EXCESS LIPID ACCUMULATION is implicated in the pathophysiology of obesity-associated insulin resistance, and many believe this is secondary to impairments in lipid disposal pathways (22, 38, 48, 52, 55, 56). Consequently, much research has focused on primary aspects involved in lipid metabolism, such as mitochondrial oxidative capacity, lipid transport, and lipid trafficking. However, an important factor that has largely been overlooked with respect to maintaining a healthy cellular lipid environment is the peroxisome. Peroxisomes are ubiquitously expressed and have a wide range of cellular functions, including a primary role in fatty acid oxidation (68). Unlike mitochondria, peroxisomal β-oxidation is incomplete and cannot chain-shorten fatty acids beyond six carbon residues (50), thus leaving a medium-chain acyl-CoA derivative as well as acetyl-CoA residues. Since peroxisomes lack a tricarboxylic acid cycle and electron transport system, the products of peroxisomal oxidation are not linked directly to energy production. Instead, they are released into the cytosol, where they can be either incorporated into biosynthetic pathways or partitioned toward the mitochondria for complete lipid disposal. It is clinically important to determine the fate of these products as conditions such as obesity, insulin resistance, and diabetes are characterized by imbalances between the synthetic and catabolic pathways of lipid metabolism.

Peroxisomal fatty acid entry is not carnitine dependent (43, 57, 61, 64, 66) or sensitive to inhibitors of carnitine palmitoyl-transferase I (CPT I) (33, 54, 58, 61). Furthermore, although there is debate as to whether the products of peroxisomal oxidation are released into the cytosol as unbound carbon moieties (21, 28, 51) or as carnitine derivatives (35, 46, 62, 67), this controversy may be inconsequential, as either form is believed to enter mitochondria independently of CPT I, thus bypassing the rate-limiting step of mitochondrial fat oxidation (54). The potential for peroxisomes to aid lipid disposal by circumventing CPT I makes the study of this organelle’s potential for attenuating dysfunctions in lipid metabolism that are often observed in obese and diabetic conditions intriguing.

To date, investigations targeted at elucidating the role of peroxisomes in diabetes have been performed primarily in rodent models of type 1 diabetes and generally indicate elevated peroxisomal β-oxidative capacity (4, 15–17, 33, 42, 44, 60, 72). Although the clinical manifestations of type 1 and type 2 diabetes are similar, the pathophysiology underlying these diseases is quite different. Investigations using animal models of insulin resistance are sparse but have shown diminished hepatic peroxisomal oxidative capacity in obese rats (11, 40). With respect to peroxisomal responses to changes in lipid content in extrahepatic tissues, skeletal muscle from hHTg rats (nonobese model of hypertriglyceridermia and insulin resistance) exhibits elevated acyl-CoA oxidase activity (63), whereas animals treated with hypolipidemic agents tend to exhibit increases in myocardial and skeletal muscle peroxisomal activity (32, 75). Although these studies give some indication of peroxisomal responses under pathological conditions of lipid overload, there exists no direct evidence regarding peroxisomal contributions to mitochondrial fatty acid oxidation to assess the potential for this organelle to assist in complete lipid disposal (CO₂ production) within these disease states. Due to these deficiencies in the literature, the purpose of
this investigation was to determine the peroxisomal contribution to complete lipid disposal in three major tissue sites of lipid metabolism (liver, heart, and skeletal muscle) in a rodent model of obesity-associated insulin resistance. Since skeletal muscle and heart use lipids primarily as a source of metabolic fuel we hypothesized that peroxisomes would aid primarily in complete lipid disposal in these tissues, whereas the products of hepatic peroxisomal oxidation would not be as readily utilized by the mitochondria. We further predicted that peroxisomal oxidation would be elevated in the heart and skeletal muscle and decreased in the liver in response to obesity-associated insulin resistance.

MATERIALS AND METHODS

Animals. Age-matched male Zucker lean (292 g; n = 13) and fatty (fa/fa, 507 g; n = 13) rats (Harlan Laboratories, Indianapolis, IN) were used to determine organelle intactness and peroxisomal contributions to complete lipid disposal in insulin resistance. Male Sprague-Dawley rats (n = 12; Harlan Laboratories) were used to assess the effect of increasing peroxisomal content on lipid disposal as well as to test endogenous lipid effects on fatty acid oxidation. Animals were housed in a temperature-controlled environment with a 12:12-h light-dark cycle and provided standard rat chow and water ad libitum. Oral glucose tolerance tests were performed on lean and fatty Zucker rats after an overnight fast as previously described (41). On days of in vitro experimentation, food was removed 4 h prior to the rats being killed. This protocol was chosen because lipids become the primary fuel we hypothesized that peroxisomes would aid primarily in lipid metabolism (liver, heart, and skeletal muscle) in a rodent model of obesity-associated insulin resistance. Since skeletal muscle and heart use lipids primarily as a source of metabolic fuel we hypothesized that peroxisomes would aid primarily in complete lipid disposal in these tissues, whereas the products of hepatic peroxisomal oxidation would not be as readily utilized by the mitochondria. We further predicted that peroxisomal oxidation would be elevated in the heart and skeletal muscle and decreased in the liver in response to obesity-associated insulin resistance.

Fatty acid oxidation procedures. Experiments utilizing [1-14C]palmitate, [U-14C]octanoate, [1-14C]octanoylcarnitine, [1-14C]palmitoylcarnitine, and [1-14C]lignoceric acid were performed to study the pathways of mitochondrial and peroxisomal fatty acid oxidation. Lignoceric acid (25 μM) was solubilized using α-cyclohex dextrin (10 mg/ml final concentration), whereas all other fatty acids (200 μM) were bound to 0.5% bovine serum albumin (3.3 molar ratio of fatty acid:albumin). Specific activities for 14C fatty acid substrates at final concentrations of 200 μM were ~8,000–10,000 dpm/nmol (0.5 μCi/ml), and that of lignoceric acid (25 μM) was ~30,000–40,000 dpm/nmol (0.5 μCi/ml). Once solubilized, all fatty acid substrates were brought up in reaction buffer to yield the following final concentrations: 100 mM sucrose, 10 mM Tris-HCl, 10 mM KPO4, 100 mM KCl, 1 mM MgCl2·6H2O, 1 mM EDTA, 1 mM ATP, pH = 7.4). Homogenates were prepared according to the methods of Kim et al. (23). Briefly, ~50–100 mg of tissue was thoroughly minced with scissors in 200 μl of SET buffer (250 mM sucrose, 1 mM EDTA, 10 mM Tris·HCl, and 2 mM ATP, pH = 7.4), and then the buffer volume was brought up to yield a 20-fold (wt/vol) dilution. Tissue suspensions were transferred to a 3-ml Potter-Elvehjem glass mortar and homogenized on ice with a Teflon pestle at 10 passes over 30 s at 1,200 rpm. Sample volumes were brought up to yield the appropriate dilutions required for the fatty acid oxidation procedures [1:20 wt/vol for all tissues used for lignoceric acid oxidation; all other oxidation experiments were performed using the following dilutions: MG (1:20), liver (1:40), and LV (1:60)]. Homogenates were kept on ice until oxidation experiments were performed (<10 min elapsed time between beginning of homogenization procedure and start of the incubation of the final oxidation plate).

