

Food Deprivation Changes Peroxisomal β -Oxidation Activity but Not Catalase Activity during Postnatal Development in Pig Tissues^{1,2,3}

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ABSTRACT Peroxisomal β -oxidation and catalase activity were investigated in liver, kidney and heart from pigs at the following timepoints: within 0.5 h after birth (0 h, unfed) and at 24 h (suckled or unsuckled), 10 d (suckled or 24-h food-deprived), 21 d (suckled or 24-h food-deprived) and 5 mo (overnight food-deprived). In liver, peroxisomal β -oxidation increased about twofold at 24 h for suckled pigs ($P < 0.001$) but did not change for unsuckled pigs. The rate was further increased in 21-d-old pigs compared with 0- ($P < 0.001$) or 24-h-old ($P < 0.05$) pigs, but was lower at 5 mo than at 10 or 21 d ($P < 0.01$). The rate was higher for food-deprived pigs than suckled pigs at 10 d ($P < 0.001$) of age. In kidney, peroxisomal β -oxidation was unchanged during the first 24 h but was higher ($P < 0.05$) at 10 d for suckled pigs and at 21 d than at 0 h. Nutritional state did not influence renal peroxisomal β -oxidation. In heart, peroxisomal β -oxidation did not change with age or nutritional state. The developmental pattern of fatty acyl-CoA oxidase activity was similar to that of peroxisomal β -oxidation in each tissue. Developmental increases of peroxisomal β -oxidation were greater than those for first-cycle peroxisomal β -oxidation reported earlier, suggesting that peroxisomal β -oxidation became more complete in older pigs. Catalase activity did not change during the first 24 h after birth but then increased 10.5-, 2.9-fold and 33% at 10 d in liver, kidney and heart, respectively. The concentration of catalase mRNA was only 1.1- and 1.3-fold higher at 10 d than at 24 h in liver and kidney, respectively. Catalase activity was not affected by food deprivation. We concluded the following: 1) peroxisomal β -oxidation develops rapidly after birth and may be important for piglets to oxidize milk fatty acids; 2) food is required for the initial induction after birth; and 3) rapidly increased catalase activity during the first 10 d of life resulted from both pretranslational and post-translational regulation. *J. Nutr.* 128: 1114–1121, 1998.

KEY WORDS: • piglets • peroxisomes • β -oxidation • development • catalase

Peroxisomal β -oxidation of fatty acids occurs in a variety of tissues, including liver, kidney, heart, intestine, adipose tissue, muscle and brain of many species (see Bieri 1993). Fatty acyl-CoA oxidase (FAO; EC 1.3.99.3) catalyzes the first oxidation step in the peroxisomal pathway by transferring electrons to molecular oxygen to produce H_2O_2 . In rodents, FAO is the rate-limiting enzyme in this pathway (Reubsæet et al. 1988). Rodent FAO oxidizes medium-, long- and very-long-chain fatty acids with high affinity but is inactive with short-chain acyl-CoA (Vamecq and Draye 1987). Consequently, peroxi-

somal β -oxidation of fatty acids usually is incomplete. The extent of chain shortening is influenced by many factors, including the administration of hypolipidemic drugs and the type, concentration and degree of saturation of fatty acids (for review see Osmundsen et al. 1991).

Catalase (EC 1.11.1.6), a tetrameric enzyme containing ferriprotoporphyrin IX, is present in peroxisomes and degrades the H_2O_2 produced by FAO during peroxisomal β -oxidation of fatty acids. Catalase also degrades H_2O_2 generated from other metabolic pathways such as D-amino acid oxidase and uricase (Vamecq et al. 1993). Although often used as a marker for peroxisomes, catalase also is found in other intracellular compartments (Chance et al. 1979) of aerobic eukaryotic cells. The activity of catalase has been shown to be variable among species (Feinstein 1970), as well as among strains (Novak et al. 1978), tissues (Novak et al. 1978) and even developmental stages (Holmes 1971) within species.

Postnatal development of peroxisomal β -oxidation and its associated enzymes, including catalase, has not been well characterized in swine. In rodents, peroxisomal β -oxidation and its associated enzymes develop rapidly after birth (Krahling et al. 1979, Périchon and Bourre 1995, Veerkamp and van Moerkerk 1986). A morphometric study that used electron

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microscopy showed that the number of peroxisomes per unit of piglet liver increased during the first 28 d of life (Laging et al. 1990). Therefore, we postulated that the peroxisomal capacity for β -oxidation of fatty acids in pigs, as in rats, might develop rapidly after birth and might be an important component of postnatal adaptations in lipid metabolism. We found that the proportional contribution of peroxisomal β -oxidation to total β -oxidation was greater in neonatal pigs than in adult pigs, and both were greater than in adult rats (Yu et al. 1997b).

In a previous report (Yu et al. 1997a), we examined rates of total, mitochondrial and peroxisomal β -oxidation in liver, kidney and heart from piglets at various ages after birth. These measurements were made by incubating tissue homogenates with [$1\text{-}^{14}\text{C}$]-palmitate in either the absence or presence of antimycin A and rotenone as mitochondrial respiratory inhibitors. We found that rates of total, mitochondrial and peroxisomal β -oxidation generally increased with postnatal age. Food deprivation increased hepatic total oxidation at 10 d of age and decreased hepatic peroxisomal β -oxidation at 24 h of age but did not affect β -oxidation in kidney or heart.

Measuring oxidation of [$1\text{-}^{14}\text{C}$]-palmitate in the presence of mitochondrial respiratory inhibitors measures only the initial β -oxidation cycle of peroxisomes (Yu et al. 1997a). Use of assays that measure products from all cycles of peroxisomal β -oxidation may provide additional information about the extent of this pathway and the degree of chain-shortening activity. Therefore, we report herein data for rates of peroxisomal β -oxidation measured as the palmitoyl-CoA-dependent, cyanide-insensitive reduction of NAD^+ in tissues from the experiment reported earlier (Yu et al. 1997a). Activities of the putative rate-limiting enzyme, FAO, and the associated enzyme catalase also were measured.

Together with data reported previously (Yu et al. 1997a), our results indicate that peroxisomal β -oxidation develops rapidly in a tissue-specific manner after birth and that development of peroxisomal β -oxidation capacity in liver is dependent on food intake. Furthermore, a comparison of these data with the companion data reported previously (Yu et al. 1997a) suggests that the completeness of peroxisomal β -oxidation may increase with postnatal age.

