Relationship between hepatic fatty acid oxidation and gluconeogenesis in the fasting neonatal pig

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Hepatocytes were isolated from sixteen fasting neonatal pigs and used in two experiments: (1) to determine the effect of various factors on the ability for hepatic oxidation of fatty acids and (2) to clarify the relationship between fatty acid oxidation and glucose synthesis. In Expt 1, newborn pigs were either fasted from birth for 24 h or allowed to suck *ad lib.* for 3 d followed by a 24 h fast. In the presence of pyruvate, oxidation of octanoate (2 mM) was about 30-fold greater than oleate (1 mM) regardless of age, but glucose synthesis was not enhanced beyond that observed for pyruvate alone. Inclusion of carnitine (1 mM), glucagon (100 nM) or dibutryl cAMP (50 μM) in the incubation media did not stimulate either fatty acid oxidation (octanoate or oleate) or glucose synthesis. Extending the period of fasting to 48 h (Expt 2) failed to enhance the fatty acid oxidative capacity or glucose synthesis rate. Likewise, the redox potential of the gluconeogenic substrate (lactate *v.* pyruvate) did not influence glucose synthesis regardless of the oxidative capacity exhibited for fatty acids. These data indicate that fatty acid oxidative capacity is not the first limiting factor to full expression of gluconeogenesis in hepatocytes isolated from fasted newborn pigs.

Hepatocytes: Fatty acid oxidation: Gluconeogenesis: Neonatal pig

The newborn pig that does not establish a regular pattern of sucking develops hypoglycaemia within 24 h after birth (Swiatek *et al.* 1968; Gentz *et al.* 1970). Hepatic glycogen reserves are relatively abundant and mobilizable (Mersmann, 1971; Boyd *et al.* 1978; Martin *et al.* 1980; Pegorier *et al.* 1981), but the capacity for gluconeogenesis is constrained unless the animal is allowed to suck *ad lib.* (Pegorier *et al.* 1982). The fact that gluconeogenic capacity of the nursing newborn pig is comparable with older counterparts and sufficient to maintain normal blood glucose concentrations suggests that some limiting factor(s) is provided via colostrum or milk. Possible limitations to gluconeogenesis in the fasting newborn pig include fatty acid supply, fatty acid oxidative capacity and/or failure to adequately cycle glucogenic precursors.

Innovative studies have been reported relative to the partition of fatty acids toward the esterification *v.* oxidative pathways and the impact of oxidation (or lack thereof) on gluconeogenesis in neonatal pigs (Pegorier *et al.* 1983; Duee *et al.* 1985). However, a systematic evaluation of the various factors that potentially limit both processes in the newborn pig is lacking. Previous investigations in our laboratory demonstrated that even a very limited intake of colostrum or medium-chain triacylglycerols improves the glucoregulatory hormone pattern and glucose status of the newborn relative to fasted pigs (Lepine *et al.* 1989*a*, *b*). Furthermore, colostrum or milk intake enhanced hepatic glucose

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synthesis (Pegorier *et al.* 1982; Lepine *et al.* 1991) but the mechanism for this response remains unknown. The objective of this research was to evaluate potential limitations in glucose synthesis by (1) systematically investigating factors that potentially affect fatty acid oxidation and gluconeogenesis and (2) clarifying the relationship between fatty acid oxidation and hepatic gluconeogenesis in fasting neonatal pigs.

MATERIALS AND METHODS

Animals and treatments

Expt 1. Parturition was induced in four crossbred sows (Yorkshire × Landrace females mated to Duroc sires) by a 10 mg intramuscular injection of prostaglandin $F_{2\alpha}$ (Lutalyse; The Upjohn Co., Kalamazoo, MI, USA) at 08.00 hours on day 110 of gestation followed approximately 24 h later by a 40 IU intramuscular injection of oxytocin. One neonatal pig (1·32–1·41 kg body weight) was removed from each sow before sucking, weighed, housed individually in an environmentally controlled room ($32 \pm 1°$) and fasted for 24 h. One additional contemporary neonatal pig from each sow was weighed at birth, allowed to suck from the sow *ad lib.* for 3 d and subsequently housed in an environmentally controlled room for 24 h fasting period as previously described. All pigs were provided water *ad lib.* during fasting. Isolated hepatocytes were prepared from each pig following the completion of the fasting period.

