

## IDIOSYNCRASIES OF PIGLET LIPID METABOLISM AND THEIR RELATIONSHIP TO POSTNATAL MORTALITY

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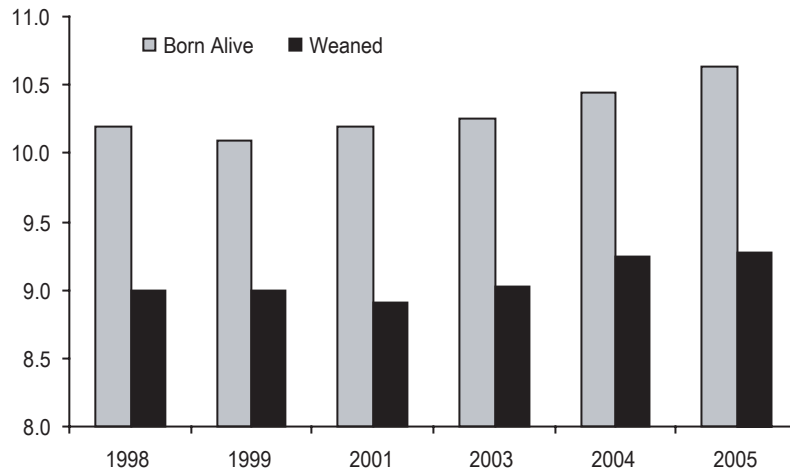
### Introduction

Postnatal mortality represents a significant problem for the global pig industry (NAHMS, 1997, 2000; Herpin, Damon and Le Dividich, 2002), claiming 0.15 - 0.20 of piglets born alive. In some less-developed countries, the rate may exceed 0.30 (Lay, Matteri, Carroll, Fangman and Safranski, 2002; Losinger, 2005). While the number of piglets born alive and the number weaned per litter have increased since 1998, the mortality rate has remained unchanged (Figure 11.1). On average 0.75 of the deaths occur within three days of birth, emphasizing the need for early intervention. The underlying etiology is complex, but data indicate that inadequate nurture is among the leading causes, accounting for 0.20 of deaths (Figure 11.2). The impact of inadequate nutrition is even greater than direct estimates suggest because piglets suffering from starvation are more susceptible to crushing by the dam which is reported as the predominant cause of death (Figure 11.2). While the importance of early nutrition has been recognized (Varley, 1995), practical improvements in mortality rate are yet to be realized possibly due to technical difficulties in implementation and in potential costs/benefit constraints.

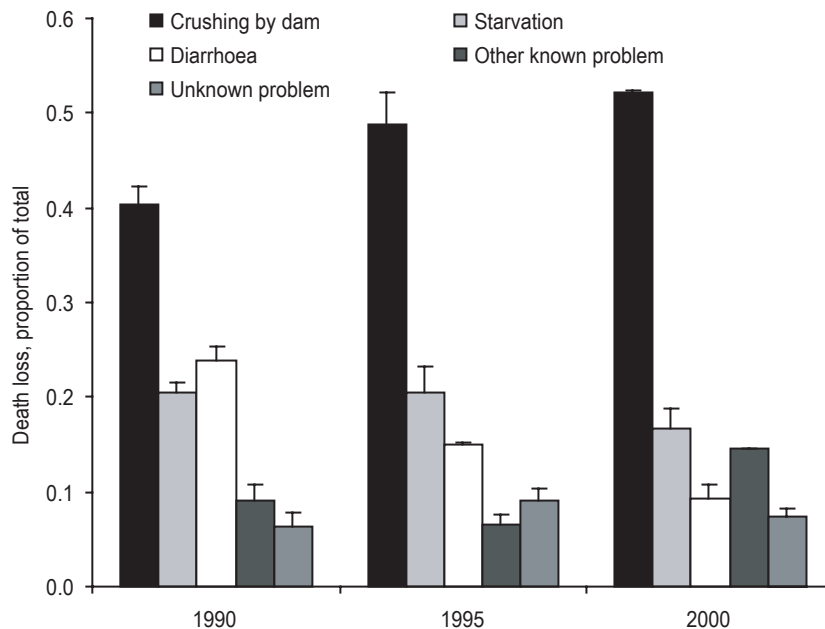
Starvation, although a consequence of inadequate nutrient supply, also can result from inadequate nutrient utilization by the neonates. Because newborn pigs have very low energy reserves (< 0.02 body fat; Mannaert and McCrea, 1963) with no immediate and appropriate nutrition at birth (Varley, 1995), milk lipids become the principal substrate for oxidative metabolism after birth, comprising 0.60 of dietary energy (Girard, Ferré and Duée, 1992). Furthermore, the energy requirement of the piglet is maximum in the immediate postnatal period due to their high relative growth rate, high relative surface area and the need for thermoregulation (Le Dividich and Sève, 2000). Therefore, piglet survival greatly depends on their rapid metabolic adaptation to utilize milk fat as their primary postnatal fuel. However, metabolic studies suggest that neonatal piglets have a limited capacity to catabolize fatty acids. Limitations may stem

## 2 Idiosyncrasies of piglet lipid metabolism

from low gene expression as well as from unique characteristics of key enzymes in the fatty acid oxidative pathway, suggesting that regulation of fatty acid metabolism may differ in pigs compared with other mammals.



