Differential induction of peroxisomal β-oxidation enzymes by clofibrac acid and aspirin in piglet tissues

XING XIAN YU,1 JACK ODLE,1,2 AND JAMES K. DRACKLEY1,2

1Division of Nutritional Sciences and 2Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

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Yu, Xing Xian, Jack Odle, and James K. Drackley. Differential induction of peroxisomal β-oxidation enzymes by clofibrac acid and aspirin in piglet tissues. Am J Physiol Regulatory Integrative Comp Physiol 281: R1553–R1561, 2001.—Peroxisomal β-oxidation (POX) of fatty acids is important in lipid catabolism and thermogenesis. To investigate the effects of peroxisome proliferators on peroxisomal and mitochondrial β-oxidation in piglet tissues, newborn pigs (1–2 days old) were allowed ad libitum access to milk replacer supplemented with 0.5% clofibrac acid (CA) or 1% aspirin for 14 days. CA increased ratios of liver weight to body weight (P < 0.07), kidney weight to body weight (P < 0.05), and heart weight to body weight (P < 0.001). Aspirin decreased daily food intake and final body weight but increased the ratio of heart weight to body weight (P < 0.01). In liver, activities of POX, fatty acyl-CoA oxidase (FAO), total carnitine palmitoyltransferase (CPT), and catalase were 2.7-, 2.2-, 1.5-fold, and 33% greater, respectively, for pigs given CA than for control pigs. In heart, these variables were 2.2-, 4.1-, 1.9-, and 1.8-fold greater, respectively, for pigs given CA than for control pigs. CA did not change these variables in either kidney or muscle, except that CPT activity was increased ~110% (P < 0.01) in kidney. Aspirin increased only hepatic FAO and CPT activities. Northern blot analysis revealed that CA increased the abundance of catalase mRNA in heart by ~2.2-fold. We conclude that 1) POX and CPT in newborn pigs can be induced by peroxisomal proliferators with tissue specificity and 2) the relatively smaller induction of POX in piglets (compared with that in young or adult rodents) may be related to either age or species differences.

Peroxisomes are ubiquitous subcellular organelles present in all eukaryotic organisms and almost all mammalian cells (for review, see Ref. 49). Peroxisomes contain a fatty acid β-oxidation system that is distinct from the mitochondrial β-oxidation system (21, 31). The first oxidation step of peroxisomal β-oxidation is catalyzed by fatty acyl-CoA oxidase (FAO), which generates 2-trans-enoyl-CoA and H₂O₂; FAO was found to be the rate-limiting enzyme of this system (9, 24). The H₂O₂ produced is degraded by catalase, a reaction that releases heat. Peroxisomes not only can β-oxidize medium- and long-chain fatty acids (MCFA and LCFA), but also can β-oxidize very long chain fatty acids (VLCFA) that are poorly oxidized by mitochondria (see Ref. 49).

One outstanding feature of peroxisomal β-oxidation is the dramatic induction in liver and other organs when animals are treated with hypolipidemic drugs (e.g., clofibrate and its analogs) and other structurally diverse xenobiotic agents, all of which are termed peroxisome proliferators (22, 23, 41, 49). In contrast, mitochondrial β-oxidation usually is induced less strongly by peroxisome proliferators (22, 43). Induction by fibrates is mediated by interaction of the peroxisome proliferator with a nuclear receptor, peroxisome proliferator-activated receptor-α (PPAR-α), which when activated binds to specific sequences (peroxisome proliferator response elements (PPRE)) in the promoter region of target genes and activates their transcription (see Refs. 11 and 47 for reviews). Sensitivity to peroxisomal induction by peroxisomal proliferators varies greatly among animal species and tissues (22, 23, 52). Rats and mice are highly sensitive to induction by peroxisome proliferators (19, 31, 52), hamsters are intermediate (17, 29), and guinea pigs, primates, and humans are relatively insensitive (7, 20, 23, 39, 52). In rodents, liver is most sensitive; kidney, heart, and intestinal mucosa are moderately sensitive; and muscle is insensitive (25, 35, 48). The molecular basis for species and tissue differences remains unclear (4, 23, 44). Few data are available on the relative efficacy of peroxisome proliferators for neonates of any species. Furthermore, no data are available on the induction of peroxisomal β-oxidation in swine, a species of both agricultural and biomedical importance.

