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

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Yu, Xing Xian, Jack Odle, and James K. Drackley. Differential induction of peroxisomal β-oxidation enzymes by clofibric 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% clofibric 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.

pigs; fatty acids; peroxisome proliferators; carnitine palmitoyltransferase; fatty acyl-coenzyme A oxidase

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 H_2O_2; FAO was found to be the rate-limiting enzyme of this system (9, 24). The H_2O_2 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

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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, clofibric 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% H_2O_2, homovanillate, horseradish peroxidase (type II), glycylglycine, Tris·HCl, EDTA, HEPES, triethanolamine HCl, isocitrate, clofibric 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 (Ridglan 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/wt) clofibric 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 clofibric acid and aspirin were selected on the basis of previous studies with rodents in which diets containing 0.5% (wt/wt) clofibric 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 N_2 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 N_2 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 Lazarow (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 H_2O_2; 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 spectrofluorometrically. 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_2O_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_2O_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 mM/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), DL-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 mM/cm. All assays were performed within their linear ranges.

Extraction of total RNA and assay of specific mRNA. Total RNA was extracted by using a kit (Biotecx Laboratories, Houston, TX) according to the manufacturer's instructions. Briefly, tissue samples were homogenized with Ultraspec RNA reagent (0.1 g tissue/ml reagent), and 0.2 ml of chloroform per ml of RNA reagent was added to the resulting homogenate. The mixture was centrifuged at 12,000 *g* for 15 min. The resulting aqueous phase was mixed thoroughly with 0.5 volume of isopropanol and 0.05 volume of RNA Tack Resin, and the mixture was centrifuged for 1 min in a tabletop minicentrifuge. The pellet was washed twice with 75% ethanol. Finally, the pellet was resuspended in H_2O by vortexing vigorously; RNA was separated from the resin by centrifugation for 1 min and then stored at −70°C.

For determination of specific mRNA abundance, 20 μg of total RNA was subjected to gel electrophoresis in 1.2% agarose gels under denaturing conditions. The RNA was transferred to MagnaGraph nylon transfer membranes (MSI, Westboro, MA) by capillary blotting and immobilized by UV irradiation.

About 25 ng of the purified coding region of human cDNA for catalase was labeled with 6.66 Gbq of [α-^{32}P]dCTP (DuPont NEN, Boston, MA) to a specific activity >60 MBq/μg by using the RadPrime DNA labeling system (Life Technologies, Gaithersburg, MD) as described (61). The cDNA probe was generously provided by Dr. Inderjit Singh (Medical University of South Carolina, Charleston, SC). The membranes were prehybridized with QuikHyb hybridization solution (Stratagene, La Jolla, CA) for 1.5–2 h at 42°C and then were hybridized with labeled denatured probe and 75 μg/ml denatured salmon sperm DNA in the presence of the hybridization solution for 1.5–2.0 h at 42°C, according to the Stratagene procedures. Following hybridization, the membranes were washed in 2 × sodium chloride-sodium citrate (SSC)-0.1% SDS (wt/vol) solution at room temperature twice for 15 min each, and in 0.1 × SSC-0.1% SDS (wt/vol) solution at 50°C one to three times for 5–15 min total. The [^{32}P]cDNA/mRNA hybrids then were visualized by autoradiograph, and the catalase mRNA abundance was quantified by densitometric scanning of the autoradiographs (Molecular Dynamics ImageQuant 3.0, model 300A). Data were normalized to ethidium bromide-stained 28S rRNA. Northern blots were run in triplicate for liver and heart from each treatment group. Northern assay to determine the FAO mRNA content was also conducted but failed probably due to an insufficient sensitivity of the assay.

Statistical analysis. Data were subjected to one-factor ANOVA; least-squares means for treatment groups were separated by multiple *t*-tests (50) using the probability of differences (PDIFF) option of the General Linear Models procedure of SAS (46). Probability values of $P < 0.10$ were considered to be statistically significant.