Expt 2. Neonatal pigs were obtained from first, second and third parity crossbred sows induced to farrow as described in Expt 1. Four neonatal pigs were removed from the sow, weighed, housed individually in an environmentally controlled room ($32 \pm 1°$) and fasted for 24 h. Four additional neonatal pigs were weighed at birth, allowed to suck from the sow *ad lib.* for 3 d then fasted for 48 h in an environmentally controlled room. Water was provided *ad lib.* during the fasting period. Isolated hepatocytes were prepared from each pig following fasting.

Hepatocyte preparation

The procedure for hepatocyte isolation was based on the original method of Berry & Friend (1969) and was similar to that described by Clark *et al.* (1976). Neonatal pigs were secured in a dorsal recumbent position following general anaesthesia with 2-bromo-2-chloro-1,1,1-trifluoroethane (Halothane; Ayerst Laboratory Inc., New York, NY, USA). The peritoneal cavity was exposed by a lateral incision beginning at the lower abdomen proceeding anteriorly to the diaphragm, traversing contralaterally along the margin of the rib cage, then proceeding inferiorly to the lower abdomen. The diaphragm was not punctured during this procedure. Heparin (500 USP units/kg body weight) was injected into the portal vein via a 25-gauge needle. The pleural cavity was rapidly opened allowing two loose ligatures to be placed around the inferior vena cava. A cannula (polyethylene tubing 3 mm o.d.) was inserted into the inferior vena cava through a small incision anterior to the ligatures and secured by tightening the ligatures. Severing the portal vein allowed a non-recirculating, retrograde perfusion of Ca-free Krebs–Henseleit bicarbonate buffer (equilibrated with O_2–CO_2 (95:5, v/v) to flush the blood from the liver *in situ*. The liver was gently massaged to expedite the flushing of blood. About 1 min elapsed from the opening of the pleural cavity to flushing of the liver with oxygenated Ca-free Krebs–Henseleit perfusion buffer, thereby minimizing the potential for hepatocyte anoxia. The liver was carefully dissected from the pig and placed on the supporting platform of a perfusion apparatus, similar to that described by Krebs *et al.* (1974), which comprised a water-jacketed oxygenator for warming and oxygenating the perfusion media, a peristaltic pump (Masterflex Variable Speed Pump; Cole-Parmer Instrument Co., Chicago, IL, USA) and a water circulator

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(1419E Thermomix Constant Temperature Circulator; Ranin Instrument Co. Inc., Woburn, MA, USA). The perfusion was modified to a recirculating perfusion, with fresh, oxygenated, Ca-free Krebs–Henseleit bicarbonate buffer containing collagenase (*EC* 3.4.24.3) (Worthington Life Science Products, Malvern, PA, USA) at a final concentration of 0·25 mg/ml. The liver was perfused (90 ml/min, 37°) for 20–25 min until adequately digested, as evidenced by a jelly-like consistency. The digested liver was minced in a beaker containing fresh, Ca-free Krebs–Henseleit bicarbonate buffer and filtered through a 250 μm nylon mesh to remove any undigested tissue. The resulting cell suspension was centrifuged at low speed (320 ***g***) for about 30 s, the supernatant discarded and the cell pellet washed three times in Ca-containing Krebs–Henseleit bicarbonate buffer to remove non-parenchymal cells, collagenase and residual blood. The final cell pellet was weighed and resuspended with eight volumes of Ca-containing Krebs–Henseleit bicarbonate buffer.

In vitro *treatments*

Expt 1. Hepatocytes (about 6–8 $\times$ 10^6 cells) were incubated with selected substrates and Ca-containing Krebs–Henseleit bicarbonate buffer in a final volume of 3 ml in 25 ml polycarbonate Erlenmeyer flasks. Substrate concentrations used were previously determined as optimal by concentration response curves (Lepine, 1988). Glucose synthesis was determined in flasks containing 10 mM-pyruvate and 0·25 μCi [3-^{14}C]pyruvate (Amersham Corp., Arlington Heights, IL, USA). Selected flasks contained either octanoate (2 mM) or oleate (1 mM) in the presence or absence of glucagon (100 nM), L-carnitine (1 mM) or dibutryl cAMP (50 μM) (Sigma Chemical Co., St Louis, MO, USA). Oleate was bound to defatted bovine serum albumin (BSA; Sigma Chemical Co.) by the methods of Chen (1967) and Spector & Hoak (1969) before addition to the incubation media. All flasks contained defatted BSA at a final concentration of 25 mg/ml.

