Acetogenesis does not replace ketogenesis in fasting piglets infused with hexanoate

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Adams, Sean H., and Jack Odle. Acetogenesis does not replace ketogenesis in fasting piglets infused with hexanoate. *Am. J. Physiol.* 274 (*Endocrinol. Metab.* 37): E963–E970, 1998.—The current studies were performed to better understand the physiological relevance of acetate in the poorly ketogenic piglet and to determine if endogenous acetogenesis rises with increased mitochondrial fatty acid β-oxidation, analogous to ketogenesis. Plasma acetate concentration values in newborn, fasted, or suckled piglets (230–343 μM) were at least 10-fold higher than the ketone bodies, a pattern opposite to that in 24- to 48-h suckled rats (77–175 μM). Employing continuous infusion techniques with sodium [^{3}H]acetate tracer in fasting ~40-h-old piglets, acetate rate of appearance (R_a) was found to be 34 ± 4 μmol·min^{-1}·kg body wt^{-1}. This basal R_a was double that observed in animals coinfused with sodium [1-^{14}C]hexanoate ($P < 0.001$), despite active oxidation of the latter as determined by $^{14}CO_2$ production. Active acetogenesis in vivo and relatively abundant acetate in piglet blood are consistent with the hypothesis that acetate plays an important physiological role in piglets. However, the negative impact of hexanoate oxidation upon acetate R_a and the lack of significant changes in circulating acetate in newborn, suckled, and fasted piglets draws into question the extent of analogy between acetogenesis and ketogenesis in vivo.

β-oxidation; newborns; ketone bodies; medium-chain fatty acids

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THE SUCKLING PERIOD, bridging intrauterine life and the nutritional independence of weaning, is characterized by a heavy reliance upon milk-derived lipids to cover the energetic and growth requirements of the neonate. The postpartum transition toward a lipid-based metabolism has been best characterized in the rat, a species in which birth triggers mechanisms working in concert to support fatty acid oxidation and ketogenesis (reviewed in Ref. 10). In particular, carnitine palmitoyltransferase I (CPT-I, EC 2.3.1.21) and mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase (HMG-CoA synthase, EC 4.1.3.5), enzymes central to ketogenesis, are upregulated in newborn suckled rat liver (10, 32) and small intestine (11). The dramatic fall of insulin coupled to rising glucagon and nonesterified fatty acids (NEFA) in the circulation of rat pups after birth (10) appears to spur gene expression for these enzymes, as the genes encoding these proteins are sensitive to the prevailing hormonal and fatty acid milieu (5, 11, 32).

The extent to which this model applies across mammalian taxa is not firmly established. The suckling piglet, for example, does not develop the neonatal ketonemia evident in some species (2, 27) and may not display a postpartum pancreatic hormone shift equivalent to the rat (27, 37). The piglet's negligible capacity for hepatic ketogenesis (1, 2, 8, 20, 24, 28) could be the result of unique differences in liver activities of mitochondrial HMG-CoA synthase and/or CPT-I, the former being negligible around birth (3, 8) and the latter being particularly sensitive to malonyl-CoA inhibition (8). Interestingly, recent characterization of the end products derived from medium- (MCFA) or long (LCFA)-chain fatty acid β-oxidation in piglet liver revealed that acetogenesis far surpasses ketogenesis in vitro (1, 20, 24), a pattern opposite that observed in rats (1, 35). However, the physiological relevance of acetogenesis vs. ketogenesis during the neonatal period of piglets could not be determined from those studies. Furthermore, despite evidence of in vitro fatty acid carbon flux to acetate in the liver of piglets (1, 20, 24) and rats (19, 25, 34, 35), the prospect that mitochondrial acetogenesis is analogous to ketogenesis has not been fully explored.

From a physiological standpoint, active acetogenesis and low ketogenesis in vitro suggest that acetate turnover more profoundly impacts piglet metabolism than do the ketone bodies. If true, this scenario would differ substantially from models in which ketone bodies serve as important glucose-sparing fuels and potential brain lipid precursors during neonatal development (see Ref. 10). The current studies were designed to gain insight into the physiological roles of acetate in the newborn piglet model and to explore the possibility that mitochondrial acetogenesis (like hepatic mitochondrial ketogenesis in rats) increases concomitant with elevated mitochondrial β-oxidation in vivo. As a first approach, plasma concentrations of acetate and ketone bodies were compared during the first 48 h of life in piglets. Circulating acetate levels were also determined in neonatal rats, animals with substantial ketogenic capacity during the neonatal period (10). A second set of experiments measured the kinetics of endogenous (nonfermentative) acetate in piglets determined in the absence or presence of the mitochondrial fuel hexanoate to measure basal acetate rate of appearance (R_a) and the impact of mitochondrial β-oxidation upon R_a, respectively. Results showing comparatively elevated plasma acetate and a basal acetate R_a reflecting substantial production/utilization are consistent with an important physiological role for acetate vs. ketone bodies in piglets. However, some aspects of acetate metabolism in piglets draw into question the extent of analogy between acetogenesis and ketogenesis under the current experimental conditions.