Enzyme activity assays. Mitochondrial citrate synthase (CS) activity was analyzed using the spectrophotometric detection of reduced DTNB at a wavelength of 412 nm according to the methods of Srere (59). Peroxisomal catalase activity was determined by the spectrophotometric (520 nm) oxidative coupling of 3,5-dichlorobenzene-sulfonic acid to 4-aminophthaldialdehyde and hydrogen peroxide (Sigma).

Organelle intactness. Whole tissue homogenates do not contain 100% intact organelles; therefore, we conducted experiments to determine mitochondrial and peroxisomal intactness under our experimental conditions in a subset of age-matched lean (n = 6) and fatty (n = 6) Zucker rats. Fresh tissue homogenates were partitioned as two equal aliquots. One aliquot was sonicated (5 x 1 s at 2.5 Hz on ice) to determine total mitochondrial or peroxisomal activity as CS or catalase enzyme activity, respectively. Aliquot 2 was centrifuged at 12,000 g (mitochondria) or 16,000 g (peroxisome), and the supernatant (disrupted organelles) was analyzed for CS or catalase activity as appropriate. Organelle intactness was determined as [(catalase or CS activity from sonicated samples / total enzyme activity) – (catalase or CS activity from the supernatant / disrupted organelles following centrifugation)] / (total catalase or CS activity from sonicated samples) x 100.

Oxidation studies measured 14C-labeled CO2 and acid-soluble metabolite (ASM) production over the course of 30 min (4 h for lignoceric acid) according to the methods of Kim et al. (23). Radioactivity of CO2 and ASM fractions was determined by liquid scintillation counting using 4 ml of Unisint BD (National Diagnostics, Atlanta, GA). Fatty acid oxidation was quantified using the following formula: [{(dpm – BL)/SA} / [g of tissue wet wt/well × time (min) of reaction incubation)] x 60/min, where BL is dpm of blank wells and SA is fatty acid-specific radioactivity. Data are expressed as nanomoles of substrate oxidized per gram tissue wet weight per hour.

Effect of differential endogenous lipid content on rates of fatty acid oxidation in whole tissue homogenates. There is the potential for differences in endogenous (nonlabeled) lipids to differentially affect the specific activity of the isotopes, which could then alter our understanding of how lean and obese insulin-resistant subjects compared with respect to the oxidative handling of the isotopes employed. Consequently, experiments were conducted to determine the effects of differential fatty acid loads on rates of fatty acid oxidation in our system. Fresh MG, liver, and LV from Sprague-Dawley animals were homogenized under identical conditions, as described above. In addition, similar masses of MG, liver, and LV from lean and fatty Zucker rats were homogenized, lysed in liquid nitrogen, and then added in an equal vol/vol ratio to the fresh tissue homogenates from Sprague- Dawley rats and assayed for rates of fatty acid oxidation. The purpose was to add similar, nonradioactive lipid as would be encountered in the experimental conditions in the homogenates obtained from the lean and fatty Zucker rodent subjects to determine whether the differences in endogenous lipid content detected between lean and fatty Zucker rats resulted in a differential radiolabel dilution effect that would alter our assessment of rates of oxidation. Fatty acid oxidation rates from radiolabeled CO2 and ASMs were determined as described above.
Organelle isolation procedure. Sprague-Dawley rats (n = 12) were anaesthetized as described above, and ~10 g of liver was collected for peroxisome isolation while whole gastrocnemius and whole quadriceps were combined (~10 g total) to isolate skeletal muscle mitochondria. Tissues were excised with blood flow intact and placed in homogenization medium (250 mM sucrose, 1 mM EDTA, 5 mM MOPS, 0.1% ethanol, pH = 7.2) to make a twofold dilution (wt/vol) and minced for 3 min with Miltex (York, PA) scissors. The solution was brought up to a 10-fold dilution (wt/vol) and homogenized on ice using an Ultra-Turrax T-25 homogenizer (IKA, Wilmington, NC) at 9,500 rpm for 15 s. The suspension was transferred to a Potter Elvehjem glass mortar and further homogenized using a Yamato Labo-Stirrer Model LR-41D homogenizer (Tokyo, Japan) with an attached Teflon pestle at setting seven over 10 passes. The homogenate was transferred to a polycarbonate centrifuge tube and spun at 800 g for 10 min at 4°C. The supernatant was transferred into a separate polycarbonate tube and spun at 17,000 g for 10 min at 4°C. The resultant pellet was washed and gently resuspended in 3 mL of homogenization medium, transferred to a Dounce mortar, and homogenized by hand over three passes. This suspension was passed through a 20-ml continuous (10–40%) Optiprep density gradient (Axis-Shield PoC, Oslo, Norway) by centrifugation at 105,000 g for 65 min at 4°C to separate organelle fractions. Fractions were collected in 1-ml segments, and each was assayed for CS and catalase activity to determine mitochondrial and peroxisomal content/purity, respectively. The three fractions with the highest enzyme activity and purity for a given organelle were combined and brought up to 6 mL with homogenization medium. These isolates were then centrifuged at 17,000 g for 10 min at 4°C, and the pellets were gently resuspended in 900 μL of SET buffer and used immediately for oxidation experiments.

Immunofluorescence. Approximately 20 mg of tissue was fixed in a 4% paraformaldehyde-0.1% glutaraldehyde solution for 3.5 h. Following fixation, tissues were rinsed twice for 10 min each in 0.1 M NaPO₄ (pH = 7.3) and then stored at 4°C for 24 h in a 30% sucrose and 0.1 M NaPO₄ solution. Tissues were subsequently frozen in optimal cutting temperature compound and stored at −80°C. Serial sections were cut at 11 μm, mounted on slides, and stored at −80°C until immunofluorescence was performed.