**Figure 11.1** Estimated piglet mortality rates in the USA. While the number of piglets born alive and the number weaned per litter show slight upward trends, mortality rates have not changed appreciably over the past eight years. Data compiled from the *Pigchamp* database.



**Figure 11.2** Suggested causes of postnatal piglet mortality in the USA. This book chapter examines a biochemical component of “starvation”, estimated to account for about 20% of deaths. Data compiled from reports by the National Animal Health Monitoring System (NHAMS).

Recent developments in cellular and molecular biology have motivated detailed investigation of the attenuated metabolic development in neonatal piglets and have illuminated interesting molecular idiosyncrasies that merit further examination. Nutritional and pharmacologic modulation of key lipid-metabolizing enzymes that likely regulate development of postnatal fatty acid oxidation may eventually lead to improved efficiency of energy utilization and reduced postnatal mortality. Accordingly, the current chapter will highlight the nutritional and metabolic idiosyncrasies of lipid metabolism that underlie piglet postnatal morbidity and mortality.

### **Timely energy support is vital for survival and growth**

Abrupt and dramatic changes in the bioenergetics and nutrition of the piglet at birth necessitate immediate adaptation if the animal is to thrive. The piglet's metabolic rate (energy demand) increases by 2 fold at birth, congruent with an increase in the relative body surface area, the need to evaporate birth fluids, and the need to retain homeothermy in an environment that is often more than 10 °C below its lower critical temperature (Stanier, Mount and Bligh, 1984). Survival therefore hinges on adequate nurture, including both 1) adequate fuel intake and 2) adequate fuel metabolism. Regarding the first, while nature endows the piglet with modest glycogen stores to temporarily buffer the postnatal energy demand, the animal simply cannot sustain the elevated metabolic rate without exogenous fuel intake (i.e., milk consumption). Regarding the second, newborn piglets have a limited protein oxidative capacity in the first week after birth (Marion and Le Dividich, 1999), even under conditions of starvation or cold stress (Benevenga, Steinman-Goldsworthy, Crenshaw and Odle, 1989; Herpin, Le Dividich and van Os, 1992). Therefore, rapid biochemical adaptations (described later) are needed to utilize milk-fat for energy which adds further "metabolic stress" to the piglet. Both of these important stressors are related to the post mortality directly and merit further discussion.

#### **INADEQUATE INTAKE**

Immediately after birth, the piglet must successfully engage instinctive suckling behavior in a competitive environment with multiple littermates. Behavioral research (Rohde Parfet and Gonyou, 1988) has shown that > 30 minutes may lapse between birth and first-suckling. Lower birth weight piglets with lower glycogen reserves take longer to establish a successful suckling rhythm, thereby further predisposing them to morbidity and mortality. Inadequate consumption of milk predictably lowers piglet heat production and body temperature drops accordingly (Le Dividich & Noblet, 1984). Inadequate intake of colostrum-

#### 4 *Idiosyncrasies of piglet lipid metabolism*

derived IgG compromises passive immunity as well. In a weakened state, the piglet is less able to compete for milk, so intake further declines leading to the negative spiral that ultimately results in death (Mount, 1968; Tuchscherer, Puppe, Tuchscherer and Tiemann, 2000). To combat this problem of inadequate energy intake, we investigated two direct intervention approaches to supply supplemental energy to the piglets. In the first approach (see Veum & Odle, 2001 for review), previous efforts (Braude, Mitchell, Newport, and Poter, 1970; Lecce, 1975) were extended to supply supplemental milk replacer to needy piglets. While this approach shows some promise, especially when supplemental milk is supplied to heat-stressed dams (Azain, Tomkins, Sowinski, Arentson and Jewell, 1996), adoption by the industry has been hampered by high cost of milk ingredients and the mechanical challenges of operating the feeding systems in a production environment. In the second approach, the use of medium-chain triglycerides (MCT) as a supplemental exogenous fuel (Odle, Lin, van Kempen, Drackely and Adams, 1994; Odle, 1997) that can be directly gavaged to compromised piglets was explored extensively. This research led to identifying and understand an underlying impairment in the piglet's ability to oxidize fatty acids and produce ketone bodies (discussed later).

