Clofibrate Increases Long-Chain Fatty Acid Oxidation by Neonatal Pigs¹–³

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Abstract

Background: Utilization of energy-dense lipid fuels is critical to the rapid development and growth of neonates.

Objective: To increase efficiency of milk fat utilization by newborn pigs, the effect of clofibrate on in vivo and in vitro long-chain fatty acid (LCFA) oxidation was evaluated.

Methods: Newborn male pigs were administered 5 mL of vehicle (2% Tween 80) with or without clofibrate (75 mg/kg body weight) once daily via i.g. gavage for 4 d. Total LCFA oxidative capacity was measured in respiration chambers after gastric infusion (n = 5 per treatment) with isoenergetic amounts of [1-¹⁴C]triglycerides (TGs), either oleic acid (18:1n–9) TG [3.02 mmol/kg body weight (BW)⁰.⁷⁵] or erucic acid (22:1n–9) TG [2.46 mmol/kg BW⁰.⁷⁵]. Total expired ¹⁴CO₂ was collected and quantified at 20-min intervals over 24 h. Hepatic in vitro LCFA oxidation was determined simultaneously using [¹⁴C]oleic acid and erucic acid substrates.

Results: The in vivo 24-h accumulative [¹⁻¹⁴C]TG oxidation (percentage of energy intake/kg BW⁰.⁷⁵) tended to increase with clofibrate supplementation (P = 0.10), although there was no difference in the peak or mean utilization rate. The maximal extent of oleic acid TG oxidation was 1.6-fold of erucic acid TG (P < 0.006). Hepatic in vitro LCFA oxidation increased 61% with clofibrate (P < 0.0008). The increase in mitochondria was 4-fold greater than in peroxisomes. The relative abundance of mRNA increased 2- to 3-fold for hepatic peroxisome proliferator-activated receptor α and its target genes (fatty acyl-coenzyme A oxidase and carnitine palmitoyltransferase) in the pigs that were administered clofibrate (P < 0.04).


Introduction

The early postnatal period poses the greatest challenge to the energy balance of neonates, which must rapidly adapt from the utilization of carbohydrates supplied by the mother in utero to lipids supplied via milk. To cope with this challenge, the ability of neonates to oxidize FAs from endogenous lipid sources or from milk fat develops rapidly in various tissues and remains high throughout the suckling period (1,2). Ketogenesis emerges in the liver after the increased FA oxidation and provides a fuel source for extrahepatic tissues. This adaptive evolution occupies an important position in mammalian survival and is critical to the rapid development and growth of neonates after birth. However, the capacity of newborn pigs to oxidize FAs is only ~32% of 24-d-old pigs (3). Furthermore, >90% of oleate taken up by piglet hepatocytes is re-esterified with limited flux through β-oxidation (4), and suckling pigs remain hypoketone-mic despite elevated dietary fat (5,6). Studies in liver tissue demonstrated that at least 75% of the acetyl-CoA generated from hepatic FA β-oxidation is converted to acetate rather than ketone bodies (7). These observations imply unique aspects of FA metabolism and its regulation in neonatal pigs.

The pivotal role of PPARα in the regulation of FA oxidation was highlighted greatly in the past 2 decades. This transcription factor controls expression of genes encoding carnitine palmitoyltransferase (CPT)⁵ I, mitochondrial 3-hydroxy-3-methyl-glutaryl-CoA synthase (mHMGCS), and acyl-CoA oxidase (ACO), thereby inducing FA catabolism. PPARα can be activated by either its endogenous ligands, such as long-chain FAs (LCFAs) and eicosanoids, or pharmacologic agonists.

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including fibrates (8). Clofibrate, as a PPARα agonist, was used extensively in studies examining FA metabolism and regulation. Administration of clofibrate increases cellular FA uptake, transport, and oxidation in rodent species specifically for the very-long-chain FAs (VLCFAs) that are oxidized initially in peroxisomes. For instance, the oxidation of erucic acid (22:1n–9) is increased 5-fold in hepatocytes isolated from rats fed clofibrate (9). The effects of clofibrate on gene expression and enzyme activities were well documented in the review by Bremer (10).