The gluconeogenic rate for 4-d-old pigs was determined only in incubation flasks containing [^{14}C]pyruvate alone or in the combinations containing carnitine (see Table 2) since it was anticipated that this would provide the most valuable age-related comparison. Fatty acid oxidation and acid-soluble product (ASP) formation were determined in flasks containing the substrates as described above, except that the radioactive label was either 0·44 μCi [1-^{14}C]oleic acid or 0·60 μCi [1-^{14}C]octanoic acid per flask (Amersham Corp.). Oxidation rate and ASP production for hepatocytes isolated from 4-d-old pigs were determined only in incubation flasks which included carnitine (see Table 1) for the reason previously indicated for gluconeogenic rate. Incubation flasks were flushed with O_2–CO_2 95:5, v/v) and sealed with a rubber stopper (gluconeogenic rate flasks) or a rubber stopper containing a centre well and filter paper (fatty acid oxidation flasks) and incubated at 37° for 60 min in a reciprocating water bath (100 oscillations/min).

Incubations were terminated by the addition of 3 M-$HClO_4$ (0·60 ml) to the incubation media. CO_2 in the fatty acid oxidation rate flasks was trapped by injecting 3·6 M-KOH (0·20 ml) through the stopper into the centre well containing a filter paper and allowing an additional 60 min incubation. The filter paper was removed to a scintillation vial containing 10 ml scintillation fluid (ACS II; Amersham Corp.) and counted in a liquid scintillation spectrophotometer (Isocap/300 6868 Liquid Scintillation System; Searle Analytical Inc., Des Plaines, IL, USA) for determination of $^{14}CO_2$ production. The contents of the incubation flasks were centrifuged at 1800 ***g*** for 10 min at 4°. A portion (0·5 ml) from incubations containing [1-^{14}C]oleate was removed to a scintillation vial containing 10 ml scintillation fluid for the determination of ASP formation. Incubation medium containing [1-^{14}C]octanoate was subjected to ether extraction to remove any unoxidized, non-BSA-bound [1-^{14}C]octanoate prior to ASP determination based on the procedure of Mannaerts *et al.* (1979). The supernatant from the gluconeogenic rate flasks was neutralized by the

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Table 1. *Effect of age and* in vitro *treatment on oxidative capacity and ketogenesis (acid soluble products; ASP) in hepatocytes from neonatal pigs fasted for 24 h (Expt 1)**

(Mean values for four pigs in each subgroup with their pooled standard errors)

	FA conversion to CO_2 (nmol FA/h per mg DNA)		FA conversion to ASP (nmol FA/h per mg DNA)	
Age of pig (d)...	1	4	1	4
[^{14}C]Oleate (18:1):				
+PYR	0·024	ND	0·040	ND
+PYR+glucagon	0·023	ND	0·040	ND
+PYR+carnitine	0·041	0·064	0·075	0·158
+PYR+glucagon +carnitine	0·047	0·059	0·082	0·142
+PYR+cAMP +carnitine	0·049	0·065	0·086	0·152
Pooled SEM	0·007		0·019	
[^{14}C]Octanoate (8:0)				
+PYR	0·731	ND	0·991	ND
+PYR+glucagon	0·738	ND	0·814	ND
+PYR+carnitine	0·712	1·052	0·756	1·343
+PYR+glucagon +carnitine	0·706	1·014	0·821	1·620
+PYR+cAMP +carnitine	0·763	0·994	0·774	1·577
Pooled SEM	0·089		0·389	
Statistical significance of the effect of fatty acid chain length (18:1 *v.* 8:0): *P*	< 0·001		< 0·01	

ND, not determined; FA, fatty acid; PYR, pyruvate; cAMP, dibutryl cAMP.
* For details of procedures, see pp. 82–85.

addition of 0·2 M-KOH in the presence of a pH indicator solution (Fisher Universal Indicator Solution: Fisher Scientific Co., Fair Lawn, NJ, USA). A portion (0·5 ml) was applied to an ion exchange column (0·8 × 10 cm, Dowex 1-8X, 100–200 mesh, Cl^- form) which was attached to a cation exchange column (0·8 × 10 cm, Dowex 50–8, 100–200 mesh, H^+ form) (Bio-Rad Laboratories, Richmond, CA, USA). Eluted glucose (total volume 7·5 ml following 2·5 ml void volume) was lyophilized, reconstituted to 1 ml volume and radioactivity determined using a liquid scintillation spectrophotometer. Ion exchange recovery rates for [^{14}C]glucose were in excess of 99%.