#### INADEQUATE METABOLISM

In addition to inadequate fuel intake, the problem of negative energy balance may be exacerbated by inadequate fuel metabolism by the neonate. Indeed, the piglet's ability to switch rapidly /adapt from carbohydrate to fat oxidation is paramount for survival. The permeability of the placenta to fatty acids is limited and, therefore, the primary energy substrate for the developing fetal pig is glucose (Battaglia and Meschia, 1988). However, during the postnatal period, milk lipids become the principal substrate for oxidative metabolism, comprising 0.60 of dietary energy (Girard *et al.*, 1992). To buffer this transition from carbohydrate- to lipid-based metabolism, the piglet is born with a reserve of hepatic glycogen that precipitously declines by 48 h (Pégorier, Duée, Assan, Peret and Girard, 1981). This fuel utilization profile is further confirmed by reports of high respiratory quotients immediately postpartum (Noblet and Le Dividich, 1981). In addition, the piglet lacks an appreciable fat depot (<0.02 of body weight; Mannaerts and McCrea, 1963). Therefore, piglet survival also hinges on their rapid metabolic adaptation to utilize milk fat as their primary postnatal fuel. Although postnatal increases in fatty acid oxidation were reported (Wolfe, Maxwell and Nelson, 1978), they must be interpreted in light of the general increase in metabolic rate which occurs after birth (Odle, Benevenga and Crenshaw, 1991a). For example, the increase is consistent with an increase of mitochondrial proliferation and respiration which is supported by the increased oxygen consumption rate. Odle, Benevenga and Crenshaw (1991b)

also observed that the increase of hepatic fatty acid oxidation occurred in both small and normal birth-weight pigs during the first 48 h of life.

### **Idiosyncrasies of hepatic fatty acid oxidative metabolism in neonatal pigs**

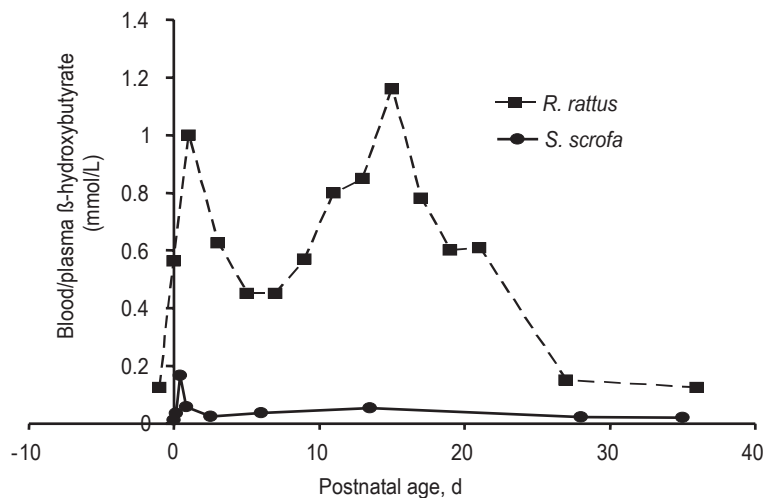
Lipid metabolism in rodents has been well documented, and is often used as a reference standard when characterizing the pathways involved; however, important species differences do exist. Indeed, research has revealed a number of idiosyncrasies in the neonatal pig that make extrapolation from rodent data inappropriate. These differences and their ramifications for fuel homeostasis in the piglet will be highlighted.

Like other mammalian neonates the pig must rapidly adapt to a change in fuel source that is initiated at birth. These adaptations include an up-regulation of gluconeogenesis, fatty acid oxidation and ketogenesis all of which serve to ensure adequate energy supply. The neonatal pig is extremely susceptible to hypoglycaemia within 24 h after birth. During this time, liver glycogen in both the fed and starved animal decreases precipitously and, unless suckling ensues, ~~animals~~ will become exhausted within 48 h (Pégorier *et al.*, 1981). Therefore, the use of milk fat as energy substrate is a prerequisite of utmost importance for successful transition to extra-uterine life.

However, at least two lines of evidence confirm that the neonatal piglet is limited in its capacity to catabolize dietary fatty acids. First, the capacity of the one-day-old piglet to oxidize fatty acids is only 0.32 of the rate of the 24-d-old piglet (Bieber, Markwell, Blair and Helmarath, 1973), and the rates of  $\beta$ -oxidation in liver preparations are markedly lower compared to that in liver from other species such as rabbits (Duée, Pégorier, Manoubi, Herbin, Kohl and Girard, 1985; Pégorier, Garcia-Garcia, Prip-Buus, Duée, Kohl, and Girard, 1989) and rats (Duée, Pégorier, Quant, Herbin, Kohl and Girard, 1997). This low hepatic capacity to oxidize fatty acids has been contrasted to a high capacity for esterification. Indeed, Pégorier, Duée, Girard and Peret (1983) reported that 0.90 of oleate taken up by piglet hepatocytes was re-esterified with limited flux through  $\beta$ -oxidation, regardless of age or nutritional status. Second, suckling piglets do not display a hyperketonemia despite elevated plasma non-esterified fatty acid (Bengtsson, Gents, Harkkarainen, Hellström and Persson, 1969; Pégorier *et al.*, 1981, Adams, Lin, Yu, Odle and Drackley, 1997a). This starkly contrasts other mammalian species (e.g., rats, rabbits, etc) which show pronounced hyperketoemia during suckling (Foster and Bailey, 1976; Figure 11.3). For example, plasma ketones may exceed 2 mM in the rat (Robles-Valdes, McGarry and Foster, 1976), but the concentration in newborn pigs is less than 0.25 mM (Pégorier *et al.*, 1981). The lower ketone concentration is attributed to reduced synthesis and not increased utilization as Tetrick, Adams, Odle and Benevenga (1995) showed that, at physiological concentrations,  $\beta$ -