To understand the inadequacy of FA oxidation in neonatal pigs after birth, we investigated the regulatory role of PPARα on the key enzymes that regulate FA β-oxidation by providing exogenous clofibrate stimulation. We found that the activities of CPT, ACO, and catalase in liver were significantly increased in neonatal pigs fed clofibrate via milk replacer for 14 d (11). We further tested the in vitro effects of clofibrate on hepatic FA oxidation using radiolabeled palmitic acid (16:0). Clofibrate increased rates of hepatic peroxisomal and mitochondrial β-oxidation by 60% and 186%, respectively (12). The increase of FA β-oxidation is consistent with the increase in the key enzyme activities, demonstrating that clofibrate effectively induces the expression of key enzymes related to FA oxidation and that induction is mediated by PPARα in neonatal pigs. Actually, PPARα is likely to play a central role not only in the nutritional transition period in newborn pigs but also in the adaptation to food deprivation in weaning pigs (13).

Although data from metabolic studies in vitro verified the role of clofibrate in increasing FA oxidation by activation of PPARα, to our knowledge, the effectiveness of administrating clofibrate on dietary TG utilization in vivo was never tested in neonatal animals. Moreover, clofibrate has been used in humans for lowering blood TGs and cholesterol since the 1960s, but data illustrating the effects of clofibrate on in vivo FA oxidation are very limited. PPARα is highly expressed in cells that have high FA catabolic rates, including not only the liver but also the kidney, heart, and skeletal muscle (14). Therefore, we hypothesized that the increased hepatic FA oxidation observed in vitro in neonatal pigs administered clofibrate would improve the efficiency of milk fat utilization in vivo. To test our hypothesis, we studied the capacity of FA oxidation in neonatal pigs using respiration chambers after gastric infusion of control and clofibrate-treated pigs with isoennergic amounts of [1-14C]TG. Because peroxisomal β-oxidation develops rapidly after birth (15,16) and because 60% of the energy in sow milk is derived from TGs containing LCFAs and VLCFAs, the effects of clofibrate on LCFA and VLCFA oxidation in both mitochondria and peroxisomes were investigated using [1-14C]oleic acid (18:1n–9) and erucic acid TG.

**Materials and Methods**

**Pigs.** Thirty newborn male pigs (1.61 ± 0.21 kg) from 5 litters (6 pigs per litter) were used. The pigs were assigned to 2 groups (control vs. clofibrate) based on body weight (BW) and then kept with their mothers. The clofibrate-treated pigs were oorachastically gavaged with clofibrate (75 mg/kg BW) in 2 mL of 2% Tween 80, and the control pigs were gavaged with 2 mL of Tween 80 vehicle only at 0800 for each of 4 consecutive days. On day 4, all pigs were removed from the sow after the last gavage, and BW was recorded at the transfer. In vivo and in vitro FA oxidation was then assessed as described by Odle et al. (17). All procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University.

**TG synthesis.** Oleic acid TG and erucic acid TG were synthesized from glycerol and free oleic and erucic acids using β-toluenesulfonic acid as a catalyst. The syntheses were conducted in a glass reflux unit. The reactants were refluxed at 135°C under N2 for 60–72 h as described by Weeler et al. (18). The synthesized TGs were purified by alkaline/ethanol extraction, and purity (≥95%) was confirmed chromatographically using an HPLC system (Waters) equipped with a photodiode array detector (19). The synthesis of [1-14C]oleic acid and erucic acid TGs followed the same procedure as described above using [1-14C]oleic acid and erucic acid FAs as substrates. The specific activity was diluted to 14.8 kBq/mmol for [1-14C]oleic acid and 18.4 kBq/mmol for [1-14C]erucic acid using nonradioactive TG.

