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# Hepatic $\beta$ -oxidation and carnitine palmitoyltransferase I in neonatal pigs after dietary treatments of clofibric acid, isoproterenol, and medium-chain triglycerides

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**Peffer, Pasha Lyvers, Xi Lin, and Jack Odle.** Hepatic  $\beta$ -oxidation and carnitine palmitoyltransferase I in neonatal pigs after dietary treatments of clofibric acid, isoproterenol, and medium-chain triglycerides. *Am J Physiol Regul Integr Comp Physiol* 288: R1518–R1524, 2005. First published February 24, 2005; doi:10.1152/ajpregu.00822.2004.—A suckling piglet model was used to study nutritional and pharmacologic means of stimulating hepatic fatty acid  $\beta$ -oxidation. Newborn pigs were fed milk diets containing either long- or medium-chain triglycerides (LCT or MCT). The long-chain control diet was supplemented further with clofibric acid (0.5%) or isoproterenol (40 ppm), and growth was monitored for 10–12 days. Clofibrate increased rates of hepatic peroxisomal and mitochondrial  $\beta$ -oxidation of [1-<sup>14</sup>C]-palmitate by 60 and 186%, respectively. Furthermore, malonyl-CoA sensitive carnitine palmitoyltransferase (CPT I) activity increased 64% ( $P < 0.05$ ) in pigs receiving clofibrate. Increased CPT I activity was not congruent with changes in message, as elevated abundance of CPT I mRNA was not detected ( $P = 0.16$ ) when assessed by qRT-PCR. Neither rates of  $\beta$ -oxidation nor CPT activities were affected by dietary MCT or by isoproterenol treatment ( $P > 0.1$ ). Collectively, these findings indicate that clofibrate effectively induced hepatic CPT activity concomitant with increased fatty acid  $\beta$ -oxidation.

acyl-CoA oxidase; fatty acid oxidase; malonyl-CoA; peroxisome; peroxisome proliferator-activated receptor

DURING THE TRANSITION FROM intrauterine to extrauterine life, survival of the young hinges on upregulation of the enzymes required to promote efficient use of fatty acids postpartum. Although the primary energy source for the developing fetus is maternal glucose, milk lipids become the principal source of energy for postnatal growth and development (13). In support of the changing nutrient environment, many neonates, including rats, rabbits, and humans exhibit increased  $\beta$ -oxidative capacity, resulting in pronounced hyperketonemia (10, 13, 43). However, piglets do not show an appreciable accumulation of circulating ketone bodies during the suckling period (1, 7, 38), and examination of fatty acid metabolism in cultured porcine hepatocytes demonstrates a propensity toward fatty acid reesterification with limited flux through the pathway of  $\beta$ -oxidation (39). Why the piglet deviates from the accepted model of hepatic lipid metabolism in the young is not known. However, the demand for energy after birth necessitates an understanding of the etiology of attenuated hepatic  $\beta$ -oxidation in the piglet

for improving neonatal survival, as 55–60% of the energy for the newborn is derived from milk fatty acids (13).

It is well established that the carnitine palmitoyltransferase (CPT) system plays an essential role in regulating fatty acid flux through the pathway of  $\beta$ -oxidation (27). Most notably, CPT I and its regulation by malonyl-CoA governs the translocation of activated fatty acids into the mitochondria for subsequent  $\beta$ -oxidation. Data collected in neonatal pigs suggest that CPT I may contribute to the reduced rate of hepatic  $\beta$ -oxidation noted in this species (33, 35). Although CPT activity doubles between birth and 1 day of age, the activity measured at 1 day of age is equivalent to that measured at 24 days of age (5), inferring that activity expeditiously increases postpartum before it plateaus after the first day of life. However, fatty acid oxidation in the 1-day-old pig is only one-third of the rate determined for the 24-day-old pig (5). This discrepancy between CPT activity and fatty acid oxidation may be explained by enhanced sensitivity of CPT I to malonyl-CoA. Studies examining tissue specific CPT activity suggest that unlike most other mammals, hepatic CPT I in the neonatal pig is more sensitive to the inhibitory effects of malonyl-CoA (33). The high degree of sensitivity for piglet hepatic CPT I is reflected in hepatic concentrations of malonyl-CoA, which are 94% lower than rat liver concentrations (11). However, piglet CPT I maintains its high degree of sensitivity to malonyl-CoA under various physiological states (11, 44), whereas in the rodent, sensitivity to malonyl-CoA is decreased under physiological changes that elevate circulating fatty acids (40).

As part of an ongoing research effort to understand the basis of low hepatic  $\beta$ -oxidation in the young pig and the role of CPT I, the objectives of the study were to determine whether dietary intervention with clofibrate, medium-chain triglycerides (MCT), or isoproterenol would enhance hepatic  $\beta$ -oxidation and CPT activity. Clofibrate, a peroxisomal proliferator, has been shown to increase total (malonyl-CoA sensitive and insensitive) CPT activity in neonatal pigs; however, its effects on CPT I activity and mRNA abundance are not known. In addition, stimulation of peroxisomal oxidation, which has been shown to occur in piglets after clofibrate administration (50), provides an ancillary route for the oxidation of fatty acids, which may bypass CPT I regulation of mitochondrial  $\beta$ -oxi-

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dition. Second, MCT were fed to assess the response to a fatty acid energy source, which is not reliant on CPT I for mitochondrial  $\beta$ -oxidation (36). Third, it was hypothesized that the antilipogenic effects of isoproterenol (41) may reciprocally promote  $\beta$ -oxidation. The study presented here illustrates that hepatic  $\beta$ -oxidation can be enhanced, concomitant with increased CPT I activity after clofibrate supplementation, and is the first to report the effects of isoproterenol on hepatic fatty acid oxidation in neonatal pigs. Research centered on improving  $\beta$ -oxidation in the young pig may help to elucidate nutritional strategies to reduce neonatal morbidity and mortality in this species, while also providing information on an animal model of interest to biomedical research.

## MATERIALS AND METHODS

**Animals and diets.** Procedures for this study were approved by the North Carolina State University Animal Care and Use Committee. Piglets were obtained from North Carolina State University Swine Education Unit within 24–48 h after birth (suckled). Piglets were individually housed in an environmentally controlled (30°C) facility and allotted by gender and weight to one of four dietary treatments: 1) control milk replacer with soybean oil as the lipid source and formulated to contain 21% fat, 32% crude protein, and 34% lactose; 2) control milk replacer supplemented with 0.5% (wt/wt) clofibrate; 3) control milk replacer with MCT source replacing 95% of soybean oil; and 4) control milk replacer supplemented with 40 ppm isoproterenol. Milk replacer was reconstituted with water to 15% (wt/vol) dry matter and fed via gravity flow through a nipple. Milk replacer was mixed and replaced twice daily to maintain freshness, and dietary treatments were fed for 10–12 days. Additional suckled piglets (24–48 h of age) were euthanized at time of removal from dam to obtain data in the newborn.