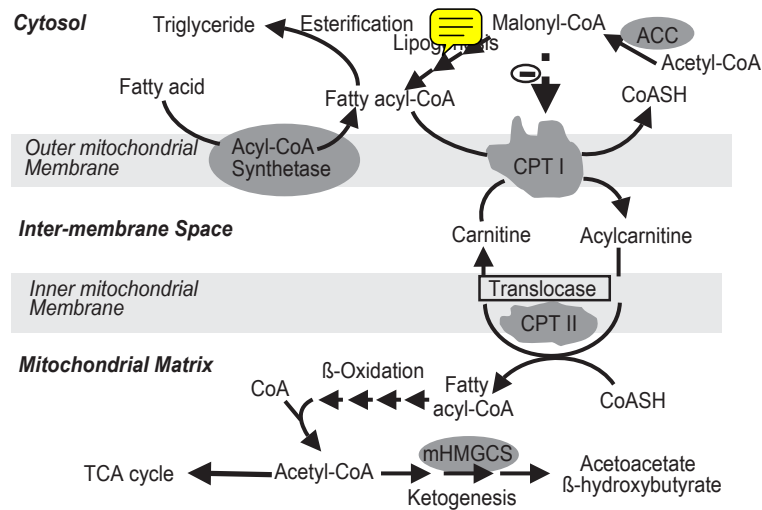
## 6 *Idiosyncrasies of piglet lipid metabolism*

hydroxybutyrate contributes less than 0.05 to the piglet's energy requirement. Because ketone bodies provide important glucose-sparing carbon, aiding otherwise glucose-dependent tissues (e.g., neural tissues), their absence may be detrimental to the survival of the piglet which is keenly susceptible to hypoglycaemia (Swiatek, Kipnis, Mason, Chao and Cornblath, 1968). Furthermore, insofar as fatty acid oxidation also is required to support active gluconeogenesis, impaired fat oxidation also could contribute indirectly to hypoglycemia.

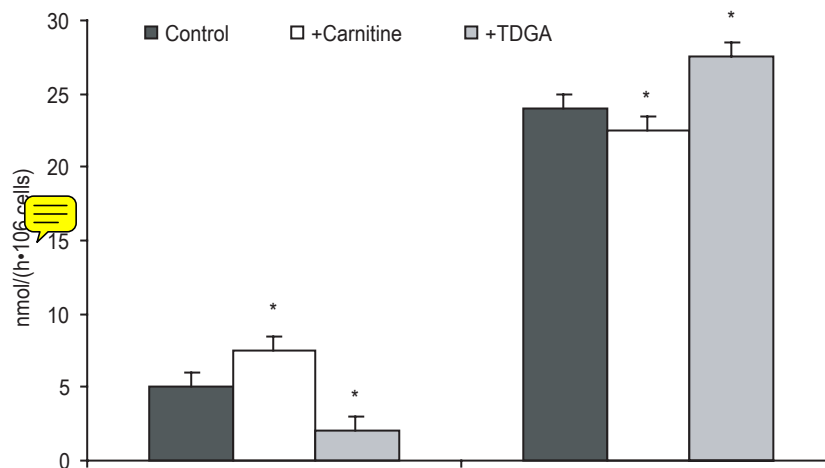


**Figure 113** Suckling hyperketonemia in *R. rattus* (rats) exceeds that in *S. scrofa* (pigs) by greater than 10-fold. Adapted from Foster & Bailey, 1976 and Bengtsson *et al.*, 1969, respectively.

The partitioning of dietary fatty acids towards esterification and away from oxidation (Pégorier *et al.*, 1983) suggests that carnitine palmitoyltransferase I (CPT I) activity is connected to the low oxidative capacity of the newborn pig liver (Figure 11.4). This enzyme is well known for its central role in the regulation of oxidative lipid metabolism (McGarry, 2001), controlling the shuttle of long-chain fatty acids into the mitochondrion where they are subjected to the pathway of  $\beta$ -oxidation. During physiological states in which lipogenesis is occurring, acetyl-CoA carboxylase is activated and the attendant high level of malonyl-CoA inhibits CPT I thereby ablating the simultaneous (and futile) fatty acid oxidation by preventing entry into the mitochondria. Therefore, regulation of CPT I is thought to function in directing fatty acyl-CoA between esterification and oxidation fates. Indeed, we were able to redirect carbon flux of palmitate through the oxidative pathway and concomitantly reduce the rate of esterification by addition of L-carnitine, a co-factor for CPT I (Odle, Lin, van Kempen, Drackely and Adams, 1995; Figure 11.5). Evidence indicates that the activity of CPT I develops rapidly in the neonatal pig, and this increase coincides with increased tissue carnitine concentrations (Beiber *et al.*, 1973).



