

## Rates of Mitochondrial and Peroxisomal $\beta$ -Oxidation of Palmitate Change during Postnatal Development and Food Deprivation in Liver, Kidney and Heart of Pigs<sup>1,2,3</sup>

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**ABSTRACT** We measured total, mitochondrial and peroxisomal capacities for  $\beta$ -oxidation of [1-<sup>14</sup>C]palmitate in homogenates of liver, kidney and heart from pigs within 0.5 h after birth (0 h, unfed) and at 24 h (suckled or unfed), 10 d (suckled or 24-h food-deprived), 21 d (suckled or 24-h food-deprived) and 5 mo (overnight food-deprived) of age. Assays were conducted in the absence (total  $\beta$ -oxidation) or presence (peroxisomal  $\beta$ -oxidation) of antimycin A and rotenone. Mitochondrial  $\beta$ -oxidation was calculated as total minus peroxisomal  $\beta$ -oxidation. Acid-soluble products (ASP) from incubation of tissue homogenates from 24-h-old unfed pigs with [1-<sup>14</sup>C]palmitate were analyzed by radio-HPLC. Total and mitochondrial  $\beta$ -oxidation capacities were greater ( $P < 0.05$ ) at 24 h after birth in liver, and at 10 d in kidney and heart, than at 0 or 24 h. Peroxisomal  $\beta$ -oxidation capacity was increased ( $P < 0.05$ ) at 24 h after birth in liver and at 10 and 21 d in heart; in kidney, the capacity was higher during the preweaning period than in adults. Across ages, peroxisomal  $\beta$ -oxidation capacity represented 37 to 51%, 28 to 41%, and 26 to 31% of total  $\beta$ -oxidation capacity in liver, kidney, and heart, respectively. Food deprivation increased hepatic total  $\beta$ -oxidation at 10 d and decreased peroxisomal  $\beta$ -oxidation at 24 h but had no effect in kidney and heart. Regardless of the presence of respiratory inhibitors, 32%, 31 to 40%, and 45 to 50% of palmitate carboxyl carbon in acid-soluble products was accumulated in acetate in liver, kidney, and heart, respectively. We suggest that a high percentage contribution of peroxisomal  $\beta$ -oxidation may act as a compensatory mechanism for piglets to oxidize milk fatty acids during postnatal development. Furthermore, acetogenesis may be an important fate of acetyl-CoA from  $\beta$ -oxidation of fatty acids in various piglet tissues. *J. Nutr.* 127: 1814–1821, 1997

**KEY WORDS:** • piglets •  $\beta$ -oxidation • fatty acid • development • peroxisomes

Successful adaptation of newborn pigs to the dramatic changes of nutrition and ambient temperature after birth requires rapid modifications of energy metabolism. The change from carbohydrate fuel in utero to a high fat and low carbohydrate nutrient source (i.e., colostrum and milk) after birth

requires an increased capacity for fatty acid oxidation. Understanding these developmental changes and their mechanisms is important for improving neonatal survival.

Several studies have documented rapid increases of  $\beta$ -oxidation in pig liver during early postnatal development. Mersmann et al. (1972) observed that hepatic mitochondrial capacities for oxidation and phosphorylation increased markedly 2 d after birth in pigs. The rate of oxidation of palmitate to CO<sub>2</sub> or acid-soluble products (ASP)<sup>7</sup> by homogenates of liver from newborn pigs was low, but increased fourfold by d 7 before declining to the lowest rate by d 50 (Mersmann and Phinney 1973). Odle et al. (1991) also observed developmental increases in hepatic fatty acid oxidation during the first 48 h of life in both low-birth-weight and normal-birth-weight pigs. Evidence also indicates that the capacity for mitochondrial  $\beta$ -oxidation of long-chain fatty acids is limited in piglets (Adams and Odle 1993, Pégrier et al. 1983).

In contrast to data for the liver, data for postnatal changes of  $\beta$ -oxidation in other organs of pigs are limited and inconclusive. Oxidation of palmitate by heart homogenates was unchanged in pre-weaned pigs but declined somewhat by d 50 (Mersmann and Phinney 1973). Wolfe et al. (1978) found that the rate of palmitate oxidation increased with age in liver

<sup>1</sup> Portions of this work were presented in abstract form at Experimental Biology 95, Atlanta, GA [Yu, X. X., Lin, X., Drackley, J. K. & Odle, J. (1995) Fatty acid oxidation in pig liver and kidney during postnatal development: the role of peroxisomes. *FASEB J.* 9: A553 (abs.)] and at the 1995 International Symposium on Swine in Biomedical Research, College Park, MD [Yu, X. X., Drackley, J. K. & Odle, J. (1995) Developmental changes of palmitate oxidation capacity in pig heart, liver and kidney. In: *Proc. Int. Symp. Swine in Biomed. Res.*, p. 131, University of Maryland, College Park, MD (abs.)].

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<sup>7</sup> Abbreviations used: ASP, acid-soluble products; BSA, bovine serum albumin; CPT, carnitine palmitoyltransferase; Mito, mitochondrial  $\beta$ -oxidation; Perox, peroxisomal  $\beta$ -oxidation.

and kidney from neonatal pigs, reaching a maximum at 7 d in liver and at 21 d in kidney, but palmitate oxidation in the heart tended to decrease with age. However, Ascuitto et al. (1989) found that the palmitate oxidation rate ( $\text{CO}_2$  production) in the isolated perfused piglet heart increased by 61 to 68% at an age of 9.5 d compared with rates at 0.6 or 3.3 d of age.

Peroxisomal  $\beta$ -oxidation is an alternate pathway of metabolism of fatty acids that has not been characterized in swine. Peroxisomal  $\beta$ -oxidation is present in a variety of tissues, including liver, kidney, heart, intestinal mucosa and skeletal muscle of many species (for reviews, see Osmundsen et al. 1991, Van den Bosch et al. 1992). Peroxisomes can  $\beta$ -oxidize medium- and long-chain fatty acids, as well as very-long-chain fatty acids that are poor substrates for mitochondrial  $\beta$ -oxidation (Osmundsen et al. 1991, Van den Bosch et al. 1992). In addition, peroxisomal  $\beta$ -oxidation may play a role in thermogenesis (Goglia et al. 1989) because the first oxidation step catalyzed by fatty acyl-CoA oxidase (EC 1.3.99.3) is not coupled to ATP production and the energy is released as heat.