**Measurement of TG utilization in vivo.** After removal from the sow, pigs from the control and clofibrate groups were gastrointestinally intubated with isoennergic amounts (97.7 kJ/kg BW0.75) of either [1-14C]oleic acid (2.94 mmol/kg BW0.75) or erucic acid (2.44 mmol/kg BW0.75) TG in fresh oil-in-water emulsions (30% lipid, v/v) for each group (20), resulting in 4 treatments: 1) control pigs with [1-14C]oleic acid; 2) pigs administered clofibrate with [1-14C]oleic acid; 3) control pigs with [1-14C]erucic acid; and 4) pigs administered clofibrate with [1-14C]erucic acid. The pigs were subsequently positioned in 1 of 4 respiration chambers equipped for continuous collection of expired CO2 (21). The schedule and duration of the expired CO2 collection was patterned following the study by Heo et al. (20), and the pigs were killed by American Veterinary Medical Association-approved electrocution when the experiment ended, after 24 h of collection. The rate and extent of FA oxidation were computed from the kinetics of 14CO2 expiration measured continuously over 24 h (22). TG utilization rates were expressed in terms of ATP yield [μmol ATP/(kg BW0.75 min)], which corrected for differences in molar energy content between oleic acid TG and erucic acid TG (20).

**Measurement of FA oxidation in vitro.** For measurement of in vitro FA oxidation, fresh liver samples from control and clofibrate-treated pigs (n = 5) were homogenized in buffer containing 220 mmol/L mannitol, 70 mmol/L sucrose, 2 mmol/L HEPES, and 0.1 mmol/L EDTA (pH 7.2) at a ratio of 1:7 (gramsmilliliters). FA oxidation was measured in vitro using the procedure described by Peffer et al. (12). Briefly, duplicate liver homogenates (35 mg in 0.25 mL) were incubated in 25-mL Erlenmeyer flasks containing 1.5 mL of incubation medium with 1 mmol/L [1-14C]oleic acid (1.0 mBq/mmol) or erucic acid (1.37 mBq/mmol) in the presence or absence of antimycin A (50 μmol/L) and rotenone (10 μmol/L) in a water shaker bath at 37°C for 30 min. The possible effect of endogenous oleic acid and erucic acid was not corrected, because the tissue amount used was only 35 mg in wet weight and the lipid in the tissue is very low (negligible) in neonatal pigs. The incubation medium contained 50 mmol/L sucrose, 150 mmol/L Tris-HCl, 20 mmol/L KH2PO4, 10 mmol/L MgCl2 · 6H2O, 2 mmol/L EDTA, 1 mmol/L l-carnitine, 0.2 mmol/L CoA, 2 mmol/L NAD, 0.1 mmol/L malate, and 10 mmol/L ATP (pH 7.4) . Incubations were terminated at 30 min by injecting 0.5 mL of 30% HClO4, and the accumulation of radioactivity in CO2 and acid-soluble products (ASPs) was determined using liquid scintillation spectrometry (Beckman Coulter).

**Enzyme activity and mRNA expression.** Activity of CPT I was determined in isolated mitochondria (23). Liver mitochondria were prepared from the fresh liver samples (24), and intactness of mitochondria was evaluated using the respiratory control ratio method (25). ACO activity was determined fluorometrically in liver homogenates (26). Homogenates were prepared in an ice-cold buffer containing 250 mmol/L sucrose, 1 mmol/L EDTA, and 1% ethanol. The assay was conducted with homogenates (0.6 ± 0.035 mg of protein) cultured in a dark room at 37°C in 0.5 mL (final volume) of a medium with or without 35 μmol/L palmityl-CoA for 20 min. The standard medium contained 60 mmol/L Tris-HCl, 50 mmol/L FAD, 170 mmol/L CoA, 1 mmol/L scorpion, and 6% BSA. After termination with 2 mL of borate buffer (0.1 mol/L, pH 10), 200 μL of the incubated medium was transferred into a 96-well plate and measured in a BioTek reader (Synergy HT) with emission at 460 nm and excitation at 360 nm (BioTek Instruments). The standard curve was established using H2O2 (30%, wt:wt) and 150 IU of peroxidase under the same incubation conditions and measurement.