**Reagents and chemicals.** L-[N-Methyl- $^3$ H]carnitine (2.5 GBq/mol), [1- $^{14}$ C]-palmitate (2.0 GBq/mmol), and [1- $^{14}$ C]-glucose (2.0 GBq/mmol) were purchased from American Radiolabeled Chemicals (St. Louis, MO). Palmitoyl-CoA, antimycin A, rotenone, and other chemicals were purchased from Sigma Chemicals (St. Louis, MO). Scintillation fluid (Scintisafe) was purchased from Fisher Scientific (St. Louis, MO).

**Collection of tissue samples.** Pigs were euthanized via American Veterinary Medical Association approved electrocution and had access to feed before euthanasia. Liver and a portion of muscle (semitendons) tissue were excised immediately. Liver was weighed, and a portion was placed in ice-cold isolation buffer containing (in mmol/l) 220 mannitol, 70 sucrose, 2 HEPES, and 0.1 EDTA (pH 7.4). Liver was blotted, minced, and manually homogenized in 10 volumes of the isolation buffer using a Potter-Elvehjem homogenizer. Homogenate and mitochondrial fractions were obtained by differential centrifugation (29). Respiratory control ratios were determined on the mitochondrial fraction to assess mitochondrial integrity (2). Protein was determined by the biuret method (15) in both the homogenate and mitochondrial fractions. Additional samples of liver and muscle were frozen immediately in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ .

**Analysis of carnitine palmitoyltransferase-I activity.** Liver mitochondria were used to assess CPT activity as previously described by Bremer (7). The assay measured the rate of formation of palmitoyl-carnitine from palmitoyl-CoA and carnitine. The reaction medium contained 75 mmol/l KCL, 50 mmol/l HEPES, 0.2 mmol/l EGTA, 1% fatty acid free bovine albumin, and 80  $\mu\text{mol/l}$  of palmitoyl-CoA for determination of total CPT activity. Activity of CPT was further assessed as malonyl-CoA-sensitive or insensitive activity. Malonyl-CoA-insensitive activity was determined by the addition of 250  $\mu\text{mol/l}$  of malonyl-CoA to the reaction medium. Malonyl-CoA-sensitive CPT activity was determined as the difference between total activity and malonyl-CoA-insensitive activity and representative of

CPT I activity. Reactions were initiated by the addition of 1 mmol/l of L-[N-methyl- $^3$ H]-carnitine (0.05  $\mu\text{Ci}/\text{reaction}$ ), and terminated by the addition of 6%  $\text{HClO}_4$  after 6 min. The labeled palmitoyl-carnitine was extracted with butanol and quantified by liquid scintillation spectrometry.

**Assays of mitochondrial and peroxisomal  $\beta$ -oxidation.** Rates of first-cycle total, mitochondrial, and peroxisomal  $\beta$ -oxidation of [1- $^{14}$ C] palmitate and oxidation of [1- $^{14}$ C] glucose were measured in liver homogenates by a modification of the procedures as previously described (48). In duplicate reactions, aliquots of homogenate (300  $\mu\text{l}$ ) were added to Erlenmeyer flasks containing 1.7 ml of a reaction medium (in mmol/l): 50 sucrose, 150 Tris $\cdot$ HCl, 20  $\text{KH}_2\text{PO}_4$ , 10  $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ , 2 EDTA, 1 L-carnitine, 0.2 CoA, 2 NAD, 0.1 malate, 10 ATP (pH 7.4). The reaction medium contained either: 1) [1- $^{14}$ C]-palmitate, 2) [1- $^{14}$ C]-glucose, 3) [1- $^{14}$ C]-palmitate plus antimycin/rotenone, 4) [1- $^{14}$ C]-palmitate plus glucose, or 5) [1- $^{14}$ C]-glucose plus palmitate. Each reaction contained 0.5  $\mu\text{Ci}$  of either [1- $^{14}$ C]-palmitate or [1- $^{14}$ C]-glucose. Palmitate was added to the reaction medium as Na-palmitate bound to fatty acid-free albumin (complexed in a 5:1 molar ratio) to a final concentration of 1 mmol/l. Glucose was added to the reaction medium to achieve a final concentration of 5 mmol/l. Antimycin (50  $\mu\text{mol/l}$ ) and rotenone (10  $\mu\text{mol/l}$ ) were added to inhibit mitochondrial oxidation for indirect determination of peroxisomal oxidation.

Reactions were initiated by the addition of the homogenate and allowed to proceed for 30 min in a shaking water bath at  $37^{\circ}\text{C}$ . Incubations were terminated by the addition of 1 ml of 3 mol/l  $\text{HClO}_4$  to the reaction medium. After termination of the reaction, flasks were incubated at room temperature (2 h) for collection of  $^{14}\text{CO}_2$  into a suspended well containing 500  $\mu\text{l}$  of ethanolamine. When [1- $^{14}$ C]-palmitate was contained in the reaction medium, acid-soluble products (ASP) were collected following centrifugation of flask contents to precipitate protein and nonmetabolized palmitate. Blanks were prepared by immediate acidification of the reaction medium after the addition of the homogenate. Radioactivity in  $\text{CO}_2$  and ASP was quantified by liquid scintillation spectrometry.

The rate of total  $\beta$ -oxidation was measured as the rate of accumulation of  $^{14}\text{C}$  in  $^{14}\text{CO}_2$  plus [1- $^{14}$ C]-JASP. The rate of accumulation of  $^{14}\text{C}$  into [1- $^{14}$ C]-ASP after the addition of the inhibitors antimycin and rotenone was reported as peroxisomal  $\beta$ -oxidation. Negligible  $^{14}\text{CO}_2$  production was measured in the presence of the inhibitors (data not shown). Mitochondrial  $\beta$ -oxidation was calculated to be the difference between total  $\beta$ -oxidation and peroxisomal  $\beta$ -oxidation.

**Extraction of total RNA.** Total RNA was extracted from liver and muscle tissue using guanidine isothiocyanate and phenol (TRI Reagent solution; Sigma-Aldrich.). Briefly, tissue was homogenized (0.1 g tissue/ml TRI Reagent), and 0.2 ml of chloroform (per 1 ml TRI Reagent) was added to the homogenate. After centrifugation (12,000 g, 15 min), the RNA was precipitated from the aqueous phase by the addition of isopropanol (0.5 ml/ml TRI Reagent) followed by centrifugation (12,000 g, 10 min). The resulting pellet was washed with 75% ethanol and resuspended in RNase-free water. Gel electrophoresis with ethidium bromide staining was used to assess RNA integrity. The RNA was quantified and purity assessed by UV spectrophotometric analysis of  $A_{260}$  and  $A_{260}/A_{280}$  ratio ( $>1.7$ ), respectively.