The ontogeny of peroxisomal  $\beta$ -oxidation in neonatal mammals has received little attention. Hepatic peroxisomes in rats (Tsukada et al. 1968) and mice (Masters and Holmes 1977) were essentially absent until the late fetal stage but increased rapidly after birth, reaching adult levels by d 6 of age. Peroxisomal  $\beta$ -oxidation in rat liver and heart, measured as antimycin-insensitive [ $1\text{-}^{14}\text{C}$ ]palmitate oxidation per gram of tissue, increased from birth to weaning and then decreased (Veerkamp and van Moerkerk 1986). Horie et al. (1981) measured a 2.5-fold increase in hepatic palmitoyl-CoA oxidase activity from 1 d before birth to 1 d after birth; rates then gradually decreased until d 7 of age. Krahling et al. (1979) determined that fatty acid oxidation in peroxisomes and mitochondria of rat liver reached maximum activities (per gram of liver) between 1 and 2 wk of age and then decreased to adult levels at 2 wk of age. Peroxisomal  $\beta$ -oxidation represented 10% of the total  $\beta$ -oxidation in "young" rats but increased to 30% for adult females and 20% for adult males (Krahling et al. 1979).

Developmental changes and roles of peroxisomal  $\beta$ -oxidation, as well as the effect of nutritional state on peroxisomal  $\beta$ -oxidation, are unknown for pigs. A morphometric study by electron microscopy reported that the number of peroxisomes per unit of piglet liver increased during the first 28 d of life (Laging et al. 1990). Porcine milk fat constitutes about 60% of the total energy in milk and is composed almost entirely of fatty acids with chain lengths of 14 carbon atoms or greater (Jenness 1985) that are good substrates for peroxisomal  $\beta$ -oxidation (Osmundsen et al. 1991).

Because of the large demand for energy after birth, the abundant supply of long-chain fatty acids from milk and the limited capacity for ketogenesis in neonatal pigs, we hypothesized that peroxisomal  $\beta$ -oxidation of fatty acids might be an important metabolic adaptation in neonatal pigs. We recently obtained evidence indicating that peroxisomal  $\beta$ -oxidation represents a greater proportion of total oxidation in piglets than in adult rats (Drackley et al. 1995, Yu et al. 1997). In the present study, we measured capacities for the total, mitochondrial and peroxisomal  $\beta$ -oxidation of palmitate in homogenates of liver, kidney and heart from pigs at different postnatal ages. Comparisons also were made between fed and unfed states. In addition, the ASP generated from  $\beta$ -oxidation of palmitate were identified and quantified in liver, kidney and heart from newborn pigs, because previous work in our laboratory has demonstrated that ketogenesis is limited in piglets but that acetogenesis may be a major fate of acetyl-CoA,

at least in liver (Lin et al. 1996, Odle et al. 1995). These are the first data to describe developmental and nutritional changes in peroxisomal  $\beta$ -oxidation and its potential contribution to total  $\beta$ -oxidation capacity in swine.

## MATERIALS AND METHODS

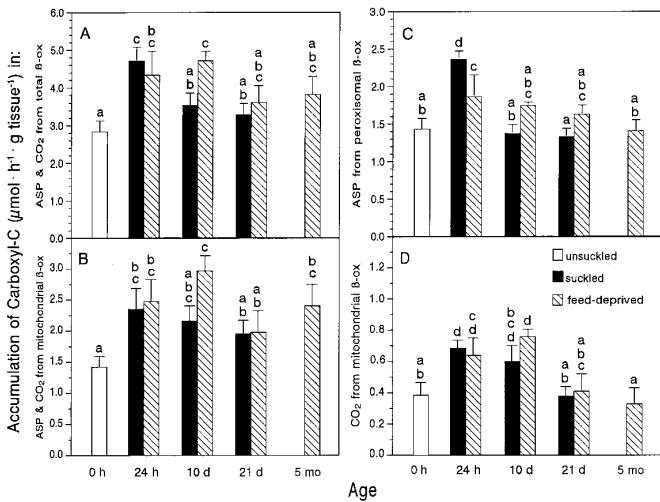
**Reagents.** Antimycin A, ATP, bovine serum albumin (BSA), L-carnitine HCl, coenzyme A, EDTA, malate, NAD, Na palmitate, rotenone, HEPES, D-mannitol and Tris-HCl were purchased from Sigma Chemical (St. Louis, MO). Sucrose was purchased from Mallinckrodt (St. Louis, MO). [ $1\text{-}^{14}\text{C}$ ]Palmitate was purchased from American Radiolabeled Chemicals (St. Louis, MO). Scintillation fluid (Scinti Verse II) was purchased from Fisher Scientific (St. Louis, MO). Phosphoric acid for HPLC work was purchased from Fisher Scientific (Fair Lawn, NJ). All other chemicals were of reagent or higher grade.

**Animals and collection of tissue samples.** Procedures for this study were reviewed and approved by the Laboratory Animal Care Advisory Committee of the University of Illinois. Commercial cross-bred pigs of normal body weight were obtained from the University of Illinois Swine Farms within 0.5 h after birth (0 h, unsuckled) and at 24 h (suckled or 24-h unfed), 10 d (suckled or 24-h food-deprived), 21 d (just before weaning; suckled or 24-h food-deprived) and 5 mo (adults, overnight food-deprived) of age. The animals were anesthetized with pentobarbital at a dose of about 25 mg/kg body wt 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 containing 220 mmol/L mannitol, 70 mmol/L sucrose, 2 mmol/L HEPES and 0.1 mmol/L EDTA (pH 7.2). After washing off the blood, a portion of the tissues was blotted, minced and homogenized manually in 10 (for liver and kidney) or 5 (for heart) volumes of the same ice-cold buffer using two strokes of a Potter-Elvehjem homogenizer for each sample. Homogenates were used immediately in incubations to measure palmitate oxidation rates.