The abundances of mRNA in peroxisome proliferator-activated receptor α (PPARα), CPT I, CPT II, ACO, and mHMGCS in the liver were analyzed using real-time PCR. Total RNA was isolated from the liver samples using Qiagen RNeasy Mini Kit. The integrity of isolated RNA was confirmed using gel electrophoresis with SYBR Safe DNA gel stain.
stain (Invitrogen Life Technologies). The extracted RNA was treated with TurboD Nase (Ambion) and then transcribed using an iScript Select cDNA Synthesis Kit (Bio-Rad Laboratories). Real-time PCR was performed using the MyiQ Single Color Real-Time PCR Detection System (Bio-Rad Laboratories). All samples were analyzed in triplicate. Each reaction contained cDNA (200–500 ng) and reverse and forward primers (200–600 nM). All templates were amplified for 40 cycles under the following conditions: denaturation for 30 s at 95°C, primer annealing and extension for 30 s at 60°C. Data were collected at the end of each elongation phase. The primer pairs used are shown in **Supplemental Table 1**. qPCR efficiencies were acquired by the amplification of a dilution series of cDNA, and efficiencies of all target genes and the housekeeping gene (GAPDH) used in this study approximated 100%. At the end of the amplification, melt curve analysis was used to validate the absence of nonspecific products and primer dimmers. Data were calculated and analyzed for the relative changes in gene expression using the 2^(-DDCT) method (27). Relative mRNA abundance of each target gene was normalized to the control group.

**Chemicals.** The [1-14C]oleic acid and erucic acid FAs were purchased from American Radiolabeled Chemicals. The clofibrate was purchased from Cayman Chemicals. NaOH, HClO4, and HCl were purchased from Thermo Fisher Scientific, and all other chemicals were obtained from Sigma-Aldrich.

**Statistical analysis.** The BW and daily gain of control and clofibrate-treated pigs were analyzed using the t test procedure of SAS. Data from the measurements of TG utilization in vivo were calculated as described by Heo et al. (20). Enzyme-specific activities and mRNA expressions were subjected to analysis of variance according to a randomized complete block design with 2 treatments, with and without clofibrate, blocked by replicate. A simple linear regression was performed for the CO2 expiration rate with time, and the parameters were estimated using the proc REG procedure of SAS. The sigmoidal substrate saturation kinetics analysis for accumulative TG utilization was conducted using the logistic model. The maximal utilization and the time of reaching half-maximal utilization were estimated with the equations obtained by the NLIN procedure of SAS (20). Data for utilization rate, maximal utilization, and in vitro FA oxidation were analyzed with a split-plot design, including a main plot (control vs. clofibrate) of randomized blocks and a subplot modeling TG (tri-oleic acid vs. tri-erucic acid) effects, time effects, and interactions. Multiple comparisons among the 4 treatments were performed using a Tukey’s test, with significance declared when P ≤ 0.05 and tendencies noted when 0.05 ≤ P ≤ 0.1.

**Results**

**Growth performance.** The BW of the pigs increased 25% by the end of the experiment (4 d, 2.00 ± 0.08 kg) compared with the initial BW (1.61 ± 0.06 kg, P < 0.05), but clofibrate had no effect on either the BW or the daily gain (P > 0.12). The mean ± SEM BW and daily gain were 2.01 ± 0.08 and 0.14 ± 0.02 kg for the control pigs and 2.0 ± 0.08 and 0.12 ± 0.02 kg for the clofibrate-treated pigs.

**TG utilization.** The expiration rate of CO2 (Fig. 1A) decreased linearly with time (r² = 0.65, P < 0.01). There was no treatment effect on the rate of CO2 expiration over the 24-h experiment (P = 0.67). The instantaneous oxidative utilization [the time course of TG oxidative utilization, μmol ATP/[kg0.75 · min]] of oleic acid TG was greater than that of erucic acid TG (P < 0.006) for both control and clofibrate-treated pigs (Fig. 1B). There was no statistical difference in peak oxidation rate (P = 0.23) and mean rate (P = 0.14) between the clofibrate-treated and control groups. The oxidative utilization in the pigs increased with time after feeding and peaked at ~5 h for oleic acid TG [436 μmol ATP/[kg0.75 · min]], and the utilization rate gradually decreased after 7 h (Fig. 1B) and at ~4 h for erucic acid TG [179 μmol ATP/[kg0.75 · min]]. Consistent with the instantaneous oxidative utilization, the accumulative oxidative utilization [percentage of the energy intake/[kg0.75 · min]] (Fig. 1C) was greater for oleic acid than erucic acid TG (P < 0.001). Although there was no difference in instantaneous oxidative utilization at the peak and mean rates between the 2 groups, the accumulative utilization of the TGs tended to increase in clofibrate-treated pigs (P = 0.10). The estimated TG utilization by clofibrate-treated pigs using the logistic regression model for oleic acid and erucic acid TGs (Table 1) exceeded controls by 1.5- and 1.3-fold, respectively. Neither clofibrate nor FA chain length had any effect on the time required to reach half-utilization of the TG.