RESULTS

Initial body weights did not differ among the three groups (Table 1). Clofibric acid did not significantly affect daily food intake (Table 1), and the final body weight did not differ between controls and pigs given clofibric acid. Aspirin decreased daily food intake by ~17% ($P < 0.05$) but did not significantly affect feed

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

	Control (n = 5)	Clofibric Acid (n = 6)	Aspirin (n = 4)
Initial body wt, kg	2.057 ± 0.130	2.084 ± 0.120	2.090 ± 0.150
Final body wt, kg	6.295 ± 0.400	5.612 ± 0.370	4.776 ± 0.450*
Daily food intake, liters	1.649 ± 0.059	1.459 ± 0.067	1.369 ± 0.070*
Body wt gain/feed, g/l	185.7 ± 2.5	171.5 ± 21.5	167.4 ± 19.1
Liver wt, g	205.0 ± 21.8	219.3 ± 19.9	166.0 ± 24.3
Liver wt/final body wt, %	3.26 ± 0.22	3.83 ± 0.20	3.48 ± 0.24
Kidney wt, g	48.8 ± 4.9	53.9 ± 4.5	38.6 ± 5.5
Kidney wt/final body wt, %	0.78 ± 0.05	0.95 ± 0.05*	0.81 ± 0.06
Heart wt, g	33.9 ± 2.7	40.4 ± 2.4	29.8 ± 3.0
Heart wt/final body wt, %	0.54 ± 0.01	0.72 ± 0.01‡	0.63 ± 0.02†

Values are least-squares means ± SE. *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$ vs. control.

Table 2. *Activities of peroxisomal and mitochondrial enzymes in liver of piglets after different treatments*

Activity	Control ($n = 5$)	Clofibric Acid ($n = 6$)	Aspirin ($n = 4$)
Peroxisomal β-oxidation, μmol NADH·h^{-1}·g^{-1}	2.53 ± 0.81	9.46 ± 0.74†	4.62 ± 0.91
FAO, μmol H_2O_2·h^{-1}·g^{-1}	1.55 ± 0.44	4.89 ± 0.40†	3.24 ± 0.49*
Catalase, mmol H_2O_2·min^{-1}·g^{-1}	36.29 ± 3.03	48.21 ± 2.77*	42.28 ± 3.39
CPT, μmol CoA·h^{-1}·g^{-1}	20.16 ± 3.00	51.17 ± 2.74†	33.14 ± 3.36
ICDH, μmol NADH·h^{-1}·g^{-1}	10.77 ± 1.47	7.65 ± 1.34	6.72 ± 1.65

Values are least-squares means ± SE. *$P < 0.05$ and †$P < 0.001$ vs. control. FAO, fatty acyl-CoA oxidase; CPT, carnitine palmitoyltransferase; ICDH, isocitrate dehydrogenase. All activities are expressed per gram of wet tissue.

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

Table 3. *Activities of peroxisomal and mitochondrial enzymes in heart of piglets after different treatments*

Activity	Control ($n = 5$)	Clofibric Acid ($n = 6$)	Aspirin ($n = 4$)
Peroxisomal β-oxidation, μmol NADH·h^{-1}·g^{-1}	0.06 ± 0.02	0.20 ± 0.02†	0.06 ± 0.03
FAO, μmol H_2O_2·h^{-1}·g^{-1}	0.02 ± 0.01	0.10 ± 0.01‡	0.02 ± 0.01
Catalase, mmol H_2O_2·min^{-1}·g^{-1}	0.65 ± 0.18	1.79 ± 0.16‡	0.90 ± 0.20
CPT, μmol CoA·h^{-1}·g^{-1}	1.35 ± 0.44	3.96 ± 0.40‡	1.34 ± 0.49
ICDH, μmol NADH·h^{-1}·g^{-1}	2.82 ± 0.22	3.54 ± 0.20*	1.23 ± 0.24‡

Values are least-squares means ± SE. *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$ vs. control. All activities are expressed per gram of wet tissue.

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

Activity	Control ($n = 5$)	Clofibric Acid ($n = 6$)	Aspirin ($n = 4$)
Peroxisomal β-oxidation, μmol NADH·h^{-1}·g^{-1}	1.96 ± 0.27	2.09 ± 0.24	1.70 ± 0.30
FAO, μmol H_2O_2·h^{-1}·g^{-1}	0.98 ± 0.15	1.14 ± 0.14	0.71 ± 0.17
Catalase, mmol H_2O_2·min^{-1}·g^{-1}	19.56 ± 3.86	20.41 ± 3.53	20.09 ± 4.32
CPT, μmol CoA·h^{-1}·g^{-1}	5.88 ± 1.20	12.23 ± 1.10*	7.98 ± 1.34
ICDH, μmol NADH·h^{-1}·g^{-1}	3.63 ± 0.47	3.35 ± 0.43	3.31 ± 0.53

Values are least-squares means ± SE. *$P < 0.01$ vs. control. All activities are expressed per gram of wet tissue.