Lipid accumulation was determined using Oil Red O staining as previously described (41). For peroxisome staining, slides were rehydrated for 15 min in phosphate-buffered saline (PBS) and incubated in blocking solution (PBS, 0.3% Triton X-100, 10% normal donkey serum) for 90 min, both at room temperature. Slides were then incubated in 1:750 (MG) and 1:1,000 (liver and LV) diluted peroxiosomal membrane protein-70-kDa primary antibody (PMP-70; Zymed Laboratories, San Francisco, CA) overnight at room temperature and rinsed (3 × 7 min) in Triton-Tris buffer solution while being gently agitated. Afterward, slides were incubated in 1:200 (MG) and 1:500 (liver and LV) diluted Cy2 secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) for 60 min at room temperature and rinsed (3 × 15 min) in Triton-Tris buffer solution, before nonphenylenediamine containing mounting medium was applied, (Kirkegaard & Perry Laboratories, Gaithersburg, MD) and covered with a coverslip. Negative controls (secondary antibody only) for each staining session were used to ensure specificity of fluorescence.

Images were digitized with a Nikon E600 fluorescence microscope fitted with an Orca II charge-coupled device camera (Hamamatsu, Bridgewater, NJ). A 10 × 10 grid reticle (Klarmann Rulings, Litchfield, NH) numbered 1–100 was placed in the eyepiece to determine the sampling regions (grids) for images to be taken for all microscopy sessions. Determination of the sampling regions was done prior to experimentation and held constant during all microscopy sessions for their respective stains. Images for all peroxisome slides were captured in grids 13, 19, 56, 73, and 79 and grids 23, 28, 73, and 78 for all lipid slides. The initial field of view was determined using the ×10 objective to position the slide, where tissue “filled” the entire grid reticle. Images were subsequently obtained within each predetermined grid using the ×20 and ×100 objectives for lipids and peroxisomes, respectively.

Image analysis was performed using ImagePro Plus 4.5.1 software (Media Cybernetics, Silver Spring, MD). Peroxisome image analysis was performed on four slides from each tissue for each rat (n = 3 model). One image was taken within each of the five sampling regions (grids 13, 19, 56, 73, and 79) for each slide and quantified for peroxisome density per μm² tissue. Thus, a total of 60 images per tissue were analyzed for both the lean and fatty Zucker rats.

Statistical analysis. All data are presented as means ± SE and were analyzed using SigmaStat software (Systat Software, Point Richmond, CA). Inhibition induced by malonyl-CoA was analyzed using a repeated-measures analysis of variance, whereas between-group differences were determined using a one-way analysis of variance. A Tukey’s post hoc test was performed where appropriate. Significance level was established a priori at P ≤ 0.05.

RESULTS

Oral glucose tolerance test. No difference in resting glucose values was observed between lean and fatty Zucker rats (Fig. 1A), although fatty rats demonstrated basal hyperinsulinemia (Fig. 1B). In response to the oral glucose challenge, serum glucose (Fig. 1A) and insulin (Fig. 1B) levels were significantly higher in fatty rats than their lean counterparts at all time points. The finding that fatty rats had more difficulty clearing the glucose load than their lean counterparts despite having higher insulin levels is indicative of insulin resistance in fatty Zucker rats.

![Fig. 1](http://ajpendo.physiology.org/)

Fig. 1. Oral glucose tolerance test. No difference in resting glucose values was observed between groups (A), but fatty rats demonstrated basal hyperinsulinemia (B). Following an oral glucose challenge, fatty rats had more difficulty clearing the glucose load than their lean counterparts despite higher insulin levels at all time points. These results indicate that fatty rats were insulin resistant. *P < 0.05 between lean (n = 7) and fatty (n = 7) Zucker rats.
Endogenous lipids. Quantification of tissue lipid accumulation using Oil Red O staining is displayed in Fig. 2. Fatty Zucker rats exhibited excess lipid accumulation in MG (3.8-fold) and liver (1.6-fold) compared with lean controls. No differences were detected between strains in LV tissue. Since the experimental design employed the use of tissue homogenates from lean and fatty Zucker rats to explore rates of fatty acid oxidation, it is reasonable to suspect that the greater lipid content in homogenates from fatty Zucker vs. lean rats may differentially alter (i.e., lower) fatty acid oxidation rates by competing with the exogenous fatty acids added in these experiments. Therefore, it was important to perform control experiments (described in MATERIALS AND METHODS) to determine whether differences in endogenous lipids between these strains may have resulted in a label dilution effect. Tissue homogenates obtained from lean and fatty Zucker rats were lyzed in liquid nitrogen exhibited no capacity to produce 14CO₂, thus indicating complete disruption of functional mitochondria (Fig. 3, A, C, and E). Titration of these samples, which would retain differences in endogenous lipids, upon intact homogenates obtained from Sprague-Dawley rats revealed that neither 14CO₂ (Fig. 3, A, C, and E) nor 14C-ASM (Fig. 3, B, D, and F) production was diminished in any tissue tested. Importantly, this was consistent between strains. These results globally indicate that there were no detectable label dilution effects incurred by excess endogenous lipids in fatty Zucker rat samples upon our assessment of rates of fatty acid oxidation. Furthermore, given the dilution of endogenous lipids due to the homogenization procedure (1:20 in MG, 1:40 in liver, and 1:60 in LV) and the high concentration of exogenous fatty acid added within our incubations, we are confident that the differences in rates of oxidation measured are valid and support the conclusions offered.

Malonyl-CoA vs. etomoxir. A central tenet to the current study is that, when mitochondrial CPT I is inhibited, long-chain fatty acids (LCFAs) can be redistributed toward peroxisomes that chain-shorten these lipids and ultimately yield products that are capable of entering the mitochondria independently of CPT I. Since inhibition of CPT I is conceptually important to the experimental design of this study, we com-

Fig. 2. Tissue lipid accumulation in lean and fatty Zucker rats. Lipid density was quantified (72 images per tissue per strain) using Oil Red O in liver, left ventricle, and mixed gastrocnemius sections. Objective magnification: ×20. *P < 0.05 between lean (n = 3) and fatty (n = 3) Zucker rats.
pared the maximal degree of CPT I inhibition induced by 100 μM malonyl-CoA (physiological inhibitor) vs. 100 μM etomoxir (irreversible, covalent inhibitor) using 14CO2 production from [1-14C]palmitate as an output measure. Figure 4 shows that, in all tissues studied, the inhibition of palmitate oxidation was significantly greater using 100 μM malonyl-CoA than a similar dose of etomoxir; therefore, we chose to use 100 μM malonyl-CoA to inhibit CPT I activity in all subsequent experiments. Since malonyl-CoA can be metabolized by the enzymatic action of malonyl-CoA decarboxylase, which remains in tissue homogenates, it is important to consider the potential ramifications of malonyl-CoA catabolism on the results of experiments using this inhibitor. With this in mind, the finding that the inhibition induced by malonyl-CoA was greater than that of etomoxir suggests that malonyl-CoA concentrations did not drop below that which is required to attain maximal inhibition. In addition, it is unlikely that malonyl-CoA catabolism was enough to supply a competing substrate pool, since oxidation of the substrate that is most susceptible to such contamination, acetylcarnitine, was not significantly diminished in any of the tissue homogenates studied (Figs. 5D, 6D, and 7D).

