approved by the Garvan Institute/St. Vincent’s Hospital Animal Experimentation Ethics Committee and were in accordance with the National Health and Medical Research Council (NHMRC) of Australia Guidelines on Animal Experimentation.

In vivo electrophoresis. The purified CPT1 plasmid was directly injected into rat tibialis anterior muscle by a previously described protocol (23,25,26). The tibialis anterior muscle was chosen because it can be electroporated easily without the requirement for any surgical intervention and the fibres composition is representative of the entire musculature of the rat. We have previously reported that this protocol results in a transfection rate of ~50% of fibers (25,26). Hyperinsulinemic-euglycemic clamps (subsequently described) were performed on the total membrane fraction, was collected, and the protein content of both the mitochondrial and cytosolic fractions obtained from subcellular fractionation (described above) were determined. Unless otherwise stated, the muscles were rapidly freeze-clamped using precooled tongs and stored at −80°C.

PCR and Western blot analysis. The mRNA abundance of genes encoding the proteins involved in electron transfer/oxidative phosphorylation (ET/OnPhos), peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1α), nuclear respiratory factor-1 (NRF-1), mitochondrial transcription factor A (tFAM), and protein abundance were analyzed using real-time RT-PCR and Western blot analysis as previously described (27,28).

CPT1 activity and palmitate metabolism. Mitochondria were isolated using a previously described protocol (23,29,30). The forward radioisotope assay for the determination of CPT1 (EC 2.3.1.121) activity was used as described by McGarry et al. (31) with minor modifications (23). To examine palmitate metabolism in control and transfected muscles, the EDL muscle was carefully dissected into longitudinal strips from tendon to tendon by use of a 27-gauge needle. Strips were removed and assayed as described previously (23).

Glucose uptake and insulin signaling in isolated EDL muscle. Glucose uptake in isolated EDL muscle strips was assessed in sealed flasks containing preassembled (95%/5% O2/CO2) Krebs-Henseleit bicarbonate buffer (pH 7.4), supplemented with 2 mmol/l sodium pyruvate, 8 mmol/l mannitol, and 0.1% wt/vol BSA at 30°C. Muscles were allowed to recover for 30 min and were incubated without or with insulin (1 mU/ml) for 30 min. Glucose uptake was assayed for 15 min using [3H]-2-deoxy-D-glucose (1 mmol/l; 0.128 μCi/ml) as described previously (33). For analysis of insulin signaling, isolated EDL muscles were preincubated for 30 min and were stimulated for 15 min with 1 mU/ml insulin. Muscles were rinsed with ice-cold saline, blotted, and freeze-clamped for subsequent analysis.

Hyperinsulinemic-euglycemic clamp. Conscious rats were studied after 5–7 h of fasting. Between 0900 and 1000 h, jugular cannulae were either connected to a sampling line or an infusion line, and after 30–40 min, hyperinsulinemic-euglycemic clamps commenced (34), incorporating administration of a bolus injection of 2-deoxy-o-[2,6-3H]-glucose (GE Healthcare Life Sciences, Buckinghamshire, U.K.). Insulin was infused at a rate of 0.25 units · kg⁻¹ · h⁻¹. After 120 min, rats were killed by intravenous injection of sodium pentobarbital, and the tibial anterior and posterior muscles were rapidly dissected and freeze-clamped. The area under the tracer disappearance curve of 2-deoxy-o-[2,6-3H]-glucose together with the counts of phosphorylated [3H]deoxyglucose from individual muscles were used to calculate the glucose metabolic index (R'). During the clamp procedure, plasma glucose was determined every 10 min.

Subcellular fractionation and PKC analysis and intramuscular lipid analysis. Tibialis anterior muscle was homogenized in ice-cold buffer containing 20 mmol/l HEPES (pH 7.4), 2 mmol/l EGTA, 50 mmol/l 8-glycerophosphate, 1 mmol/l dithiothreitol, 1 mmol/l Na3VO4, 10% glycerol, 3 mmol/l benzamidine, 10 μmol/l leupeptin, 5 μmol/l pepstatin A, and 1 mmol/l phenylmethylsulfonylfluoride. The homogenate was centrifuged at 92,000 × g for 30 min at 4°C, and the supernatant was collected as the cytosolic fraction. The pellet was resuspended in ice-cold homogenization buffer to which 1% NP-40 was added. The resuspended pellet was incubated on ice for 30 min and was centrifuged at 50,000 × g for 60 min at 4°C. The supernatant, representing the total membrane fraction, was collected, and the protein content of both fractions determined. Western blotting was performed using antibodies specific for PKCθ, -α, or -β (Cell Signalling Technology). TAG content was determined in the tibialis anterior muscles using an enzymatic colorimetric technique as previously described (25). DAG and ceramide were extracted and quantified using a fluorophotometric technique of Bress et al. (35). DAG and ceramide content was also determined in the membrane and cytosolic fractions obtained from subcellular fractionation (described above) to examine the intracellular partitioning of these lipids.