MATERIALS AND METHODS

Reagents. Palmitoyl-CoA, coenzyme A, NAD^+ , EDTA, FAD, dithiothreitol, Triton X-100, Brij 58, H_2O_2 , homovanillate, horseradish peroxidase (type II), glycylglycine, Tris-HCl and HEPES were purchased from Sigma Chemical (St. Louis, MO). Potassium cyanide was purchased from Mallinckrodt (Paris, KY). All other chemicals were of reagent or higher grade.

Animals and collection of tissue samples. Procedures for this study were approved by the Laboratory Animal Care Advisory Committee of the University of Illinois. Piglets used were males, were of normal body weight and resulted from matings of Camborough-15 females to Line-326 males (Pig Improvement, Franklin, KY). Piglets were obtained from the University of Illinois Swine Research Farms within 0.5 h after birth (0 h, unsuckled; $n = 4$) and at 24 h (suckled or 24-h unfed; $n = 4$ for each nutritional state), 10 d (suckled or 24-h food-deprived; $n = 4$ for each nutritional state), 21 d (just before weaning; suckled or 24-h food-deprived; $n = 4$ for each nutritional state) and 5 mo (adults; overnight food-deprived; $n = 3$) of age. The animals were anesthetized with pentobarbital at a dose of ~ 25 mg per kg of body weight via intraperitoneal injection, except for the adults, which were killed by electrical shock. Liver, kidney and heart were removed immediately and placed in ice-cold isolation buffer (pH 7.2) containing 220 mmol/L mannitol, 70 mmol/L sucrose, 2 mmol/L HEPES and 0.1 mmol/L EDTA. After the blood was washed off, a portion of the tissues was frozen in liquid N_2 and then stored at -70°C .

Preparation of a peroxisome-enriched fraction from tissues. Peroxisome-enriched fractions were prepared essentially as described by Goglia et al. (1989). Using a Potter-Elvehjem homogenizer, samples (~ 0.5 g) of tissue were homogenized manually in five volumes of ice-cold homogenization buffer (pH 7.4) containing 0.3 mol/L sucrose, 20 mmol/L Tris-HCl and 0.5 mmol/L EDTA. The homogenates were subjected to differential centrifugation using a Beckman J2-21 centrifuge and JA-18.1 rotor (Beckman Instruments Inc., Fullerton, CA). A combined fraction containing debris, nuclei and mitochondria was sedimented by centrifugation at $3000 \times g$ for 10 min. The resulting supernatant was centrifuged at $27,000 \times g$ for 10 min to sediment a peroxisome-enriched fraction. The peroxisome-enriched fractions from the tissues were resuspended in 0.5 mL (for liver and kidney) or 0.25 mL (for heart) of the same ice-cold homogenization buffer per gram of tissue, and samples were immediately frozen and stored in liquid N_2 until determinations of peroxisomal β -oxidation rate and FAO activity.

Assays of peroxisomal β -oxidation and enzyme activities. Peroxisomal β -oxidation rate was determined spectrophotometrically at 340 nm and 37°C as the palmitoyl-CoA-dependent, KCN-insensitive reduction of NAD^+ as described by Lazarow (1981), except that 0.1 g/L Brij 58 was added to the incubation medium and bovine serum albumin was omitted (Kvannes et al. 1994). The final concentration of KCN in the reaction mixture was 1.0 mmol/L. Contents of sample and reference cuvettes were identical except for palmitoyl-CoA, which was added to the sample cuvette only.

Activity of FAO was assayed at room temperature by using a modification of the procedure of Vamecq (1990). In this method, the release of H_2O_2 is determined spectrofluorometrically from a coupled peroxidative reaction, using homovanillate as electron donor in the presence of horseradish peroxidase to produce a fluorescent oxidation dimer. The reaction mixture contained, in a total volume of 0.5 mL, 80 mmol/L glycylglycine buffer (pH 8.3), 5 mmol/L homovanillate, 0.1 g/L Brij 58, 0.1 mg horseradish peroxidase (type II), 0.02 mmol/L FAD, 0.1 mmol/L palmitoyl-CoA and 10 μL (for liver and kidney) or 20 μL (for heart) of the peroxisome-enriched fraction. The reaction was started by adding palmitoyl-CoA. At 2-min intervals, beginning at 4 min after the reaction was started, a 75- μL aliquot was removed and mixed with 1.5 mL of 0.5 mol/L carbonate-bicarbonate buffer (pH 10.7). The fluorescence produced was measured in a spectrofluorometer at wavelengths of 325 nm for excitation and 425 nm for emission.

For assay of catalase activity, samples (0.2–0.5 g) of tissue were homogenized in 5 volumes of ice-cold 0.05 mol/L phosphate buffer (pH 7.4) containing 0.02 g/L Triton X-100 using a Potter-Elvehjem homogenizer. The resulting homogenates were frozen and thawed three times. The homogenates then were centrifuged at $10,000 \times g$ for 10 min, and the supernatants were used for the assay. Catalase activity was determined spectrophotometrically using the UV assay method of Aebi (1974) at room temperature. In this system, the decomposition of H_2O_2 is tracked by measuring 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 stock solution of 30% H_2O_2 . All assays were conducted within their linear ranges with respect to amounts of tissue and time.

Extraction of total RNA and assay of catalase mRNA. Total RNA was extracted with the use of a total RNA extraction kit (Bio-tex Laboratories, Houston, TX) according to the manufacturer's instructions. Briefly, by using a VirTishear polytron homogenizer (The Virtis Company, Gardiner, NY) at the highest speed for 45–60 s, tissue samples were homogenized with Ultraspec RNA reagent (0.1 g tissue per mL reagent), and 0.2 mL of chloroform per mL of RNA reagent was added to the resulting homogenate. The sample was centrifuged at $12,000 \times g$ for 15 min. The resulting aqueous phase was mixed thoroughly with 0.5 volume of isopropanol and 0.05 volume of RNATack Resin, and the mixture was centrifuged for 1 min in a table-top minicentrifuge. The pellet was washed twice with 75% ethanol by vortexing and centrifugation. Finally, the pellet was resuspended in water by vortexing vigorously; RNA was separated from the resin by centrifuging for 1 min and was stored at -70°C .

For determination of specific mRNA contents, 20 μg of total

RNA was subjected to gel electrophoresis in 1.2% agarose gels under denaturing conditions [formamide, formaldehyde and 2-(*N*-morpholino)propanesulfonic acid (MOPS) buffer]. 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 (Singh et al. 1996) was labeled with 6.66 GBq of [α - 32 P]dCTP (Dupont NEN, Boston, MA) to a specific activity >60 MBq/ng by using RadPrime DNA labeling system (Life Technologies, Gaithersburg, MD). The cDNA probe was generously provided by Inderjit Singh (Medical University of South Carolina, Charleston, SC). The unincorporated [α - 32 P]dCTP, other nucleotides and contaminants were removed by purifying the cDNA using an Elutip minicolumn (Schleicher & Schuell, Keene, NH) according to the manufacturer's instructions.