**Reverse transcription.** The RNA (10  $\mu\text{g}/50 \mu\text{l}$ ) was treated with 4 units of TurboDNase (Ambion, Austin, TX), according to the manufacturer's instructions for removal of genomic DNA. Phenol:chloroform extraction was used to recover the RNA, and 2  $\mu\text{g}$  was reverse-transcribed using Omniscript reverse-transcription kit, according to the manufacturer's instructions (Qiagen, Valencia, CA). First-strand cDNA was synthesized at  $42^{\circ}\text{C}$  for 60 min by priming with oligo-dT (Roche Diagnostics, Basel, Switzerland). A negative control (using pooled RNA) containing all of the reverse-transcription reagents in the absence of RT enzyme was included to verify absence of genomic DNA during PCR (no RT control).

Table 1. Primers used for amplification of *HPRT*, *LCPT I*, *MCPT I*, and *ACO* and expected amplicon size following SYBR Green RT-PCR

| Gene    | Primer sequence (5' → 3')   | Amplicon Size, bp | Accession* |
|---------|-----------------------------|-------------------|------------|
| HPRT    | F: CCATGACATCGTAGCCCTCTG    | 172               | AF143818   |
|         | R: TACTTTTATATCGCCCGTTGACTG |                   |            |
| L CPT I | F: GGACCGCCACCTGTCTGCCTCTA  | 175               | AF288789   |
|         | R: GCCCCCTCCGCTCGACACATAC   |                   |            |
| M CPT I | F: GCACGCCAGGCCTTCTTCAGC    | 121               | AY181062   |
|         | R: TGGCCTCGTCTCCGGGTCATAGT  |                   |            |
| ACO     | F: ACCACGGTGAAGAAGATAAGG    | 181               | AF185048   |
|         | R: TCCAGCGGGCATGAAGAAGC     |                   |            |

\*Accession numbers listed reference published GenBank pig-specific sequences that were used to design forward and reverse primers. HPRT, hypoxanthine phosphoribosyltransferase; LCPT I, liver isotype of carnitine palmitoyltransferase I; MCPT I, skeletal muscle isotype of carnitine palmitoyltransferase I; ACO, acyl-CoA oxidase.

**Primer design.** The primer design program Lasergene Primer Select (DNASTar) was used to design pig-specific primers for hypoxanthine phosphoribosyltransferase (HPRT), CPT I (liver and skeletal muscle isoforms), and acyl-CoA oxidase (ACO) (Table 1). Primer pairs were selected for optimum annealing temperatures and negligible secondary structure. Primers were purchased through Sigma-Genosys, and resuspended in  $1 \times$  Tris/EDTA. The PCR amplicons were sequenced to verify primer accuracy and specificity.

**Real-time RT-PCR with SYBR Green.** Real-time RT-PCR was performed using the DNA Engine Opticon (MJ Research,). Real-time fluorescence detection was performed in 96-well plates using Quantitect SYBR Green buffer (Qiagen). Samples were assayed in triplicate. Each 20- $\mu$ l reaction contained cDNA (equivalent to 100-ng RNA) and 0.3  $\mu$ M each of the forward and reverse primers. After the initial 15-min. incubation at 95°C for activation of the Hot Start Taq DNA polymerase, all templates were amplified for 40 cycles using the following protocol: denaturation for 15 s at 94°C, primer annealing for 30 s at 60°C, and elongation for 30 s at 72°C. Fluorescence data were acquired at the end of each elongation phase. After the amplification program, melting curves were generated by measuring the fluorescence as the temperature was raised from 50°C to 95°C in 0.2°C increments to verify specificity of fluorescence detection. Specificity of amplification products was further verified by gel electrophoresis and ethidium bromide staining. Background fluorescence was set as the average fluorescence between cycles 3 and 15, and cycle threshold (Ct) was determined on the basis of 10 SD above background fluorescence (14). Positive (no template reaction) and negative (no reverse transcriptase) controls were run in triplicate and included in each PCR assay.

**Analysis of real-time RT-PCR data.** Standard curves for the reference gene HPRT and the target genes of interest for liver (LCPT I and ACO) and muscle (MCPT I) were generated from pooled cDNA (equivalent to 200, 100, 50, 25, and 12.5 ng of total RNA). The Ct values were regressed on the log of template cDNA concentration. The quality of the standard curves was assessed according to the correlation coefficient ( $r^2 > 0.95$ ), and the slope of the lines were used to determine efficiency of amplification according to the equation  $E = 10^{-1/\text{slope}}$  (42; Fig. 1, Table 2). Because of unequal amplification efficiencies between the reference gene and target genes, a relative expression ratio indicating fold change was determined according to the method by Pfaffl (42) for assessment of treatment differences:

$$\text{Expression Ratio} = \frac{E^{\Delta C_t}_{\text{target}}}{E^{\Delta C_t}_{\text{reference}}}$$

where  $\Delta C_t$  = sample – control for the target gene and reference gene. Differences in Ct values for pigs fed clofibrate (sample) were assessed

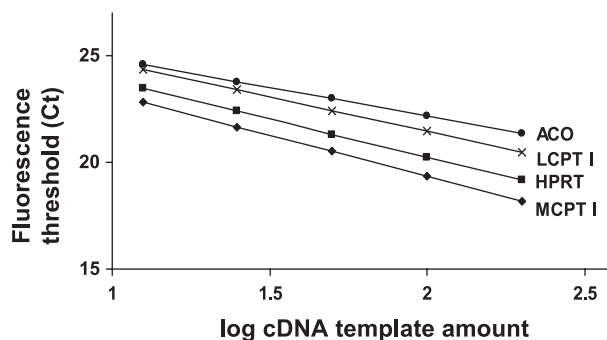


Fig. 1. Relationship between cDNA template concentration and fluorescence threshold cycle threshold (Ct). Linear regression of log concentration of template vs. Ct. Template concentrations were equivalent to 200, 100, 50, 25, and 12.5 ng RNA. Values are means of triplicate readings for the genes acyl-CoA oxidase (ACO; ●), hypoxanthine phosphoribosyltransferase (HPRT; ■), skeletal muscle carnitine palmitoyltransferase I (MCPT I; ◆), and liver carnitine palmitoyltransferase I (LCPT I; ×).

against Ct values obtained from control pigs for HPRT and the target genes of interest (LCPT I, MCPT I, or ACO) to determine  $\Delta C_t$  values. The  $\Delta C_t$  was used to determine abundance of mRNA using the efficiency of amplification for target genes and the reference gene. The target gene was corrected for differences in reference gene amplification to obtain the expression ratio. Variance of the expression ratio was determined according to Marino and colleagues (26):

$$\text{Variance}_{\ln R} = \left(\frac{sCt_1}{\beta}\right)^2 + \left(\frac{sCt_2}{\beta}\right)^2 + \left(\frac{(Ct_2 - Ct_1) s_\beta}{\beta^2}\right)^2$$

where  $Ct_1$  = sample,  $Ct_2$  = control and  $sCt_1$  and  $sCt_2$  = estimate of SE (average of triplicate readings) for the sample and control (respectively),  $\beta$  = slope of the gene being evaluated and  $s_\beta$  = error of the slope. Log variance was determined for the target genes and the reference gene. Target gene variance was corrected for differences in the reference gene using the following equation:

$$\text{corrected variance}_{\ln R} = \text{variance}_{\ln R \text{ Target}} + \text{variance}_{\ln R \text{ Reference}}$$

The corrected variance was linearized and the SE determined.