Peroxisomal β-oxidation is believed to play a role in thermogenesis (16) because the first oxidation step is not coupled to ATP production and the energy is dissipated as heat. Newborn pigs and other mammalian neonates encounter great nutritional and metabolic challenges immediately after birth due to the dramatic change of living environment; one of the major challenges is to maintain body temperature. The rapid postnatal development of peroxisomal β-oxidation that we have measured in piglets (60, 61) and that others
have determined in rats (54, 58) may be a physiologically adaptive mechanism for neonatal survival and growth. The significance of peroxisomal β-oxidation is underscored in peroxisomal genetic disorders, such as neonatal adrenoleukodystrophy, that are characterized by a deficient peroxisomal β-oxidation activity and thereby a resultant accumulation of VLCFA, which results in progressive anatomical and functional defects (see Ref. 49). However, the physiological significance of peroxisomal β-oxidation of fatty acids and the functional coordination between peroxisomal β-oxidation and mitochondrial β-oxidation in neonates are incompletely understood.

Piglets, like many other neonates, rely heavily on fatty acids for survival because fatty acids constitute ~60% of the total energy in porcine milk (15). However, capacities for ketogenesis from fatty acids and for mitochondrial β-oxidation of fatty acids have been clearly demonstrated to be limiting in piglets (1, 2, 13, 37, 40). We recently demonstrated a relatively high activity of peroxisomal β-oxidation in piglet tissues and a rapid postnatal increase of the activity in piglet liver, which we proposed acts as a compensatory mechanism for piglets to β-oxidize the milk fatty acids (60–62). The increased hepatic peroxisomal β-oxidation was seen in 24-h-old suckled piglets but not in 24-h-old unsuckled piglets (60, 61). Given that some LCFA and VLCFA, like peroxisomal proliferators, can induce peroxisomal β-oxidation via a receptor-mediated mechanism (11, 12, 47, 57) and that >95% of porcine milk fatty acids are LCFA and VLCFA (32), we speculated that the increased peroxisomal β-oxidation in suckled piglets was induced by the milk fatty acids (61). If true, therefore, peroxisomal β-oxidation should be inducible by peroxisomal proliferators in newborn pigs.

In the present study, we tested the hypothesis that peroxisomal β-oxidation can be induced in newborn piglets by administration of classical peroxisome proliferators. To accomplish our objectives, we treated newborn piglets with two compounds known to induce peroxisomal β-oxidation, clofibrin acid and aspirin, and examined their effects on the activities of peroxisomal and mitochondrial β-oxidation in several piglet tissues. Our results indicate that peroxisomal β-oxidation can be readily induced in piglets with tissue specificity and that this induction may differ compared with data published for other species, especially rodents.

MATERIALS AND METHODS

Chemicals. Palmitoyl-CoA, CoA, NAD, FAD, dithiothreitol, Triton X-100, Brij 58, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 30% H2O2, homovanillate, horseradish peroxidase (type II), glycolyglycine, Tris·HCl, EDTA, HEPES, triethanolamine HCl, isocitrate, clofibrin acid, and aspirin were purchased from Sigma (St. Louis, MO). Potassium cyanide (KCN) was obtained from Mallinckrodt (Paris, KY). All other chemicals indicated below were of reagent or molecular biology grade.

Animal and diets. All procedures were conducted under protocols approved by the University of Illinois Laboratory Animal Care Advisory Committee. Commercial crossbred piglets (Chrisman Feeder pigs, Chrisman, IL) of normal body wt (2.08 ± 0.23 kg) were obtained at 1–2 days of age. Pigs were housed individually in racks with Plexiglas cages with coated expanded stainless steel flooring (Ridgian Animal Care System, Mt. Horeb, WI) in an environmentally controlled room (23°C) with a 12:12-h light-dark cycle. Additional radiant heaters (Kalglo Electronics, Bethlehem, PA) were used to maintain an ambient temperature of 25–30°C, depending on the age of the animal.