(23, 41). 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 was 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 some 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 clofibric 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. Reinhart 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 clofibric 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 mitochon-

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

Activity	Control ($n = 5$)	Clofibric Acid ($n = 6$)	Aspirin ($n = 4$)
Peroxisomal β-oxidation, μmol NADH·h^{-1}·g^{-1}	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
Catalase, mmol H_2O_2·min^{-1}·g^{-1}	0.61 ± 0.09	0.88 ± 0.09	0.79 ± 0.11
CPT, μmol CoA·h^{-1}·g^{-1}	2.62 ± 0.82	4.94 ± 0.75	4.23 ± 0.92
ICDH, μmol NADH·h^{-1}·g^{-1}	11.23 ± 1.04	12.68 ± 0.95	11.33 ± 1.16

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

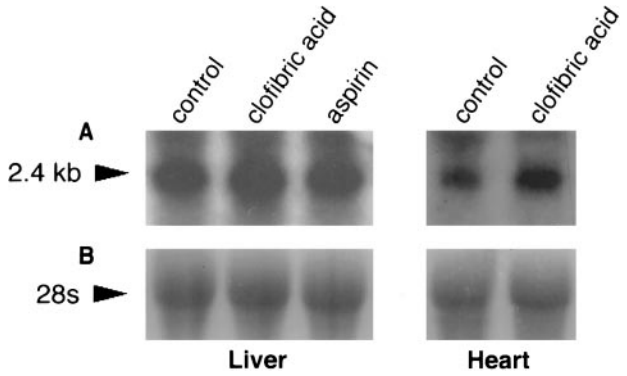

Fig. 1. *A*: Northern blot analysis of catalase mRNA in liver and heart from neonatal (1–2 days old) pigs fed on a control milk replacer diet, control plus 0.5% clofibric acid, or control plus 1% aspirin for 14 days. *B*: the 28s RNA bands photographed from the corresponding ethidium bromide-stained 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). Clofibric 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, clofibric 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-ethylexyl)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 $mg \cdot kg^{-1} \cdot day^{-1}$) 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 clofibric 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

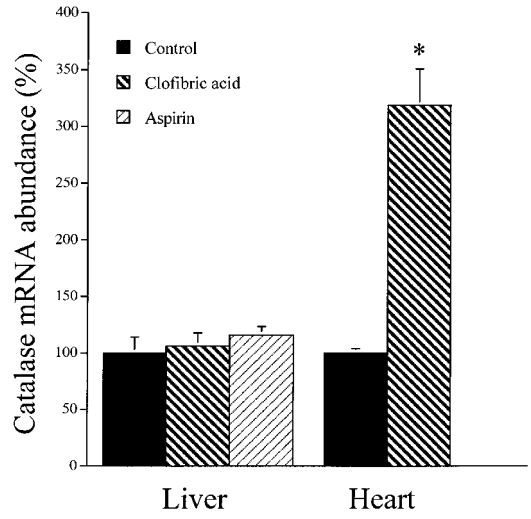

Fig. 2. Relative changes in catalase mRNA abundance in liver (n = 3) and heart (n = 3) from neonatal (1–2 days old) pigs fed on a control milk replacer diet, control plus 0.5% clofibric acid, or control plus 1% aspirin for 14 days. Data are expressed as percentages of control values that are normalized to 100% in each tissue. Bars represent means ± SE. *$P < 0.05$ vs. control for the same tissue.

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.

Peroxisomal 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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REFERENCES