**Statistical analysis.** Data were subjected to ANOVA using the general linear models procedure of SAS (SAS, Cary, NC) appropriate for a completely randomized design (CRD). Initial body weight was used as a covariate in analysis of growth parameters. Effects of substrate (palmitate or glucose) oxidation were analyzed as a subplot within the CRD. A one-sided Student's *t*-test was used for determining significance in fold change of hepatic ACO and LCPT I, and MCPT I expression for clofibrate supplementation relative to control.

## RESULTS

At the end of the treatment period control, clofibrate, MCT, and isoproterenol-supplemented pigs weighed 5138 g ( $\pm 135$ ),

Table 2. Efficiency of amplification of *HPRT*, *LCPT I*, *MCPT I*, and *ACO*

| Gene   | Slope | $r^2$ | Efficiency* | Average Ct      |
|--------|-------|-------|-------------|-----------------|
| HPRT   | -3.60 | 0.99  | 1.89        | 22.4 $\pm$ 2.00 |
| LCPT I | -3.21 | 1.00  | 2.04        | 23.5 $\pm$ 2.43 |
| MCPT I | -3.80 | 0.99  | 1.82        | 21.5 $\pm$ 1.33 |
| ACO    | -2.68 | 0.99  | 2.34        | 24.3 $\pm$ 1.73 |

\*Efficiency calculated as  $E = 10^{-1/\text{slope}}$ . Average cycle threshold (Ct) values are representative of starting template concentration equivalent to 100 ng RNA for control pigs.

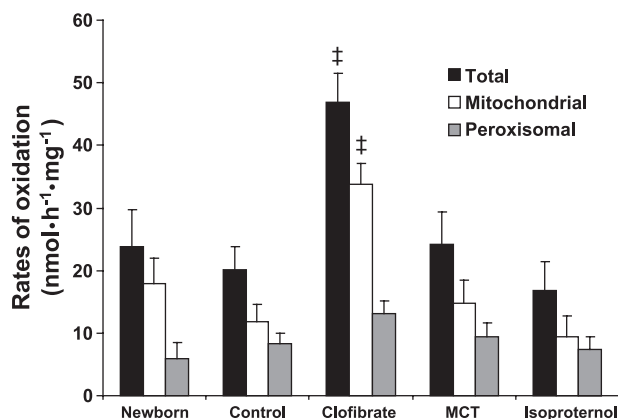


Fig. 2. Rates of total, mitochondrial, and peroxisomal  $\beta$ -oxidation ( $^{14}\text{CO}_2$  and [ $^{14}\text{C}$ ]-ASP accumulation); and mitochondrial [ $^{14}\text{C}$ ]-ASP production ( $\text{nmol}\cdot\text{h}^{-1}\cdot\text{mg protein}^{-1}$ ). Dietary treatments listed on the *x*-axis were fed 10–12 days before data collection. Bars represent means ( $n = 5\text{--}9$ )  $\pm$  SE.  $\ddagger\ddagger$ Different from control ( $P < 0.01$ ); comparison was made between similar coded bars.

4743 g ( $\pm 182$ ), 4539 g ( $\pm 197$ ), and 4678 g ( $\pm 193$ ), respectively. Compared with control pigs, MCT supplemented pigs weighed less ( $P = 0.02$ ), whereas no differences were detected between control and clofibrate or isoproterenol treatments. Liver weights were 165 g ( $\pm 7$ ), 228 g ( $\pm 10$ ), 179 g ( $\pm 10$ ), and 142 g ( $\pm 10$ ) for control, clofibrate, MCT, and isoproterenol-supplemented pigs, respectively. Clofibrate supplementation increased liver weight by 38% ( $P < 0.0001$ ). There was no difference in liver weight after MCT or isoproterenol supplementation. The liver weight/body weight ratio was greater for clofibrate and MCT treatments ( $4.7 \pm 0.17$  and  $4.1 \pm 0.19$ , respectively) than control pigs ( $3.3 \pm 0.13$ ); however, there was no difference between control and isoproterenol treatment ( $3.0 \pm 0.18$ ).

Total, mitochondrial, and peroxisomal first-cycle  $\beta$ -oxidation rates of [ $^{14}\text{C}$ ]-palmitate, were similar between suckled newborn pigs, control pigs, and pigs supplemented with MCT or isoproterenol (Fig. 2; Table 3). Total and mitochondrial oxidative capacities were increased 134 and 186%, respectively, in liver homogenates from clofibrate-supplemented pigs compared with similar-age, control pigs ( $P < 0.05$ ; Fig. 2). Peroxisomal  $\beta$ -oxidation of [ $^{14}\text{C}$ ]-palmitate accounted for 41, 28, 39, and 43% of total  $\beta$ -oxidative capacity for control, clofibrate, MCT, or isoproterenol-supplemented pigs, respec-

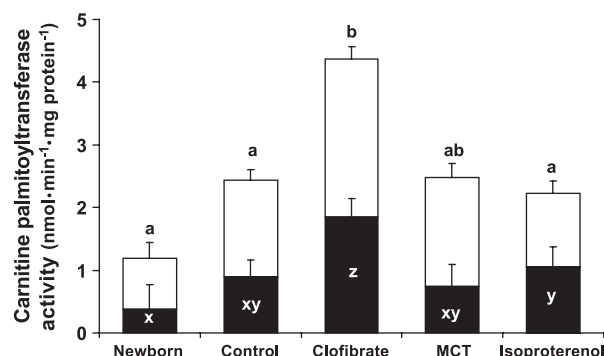


Fig. 3. Malonyl-CoA-sensitive and malonyl-CoA-insensitive hepatic carnitine palmitoyltransferase activity (CPT). Activity was measured at the end of a 10- to 12-day feeding period of the dietary treatments listed on the *x*-axis. Total activity is represented by summation of malonyl-CoA sensitive (CPT I) and insensitive activities. Bars represent means  $\pm$  SE. Different letters (a,b) denote significant difference between dietary treatment for malonyl-CoA sensitive CPT activity ( $P < 0.05$ ). Different letters (x,y,z) denote significant difference between dietary treatment for malonyl-CoA insensitive CPT activity ( $P < 0.05$ ).

tively. Peroxisomal oxidation increased 60% with clofibrate supplementation ( $P = 0.06$ ); however, it represented less of the total oxidation as a result of increased mitochondrial oxidative capacity. Production of  $^{14}\text{CO}_2$  measured in liver homogenates from clofibrate, MCT, or isoproterenol-supplemented pigs did not differ from control pigs, but the rate of  $^{14}\text{C}$  accumulation into ASP from mitochondrial oxidation was 137% greater after clofibrate supplementation ( $P < 0.001$ ; Fig. 2).