The piglets were assigned randomly to three groups that were fed one of three diets for ad libitum intake: 1) control milk replacer (Milk Specialties, Dundee, IL), which contained 25.0% protein, 13.0% fat, 47.9% carbohydrate (lactose), and sufficient vitamins and minerals to meet established requirements (34), 2) control plus 0.5% (wt/vt) clofibrin acid, or 3) control plus 1% (wt/wt) aspirin. Milk replacer was reconstituted to 20% (wt/vol) solids in water. The reconstituted milk replacers were fed through a nipple connected to a gravity-flow reservoir. Milk replacer was mixed and replaced twice daily to keep it fresh, at which times the wastage was measured and intake was recorded. Dosages of clofibrin acid and aspirin were selected on the basis of previous studies with rodents in which diets containing 0.5% (wt/vt) clofibrin acid (25) or 1% (wt/wt) aspirin (10) significantly increased the activities of peroxisomal β-oxidation or FAO.

After piglets were fed for 14 days, they were weighed after feed was withheld for 4 h and then were anesthetized with pentobarbital sodium at a dose of ~25 mg/kg body wt. Liver, kidney, heart, and a representative muscle (psoas major) were removed and placed in ice-cold saline (0.9% NaCl). After the blood was washed off, the organs were blotted and weighed. Portions of liver, kidney cortex, heart ventricles, and muscle were frozen immediately in liquid N2 and then stored at ~70°C for laboratory assays. All tissues were obtained between 1430 and 1630.

Preparation of a peroxisome-enriched fraction from tissues. A peroxisome-enriched fraction was prepared from pig tissues essentially as described (61). Briefly, using a Potter-Elvejhem homogenizer, frozen tissues were homogenized manually in five volumes of ice-cold homogenization buffer (pH 7.4) containing 0.3 M sucrose, 20 mM Tris·HCl, and 0.5 mM EDTA. The homogenates were subjected to differential centrifugation by using a Beckman J2-21 centrifuge and JA-18.1 rotor. A combined fraction containing cellular debris, nuclei, and mitochondria was sedimented by centrifugation at 3,000 g for 10 min. The resulting supernatant was centrifuged at 27,000 g for 10 min to sediment the peroxisomal fraction. The peroxisome-enriched fractions from the tissues were resuspended in 0.5 ml (for liver and kidney cortex) or 0.25 ml (for heart ventricles and skeletal muscle) of the same ice-cold homogenization buffer per gram of tissue, and samples were frozen immediately in liquid N2 until determinations of peroxisomal β-oxidation and FAO activities.

Assays of peroxisomal β-oxidation and enzyme activities. The peroxisomal β-oxidation rate was determined spectrophotometrically at 340 nm and 37°C as palmitoyl-CoA-dependent KCN-insensitive reduction of NAD, as described by Lazarov (30), except that 0.01% (wt/vol) Brij 58 was added in the incubation medium and bovine serum albumin was omitted (61). The contents of sample and reference cuvettes were identical except for palmitoyl-CoA, which was added to the sample cuvette only.

Activity of FAO (EC 1.3.99.3) was assayed by the procedure of Vamecq (55) after modification (61). In this assay, palmitoyl-CoA was used as substrate and activity of FAO was determined based on the release of H2O2; the latter was determined by a coupled peroxidative reaction using homovanillate as electron donor in the presence of horseradish per-
oxidase to produce a fluorescent oxidation dimer, the amount of which was measured spectrophotometrically. Tissue catalase (EC 1.11.1.6) activity was determined spectrophotometrically by using the ultraviolet (UV) assay method of Aebi (3) at room temperature. In this system, the decomposition of H$_2$O$_2$ is followed by observing the decrease in absorbance at 240 nm with time. Reaction rates for FAO and catalase were determined from calibration curves consisting of a series of diluted H$_2$O$_2$ solutions prepared from a 30% (wt/wt) stock solution.

For assays of total carnitine palmitoyltransferase (CPT; EC 2.3.1.23) and isocitrate dehydrogenase (ICDH; EC 1.1.1.42), tissues were homogenized in five volumes of ice-cold buffer (pH 7.4) containing 220 mM mannitol, 70 mM sucrose, 2 mM HEPES, and 0.05% Triton X-100. The homogenates were centrifuged at 800 g for 10 min to sediment the cell debris. The resulting supernatants were frozen and thawed three times and then used for assays. Total CPT activity was measured spectrophotometrically by using the procedure of Bieber et al. (6). The reaction mixture (1 ml final volume) contained 0.1 M Tris-HCl, 0.2 mM DTNB, 0.09% (wt/vol) Triton X-100, 50 μM palmitoyl-CoA, and 20 μl of supernatant. The reaction was initiated by addition of 10 μl of 25 mM L-carnitine, and the changes in absorbance at 412 nm were recorded. The enzyme activity was calculated by using an extinction coefficient of 13.6 M/cm.