**Measurement of fatty acid oxidation.** Incubations to measure the rates of total and peroxisomal  $\beta$ -oxidation of [ $1\text{-}^{14}\text{C}$ ]palmitate were performed using the methods of Grum et al. (1994) as modified (Yu et al. 1997). Briefly, aliquots of homogenate (300  $\mu\text{L}$ ) were added to 25-mL Erlenmeyer flasks that contained incubation buffer (Yu et al. 1997) at pH 7.4. The substrate was 1.0 mmol/L [ $1\text{-}^{14}\text{C}$ ]palmitate ( $\sim 46$  kBq/ $\mu\text{mol}$ ), which was complexed with BSA in a 5:1 molar ratio. Some flasks also contained antimycin A (50  $\mu\text{mol/L}$  final concentration) and rotenone (10  $\mu\text{mol/L}$  final concentration) to inhibit mitochondrial  $\beta$ -oxidation. Homogenates were incubated in a total volume of 2.0 mL for 30 min at 37°C in a shaking water bath. Incubations were terminated by addition of 1 mL of 3 mol/L  $\text{HClO}_4$  to the medium, and 0.1 mL of 30% NaOH was injected into a hanging well containing a folded filter paper to collect  $^{14}\text{CO}_2$ . After termination, flasks were shaken continuously for 2 h to ensure quantitative collection of  $^{14}\text{CO}_2$ . Blanks were prepared by immediate acidification of flasks after addition of homogenate. Radioactivity in  $\text{CO}_2$  and ASP was quantified by liquid scintillation as described (Yu et al. 1997).

The rate of total first-cycle  $\beta$ -oxidation of palmitate was calculated as the rate of accumulation of palmitate carboxyl carbon in ASP plus  $\text{CO}_2$  following incubation without respiratory inhibitors. The rate of the initial cycle of peroxisomal  $\beta$ -oxidation was assumed to be the rate of accumulation of palmitate carboxyl carbon in ASP following incubation of homogenate with antimycin A and rotenone. This rate was corrected by the ratio of  $^{14}\text{CO}_2$  production during inhibition to the total  $^{14}\text{CO}_2$  production without inhibitors. Negligible amounts of  $^{14}\text{CO}_2$  were produced in the presence of antimycin A and rotenone for all tissues and ages. The difference between total  $\beta$ -oxidation and peroxisomal  $\beta$ -oxidation was considered to be mitochondrial  $\beta$ -oxidation. Concentrations of palmitate and cofactors were shown previously (Yu et al. 1997) to maximize both mitochondrial  $\beta$ -oxidation rate (mitochondrial  $\beta$ -oxidation capacity) and peroxisomal  $\beta$ -oxidation rate (peroxisomal  $\beta$ -oxidation capacity).

**Radio-HPLC analysis of acid-soluble products.** Liver homogenates from 24-h-old unfed pigs were also incubated as described above with different concentrations of [ $1\text{-}^{14}\text{C}$ ]palmitate (0.2, 0.5 and 1.0



**FIGURE 1** Liver metabolism: Total (panel A), mitochondrial (panel B) and peroxisomal (panel C) capacities for [ $1\text{-}^{14}\text{C}$ ]palmitate  $\beta$ -oxidation ( $\beta$ -ox) and rate of  $\text{CO}_2$  production from [ $1\text{-}^{14}\text{C}$ ]palmitate (panel D) in pig liver during development and during fed or food-deprived states. Rates were calculated as the accumulation of carboxyl carbon in  $\text{CO}_2$ , acid-soluble products (ASP) or both after incubation of liver homogenate for 30 min in the absence or presence of antimycin A and rotenone (see text for details). Bars represent means  $\pm$  SEM for  $n = 4$  pigs per treatment group ( $n = 3$  for adult pig group). Values with different letters above the bars are different ( $P < 0.05$ ).

mmol/L). Kidney and heart homogenates from these pigs were incubated as described above with 1.0 mmol/L [ $1\text{-}^{14}\text{C}$ ]palmitate. The specific radioactivity in these incubations was maintained at  $\sim 46$  kBq/ $\mu\text{mol}$ . Acidified media were centrifuged for 10 min at  $700 \times g$  to pellet the residual substrate, denatured BSA, and other proteins. The supernatants were subjected to reversed-phase ion-pairing HPLC (Beckman System Gold, Beckman Instruments, San Ramon, CA) connected to an in-line  $\beta$ -radiochromatography detector (Radiomatics Flo-one/Beta Series, model A-265, Packard Instruments, Meridian, CT) to identify the radioactive peaks associated with acetate and ketone bodies (Lin et al. 1996). The organic acids and ketone bodies were separated using a mobile phase of 0.3%  $\text{H}_3\text{PO}_4$  (pH 2.1, 0.65 mL/min) with a Beckman Ultrasphere IP column ( $5 \mu\text{m}$ ,  $4.6 \times 250$  mm), and volumes of eluent with retention times corresponding to radioactive peaks of interest were retrieved by a fraction collector. Radioactivity in these samples was quantified using liquid scintillation.

**Statistical analyses.** Data for  $\beta$ -oxidation capacities at different ages and nutritional states were subjected to ANOVA for a completely random design. Means for treatment groups were separated by multiple  $t$  tests (Steel and Torrie 1980) using the PDIF statement within the general linear models procedure of SAS (1985). Data for production of acetate and ketone bodies from [ $1\text{-}^{14}\text{C}$ ]palmitate by homogenates of liver from 24-h-old pigs were subjected to ANOVA for a split-plot design, with palmitate concentrations as the main plot and antimycin-rottenone treatment as the subplot. Data for production of acetate from [ $1\text{-}^{14}\text{C}$ ]palmitate by homogenates of liver, kidney and heart from 24-h-old pigs were subjected to ANOVA for a split-plot design, with tissues as the main plot and antimycin-rottenone treatment as the subplot. All computations were made using the general linear models procedure of SAS (1985). Values presented are means  $\pm$  SEM or pooled SEM as noted in legends. Differences were declared statistically significant when  $P < 0.05$ .