Glucose synthesis was calculated as radioactivity recovered in glucose relative to the initial specific activity of the precursor and was expressed per unit of DNA. DNA content of hepatocyte preparations was determined according to Burton (1956) and Tedesco & Mellman (1966). Cornell (1983) demonstrated that a decline in biochemical competence of isolated heopatocytes occurred with compromise of membrane integrity. The latter is manifested, for example, through an increase in the activity of the cytosolic enzyme lactate dehydrogenase (LDH; *EC* 1.1.1.27) in incubation media. This association was validated for hepatocytes prepared in our laboratory by the comparison of cellular ATP concentration and extracellular LDH activity through 105 min incubation (Lepine, 1988). Accordingly, metabolic competence of isolated hepatocyte preparations was quantitatively monitored by the determination of LDH activity pre- and post-incubation using the

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method of Bergmeyer & Bernt (1974). All hepatocyte preparations had extracellular LDH activities less than 10% and on this basis were considered viable.

Expt 2. Hepatocytes were isolated as previously described. Gluconeogenic rate was determined in flasks containing 10 mM-pyruvate or 10 mM-L-lactate with 0·25 μCi of [3-^{14}C]pyruvate or L-[U-^{14}C]lactate (Amersham Corp.). Selected incubations contained oleate (1 mM) or octanoate (2 mM) in combination with carnitine (1 mM). Fatty acid oxidation rate flasks contained radioactively labelled octanoate or oleate as described for Expt 1. Methods of sample assay were also identical.

Statistical analysis

Data were analysed by analysis of variance for the randomized block design using the Statistical Analysis System (SAS Institute Inc., 1982). Sources of variation were pig age, *in vitro* treatment and the respective interaction. *In vitro* treatments were factorially arranged within two main effects: fatty acid length (18:1 *v.* 8:0) and glucogenic substrate (pyruvate *v.* lactate). Treatment combinations were compared using pre-determined, non-orthogonal contrasts. In Expt 1, contrasts for fatty acid oxidation to CO_2 and ASP were: 18:1 *v.* 8:0, and sequential comparisons of pyruvate *v.* each sub-treatment within fatty acid type. Contrasts for glucose synthesis also included the comparison pyruvate *v.* each combination in sequence. In Expt 2, contrasts for glucose synthesis were: main effect of pyruvate *v.* lactate, and sequential comparisons of pyruvate or lactate alone *v.* individual subtreatments within each substrate. Fatty acid oxidation was compared by testing the main effect of chain length and then individual treatments within.

RESULTS

Expt 1. Oxidation of fatty acids were primarily a function of fatty acid chain length (Table 1). Production of CO_2 and ketone products (estimated from ASP as reported by Pegorier *et al.* 1983) from octanoate was 9–30-fold greater ($P < 0·001$) than from oleate regardless of age. Due to the difference in chain length, $^{14}CO_2$ and [^{14}C]ASP production from [^{14}C]octanoate would be approximately 2·25-fold greater than from [^{14}C]oleate at equal rates of fatty acid oxidation. Therefore, the data overestimate the magnitude of the difference but when this is taken into account the residual difference is considerable. Inclusion of carnitine in the incubation media approximately doubled the rate of CO_2 and ASP production from oleate, although this trend was not statistically ($P > 0·10$) significant.

Although octanoate was relatively oxidizable, glucose synthesis was not elevated beyond that observed for pyruvate alone (Table 2). Furthermore, additions of glucagon, dibutryl cAMP, and/or carnitine were similarly without effect ($P > 0·10$) for either 1- or 4-d-old pigs fasted for 24 h (Table 2).

Expt 2. It is possible that a 24 h fast following a 3 d nursing period was insufficient to evoke physiological changes sufficient for maximum expression. Consequently, a 48 h fast was imposed. The hepatic capacity for oleate oxidation and ketone production remained low at both 1 and 5 d of age, but tended to increase ($P > 0·05$, about 3–4-fold) for the older pigs (Table 3). In contrast, the rate of octanoate metabolism was considerably greater ($P < 0·001$) than for oleate and was unaffected by increasing age. The redox potential of the gluconeogenic precursor did not affect the rate of fatty acid oxidation.