MATERIALS AND METHODS

Neonatal Changes in Circulating Acetate and Ketone Bodies

Animals and blood sampling. All work was approved by the University of Illinois Laboratory Animal Care Advisory Com-

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mittee. For the study of changes in acetate and ketone body concentrations over the first 2 days postpartum in swine, commercial crossbred piglets (birthweight 1.85 ± 0.07 kg) were identified from attended farrowings and assigned randomly to the following groups: newborn unsuckled (0.5–2 h old), 24 h old unsuckled, 24 h old suckled, and 48 h old suckled. Animals left unsuckled 24 h were confined to farrowing crates with access to a heat lamp. At the respective time points, blood was sampled by jugular venipuncture into heparinized Vacutainer tubes (Becton-Dickinson, Rutherford, NJ). Blood was placed in ice until centrifugation at 5,000 *g* (4°C) to obtain plasma, which was stored at −75°C. Aliquots were processed for determination of acetoacetate (AcAc; see below) before freezing. For comparative analyses of circulating acetate concentrations, pooled serum samples derived from 24- and 48-h-old suckling rat pups also were obtained after decapitation.

Analysis of volatile fatty acids and ketone bodies. Samples were analyzed using a slight modification of the gas-liquid chromatography (GLC) method of Rémésy and Demigné (33). To precipitate protein and extract volatile fatty acids (VFA), ~2–3 vol of ice-cold 100% ethanol were added to samples (450–700 µl) combined with 0.1 µmol of isobutyric acid internal standard. After centrifugation (10 min, 13,000 *g*, 4°C), the supernatant fractions were transferred to microcentrifuge tubes containing 6 µmol of NaOH, yielding a final pH of ~8. Contents were dried using a Speedvac (Savant, Farmingdale, NY). Just before GLC analysis, samples were redissolved in 80 µl of ice-cold 6.25% metaphosphoric acid and clarified by centrifugation (15 min, 4,000 *g*, 4°C) through 0.45-µm nylon microcentrifuge tube filters (Lida Manufacturing, Kenosha, WI). Samples were transferred to 200-µl autosampler vial inserts placed in crimp-top vials and loaded into an HP 7673 autosampler attached to an HP 5890 Series II GLC instrument (Hewlett-Packard, Wilmington, DE).

Samples (2 µl) were injected into the gas chromatograph, which employed the following conditions: a Supelco (Bellefonte, PA) GP 10% SP-100/1% H_3PO_4 on 80/100 Chromosorb WAW column (6 ft × 4 mm ID), N_2 carrier (73 ml/min), injector temperature 175°C, oven temperature 125°C, and a flame-ionization detector (FID) set at 180°C. Conversion factors (µM/FID response peak area) and assay linearity were determined each day from authentic VFA mixed standards (fatty acid chain lengths C2-C5, including isobutyric acid) covering the range of sample concentrations. Water blanks were chromatographed between each sample. No loss of acetate or isobutyrate was observed in samples through at least 12 h or in mixed standards in crimp-top vials over 5 days at room temperature (data not shown). The method separated VFA and MCFA through at least hexanoate with high resolution (not shown). The coefficient of variation of duplicate sample analyses averaged 5.4 ± 0.4%.

To assess the potential for artifactual elevation of acetate concentration due to contamination from reagents or other sources, equivalency of acetate and isobutyrate recovery was checked via the following procedures. Mixed standards were processed using the normal protocol, and recovery of individual VFAs was calculated by comparison with directly injected standards. Recovery was consistently 10% higher for acetate vs. isobutyrate, confirmed by recovery of radioactivity in a subset in which tracer sodium [1-^{14}C]acetate (Research Products International, Mt. Prospect, IL) was added with standards before processing. Thus all reported values have been adjusted to account for this difference, with mean adjusted acetate recovery equal to 74 ± 1% over all samples (average recovery of radioactivity in 2 plasma samples spiked with sodium [1-^{14}C]acetate was 72%). No contamination of acetate in assay or blood collection reagents was detected by GLC. Using this method of VFA analysis, plasma acetate concentration in samples obtained from overnight-fasted adult humans (71 ± 6 kg; $n = 4$) was consistent with the literature (73 ± 8 µM; e.g., see Ref. 31).

Plasma β-hydroxybutyrate (β-OHB) concentration in piglets was measured as described previously (2), with AcAc concentration measured as the difference in β-OHB concentration between assays of matched samples with or without treatment by β-OHB dehydrogenase plus NADH.

Acetate Kinetics

Animals and surgery. To facilitate measurement of acetate kinetics and hexanoate oxidation, neonatal piglets were fitted with infusion and sampling catheters (see below). On the morning of surgery (~0900), six normal-weight commercial crossbred piglets from a litter born the previous night were randomly selected, transported to the lab, and maintained in boxes with access to a heat lamp. Surgeries were completed by 1800 when animals were ~15–21 h old, and piglets were fasted until initiation of experiments the following afternoon at 1500 (~36–42 h of age; mass 1.55 ± 0.09 kg). Animals were provided with 20–30 ml of water by gastric intubation the evening before and morning of infusions.