**Figure 11.4** Central dogma of hepatic long-chain fatty acid metabolism. Following activation to their Co-A esters by *acyl-CoA synthetase*, fatty acids may be esterified in the cytosol, or may be shuttled into the mitochondria via the *carnitine palmitoyltransferase* (CPT) system. The shuttle is subject to acute allosteric regulation in that CPT1 is inhibited by malonyl-CoA which is the first committed metabolite formed in the cytosolic pathway of lipogenesis by *acetyl-CoA carboxylase* (ACC). Within the mitochondria, fatty acids are subjected to the pathways of  $\beta$ -oxidation and ketogenesis. As discussed in the text, piglets show the following idiosyncrasies: 1) negligible lipogenesis, with low concentrations of malonyl-CoA, 2) comparatively substantial esterification capacity, 3) a chimeric form of CPT1 that is highly sensitive to malonyl-CoA inhibition but has a high affinity for carnitine, 4) limited ketogenesis owing to low activity of *3-hydroxy-3-methylglutaryl-CoA synthase* (mHMGCS), and 5) comparatively high rates of peroxisomal  $\beta$ -oxidation (not illustrated). Adapted from Heo, 2000.



**Figure 11.5** Radiolabeled palmitate metabolism by piglet hepatocytes. As a co-substrate for CPT1, supplemental carnitine increased oxidation and reciprocally reduced esterification; whereas, TDGA (a specific inhibitor of CPT1) produced the opposite effects. These data illustrate the ability of CPT1 to channel fatty acids between catabolic and anabolic fates. Adapted from Odle *et al.*, 1995.

## 8 *Idiosyncrasies of piglet lipid metabolism*

In pigs, like other mammalian neonates, CPT I is reversibly inhibited by malonyl-CoA. Malonyl-CoA is produced by acetyl-CoA carboxylase (ACC), specifically the  $\alpha$  isoform in lipogenic tissues and the  $\beta$  isoform, with a mitochondrial leader sequence, in non-lipogenic tissues (Kim, 1997). In non-lipogenic tissues like muscle, malonyl-CoA presence may serve only to regulate CPT I activity. In muscle, the concentration of malonyl-CoA required for 0.50 inhibition of CPT I ( $IC_{50}$ ) is significantly less than that of liver in many mammals. The pig, however, is considerably different in regards to CPT I regulation by malonyl-CoA in both liver and muscle. In fact, the  $IC_{50}$  for inhibition of CPT I by malonyl-CoA in pigs is greater in muscle (5.48–6.34  $\mu$ M) than in liver (0.10  $\mu$ M) (Schmidt and Herpin, 1998). This hierarchy is opposite to that in rats and humans. Furthermore, this high degree of sensitivity occurs in both fed and fasted piglets (Duée *et al.*, 1994; Lin and Odle, 1995; Schmidt and Herpin, 1998). The finding that pig liver was 20 times more sensitive to inhibition by malonyl-CoA ultimately led to the characterization of pig CPT I. Nicot, Hegardt, Woldegiorgis, Haro and Marrero (2001) were the first to determine that pig CPT I is structured as a natural chimera of rat liver and muscle isoforms. The hybrid pig protein contains elements resembling both liver- (L) and muscle- (M)-CPT I isotypes, with the L-CPT I binding site for acyl-CoA and the M-CPT I binding sites for carnitine and malonyl-CoA. Accordingly, pig L-CPT I exhibits saturation kinetics similar to the rat for carnitine (126  $\mu$ M) and palmitoyl-CoA (35  $\mu$ M); however, the  $IC_{50}$  for malonyl-CoA (140 nM), is similar to human M-CPT I (Nicot *et al.*, 2001). Although the liver is not a lipogenic organ for the pig (Mersmann, Goodman, Houk and Anderson, 1973), which in part could account for a higher sensitivity to malonyl-CoA, it is unknown what the direct consequences of this increased sensitivity are toward fuel homeostasis of the neonatal pig. Related observations by Dyck, Cheng, Stanley, Barr, Chandler, Brown, Wallace, Arrhenius, Harmon, and Yang, Nadzan and Lopaschuk (2004) showed that inhibiting malonyl-CoA decarboxylase (MCD) increased myocardial malonyl-CoA concentrations and decreased fatty acid oxidation in pig hearts *in vivo*, suggesting that MCD might have a role in the regulation of fatty acid oxidation by changing malonyl-CoA concentrations. Furthermore, gene knockout studies also have shown that blood ketone bodies were significantly increased by overnight fasting of rats lacking acetyl-CoA carboxylase  $\beta$ , (Abu-Elheiga, Matzuk, Abo-Hashema and Wakil, 2001), suggesting that ACC $\beta$  controls fatty acid oxidation in liver and its regulation may be different in liver and muscle.