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 mg/L of denatured salmon sperm DNA in the presence of hybridization solution for 1.5–2.0 h at 42°C according to the Stratagene procedures. After hybridization, the membranes were washed in $2 \times$ SSC/0.01 g/L SDS solution at room temperature twice for 15 min each and in $0.1 \times$ SSC/0.01 g/L SDS solution at 50°C one to three times for a total of 5–15 min. The [32 P]cDNA probe/mRNA hybrids were visualized by autoradiography, and the catalase mRNA abundance was quantified by densitometric scanning of the autoradiographs (ImageQuant 3.0, Model 300A, Molecular Dynamics, Sunnyvale, CA).

Statistical analysis. Peroxisomal β -oxidation rate, FAO activity and catalase activity data were subjected to ANOVA for a completely random design with ages and nutritional states as treatments. Computations were performed with the general linear models procedure of SAS (1985). Treatment means were separated by a protected least significant difference test (Steel and Torrie 1980) using the PDIFF statement within the general linear models procedure of SAS (1985). Because no differences were found for peroxisomal β -oxidation and FAO activity between the two nutritional states at any age in heart, or for catalase activity between nutritional states at any age in all three tissues, the data were pooled at each age and reanalyzed. Means for catalase mRNA abundance were compared by *t* test. Statistical significance was declared when $P < 0.05$.

RESULTS

In liver, peroxisomal β -oxidation rate increased about two-fold in suckled pigs at 24 h after birth ($P < 0.001$) but did not change in unsuckled pigs (Fig. 1A). Therefore in 24-h-old pigs, this rate for suckled pigs was more than twice that of unsuckled pigs ($P < 0.001$). The peroxisomal β -oxidation rate increased further in 21-d-old pigs compared with 0- ($P < 0.001$) or 24-h-old pigs ($P < 0.05$). However, the rate at 5 mo was lower than that at 10 d or 21 d ($P < 0.01$). In contrast to 24-h-old pigs, the peroxisomal β -oxidation rate was higher for food-deprived pigs than suckled pigs at 10 d ($P < 0.001$) and was not affected by food deprivation at 21 d ($P > 0.05$) of age. Changes of FAO activity showed a pattern similar to that of peroxisomal β -oxidation during development and different nutritional states (Fig. 1B). Absolute rates for FAO were lower than for peroxisomal β -oxidation, probably because of the difference in assay temperatures (37°C for peroxisomal β -oxidation vs. room temperature for FAO).

In kidney, the peroxisomal β -oxidation rate also increased after birth (Fig. 2A), but not as rapidly or to the same extent as in liver. The rate did not change during the first 24 h after birth. At 10 d, the rate in suckled pigs was higher than that at 0 h ($P < 0.05$), but the rate for food-deprived pigs did not differ significantly from other ages. The rate was significantly higher at 21 d during either nutritional state than at 0 h ($P < 0.05$). However, the rate at 5 mo was intermediate and not

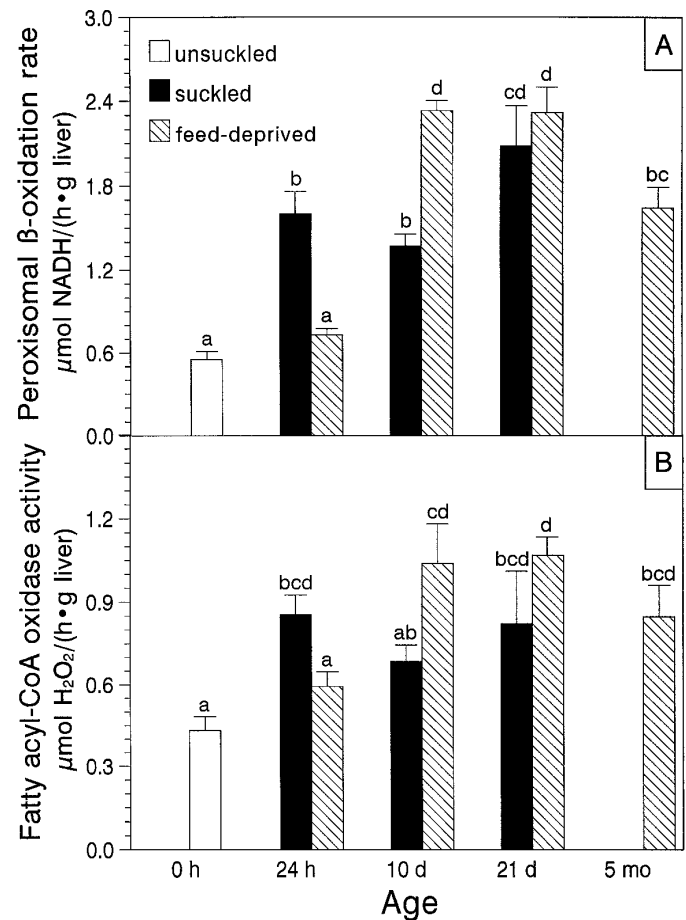


FIGURE 1 Changes of peroxisomal β -oxidation (panel A) and fatty acyl-CoA oxidase activity (panel B) in liver of pigs during development and during fed or food-deprived states. Bars represent mean \pm SEM for $n = 4$ pigs per treatment group ($n = 3$ for 5-mo-old group). Values with different letters are different, $P < 0.05$.

different from other ages. Nutritional state did not influence the peroxisomal β -oxidation rate significantly at any age.

Developmental changes of renal FAO also showed a pattern similar to that of renal peroxisomal β -oxidation (Fig. 2B). The FAO activity did not change significantly at 24 h after birth but increased significantly at 10 and 21 d of age. Nutritional state did not affect FAO activity.

In heart, neither peroxisomal β -oxidation rate nor FAO activity were affected by age or nutritional state except for a higher rate of peroxisomal β -oxidation at 10 d than at 24 h ($P < 0.05$; Fig. 3). Rates of both peroxisomal β -oxidation and FAO were approximately an order of magnitude less than corresponding rates in liver and kidney.

In contrast to activities of peroxisomal β -oxidation and FAO, developmental changes of catalase activity among these three organs showed the same pattern (Table 1). The activity did not change during the first 24 h after birth but then increased sharply at 10 d (increased 10.5-fold, 2.9-fold and 33% from 24 h to 10 d of age in liver, kidney and heart, respectively); thereafter, the activity increased more slowly until 5 mo of age. The absolute rates and the degree of increase were greatest in the liver and least in the heart (e.g., the activity was 12.5-fold greater at 0 h, but 73.5-fold greater at 5 mo in liver than in heart). Activities were similar in liver and kidney at 0 and 24 h.