For surgery, piglets were anesthetized with 2% isoflurane (Anaquest, Madison, WI) in O_2 (15). A 3½ French umbilical catheter (Sherwood Medical, St. Louis, MO) was inserted into the umbilical artery and advanced 22 cm up the abdominal aorta so that the tip was located at the aortic arch (23). The catheters were subsequently flushed with heparinized physiological saline (5 U/ml) and secured to the umbilicus using suture. This line was used for infusion. A blood sampling catheter was inserted into the left jugular vein to the level of the heart via a cut down to this vessel (38). All procedures were carried out under semisterile conditions using pividone-iodine 10% and ethanol sterilization of surgical fields and instruments. Both catheters were placed along the back of the animal and secured using surgical wrap and tape. Catheters were maintained with heparinized saline, and internal placement of each catheter was confirmed by visual inspection upon termination of the experiment.

Infusion protocol. The aim of this study was to determine steady-state, nonfermentative acetate R_a, as well as alterations of acetate R_a induced by mitochondrial MCFA β-oxidation in neonatal fasted piglets. Piglets from a given litter were randomly assigned to receive a continuous infusion of a tracer dose of sodium [^{3}H]acetate (controls; ICN Biomedicals, Irvine, CA) or sodium [^{3}H]acetate coinfused with sodium [1-^{14}C]hexanoate (3 piglets/treatment). The experiment was replicated using two litters on separate days for a total of six piglets per treatment. Animals were placed in a warmed six-chamber metabolic apparatus, and catheters were connected to lines exiting the chambers through airtight septa (14). The infusion (umbilical) lines were attached to an eight-channel peristaltic pump (Cole-Parmer, Niles, IL) set to deliver 10 ml/h from attached intravenous bags (Baxter Healthcare, Deerfield, IL) containing infusate. Animals were infused with physiological saline for a 1-h equilibration period before initiation of tracer continuous infusion. Blood was sampled at intervals from the jugular catheter (limited to 13 ml withdrawn/pig for the entire experiment). Blood was maintained on ice and promptly centrifuged at 4°C to obtain plasma, which was frozen at −75°C.

Preliminary experiments indicated that plasma acetate specific activity (SA) in fasted piglets reaches steady state by 120 min upon initiation of a continuous infusion of sodium [^{3}H]acetate (not shown), so blood collection for determination

of acetate SA (see below) was begun at 90 min of infusion. Collections were then carried out at 130, 170, 210, 250, 290, 330, 370, and 410 min, which corresponded to the midpoint of each 20-min CO_2 collection (see below). Animals were infused for 420 min with a tracer (48 $\mu Ci \cdot h^{-1} \cdot kg^{-1}$) of sodium [^{3}H]acetate in physiological saline or coinfused with sodium [^{3}H]acetate plus sodium [1-^{14}C]hexanoate (American Radiolabeled Chemicals, St. Louis, MO) tracer diluted with nonradioactive sodium hexanoate (SA of the latter was 226 µCi/mol, delivered at 0.74 $\mu Ci \cdot h^{-1} \cdot kg^{-1}$). Sodium hexanoate substrate was prepared by neutralizing the free acid of hexanoate (Sigma, St. Louis, MO) with an equimolar amount of NaOH in water at a final concentration of 474 mM and adjusted to pH 7.4. The infusion rate of sodium hexanoate (3.27 $mmol \cdot h^{-1} \cdot kg^{-1}$) was estimated to provide 100% of the expected metabolic rate (MR; 141 mmol $ATP \cdot h^{-1} \cdot kg^{-1}$ = 11.2 $kJ \cdot h^{-1} \cdot kg^{-1}$; see Refs. 14 and 23) of a typical neonatal MCFA-infused piglet weighing 1.5 kg, if all hexanoate were completely catabolized to CO_2. Oxidation and infusion rates of hexanoate in energy equivalents were calculated assuming a stoichiometry of 44 mmol ATP/mmol hexanoate and 79.5 J/mmol ATP (23). The 7-h time frame was chosen because steady-state oxidation of MCFA in fasted piglets is typically within 5 h of continuous infusion of MCFA (23).

Acetate SA and turnover. SA of plasma acetate was determined in two steps. Plasma acetate concentrations were determined by the GLC protocol described above. Samples were subsequently maintained at 4°C in crimp-top sealed vials until HPLC separation of acetate was performed ≤24 h later. Separation of radiolabeled acetate from other plasma metabolites (i.e., ketone bodies) was achieved through reversed-phase HPLC using chromatography conditions identical to those used previously (1, 20). Samples (40–45 µl) were manually loaded onto a 50-µl injection loop of the HPLC (Beckman System Gold HPLC Workstation; Beckman Instruments, San Ramon, CA) and subjected to chromatographic separation using isocratic elution with 0.3% H_3PO_4, pH 2.1 (flow rate 0.65 ml/min), and a Beckman Ultrasphere IP column (5 µm, 4.6 × 250 mm). For each sample, the eluent fraction containing acetate (1, 20) was collected into a scintillation vial using a fraction collector (ISCO Retriever II, Lincoln, NE), and ^{3}H radioactivity was determined after mixture with 5 ml scintillation cocktail (Bio-Safe II; Research Products International). Two additional fractions of equal volume were obtained at the beginning (2–3.5 min) and end (33–34.5 min) of each 40-min run and served as blanks. Radioactivity was measured concurrently in the ^{3}H and ^{14}C windows of a Beckman LS 6000IC scintillation spectrometer. In data not shown, it was demonstrated that determinations of ^{3}H radioactivity in acetate were not affected by [^{14}C]hexanoate radioactivity because *1*) in a subset of samples derived from hexanoate-infused animals, one distinct radioactive peak (visualized with in-line scintillation spectrometry; Radiomatics Flo-one; Packard Instruments, Meridian, CT) was observed with a retention time corresponding to acetate, and *2*) ^{14}C radioactivity values recorded in the acetate eluent fraction from samples derived from hexanoate-infused animals were negligible, as determined using dual-isotope counting procedures.