Activity of ICDH (EC 1.1.1.42) was measured spectrophotometrically at room temperature by employing the UV assay method of Bernt and Bergmeyer (5) with modifications. The reaction mixture (1 ml final volume), containing triethanolamine (80 mM final concentration), 0.1-isocitrate (3.7 mM final concentration), NaCl (42 mM final concentration), and 0.1 ml of supernatant, was incubated at room temperature for 3–4 min, and then the reaction was initiated by addition of 30 μl of NAD-MnSO$_4$ solution (0.32 and 3.9 mM final concentrations, respectively). The changes in absorbance at 340 nm were recorded. The enzyme activity was calculated by using an extinction coefficient of 6.22 M/cm. All assays were performed within their linear range.

**Table 1. Daily food intake, body weight of piglets before and after treatment, and organ weight after treatments**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 5)</th>
<th>Clofibric Acid (n = 6)</th>
<th>Aspirin (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body wt, kg</td>
<td>2.057 ± 0.130</td>
<td>2.084 ± 0.120</td>
<td>2.090 ± 0.150</td>
</tr>
<tr>
<td>Final body wt, kg</td>
<td>6.295 ± 0.400</td>
<td>5.612 ± 0.370</td>
<td>4.776 ± 0.450</td>
</tr>
<tr>
<td>Daily food intake, liters</td>
<td>1.649 ± 0.059</td>
<td>1.459 ± 0.067</td>
<td>1.369 ± 0.070</td>
</tr>
<tr>
<td>Body wt gain/feeding, g/l</td>
<td>185.7 ± 2.5</td>
<td>171.5 ± 21.5</td>
<td>167.4 ± 19.1</td>
</tr>
<tr>
<td>Liver wt, g</td>
<td>205.0 ± 21.8</td>
<td>219.3 ± 19.9</td>
<td>166.0 ± 24.3</td>
</tr>
<tr>
<td>Liver wt/final body wt, %</td>
<td>3.26 ± 0.22</td>
<td>3.83 ± 0.20</td>
<td>3.48 ± 0.24</td>
</tr>
<tr>
<td>Kidney wt, g</td>
<td>48.8 ± 4.9</td>
<td>53.9 ± 4.5</td>
<td>38.6 ± 5.5</td>
</tr>
<tr>
<td>Kidney wt/final body wt, %</td>
<td>0.78 ± 0.05</td>
<td>0.95 ± 0.05</td>
<td>0.81 ± 0.06</td>
</tr>
<tr>
<td>Heart wt, g</td>
<td>33.9 ± 2.7</td>
<td>40.4 ± 2.4</td>
<td>29.8 ± 3.0</td>
</tr>
<tr>
<td>Heart wt/final body wt, %</td>
<td>0.54 ± 0.01</td>
<td>0.72 ± 0.01</td>
<td>0.65 ± 0.02</td>
</tr>
</tbody>
</table>

Values are least-squares means ± SE. *P < 0.05, †P < 0.01, and ‡P < 0.001 vs. control.
efficiency (body wt gain/food intake; Table 1). Therefore, the 24% lower final body weight for aspirin-treated pigs than for control pigs ($P < 0.01$; Table 1) was primarily attributable to the decreased food intake. Although not significant, clofibric acid increased liver weight by 7.0%, kidney weight by 10.4%, and heart weight by 19.3%, which resulted in higher ratios of liver weight to body weight ($P = 0.07$), kidney weight to body weight ($P < 0.05$), and heart weight to body weight ($P < 0.001$) compared with controls. Aspirin did not significantly affect liver, kidney, or heart weight. As a result, the ratios of organ weight to body weight did not differ, except for a greater ratio of heart weight to body weight ($P < 0.01$) in aspirin-treated pigs than in control pigs.