1. **Adams SH, Lin X, Yu XX, Odle J, and Drackley JK.** Hepatic fatty acid metabolism in pigs and rats: evidence for major differences in end-products, O_2 uptake, and β- oxidation. *Am J Physiol Regulatory Integrative Comp Physiol* 272: R1641–R1646, 1997.
2. **Adams SH and Odle J.** Plasma β-hydroxybutyrate after octanoate challenge: attenuated ketogenic capacity in neonatal swine. *Am J Physiol Endocrinol Metab* 265: E761–E765, 1993.
3. **Aebi H.** *Methods of Enzymatic Analysis*. Weinheim: Verlag Chemie, 1974, p. 673–678.
4. **Bell AR, Savory R, Horley NJ, Choudhury AI, Gray TJB, Salter AM, and Bell DR.** Molecular basis of non-responsiveness to peroxisome proliferators: the guinea-pig PPARα is functional and mediates peroxisome proliferator-induced hypolipidaemia. *Biochem J* 332: 689–693, 1998.
5. **Bernt E and Bergmeyer HU.** *Methods of Enzymatic Analysis*. Weinheim: Verlag Chemie, 1974, p. 624–627.
6. **Bieber LL, Markwell MAK, Blair M, and Helmrath TA.** Studies on the development of carnitine palmitoyltransferase and fatty acid oxidation in liver mitochondria of neonatal pigs. *Biochim Biophys Acta* 326: 145–154, 1973.
7. **Blümcke S, Schwartzkopff W, Lobeck H, Edmondson NA, Prentice DE, and Blane GF.** Influence of fenofibrate on cellular and subcellular liver structure in hyperlipidemic patients. *Atherosclerosis* 46: 105–116, 1983.
8. **Brandt JM, Djouadi F, and Kelly DP.** Fatty acids mediate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor α. *J Biol Chem* 273: 23786–23792, 1998.
9. **Bronfman M, Inestrosa NC, Nervi FO, and Leighton F.** Acyl-CoA synthetase and the peroxisomal enzymes of β-oxidation in human liver. *Biochem J* 224: 709–720, 1984.
10. **Cai YN, Sohlenius AK, Andersson K, Sundberg C, and DePierre JW.** Effects of acetylsalicylic acid on parameters related to peroxisome proliferation in mouse liver. *Biochem Pharmacol* 47: 2213–2219, 1994.
11. **Desvergne B and Wahli W.** Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 205: 649–688, 1999.
12. **Dreyer C, Keller H, Mahfoudi A, Laudet V, Krey G, and Wahli W.** Positive regulation of the peroxisomal β-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR). *Biol Cell* 77: 67–76, 1993.
13. **Duée PH, Pégorier JP, Quant PA, Herbin C, Koul C, and Girard J.** Hepatic ketogenesis in newborn pigs is limited by low mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase activity. *Biochem J* 298: 207–212, 1994.
14. **Gear ARL, Albert AD, and Bednarek JM.** The effect of the hypocholesterolemic drug clofibrate on liver mitochondrial biogenesis: a role for neutral mitochondrial proteases. *J Biol Chem* 249: 6495–6504, 1974.