Blood analysis. Blood was transferred into tubes containing EDTA and centrifuged, and the plasma was stored at −80°C for later analysis. Plasma glucose was determined using an automated glucose analyzer (YSI 2300; YSI, Yellow Springs, OH). Plasma free fatty acid (FFA) was assayed using an enzymatic colorimetric method (Triglycerides GPO-PAP, Roche Diagnostics, Indianapolis, IN). Basal insulin was assessed using a rat/mouse insulin enzyme-linked immunosorbent assay (Linco, St. Charles, MO), and clamp insulin was assessed using human ELISA (Merckodia, Uppsala, Sweden).

Acylcarnitine analysis. Muscle acylcarnitines were analyzed according to the method of van Vlies et al. (36). Muscle (−60 mg) was lyophyllized and powdered before addition of stable isotope internal standards (NeoGen Cambridge Isotope Laboratory, Andover, MA) in 1 ml 80% acetonitrile. After sonication and centrifugation, the supernatant was dried under nitrogen, and the acylcarnitines were converted to their butyl esters with butanolic HCl. After evaporation of the butanol, the residue was dissolved in 100 μl 50% acetonitrile, and tandem mass spectrometric analysis was performed (Quattro LC; Waters, Milford, MA).

Statistics. Data are reported as means ± SE. Comparisons between data from multiple treatment groups were made using factorial ANOVA followed by Tukey’s post hoc analysis. To specifically examine differences in insulin sensitivity and signaling between control and transfected muscles in animals fed a high-fat diet, data were analyzed with paired t tests. Statistical significance was accepted at P < 0.05.

RESULTS

CPT1 overexpression increases CPT1 activity and fatty acid oxidation and reduces fatty acid incorporation into TAG. The physiological characteristics of the chow and high-fat diet–treated animals are presented in Table 1. Because the CPT1 antibody cross-reacts with both mouse and human CPT1, it recognized both the endogenous and electroporated forms of the protein. In vivo electroporation resulted in CPT1 being expressed above endogenous levels by ~100% in the tibialis anterior and EDL (Fig. 1A and B; P < 0.05) of chow animals. CPT1 protein was increased ~40% (Fig. 1A; P < 0.05) in the tibialis anterior and ~30% in the EDL (Fig. 1B; P < 0.05) in
high-fat diet–fed rats. CPT1 protein and activity were increased by the high-fat diet (Fig. 1A and B; \( P < 0.05 \)). CPT1 activity was increased by \(~40\%\) by CPT1 overexpression in chow animals and \(~20\%\) in high-fat diet–fed animals (Fig. 1C; \( P < 0.05 \)). Although fatty acid oxidation tended to be elevated by high-fat diet, the increase was not statistically significant (Fig. 1D; \( P = 0.07 \)). Importantly, CPT1 overexpression increased fatty acid oxidation in EDL muscles obtained from chow and high-fat diet–fed animals (Fig. 1D; \( P < 0.05 \)). Palmitate incorporation into DAG was unchanged in muscles that overexpressed CPT1 (chow empty vector 11.7 \( \pm \) 1.0 nmol/g; chow CPT1 vector 13.4 \( \pm \) 0.5 nmol/g; high-fat diet empty vector 11.6 \( \pm \) 1.8 nmol/g; high-fat diet CPT1 vector 10.3 \( \pm \) 1.2 nmol/g). However, palmitate incorporation into TAG was increased with high-fat diet (\( P < 0.05 \)) but was reduced in muscles overexpressing CPT1 to levels comparable with chow animals (Fig. 1E).