In liver (Table 2), clofibric acid increased peroxisomal β-oxidation activity by ~2.7-fold compared with controls ($P < 0.001$) and increased FAO activity ~2.2-fold compared with controls ($P < 0.001$). Hepatic catalase activity was increased by 33% ($P < 0.05$) in pigs given clofibric acid. In addition, clofibric acid increased hepatic CPT activity by 1.5-fold over that of controls ($P < 0.001$). However, clofibric acid did not change hepatic ICDH activity (Table 2). Hepatic peroxisomal β-oxidation and FAO activities were 82.3% ($P < 0.1$) and 108% higher ($P < 0.05$) in aspirin-treated pigs than in controls, respectively. Hepatic CPT activity also was increased significantly in aspirin-treated pigs, but hepatic catalase and ICDH activities were unchanged (Table 2).

In heart (Table 3), clofibric acid increased peroxisomal β-oxidation, FAO, and catalase activities by ~2.2-, 4.1-, and 1.8-fold ($P < 0.01, 0.001$, and 0.001), respectively, compared with those of controls. In addition, clofibric acid increased cardiac CPT activity (1.9-fold higher, $P < 0.001$) and ICDH activity ($P < 0.05$). Aspirin did not change cardiac activities of peroxisomal β-oxidation, FAO activity, catalase, or CPT, but significantly decreased ICDH activity (Table 3).

In kidney, neither clofibric acid nor aspirin affected enzyme activities except for an increased CPT activity (~1.1-fold higher, $P < 0.01$) in pigs given clofibric acid (Table 4). Neither clofibric acid nor aspirin significantly affected metabolic variables in skeletal muscle (Table 5). Values for FAO activity in muscle were close to the limit for detection and are not reported.

Northern blot analysis showed that neither clofibric acid nor aspirin significantly changed the abundance of catalase mRNA in liver (Figs. 1 and 2). However, catalase mRNA was ~2.2-fold higher ($P < 0.05$) in heart from pigs given clofibric acid than in controls (Figs. 1 and 2).

### DISCUSSION

To our knowledge, our data for pigs are the first report of induction of peroxisomal β-oxidation by classical peroxisomal proliferators in an ungulate species. Induction of peroxisomal β-oxidation has been widely investigated in rodents and other mammals by using different peroxisome proliferators. Generally, the induction of β-oxidation by clofibrate and other peroxisome proliferators reaches a maximum after 2 wk of treatment (41). Rodent hepatic peroxisomal β-oxidation and FAO activity are induced by >10-fold after 10–14 days of treatment with clofibrate or related compounds (19, 28, 31). In contrast, renal and cardiac peroxisomal β-oxidation and FAO activity are relatively refractory to treatment and usually are induced by less than fivefold (25, 28, 48). Catalase activity in rodents also is induced by fibrate compounds, but usually by less than or equal to twofold (31, 35).

In the present study, we found that administration of clofibric acid to piglets for 14 days caused a relative enlargement of liver, heart, and kidney. Hepatomegaly often has been found in association with dramatic increases of hepatic peroxisomal β-oxidation and FAO activity in rats treated with peroxisome proliferators.
The hepatic enlargement has been ascribed to an increased number and average size of peroxisomes and associated smooth endoplasmic reticulum, which resulted in hyperplasia and hypertrophy (22). Clofibrate treatment also found to increase the number of peroxisomes in heart and kidney (36, 56), but a changed heart or kidney weight has been reported less frequently, probably because of the relatively lower volume fraction of peroxisomes in the cytosol (28) and smaller increases of peroxisomal β-oxidation (usually ≤5-fold after treatment). However, in our study, we found that the increased organ weights in piglets occurred with relatively smaller increases of peroxisomal β-oxidation or FAO activity (<5-fold increase in both liver and heart) or with no change of peroxisomal β-oxidation or FAO activity (in kidney). These results indicate that increased organ weight in piglets given clofibrate is attributable not only to proliferation or enlargement of peroxisomes but perhaps also to proliferation or enlargement of other cellular organelle(s), which might occupy relatively greater percentages of cell volume in tissues of piglets than in rodents. Our previous finding of a relatively higher peroxisomal β-oxidation activity in piglet liver, heart, and kidney than in rat (2, 61) indirectly supports this possibility, but histological studies are needed in verification.