**Hepatic FA oxidation.** Hepatic mitochondrial FA oxidation was increased in pigs treated with clofibrate (Fig. 2A). The 14C accumulations in CO2, ASP, and CO2 + ASP from oleic acid were 0.6-, 0.8-, and 0.8-fold greater in pigs given clofibrate than in control pigs (P < 0.01), respectively. There was no significant difference in the 14C accumulation in CO2 for erucic acid between clofibrate-treated and control pigs, but 14C accumulation in ASP (and CO2 + ASP) increased 260% (P < 0.005). The β-oxidation rate of oleic acid was 1.8-fold greater than that of erucic acid (P < 0.001). No interaction was observed between FA chain length and clofibrate (P = 0.4).

In contrast with the FA oxidation in mitochondria, the effect of clofibrate on oxidation in peroxisomes differed for oleic acid and erucic acid substrates (Fig. 2B). The 14C accumulation in ASP (and CO2 + ASP) from oleic acid was 51% greater in clofibrate-treated pigs than in controls (P < 0.07), whereas 14C accumulation from
erucic acid was unaltered by clofibrate ($P = 0.45$). In addition, there was no difference in peroxisomal β-oxidation between the 2 FAs ($P = 0.46$). Again, no interaction was detected between FA chain length and clofibrate treatment ($P = 0.36$), and accumulation of $^{14}$C in CO$_2$ from both treatments was negligible ($<0.37\%$).

There were no significant differences in the total accumulation of $^{14}$CO$_2$ in either mitochondria or peroxisome incubations (Fig. 2C). Clofibrate increased total β-oxidation rate (radioactivity accumulated in CO$_2$ + ASP) by 67% and 77% for oleic acid and erucic acid, respectively ($P < 0.001$), and the increases were primarily due to the $^{14}$C accumulation in ASP. The $^{14}$C accumulation in CO$_2$, ASP, and CO$_2$ + ASP was higher for oleic acid substrate than for erucic acid substrate ($P < 0.005$). The total β-oxidation rate was 0.7-fold greater for oleic acid than for erucic acid. No interaction was detected between the FA chain length and clofibrate ($P = 0.59$).

The contribution of peroxisomal β-oxidation (Fig. 3) to the total FA β-oxidation (percentage) was 0.8-fold greater from erucic (82%) than oleic acid (46%) in control pigs. A significant interaction was observed between FA chain length and clofibrate ($P < 0.015$). Feeding clofibrate reduced the contribution of peroxisomal β-oxidation for erucic acid by 32% (from 82% to 56%; $P = 0.0005$) but had no impact on the contribution of oleic acid ($P = 0.28$). The influence of FA chain length and clofibrate on the distribution of peroxisomal β-oxidation was due to the $^{14}$C accumulation in ASP because the proportion of peroxisomal β-oxidation to CO$_2$ was low (<7%).

**Enzyme activity and mRNA expression.** The enzyme activities [μmol/(h · g protein)] were 0.9-fold greater for CPT I measured in mitochondria and 1.2-fold greater for ACO in liver homogenates from clofibrate-treated pigs (170.3 ± 7.8 and 65.5 ± 5.2) than control pigs (90.4 ± 7.8 and 29.5 ± 5.2), respectively (means ± SEMs; $P < 0.01$). Consistent with the enzyme activities, the relative abundances of mRNA of CPT I and ACO (Fig. 4) also were increased by 3.51- and 2.83-fold, respectively, and the liver of clofibrate-treated pigs ($P < 0.04$). The induction of mRNA expression of PPARα was observed in the liver of clofibrate-treated pigs ($P < 0.03$). There was no effect of clofibrate on CPT II ($P = 0.15$).