Liver homogenates from control, clofibrate, MCT, or isoproterenol-supplemented pigs exhibited no preference toward metabolic fuel substrate as evidenced by similar rates of  $\text{CO}_2$  and ASP production with the addition of glucose to [ $^{14}\text{C}$ ]-palmitate or palmitate to [ $^{14}\text{C}$ ]-glucose (Table 3). However, in newborn pigs there was a 55% reduction in oxidative products ( $\text{CO}_2$  plus ASP) when glucose was added to [ $^{14}\text{C}$ ]-palmitate.

Differences in mean respiratory control ratios for isolated mitochondria were not detected. Values were 3.0 for newborn, control, and clofibrate-treated pigs; and 3.7 and 3.1 for MCT and isoproterenol-treated pigs, respectively. Supplementation with clofibrate increased both malonyl-CoA sensitive (CPT I) and insensitive CPT activity by 64 and 105%, respectively (Fig. 3). Supplementation with MCT or isoproterenol had no

Table 3. Competitive oxidation of palmitate and glucose in liver homogenates of newborn pigs and pigs fed clofibrate, MCT, or isoproterenol: rates of carbon accumulation into oxidative products after incubations with [ $^{14}\text{C}$ ]-palmitate or [ $^{14}\text{C}$ ]-glucose with or without the addition of unlabeled glucose or palmitate, respectively

|               | [ $^{14}\text{C}$ ]-Palmitate                                 | [ $^{14}\text{C}$ ]-Palmitate + Glucose | [ $^{14}\text{C}$ ]-Glucose | [ $^{14}\text{C}$ ]-Glucose + Palmitate |
|---------------|---|---|-----------------------------|---|
|               | $\text{nmol}\cdot(\text{h}\cdot\text{mg protein})^{-1}\cdot*$ |   |                             |   |
| Newborn       | 23.9 $\pm$ 3.4  | 10.8 $\pm$ 3.4 <sup>ax</sup>            | 27.8 $\pm$ 3.4              | 21.3 $\pm$ 3.4                          |
| Control       | 20.0 $\pm$ 2.3  | 20.3 $\pm$ 2.3 <sup>b</sup>             | 21.6 $\pm$ 2.3              | 21.7 $\pm$ 2.3                          |
| Clofibrate    | 46.9 $\pm$ 2.6 <sup>ax</sup>                                  | 45.3 $\pm$ 2.6 <sup>cx</sup>            | 18.0 $\pm$ 2.6 <sup>a</sup> | 17.0 $\pm$ 2.6                          |
| MCT           | 24.1 $\pm$ 3.0  | 21.1 $\pm$ 3.0 <sup>b</sup>             | 27.7 $\pm$ 3.0              | 27.0 $\pm$ 3.0                          |
| Isoproterenol | 16.7 $\pm$ 2.8 <sup>x</sup>                                   | 15.8 $\pm$ 2.8 <sup>abx</sup>           | 26.5 $\pm$ 2.8              | 22.9 $\pm$ 2.8                          |

Substrates for oxidation were included in incubations as 1 mmol/l of [ $^{14}\text{C}$ ]-palmitate or palmitate and/or 5 mmol/l of [ $^{14}\text{C}$ ]-glucose or glucose. \*For palmitate-oxidative products, the carbon accumulated is the sum of  $\text{CO}_2$  plus acid-soluble products; for glucose-oxidative products, the carbon accumulated represents  $\text{CO}_2$  only. <sup>abc</sup> Means within a column lacking a common superscript differ ( $P < 0.05$ ). <sup>xyz</sup> Means within a row lacking a common superscript differ ( $P < 0.05$ ). MCT, medium-chain triglycerides.

effect on CPT activity. For pigs receiving clofibrate supplementation, the relative abundance of liver CPT I, skeletal muscle CPT I, and ACO mRNA also was determined. As assessed by real-time RT-PCR, effects of clofibrate on relative transcript amount of ACO or hepatic CPT I and skeletal muscle CPT I in the respective tissues, were not detected (Fig. 4).

## DISCUSSION

Studies have demonstrated that the rate of hepatic  $\beta$ -oxidation in young pigs is attenuated compared with other mammals (1, 39). With fatty acids serving as the principal energy substrate for oxidative metabolism during the suckling period (13), the lower  $\beta$ -oxidative capacities noted in this species may indicate impaired nutrient handling. Consequently, improving neonatal nutrition in the piglet hinges on understanding the fate of fatty acids and their metabolic regulation by CPT I.

In this study, rates of palmitate oxidation did not differ between the suckled newborn and 12-day-old pig, nor did CPT I activity. Developmental changes in hepatic CPT activity have been reported to occur within the initial 24 h of life in neonatal pigs (5). Activity increases expeditiously after birth, and at 1 day of age, hepatic CPT activity is analogous to that measured at 24 days of age (5). Equivalent rates of *in vivo* fatty acid oxidation also have been reported between 1 and 7 days of age in young pigs (31). However, studies suggest that  $\beta$ -oxidative capacity of pig liver homogenates increases approximately fourfold between birth and 7 days of age (28). It was proposed that postnatal increases in hepatic  $\beta$ -oxidative capacity of the liver occurs within the first 24 h of life (34, 48). This postnatal increase in fatty acid oxidation measured during the initial day of life may be a consequence of increased cellular respiration and not necessarily increased reliance on fatty acids for energy (1, 34), as this period is marked by significant mitochondrial hyperplasia (29). Furthermore, postpartum peroxisomal proliferation has been measured in the young pig (21) and may further contribute to increased rates of hepatic fatty acid oxidation.