In addition to the unique structure and regulation of CPT I, the activity of mitochondrial 3-hydroxy-3-methylglutaryl-Coenzyme A synthase (mHMGCS), the putative rate limiting enzyme of ketogenesis, is extremely low in piglets, being 70% lower than in neonatal rabbits (Adams and Odle, 1993) or adult rats (Duée *et al.*, 1994). Ketogenesis represents significant carbon flux during the neonatal period of most mammalian neonates. In rats, ketogenesis increases rapidly during postnatal development (Figure 11.3) (Girard *et al.*, 1992) or fasting (McGarry and Foster, 1980). The increase is ascribed in part to increased mRNA abundance,

protein abundance and activity of mHMGCS (Thumelin, Forestier, Girard and Pégrier, 1993; Quant, 1994). The critical role of mRNA abundance also has been demonstrated in sheep during development (Lane, Baldwin and Jesse, 2002) and in rats by using a ketogenic diet (Cullingford, Eagles and Sato, 2002). In contrast, there is no substantial mRNA detected in piglets during the first two weeks after birth, although the abundance does increase later in life (Adams, Alho, Asins, Hegardt and Marrero, 1997b). This observation suggests that the unusually low ketogenic capacity and lack of hyperketonemia in postnatal piglets is associated with low expression of the mHMGCS gene during development. In addition, the gene expression can be highly induced by fasting during this stage, while suckled rat pups have no such changes in mRNA level of mHMGCS when fasted (Adams *et al.*, 1997b). Consistent with the increase in mRNA abundance, enzyme activity also is increased by 27-fold, but it still remains 50 fold lower than observed in fasted adult and suckling rats. These data imply that a post-transcriptional mechanism could be involved in the expression of mHMGCS. Indeed, Barrero, Alho, Ortiz, Hegardt, Haro and Marrero (2001) demonstrated that the attenuated activity of mHMGCS in piglets is not associated with low mRNA expression per se, but rather a low rate of translation.

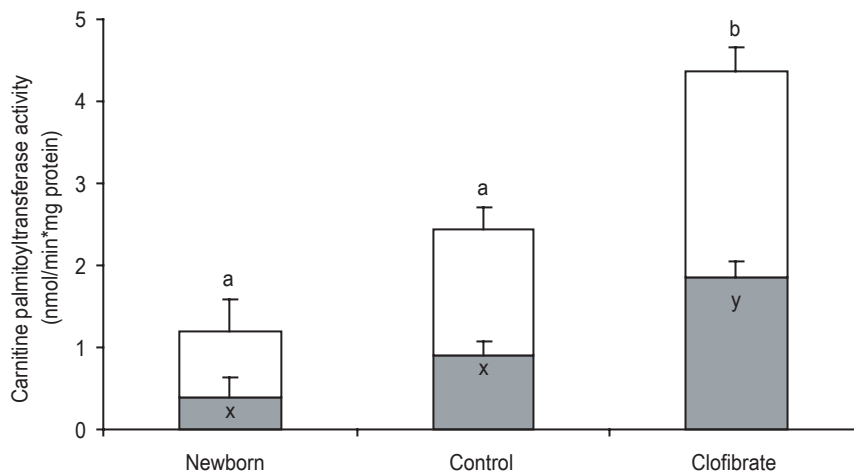
### Peroxisomal $\beta$ -oxidation in pigs

Peroxisomal  $\beta$ -oxidation is an alternate pathway of metabolism of fatty acids that has been well characterized in rodents and other species. In general, oxidation occurring in the peroxisome is considered ancillary to mitochondrial oxidation. The peroxisome is, however, essential for the oxidation of medium- and very-long-chain fatty acids that are not oxidized very well ~~oxidized~~ in the mitochondrion (Osmundsen, Bremer and Pedersen, 1991; Van den Bosch, Schrakamp, Hardeman, Zomer, Wanders and Schutgens, 1993; Wanders, Vreken, Ferdinandusse, Jansen, Waterham, van Roermund and Van Grunsven, 2001). Because the peroxisome lacks an electron transport chain, electrons are transferred directly to molecular oxygen (Mannaerts, Debeer, Thomas and De Schepper, 1979). Thus, the first step in peroxisomal  $\beta$ -oxidation, catalyzed by fatty acyl-CoA oxidase (~~FAO~~), is not coupled to ATP production, but rather, heat is generated and released. Therefore, peroxisomal  $\beta$ -oxidation may play an important role in thermogenesis (Goglia, Liverini, Lanni, Lossa and Barletta, 1989 ab). In addition, ACO activity is greatest with longer carbon chain lengths (Vanhove, van Veldhoven, Fransen, Denis, Wanders, Eyssen, and Mannaerts, 1993). Thus, fatty acids are only chain shortened (not fully combusted) to acetyl-CoA and peroxisomal  $\beta$ -oxidation produces approximately 30% less energy compared to mitochondrial  $\beta$ -oxidation (Reddy and Mannaerts, 1994).