**Discussion**

The stimulatory effects of clofibrate, as a pharmaceutical PPARα agonist, on in vitro FA oxidation have been studied extensively, but, to our knowledge, the effects on in vivo FA oxidation were never evaluated in living animals by providing TGs as the FA source. Paul and Adibi (28) demonstrated that clofibrate increased $^{14}$CO$_2$ production in expired air by 40% after i.v. injection of a trace dose of [$^{14}$C]palmitate, suggesting that clofibrate indeed stimulates in vivo FA oxidation. In contrast, we found that the TG utilization rate (calculated from expired $^{14}$CO$_2$) in clofibrate-treated pigs did not significantly differ from control pigs ($P = 0.14$), although the utilization rate was 1.5-fold

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**TABLE 1** Effects of clofibrate and infused FA chain length on the rate and extent of TG utilization by male neonatal pigs$^1$

<table>
<thead>
<tr>
<th></th>
<th>Control 18:1</th>
<th>Clofibrate 18:1</th>
<th>Clofibrate 22:1</th>
<th>Pooled SEM</th>
<th>FA</th>
<th>Clo</th>
<th>FA × Clo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal pigs, n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>TG intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG, mmol/kg BW$^{0.75}$</td>
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<td>2.51</td>
<td>3.00</td>
<td>2.56</td>
<td>0.1</td>
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<td>—</td>
</tr>
<tr>
<td>ATP, mmol/kg BW$^{0.75}$</td>
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<td>1390</td>
<td>1360</td>
<td>1420</td>
<td>63.9</td>
<td>0.55</td>
<td>0.97</td>
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<tr>
<td>Utilization rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Peak ATP, mmol/(kg BW$^{0.75}$ · min)</td>
<td>0.86</td>
<td>0.32</td>
<td>1.18</td>
<td>0.38</td>
<td>0.19</td>
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<td>Mean ATP, mmol/(kg BW$^{0.75}$ · min)</td>
<td>0.37</td>
<td>0.11</td>
<td>0.49</td>
<td>0.18</td>
<td>0.07</td>
<td>0.002</td>
<td>0.14</td>
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<tr>
<td>TG accumulative utilization</td>
<td>ATV, mmol/kg BW$^{0.75}$</td>
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<td>107</td>
<td>442</td>
<td>162</td>
<td>62.3</td>
<td>0.001</td>
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<tr>
<td>Intake, %</td>
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<td>8.7</td>
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<td>11.4</td>
<td>4.36</td>
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<tr>
<td>Time to reach half of total, h</td>
<td>3.80</td>
<td>4.24</td>
<td>3.91</td>
<td>4.14</td>
<td>0.25</td>
<td>0.98</td>
<td>0.19</td>
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</table>

$^1$ Values are least-squares means unless otherwise indicated. Utilization refers to the composite of digestion, absorption, and oxidation to CO$_2$ after feeding TGs. BW, body weight; Clo, clofibrate.

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**FIGURE 2** Effects of feeding clofibrate on hepatic FA β-oxidation (accumulation of $^{1-14}$C in CO$_2$, ASP, and CO$_2$ + ASP) in mitochondria (A), peroxisomes (B), and both mitochondria and peroxisomes (C) of neonatal male pigs. Values are least-squares means ± pooled SEMs, n = 5. Labeled means for a variable without a common letter differ, $P < 0.05$. ASP, acid-soluble product; Clo, clofibrate; Con, control.
greater at peak and 1.3-fold higher over the entire 24-h collection time compared with controls. We showed previously (11) that CPT I, ACO, and catalase increased in liver but not in muscle from neonatal pigs fed clofibrate for 14 d, indicating that the stimulation of FA oxidation in skeletal muscle is less than in the liver. Because skeletal muscle comprises >50% of BW, the utilization of the TGs ingested would occur primarily in the muscle. Thus, the mild response of the muscle to clofibrate stimulation could result in attenuation of whole-body TG utilization in response to clofibrate treatment. In support of this, we did observe in vitro tissue-specific responses to clofibrate as expected and shown in other species (29,30).

The FA chain length used in our study may also have influenced our results. Indeed, the accumulative utilization of oleic acid but not erucic acid TG was significantly greater in clofibrate-treated pigs than in control pigs after 12 h (Fig. 1). Induction of peroxisomal β-oxidation by clofibrate is relatively small in pigs compared with that in young or adult rodents (11). Our in vitro data showed that >80% of the erucic acid was oxidized in peroxisomes, and clofibrate did not alter the peroxisomal oxidation rate. It is very interesting that oleic acid oxidation also was substantial in peroxisomes (≥46%). Because of the high proportion of peroxisomal oxidation and the low response of peroxisomes to clofibrate induction, the relative contribution of peroxisomal β-oxidation to the total FA oxidation was either reduced from erucic acid or unchanged from oleic acid in response to clofibrate (Fig. 3). Collectively, we infer that the observed in vivo oxidation rates of oleic acid and erucic acid TGs reflected primarily hepatic effects of clofibrate.