Steady-state R_a of acetate ($\mu mol \cdot min^{-1} \cdot kg^{-1}$) was calculated using an equation based on single-pool metabolite kinetics and using the constant infusion technique (39), calculated as

$$R_a = ([^3\text{H}]\text{acetate IR}) \div (\text{plasma acetate SA}) \quad (1)$$

where IR is infusion rate (µCi [^{3}H]acetate$\cdot min^{-1} \cdot kg^{-1}$) and SA is the individual animal's mean acetate SA (µCi/µmol acetate) at steady state. Steady state was attained when a plateau of acetate SA was observed at ≥130 min of infusion (see RESULTS).

CO_2 collections and hexanoate oxidation rate. The rate of hexanoate oxidation to CO_2 was assessed by measuring the quantity and SA of expired CO_2, assuming the 1-^{14}C-labeled carboxyl carbon marked the fate of the entire fatty acid molecule. As detailed elsewhere (14), air (2 l/min) was drawn through each respiration chamber after being cleared of atmospheric CO_2 via a sodasorb canister. At selected intervals, expired CO_2 was collected over 20 min and trapped in 75 ml of 1.8 M NaOH. Between CO_2 collections, airflow was diverted to empty columns, and NaOH was retrieved and replaced in the trapping columns. Collections were made during the following intervals after commencement of continuous tracer infusion (in minutes): 80–100, 120–140, 160–180, 200–220, 240–260, 280–300, 320–340, 360–380, and 400–420. The total amount of trapped CO_2 over 20 min and its SA (µCi/µmol) were determined after $BaCO_3$ precipitation of NaOH aliquots, and CO_2 production rate ($\dot{V}CO_2$, µmol/min) was calculated. The CO_2 content of air collected over 20 min from an empty metabolic chamber served as a blank. A transfer quotient (TQ), defined as the fraction of CO_2 derived from the oxidation of hexanoate, was estimated from SA values as follows

$$\text{TQ} = (\text{CO}_2 \text{ SA at steady state}) \div (\text{infusate hexanoate SA}) \quad (2)$$

Hexanoate oxidation (µmol/min) was calculated by multiplying the $\dot{V}CO_2$ by the TQ. Steady state was reached when a plateau of CO_2 SA was achieved at ≥280 min of infusion (see RESULTS), and each piglet's mean CO_2 SA and CO_2 production over this period were used to calculate individual hexanoate oxidation rates.

MR. An assessment of MR in piglets was necessary to estimate relative hexanoate oxidation and the potential contribution of acetate toward meeting the piglet's energy demands in vivo. Based on measured $\dot{V}CO_2$ in hexanoate-infused pigs and with the assumption of a respiratory quotient (RQ) of 0.75 for piglets oxidizing fatty acids, O_2 consumption ($\dot{V}O_2$, µmol/min) was calculated as follows

$$\dot{V}O_2 = \dot{V}CO_2 \div RQ \quad (3)$$

where RQ is defined as $\dot{V}CO_2/\dot{V}O_2$. For control piglets receiving no hexanoate, $\dot{V}O_2$ was estimated by measuring $\dot{V}CO_2$ in a subset of three animals and assuming an RQ of 0.929 for fasting 1-day-old piglets (12). From $\dot{V}O_2$ estimates, MR in terms of joules (J/min) or ATP turnover (mmol ATP/min) was calculated using the following conversion factors: 22.4 ml O_2/mmol O_2, 19.2 J/ml O_2, and 79.5 J/mmol ATP. Determinations of potential oxidation rate of acetate using R_a values assumed an energy content of 10 mmol ATP/mmol acetate if all acetate turned over was activated to acetyl-CoA and directly combusted in the tricarboxylic acid cycle.

Statistics. Differences in circulating metabolite concentrations across ages and/or species were assessed using a general linear models ANOVA procedure, followed by mean comparisons using a protected Fisher's least significant difference test when appropriate (SAS Institute, Cary, NC). Differences in various parameters between control and hexanoate-infused animals were assessed using *t*-tests. Means ± SE are reported.