Because of the characteristics of peroxisomal  $\beta$ -oxidation, its ontogeny and putative role in postnatal thermoregulation has received research attention in last 10 years. Interestingly, we have showed that the pig has a high proportion of

## 10 Idiosyncrasies of piglet lipid metabolism

peroxisomal fatty oxidation that represents a greater percentage (40-50%) of total  $\beta$ -oxidation, independent of the administration of peroxisome proliferators (Yu, Drackley, Odle and Lin, 1997a; Yu, Drackley and Odle, 1997b). The induction of peroxisomal oxidation occurs immediately postpartum, is greater in the suckled versus fasted piglet (Yu *et al.*, 1997b; Yu, Drackley and Odle, 1997c), and is reliant on the initiation of suckling (Yu *et al.*, 1997c). The control of peroxisomal  $\beta$ -oxidation appears to be correlated with the developmental pattern of fatty acyl-CoA oxidase activity. The rapid postnatal development of peroxisomal  $\beta$ -oxidation that we have measured in piglets and that others have determined in rats (Tsukada, Mochizuki and Konishi, 1968; Veerkamp and van Moerkerk, 1986) may be a physiologically adaptive mechanism for neonatal survival and growth. In addition, peroxisomal oxidation may have a great contribution to the acetate pool (70%) which predominates the acid soluble products pool (ASP) during *in vitro*  $\beta$ -oxidation (Lin *et al.*, 1995), presumably because of high acetyl-CoA hydrolase activity present in peroxisomes. However, we have noticed that inhibition of CPT I significantly reduces acetate production (Lin *et al.*, 1995). Also, induction of peroxisomal  $\beta$ -oxidation by stimulating peroxisome proliferator activated receptor  $\alpha$  (PPAR $\alpha$ ) resulted in a great increase of CPT I and ACO activities and  $\beta$ -oxidation in both peroxisome and mitochondria (Yu *et al.*, 1997a; Lyvers Peffer, Lin, and Odle, 2005; Figure 11.6). From this we infer that the regulation of fatty acid oxidative metabolism is different in pigs compared to other mammals. The physiological significance of peroxisomal  $\beta$ -oxidation of fatty acids and the functional coordination between peroxisomal and mitochondrial  $\beta$ -oxidation in neonates merits further investigation.



**Figure 11.6** Malonyl-CoA sensitive and malonyl-CoA insensitive hepatic carnitine palmitoyltransferase activity (CPT) in piglet liver. Activity was measured at the end of a 10-12 d feeding period of  $\pm$  clofibrate (a PPAR $\alpha$  agonist). Total activity is represented by summation of malonyl-CoA sensitive (CPT I) and insensitive activities. Different letters (a,b) denote significant difference between treatments for malonyl-CoA sensitive CPT activity ( $p < 0.05$ ). Different letters (x,y) denote significant difference between dietary treatment for malonyl-CoA insensitive CPT activity ( $p < 0.05$ ). Modified from Lyvers Peffer *et al.*, 2005.

### The role of PPAR in regulation of fatty acid oxidation in neonatal pigs

The key regulatory enzymes in hepatic peroxisomal and mitochondrial  $\beta$ -oxidation (ACO, CPT I, mHMGCS and MCD) all are under PPAR $\alpha$  regulation. A member of the nuclear receptor superfamily, this transcription factor was first discovered as an inducer of peroxisome proliferation (as reviewed by Schoonjans, Staels and Auwerx, 1996). This involves the up-regulation of peroxisomal genes (mainly those involved with  $\beta$ -oxidation), an increase in peroxisomal size, and the production of more peroxisomes (Purdue and Lazarow, 2001). In mammals, peroxisomal proliferation is induced by both physiological stimuli (fatty acids) and a variety of xenobiotics through their direct binding with PPAR $\alpha$  (Kliwer, Xu, Lambert, and Willson, 2001; Totland, Madsen, Klementsens, Vahrennes, Kryvi, Frøyland, Hexeberg and Berge, 2000). In mice and rats, PPAR $\alpha$  is highly expressed in cells that have high fatty acid catabolic rates including the liver, kidney, heart, and skeletal muscle (Braissant, Foufelle, Scotto, Dauca and Wahli, 1996). However, only the liver and to a lesser degree, the kidney, undergo peroxisome proliferation (Schoonjans *et al.*, 1996). Proliferation also is species dependent; while the rat is quite responsive to peroxisome proliferation, humans and pigs remain unresponsive despite the peroxisome being an essential organelle (Vamecq and Draye, 1989; Cheon, Nara, Band, Beever, Wallig and Nakamura, 2005).