At birth,  $\sim 50\%$  of piglet hepatic  $\beta$ -oxidative capacity has been ascribed to peroxisomal oxidation. This value increased to 66% by 1 day of age and then decreased to 40% by 10 days of age (47, 48). This is in agreement with the current study, in which peroxisomal oxidation represented 41% of total oxida-

tion in 12-day-old control pigs. In contrast, Yu and colleagues (48) reported a decrease in peroxisomal oxidation between 24 h of age and 10 days of age, whereas, we did not detect differences in peroxisomal oxidation between the suckled newborn and 12-day-old piglet. Although it is not known why these differences occurred, piglets in the current study received a diet formulated to contain 21% fat throughout the feeding period, while Yu et al. (48) obtained piglets directly from the dam before sampling. Fluctuations in milk fat content could have contributed to differences in peroxisomal oxidation, as Yu et al. (48) suggested that peroxisomal oxidation in pigs reflects the availability of long-chain fatty acids. It is important to note that although hepatic peroxisome number increases rapidly after birth in rodents, peroxisomal oxidation represents only 10% of total oxidation (19). In the neonatal pig, the greater contribution of peroxisomal oxidation to total oxidation may provide an alternative route for catabolism of milk lipids (47, 48, 49), which by-passes regulation imposed at the level of CPT I for mitochondrial oxidation.

*Oxidative capacity of the liver after clofibrate supplementation.* Clofibrate supplementation increased liver size 38%. Similar observations have been noted in the rodent (18) and attributed to increases in peroxisomal number and size (22). Although neither peroxisomal hyperplasia nor hypertrophy were determined in this study, Cheon and colleagues (9) did not detect changes in peroxisomal nor hepatocyte morphology after clofibrate supplementation. It should be noted that in the latter study, liver size was unchanged after clofibrate supplementation, contrary to our findings.

Only a modest effect (60% increase;  $P = 0.06$ ) on the rate of peroxisomal oxidation was detected, as differences in ACO mRNA approached significance ( $P = 0.08$ ) with a 3.7-fold induction. A previous study conducted in neonatal pigs (50) suggested a more robust effect after clofibrate supplementation, resulting in a 2.7-fold increase in peroxisomal oxidation. This discrepancy may be a result of methodology used to measure peroxisomal oxidation. While in the current study, peroxisomal oxidation was assessed by rotenone- and antimycin-insensitive  $\beta$ -oxidation of [ $1\text{-}^{14}\text{C}$ ]-palmitate, which measures only the initial round of  $\beta$ -oxidation, Yu and colleagues (50) used the palmitoyl-CoA-dependent, KCN-insensitive reduction of  $\text{NAD}^+$ , which quantitates all cycles of peroxisomal  $\beta$ -oxidation.

In comparison to the effects observed in the rat, where hypolipidemic drugs have induced a 20-fold increase in peroxisomal oxidation (22), the effects of clofibrate supplementation are attenuated in the pig. It is generally accepted that peroxisome proliferator-activated receptor (PPAR) $\alpha$  mediates the effects of clofibrate. In humans, lower transcript amounts of ACO have been attributed to lower hepatic expression of PPAR $\alpha$  (2, 37). Although hepatic PPAR $\alpha$  mRNA is abundant in 5-wk-old pigs (9, 46), expression is reportedly low in certain breeds of neonatal pigs (46). Furthermore, the presence of an alternatively spliced PPAR $\alpha$  isoform, which lacks the ligand-binding domain could contribute to the lower responsiveness of the pig to clofibrate (46).

Despite only a modest increase in peroxisomal oxidation, total oxidation increased approximately twofold as a result of greater mitochondrial oxidative rates. Clofibrate treatment in rats increases hepatic mitochondrial number (20) and mitochondrial oxidation (25). Although mitochondrial mass was

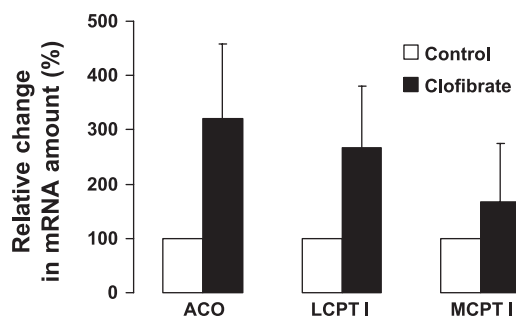


Fig. 4. Relative hepatic ACO, LCPT I, and MCPT I mRNA amounts. Amounts of mRNA for control pigs are set as 100%, and data are reported as % change for clofibrate-supplemented pigs relative to control pigs. Data were corrected for changes in reference gene expression, according to Pfaffl (42). Bars represent means ( $n = 4\text{--}5$ )  $\pm$  SE;  $P = 0.08, 0.16,$  and  $0.3$  for ACO, LCPT I, and MCPT I, respectively, for clofibrate-supplemented vs. control pigs.

not determined in the current study, increased mitochondrial number is correlated with increased rates of mitochondrial oxidation (30) and could account for increased rates of  $\beta$ -oxidation measured. Alternatively, data in rodents suggest that clofibrate treatment increases CPT I expression and activity (8, 25). Indeed, concomitant with increased mitochondrial flux of palmitate, CPT I activity was increased; however, we failed to detect a difference in hepatic mRNA abundance. Likewise, an effect of clofibrate supplementation on expression of skeletal muscle CPT I was not detected. If the effects of clofibrate on CPT I expression are mediated via PPAR $\alpha$ , then the low abundance of PPAR $\alpha$  in the liver, which also has been observed in piglet skeletal muscle (46), may have contributed to the lack of CPT I upregulation in the current study. Recently, evidence has emerged, which supports the role of PPAR $\alpha$  in mediating the effects of clofibrate in young pigs (9). Contrary to our findings, Cheon and colleagues (9) reported a fivefold induction in hepatic CPT I following clofibrate supplementation, coincident with elevated mRNA abundance of PPAR $\alpha$ . Research indicates changes in PPAR $\alpha$  mRNA with age (45) could account for the differences noted.

*Oxidation of palmitate and glucose after MCT feeding.* The use of MCT as an alternative metabolic fuel for the neonatal pig has been researched extensively in light of the pigs' ability to digest, absorb, and utilize medium-chain fatty acids released from this energy source (36). Compared with long-chain fatty acids, the rate and extent of MCT utilization was reported to be three- and four-fold greater, respectively (17). Greater oxidative flux of carbon has been measured in cultured porcine hepatocytes after MCT incubation (34); however, we were unable to detect differences in palmitate oxidation or CPT I activity after MCT feeding. Data collected from rodents also suggests that MCT feeding has no effect on subsequent flux of long-chain fatty acids through  $\beta$ -oxidation (40). Furthermore, final weight of pigs receiving MCT was lower than control pigs. It has been reported that although the piglet can use MCT, growth is not improved (24).

It is interesting to note that MCT supplementation had no deleterious effects on peroxisomal oxidation. Medium-chain fatty acids are poor substrates for oxidation by the peroxisome, because of the lower affinity of acyl-CoA oxidase toward acyl substrates of eight or fewer carbons (16). Furthermore, peroxisomal oxidative capacity is responsive to high-fat feeding of long-chain fatty acids (32). Although suckling is important for the establishment of optimal peroxisomal  $\beta$ -oxidative capacities (48, 49), it appears that maintaining high rates of peroxisomal  $\beta$ -oxidation is not dependent on a continual source of long-chain fatty acids in the young pig.