**TABLE 1**

| Characteristic                  | Chow            | High fat       |
|--------------------------------|-----------------|----------------|
| Body mass (g)                  | 316 \( \pm \) 13 | 340 \( \pm \) 8 |
| Plasma glucose (mmol/l)        | 7.1 \( \pm \) 0.3 | 7.6 \( \pm \) 0.3 |
| Plasma insulin (pmol/l)        | 187 \( \pm \) 22  | 285 \( \pm \) 51* |
| Plasma FFA (\( \mu \)mol/l)    | 0.56 \( \pm \) 0.05 | 0.74 \( \pm \) 0.07 |
| Plasma TAG (\( \mu \)mol/l)    | 0.94 \( \pm \) 0.03 | 1.05 \( \pm \) 0.24 |
| Epididymal fat (g)             | 0.95 \( \pm \) 0.12 | 1.60 \( \pm \) 0.15* |
| Subcutaneous fat (g)           | 1.49 \( \pm \) 0.14 | 2.36 \( \pm \) 0.21* |
| Glucose infusion rate (mg \( \cdot \) kg \( ^{-1} \) \( \cdot \) min \( ^{-1} \)) | 40.1 \( \pm \) 2.6 | 25.4 \( \pm \) 2.0* |

Data are means \( \pm \) SE. *Significantly different from chow (\( P < 0.05 \)), \( n = 9–12 \).

**FIG. 1.** CPT1 overexpression increases CPT1 activity and fatty acid oxidation and reduces fatty acid incorporation into TAG. Representative immunoblots and quantification of CPT1 protein expression in tibialis anterior (A), representative immunoblots and quantification of CPT1 protein expression in EDL muscle (B), CPT1 activity in isolated mitochondria from tibialis anterior muscles (C), fatty acid oxidation rate in isolated EDL muscles ex vivo (D), and fatty acid incorporation into TAG in isolated EDL muscles ex vivo (E) from a leg electroporated with an empty vector (■) or CPT1 vector (■) in animals placed either on a standard chow diet or high-fat diet for 4 weeks. Data are means \( \pm \) SE; \( n = 8 \). *\( P < 0.05 \) main effect for CPT1 expression; †\( P < 0.05 \) main effect for diet; #\( P < 0.05 \) vs. high-fat diet control leg.
The effect of CPT1 overexpression on markers of oxidative capacity and mitochondrial function. We observed a high-fat diet–induced increase in the activity of β-hydroxyacyl-CoA-dehydrogenase (β-HAD; Table 2; \(P < 0.05\)) and a trend for increased PGC-1α protein expression in tibialis anterior (supplementary Fig. 1, available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db08-1078/DC1), results consistent with a previous study from our group (37). Neither the high-fat diet nor CPT1 overexpression affected the expression of the ET/OxPhos genes in tibialis anterior muscles (Table 2). We also measured the maximal activity of another important mitochondrial enzyme, citrate synthase (Table 2), and the protein abundance of complex I, II, and V proteins in the ET/OxPhos chain (supplementary Fig. 1) and observed no significant effect of either high-fat diet or CPT1 overexpression.

To investigate whether increased flux of fatty acid into the mitochondria would impair β-oxidation via changes in key metabolite levels, we determined the effect of a high-fat diet and increased CPT1 activity on acylcarnitine levels and the ratio of fatty acid oxidation to CO₂ and acid soluble metabolites (ASMs) in EDL muscles. Using tandem mass spectrometry we analyzed several muscle acylcarnitine species ranging from C₂ to C₁₆. Free carnitine levels were not altered by diet or CPT1 overexpression (supplementary Fig. 2, available in the online appendix). We showed that most muscle acylcarnitine species were affected by neither high-fat diet nor CPT1 overexpression. However, we observed a small increase in C₁₆ acylcarnitine in high-fat diet–fed animals (\(P < 0.05\), a result consistent with previous observations (18). Importantly, however, CPT1 overexpression did not exacerbate C₁₆ acylcarnitine levels in high-fat diet–fed rats (Fig. 2A). To assess whether this small increase in C₁₆ acylcarnitine levels in high-fat diet–fed rats affected markers of mitochondrial metabolic control, we measured fatty acid oxidation to CO₂ and ASM fractions, because if fatty acids are incompletely oxidized, the percentage of oxidation to CO₂ should fall, leading to a concomitant increase in the percentage of fatty acid metabolites in the ASM fraction. However, we show that neither the high-fat diet nor CPT1 overexpression affected the percentage of fatty acid oxidation in either the ASM or CO₂ fraction (Fig. 2B), although both high-fat diet and CPT1 overexpression increased the total oxidation of fatty acids by EDL ex vivo (Fig. 1C).