RESULTS

Metabolites

Ketone bodies and acetate. Plasma acetate concentrations in piglets (Table 1) were between one and two orders of magnitude higher than total ketone body

Table 1. *Changes in circulating acetate and ketone body concentrations in neonatal piglets*

	Acetate, µM	β-Hydroxybutyrate, µM	Acetoacetate, µM	β-Hydroxybutyrate + Acetoacetate, µM
Newborn	311 ± 18 (6)	6 ± 1[a] (5)	≤1[a] (5)	7 ± 1[a] (5)
24 h Suckled	234 ± 75 (5)	14 ± 3[a] (4)	6 ± 1[b]* (4)	20 ± 2[b]† (4)
24 h Fasted	276 ± 54 (5)	8 ± 1[a] (8)	≤1[a] (8)	9 ± 2[a] (8)
48 h Suckled	343 ± 56 (5)	27 ± 5[b] (5)	9 ± 3[b] (5)	36 ± 7[c] (5)

Values are means ± SE; sample sizes are in parentheses. Values sharing the same letter within a column are not significantly different at $P < 0.05$. * and †, $P < 0.1$ vs. newborn and fasted or fasted, respectively.

concentrations over the first 48 h of life, with ketone bodies remaining remarkably low (<40 µM) regardless of suckling status. There was a significant rise in ketone bodies with suckling and age in piglets. Mean circulating acetate concentrations in suckled rat neonates aged 24 h (77 ± 9 µM) and 48 h (175 ± 41 µM) were lower ($P < 0.05$) than those determined for age-matched piglets (Table 1) and were of similar magnitude to those reported previously for rat pups (4, 16). Acetate concentration in piglets was not significantly different across sampling times.

Acetate Kinetics and Hexanoate Oxidation

Acetate R_a and potential contribution to MR. The temporal changes in plasma acetate SA in control and hexanoate-infused piglets are illustrated in Fig. 1*A*. Steady-state acetate turnover was achieved by ≥130 min, with individual coefficients of variation for steady-state SA averaging 8.9 ± 0.7%. It is notable that one hexanoate-infused piglet (*animal 4–2*) displayed a steady-state acetate SA markedly lower (~3-fold) than its counterparts (not shown), reflective of a much higher acetate R_a for that individual (Table 2). Despite this fact, mean acetate R_a in piglets oxidizing hexanoate remained just one-half that determined in controls (Table 2; mean R_a for hexanoate-infused piglets drops to 40% of controls if *animal 4–2* is excluded). Plasma acetate concentrations during the period of steady state for acetate were equivalent ($P > 0.1$) in controls (79 ± 4 µM) and hexanoate-infused piglets (109 ± 23 µM).

At steady state, the R_a of a substrate equals the rate of disposal (R_d; see Ref. 39). Should R_d reflect direct and complete combustion of acetate (see DISCUSSION), then acetate catabolism delivered a mean of 0.34 mmol $ATP \cdot min^{-1} \cdot kg^{-1}$ (26.8 $J \cdot min^{-1} \cdot kg^{-1}$) to control piglets (Table 2). This would correspond to almost 20% of the MR of the animals, with up to 28% in one subject. Estimated energy derived from acetate oxidation in hexanoate-infused piglets (0.16 mmol $ATP \cdot min^{-1} \cdot kg^{-1}$ or 12.6 $J \cdot min^{-1} \cdot kg^{-1}$) was far lower, accounting for just 7% of the energy consumption rate of this group. With the use of RQ estimates of 0.929 (12) and 0.75 for fasted control and hexanoate-infused piglets, respectively, calculated MR over the course of the entire experiment was somewhat lower in controls (Table 2).

Hexanoate oxidation. The infusion rate of hexanoate (equivalent to 2.35 mmol $ATP \cdot min^{-1} \cdot kg^{-1}$) was designed to deliver 100% of a typical fasted piglet's energy demands (see MATERIALS AND METHODS). Indeed, the infusion rate closely matched estimated MRs of hexanoate-infused piglets in this study (2.38 mmol $ATP \cdot min^{-1} \cdot kg^{-1}$, Table 2). Hexanoate combustion to CO_2 reached steady-state by 5 h of constant infusion, as reflected in the stable CO_2 SA observed in all hexanoate-