Organelle intactness assay. Results presented in Table 1 show that 59–73% of mitochondria and 24–48% of peroxisomes are intact following the homogenization procedure in all tissues. These results are comparable with past reports in the literature (3, 65). Importantly, no strain-specific differences in organelle intactness were detected within any given tissue.

Liver fatty acid oxidation. Figure 5A shows that CS activity in liver homogenates was significantly lower in fatty Zucker rats vs. their lean counterparts. Additionally, 14CO2 production from [1-14C]palmitate (Fig. 5B), [U-14C]palmitate (Fig. 5C), octanoate (Fig. 5E), and octanoylcarnitine (Fig. 5F) was also significantly reduced in fatty liver homogenates. Alternatively, incomplete oxidation (ASM production) from palmitate substrates (Fig. 5, B and C) was similar between strains. These data indicate a generalized decrease in complete hepatic mitochondrial lipid disposal in fatty Zucker rats. It is important to note that malonyl-CoA did not have an inhibitory effect on 14CO2 production from acetylcarnitine (Fig. 5D), octanoate (Fig. 5E), or octanoylcarnitine (Fig. 5F) in either strain, suggesting that these lipid moieties enter hepatic mitochondria independently of CPT I. Interestingly, however, although oxidation rates of these short- and medium-chain lipid species...
were unaltered in the presence of malonyl-CoA in lean animals, these rates were significantly elevated in fatty liver homogenates. Since data presented in Fig. 3 indicate that this is not likely an artifact due to a compensatory increase in response to a decrease in the oxidation of endogenous lipids, these results seem to suggest yet to be determined biological alterations in the capacity to handle chain-shortened fatty acids in the presence of malonyl-CoA by liver mitochondria experiencing chronic lipid overload.

Hepatic catalase activity was not different between strains (Fig. 5G); however, immunofluorescent staining of PMP-70 indicated that peroxisomal density was 1.8-fold greater in fatty Zucker livers vs. their lean counterparts (Fig. 5H). As depicted in Fig. 5I, complete oxidation of lignoceric acid (CO₂ production) was not different between strains, whereas incomplete oxidation (ASM production) was significantly elevated in liver homogenates from fatty Zucker rats. Interestingly, experiments using a dose of malonyl-CoA (100 μM) that has been reported
to inhibit the activity of the liver isoform of CPT I >80% (2, 37) revealed that CO₂ production was uninhibited using [1-14C]palmitate and was significantly elevated (represented graphically as a negative %inhibition) when using [U-14C]palmitate (Fig. 5J). The significant difference between [1-14C]- vs. [U-14C]palmitate in response to malonyl-CoA likely represents the peroxisomal contribution of medium-chain fatty acid (MCFA) derivatives to the mitochondria for use as metabolic fuel, which can be tracked using [U-14C]palmitate. With this in mind, the evidence presented in Fig. 5J shows that the percent inhibition of each uniquely radiolabeled palmitate substrate was not different between lean and fatty Zucker liver homogenates, suggesting that the peroxisomal contribution of MCFA derivatives to mitochondria was not different between strains. These data support findings using lignoceric acid (Fig. 5I) and suggest that the peroxisomal contribution to complete lipid disposal in liver was not different between strains.

Heart fatty acid oxidation. CS activity in the LV was similar between lean and fatty Zucker rats (Fig. 6A). Additionally, oxidation rates of all fatty acid substrates were similar between strains (Fig. 6, B–F). These data suggest that the heart’s inherent mitochondrial oxidative capacity is not different between lean and fatty rats. Importantly, malonyl-CoA did not inhibit complete oxidation (14CO₂ production) of acetyl carnitine (Fig. 6D), octanoate (Fig. 6E), or octanoylcarnitine (Fig. 6F), supporting the notion that, like the liver, these substrates enter myocardial mitochondria independently of CPT I.

Catalase activity (Fig. 6G) and PMP-70 immunofluorescent staining (Fig. 6H) indicated that cardiac peroxisomal density was similar between lean and fatty Zucker rats. Results from experiments using lignoceric acid revealed that, unlike the liver, partitioning of the products of peroxisomal β-oxidation went exclusively toward mitochondria for complete lipid disposal since CO₂ was the only product recovered (Fig. 6I). Oxidation of lignoceric acid, however, was not different between strains. Figure 6J indicates that, regardless of strain, there was a significant difference in the degree of malonyl-CoA inhibition of 14CO₂ production between [1-14C]- vs. [U-14C]palmitate, suggesting partitioning of peroxisome-derived MCFA moieties to the mitochondria. However, the degree of inhibition of each radiolabeled palmitate substrate was consistent between lean and fatty rats, signifying that there was no difference in the peroxisomal contribution of MCFA derivatives toward complete oxidation between strains. These results consistently suggest the peroxisomal oxidative capacity in the heart is similar between lean vs. fatty Zucker rats.