Extending the fasting period to 48 h did not enhance the rate of hepatic glucose synthesis from either lactate or pyruvate (Table 4). It is not possible to compare the rates of precursor conversion to glucose since the ^{14}C-labelling of lactate and pyruvate carbon atoms was different. However, additions of dibutryl cAMP, octanoate or oleate (+carnitine) to the incubation media failed to improve glucose synthesis from either substrate. Furthermore, the presence of fatty acids tended to decrease gluconeogenic rates.

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Table 2. *Effect of age and* in vitro *treatment on glucose synthesis in hepatocytes from neonatal pigs fasted for 24 h (Expt 1)**

(Mean values for four pigs in each subgroup with the pooled standard error)

	Pyruvate conversion to glucose (μmol pyruvate/h per mg DNA)	
Age of pig (d)...	1	4
[^{14}C]PYR	7·12	5·40
[^{14}C]PYR + glucagon	7·96	ND
[^{14}C]Oleate (18:1):		
+ PYR	5·56	ND
+ PYR + glucagon	6·85	ND
+ PYR + carnitine	5·54	4·35
+ PYR + glucagon + carnitine	5·76	4·58
+ PYR + cAMP + carnitine	5·70	4·30
[^{14}C]Octanoate (8:0):		
+ PYR	5·08	ND
+ PYR + glucagon	4·75	ND
+ PYR + carnitine	4·95	4·09
+ PYR + glucagon + carnitine	5·35	4·66
+ PYR + cAMP + carnitine	5·12	4·92
Pooled SEM	0·85	
Statistical significance: effect of fatty acid chain length (18:1 *v.* 8:0)	$P > 0·10$	
effect of other treatments within fatty acid type	$P > 0·10$	

ND, Not determined; PYR, pyruvate; cAMP, dibutryl cAMP.
* For details of procedures, see pp. 82–85.

DISCUSSION

Fatty acid oxidation is low in the newborn of a number of species and increases after birth (Girard & Ferre, 1982). Similarly, oxidation of oleate and octanoate tended to increase in 4-d-old, 24 h fasted (Table 1) and 5-d-old, 48 h-fasted (Table 3) pigs compared to 1-d-old fasted pigs. Mersmann & Phinney (1973) reported that the capacity of the newborn pig to oxidize fatty acids is only 23% of that observed by 7 d of age, while Bieber *et al.* (1973) observed that the rate in the 1-d-old pig is only 32% of that exhibited by 24 d of age. This relative inability for fatty acid oxidation in the newborn pig is apparently a function of both a deficiency in mitochondrial numbers at birth and a lower capacity for long-chain fatty acid oxidation in general (Bieber *et al.* 1973). Mitochondrial proliferation occurs predominantly between 24 and 48 h after birth (Mersmann *et al.* 1972), while oxidative capacity increases markedly during the first 7 d (Mersmann & Phinney, 1973; Wolfe *et al.* 1978).

Fatty acid oxidation and ketone production from octanoate was 10–50-fold greater than from oleate (Tables 1 and 3) regardless of age or duration of fasting for neonatal pigs. Medium-chain fatty acids (e.g. octanoate) are not dependent on the carnitine palmitoyltransferase (*EC* 2.3.1.21) transport system for mitochondrial uptake, thereby suggesting that factors associated with transport (e.g. carnitine and glucagon) may limit oleate oxidation. Carnitine concentration is low in the liver of the newborn pig (Kerner *et*

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Table 3. *Effect of age, fatty acid (FA) length and glucogenic precursor redox potential on oxidative capacity and ketogenesis (acid soluble products, ASP) in hepatocytes from 1- and 5-d-old neonatal pigs fasted for 24 and 48 h respectively (Expt 2)**

(Mean values for four pigs in each subgroup with their pooled standard errors)

	FA conversion to CO_2 (nmol FA/h per mg DNA)		FA conversion to ASP (nmol FA/h per mg DNA)	
Age of pig (d)...	1	5	1	5
[^{14}C]Oleate (18:1):				
+PYR+carnitine	0·011	0·036	0·025	0·098
+LAC+carnitine	0·011	0·036	0·022	0·073
Pooled SEM	0·002		0·006	
[^{14}C]Octanoate (8:0):				
+PYR+carnitine	0·594	0·459	0·547	1·054
+LAC+carnitine	0·371	0·444	0·514	0·846
Pooled SEM	0·031		0·083	
Statistical significance of 18:1 *v.* 8:0; *P*	< 0·001		< 0·001	

PYR, pyruvate; LAC, lactate.
* For details of procedures, see pp. 82–85.