Rats and other animals have a limited capacity to metabolize MUFAs with 22 carbons, such as erucic acid, because of the high TG accumulation rate in extrahepatic tissues and low oxidation rate in the liver (31). Erucic acid is naturally present in rapeseed oil or hydrogenated fish oil. The digestibility of rapeseed oil and its effects on lipid profile in tissues and histopathology in organs were evaluated in swine (32–35), but there is no information on erucic acid oxidation. Swine have high digestion and absorption rates of rapeseed oil and a high proportion of peroxisomal oxidation compared with rodent species (11). Even so, the data from this study showed that the oxidative utilization of erucic acid TG in vivo was only ∼40% of oleic acid TG. This was consistent with our in vitro data in which the hepatic FA oxidation of erucic acid was only 58% of the oleic acid. Considering that neonatal pigs have a high LCFA esterification rate during the suckling period (4), we believe that neonatal pigs might also have a high erucic acid accumulation rate in the tissues, as observed in rodent species (31).

Hepatic in vitro FA oxidation was increased by clofibrate in both mitochondria and peroxisomes. The increase was consistent with increased mRNA and activity of CPT I and ACO, confirming our previous observations in suckling pigs (12). These data indicate that the hepatic FA oxidation capacity was enhanced in clofibrate-treated pigs, and the results imply that at least part of the increased oxidative utilization of TG observed in vivo was contributed by the increased hepatic FA oxidation. However, the contribution of LCFA and VLCFA in mitochondria was apparently different from that in peroxisomes. Clofibrate increased mitochondria oxidation of both LCFA and VLCFA, but the relative increase was greater for VLCFA (3.6-fold) than for LCFA (1.8-fold). In contrast with mitochondria, clofibrate also increased LCFA oxidation in peroxisomes but had no impact on VLCAFA oxidation. Thus, the relative contribution of peroxisomal oxidation to the total oxidation was decreased for VLCAFA (Fig. 3). Dietary LCFA are oxidized primarily in the mitochondria. Data from previous rat studies indicate that the contribution of hepatic peroxisomal oxidation to total β-oxidation ranges from 20% to 35% for palmitate or oleate (36). In contrast to rats, pigs have a greater proportion of peroxisomal β-oxidation for LCFA (40–47%). Our data showed that 46% of oleic acid was oxidized in peroxisomes, and the oxidation rate was surprisingly similar to the rate of erucic acid oxidation (Fig. 2B). Furthermore, clofibrate stimulated VLCAFA oxidation in mitochondria and increased the energy utilization efficiency of VLCAFA.

Neonatal pig survival depends on its rapid metabolic adaptation to utilize FAs as its primary postnatal fuel because FAs represent 60% of the total energy in sow milk (37). The greater peroxisomal β-oxidation capacity observed in pigs and its rapid increase in liver after birth might act as a compensatory mechanism to enhance milk fat oxidation and thermo-adaptation (11,15). One of the differences between mitochondrial and peroxisomal β-oxidation is energy generation. Peroxisomal β-oxidation is less coupled to ATP synthesis and therefore more thermogenic (38). Accordingly, the efficiency of energy generation for complete FA oxidation in peroxisomes could be only 71% of that in mitochondria. Despite this low FA oxidative efficiency, peroxisomal β-oxidation is an important metabolic pathway for multiple functions (39) and is of significance for maintaining thermogenesis (40–42). The increase in FA oxidation elicited by clofibrate not only improved the efficiency of energy production from VLCAFA but also increased the efficiency of thermogenesis by VLCAFA.

In conclusion, clofibrate increased accumulative in vivo FA oxidation in the same manner as it was shown to increase in vitro hepatic FA oxidation. Administration of clofibrate significantly increased VLCAFA oxidation in mitochondria and LCFA oxidation in peroxisomes. The increases might promote both energy production and chemical thermogenesis. Therefore, natural or
References