Located upstream of both L-CPT I and mHMGCS genes is the response element (PPRE; Ortiz, Mallolas, Nicot, Bofarull, Rodriguez, Hegardt, Haro and Marrero, 1999), implying that expression of both genes may be regulated through the use of peroxisome proliferators. We (Yu, Odle and Drackley, 2001) showed a >2 fold increase in total CPT activity when pigs were fed clofibrate for 2 wk, and a 3 fold increase in peroxisomal  $\beta$ -oxidation of the liver. The increase of hepatic CPT activity and peroxisomal  $\beta$ -oxidation appears to be associated with an increase of mRNA expression by clofibrate (Luci, Giemsa, Kluge and Eder, 2007)

Most recently, the PPAR distribution in pig tissues was investigated at 1 day, 5 weeks and 25 weeks of age. The deduced amino acid sequence of PPAR $\alpha$  in pigs revealed an evolutionary distance to rodent species, suggesting that the difference could account for the species-dependent response to peroxisome proliferators, such as clofibrate. Pig PPAR $\alpha$  mRNA is predominately expressed in kidney and liver (Sunvold, Grindflek and Lien, 2001) and expression of PPAR $\alpha$  mRNA and its target gene ACO also were observed in adipose tissue from two genetic populations of pigs (Ding, Schinckel, Weber and Mersmann, 2000). Clofibrate induces genes involved in mitochondrial fatty acid oxidation and ketogenesis in 21-day old fasted pigs (Cheon *et al.*, 2005) and 56-day old pigs (Luci *et al.*, 2007) without liver hyperplasia or hepatomegaly. However, liver glycogen was reduced by clofibrate. These data suggested that PPAR $\alpha$  plays a central role in metabolic adaptation in pig liver (Cheon *et al.*, 2005).

## Conclusion

Inadequate nurture remains one of the leading causes of piglet mortality. Increasing energy supply and utilization will play an essential role in preventing the serious losses stemming from inadequate nurture. At birth, the piglet is poorly adapted to use milk lipids, and the attenuated production of ketone bodies exacerbates the problem for extrahepatic tissues. Because ketone bodies function as glucose-sparing carbon for otherwise glucose-dependent tissues, lack of ketone bodies may be detrimental to the survival of piglets. In addition, because fatty acid oxidation is required to support active gluconeogenesis, impaired fatty acid oxidation also could contribute indirectly to hypoglycemia. The enzymologic basis for attenuated ketogenesis and low hepatic  $\beta$ -oxidation in piglets remains an active area of research. Modulation of CPT I by malonyl-CoA inhibition is generally accepted as the predominate control site for ketogenesis and fatty acid oxidation in liver. This system may be important in neonatal swine because pig CPT-I is particularly sensitive to malonyl-CoA inhibition which appears to be associated with the unique structure of the pig enzyme. An intramitochondrial regulation site of ketogenesis also has been suggested which is an idea strongly supported by reports of negligible activity of the ketogenic enzyme mitochondrial HMG-CoA synthase in pig liver. The low activity of this enzyme results from suppressed expression in neonatal pigs and may be related to post transcriptional modification and /or specific differences in enzyme kinetics. It remains unclear if the low mitochondrial HMG-CoA synthase activity in pigs controls the hepatic  $\beta$ -oxidation flux in this species. Because PPAR $\alpha$  is largely expressed in liver and induces genes involved in both peroxisomal and mitochondrial fatty acid oxidation, PPAR $\alpha$  is now considered to be an essential transcription factor in regulating hepatic fatty acid oxidation. Transcriptional regulation of enzymes by PPAR $\alpha$  activation is required in the  $\beta$ -oxidative pathway of the mitochondria and peroxisomes including CPT I and CPT II (Chatelain, Kohl, Esser, McGarry, Girard and Pégrier, 1996; Mascaró, Acosta, Ortiz, Marrero, Hegardt and Haro, 1998), mHMGCS (Rodriguez, Gil-Gomez, Hegardt and Haro, 1994; Cullingford, Dolphin and Sato, 2002b), MCD (Lee, Kim, Zhao, Cha and Kim, 2004), ACO and catalase. Clearly, with the recent progress of clarifying the physiological idiosyncrasies of lipid metabolism in neonatal piglets at molecular levels, the control of fatty acid oxidation via gene regulation may be an effective tool to improve the utilization of milk fat and lead to improved piglet survivability.



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16 *Idiosyncrasies of piglet lipid metabolism*

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18 *Idiosyncrasies of piglet lipid metabolism*

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