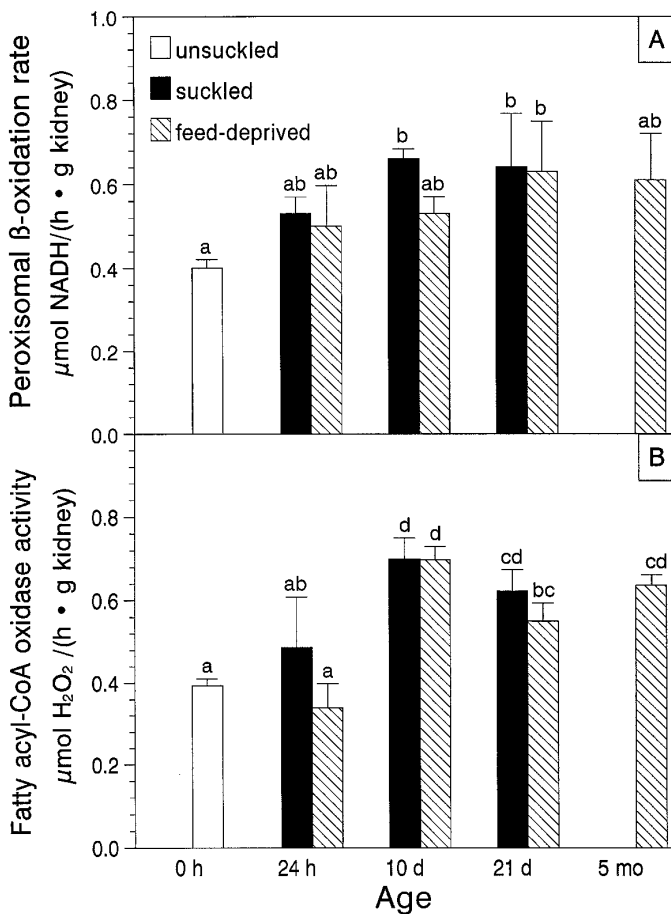


FIGURE 2 Changes of peroxisomal β -oxidation (panel A) and fatty acyl-CoA oxidase activity (panel B) in kidney of pigs during development and during fed or food-deprived states. Bars represent mean \pm SEM for $n = 4$ pigs per treatment group ($n = 3$ for 5-mo-old group). Values with different letters are different, $P < 0.05$.

Northern blot analysis showed that the abundance of catalase mRNA was only ~ 1.1 - ($P < 0.05$) and 1.3-fold ($P < 0.05$) greater at 10 d than at 24 h of age in liver and kidney, respectively, and did not differ between these two ages in heart (Figs. 4 and 5).

DISCUSSION

In this study, peroxisomal β -oxidation activity in liver, kidney and heart from pigs at different postnatal developmental stages was determined by measuring the palmitoyl-CoA-dependent, KCN-insensitive reduction of NAD^+ and by measuring FAO activity. Activities were low and relatively unchanged during development in heart. In liver and kidney, the activity of both peroxisomal β -oxidation and FAO increased rapidly during the postnatal period, especially in liver. This result is in accordance with data from a morphometric study in which the number of peroxisomes per unit of piglet liver was found to increase during the first 28 d of life (Laging et al. 1990). A rapid increase of FAO activity in kidney cortex was found previously in rats during the first month of life (Stefanini et al. 1994).

Postnatal increases of peroxisomal β -oxidation and FAO activity in hepatic and renal tissue were much more pronounced than the increases of rotenone- and antimycin-insen-

sitive β -oxidation of $[1\text{-}^{14}\text{C}]$ -palmitate measured in homogenates of the same tissues (Yu et al. 1997a). The assays used in this study measure all cycles of β -oxidation, whereas β -oxidation of $[1\text{-}^{14}\text{C}]$ -palmitate measures only the initial cycle of β -oxidation. The increases in total peroxisomal β -oxidation activity at 10 and 21 d in this study, combined with an unchanged activity of the initial cycle of β -oxidation at the same ages (Yu et al. 1997a), could indicate that peroxisomal β -oxidation was becoming more complete as pigs aged. Thus, capacities for activation of palmitate to palmitoyl-CoA and the initial peroxisomal β -oxidation cycle may have been unchanged, but capacities for subsequent cycles of β -oxidation may have been increased, which would result in greater amounts of NADH or H_2O_2 produced and detected by the methodologies used here.

In adult animals, liver, kidney cortex and cardiac muscle use fatty acids as the primary energy substrate (see Krebs 1972). About 60% of the total energy in sow's milk is in fatty acids (Girard et al. 1992), 97.5% of which are 14 carbons or longer (Lucas and Lodge 1961). Teleologically, a rapid postnatal development of peroxisomal β -oxidation in pig liver and kidney, and the presence at birth of adult-like activity in the heart, seem likely to be of substantial physiologic significance for oxidation of milk fatty acids by piglets, considering their limited mitochondrial capacity for fatty acid β -oxidation (Pégrier et al. 1983) and ketogenesis (Adams and Odle 1993, Adams et al. 1997, Odle et al. 1995). By measuring the rate of the first cycle of $[1\text{-}^{14}\text{C}]$ -palmitate β -oxidation in the presence or absence of antimycin A and rotenone, we found that the relative percentage contribution of peroxisomal β -oxidation to total β -oxidation in pig liver was much higher than that in rat liver (Yu et al. 1997b), leading to the hypothesis that a greater activity of peroxisomal β -oxidation in neonatal pigs may act as a compensatory mechanism to oxidize milk fatty acids (Yu et al. 1997a and 1997b). This speculation is further supported by the finding that hepatic peroxisomal β -oxidation increased at 24 h of age only in fed pigs and not in food-deprived pigs.

