HEPATIC FATTY ACID OXIDATION AND KETOGENESIS IN YOUNG PIGS[1]

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[1]Supported in part by a grant from the USDA-NRI, #98–35206–6645.

Abstract. A primary limitation to efficient pork production is morbidity and mortality during the perinatal period. Because pigs are born with low energy reserves, their survival hinges on timely consumption of milk. In contrast to carbohydrate-based fetal metabolism, the transition to a milk-based diet necessitates rapid biochemical adaptations to accommodate the oxidation of fatty acids which comprise more than 60% of milk energy. From research reported to date, the degree to which neonatal pigs make these adaptations is questionable. In stark contrast to other mammalian neonates, piglets do not demonstrate elevated ketogenesis despite high milk-fat intake. Ketone bodies play a pivotal role in the transition from carbohydrate-based metabolism to fat-based metabolism, providing an important alternative fuel for glucose-dependent tissues. Impaired adaptation limits the piglets' ability to oxidize fat which likely contributes to the etiology of mortality. Therefore, this review considers the developmental aspects of lipid oxidation in the young pig. The key regulatory enzymes previously elucidated in rodents are reviewed, with inclusion of the limited knowledge available in pigs. Further research in this area will hopefully assist in development of strategies (via nutritional and/or exogenous hormonal manipulation) to enhance development of fatty acid oxidation and ultimately improve piglet survival and growth.

1. INTRODUCTION

Impaired growth and high mortality of neonatal pigs pose significant challenges to the swine industry. Postnatal mortality varies among production units, but has been recently estimated by the Agricultural Statistics Service of the United States Department of Agriculture to average approximately 12% of live births, and has shown only modest improvement over the past 20 years (USDA, 2002). In addition, it is estimated that prenatal (embryo and fetal) mortality in swine may be as high as 25% so that, collectively, these data imply that the number of piglets weaned per litter currently may be less than 65% of true potential. Associated problems of slow growth (morbidity) further add to the inefficiency. The combined economic impact of these losses is enormous. The cost is ultimately carried by the consumer in the price paid for pork. For this reason, there is great impetus for identifying and studying the stressors responsible for the high postnatal mortality. The etiology is complex, as a number of factors may contribute, including nutritional deficiency, low immunocompetence and disease resistance, hypothermia, and crushing by the dam. Many retrospective survey studies have attempted to determine the relative importance of these stressors, but epidemiological/survey approaches have contributed little useful information because the cause of death is difficult to determine precisely, and interactions among the factors complicate interpretation. Consequently, if progress is to be made, controlled experimentation is needed to better understand the developing piglet's nutritional,

immunological and behavioral responses to its environment.

This review addresses a metabolic component of this multi-factorial etiology, examining the biochemical competency of piglets to oxidize fatty acids during early postnatal life. In particular, the ontogeny and regulation of hepatic fatty acid oxidation is highlighted owing to the dramatically low level of ketogenesis expressed in neonatal pigs compared to other species.

2. THE NEED FOR RAPID DEVELOPMENT OF FATTY ACID OXIDATION

Prior to birth, the fetus oxidizes predominantly glucose, lactate and amino acids (Battaglia and Meschia, 1978). At parturition, the newborn must elicit the behavioral responses necessary to acquire milk from the dam. This requires effective competition among littermates and occurs in a thermal environment that may be more than 10ºC below the animals critical temperature (Stanier et al., 1984). Rohde and Gonyou (1988) have shown that > 30 min may lapse before the first milk is consumed and considerably longer time is required before positive energy balance is regained. Due to limited body reserves at birth, negative energy balance quickly becomes life threatening to the piglet. Survival therefore hinges on the timely consumption of the dam's milk which provides 60% of its calories as fat (Ferre et al., 1986). These events necessitate rapid metabolic adaptations to shift from carbohydrate-based fetal metabolism to fat-based postnatal metabolism.