CPT1 overexpression restores insulin-stimulated glucose uptake and insulin signaling due to diet-induced obesity. Insulin increased glucose uptake (\(≈\)twofold, \(P < 0.05\)), phosphorylation of IRS1 (Tyr\(_{612}\), twofold, \(P < 0.05\)) and Akt (Ser\(_{473}\), 10-fold, \(P < 0.05\)) in the EDL. CPT1 overexpression had no effect on these measures in chow-fed animals (supplementary Fig. 3, available in the online appendix). However, although the high-fat diet reduced insulin-stimulated glucose uptake in the control leg by \(−30\%\), overexpression of CPT1 in the EDL of high-fat diet–fed animals increased insulin-stimulated glucose uptake to levels comparable with control chow-fed animals (Fig. 3A). Whereas the high-fat diet reduced the phosphorylation of IRS-1 and Akt under insulin-stimulated conditions (\(P < 0.05\), CPT1 overexpression in the EDL of high-fat diet–fed animals restored insulin-stimulated phosphorylation of IRS-1 (Fig. 3B) and

### Table 2: Markers of oxidative metabolism

|                         | Chow empty vector | CPT1 vector | High fat empty vector | CPT1 vector |
|-------------------------|-------------------|-------------|-----------------------|-------------|
| Citrate synthase (\(\mu\)mol·min\(^{-1}\)·g\(^{-1}\) wet wt) | 3.8 ± 0.4         | 3.7 ± 0.3   | 4.0 ± 0.6             | 3.6 ± 0.4   |
| B-HAD (\(\mu\)mol·min\(^{-1}\)·g\(^{-1}\) wet wt)* | 2.3 ± 0.2         | 2.2 ± 0.4   | 5.1 ± 0.4             | 4.7 ± 0.6   |
| PGC1α mRNA              | 1.0 ± 0.2         | 1.3 ± 0.7   | 1.2 ± 0.3             | 1.1 ± 0.2   |
| NRF-1 mRNA              | 1.0 ± 0.2         | 1.6 ± 0.9   | 1.3 ± 1.0             | 1.6 ± 0.6   |
| tFAM mRNA               | 1.0 ± 0.2         | 1.2 ± 0.8   | 1.1 ± 0.4             | 0.7 ± 0.2   |

Data are means ± SE. Values for mRNA expression are expressed relative to the value obtained from the control leg of chow-fed animals using ribosomal 18S as a housekeeping gene. *Significant main effect for diet (\(P < 0.05\)), \(n = 5–7\).

**FIG. 2.** Muscle acylcarnitine levels and the ratio of fatty acid oxidation to CO₂ and ASMs in overnight fasted animals. Acylcarnitine levels in tibialis anterior muscle (A) and the ratio of fatty acid oxidation to CO₂ and ASMs (B) in EDL muscle from a leg electroporated with an empty vector or CPT1 vector, in animals placed either on a standard chow diet or high-fat diet (HFD) for 4 weeks. Data are means ± SE; \(n = 8\).
Akt (Fig. 3C) to levels observed in control chow-fed animals.

Lipid handling by muscle and its role in the etiology of insulin resistance is a process that involves tissue cross-talk that is highly influenced by circulatory factors and flux of fatty acids across the plasma membrane. Therefore, we examined the effect of CPT1 overexpression on insulin action in vivo, by conducting hyperinsulinemic-euglycemic clamps with the infusion of radiolabeled 2-deoxyglucose to measure glucose uptake (Rg') in tibialis anterior muscles. The high-fat diet increased fat pad mass and induced whole-body insulin resistance, as shown by an increase in basal insulin levels (Table 1) and a reduction in the glucose infusion rate during the clamp. Fasting plasma glucose and FFA were not different after high-fat diet (Table 1). Rg' was reduced in the tibialis anterior of high-fat diet–fed animals (32%, \( P < 0.05 \); Fig. 4A), but overexpression of CPT1 in the tibialis anterior of chow-fed animals had no effect on Rg' (supplementary Fig. 4, available in the online appendix). In contrast, overexpression of CPT1 in high-fat diet–fed animals prevented the decrease in Rg', restoring rates comparable with the control leg of chow-fed animals (24.3 ± 3.3 vs. 22.2 ± 3.3 \( \mu \text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \) for chow and high-fat diet, respectively; Fig. 4A). Consistent with these findings, the phosphorylation of Akt (Ser^473) was enhanced in the tibialis anterior muscles of high-fat diet–fed animals overexpressing CPT1 (Fig. 4B).
CPT1 overexpression decreases high-fat diet–induced accumulation of TAG, the membrane-to-cytosolic ratio of DAG, serine phosphorylation of IRS-1, and activation of PKCθ and JNK.