## RESULTS

**Palmitate  $\beta$ -oxidation capacities.** In liver, total, mitochondrial and peroxisomal  $\beta$ -oxidation capacities and  $^{14}\text{CO}_2$  production rate were greater in either suckled or unfed 24-h-old pigs than in 0-h-old pigs (Fig. 1). These variables returned

to 0-h rates in 10-d-old and older pigs, except for total and mitochondrial  $\beta$ -oxidation and  $^{14}\text{CO}_2$  production in 10-d-old food-deprived pigs. Thus, total, mitochondrial and peroxisomal  $\beta$ -oxidation capacities did not differ among the three older groups (d 10, d 21 and 5 mo) except for greater mitochondrial  $\beta$ -oxidation for 10-d-old than 21-d-old pigs during food deprivation. However, from 10 d to 5 mo, the  $^{14}\text{CO}_2$  production rate decreased gradually, which caused the ratio of  $^{14}\text{CO}_2$  production to mitochondrial oxidation ( $\text{CO}_2/\text{Mito}$ ; panel D/panel B, Fig. 1) to decrease by 28% for suckled pigs and 23% for food-deprived pigs at 21 d of age, and by 49 to 52% for 5-mo-old pigs when compared with those at 10 d (Table 1). Across all ages, the  $\text{CO}_2/\text{Mito}$  ratio was less than 0.31 in liver, indicating that most of the carboxyl carbon of palmitate accumulated in ASP.

Peroxisomal  $\beta$ -oxidation capacity was lower ( $P < 0.05$ ) in liver from 24-h-old unfed pigs than from 24-h-old suckled pigs, but mitochondrial  $\beta$ -oxidation capacity did not differ between nutritional states at 24 h of age (Fig. 1B, C). Food deprivation increased total  $\beta$ -oxidation capacity at 10 d but not at 21 d (Fig. 1A). The ratio of peroxisomal  $\beta$ -oxidation capacity to total  $\beta$ -oxidation capacity (Perox/Total; panel C/panel A, Fig. 1) in 0-h and 24-h-old suckled pigs was higher than that in other groups, except for that in 21-d-old food-deprived pigs (Table 1). The Perox/Total ratio ranged from 0.37 to 0.51 in liver across all ages.

In the kidney, total and mitochondrial  $\beta$ -oxidation capacities and  $^{14}\text{CO}_2$  production rate (Fig. 2A, B and D) showed similar developmental patterns; however, no changes were observed between 0 h and 24 h. At 10 d, these rates increased dramatically ( $P < 0.001$ ); at 21 d, rates tended to decrease but still were higher than those at 0 or 24 h ( $P < 0.01$ ). In 5-mo-old pigs, these rates had decreased to values comparable to rates at 0 h. Peroxisomal  $\beta$ -oxidation capacity did not change from birth to 21 d of age but generally was higher during the preweaning period than at 5 mo (Fig. 2C). Similar to the ratio in the liver, the ratio of Perox/Total in the kidney (Table 1) did not change at 24 h after birth, but then decreased at other ages compared with 0 or 24 h ( $P < 0.01$ ). Although the Perox/Total ratios were relatively smaller than those for liver, peroxisomal  $\beta$ -oxidation capacity still represented 28 to 40% of the total  $\beta$ -oxidation capacity in kidney across all ages (Table 1). The ratio of  $\text{CO}_2/\text{Mito}$  in the kidney was more than 0.42 across all ages, which was substantially higher than in the liver. The  $\text{CO}_2/\text{Mito}$  ratio was not affected by age or nutritional state (Table 1).

In heart, total and mitochondrial  $\beta$ -oxidation capacities and  $^{14}\text{CO}_2$  production rate (Fig. 3A, B and D) did not change 24 h after birth. At 10 d, total  $\beta$ -oxidation was higher than at 24 h for suckled pigs ( $P < 0.05$ ), and total and mitochondrial  $\beta$ -oxidation for food-deprived pigs were higher than at 0 h ( $P < 0.01$ ). Peroxisomal  $\beta$ -oxidation capacity was higher ( $P < 0.05$ ) at either 10 or 21 d than at 0 h regardless of nutritional state (Fig. 3C). Except for the  $\text{CO}_2/\text{Mito}$  ratio at 24 h during food deprivation, both  $\text{CO}_2/\text{Mito}$  and Perox/Total ratios did not change with age or nutritional state (Table 1). The Perox/Total ratio in the heart was lower than that in liver or kidney, and the  $\text{CO}_2/\text{Mito}$  ratios generally were intermediate to those for liver and kidney.

**Acetate and ketone body production in piglet tissues.** In either the absence or presence of antimycin A and rotenone, 30 to 35% of the palmitate carboxyl carbon that accumulated in ASP from incubation of liver homogenate was recovered in acetate, and neither the inhibitors nor substrate concentration affected this percentage (Table 2). Changing palmitate concentration also did not affect the absolute accumulation rates

TABLE 1

The ratios of CO<sub>2</sub> production to mitochondrial β-oxidation (CO<sub>2</sub>/Mito) and peroxisomal to total β-oxidation (Perox/Total) of [1-<sup>14</sup>C]palmitate in homogenates of liver, kidney and heart from pigs at different ages and nutritional states<sup>1</sup>