The increased organ weight for piglets might result, partially or totally, from proliferation or enlargement of mitochondria. A one- to twofold increase of total CPT activity was observed in liver, heart, and kidney of pigs given clofibrate acid. Although total CPT activity as measured in our studies includes CPT isoforms in mitochondria, peroxisomes, and microsomes (63), the greatest activity is found in mitochondria as CPT-I and CPT-II (33). If this increased CPT activity is related to an increased size or number of mitochondria, similar to the relationship between FAO and peroxisomes in rodents after clofibrate treatment, then mitochondrial mass may have been increased, which may have contributed to the increased organ size. However, the lack of similar increases in ICDH activity suggests that clofibrate did not cause a generalized increase of mitochondrial mass. In clofibrate-treated rats, a 50–100% increase in the hepatic content of mitochondria has been reported (27); the maximal increase was seen at 2–4 days after clofibrate administration (14). After summarizing the literature, Hawkins et al. (22) concluded that mitochondrial β-oxidation was increased along with peroxisomal β-oxidation after administration of peroxisome proliferators and that this increase was accompanied by mitochondrial proliferation. Reinhardt et al. (42) found that, after dietary treatment of weanling pigs with clofibric acid (0.5% wt/wt) for 2 wk, total hepatic cytosolic protein and the amount of protein per gram of liver were greater in clofibrate acid-fed pigs than in control pigs, although the treatment did not increase liver weight.

The increased total CPT activity might indicate an increased capacity for mitochondrial β-oxidation of fatty acids. In newborn piglets, CPT-I probably functions as the rate-limiting enzyme for mitochondrial β-oxidation (37), and the amount of CPT-I was reported to be about the same as that of CPT-II in mitochondria of piglet liver (6). Actual flux through CPT-I, however, depends on the concentration of malonyl-CoA and the sensitivity of CPT-I to inhibition by malonyl-CoA (33, 63). The concentration of malonyl-CoA was found to be very low in piglets because of low lipogenic activity (40). Studies have demonstrated that various hypolipidemic drugs and other xenobiotics that induced peroxisomal proliferation and β-oxidation in rodents also increased CPT activity and the rate of mitochondrial β-oxidation of fatty acids (43, 57). The gene for muscle-type CPT-I in cardiac myocytes was shown to contain a PPRE that was activated by PPAR-α (8). A concomitant induction of mitochondrial total CPT activity with induction of peroxisomal β-oxidation and its FAO activity found in piglet liver and heart in our study supports the idea that the peroxisomal and mitochondrial

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**Table 4. Activities of peroxisomal and mitochondrial enzymes in kidney of piglets after different treatments**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Control (n = 5)</th>
<th>Clofibrin Acid (n = 6)</th>
<th>Aspirin (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxisomal β-oxidation, µmol NADH h⁻¹·g⁻¹</td>
<td>1.96 ± 0.27</td>
<td>2.09 ± 0.24</td>
<td>1.70 ± 0.30</td>
</tr>
<tr>
<td>FAO, µmol H₂O₂ h⁻¹·g⁻¹</td>
<td>0.98 ± 0.15</td>
<td>1.14 ± 0.14</td>
<td>0.71 ± 0.17</td>
</tr>
<tr>
<td>Catalase, mmol H₂O₂·min⁻¹·g⁻¹</td>
<td>19.56 ± 3.86</td>
<td>20.41 ± 3.53</td>
<td>20.09 ± 4.32</td>
</tr>
<tr>
<td>CPT, µmol CoA h⁻¹·g⁻¹</td>
<td>5.88 ± 1.20</td>
<td>12.23 ± 1.10</td>
<td>7.98 ± 1.34</td>
</tr>
<tr>
<td>ICDH, µmol NADH h⁻¹·g⁻¹</td>
<td>3.63 ± 0.47</td>
<td>3.35 ± 0.43</td>
<td>3.31 ± 0.53</td>
</tr>
</tbody>
</table>

Values are least-squares means ± SE. All activities are expressed per gram of wet tissue.