CPT1 overexpression decreases high-fat diet–induced accumulation of TAG, the membrane-to-cytosolic ratio of DAG, serine phosphorylation of IRS-1, and activation of PKCθ and JNK. The high-fat diet increased TAG, whereas overexpression of CPT1 decreased TAG in both chow and high-fat diet conditions (Fig. 5A). Both DAG and ceramide content in the tibialis anterior muscle were increased with high-fat diet, but overexpression of CPT1 affected neither DAG nor ceramide content (data not shown). However, the bioactivity of deleterious lipid species is critically dependent on their cellular localization. We, therefore, measured the membrane-to-cytosolic ratio of DAG and ceramide. The membrane-to-cytosolic ratio of DAG was reduced in muscles from high-fat diet–fed animals overexpressing CPT1 (Fig. 5B). In addition, although not statistically significant, there was a trend (P = 0.07) for the same to occur in the membrane-to-cytosolic ratio of ceramide (Fig. 5C).

Given that ceramide and DAG act as second messengers, we next measured a raft of signal transduction pathways known to affect insulin action. The high-fat diet increased phosphorylation of IRS-1 (Ser307; Fig. 6A), the membrane-to-cytosolic ratio of PKCθ (Fig. 6B), and the phosphorylation of JNK (Thr183/Tyr185). However, these
effects were ameliorated by CPT1 overexpression (Fig. 6A–C). There were no effects of either CPT1 overexpression or high-fat diet on FAT/CD36 expression, the phosphorylation status and protein expression of AMPK, ACC, IkBα, and MLK3, and membrane-associated PKCα/β or -δ (supplementary Fig. 5).

**DISCUSSION**

Although it has been known for many years that excess lipid leads to insulin resistance, the precise molecular mechanisms linking lipid oversupply to defective insulin action are not fully elucidated. It has been suggested that enhancing fatty acid flux through the mitochondria in the absence of a concomitant “metabolic remodeling” of mitochondrial machinery may lead to deleterious effects on insulin action (38), whereas other studies report that increasing fatty acid oxidation can ameliorate insulin resistance by reducing intracellular lipid levels (22,39).

Here, we report that a physiologically relevant increase in skeletal muscle CPT1 protein expression was sufficient to increase fatty acid oxidation and to prevent high-fat diet–induced fatty acid esterification into intracellular lipids, subsequently leading to enhanced muscle insulin sensitivity in high-fat–fed rats in the absence of major changes in markers of downstream capacity of the TCA cycle and ET/OxPhos pathways. The insulin-sensitizing effect of CPT1 overexpression was associated with a lower membrane-to-cytosolic ratio of DAG and reduced PKCδ/H11002 and JNK activation (Fig. 6), resulting in a trend toward increased fatty acid oxidation. In our study, we used a technique in which we selectively overexpressed CPT1 in one hind limb of a rat and then were able to compare muscles from this hind limb with those obtained from the sham-treated, contralateral limb to see whether there were alterations to pre-existing, high-fat diet–induced insulin resistance. This technique has many advantages. First, the comparable muscles are subjected to exactly the same circulatory factors. Second, we were able to increase the activity of CPT1 in a skeletal muscle to levels seen with physiological interventions such as exercise training (29). Of note, we have consistently reported a transfection efficiency of ~50% in the tibialis anterior muscle (25,26). We did not determine transfection efficiency of the CPT1 plasmid, but based on our previous reports (25,26), we expect that 40–50% of fibers would be overexpressing CPT1. However, given that CPT1 protein was only increased by ~40%, it is possible that the transfection efficiency of CPT1 was lower than in our previous studies, or it may indicate that CPT1 protein has a rapid turnover rate when overexpressed. Nonetheless, the critical aspect of the study is that we achieved a physiological increase in CPT1 protein and activity that functionally translated into enhanced rates of fatty acid oxidation and, importantly, suppressed fatty acid esterification into TAG. Having performed hyperinsulinemic-euglycemic clamps with 2-deoxyglucose tracers, we were able to demonstrate that CPT1 overexpression improves insulin action in diet-induced obese models. Our results are entirely consistent with previous work in vitro, where it has been reported that a two- to threefold overexpression of CPT1 in L6 myotubes increased oxidation of the long-chain fatty acids palmitate and oleate and increased insulin stimulation of [14C]glucose incorporation into glycogen by 60% (21,22).