The substantial activity of peroxisomal β -oxidation detected in all three tissues immediately after birth in this study suggests that peroxisomes and the peroxisomal β -oxidation system probably begin to develop before birth in pigs. Such

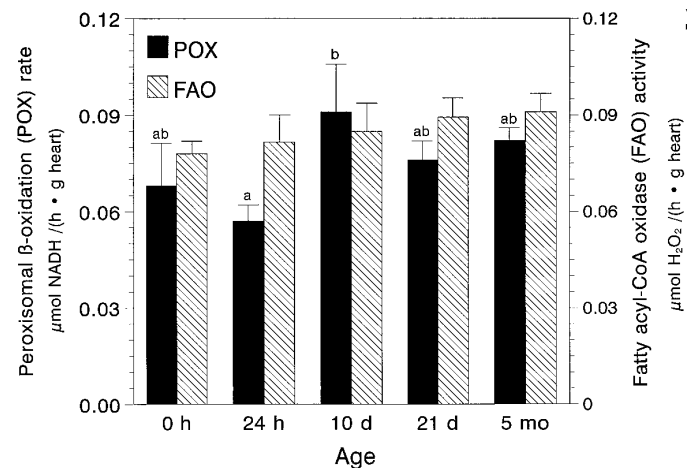


FIGURE 3 Changes of peroxisomal β -oxidation and fatty acyl-CoA oxidase activity in heart of pigs during development. Bars represent mean \pm SEM for $n = 8$ pigs per treatment group ($n = 3$ for 5-mo-old group). Values with different letters are different, $P < 0.05$.

TABLE 1

Catalase activities in liver, kidney and heart from pigs of varying ages¹

| <i>n</i> | 0 h 5 | 24 h 9 | 10 d 8 | 21 d 8 | 5 mo 3 |
|----------|---|--------------------------|---------------------------|---------------------------|---------------------------|
| | <i>mmol H₂O₂/(min · g tissue)</i> | | | | |
| Liver | 3.49 ± 0.12 ^a | 3.15 ± 0.22 ^a | 36.41 ± 0.84 ^b | 38.48 ± 0.99 ^b | 41.95 ± 1.28 ^c |
| Kidney | 3.90 ± 0.19 ^a | 4.05 ± 0.13 ^a | 15.83 ± 0.73 ^b | 18.05 ± 1.48 ^b | 26.33 ± 3.02 ^c |
| Heart | 0.28 ± 0.02 ^a | 0.33 ± 0.02 ^a | 0.44 ± 0.04 ^b | 0.44 ± 0.04 ^b | 0.57 ± 0.03 ^c |

¹ Values represent means ± SEM pooled across nutritional states at each age. Values in a row with different superscript letters are different, *P* < 0.05.

embryonic development of peroxisomal β -oxidation might be important for the metabolism of arachidonic and docosahexaenoic acids, which were found to be present in relatively large amounts in plasma of fetal piglets during late gestation (Elphick et al. 1980). Hepatic peroxisomes appeared in significant numbers late in gestation in rats (Stefanini et al. 1989) and mice (Masters and Holmes 1977), with the period of maximum increase from immediately before to 1 wk after birth (Stefanini et al. 1989).

Data for the postnatal developmental changes of peroxisomal β -oxidation in other species have been variable and

contradictory. Our data for liver, but not those for heart, agree with previous observations that peroxisomal β -oxidation, measured as oxidation of [1-¹⁴C]-long-chain fatty acids with mitochondrial β -oxidation inhibited by KCN or antimycin A and rotenone, increased from birth to weaning and then decreased in mouse liver (Périchon and Bourre 1995) and in rat liver and heart (Veerkamp and van Moerkerk 1986). However, Krahling et al. (1979) found that peroxisomal β -oxidation, measured as palmitoyl-CoA dependent O₂ uptake by isolated peroxisomes, increased only during the first 2 wk of life and then decreased. Sartori et al. (1992) found no change in the rate of peroxisomal β -oxidation in rat liver, measured as palmitoyl-CoA-dependent KCN-insensitive reduction of NAD⁺, during the first week of life except for a slight increase at d 1.

These variable results among studies for developmental changes of hepatic peroxisomal β -oxidation in rats probably have resulted, at least partially, from the different methodologies employed. Incubation of tissue preparations with [1-¹⁴C]-long-chain fatty acids under conditions of mitochondrial respiratory inhibition detects the rate of only the first cycle of peroxisomal β -oxidation rather than the overall peroxisomal β -oxidation. Therefore, if the completeness of peroxisomal β -oxidation (i.e., the extent of chain shortening) changes with age during the postnatal period, the results obtained from this

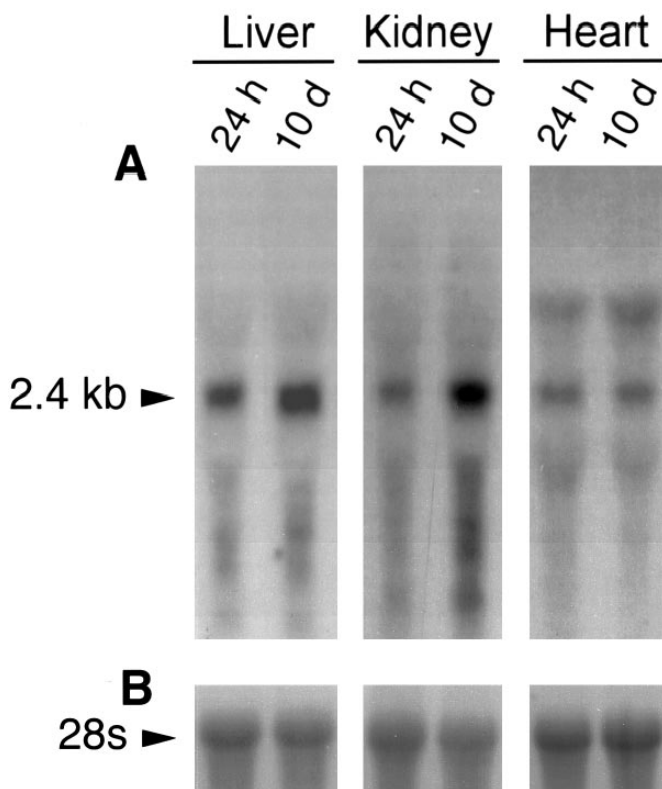


FIGURE 4 Panel A: Northern blot analysis of catalase mRNA in liver (lanes 1 and 2), kidney (lanes 3 and 4) and heart (lanes 5 and 6) from suckled pigs at 24 h (odd numbered lanes) and 10 d (even numbered lanes) of age. Panel B: the 28S RNA bands photographed from the corresponding ethidium bromide-stained agarose gels on a UV transilluminator, before transferring to a nylon filter. Densitometric scans of the 28S RNA were used to control for differences in sample loading and were used to calculate the relative abundance of catalase mRNA in each sample.