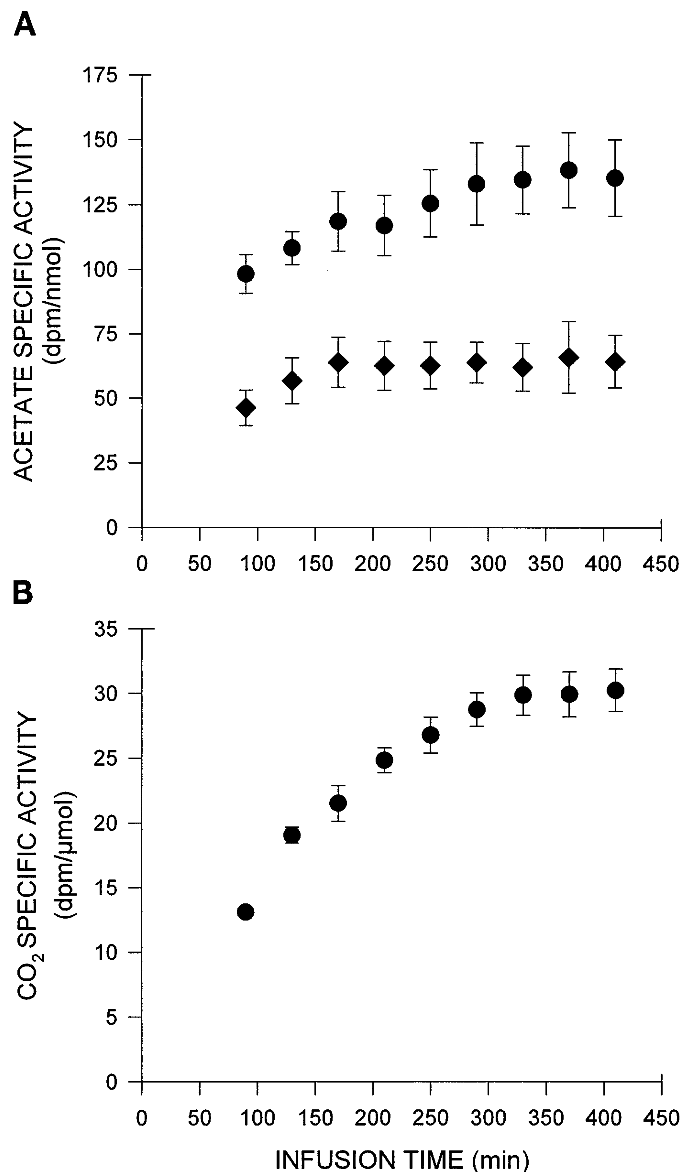

Fig. 1. Changes in specific activity (SA) of ^3H and ^{14}C radioactivity in plasma acetate (*A*) and expired CO_2 (*B*), respectively, after a continuous infusion of sodium [^3H]acetate (controls, ◆) or sodium [^3H]acetate plus sodium [1-^{14}C]hexanoate (●) in fasted piglets aged ~40 h (see MATERIALS AND METHODS for details). Steady-state SA for acetate was evident at ≥130 min for both groups, and steady-state CO_2 SA plateaued by ≥280 min postinfusion in animals given hexanoate. Values are means ± SE for each time point ($n = 6$).

Table 2. *Acetate kinetics, metabolic rate, and potential contribution of acetate oxidation toward meeting the energy needs of fasted neonatal piglets*

	R_a Acetate, $\mu mol \cdot min^{-1} \cdot kg^{-1}$	CO_2 Production, $\mu mol \cdot min^{-1} \cdot kg^{-1}$	Estimated Metabolic Rate, $J \cdot min^{-1} \cdot kg^{-1}$ (mmol ATP $\cdot min^{-1} \cdot kg^{-1}$)	Potential Acetate Energy: $J \cdot min^{-1} \cdot kg^{-1}$ (mmol ATP $\cdot min^{-1} \cdot kg^{-1}$)	Potential Acetate Energy: % metabolic rate
Controls					
Mean	33.6*	322	149.2† (1.88)†	26.8* (0.34)*	18.0*
SE	4.0	6	3.0 (0.04)	2.9 (0.04)	2.2
Range	22.5–50.9	308–335	142.6–155.1 (1.79–1.95)	17.9–40.5 (0.23–0.51)	11.5–28.4
Hexanoate-infused					
Mean	15.9	329	189 (2.38)	12.6 (0.16)	6.9
SE	2.7	22	12 (0.16)	2.0 (0.02)	1.2
Range	9.4–27.9	277–431	154–247 (1.94–3.11)	7.5–15.0 (0.09–0.28)	3.8–11.7

Values are for 6 piglets/group, but CO_2 production in control animals was determined in a subset of 3 animals. R_a, rate of appearance. Mean CO_2 production or metabolic rate was measured over the entire infusion period. See text for calculations of estimated metabolic rate and potential acetate energy ("potential" denotes values expected if R_a = direct combustion of acetate to form ATP, after activation to acetyl-CoA). * $P < 0.01$ and † $P < 0.05$ vs. hexanoate-infused group.

infused animals between 280 and 420 min of collection (Fig. 1*B*), throughout which the individual coefficient of variation for CO_2 SA averaged 3.3 ± 0.8%. Hexanoate oxidation estimated from CO_2 SA provided an estimated 30–40% of the energy needs of the piglets (Table 3), despite infusion at a rate that delivered an amount of hexanoate energy equivalents matching the MR of the piglets (see above). The lack of equivalence between the infused rate of 1-^{14}C fatty acid carbon and its subsequent recovery CO_2 in piglets (this study and Refs. 14, 15, 23) may in part be explained by factors such as CO_2 recycling, incorporation of radiolabel into metabolic pools with slow turnover, or incomplete oxidative catabolism of MCFA in the fasting piglet model. The first two possibilities are discussed in greater detail elsewhere (23, 39).

DISCUSSION

An increasing body of evidence supports the idea that certain aspects of fatty acid metabolism in the neonatal piglet stray significantly from traditional paradigms. Of particular note is the ketogenic deficit in piglets (1, 2, 8, 20, 24, 28), which would be expected to promote "alternative," nonketogenic pathways of fuel carbon flow in the liver. Our efforts to characterize such phenomena through analyses of metabolic end products of hepatic fatty acid oxidation in vitro have demonstrated that ketogenesis is supplanted by acetogenesis and other routes that await further clarification (1, 20, 24). The current set of experiments represents a first attempt to pinpoint the relevance of acetogenesis vs. ketogenesis for neonatal piglets in vivo and to probe the

Table 3. *Steady-state hexanoate (C6:0) oxidation in fasted piglets during infusion of sodium [1-^{14}C]hexanoate over 7 h (see text for details)*