MG fatty acid oxidation. Skeletal muscle from Zucker fatty rats had significantly higher CS activity (Fig. 7A) as well as elevated 14CO₂ production from [1-14C]palmitate (Fig. 7B), acetyl carnitine (Fig. 7D), octanoate (Fig. 7E), and octanoylcarnitine (Fig. 7F). In addition, ASM production from both [1-14C]- and [U-14C]palmitate (Figs. 7, B and C, respectively) was higher in MG from fatty Zucker rats compared with lean controls. These data indicate that skeletal muscle mitochondrial fatty acid oxidative capacity is elevated in fatty Zucker rats. Malonyl-CoA did not alter 14CO₂ production from acetyl carnitine (Fig. 7D) or octanoylcarnitine (Fig. 7F) but did significantly inhibit 14CO₂ production from octanoate (Fig. 7E), suggesting that skeletal muscle is unique (compared with liver or LV) in that MCFA oxidation is at least partially carnitine dependent.
Fig. 5. Liver fatty acid oxidative capacity. Mitochondrial oxidative capacity was determined by using citrate synthase activity (A) as well as measuring oxidation rates of \([1-^{14}C]\)palmitate (B), \([U-^{14}C]\)palmitate (C), acetylcarnitine (D), octanoate (E), and octanoylcarnitine (F). Acetylcarnitine, octanoate, and octanoylcarnitine oxidation were measured as \(^{14}CO_2\) production per 100 \(\mu\)M malonyl-CoA. Catalase activity (G) and peroxisomal membrane protein-70-kDa primary antibody (PMP-70) immunofluorescence (H) were used to assess peroxisomal content. \([1-^{14}C]\)lignoceric acid oxidation (I) was used to track the fate of peroxisomal-derived C\(_2\) units, whereas the difference in \%'inhibition of \(^{14}CO_2\) production between \([1-^{14}C]\)- vs. \([U-^{14}C]\)palmitate induced by 100 \(\mu\)M malonyl-CoA (J) served as an index of peroxisomal-derived medium-chain fatty acid (MCFA) moieties that were shuttled to mitochondria for complete lipid disposal. \(^*P<0.05\) between lean (n = 7) and fatty (n = 7) Zucker rats; \(^#P<0.05\) due to malonyl-CoA; \(^$P<0.05\) between \%'inhibition of \(^{14}CO_2\) production induced by malonyl-CoA of \([1-^{14}C]\)palmitate and \([U-^{14}C]\)palmitate.
Mitochondria

**A** Citrate Synthase Activity

- **B**: 1-14C Palmitate
- **C**: U-14C Palmitate

Peroxisomes

**D**: Acetyl carnitine

- **E**: Octanoate
- **F**: Octanoylcarnitine

**G**: Catalase Activity

- **H**: Peroxisomal Density

**I**: Lignoceric Acid

- **J**: Palmitate % Inhibition of CO2

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Fig. 6. LV fatty acid oxidative capacity. Mitochondrial oxidative capacity was determined by using citrate synthase activity (A) as well as measuring oxidation rates of [1-14C]palmitate (B), [U-14C]palmitate (C), acetyl carnitine (D), octanoate (E), and octanoylcarnitine (F). Acetyl carnitine, octanoate, and octanoylcarnitine oxidation were measured as 14CO2 production ± 100 μM malonyl-CoA. Catalase activity (G) and PMP-70 immunofluorescence (H) were used to assess peroxisomal content. [1-14C]lignoceric acid oxidation (I) was used to track the fate of peroxisomal-derived C2 units, whereas the difference in %inhibition of 14CO2 production between [1-14C]- vs. [U-14C]palmitate induced by 100 μM malonyl-CoA (J) served as an index of peroxisomal-derived MCFA moieties that were shuttled to mitochondria for complete lipid disposal. *P* < 0.05 between %inhibition of 14CO2 production induced by malonyl-CoA of [1-14C]palmitate and [U-14C]palmitate.
Mitochondria

A  Citrate Synthase Act

B  1-14C Palmitate

C  U-14C Palmitate

D  Acetylcaritnine

E  Octanoate

F  Octanoylcarnitine

Peroxisomes

G  Catalase Act

H  Peroxisome Density

I  Lignoceric Acid

J  Palmitate % Inhibition of CO2

Fig. 7. MG fatty acid oxidative capacity. Mitochondrial oxidative capacity was determined by using citrate synthase activity (A) as well as measuring oxidation rates of [1-14C]palmitate (B), [U-14C]palmitate (C), acetylcaritnine (D), octanoate (E), and octanoylcarnitine (F). Acetylcaritnine, octanoate, and octanoylcarnitine oxidation were measured as 14CO2 production ± 100 µM malonyl-CoA. Catalase activity (G) and PMP-70 immunofluorescence (H) were used to assess peroxisomal content. [1-14C]lignoceric acid oxidation (I) was used to track the fate of peroxisomal-derived C2 units, whereas the difference in %inhibition of 14CO2 production between [1-14C]- vs. [U-14C]palmitate induced by 100 µM malonyl-CoA (J) served as an index of peroxisomal-derived MCFA moieties that were shuttled to mitochondria for complete lipid disposal. #P < 0.05 between lean (n = 7) and fatty (n = 7) Zucker rats; *P < 0.05 due to malonyl-CoA; $P < 0.05 between %inhibition of 14CO2 production induced by malonyl-CoA of [1-14C]palmitate and [U-14C]palmitate.
Catalase activity (Fig. 7G) and PMP-70 immunofluorescence (Fig. 7H) were both significantly elevated in MG from fatty Zucker rats, suggesting elevated peroxisomal density. Perhaps the most provocative result of the current study was that lignoceric acid oxidation was completely undetectable in MG from lean rats, whereas this was distinctly present in MG from fatty rats (Fig. 7I). In addition, lignoceric acid oxidation in this tissue was detected only as $^{14}$CO$_2$, indicating complete partitioning of peroxisomal products toward the mitochondria for lipid disposal. Also, the percent inhibition of $^{14}$CO$_2$ production from [1-$^{14}$C]-vs. [U-$^{14}$C]palmitate induced by malonyl-CoA was identical in MG obtained from lean rats but was distinctly different in fatty rats (Fig. 7J). The fact that the differences in the inhibitory characteristics between [1-$^{14}$C]- vs. [U-$^{14}$C]palmitate were detected solely in samples that exhibited lignoceric acid oxidation (Zucker fatty MG) lends credence to the concept that the difference in percent inhibition between these two uniquely radiolabeled palmitate substrates is indicative of peroxisomal contributions of MCFA derivatives toward complete lipid disposal. With this in mind, the degree of malonyl-CoA inhibition of $^{14}$CO$_2$ production was significantly lower in fatty vs. lean MG for both [1-$^{14}$C]palmitate (80 vs. 92%) and [U-$^{14}$C]palmitate (72 vs. 92%), indicating enhanced distribution of peroxisomal products toward the mitochondria only in fatty Zucker skeletal muscle, which is consistent with findings using lignoceric acid as a substrate.