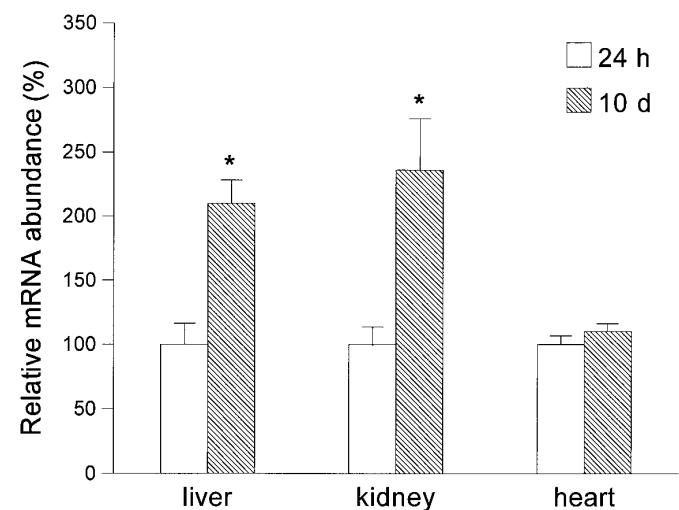


FIGURE 5 Relative changes in catalase mRNA abundance in liver (*n* = 3), kidney (*n* = 3) and heart (*n* = 4) from suckled pigs at 24 h and 10 d of age. Data are expressed as percentages of the values in the 24-h-old group for each tissue, which were normalized to 100%. Bars represent mean ± SEM. **P* < 0.05, 10 d vs. 24 h for the same tissue.

methodology will not completely reflect the developmental changes of the peroxisomal β -oxidation system.

Furthermore, different approaches used to estimate peroxisomal β -oxidation are subject to their own methodological limitations. Incomplete inhibition of electron transport chain activity, and thus mitochondrial β -oxidation, by respiratory inhibitors results in an overestimation of peroxisomal β -oxidation rate (Chu et al. 1994, Guzman et al. 1995). Inhibition of electron transport chain activity by rotenone and antimycin A in our studies (Yu et al. 1997a and 1997b), as assessed by the inhibition of $^{14}\text{CO}_2$ production, was maximized by the concentrations of mitochondrial inhibitors used and was always >95%. The existence of other intramitochondrial pathways that also can reoxidize NADH, such as the ketogenic pathway (i.e., the conversion of acetoacetate to 3-hydroxybutyrate), in the presence of mitochondrial respiratory inhibitors also could lead to overestimation of peroxisomal β -oxidation by this methodology. On the other hand, an underestimation of peroxisomal oxidation measured as substrate-dependent KCN-insensitive reduction of NAD^+ cannot be ruled out completely because of the possible inhibitory effects of intermediates and cofactors, such as that reported for CoA (Hovik and Osmundsen 1993). The activity of FAO also is subject to underestimation because of incomplete reaction of H_2O_2 with the dye or inorganic acid, attributable either to the instability of H_2O_2 or to competing reactions that use H_2O_2 (Chu et al. 1994).

At some postnatal ages, rates of peroxisomal β -oxidation estimated as antimycin- and rotenone-insensitive $[1-^{14}\text{C}]$ -palmitate oxidation (Yu et al. 1997a) were higher than the rates obtained using the methodology reported here, which should not be possible in theory. In addition, we found that accumulation of palmitoyl carboxyl-carbon in ketone bodies remained at 25–35% of control values in piglet liver homogenates exposed to antimycin A and rotenone (Yu et al. 1997a) even though the CO_2 production was inhibited by >95%. Similar results were obtained for piglet and rat liver by Adams et al. (1997), who, in addition, measured a greater rate of O_2 consumption by liver homogenates in the presence of the respiratory inhibitors than could be attributed to peroxisomal β -oxidation. Therefore, it is likely that the activity of peroxisomal β -oxidation was overestimated when using antimycin A/rotenone-insensitive oxidation of $[1-^{14}\text{C}]$ -long-chain fatty acids, especially in tissues with high ketogenesis or other intramitochondrial NAD^+/NADH recycling pathways (e.g., liver) or those with low peroxisomal β -oxidation activity (e.g., heart). The degree of such an overestimation, however, would not be expected to vary across the developmental and nutritional states studied in our experiments. Consequently, relative comparisons among ages and nutritional states within each method should be valid. Differences in developmental patterns between the isotopic approach (Yu et al. 1997a) and the present methods most likely reflect differences in the degree of completeness (or chain shortening) of peroxisomal β -oxidation.

A striking finding in this study was that food intake is required for the initial induction of hepatic peroxisomal β -oxidation. Pigs not fed during the 24 h after birth (24-h-old unsuckled pigs) had lower hepatic activities of peroxisomal β -oxidation and FAO than their suckled counterparts. Similar findings were reported earlier (Yu et al. 1997a). Differences between suckled and unsuckled 24-h-old pigs for peroxisomal β -oxidation in liver did not appear to be attributable to differences in liver size, because relative liver weights were similar (2.6 and 2.7% of body weight for suckled and unsuckled pigs,

respectively). How long this initial induction could be prevented by undernutrition is an unanswered question. In contrast, 24 h of food deprivation increased peroxisomal β -oxidation and FAO activities in 10-d-old pigs but had no significant effect in 21-d-old pigs. Food deprivation did not change renal and cardiac peroxisomal β -oxidation and FAO activity at any age. These findings agree with the radioisotopic data from this experiment reported previously (Yu et al. 1997a).

As discussed earlier (Yu et al. 1997a), differential responses to food deprivation among 24-h-old, 10-d-old and 21-d-old pigs may relate to the availability of long-chain fatty acids from milk or from the increased stores of body fat available for mobilization in older pigs. In rodents, high fat diets as well as physiologic or pathophysiologic conditions characterized by greatly increased availability of long-chain fatty acids stimulated peroxisomal proliferation and increased activities of peroxisomal, mitochondrial and microsomal systems for fatty acid metabolism (Bieri 1993, Schoonjans et al. 1996). These effects are believed to be caused by interaction of fatty acids or their metabolites with peroxisomal proliferator-activated receptors, which in turn interact with response elements of genes encoding enzymes of fatty acid metabolism (Schoonjans et al. 1996). Thus fatty acids from porcine milk are the most likely nutrients involved in the early proliferation of peroxisomal β -oxidation in suckled 24-h-old pigs. Similarly, greater body fat in 10-d-old pigs results in large increases in mobilization of fatty acids during food deprivation; these mobilized nonesterified fatty acids may serve as the ligand to increase activities of peroxisomal β -oxidation (Yu et al. 1997a). At 21 d, the duration of food deprivation may not have been sufficient to increase fatty acid mobilization to the extent necessary to induce peroxisomal β -oxidation (Yu et al. 1997a).

Food deprivation did not change activities of peroxisomal β -oxidation or FAO in kidney or heart at any age. The tissue-specific patterns of peroxisomal β -oxidation and FAO activity during development and food deprivation likely reflect differences in concentrations of peroxisome proliferator-activated receptors, different isoforms of these receptors or tissue-specific differences in the response elements of the genes encoding enzymes of fatty acid metabolism (Schoonjans et al. 1996).