	0 h		24 h		10 d		21 d		5 mo	Pooled SEM
	Unsuckled	Suckled	24-h Food-deprived	Suckled	24-h Food-deprived	Suckled	24-h Food-deprived	Overnight food-deprived		
ratio (%)										
CO <sub>2</sub> /Mito in										
Liver	28.4 <sup>bc</sup>	30.2 <sup>c</sup>	25.5 <sup>bc</sup>	27.5 <sup>bc</sup>	25.7 <sup>bc</sup>	19.9 <sup>ab</sup>	19.8 <sup>ab</sup>	13.1 <sup>a</sup>		3.2
Kidney	49.0	42.4	44.1	49.9	51.2	47.6	51.4	44.5		2.9
Heart	40.3 <sup>b</sup>	24.1 <sup>ab</sup>	20.1 <sup>a</sup>	30.7 <sup>ab</sup>	28.6 <sup>ab</sup>	32.8 <sup>ab</sup>	29.4 <sup>ab</sup>	ND		6.1
PeroX/Total in										
Liver	51.0 <sup>c</sup>	51.5 <sup>c</sup>	42.9 <sup>ab</sup>	39.1 <sup>ab</sup>	37.3 <sup>a</sup>	41.0 <sup>ab</sup>	46.1 <sup>bc</sup>	37.2 <sup>a</sup>		2.4
Kidney	40.0 <sup>c</sup>	37.1 <sup>bc</sup>	41.6 <sup>c</sup>	28.7 <sup>a</sup>	28.0 <sup>a</sup>	28.3 <sup>a</sup>	32.5 <sup>ab</sup>	29.2 <sup>a</sup>		1.7
Heart	29.0	28.4	26.4	27.8	25.9	30.9	30.3	ND		2.0

<sup>1</sup> Values are means, n = 4, except for overnight food-deprived at 5 mo (n = 3). Within a row, values with different superscripts are significantly different (P < 0.05). ND = not determined.

(1.1 to 1.3 μmol palmitate carboxyl carbon · h<sup>-1</sup> · g tissue<sup>-1</sup> in the absence of inhibitors, 0.4 to 0.5 μmol palmitate carboxyl carbon · h<sup>-1</sup> · g tissue<sup>-1</sup> in the presence of inhibitors). However, incubation with inhibitors significantly decreased the accumulation rate to 34 to 44% of that without inhibitors at all substrate concentrations. Only about 3 to 4% of the palmitate carboxyl carbon that accumulated in ASP was recovered in ketone bodies (acetoacetate plus β-hydroxybutyrate) regardless of substrate concentration or presence of respiratory inhibitors (Table 2).

Substantial palmitate carboxyl carbon of ASP also was accumulated in acetate in kidney (31 to 40%) and heart (45 to 50%, Table 3). There was a significant difference in the absolute rate of accumulation among the three tissues in the ab-

sence of inhibitors, with the highest rate in heart and the lowest in kidney, but no difference in the presence of inhibitors. As a result, the ratio of the accumulation of acetate in the presence of inhibitors to that in the absence of inhibitors was about twice as high in kidney (80.7%) as in liver (44.1%) or heart (35.7%) (Table 3).

DISCUSSION

We report here the first description of developmental changes of peroxisomal β-oxidation and its potential contributions to total β-oxidation capacity in young and growing pigs. Developmental patterns of mitochondrial and peroxisomal β-oxidation differed among the three tissues studied. Hepatic

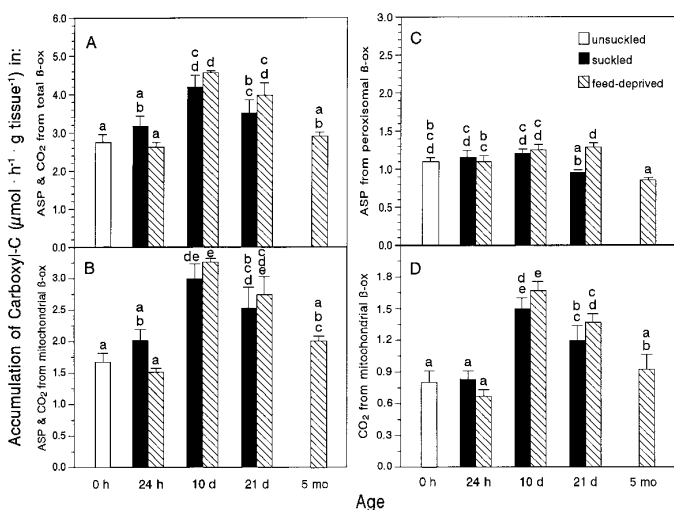


FIGURE 2 Kidney metabolism: Total (panel A), mitochondrial (panel B) and peroxisomal (panel C) capacities for [1-<sup>14</sup>C]palmitate β-oxidation (β-ox) and rate of CO<sub>2</sub> production from [1-<sup>14</sup>C]palmitate (panel D) in pig kidney during development and during fed or food-deprived states. Rates were calculated as the accumulation of carboxyl carbon in CO<sub>2</sub>, acid-soluble products (ASP) or both after incubation of kidney homogenate for 30 min in the absence or presence of antimycin A and rotenone (see text for details). Bars represent means ± SEM for n = 4 pigs per treatment group (n = 3 for adult pig group). Values with different letters above the bars are different (P < 0.05).

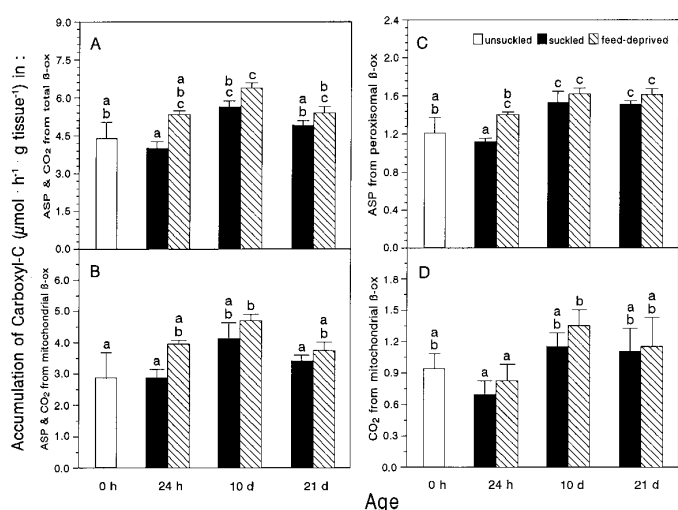


FIGURE 3 Heart metabolism: Total (panel A), mitochondrial (panel B) and peroxisomal (panel C) capacities for [1-<sup>14</sup>C]palmitate β-oxidation (β-ox) and rate of CO<sub>2</sub> production from [1-<sup>14</sup>C]palmitate (panel D) in pig heart during development and during fed or food-deprived states. Rates were calculated as the accumulation of carboxyl carbon in CO<sub>2</sub>, acid-soluble products (ASP) or both after incubation of heart homogenate for 30 min in the absence or presence of antimycin A and rotenone (see text for details). Bars represent means ± SEM for n = 4 pigs per treatment group. Values with different letters above the bars are different (P < 0.05).