Peroxisomal titration experiments. To support and extend the results above, we decided to test the effect of increasing peroxisomal content on complete fatty acid oxidation using varying amounts of isolated hepatic peroxisomes titrated upon a set amount of mitochondria isolated from skeletal muscle from Sprague-Dawley rats. These organelle fractions were isolated from different tissues because 1) each tissue chosen maximized the yield of the selected organelle and 2) the muscle isoform of CPT I is the most sensitive to malonyl-CoA inhibition and is therefore best suited to test the peroxisomal involvement in LCFA oxidation under conditions where CPT I activity is diminished. Optiprep gradients were collected in

![Fig. 8. Effect of artificial enhancement of peroxisomal content on fatty acid oxidation.](image-url)

Table 1. *Organelle intactness (%)*  

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<th>Mitochondria</th>
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Values are means ± SE. MG, mixed gastrocnemius; LV, left ventricular. Mitochondrial and peroxisomal intactness were assessed using citrate synthase and catalase activity latency, respectively, in tissue homogenates from a subset of age-matched lean (n = 6) and fatty (n = 6) Zucker rats.
It was necessary to verify that the peroxisomal contamination of the purified skeletal muscle mitochondrial suspension did not represent a large percentage relative to the amount of hepatic peroxisomes that were to be included in subsequent titration experiments and vice versa. For this reason, Fig. 8A and B, was plotted using the same scale and reveals that such potential contamination represented <1% of the total amount of enzymatic activity that was present in the titration experiments. These data verify that the chosen fractions were indeed enriched in the organelle of choice and exhibited minimal contamination.

Oxidation studies using lignoceric acid confirm that isolated mitochondria were unable to utilize lignoceric acid, since neither 14CO2 (Fig. 8C) nor 14C-ASMs (Fig. 8D) were detected when peroxisomes were not included in the incubation. Furthermore, when only peroxisomes were present, 14C-ASM production from lignoceric acid was very high (Fig. 8D), yet 14CO2 production was undetectable (Fig. 8C). Upon inclusion of mitochondria, 14C-ASM accumulation dramatically dropped in coordination with the appearance of 14CO2. These results confirm that lignoceric acid must first be metabolized by peroxisomes, yet mitochondria must be present to accept peroxisomal products and utilize them as metabolic fuel. These findings not only confirm the purity of the organelle fractions but also validate the use of 14CO2 production from lignoceric acid to assess peroxisomal-mitochondrial interactions. Since the purpose of this study was to determine the effect of increasing peroxisomal content on fatty acid oxidation, it is important to note that artificially increasing the peroxisomal content upon a set amount of mitochondria resulted in a linear increase in 14CO2 production from lignoceric acid (Fig. 8C). Interestingly, with regard to basal palmitate oxidation, peroxisomal titration resulted in increasing amounts of 14C-ASMs (Fig. 8F), yet there was no significant difference detected in 14CO2 liberation (Fig. 8E). These data suggest that peroxisomes metabolized LCFAAs, but the chain-shortened products were not shunted to the mitochondria for complete lipid disposal in the basal state. Conversely, increasing peroxisomal content did significantly diminish the inhibitory effects of malonyl-CoA on both 14CO2 and 14C-ASM production from palmitate. Because it is suspected that chain-shortened products of peroxisomal oxidation can enter the mitochondria independently of CPT I, these results suggest that the presence of peroxisomes can help maintain a supply of fatty acid-derived substrates to the mitochondria under conditions when malonyl-CoA levels are high.

DISCUSSION

The purpose of this investigation was to determine the peroxisomal contribution to complete lipid disposal in various tissues in the obese, insulin-resistant condition. To address this topic, tissue homogenates were studied because they contain both mitochondrial and peroxisomal organelle fractions without the confounding effects of cellular transport and lipid trafficking. It is known, and has been confirmed in the present investigation, that peroxisomal β-oxidation of long- and very long-chain fatty acids yields chain-shortened products but does not intrinsically yield CO2. Consequently, any production of CO2 using these substrates is ultimately derived from mitochondria. Mitochondrial CO2 production is indicative of complete substrate oxidation; therefore, liberation of CO2 from substrates derived from peroxisomal β-oxidation can be used as a measure of peroxisomes supplying chain-shortened products to the mitochondria for complete lipid disposal. Peroxisomal β-oxidation is incomplete and yields medium-chain acyl-CoA residues as well as acetyl-CoA (50), yet these products are not released into the cytosol as CoA esters. For this reason, we chose to measure CO2 production from acetyl carnitine, octanoylcarnitine, and octanoyl carnitine in the presence and absence of malonyl-CoA to directly assess the mitochondrial capacity to utilize potential chain-shortened peroxisomal products. These experiments confirmed that, with the exception of octanoate in skeletal muscle, these short- and medium-chain derivatives do enter mitochondria independently of CPT I. The fact that the carnitine dependence of octanoyl oxidation was unique to skeletal muscle was unexpected and intriguing, and experiments are being performed to further characterize this in human and rodent models.

Oxidation studies using isolated organelle fractions revealed that peroxisomes do chain-shorten palmitate, yet it appears that these products are not used by mitochondria as metabolic fuel unless malonyl-CoA is present (i.e., when CPT I activity was inhibited, artificially increasing peroxisomal content significantly alleviated the decrease in CO2 production from palmitate). These studies, therefore, offer evidence to suggest that the metabolism of fatty acids through peroxisomes is indeed a potential pathway that can circumvent the malonyl-CoA regulation of mitochondrial LCFA oxidation. Although this is certainly an intriguing concept, the mechanism remains unclear. In this regard, Joly et al. (20) have recently shown high malonyl-CoA decarboxylase content in peroxisomes, suggesting that this organelle may simply be serving a function to reduce malonyl-CoA levels. However, data from the current set of experiments do not support this possibility because, regardless of varying degrees of peroxisomal content, 1) malonyl-CoA inhibited palmitate oxidation to a greater extent than etomoxir in all tissues and 2) the catabolism of malonyl-CoA did not result in a substrate dilution effect in any tissue or strain of the animals studied. As an alternative explanation, many investigations suggest that peroxisomal fatty acid entry is not carnitine dependent (43, 57, 61, 64, 66) or sensitive to inhibitors of CPT I (33, 54, 58, 61). In addition, as mentioned above, results from the current investigation showed that, with the exception of octanoyl in skeletal muscle, the potential chain-
shortened products of peroxisomal oxidation do indeed enter mitochondria independently of CPT I. These findings suggest that peroxisomes supply a pathway of fatty acid metabolism that results in complete LCFA disposal, which is independent of malonyl-CoA regulation. This proposed function of peroxisomes may be particularly relevant to the field of obesity and diabetes research, as previous investigations (6, 26) have shown elevated malonyl-CoA levels and diminished CPT I activity in liver and skeletal muscle in these conditions.