15. **Girard J, Férre P, Pégorier JP, and Duée PH.** Adaptations of glucose and fatty acid metabolism during the perinatal period and suckling-weaning transition. *Physiol Rev* 72: 507–562, 1992.
16. **Goglia F, Liverini G, Lanni A, Iossa S, and Barletta A.** Morphological and functional modifications of rat liver peroxisomal subpopulations during cold exposure. *Exp Biol* 48: 127–133, 1989.
17. **Grey RH and De La Iglesia FA.** Quantitative microscopy comparison of peroxisome proliferation by the lipid-regulating agent gemfibrozil in several species. *Hepatology* 4: 520–530, 1984.
18. **Grindflek E, Sundvold H, Klungland H, and Lien S.** Characterization of porcine peroxisome proliferator-activated receptor γ1 and γ2: detection of breed and age differences in gene expression. *Biochem Biophys Res Commun* 249: 713–718, 1998.
19. **Grum DE, Hansen LR, and Drackley JK.** Peroxisomal β-oxidation of fatty acids in bovine and rat liver. *Comp Biochem Physiol* 109B: 281–292, 1994.
20. **Hanefeld M, Kemmer C, and Kadner E.** Relationship between morphological changes and lipid lowering action of *p*-chlorophenoxyisobutyric acid (CPIB) on hepatic mitochondria and peroxisomes in man. *Atherosclerosis* 46: 239–246, 1983.
21. **Hashimoto T.** Individual peroxisomal β-oxidation enzymes. *Ann NY Acad Sci* 386: 5–12, 1982.
22. **Hawkins JM, Jones WE, Bonner FW, and Gibson GG.** The effect of peroxisome proliferators on microsomal, peroxisomal, and mitochondrial enzyme activities in the liver and kidney. *Drug Metab Rev* 18: 441–515, 1987.
23. **Holden PR and Tugwood JD.** Peroxisome proliferator-activated receptor alpha: role in rodent liver cancer and species differences. *J Mol Endocrinol* 22: 1–8, 1999.
24. **Inestrosa NC, Bronfman M, and Leighton F.** Detection of peroxisomal fatty acyl-coenzyme A oxidase activity. *Biochem J* 182: 779–788, 1979.
25. **Katoh H, Kawashima Y, Watanuki H, Kozuka H, and Isono H.** Effects of clofibric acid and tiadenol on cytosolic long-chain acyl-CoA hydrolase and peroxisomal β-oxidation in liver and extrahepatic tissues of rats. *Biochim Biophys Acta* 920: 171–179, 1987.
26. **Kundu RK, Tonsgard JH, and Getz GS.** Induction of omega-oxidation of monocarboxylic acids in rats by acetylsalicylic acid. *J Clin Invest* 88: 1865–1872, 1991.
27. **Kurup CKR, Aithal HM, and Ramasarma T.** Increase in hepatic mitochondria on administration of ethyl-α-*p*-chlorophenoxyisobutyrate to the rat. *Biochem J* 116: 773–779, 1970.
28. **Kvannes J, Eikhom JS, and Flatmark T.** The peroxisomal β-oxidation enzyme system of rat heart. Basal level and effect of the peroxisome proliferator clofibrate. *Biochim Biophys Acta* 1201: 203–216, 1994.
29. **Lake BG, Gray TJB, Foster JR, Stubberfield CR, and Gangolli SD.** Comparative studies on DEHP induced hepatic peroxisome proliferation in rat and hamster. *Toxicol Appl Pharmacol* 72: 46–60, 1984.
30. **Lazarow PB.** *Methods in Enzymology*. New York, NY: Academic, 1981, p. 315–317.
31. **Lazarow PB and de Duve C.** A fatty acyl-CoA oxidizing system in rat liver peroxisomes: enhancement by clofibrate, a hypolipidemic drug. *Proc Natl Acad Sci USA* 73: 2043–2046, 1976.
32. **Lucas IAM and Lodge GA.** *The Nutrition of the Young Pig: A Review*. Bucks, UK: Commonwealth Agricultural Bureau, Farnham Royal, 1961.
33. **McGarry JD and Brown NF.** The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur J Biochem* 244: 1–14, 1997.
34. **National Research Council.** *Nutrient Requirements of Swine.* Washington, DC: National Academy Press, 1988.
35. **Nemali MR, Usuda N, Reddy MK, Oyasu K, Hashimoto T, Osumi T, Rao MS, and Reddy JK.** Comparison of constitutive and inducible levels of expression of peroxisomal β-oxidation and catalase genes in liver and extrahepatic tissues of rat. *Cancer Res* 48: 5316–5324, 1988.
36. **Norseth J.** Increased β-oxidation of erucic acid in perfused hearts from rats fed clofibrate. *Biochim Biophys Acta* 617: 183–191, 1980.
37. **Odle J, Lin X, Van Kempen TATG, Drackley JK, and Adams SH.** Carnitine palmitoyltransferase modulation of hepatic fatty acid metabolism and radio-HPLC evidence for low ketogenesis in neonatal pigs. *J Nutr* 121: 2541–2549, 1995.
38. **Ortiz JA, Nicot C, Bofarull J, Rodriguez JC, Hegardt FG, Haro D, and Marrero PF.** Isolation of pig mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene promoter: characterization of a peroxisome proliferator-responsive element. *Biochem J* 337: 329–335, 1999.
39. **Orton TC, Adam HK, Bentley M, Holloway B, and Tucker MJ.** Clobuzarit: species differences in the morphological and biochemical response of the liver following chronic administration. *Toxicol Appl Pharmacol* 72: 138–151, 1984.
40. **Pégorier JP, Duée PH, Girard J, and Peret J.** Metabolic fate of non-esterified fatty acids in isolated hepatocytes from newborn and young pigs. Evidence for a limited capacity for oxidation and increased capacity for esterification. *Biochem J* 212: 93–97, 1983.
41. **Reddy JK and Warren JR.** Hepatic and renal effects of peroxisome proliferators: biological implications. *Ann NY Acad Sci* 386: 81–108, 1982.
42. **Reinhart GA, Mahan DC, Lepine AJ, Simmen FA, and Moore BE.** Dietary clofibric acid increases intestinal fatty acid binding protein activity and apparent lipid digestibility in weanling swine. *J Anim Sci* 71: 2693–2699, 1993.
43. **Reubsaet FAG, Veerkamp JH, Dirven HAAM, Brückwilder MLP, Hashimoto T, Trijbels JMF, and Monnens LAH.** The effect of di(ethylhexyl)phthalate on fatty acid oxidation and carnitine palmitoyltransferase in various rat tissues. *Biochim Biophys Acta* 1047: 264–270, 1990.
44. **Rodríguez C, Noé V, Cabrero A, Ciudad CJ, and Laguna JC.** Differences in the formation of PPARα-RXR/*aco*PPRE complexes between responsive and nonresponsive species upon fibrate administration. *Mol Pharmacol* 58: 185–193, 2000.
45. **Rodríguez JC, Gil-Gómez G, Hegardt FG, and Haro D.** Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. *J Biol Chem* 269: 18767–18772, 1994.
46. **SAS Institute.** *SAS7 User's Guide*. Cary, NC: SAS Institute, 1985.
47. **Schoonjans K, Staels B, and Auwerx J.** Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res* 37: 907–925, 1996.
48. **Sharma R, Lake BG, Makowski R, Bradshaw T, Earnshaw D, Dale JW, and Gilson GG.** Differential induction of peroxisomal and microsomal fatty-acid-oxidizing enzymes by peroxisome proliferators in rat liver and kidney. *Eur J Biochem* 184: 69–78, 1989.
49. **Singh I.** Biochemistry of peroxisomes in health and disease. *Mol Cell Biochem* 167: 1–29, 1997.
50. **Steel RGD and Torrie JH.** *Principles and Procedures of Statistics*. New York, NY: McGraw-Hill, 1980.
51. **Stefanini S, Serafini B, Nardacci R, Vecchioli SF, Moreno S, and Sartori C.** Morphometric analysis of liver and kidney peroxisomes in lactating rats and their pups after treatment with the peroxisomal proliferator di-(2-ethylexy)phthalate. *Biol Cell* 85: 167–176, 1995.
52. **Stott WT, Yano BL, Williams DM, Barnard SD, Hannah MA, Cieszlak FS, and Herman JR.** Species-dependent induction of peroxisome proliferation by haloxyfop, an aryloxyphenoxy herbicide. *Fundam Appl Toxicol* 28: 71–79, 1995.
53. **Sundvold H, Grindflex E, and Lien S.** Peroxisome proliferator activated receptor α (PPARα) expression in swine: detection of an alternative transcript. In: *Proceedings 3rd Nordic Mtg on Medical and Biochemical Aspects of Lipid Metabolism.* Odense, Denmark: Univ. Southern Denmark, 2000.
54. **Tsukada H, Mochizuki Y, and Konishi T.** Morphogenesis and development of microbodies of hepatocytes of rats during pre- and post-natal growth. *J Cell Biol* 37: 231–243, 1968.