TABLE 2

Accumulation of palmitate carboxyl carbon in ketone bodies and acetate after 30-min incubations of liver homogenates from 24-h-old unfed pigs with [ $1\text{-}^{14}\text{C}$ ]palmitate<sup>1</sup>

Palmitate	Antimycin and rotenone	Ketone bodies	Ketone bodies/ASP <sup>2</sup>	Acetate	Acetate/ASP	Ratio <sup>3</sup>
mmol/L		$\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$	%	$\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$	%	%
1.0	—	0.108**	3.0	1.139**	32.2	44.1
	+	0.035	2.4	0.467	32.2	—
0.5	—	0.137**	4.0*	1.225**	35.3	40.1
	+	0.048	3.1	0.475	31.5	—
0.2	—	0.136**	3.6*	1.289**	33.4	33.6
	+	0.034	2.7	0.393	30.4	—
Pooled SEM		0.011	0.3	0.124	2.0	—

<sup>1</sup> Values are means,  $n = 3$ . Asterisks indicate a significant difference between absence and presence of inhibitors at the same palmitate concentration: \*  $P < 0.05$ , and \*\*  $P < 0.01$ .

<sup>2</sup> ASP = acid-soluble products.

<sup>3</sup> Ratio of accumulation of carboxyl carbon in acetate in presence of inhibitors to that in the absence of inhibitors.

peroxisomal  $\beta$ -oxidation increased about 66 and 31% in 24-h-old fed and 24-h-old food-deprived pigs, respectively, but by 10 d of age had returned to rates comparable to those present at birth. In kidney, little change in rates of peroxisomal  $\beta$ -oxidation was observed, except for a slightly lower rate at 5 mo of age. Peroxisomal  $\beta$ -oxidation in heart homogenates did not change significantly at 24 h of age but was increased by an average of about 29% in 10- or 21-d-old pigs, regardless of nutritional status. The rapid increase in peroxisomal  $\beta$ -oxidation in liver from 24-h-old fed pigs may indicate an adaptive response to metabolize the sudden large influx of fatty acids from ingestion of colostrum and milk, which contain almost exclusively long-chain fatty acids in swine (Jenness 1985).

Hepatic total, mitochondrial and peroxisomal  $\beta$ -oxidation capacities were increased by 24 h after birth; at 10 d, mitochondrial  $\beta$ -oxidation capacity remained relatively constant but total  $\beta$ -oxidation capacity decreased in fed pigs mainly because

of a decreased peroxisomal  $\beta$ -oxidation capacity. Mersmann and Phinney (1973) found a fourfold increase of hepatic palmitate oxidation between birth and 7 d, and Miller et al. (1971) found no difference for in vivo oxidation rates of several fatty acids in piglets at 1 or 7 d of age. From our results and those of previous studies, we infer that the postnatal increase of hepatic oxidation of fatty acids in swine mainly occurs during the first 24 h after birth. This increase would be consistent with the rapid postnatal need to oxidize fatty acids from colostrum and milk, both to obtain energy for hepatic functions and to provide short-chain oxidative fuels, such as the ketone bodies and acetate, for peripheral tissues reliant prenatally on carbohydrate as oxidative fuel.

Little is known about proliferation of peroxisomes in neonatal swine, with the exception of a morphological study that reported a general increase in peroxisomal numbers in pig liver during the first 28 d of life (Laging et al. 1990). Mersmann et al. (1972) and Mersmann (1974) indicated that hepatic organelles proliferate rapidly by about the second day postpartum and that mitochondrial proliferation in pig liver occurs by approximately 12 h postpartum. Bieber et al. (1973) found that hepatic carnitine palmitoyltransferase (CPT, EC 2.3.1.23) activity in piglets was doubled during the first 24 h of life. We recently found that the activities of both CPT I (Lin and Odle 1995) and palmitoyl CoA oxidase (Yu et al. 1996), the rate-limiting enzymes for mitochondrial and peroxisomal  $\beta$ -oxidation, respectively, increased significantly by 24 h postpartum in the liver of newborn piglets.

Renal and cardiac total  $\beta$ -oxidation capacities increased more slowly after birth, being greater by 10 d postpartum than at either 0 or 24 h. In contrast to the changes in liver, increased oxidation in kidney was mainly caused by an increase of mitochondrial  $\beta$ -oxidation capacity, because the peroxisomal  $\beta$ -oxidation capacity was virtually unchanged during the preweaning period. The increased cardiac total  $\beta$ -oxidation capacity by 21 d of age resulted primarily from an increased mitochondrial  $\beta$ -oxidation capacity, with a smaller increase of peroxisomal  $\beta$ -oxidation. Wolfe et al. (1978) reported that the rate of renal palmitate oxidation in piglets fed a diet containing 32% fat increased with age to a maximum at 21 d of age, but the oxidation rate in heart homogenate tended to decrease with age. Mersmann and Phinney (1973) found that the oxidation rate of palmitate in heart homogenate did not change with age in preweaning piglets. Our results for cardiac

TABLE 3

Accumulation of palmitate carboxyl carbon in acetate after 30-min incubation of tissue homogenates from 24-h-old pigs with 1 mmol/L [ $1\text{-}^{14}\text{C}$ ]palmitate<sup>1</sup>

Tissue	Antimycin A and rotenone	Acetate	Acetate/ASP <sup>2</sup>	Ratio <sup>3</sup>
		$\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$	%	%
Liver	—	1.095a,**	32.0a	44.1
	+	0.424	32.0a	—
Kidney	—	0.622b	30.9a,**	80.7
	+	0.491	40.0b	—
Heart	—	2.033c,**	44.9b	35.7
	+	0.681	50.0c	—
Pooled SEM		0.123	1.9	—

<sup>1</sup> Values are means,  $n = 3$ . Within a column with the same antimycin and rotenone treatment, values with different superscript letters are significantly different ( $P < 0.05$ ). \*\* Indicates a significant ( $P < 0.01$ ) difference between absence and presence of antimycin A and rotenone in the same tissue.