We found that the developmental pattern of catalase activity was the same among liver, kidney and heart but was markedly different from that of peroxisomal β -oxidation or FAO activity. Catalase is involved in peroxisomal β -oxidation of fatty acids by degrading the H_2O_2 generated from the first oxidation step, catalyzed by FAO. Inhibition of peroxisomal β -oxidation by inhibiting catalase led to widespread variation in the turnover characteristics of tissue lipids and, in particular, decreased the oxidation of fatty acids (Masters and Crane 1984). However, peroxisomal H_2O_2 can be generated not only by FAO, but also by *L*- α -hydroxy acid oxidase, *D*-amino acid oxidases, urate oxidase, polyamine oxidase, bile acyl-CoA oxidase and *L*-pipecolate oxidase (Vamecq et al. 1993). In addition, peroxisomes are not the only site of H_2O_2 production and of catalase activity in aerobic cells; catalase also is present in appreciable amounts in the endoplasmic reticulum, mitochondria and cytosol (Chance et al. 1979). Furthermore, the activity of catalase is much higher than that of peroxisomal β -oxidation, although the detoxification of H_2O_2 can involve not only catalase but also glutathione peroxidase and superoxide dismutase (Vamecq et al. 1993). Therefore it is not surprising that the developmental changes of catalase activity were different from those of peroxisomal β -oxidation in semipurified peroxisomal fractions.

Catalase activity was highest in pig liver, intermediate in

kidney and lowest in heart; for all tissues, activity was low during the 24 h after birth but increased substantially during the first 10 d of life. Others also have reported that liver catalase activity was about three to four times that of the kidney (Schisler and Singh 1991) and 40 times greater than that of heart in young mice (Feinstein 1970). Catalase activity was very low in mice at birth but increased greatly with age, especially during the first 3 wk of life (Schisler and Singh 1987).

The mechanism for the rapid increase of catalase activity during development has not been identified clearly. We found that the abundance of catalase mRNA in liver and kidney increased from 24 h to 10 d, but the magnitude of the increase was much smaller than that of the increase of catalase activity. Therefore the increased catalase activity at 10 d in these tissues must have resulted not only from the greater amount of mRNA for translation, but also from an increased efficiency of translation, an increased stability of the protein or post-translational modifications. Additional study of developmental changes of catalase between 24 h and 10 d of age should be undertaken to determine more closely when the increases of activity occur. Such study also should attempt to quantify developmental patterns of catalase found in peroxisomes and non-peroxisomal compartments.

The modulating factors for the developmental change in catalase activity might include increased blood oxygen levels with aerobic respiration after birth (Kellog and Fridovich 1976), increased aerobic metabolism (Booth et al. 1980), increased production of mitochondrial (Nohl et al. 1979) and peroxisomal H_2O_2 substrate and, in pigs, the exogenous supplementation of iron after birth, which is a component of the enzyme. Masters and Denis (1995) pointed out that there are different sensitivities and vulnerabilities to oxidative processes among tissues. Hence, different activities of catalase among liver, kidney and heart of pigs may not only reflect their individual H_2O_2 production and antioxidative capacity but also may reflect their different sensitivities and vulnerabilities to H_2O_2 .

In conclusion, results reported here demonstrate that total peroxisomal β -oxidation activity develops rapidly in liver and kidney, but not in heart, during postnatal development in pigs. Food intake is required for the initial induction of this activity in liver, but not in kidney or heart. These changes in liver are consistent with the hypothesis that peroxisomal β -oxidation plays an important role in the metabolism of milk-derived fatty acids in piglets. Food deprivation at older ages affects hepatic peroxisomal β -oxidation, but not that in kidney or heart, with the net effect likely dependent on the extent of body fat mobilization caused by food deprivation. Greater developmental increases in total peroxisomal β -oxidation activity than in first-cycle peroxisomal β -oxidation (Yu et al. 1997a) suggest that peroxisomal β -oxidation may become more complete with increasing age of piglets. Catalase activity in pig tissues increases markedly between 24 h and 10 d of age, which was a factor of both pretranslational and post-translational regulation.