Other mammalian species, faced with a similar challenge, demonstrate elevated ketogenesis during this transition (Girard et al., 1992). For example, ketogenesis measured in hepatocytes from newborn rats increases 6 fold between 0 and 16 h of age (Ferre et al., 1983), and blood ketone body concentrations may exceed 1.5 mM (Foster and Bailey, 1976) in the suckling rat. Thus, neonatal hyperketonemia plays a significant role in the energy economy of the neonate (Girard et al., 1992), sparing glucose and providing carbon for lipogenesis in neural tissue. In contrast, piglets do not display hyperketonemia (Bengtsson et al., 1969; Pegorier et al., 1981; i.e., less than 0.2 mM), and this may further compromise their survival. Available data indicate that piglets apparently digest and absorb milk fat with high efficiency (digestion coefficients >95% at 2 d of age) and that a major portion of absorbed fat is deposited in adipose tissues prior to weaning (e.g. pigs are <2% fat at birth and ~15% at weaning). However, relatively little is known regarding the oxidative fate of lipid in the piglet and even less is known regarding the regulation of lipid oxidation in this species. Before reviewing the available literature addressing development of fatty acid oxidation in piglets, the major regulatory features of fatty acid oxidation (elucidated in other species) are described below.

3. REGULATION OF FATTY ACID OXIDATION AND KETOGENESIS

The major metabolic pathways involved in hepatic fatty acid metabolism are summarized schematically in Figure 1. In animal cells there are two fatty-acid ß-oxidation systems, one located in the mitochondria and the second in the peroxisome. The mitochondria is considered the primary site for fatty acid ß-oxidation, while the peroxisome is considered an alternative pathway. Under conditions which enhance peroxisome proliferation the relative contribution of the peroxisome to total fatty acid oxidation in the liver may be as high as 30% in the rat (Kondrup and Lazarow, 1985), and 47% in the neonatal pig (Yu et al., 1997a).

The mitochondria and peroxisome are often found in close proximity to lipid droplets and are believed to work in concert (Latruffe et al., 2001). The ß-oxidation reactions of the two systems are similar beginning with an initial dehydrogenation followed by a hydration, a second dehydrogenation, and finally thiolytic cleavage to produce acetyl-CoA and an acyl-CoA shortened by two carbons (Reddy and Mannaerts, 1994 review). Although the reactions are similar, the actual proteins involved differ between the ß-oxidation systems.

3.1. Biochemical Dogma

Long chain fatty acids (LCFA) are activated to their CoA thioesters via *synthetases* (Figure 1, enzyme 1; EC 6.2.1.3) located in the ER and in the outer membrane of the mitochondria (Aas, 1971). To date, five genetic variants of the long-chain acyl-CoA synthetases have been cloned from rodent species (Oikawa et al., 1998) and their differential regulation may influence the metabolic fate of the activated fatty acids (Lewin et al., 2001). While preference is shown for LCFA substrates, those of medium-chain length (MCFA, ie., C6-C10) may be activated as well. The fatty acyl-CoA (FA-CoA) may then be esterified via *acyl-CoA transferases* (Figure 1, enzyme 3; EC 2.3.1.15) located in the ER, forming various triglycerides, cholesterol esters, phospholipids, etc., which may be exported as lipoproteins (VLDL; Coleman et al., 2000). These transferases have low affinity for MCFA-CoA's such that MCFA are obligate fuels (Bach and Babayan, 1982). The FA-CoA may alternatively be transported into the mitochondria via the coordinated activities of three membrane proteins: *carnitine acyltransferase I* (CAT, Figure 1, enzyme 4; EC 2.3.1.21) catalyzing the formation of FA-carnitine from FA-CoA outside of the mitochondrial matrix, *translocase* catalyzing the exchange/diffusion (antiport) of FA-carnitine for free carnitine across the inner mitochondrial membrane, and *carnitine acyltransferase II* (CAT II), similar to CAT I except residing on the matrix side of the membrane.