<sup>2</sup> ASP = acid-soluble products.

<sup>3</sup> Ratio of accumulation of carboxyl carbon in acetate in presence of antimycin A and rotenone to that in absence of inhibitors.

fatty acid oxidation correspond to those of Ascutto et al. (1989), who found that the palmitate oxidation rate in isolated perfused piglet hearts did not change from 0.6 to 3.3 d after birth but increased significantly by 9.5 d of age. In the absence of inhibitors, our findings of lower accumulation of palmitate carboxyl carbon in  $\text{CO}_2$  than in ASP and the differences for the  $\text{CO}_2/\text{Mito}$  ratio among organs were in accordance with the findings of Wolfe et al. (1978).

In our study, we measured capacities for the initial cycle of total  $\beta$ -oxidation and peroxisomal  $\beta$ -oxidation as the maximum rate of  $\beta$ -oxidation of [ $1\text{-}^{14}\text{C}$ ]palmitate in the absence or presence of antimycin A and rotenone. This methodology tends to overestimate the rate of peroxisomal  $\beta$ -oxidation, especially in tissues with low rates of peroxisomal  $\beta$ -oxidation, such as heart (Chu et al. 1994). This methodology also cannot indicate whether apparent increases in peroxisomal  $\beta$ -oxidation were attributable to peroxisomal proliferation or to an increased specific activity of peroxisomal  $\beta$ -oxidation enzymes. Nevertheless, qualitative patterns of development of mitochondrial and peroxisomal  $\beta$ -oxidation obtained using this methodology should be valid. Data for enzymatic activities in partially purified peroxisomal fractions from the tissues used in this study provided the same general pattern of changes among tissues (Yu 1996, Yu et al. 1996).

Using methodology similar to ours, other investigators have reported that peroxisomal  $\beta$ -oxidation represents <10% (Mannaerts et al. 1979) to >30% (Veerkamp and van Moerkerk 1986) of total  $\beta$ -oxidation capacity in rat tissues. We found a peroxisomal contribution of 37–51% in pig liver, 28–41% in pig kidney, and 26–31% in pig heart, compared with 20% in liver homogenates from adult rats (Drackley et al. 1995). Therefore, the proportional contribution of peroxisomal  $\beta$ -oxidation found in liver, kidney and heart of pigs from our study is substantially higher than that for rat tissues. Peroxisomal percentages were statistically higher at 0 and 24 h in the liver and kidney than at older ages. Neonates rely on fatty acids for survival because fatty acids represent about 60% of the total energy in sow milk (for review, see Girard et al. 1992). Therefore, considering that mitochondrial  $\beta$ -oxidation capacity may be limited in piglets, a relatively greater peroxisomal  $\beta$ -oxidation capacity and its rapid increase in liver after birth may act as a compensatory mechanism for piglets to oxidize milk fatty acids.

Data from our study suggest that the relative importance of peroxisomal  $\beta$ -oxidation compared with mitochondrial  $\beta$ -oxidation and ketogenesis is greater in newborn piglets than in mature pigs. In liver and kidney, the ratio of peroxisomal to total  $\beta$ -oxidation decreased with age of the pigs (Table 1). The relatively faster decrease of the hepatic rate of  $^{14}\text{CO}_2$  production compared with the rate of total mitochondrial  $\beta$ -oxidation from palmitate, coupled with the decrease of the hepatic ratio of  $\text{CO}_2/\text{Mito}$  from d 21, may indicate a decreased channeling of mitochondrial acetyl-CoA into the citric acid cycle as pigs aged. A decreased  $\text{CO}_2/\text{Mito}$  ratio in pig liver is suggestive of increased activity of the ketogenic pathway or other noncitric acid cycle pathways, such as more acetyl-CoA being converted into acetyl-carnitine or acetate. Pégrier et al. (1983) reported that the rate of ketone body production was about twofold greater in hepatocytes from 15-d-old suckling piglets than in those from newborn suckling piglets, and the rate was sixfold greater in hepatocytes from 15-d-old food-deprived piglets than in those from newborn food-deprived piglets.

We found that food deprivation of piglets after birth significantly attenuated the increase in peroxisomal  $\beta$ -oxidation rate in liver compared with fed pigs at 24 h of age, but did

not change the mitochondrial  $\beta$ -oxidation rate. In contrast, food deprivation increased the total oxidation rate in liver from 10-d-old pigs but not in liver from 21-d-old pigs. Pégrier et al. (1983) and Odle et al. (1995) found that rates of oxidation of fatty acids in hepatocytes isolated from food-deprived newborn piglets were decreased markedly relative to those of fed pigs. Ishii et al. (1980) observed that the response of palmitoyl-CoA oxidase to food deprivation in rats was more rapid and marked than that of mitochondrial CPT; comparable data for pigs are unavailable.

An explanation for the differential response to food deprivation between 24-h-old pigs and 10-d-old pigs may lie in differences in the availability of long-chain fatty acids to the animal. Numerous long-chain fatty acids, including those most abundant in porcine milk (palmitic, oleic, and linoleic; Jenness 1985), can activate peroxisome proliferator-activated receptors, which are nuclear receptors that in turn activate transcription of genes encoding enzymes of fatty acid metabolism (see Schoonjans et al. 1996 for review). In adult animals, food deprivation decreases concentrations of glucose and insulin in blood but increases glucagon concentration; these changes result in increased release of fatty acids from adipose tissue. However, in newborn piglets, the body fat content amounts to only 1% of body weight, most of which is structural fat (see Girard et al. 1992 for review). Following 24 h of food deprivation after birth, blood concentrations of both glucagon and free fatty acids were significantly lower in unfed pigs than in suckled pigs (Pégrier et al. 1981). Therefore it is conceivable that peroxisomal  $\beta$ -oxidation in piglet liver is regulated by blood concentrations of free fatty acids, or possibly glucagon, similar to mitochondrial  $\beta$ -oxidation. Thus normal suckling of milk by newborn piglets leads to greater concentrations of glucagon and free fatty acids in blood (Pégrier et al. 1981), which may result in higher hepatic capacities for mitochondrial and peroxisomal  $\beta$ -oxidation.