Hepatic mitochondrial density and fatty acid oxidation rates per unit of tissue mass have been shown (5, 10) to be diminished in obese, insulin-resistant animals and are related to hepatosteatosis. Findings from the current investigation support this literature, as depressed hepatic citrate synthase activity and mitochondrial oxidation rates in the obese, insulin-resistant condition were found and are indicative of diminished lipid disposal, which likely contributes to excessive lipid accumulation seen in this tissue. Previous investigations, however, have established that oxidation rates from isolated mitochondria are similar between lean and obese liver (5, 10, 70). Therefore, the diminished oxidation rates detected in the present study may not be due to inherent mitochondrial defects per se but rather may be partially explained by diminished mitochondrial content per tissue mass. This and other potential mechanisms, such as extramitochondrial factors, require future studies.

Clouet et al. (11) assessed hepatic peroxisomal capacity in the obese Zucker rat and found several parameters to be either unaltered or diminished. Unfortunately, the methodology utilized did not determine the fate of peroxisomal products. Results from the current investigation indicate that hepatic catalase activity is not different between strains, but peroxisomal content as determined by immunofluorescence of PMP-70 (a peroxisomal-specific membrane protein) is higher in fatty Zucker rats. This apparent discrepancy is likely explained by previous findings (13) showing that, although catalase is the enzyme most often associated with peroxisomes, it does not always track well with changes in peroxisomal oxidative capacity (e.g., induction by fibrates). In resolution to this incongruity, it is important to note that total lignoceric acid oxidation (CO\textsubscript{2} + ASM) was significantly greater in livers from fatty Zucker rats. Further inspection of these data, however, indicate that the heightened peroxisomal activity is not serving to enhance lipid disposal, as CO\textsubscript{2} production from lignoceric acid is not different between lean and fatty Zucker rats. Instead, ASM production accounts for all of the difference in lignoceric acid oxidation between the lean and obese strains. Therefore, it seems that the peroxisomal contribution to incomplete oxidation is elevated in the insulin-resistant liver. In this regard, previous investigations have shown that potential products of peroxisomal β-oxidation can be incorporated into phospholipids, triacylglycerols, cholesterol, ketone bodies, glutamine, glutamate, and/or glutathione. Since many of the aforementioned products have been implicated in the pathophysiology of insulin resistance, it is important for future studies to determine whether hepatic peroxisomes are reconditioning the lipid environment to alleviate lipotoxicity or whether the products of peroxisomal oxidation are being incorporated into species that exacerbate this condition.

In muscle, many lines of evidence suggest that lipid accumulation may play a causative role in the development of insulin resistance (8, 9, 18, 19, 27), and it has been suggested that excess lipid accumulation is secondary to impaired mitochondrial lipid disposal (22, 38, 48, 52, 55, 56). Data in the present study at first do not appear to support this hypothesis directly, as mitochondrial oxidative capacity was actually elevated in MG from fatty Zucker rats. Our data does, however, support previous findings indicating that skeletal muscle from fatty Zucker rats contains more intramuscular lipids (29). Therefore, it can be suggested that the mitochondrial capacity, although heightened in insulin-resistant muscle from fatty Zucker rats, is insufficient to negate intramuscular lipid accumulation. In this regard, Lowell and Shulman (30) have hypothesized that type 2 diabetes in humans may be linked to excess lipid accumulation, secondary to insufficient mitochondrial oxidation. This suggests that human skeletal muscle may be even more susceptible than rodents to conditions of lipid overload.

A novel aspect of the present study is the finding that peroxisomal oxidation of long- and very long-chain fatty acids is completely absent in skeletal muscle taken from lean rats, whereas this is distinctly detected in insulin-resistant skeletal muscle. In support, Ukropec et al. (63) noted that acyl-CoA oxidase activity and gene expression were elevated in skeletal muscle from insulin-resistant hHTg rats, although this adaptation was insufficient to prevent excess intramuscular lipid accumulation. In the present study, we noted that peroxisomes have the potential to aid in complete lipid disposal in muscle. Furthermore, the oxidation of lignoceric acid in this tissue resulted in CO\textsubscript{2} production, whereas ASM levels remained undetectable. These data imply that, unlike the liver, the products of peroxisomal oxidation in skeletal muscle are dedicated solely toward mitochondria for complete lipid disposal rather than potentially entering biosynthetic pathways. Therefore, it is likely that the functional organization of this organelle system, in a tissue that does not require peroxisomal activity in the basal condition, is an adaptive response designed to increase lipid disposal in the obese, insulin-resistant state. This adaptation, although insufficient to negate excess intramuscular lipid accumulation, may serve a valuable role in diminishing the progression and severity of insulin resistance by reconditioning the intracellular lipid environment and aiding in lipid disposal by providing mitochondria with substrates that bypass CPT I. Further research is likely to reveal important mechanisms that could be exploited to aid in resolving the incidence/severity of obesity-associated type 2 diabetes.

Cardiac function is impaired in the insulin-resistant state (1, 69, 73, 74). Previous investigations (7, 36, 69) have shown that isolated working heart preparations from insulin-resistant animals typically exhibit elevated rates of fatty acid oxidation. Evidence from the current investigation indicates this adaptation is not at the level of the mitochondria, as neither citrate synthase activity nor mitochondrial fatty acid oxidation rates were different in the obese, insulin-resistant condition. These findings suggest that alterations in fatty acid metabolism detected in the whole heart are likely due to extramitochondrial factors. Data from the present study indicate that these differences cannot be explained by alterations in peroxisomal contributions to mitochondrial oxidation, as these values were not significantly different compared with lean Zucker rats. Thus, it is apparent that intrinsic lipid disposal pathways are not altered in the heart from obese, insulin-resistant rats and argue against
The concept that remodeling of myocardial lipid disposal systems is a causative factor in the impairment of cardiac function. Indeed, the differences found in isolated working heart models are most likely attributed to enhanced sarcolemmal fatty acid uptake detected in the insulin-resistant state (14, 31), resulting in greater fatty acid presentation to lipid disposal pathways.

Although not detracting from the significance of the current findings, limitations of the present investigation should be considered. First, microsomal contributions to lipid metabolism were not assessed. Microsomes contain enzymes involved in various aspects of lipid metabolism, but of particular interest is the microsomal ω-oxidation pathway. Through a series of reactions, microsomal ω-oxidation converts fatty acids of varying chain lengths to dicarboxylyl-CoAs (49). Long-chain dicarboxylyl-CoAs are oxidized almost exclusively through peroxisomal pathways (34), thereby establishing a connection between microsomes and peroxisomes. Since the present study established heightened total peroxisomal oxidation rates in liver and skeletal muscle from fatty Zucker rats, it is certainly worthwhile to perform future studies to determine whether these results are related to, dependent upon, or in spite of the altered microsomal content/function that is often reported in insulin-resistant tissues (47, 71). This interaction, however, likely does not minimize the conclusions drawn from the current investigation regarding the role of peroxisomes in complete lipid disposal, since the products of microsomal ω-oxidation must be metabolized by peroxisomes prior to being distributed to mitochondria for complete lipid disposal.