55. **Vamecq J.** Fluorometric assay of peroxisomal oxidases. *Anal Biochem* 186: 340–249, 1990.
56. **Vamecq J, Roels F, van den Branden C, and Draye JP.** Peroxisomal proliferation in heart and liver of mice receiving chlorpromazine, ethyl 2(5(4-chlorophenyl)pentyl)oxiran-2-carboxylic acid or high fat diet: a biochemical and morphometrical comparative study. *Pediatr Res* 22: 748–754, 1987.
57. **Vamecq J, Vallee L, de la Porte PL, Fontaine M, de Craemer D, van den Branden C, Lafont H, Gratarolı R, and Nalbone G.** Effect of various n-3/n-6 fatty acid ratio contents of high fat diets on the liver and heart peroxisomal and mitochondrial β-oxidation. *Biochim Biophys Acta* 1170: 151–156, 1993.
58. **Veerkamp JH and van Moerkerk HTB.** Peroxisomal fatty acid oxidation in rat and human tissues. Effect of nutritional state, clofibrate treatment and postnatal development in the rat. *Biochim Biophys Acta* 875: 301–310, 1986.
59. **Yamoto T, Ohashi Y, Furukawa T, Teranishi M, Manabe S, and Makita T.** Change of the sex-dependent response to clofibrate in F344 rat liver during postnatal development. *Toxicol Lett* 85: 77–83, 1996.
60. **Yu XX, Drackley JK, and Odle J.** Rates of mitochondrial and peroxisomal β-oxidation of palmitate changes during postnatal development and feed deprivation in liver, kidney, and heart of pigs. *J Nutr* 127: 1814–1821, 1997.
61. **Yu XX, Drackley JK, and Odle J.** Food deprivation changes peroxisomal β-oxidation activity but not catalase activity during postnatal development in pig tissues. *J Nutr* 128: 1114–1121, 1998.
62. **Yu XX, Drackley JK, Odle J, and Lin X.** Response of hepatic mitochondrial and peroxisomal β-oxidation to increasing palmitate concentrations in piglets. *Biol Neonate* 72: 284–292, 1997.
63. **Zammit VA.** Carnitine acyltransferases: functional significance of subcellular distribution, and membrane topology. *Prog Lipid Res* 38: 199–224, 1999.