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LITERATURE CITED

- Adams, S. H., Lin, X., Yu, X. X., Odle, J. & Drackley, J. K. (1997) Hepatic fatty acid metabolism in pigs and rats: major differences in endproducts, O_2 uptake and β -oxidation. *Am. J. Physiol.* 272: R1641–R1646.
- Adams, S. H. & Odle, J. (1993) Plasma β -hydroxybutyrate after octanoate challenge: attenuated ketogenic capacity in neonatal swine. *Am. J. Physiol.* 265: R761–R765.
- Aebi, H. (1974) UV-assay of catalase. In: *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), vol. 2, pp. 673–678. Verlag Chemie, Weinheim, Germany.
- Bieri, F. (1993) Peroxisome proliferators and cellular signaling pathways. *A review. Biol. Cell* 77: 43–46.
- Booth, R.F.G., Patel, T. B. & Clark, J. B. (1980) The development of enzymes of energy metabolism in the brain of a precocial (guinea pig) and non-precocial (rat) species. *J. Neurochem.* 34: 17–25.
- Chance, B., Sies, H. & Boveris, A. (1979) Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* 59: 527–606.
- Chu, C. H., Mao, L.-F. & Schulz, H. (1994) Estimation of peroxisomal β -oxidation in rat heart by a direct assay of acyl-CoA oxidase. *Biochem. J.* 302: 23–29.
- Elphick, M. C., Flecknell, P., Hull, D. & McFadyen, I. R. (1980) Plasma free fatty acid umbilical venous-arterial concentration differences and placental transfer of [^{14}C]palmitic acid in pigs. *J. Dev. Physiol.* 2: 347–356.
- Feinstein, R. N. (1970) A catalasemia in the mouse and other species. *Biochem. Genet.* 4: 135–155.
- Girard, J., Ferré, P., Pégorier, J. P. & Duée, P. H. (1992) Adaptations of glucose and fatty acid metabolism during the perinatal period and suckling-weaning transition. *Physiol. Rev.* 72: 507–562.
- Goglia, F., Liverini, G., Lanni, A., Iossa, S. & Barletta, A. (1989) Morphological and functional modifications of rat liver peroxisomal subpopulations during cold exposure. *Exp. Biol.* 48: 127–133.
- Guzman, M., Bijleveld, C. & Geelen, M.J.H. (1995) Flexibility of zonation of fatty acid oxidation in rat liver. *Biochem. J.* 311: 853–860.
- Holmes, R. S. (1971) Ontogeny of mouse liver peroxisomes and catalase isozymes. *Nat. New Biol.* 232: 218–220.
- Hovik, R. & Osmundsen, H. (1993) Factors which affect the activity of purified rat liver acyl-CoA oxidase. *Biochem. J.* 290: 97–102.
- Kellog, E. W. & Fridovich, I. (1976) Superoxide dismutase in rat and mouse as a function of age and longevity. *J. Gerontol.* 31: 405–408.
- Krahling, J. B., Gee, R., Gauger, J. A. & Tolbert, N. E. (1979) Postnatal development of peroxisomal and mitochondrial enzymes in rat liver. *J. Cell. Physiol.* 101: 375–390.
- Krebs, H. A. (1972) Some aspects of the regulation of fuel supply in omnivorous animals. *Adv. Enz. Reg.* 10: 397–420.
- Kvannes, J., Eikhom, T. S. & Flatmark, T. (1994) The peroxisomal β -oxidation enzyme system of rat heart. Basal level and effect of the peroxisome proliferator clofibrate. *Biochim. Biophys. Acta* 1201: 203–216.
- Laging, C., Pospischil, A. & Diessecke, D. (1990) Elektronenmikroskopisch morphometrische untersuchung der entwicklung von peroxisomen bei gesunden kerkeln in den ersten 4 lebenswochen [Ultrastructural morphometric investigation of peroxisome development in piglets during the first 4 weeks of life]. *J. Vet. Med.* A37: 186–197.
- Lazarow, P. B. (1981) Assay of peroxisomal β -oxidation of fatty acids. *Meth. Enzymol.* 72: 315–317.
- Lucas, I.A.M. & Lodge, G. A. (1961) The nutrition of the young pig. A review. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England.
- Masters, C. & Crane, D. (1984) The role of peroxisomes in lipid metabolism. *Trends Biochem. Sci.* 9: 314–317.
- Masters, C. & Holmes, R. (1977) Peroxisomes: new aspects of cell physiology and biochemistry. *Physiol. Rev.* 57: 816–882.
- Masters, C. J. & Denis, I. C. (1995) On the role of the peroxisome in ontogeny, ageing and degenerative disease. *Mech. Ageing Dev.* 80: 69–83.
- Nohl, H., Hegner, D. & Summer, K. H. (1979) Responses of mitochondrial superoxide dismutase, catalase and glutathione peroxidase activities to aging. *Mech. Ageing Dev.* 11: 145–151.
- Novak, R., Matkovic, B., Varga, I. & Szabo, L. (1978) Comparison of peroxide metabolism enzymes in the organs of congenic mice. *Comp. Biochem. Physiol.* 60B: 149–152.
- Odle, J., Lin, X., Van Kempen, T.A.T.G., Drackley, J. K. & Adams, S. H. (1995) Carnitine palmitoyltransferase modulation of hepatic fatty acid metabolism and radio-HPLC evidence for low ketogenesis in neonatal pigs. *J. Nutr.* 125: 2541–2549.
- Osmundsen, H., Bremer, J. & Pedersen, J. I. (1991) Metabolic aspects of peroxisomal β -oxidation. *Biochim. Biophys. Acta* 1085: 141–158.
- Pégorier, J. P., Duée, P.-H., Girard, J. & Peret, J. (1983) 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.
- Périchon, R. & Bourre, J. M. (1995) Peroxisomal β -oxidation activity and catalase activity during development and aging in mouse liver. *Biochimie* 77: 288–293.
- Reubsæet, F.A.G., Veerkamp, J. H., Bukkens, S.G.F., Trijbels, J.M.F. & Monnens, L.A.H. (1988) Acyl-CoA oxidase activity and peroxisomal fatty acid oxidation in rat tissues. *Biochim. Biophys. Acta* 958: 434–442.
- Sartori, C., Stefanini, S., Cimini, A., Giulio, A. D. & Cerù, M. P. (1992) Liver peroxisomes in newborns from clofibrate-treated rats. II. A biochemical study of the recovery period. *Biol. Cell* 74: 315–324.
- SAS Institute Inc. (1985) SAS User's Guide: Statistics. SAS Institute, Cary, NC.
- Schisler, N. J. & Singh, S. M. (1987) Inheritance and expression of tissue-spe-

- cific catalase activity during development and aging in mice. *Genome* 29: 748–760.
- Schisler, N. J. & Singh, S. M. (1991) A quantitative genetic analysis of tissue-specific catalase activity in *Mus musculus*. *Biochem. Genet.* 29: 65–89.
- Schoonjans, K., Staels, B. & Auwerx, J. (1996) 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.
- Singh, I., Kremser, K., Ghosh, B., Singh, A. K. & Pai, S. (1996) Abnormality in translational regulation of catalase expression in disorders of peroxisomal biogenesis. *J. Neurochem.* 67: 2372–2378.
- Steel, R.G.D. & Torrie, J. H. (1980) *Principles and Procedures of Statistics*. McGraw-Hill, New York, NY.
- Stefanini, S., Mauriello, A., Farrace, M. G., Cibelli, A. & Cerù, M. P. (1989) Proliferative response of foetal liver peroxisomes to clofibrate treatment of pregnant rats. A quantitative evaluation. *Biol. Cell* 67: 299–305.
- Stefanini, S., Serafini, B., Cimini, A. & Sartori, C. (1994) Differentiation of kidney cortex peroxisomes in fetal and newborn rats. *Biol. Cell* 82: 185–193.
- Vamecq, J. (1990) Fluorometric assay of peroxisomal oxidases. *Anal. Biochem.* 186: 340–249.
- Vamecq, J. & Draye, J.-P. (1987) Interaction between the ω - and β -oxidation of fatty acids. *J. Biochem.* 102: 225–234.
- Vamecq, J., Vallée, L., Fontaine, M., Nuyts, J.-P., Lambert, D. & Poupaert, J. (1993) Preliminary studies about novel strategies to reverse chemoresistance to adriamycin regarding glutathione metabolism, peroxisomal and extraperoxisomal hydroperoxide and valproic acid metabolic pathways. *Biol. Cell* 77: 17–26.
- Veerkamp, J. H. & van Moerkerk, H.T.B. (1986) 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.
- Yu, X. X., Drackley, J. K. & Odle, J. (1997a) Rates of mitochondrial and peroxisomal β -oxidation of palmitate change during postnatal development and food deprivation in liver, kidney and heart of pigs. *J. Nutr.* 127: 1814–1821.
- Yu, X. X., Drackley, J. K., Odle, J. & Lin, X. (1997b) Response of hepatic mitochondrial and peroxisomal β -oxidation to increasing palmitate concentrations in piglets. *Biol. Neonate* 72: 284–292.