Table 4. *Effect of age, glucogenic precursor redox potential and fatty acid (FA) length on glucose synthesis in hepatocytes from 1- and 5-d-old neonatal pigs fasted for 24 and 48 h respectively (Expt 2)**

(Mean values for four pigs in each subgroup with the pooled standard error)

	Precursor conversion to glucose (μmol precursor/h per mg DNA)	
Age of pig (d)...	1	5
PYR†		
[^{14}C]PYR	3·46	2·26
[^{14}C]PYR+cAMP	3·87	3·09
[^{14}C]PYR+18:1+carnitine	2·66	2·37
[^{14}C]PYR+8:0+carnitine	2·43	2·11
LAC		
[^{14}C]LAC	3·89	4·49
[^{14}C]LAC+cAMP	4·11	4·40
[^{14}C]LAC+18:1+carnitine	3·90	3·32
[^{14}C]LAC+8:0+carnitine	3·48	3·29
Pooled SEM	0·36	
Statistical significance of effect of substrate (PYR *v.* LAC): *P*	< 0·01	

PYR, pyruvate; cAMP, dibutryl cAMP; LAC, lactate.
* For details of procedures, see pp. 82–85.
† Effect of treatment combination within gluconeogenic substrate on glucose synthesis rate: pyruvate > pyruvate+8:0+carnitine, $P < 0·05$.

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al. 1984), but substantial quantities of carnitine are provided by colostrum (Bieber *et al.* 1973). Similarly, glucagon secretory capacity in the neonatal pig is dependent on colostrum or milk intake (Pegorier *et al.* 1981; Kasser *et al.* 1982; Lepine *et al.* 1989*a*, *b*). In the present study, however, oxidation of oleate was not improved with separate or simultaneous additions of carnitine or glucagon (or dibutryl cAMP). Similarly, ketogenesis from oleate was low in hepatocytes isolated from newborn pigs fasted for 48 h and was not markedly affected by co-incubation with carnitine or glucagon (Pegorier *et al.* 1983). Furthermore, we have shown that colostrum intake does not increase the capacity for oleate oxidation by isolated hepatocytes (Lepine *et al.* 1991).

The relative inability to stimulate oleate oxidation may be the result of acylation prior to mitochondrial uptake, thereby predisposing long-chain fatty acids to esterification (Bach & Babayan, 1982). Approximately 93% of the oleate which enters hepatocytes isolated from fasting newborn pigs is partitioned toward the esterification pathway. Consequently, a very small proportion is available for oxidation (Pegorier *et al.* 1983) and the propensity for lipid deposition is, thus, emphasized for both the sucking and fasting neonatal pig. The former exhibits a 16-fold increase in whole-body triacylglycerol reserves during the initial 7 d after birth and a 100-fold increase by 21 d of age (Manners & McCrea, 1963).

The neonatal pig is analogous to the neonatal rat with respect to the presence of extremely low levels of white adipose tissue at birth (Manners & McCrea, 1963) and the poor glucoregulatory response to fasting (Pegorier *et al.* 1984). However, a significant species difference appears to exist with respect to fatty acid oxidation. In contrast to the newborn pig, ketone production from long-chain fatty acids (palmitate or oleate) increased 7-fold in hepatocytes prepared from newborn rats after fasting for 16 h (Blumenthal, 1983) and 3–4-fold in hepatocytes from adult rats after fasting for 48 h (Ferre *et al.* 1981). Oral administration of long-chain fatty acids has also been shown to increase glucose synthesis in the newborn fasting rat such that hypoglycaemia was corrected (Ferre *et al.* 1979, 1981), but similar effects have not been observed in the newborn pig provided with long-chain fatty acids shortly after birth (Helmrath & Bieber, 1975; Boyd *et al.* 1985). Glucose synthesis from lactate decreased 84% in the sucking rat when fatty acid oxidation was inhibited (Pegorier *et al.* 1977). Conversely, increasing the concentration and oxidation rate of plasma fatty acids in the fasting rat to a level comparable to the sucking rat increased glucose synthesis approximately 3-fold (Ferre *et al.* 1978). Thus, in the rat hepatic gluconeogenesis is dependent upon the oxidation of fatty acids.