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**Table 5. Activities of peroxisomal and mitochondrial enzymes in muscle (psoas major) of piglets after different treatments**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Control (n = 5)</th>
<th>Clofibrin Acid (n = 6)</th>
<th>Aspirin (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxisomal β-oxidation, µmol NADH h⁻¹·g⁻¹</td>
<td>0.07 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Catalase, mmol H₂O₂·min⁻¹·g⁻¹</td>
<td>0.61 ± 0.09</td>
<td>0.88 ± 0.09</td>
<td>0.79 ± 0.11</td>
</tr>
<tr>
<td>CPT, µmol CoA h⁻¹·g⁻¹</td>
<td>2.62 ± 0.32</td>
<td>4.94 ± 0.75</td>
<td>4.23 ± 0.92</td>
</tr>
<tr>
<td>ICDH, µmol NADH h⁻¹·g⁻¹</td>
<td>11.23 ± 1.04</td>
<td>12.68 ± 0.95</td>
<td>11.33 ± 1.16</td>
</tr>
</tbody>
</table>

Values are least-squares means ± SE. All activities are expressed per gram of wet tissue.
Fig. 1. A: Northern blot analysis of catalase mRNA in liver and heart from neonatal (1–2 days old) pigs fed on a control milk replacer diet, control plus 0.5% clofibric acid, or control plus 1% aspirin for 14 days. The 28s RNA bands photographed from the corresponding ethidium bromide-stained agarose gels on an ultraviolet transilluminator before transferring to a nylon filter. Densitometric scans of the 28s rRNA were used to normalize the abundance of catalase mRNA in each sample.

B: the 28s RNA bands photographed from the corresponding ethidium bromide-stained agarose gels on an ultraviolet transilluminator before transferring to a nylon filter. Densitometric scans of the 28s rRNA were used to normalize the abundance of catalase mRNA in each sample.

drial systems for fatty acid β-oxidation complement one another. In contrast, in kidney from piglets treated with clofibric acid, total CPT activity increased with no change in activity of peroxisomal β-oxidation or FAO, suggesting that the mitochondrial system for fatty acid β-oxidation is regulated more independently than is the peroxisomal system.

If mitochondrial β-oxidation were increased, the unchanged (in liver and kidney) or slightly increased (in heart) activity of ICDH, one of the rate-limiting enzymes in the citric acid cycle, may imply that other pathways coupled with mitochondrial β-oxidation, such as ketogenesis or acetogenesis, also were induced, which would result in an increased supply of water-soluble fuels, such as ketone bodies or acetate, for other extrahepatic tissues. Peroxisome proliferators also induce transcription of the regulatory enzyme of ketogenesis, mitochondrial hydroxymethylglutaryl-CoA synthase, the gene of which possesses a PPRE responsive to PPAR-α that is activated by clofibrate (38, 45).

Our study indicates that peroxisomal β-oxidation can be induced by clofibric acid with tissue specificity in piglets, as in rodents (19, 25, 28, 31, 48). Clofibrate acid increased peroxisomal β-oxidation by 2.7- and 2.2-fold in piglet liver and heart, respectively, and increased FAO activity by 2.2- and 4.1-fold, respectively; however, clofibrate acid did not change peroxisomal β-oxidation or FAO activity in kidney cortex and skeletal muscle. Catalase activity was increased by 1.8-fold in heart but only by 33% in liver. Induction of catalase activity in heart was primarily a consequence of increased abundance of catalase mRNA in that tissue. Compared with the effects of clofibrate observed previously in rodents (19, 25, 28, 31, 36, 58), the magnitude of induction in piglets in the present study was much smaller, especially for hepatic peroxisomal β-oxidation, FAO, and catalase activities. In addition, renal peroxisomal β-oxidation was not induced, in contrast to previous results in rodents (25, 43). Whether these differences are attributable to species differences or age effects is not known. Stefanini et al. (51) treated lactating rats with di-(2-ethylhexyl)phthalate (DEHP) from parturition to weaning and found that hepatic peroxisomal proliferation appeared to be more DEHP responsive in adults than in pups, whereas renal peroxisomal proliferation was greater in 14-day-old pups than in 21-day-old pups. Kvannes et al. (28) found that the basal FAO activity in rat heart decreased with age (34, 55, 73 days of age), whereas the induction response to 10 days of clofibrate treatment increased with age. A similar age-dependent response also was observed in the liver (28). Yamoto et al. (59) treated young rats for 7 days with clofibrate (200 mg·kg\(^{-1}\)·day\(^{-1}\)) and found that responsiveness of the treatment increased with age; increases were ~75% in 4-wk-old rats, 3.7-fold in 8-wk-old rats, and 7-fold in 12-wk-old rats.