*Effects of isoproterenol on palmitate oxidation.* This study is the first to present the effects of a  $\beta$ -agonist on hepatic fatty acid oxidation in neonatal pigs. In vitro studies in older pigs have shown a decrease in lipogenesis and an increase in lipolysis when porcine adipose tissue explants were incubated with isoproterenol (a  $\beta$ 1/ $\beta$ 2 agonist) (41). The effect on lipolysis also has been observed in vivo after isoproterenol infusion (30). Furthermore, isoproterenol has increased circulating fatty acids twofold by 7 days of age in piglets (44). It was hypothesized that a putative effect of isoproterenol would be enhanced  $\beta$ -oxidation as a consequence of increased circulating fatty acids resulting from isoproterenol's known effects on lipolysis and lipogenesis. However, differences in rates of

palmitate oxidation or hepatic CPT I activity were not detected in this study.

First-pass metabolism by the liver may limit the bioavailability of isoproterenol when fed as previously suggested (12). In rodents, isoproterenol stimulated lipolysis in isolated adipocytes; however, no effects on lipolysis were observed after the  $\beta$ -agonist was fed (12). Alternatively, desensitization and/or downregulation of the  $\beta$ -adrenergic receptor after continual exposure of the ligand are a known mode of regulation (23). Data indicate that continual exposure to  $\beta$ -agonists postpartum promotes hepatic desensitization of the receptor (4). The 12-day exposure period to isoproterenol in this study may have contributed to the lack of effect via receptor desensitization which would have abated any cellular response.

In conclusion, although nutraceutical or pharmacological benefits of isoproterenol or MCT were not evident from findings reported in the current study, the use of clofibrate for dietary intervention may provide a means of reducing neonatal morbidity and mortality in piglets. Studies implicating CPT I in reduced mitochondrial fatty acid flux suggest that increasing CPT I activity may promote increased  $\beta$ -oxidation, which is essential to the development of the newborn pig. Increased mitochondrial oxidation after clofibrate supplementation was coincident with increased CPT I in the current study; however, the effect did not appear to be due to induction of CPT I mRNA. In neonatal pigs, the effect of clofibrate is primarily through enhancement of mitochondrial oxidation, as the already substantial rates of peroxisomal oxidation were not further increased. It has been suggested that the peroxisomal oxidative system provides an alternate route for the catabolism of fatty acids (48), as peroxisomal oxidation appears to play a substantial role in the piglets' ability to adapt to the change in primary energy source encountered at birth.

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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: major differences in end products, O<sub>2</sub> uptake, and  $\beta$ -oxidation. *Am J Physiol Regul Integr Comp Physiol* 272: R1641–R1646, 1997.
2. Ammerschlaeger M, Beigel J, Klein KU, and Mueller SO. Characterization of the species-specificity of peroxisome proliferators in rat and human hepatocytes. *Toxicological Sci* 78: 229–240, 2004.
3. Aprille JR and Asimakis GK. Postnatal development of rat liver mitochondria: state 3 respiration, adenine nucleotide translocase activity, and the net accumulation of adenine nucleotides. *Arch Biochem Biophys* 201:564–575, 1980.
4. Auman JT, Seidler FJ, Tate CA, and Slotkin TA. Are developing  $\beta$ -adrenoceptors able to desensitize? Acute and chronic effects of  $\beta$ -agonists in neonatal heart and liver. *Am J Physiol Regul Integr Comp Physiol* 283: R205–R217, 2002.
5. Beiber LL, Markwell MAK, Blair M, and Helmuth TA. Studies on the development of carnitine palmitoyltransferase and fatty acid oxidation in liver mitochondria of neonatal pigs. *Biochem Biophys Acta* 326: 145–154, 1973.
6. Bengtsson G, Gents J, Hakkarainen J, Hellström R, and Persson B. Plasma levels of FFA, glycerol,  $\beta$ -hydroxybutyrate, and blood glucose during the postnatal development of the pig. *J Nutr* 97: 311–315, 1969.