Animal	Estimated Metabolic Rate, $J \cdot min^{-1} \cdot kg^{-1}$ (mmol ATP $\cdot min^{-1} \cdot kg^{-1}$)	C6:0 Oxidation Rate, $\mu mol \cdot min^{-1} \cdot kg^{-1}$	Energy from C6:0 Oxidation, $J \cdot min^{-1} \cdot kg^{-1}$ (mmol ATP $\cdot min^{-1} \cdot kg^{-1}$)	C6:0 Energy, % metabolic rate
4-1	114.0 (1.43)	12.1	42.4 (0.53)	37.2
5-1	195.6 (2.46)	19.3	67.4 (0.85)	34.4
6-1	244.9 (3.08)	20.3	71.0 (0.89)	29.0
4-2	197.3 (2.48)	21.2	74.2 (0.93)	37.6
5-2	192.1 (2.42)	22.4	78.2 (0.98)	40.7
6-2	141.7 (1.78)	14.6	51.0 (0.64)	36.0
Mean ± SE	180.9 ± 18.9 (2.28 ± 0.24)	18.3 ± 1.6	64.0 ± 5.8 (0.81 ± 0.07)	35.8 ± 1.6

Data for estimated metabolic rate based on CO_2 production during period of C6:0 steady-state oxidation (280–420 min infusion). See text for calculations of estimated metabolic rate, C6:0 oxidation rate, and energy from C6:0 oxidation.

analogy between ketogenesis and acetogenesis with enhanced provision of fatty acids in these animals.

Acetate Metabolism During the Neonatal Period

The data presented herein are fully consistent with an important physiological role for acetate (e.g., as a fuel source or lipid precursor) in neonatal piglets, especially in light of the minimal metabolic impact of ketone bodies in this model (38). Circulating acetate concentrations over the first 2 days of life, for instance, are one to two orders of magnitude higher than ketone bodies in piglets (Table 1). In contrast, postnatal acetate values in ketogenic rat pups (~100–200 μM; also see Refs. 4 and 16) are ~10-fold lower than the ketone body levels reported for these neonates (see Ref. 10). Whether comparisons of circulating acetate and ketone body levels reflect differences in utilization of these metabolites in piglets could not be directly assessed from plasma concentration alone. However, we have previously determined that, in the physiological range, plasma β-OHB concentration and its oxidation are related in a linear fashion in piglets (38). Based on the latter relationship, β-OHB was a trivial fuel in piglets used for the current study, accounting for <1% of the energy budget at the low ketone body concentrations observed (Table 1). Ketone bodies are generally considered to be important metabolites in neonatal fuel homeostasis (10).

Although the kinetics of ketone bodies in piglets have not been described, the substantial R_a of endogenously produced acetate in fasting piglets (22–51 $\mu mol \cdot min^{-1} \cdot kg^{-1}$; Table 2) rivals that of glucose in 48-h-old fasted piglets (23–31 $\mu mol \cdot min^{-1} \cdot kg^{-1}$; see Refs. 29 and 30). This signals the presence of an active acetogenic pathway in vivo, which might support interorgan fuel carbon trafficking. Should steady-state R_a values for acetate (Table 2) and glucose (29, 30) reflect oxidative disposal in fasted piglets, then acetate potentially met ~20% (Table 2), and glucose 45–60%, of the ATP requirements of piglets in the current study. The significant energy potential of fuels such as glucose, amino acids, and perhaps acetate may be especially important to the survival of fasting neonatal piglets, in which circulating NEFA (27, 37) and ketone bodies (Table 1; Refs. 2 and 27) are trifling.

Finally, it is relevant to note that energy potential calculations for acetate (Table 2) would be shifted downward if cycling between acetate and acetyl-CoA (6) is significant in piglets. Cycling mandates that multiple rounds of acetate activation occur before combustion; thus, net ATP yield per mole of acetate oxidized would fall concomitant with the degree of cycling. In control piglets, estimates of acetate oxidation as a percentage of MR (Table 2) would also be affected by differences in RQ assumptions, becoming smaller with a lower RQ. Research defining true flux of acetate carbon to CO_2 in vivo (and studies examining the magnitude of acetate/acetyl-CoA cycling) will be required to fully define the contribution of acetate oxidation toward meeting the fasting or suckling piglet's energy demands.

Etiology of Endogenous Acetogenesis in Piglets

Biogenesis of acetate, occurring in a variety of mammalian tissues, is thought to be contingent upon relative activities of acyl-CoA hydrolases and synthetases toward acetyl-CoA and acetate, respectively (4, 6, 7, 16). Although control over acetogenesis is poorly understood, acetyl-CoA hydrolysis might be expected to rise concomitant with an increase in substrate availability. Descriptions of fatty acid metabolism typically emphasize increased acetyl-CoA availability concurrent with elevated fatty acid β-oxidation in mitochondria (e.g., Ref. 21). An attendant rise in ketogenic flux may act to undergird accelerated hepatic β-oxidation through disposal of acetyl-CoA, liberation of CoASH, and exportation of abundant reducing equivalents. Does mitochondrial acetogenesis, a pathway that consumes acetyl-CoA, regenerates CoASH, and yields a readily exportable fuel, share some analogy to ketogenesis, and would such an association become more apparent in a poorly ketogenic animal? Although fatty acids can give rise to acetate (1, 19, 20, 24–26, 34, 35), a definitive correlation between acetogenesis and oxidation of these fuels has not been demonstrated.