The basis for the species or tissue differences probably lies in differences in the amount and/or type of PPAR present, differences in the gene networks that are regulated by PPAR, or differences in dimer formation or presence of inhibitory transcription factors (11, 23). The difference in the increase of catalase mRNA abundance between liver and heart found in the present study indirectly supports this point. Three different types of PPAR (α, β, and γ) have been identified in vertebrates, including humans (11), and PPAR-γ comprises at least two different isoforms (11, 47). These different types or isoforms of PPAR are differentially expressed among tissues and at different developmental stages (11, 47). The PPAR-α type is expressed primarily in liver, heart, and kidney and is responsive to fibrate drugs such as clofibrate acid (11). Therefore, the species and tissue differences in induction of peroxisomal β-oxidation found in the present study and previous studies could reflect variations in PPAR distribution or expression. The PPAR-γ isoform has been identified and characterized in swine (18), but...
this isoform is relatively unresponsive to fibrates such as clofibric acid (11). Recently, the gene for porcine PPAR-α isoform has been cloned and its mRNA abundance quantified in porcine tissues (53), which provides a molecular basis for results of our study. In humans, however, peroxisomal β-oxidation system was found to be relatively insensitive to peroxisome proliferators (7, 20) despite the presence and expression of PPAR-α. Thus species and tissue differences between our study and others also could reflect variations in dimerization of PPAR-α with the retinoic acid receptor or the presence of other transcription factors that modulate gene transcription by PPAR (11).

Aspirin has been reported to cause peroxisome proliferation (41) and hepatomegaly (26) and to increase activities of peroxisomal β-oxidation enzymes and catalase (10) in rodents. The present study found that aspirin only increased the relative weight of heart and the activities of hepatic FAO and CPT. Therefore, aspirin evidently is a much weaker peroxisome proliferator in piglet tissues than is clofibric acid, which is similar to the situation in rodents.

Perspectives

Our study demonstrated that peroxisomal β-oxidation in piglets can be induced by clofibric acid with tissue specificity. The magnitude of induction caused by clofibric acid is considerably smaller than effects seen in rats and mice. In pigs, nutritional stress and hypothermia are two of the major factors contributing to the high postnatal morbidity and mortality. Piglets, unlike rodents, lack abundant brown adipose tissue, which is an important thermogenic tissue. Newborn pigs have a limited hepatic capacity for ketogenesis (1, 2, 13, 37, 40, 62), which has been ascribed to limitations by CPT-I (37) and to a low activity of HMG-CoA synthase (13). In contrast, piglets possess a relatively high activity of peroxisomal β-oxidation, which was postulated to act as a compensatory mechanism to oxidize milk fatty acids (60, 61). In the present study, both peroxisomal β-oxidation and CPT activity in piglet liver and heart were found to be readily induced by clofibric acid. These results raise the possibility that dietary or pharmacologic manipulation of PPAR-responsive genes in young pigs could be used to increase the capacity for fatty acid oxidation and thermogenesis.

Peroxosomal β-oxidation of fatty acids is believed to play a role in thermogenesis (16) and lipid catabolism (49). Peroxisome proliferators have calorigenic effects and stimulate enzymes involved in lipid catabolism that are in many ways similar to the effects of thyroid hormones (47). Our previous studies have demonstrated a substantial activity of peroxisomal β-oxidation in tissues of pigs immediately after birth (60, 61), which indicates that the peroxisomal β-oxidation system begins to develop before birth. Therefore, we suggest that alteration of maternal diet or maternal administration of clofibric acid or other peroxisome proliferators in late gestation should be explored as a potential method to increase capacities for peroxisomal and mitochondrial β-oxidation in neonatal piglets, thereby improving their survival and growth. Developmental and comparative studies of peroxisomal and mitochondrial β-oxidation of fatty acids in different tissues of pre- and postnatal pigs may yield new insights into regulation of neonatal fatty acid metabolism.

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Present addresses: X. X. Yu, Dept. of Endocrinology and Metabolism, Genentech, South San Francisco, CA 94080; J. Odle, Dept. of Animal Sciences, North Carolina State University, Raleigh, NC 27695.

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