Fatty acid oxidation supports gluconeogenesis by elevating acetyl-CoA, an obligatory cofactor of pyruvate carboxylase (*EC* 6.4.1.1), and by providing reducing equivalents (NADH) required for substrate flux through glyceraldehyde-3-phosphate dehydrogenase (*EC* 1.2.1.12). In contrast to the rat, the relationship between fatty acid oxidation and hepatic gluconeogenesis in the neonatal pig is equivocal despite recent efforts to delineate this association (Pegorier *et al.* 1983; Boyd *et al.* 1985; Duee *et al.* 1985; Lepine *et al.* 1991). It is apparent from the present study that conditions promoting increased fatty acid oxidation (i.e. octanoate) did not support elevated rates of glucose synthesis from either lactate or pyruvate in hepatocytes isolated from fasted neonatal pigs (Tables 2 and 4). Similarly, neither glucagon nor dibutryl cAMP in combination with oleate or octanoate stimulated glucose synthesis (Tables 2 and 4).

These data suggest that neither fatty acid oxidation, glucagon, dibutryl cAMP or carnitine are primary factors limiting glucose synthesis in the fasting neonatal pig. Failure to stimulate gluconeogenesis through increased fatty acid oxidation was affirmed in another study by our group (Lepine *et al.* 1991), but is in apparent conflict with a report by Duee *et al.* (1985). The latter investigators observed a 30% reduction in glucose synthesis from lactate when fatty acid oxidation was inhibited in hepatocytes isolated from sucking pigs,

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while co-incubation of oleate, carnitine and glucagon with hepatocytes isolated from pigs fasted from birth resulted in a twofold increase in glucose synthesis (Duee *et al.* 1985). Rates of gluconeogenesis did not achieve a level comparable to the sucking pig, however. The explanation for the disparity between the present study and that of Duee *et al.* (1985), regarding the stimulatory effect of fatty acid oxidation on glucose synthesis, is unclear but may result in part from marked differences in fatty acid oxidation rates. For example, oxidation-stimulated glucose synthesis was only 28% of the rate achieved in our laboratory (herein and Lepine *et al.* 1991). Furthermore, data of Ferre *et al.* (1981) suggest that, as gluconeogenic rate increases, the stimulatory potential of enhanced fatty acid oxidation diminishes. Therefore, the potential effect of fatty acid oxidation on hepatic gluconeogenesis in the present study may be limited relative to that reported in the study of Duee *et al.* (1985).

The capacity for glucose synthesis during fasting appeared to be independent of age or duration of fast in the present study. A 48 h fast did not stimulate glucose synthesis beyond that observed following a 24 h fast, nor did colostrum consumption for 3 d enhance gluconeogenic capacity beyond that of the pig fasted from birth (Tables 2 and 4). Therefore, the effect of sucking on glucose homeostasis in the neonatal pig appears to be of relatively short duration and requires a relatively constant intake of colostrum or milk in order to be effective (Lepine *et al.* 1991). Although the mechanism of colostrum/milk stimulation is unclear, a particularly important anomaly in glucose metabolism was observed by Pegorier *et al.* (1983). They reported that a predominance of substrate flux, in the glycolytic pathway, was toward oxidation rather than glucose synthesis, resulting in a net loss of valuable precursors. Thus, the perceived sufficiency of circulating gluconeogenic substrates (Pegorier *et al.* 1981) appears to be undermined by a failure to conserve them through substrate cycling.

In conclusion, constraints to the oxidation of long-chain fatty acids and gluconeogenesis in fasting neonatal pigs remain unresolved. However, the previously documented limit of fatty acid oxidation on gluconeogenesis as observed for the neonatal rat appears not to hold for the baby pig. Although medium-chain fatty acids failed to stimulate gluconeogenesis, their relative importance as an oxidizable substrate for non-hepatic tissues and consequently whole-body glucose kinetics has not yet been determined for the neonatal pig. The potential impact of such was illustrated in neonatal rats where irreversible glucose loss was reduced by 30% (Pegorier *et al.* 1983). Response of a similar magnitude is suggested, however, since oral administration greatly improves glucose homeostasis in fasting newborn pigs (Lepine *et al.* 1989*b*).

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