A second limitation of the current investigation is that CPT I sensitivity to malonyl-CoA was not directly assessed. Previous investigations have established that malonyl-CoA’s IC₅₀ values for hepatic CPT I are increased in rodent models of streptozotocin-induced diabetes (53) and Zucker rats (12), suggesting impaired regulation of CPT I. Unfortunately, it remains unknown whether a similar paradigm exists in insulin-resistant skeletal muscle. Regardless, the present experimental design employed a maximally inhibitory dose of malonyl-CoA that far exceeds reported IC₅₀ doses for both liver (12) and skeletal muscle (25). Therefore, shifts in malonyl-CoA sensitivity would not likely confound the present interpretations. However, in the intact organism, where dynamic changes in malonyl-CoA content are believed to modulate CPT I activity, potential alterations in malonyl-CoA sensitivity could certainly impact the relative partitioning of LCFAs between mitochondria and peroxisomes. Additionally, although data from the present investigation indicate that peroxisomal metabolism of LCFAs provides a pathway that bypasses CPT I altogether, our results also suggest that peroxisomal products were distributed to mitochondria for complete lipid disposal only when malonyl-CoA was present. Further studies should be performed under dynamic conditions known to alter lipid metabolism and malonyl-CoA content to determine the physiological role of peroxisomal-mitochondrial interactions in the in vivo setting under conditions where malonyl-CoA sensitivity may be altered.

Finally, Kim et al. (24) recently described a malonyl-CoA-insensitive portion of skeletal muscle CPT I activity. Data from the present investigation revealed that a maximally inhibitory dose of malonyl-CoA (100 μM) inhibited CO₂ production from palmitate substrates 92% in skeletal muscle homogenates from lean rats, whereas 8% remained. It is important to remember that lean rats exhibited no detectable degree of peroxisomal oxidation; thus, the residual CO₂ production cannot be explained by redistribution of palmitate to peroxisomes. Therefore, present findings support the concept that a portion of skeletal muscle CPT I activity is indeed insensitive to malonyl-CoA. Whether a similar paradigm exists in other tissues or whether malonyl-CoA-insensitive CPT I activity varies in conditions where lipid metabolism is altered remains unknown. Since this is conceptually similar to the involvement of peroxisomes in palmitate oxidation (i.e., both provide pathways that bypass malonyl-CoA regulation of mitochondrial LCFA entry), the fact that malonyl-CoA-insensitive CPT I activity was not measured remains a limitation of the current investigation, and further studies should address this issue. However, potential differences in malonyl-CoA-insensitive CPT I activity cannot explain the observed differences in percent inhibition of 14CO₂ production between [1-14C]- vs. [U-14C]palmitate, nor can this explain why these differences were detected only in tissues that exhibited peroxisomal oxidation of lignoceric acid. As such, we contend that the difference in percent inhibition between [1-14C]- vs. [U-14C]palmitate is indicative of peroxisomal contributions of MCFA derivatives to the mitochondria for use as metabolic fuel. These findings are intriguing, as our data suggest that peroxisomal-derived MCFA moieties were delivered to the mitochondria despite the presence of malonyl-CoA, which has previously been shown to inhibit peroxisomal carnitine octanoyltransferase activity (39). These findings may perhaps be partially explained by results from previous investigations, which have shown that malonyl-CoA actually had a stimulatory effect on peroxisomal handling of LCFA substrates (45, 58). Clearly, studies aimed at elucidating the mechanism(s) and regulation of export of chain-shortened intermediates from peroxisomes, as well as the uptake of peroxisomal products by mitochondria, would provide key insights into the physiological importance of peroxisomal-mitochondrial cooperativity with regard to lipid metabolism.

In conclusion, excess lipid accumulation has been implicated as a causative factor in the etiology of obesity-related insulin resistance. Results from this investigation indicate that the increase in skeletal muscle mitochondrial oxidative capacity in fatty Zucker rats is insufficient to prevent intramuscular lipid accumulation, whereas the decrease in hepatic mitochondrial oxidation may contribute to the hepatosteatosis noted in this strain. Evidence in the present study provides the first direct evidence that the products of peroxisomal β-oxidation bypass the rate-limiting step of mitochondrial oxidation, CPT I. Therefore, this organelle appears to possess the potential to assist the mitochondria in lipid disposal. The present study is also the first to directly measure the potential for peroxisomes to contribute to mitochondrial oxidation in tissues (most notably in skeletal muscle) implicated in the pathophysiology of insulin resistance. Although differences were not detected in the heart, peroxisomal oxidative capacity is elevated in both liver and skeletal muscle, yet the fate of these peroxisomal products likely vary. Our data suggest that hepatic peroxisomal products are diverted toward incomplete oxidation (e.g., biosynthetic pathways) to a greater extent in the insulin-resistant condition. Because these chain-shortened deriva-
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tives can be incorporated into products implicated in the impairment of insulin signaling, more research is needed to address this issue to determine whether elevations in hepatic peroxisomal activity are alleviating a lipotoxic environment or exacerbating the insulin-resistant condition. Alternatively, peroxisomal products in skeletal muscle are dedicated solely to the mitochondria for oxidation, providing the first direct evidence supporting the hypothesis that peroxisomal fatty acid oxidation is effectively coupled to mitochondrial β-oxidation in this tissue. Given the vast quantity and importance of skeletal muscle in the etiology of insulin resistance and diabetes, peroxisomes may play a significant role in lipid disposal and may provide an intriguing therapeutic target for the treatment of lipid dysfunction diseases. By extension, studies utilizing intact tissue or whole animal models may provide additional, and valuable, information regarding whether the conclusions drawn from the current study apply to systems where cellular fatty acid uptake and intracellular lipid trafficking are intact. Finally, future studies should also be undertaken to determine mechanisms responsible for the increased peroxisomal content/activity detected in liver and skeletal muscle. With this in mind, it is interesting to note that administration of hypolipidemic drugs that act through peroxisome proliferator-activated receptor pathways does not induce peroxisomal proliferation in skeletal muscle (32). Experiments designed to explore the genes responsible for the organization of functional peroxisomes in insulin-resistant skeletal muscle may provide valuable insight into mechanisms responsible for peroxisome biogenesis/proliferation in this tissue that may be pharmacologically exploited to help alleviate a lipotoxic environment.

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