MCFA bypass regulation of β-oxidation at the level of mitochondrial CPT-I by virtue of their diffusibility, thus eliciting a robust ketogenic response in a number of animals in vivo, but not in piglets (2). Indeed, when piglets were infused with MCFA at maximal rates (23), steady-state ketone body concentrations never exceeded 0.1 mM and could not account for >2–3% of energy expenditure (38). We therefore hypothesized that, in the piglet, abundant provision of hexanoate would stimulate mitochondrial acetogenesis if tissue acetate production emulates hepatic ketogenesis. On the contrary, despite active combustion of hexanoate (Fig. 1 and Table 3), acetate R_a was curtailed to a level just one-half that of controls (Table 2). This result clearly indicates that improved fatty acid substrate availability is not associated with accelerated acetate production at this rate of hexanoate delivery in piglets, and thus under these conditions acetogenesis does not mimic ketogenesis.

Although any explanation for these results is tentative, some interesting observations about piglet metabolism warrant consideration. Exposure of piglet hepatocytes to 1 mM MCFA in vitro elicits a drop of up to 50% in total hepatocyte acetyl-CoA concentration (20). Furthermore, in vivo infusion of octanoate to piglets at a rate similar to that of hexanoate in the current work resulted in a diminution of hepatic acetyl-CoA levels to 15% of control values (15). It is thus plausible that a drop in hepatic or extrahepatic tissue acetyl-CoA concentration in animals infused with hexanoate could have occurred, thereby limiting acetyl-CoA hydrolase substrate availability and/or altering the dynamics of acetate/acetyl-CoA cycling. Further studies will be required to assess how changes in fatty acid availability impact tissue acetyl-CoA levels in pigs and to ascertain if such changes affect acetogenesis.

The fact that oxidation of hexanoate resulted in marked changes in acetogenesis (Table 2) adds to a growing body of evidence supporting an important role for mitochondria in acetate generation in the piglet. Acetyl-CoA hydrolase activity has been demonstrated in liver, heart, and kidney mitochondria of pigs (18, 22; X. Lin and J. Odle, unpublished results). In addition, chain-length restrictions for peroxisomal β-oxidation (17, 25) dictate that hexanoate is an exclusively mitochondrial fuel, yet this and other MCFA clearly impact acetogenesis in piglets (this study and also Ref. 20) and rat tissues (19, 34, 35). Finally, inclusion of tricarboxylic acid cycle or β-oxidation inhibitors with incubations of piglet liver preparations modulates acetogenesis from fatty acids upward and downward, respectively (1, 20). The cytosol (7) and peroxisomes (13, 19, 25) of tissues also carry out acetogenesis, but their contribution to acetate production in the current study is not known.

In conclusion, results from studies in piglets suggest that acetogenesis must be considered when attempting to optimize the current understanding of neonatal metabolism. The basis for the metabolic profile of piglets remains to be fully elucidated, but unique characteristics of enzyme systems and hormones appear to be involved. For instance, poor ketogenesis in piglets is likely related to the lack of a postnatal induction of mitochondrial HMG-CoA synthase in which hepatic activity (3, 8) and mRNA abundance in liver (and small intestine; see Ref. 3) remain negligible, contrasting with observations in neonatal rats (10, 11, 32). The postnatal induction of HMG-CoA synthase and liver CPT-I gene expression/activity in rats is supported by the precipitous and stable drop in the insulin-to-glucagon ratio after birth (10, 32). In the piglet, initiation of suckling over the first days of life markedly increases plasma insulin (27, 37), which may influence fuel carbon flux through alterations in gene expression and/or more direct effects upon enzyme activities.

The prospect that acetate more profoundly impacts piglet metabolism vs. ketone bodies is supported by the results of this study. Like ketone bodies, acetate could represent a viable fatty acid precursor and/or fuel source for developing piglets. With respect to its production, on the other hand, the analogy between nonfermentative acetogenesis and ketogenesis is not strong under the conditions tested. For example, initiation of suckling in rats is associated with a sharp rise in blood ketone bodies concomitant with increased fatty acid availability and β-oxidation (10). Acetate levels in piglet plasma were equivalent regardless of age or nutritional status (Table 1), and acetate R_a was substantial in animals fasted ≥30 h (Table 2), despite large differences in plasma NEFA concentration reported for newborn, suckled, and fasted piglets (27, 37). Numerous acetyl-CoA generators, notably pyruvate and lactate, could give rise to acetate. These metabolites are relatively abundant in the blood of suckling (pyruvate, ~250 μM; lactate, ~3 mM) or fasting (pyruvate, ~500 μM; lactate, ~5 mM) piglets (27), possibly maintaining acetate production independent of tissue fatty acid β-oxidation. Indeed, a dissociation between acetogenesis and ketogenesis has been reported when ketogenic flux from LCFA was altered over a wide range in perfused rat liver (9, 36). Thus it appears that endogenous mitochondrial acetogenesis may represent a pathway in which operation is dictated largely by maintenance of an adequate acetyl-CoA pool, independent of its source.

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