In the present study, high-fat diet–induced insulin resistance was associated with an increase in CPT1 activity and a trend toward elevated fatty acid oxidative capacity (Fig. 1A–C). This is consistent with previously reported data showing that rodents can adapt to a high-fat diet to coordinately increase the ability of skeletal muscle to take up (14) and utilize (37) fatty acid, this being dominant energy substrate. This is accompanied by muscle insulin resistance, which is at first glance seemingly paradoxical considering that we here demonstrate that a further enhancing of muscle CPT1 expression and fatty acid oxidation in high-fat diet–fed rats had an insulin-sensitizing effect. However, the critical difference was in the degree of fatty acid esterification (Fig. 1D). In the empty vector condition, when animals were placed on high-fat diet, fatty acid incorporation into TAG was increased compared with chow-fed rats, despite the concomitant increase in CPT1 activity and a trend toward increased fatty acid oxidation. This increase in fatty acid esterification was associated with increased lipid deposition in membranes (Fig. 6) and increases in PKδ and JNK activation (Fig. 6), resulting in impaired insulin signal transduction. These data suggest that diet-induced obesity results in an enhanced entry of fat into the mitochondria and subsequent oxidation; however, this increase is quantitatively less than the increase in fatty acid entry into the tissue, resulting in a net increase in cellular lipid storage. In contrast, by increasing CPT1 activity in fat-fed animals, in this case due to ectopic
overexpression, the exacerbated increase in fatty acid entry into the mitochondria and subsequent oxidation was sufficient to match the increased rate of fatty acid uptake into the tissue, thereby preventing any esterification into TAG and subsequent insulin resistance.

Although the total content of bioactive lipids appears to play a role in regulating insulin action in muscle, it is also emerging that the subcellular location of lipids plays a fundamental role in dictating its downstream targets and cellular responses (42). Many of the proximal steps in insulin signaling to GLUT4 translocation occur at or in close proximity to the plasma membrane (43). Thus, it is possible that excessive accumulation of lipid species, such as DAG and ceramide, in cellular membranes may affect insulin action by interfering with the activation of critical components of the insulin signaling pathway. Such an effect may be mediated by DAG and ceramide-induced displacement of cholesterol in membranes (44), which is associated with decreased Akt phosphorylation (45). In the current study, we found that the membrane-to-cytosolic ratio of ceramide was increased in muscle of high-fat–fed animals, and this was associated with impaired phosphorylation of Akt. We also observed that there was a tendency for the ceramide membrane-to-cytosolic ratio to be reduced in muscles overexpressing CPT1 from fat-fed rats, an effect that may have contributed to enhanced insulin-stimulated Akt phosphorylation. In addition, we also found that the DAG membrane-to-cytosolic ratio was decreased in muscle from fat-fed animals overexpressing CPT1.

The molecular mechanisms by which lipids induce insulin resistance in skeletal muscle remain uncertain. However, it has been suggested that lipid-induced insulin resistance in muscle is mediated by activation of a serine kinase cascade that leads to impaired insulin signaling (7). We were able to show that the high-fat diet not only induced translocation of PKCα to the membrane but also increased JNK phosphorylation, which, however, when CPT1 was overexpressed in muscle overexpressing CPT1, and this was associated with decreased Akt phosphorylation (45). In the current study, we found that the membrane-to-cytosolic ratio of ceramide was increased in muscle of high-fat–fed animals, and this was associated with impaired phosphorylation of Akt. We also observed that there was a tendency for the ceramide membrane-to-cytosolic ratio to be reduced in muscles overexpressing CPT1 from fat-fed rats, an effect that may have contributed to enhanced insulin-stimulated Akt phosphorylation. In addition, we also found that the DAG membrane-to-cytosolic ratio was decreased in muscle from fat-fed animals overexpressing CPT1.

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