7. **Bremer J.** The effect of fasting on the activity of liver carnitine palmitoyltransferase and its inhibition by malonyl-CoA. *Biochim Biophys Acta* 665: 628–631, 1981.
8. **Chatelain F, Kohl C, Esser V, McGarry JD, Girard J, and Pegorier JP.** Cyclic AMP and fatty acids increase carnitine palmitoyltransferase I transcription in cultured rat hepatocytes. *Eur J Biochem* 235: 789–798, 1996.
9. **Cheon Y, Nara TY, Band MR, Beever JE, Wallig MA, and Nakamura MT.** Induction of overlapping genes by fasting and a peroxisome proliferators in pigs: evidence of functional PPAR $\alpha$  in nonproliferating species. *Am J Physiol Regul Integr Comp Physiol* 288: R1525–R1535, 2005.
10. **Duée P, JPégrier Manoubi LE, Herbin C, Kohl C, and Girard J.** Hepatic triglyceride hydrolysis and development of ketogenesis in rabbits. *Am J Physiol Endocrinol Metab* 249: E478–E484, 1985.
11. **Duée P, JPégrier Quant PA, Herbin C, Kohl 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.
12. **Fotovati A, Hayashi T, Tsuchida N, and Ito T.** Effects of isoproterenol, a nonselective  $\beta$ -agonist, on lipid metabolism of rats: in vitro and in vivo study. *J Fac Agr, Kyushu Univ* 45: 99–108, 2000.
13. **Girard J, Ferré P, JPégrier, and Duée P.** Adaptations of glucose and fatty acid metabolism during the perinatal period and suckling-weaning transition. *Physiol Rev* 72: 507–563, 1992.
14. **Giulietti A, Overbergh L, Valckx D, Decallonne B, Bouillon R, and Mathieu C.** An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. *Methods* 25: 386–401, 2001.
15. **Gornal AG, Bardawill CJ, and David MM.** Determination of serum protein by means of the burette reaction. *J Biol Chem* 177: 751–766, 1949.
16. **Hashimoto T.** Peroxisomal  $\beta$ -oxidation: Enzymology and molecular biology. In: *Peroxisomes: Biology and role in toxicology and disease*, edited by Janardan and Reddy, New York Academy of Sciences, 1995.
17. **Heo KN, Lin X, Han IK, and Odle J.** Medium-chain fatty acids but not L-carnitine accelerate the kinetics of [ $^{14}$ C]Triacylglycerol utilization by colostrums-deprived newborn pigs. *J Nutr* 132: 1989–1994, 2002.
18. **Hess R, Staubli W, and Riess W.** Nature of the hepatomegalic effect produced by ethyl-chlorophenoxy-isobutyrate in the rat. *Nature* 208: 865–888, 1965.
19. **Krahling JB, Gee R, Gauger JA, and Tolbert NE.** Postnatal development of peroxisomal and mitochondrial enzymes in rat liver. *J Cell Physiol* 101: 375–390, 1979.
20. **Kurup CKR, Aithal HM, and Ramasarma T.** Increase in hepatic mitochondria on administration of ethyl- $\alpha$ -p-chlorophenoxyisobutyrate to the rat. *Biochem J* 116: 773–779, 1970.
21. **Laging C, Pospischil A, and Diesecke D.** Ultrastructural morphometric investigation of peroxisome development in piglets during the first 4 weeks of life. *J Vet Med Sci* A37: 186–197, 1990.
22. **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.
23. **Levitzi A.** From epinephrine to cyclic AMP. *Science* 241: 800–806, 1988.
24. **Lee HF and Chiang SH.** Energy value of medium-chain triglycerides and their efficacy in improving survival of neonatal pigs. *J Anim Sci* 72: 133–138, 1994.
25. **Mannaerts GP, Debeer LJ, Thomas J, and de Schepper PJ.** Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats. *J Biol Chem* 254: 4585–4595, 1979.
26. **Marino JH, Cook P, Miller KS.** Accurate and statistically verified quantification of relative mRNA abundances using SYBR Green I and real-time RT-PCR. *J Immunol Methods* 283: 291–306, 2003.
27. **McGarry JD and Brown NF.** The mitochondrial carnitine palmitoyltransferase system - From concept to molecular analysis. *Eur J Biochem* 244: 1–14, 1997.
28. **Mersmann HJ and Phinney G.** In vitro fatty acid oxidation in liver and heart from neonatal swine (*Sus Domesticus*). *Comp. Biochem. Physiol.* 44B: 219–223, 1973.
29. **Mersmann HJ, Goodman J, Houk JM, and Anderson S.** Studies of the biochemistry of mitochondria and cell morphology in the neonatal swine hepatocyte. *J Cell Biol* 53: 335–347, 1972.
30. **Mersmann HJ.** Acute metabolic effects of adrenergic agents in swine. *Am J Physiol Endocrinol Metab* 252: E85–E95, 1987.
31. **Miller GM, Conrad JH, Keenan TW, and Featherston WR.** Fatty acid oxidation in young pigs. *J Nutr* 101: 1343–1350, 1971.
32. **Neat CE, Thomassen MS, and Omundsen H.** Induction of peroxisomal  $\beta$ -oxidation in rat liver by high-fat diet. *Biochem J* 186: 369–371, 1980.
33. **Nicot C, Hegardt FG, Woldegiorgis G, Haro D, and Marrero PF.** Pig liver carnitine palmitoyltransferase I, with low  $K_m$  for carnitine and high sensitivity to malonyl-CoA inhibition, is a natural chimera of rat liver and muscle enzymes. *Biochemistry* 40: 2260–2266, 2001.
34. **Odle J, Benevenga NJ, and Crenshaw TD.** Postnatal age and the metabolism of medium- and long-chain fatty acids by isolated hepatocytes from small-for-gestational-age and appropriate-for-gestational-age piglets. *J Nutr* 121: 615–621, 1991.
35. **Odle J, Lin X, van Kempen T, Drackely JK, and Adams SH.** Carnitine palmitoyltransferase modulation of hepatic fatty acid metabolism and radio-HPLC evidence for low ketogenesis in neonatal pigs. *J Nutr* 125: 2541–2549, 1995.
36. **Odle J.** New insights into medium-chain triglyceride utilization by the neonate: observation from a piglet model. *J Nutr* 127: 1061–1067, 1997.
37. **Palmer NAC, Hsu MH, Griffin KJ, Raucy JL, and Johnson E.** Peroxisome proliferators activated receptor- $\alpha$  expression in human liver. *Mol Pharmacol* 53: 14–22, 1998.
38. **Pégrier J, Duée P, Assan R, Peret J, and Girard J.** Changes in circulating fuels, pancreatic hormones and liver glycogen concentration in fasting or suckling newborn pigs. *J Dev Physiol* 3: 203–217, 1981.
39. **Pégrier J, Duée P, Girard J, and Peret J.** Metabolic fate of nonesterified fatty acids in isolated hepatocytes from newborn and young pigs. *Biochem J* 212: 93–97, 1983.
40. **Pégrier J, Duée P, Herbin C, Lualan PY, Bladé C, Peret J, and Girard J.** Fatty acid metabolism in hepatocytes isolated from rats adapted to high-fat diets containing long- or medium-chain triacylglycerols. *Biochem J* 249: 801–806, 1988.
41. **Peterla TA and Scanes CG.** Effect of  $\beta$ -adrenergic agonists on lipolysis and lipogenesis by porcine adipose tissue in vitro. *J Anim Sci* 68: 1024–1029, 1990.
42. **Pfaffl MW.** A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29: 2002–2007, 2001.
43. **Robles-Valdes C, McGarry JD, and Foster DW.** Maternal-fetal carnitine relationships and neonatal ketosis in the rat. *J Biol Chem* 257: 6007–6012, 1976.
44. **Schmidt I and Herbin P.** Carnitine palmitoyltransferase I (CPTI) activity and its regulation by malonyl-CoA are modulated by age and cold exposure in skeletal muscle mitochondria from newborn pigs. *J Nutr* 128: 886–893, 1998.
45. **Stanton HC and Mueller RL.** Metabolic responses induced in neonatal swine by norepinephrine, epinephrine, and isoproterenol. *Proc Soc Exp Biol Med* 143: 492–494, 1972.
46. **Sundvold H, Grindflek E, Lien S.** Tissue distribution of porcine peroxisome proliferators-activated receptor  $\alpha$ : detection of an alternatively spliced mRNA. *Gene* 273: 105–113, 2001.
47. **Yu XX, Drackley JK, and Odle J.** Response of hepatic mitochondrial and peroxisomal  $\beta$ -oxidation to increasing palmitate concentrations in piglets. *Biol Neonate* 72: 284–292, 1997.
48. **Yu XX, Drackley JK, and Odle J.** Rates of mitochondrial and peroxisomal  $\beta$ -oxidation of palmitate change during postnatal development and food deprivation in liver, kidney, and heart of pigs. *J Nutr* 127: 1814–1821, 1997.
49. **Yu XX, Drackley JK, and Odle J.** Food deprivation changes peroxisomal  $\beta$ -oxidation activity but not catalase activity during postnatal development in pig tissues. *J Nutr* 128: 1114–1121, 1997.
50. **Yu XX, Odle J, and Drackley JK.** Differential induction of peroxisomal  $\beta$ -oxidation enzymes by clofibrate and aspirin in piglet tissues. *Am J Physiol Regul Integr Comp Physiol* 281: R1553–R1561, 2001.