In a similar way, a greater ability to mobilize fatty acids during food deprivation in the 10-d-old pigs, and the associated increase of fatty acid concentration in blood, might be associated with the tendencies for increased oxidative capacities. An increased percentage of body fat relative to that for newborn pigs was found as early as 2 d of age (Elliot and Lodge 1977). Thus food deprivation at 10 d of age would lead to large increases in fatty acid concentration in blood, which might stimulate  $\beta$ -oxidation activity. The lack of significant increases in total oxidation rate in liver from 21-d-old food-deprived pigs in our study might have been due to an insufficient duration of food deprivation. Gentz et al. (1970) found that the plasma free fatty acid concentration increased significantly in 9-d-old and 16-d-old pigs after 24 h of food deprivation and continued to increase with another 72 h of food deprivation in 16-d-old pigs but not in 9-d-old pigs. Furthermore, the decrease of blood glucose after 24 h of food deprivation was negatively correlated with the animal age after birth.

With the exception of the changes discussed for liver, food deprivation had minimal effects on capacities for total, mitochondrial and peroxisomal  $\beta$ -oxidation in kidney and heart. Other investigators have reported variable effects of food deprivation on fatty acid oxidation. Campion et al. (1986) found no difference between suckled and unfed 24-h-old piglets for palmitate oxidation by piglet muscle. Veerkamp and van Moerkerk (1986) observed that 18 h of food deprivation increased total oxidation rate in homogenates of liver and kidney of adult rats but did not change the rate in homogenates of heart and muscle. Food deprivation increased peroxisomal  $\beta$ -oxidation rate in liver homogenate, decreased peroxisomal  $\beta$ -oxidation in heart homogenate, and did not change peroxi-

somal  $\beta$ -oxidation in kidney and muscle homogenates (Veerkamp and van Moerkerk 1986).

In rodents and many other species, it is well accepted that the acetyl-CoA produced by hepatic mitochondrial  $\beta$ -oxidation is channeled either to the citric acid cycle or to ketogenesis. However, the fate of acetyl-CoA generated from peroxisomal  $\beta$ -oxidation is less clear. Furthermore, previous work (Adams and Odle 1993, Lin et al. 1996, Odle et al. 1995) demonstrated that piglet liver has a limited ketogenic capacity, which was confirmed by the present study. Only 3–4% of radioactivity in ASP from palmitate oxidation accumulated in ketone bodies. In liver from adult rats incubated similarly, we found that this value was about 55% (Adams et al. 1997). Therefore, the metabolic fate of acetyl-CoA produced from hepatic mitochondrial and peroxisomal  $\beta$ -oxidations in piglets poses an interesting question.

More than 30% of palmitate carboxyl carbon accumulated in ASP from incubation of piglet liver homogenate was in acetate regardless of whether mitochondrial  $\beta$ -oxidation inhibitors were present. In contrast, only about 5% of ASP was attributable to acetate in similar incubations of rat liver (Adams et al. 1997). These findings further support the belief that, in pigs, acetate may be one of the main "outlets" for acetyl-CoA produced from either mitochondrial  $\beta$ -oxidation or peroxisomal  $\beta$ -oxidation, as suggested previously (Lin et al. 1996, Odle et al. 1995). Cytosol, mitochondria and peroxisomes in tissues from several species, including pigs and rats, have been found to contain acetyl-CoA hydrolases (Lin et al. 1995, Söeling and Rescher 1985), which may account for the release of acetate during mitochondrial and peroxisomal fatty acid oxidations. Leighton et al. (1989) have presented evidence that peroxisomes are more important than mitochondria as sources of acetate from  $\beta$ -oxidation of fatty acids in rats.

Although not determined directly in our experiment, acetyl-carnitine might represent another substantial portion of radioactivity in ASP. Sleboda et al. (1995) observed that adding acetyl-CoA to peroxisomal incubation media inhibited rat peroxisomal  $\beta$ -oxidation of [ $U$ - $^{14}C$ ]palmitate by up to 50%, but increasing L-carnitine concentrations between 0.01 and 0.5 mmol/L stimulated hepatic peroxisomal  $\beta$ -oxidation. With increased L-carnitine concentration, acetyl-CoA concentration was decreased with a concomitant increase of acetyl-carnitine concentration. Carnitine acetyltransferase is present in peroxisomes, cytosol and mitochondria (Van den Bosch et al. 1992). Release of free acetate would constitute a mechanism to prevent sequestration of coenzyme A or secondary L-carnitine deficiency that might occur if acetyl-carnitine was the only final product of both mitochondrial and peroxisomal  $\beta$ -oxidations. Thus as ketogenesis has been considered an overflow mechanism for acetyl-CoA generated in hepatic mitochondria in other species, acetogenesis might function as an overflow mechanism for acetyl-CoA generated in peroxisomes and hepatic mitochondria of piglets.

Liver and peripheral tissues have been reported to take up and release acetate, depending on its intracellular concentration, and it has been suggested that acetate is simultaneously produced and oxidized in the liver as well as in other tissues (Herrmann et al. 1985). In our study, we found that 31 or 40% of palmitate carboxyl carbon from total or peroxisomal  $\beta$ -oxidation, respectively, accumulated in acetate in kidney, and 45 or 50% accumulated in acetate in heart; the absolute rate of accumulation was higher in heart than in liver. Whether these high rates of accumulation occur in whole-cell preparations or in vivo remains to be determined. Our results indicate that a major

source of intracellular acetogenesis in liver and other tissues may be mitochondrial and peroxisomal  $\beta$ -oxidation. Furthermore, acetogenesis may be an important metabolic fate of acetyl-CoA generated from  $\beta$ -oxidation that is not coupled with ketogenesis or that is coupled to a limited ketogenic system such as that in pigs.

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