The CAT I and II activities are catalyzed by a family of acyl-transferase enzymes. Proteins have been identified with optimum activity toward C2, C8 and C16 FA-CoAs in various tissues. The latter, referred to as carnitine palmitoyltransferase (CPT; discussed below) is likely of greatest importance in the young pig given the predominantly long-chain fatty acid composition of sow's milk. Within the mitochondrial matrix, the FA-CoA are subjected to oxidation at the ß-carbon yielding acetyl-CoA which may be further oxidized to CO_2 in the TCA cycle. Alternatively, it may exit as acetyl-carnitine (using the CAT system in reverse), may produce ketone bodies (acetoacetate, ß-OH-butyrate and acetone) or may be hydrolyzed to free acetate by *acetyl-CoA hydrolase*. *Hydroxy-methylglutaryl-CoA (HMG-CoA) synthase* (Figure 1, enzyme 6; EC 4.1.3.5) is presumably the rate limiting enzyme in the ketogenic pathway (Williamson et al., 1968). Cytosolic acetyl-CoA (derived from the mitochondria via CAT or citrate lyase) may be carboxylated to malonyl-CoA by *acetyl-CoA carboxylase* (ACC, Figure 1, enzyme 2; EC 6.4.1.2) within the lipogenic pathway wherein FA-CoA is synthesized *de novo*.

Very long-chain and long-chain fatty acids are also activated to their CoA thioesters preceeding catabolism in the peroxisome. The peroxisomal membrane contains two *synthetases,* a long-chain fatty acid synthetase positioned on the cytosolic side of the membrane (Figure 1, enzyme 8), and a very-long chain fatty acid (VLCFA) synthetase positioned toward the peroxisomal matrix (Figure 1, enzyme 9). The location of the VLCFA synthetase is responsible for the substrate specificity between the peroxisome and the mitochondria. While the mitochondria can oxidize long, medium, and short-chain fatty acids, VLCFA are either poorly or not at all oidized by this organelle (Lazo et al., 1990).

Following CoA activation, the initial dehydrogenation step in the peroxisome is catalyzed by multiple *acyl-CoA oxidases* (Figure 1, enzyme 10) - two have been identified in the human (Wanders et al., 2001) and three in the rat (Van Veldhoven et al., 2001). While acyl-CoA dehydrogenase, the first enzyme in mitochondrial ß-oxidation, produces two ATP as electrons are donated directly to coenzyme Q of the electron transport chain (ETC), the first step in peroxisomal ß-oxidation catalyzed by acyl-CoA oxidase results in the production of H_2O_2 as electrons are passed directly to molecular oxygen. As a result, the peroxisome is approximately half as efficient as the mitochondria in producing energy from the ß-oxidation of fatty acids. Acyl-CoA oxidase shows very little affinity for medium and short chain fatty acids (Reddy and Mannaerts, 1994 review), as a result fatty acids are only chain shortened in the peroxisome. In addition, the peroxisome, lacking TCA cycle enzymes, cannot metabolize the acetyl-CoA to CO_2, nor can it produce ketone bodies because the enzymes of ketogenesis are also absent. Therefore, the end products of peroxisomal fatty acid oxidation include acetyl-CoA and chain shortened acyl-CoA.

The transport of fatty acids and the subsequent end products of their oxidation across the peroxisomal membrane is a subject of much debate. It was originally believed that the peroxisomal membrane was highly permeable. However, isolation of the peroxisome results in a loss of the structural integrity of the membrane (Wanders et al., 2001), therefore, many earlier studies involving peroxisome permeability may have been misleading. Studies involving *S. Cerevisae* provide evidence for the involvement of transport proteins (Hettema and Tabak, 2000 review), in addition peroxisomal half transporters have been identified in humans, although their definitive role in the transport of fatty acids has not been fully elucidated (Wanders and Tager, 1998).

Although a CAT protein has been identified in the peroxisome, it is not membrane bound and is therefore not implicated in the transport of FA across the membrane. The CAT identified in the peroxisome, or *carnitine ocatnoyltransferase* (COT, Figure 1, enzyme 11) as it is often referred to in literature, has optimum activity toward fatty acids of medium chain length. It is speculated that the peroxisomal COT catalyzes the conversion of the end products of peroxisomal ß-oxidation to their carnitine esters. Subsequently, these carnitine esters may exit the peroxisome and be directed toward the mitochondria where they may undergo complete oxidation to CO_2. Studies have shown that 4,8-dimethylnonanoyl-CoA derived from the incomplete oxidation of pristanic acid in the peroxisome is indeed translocated to the mitochondria for complete oxidation (Verhoeven et al., 1998). Furthermore, the concerted actions of the two beta-oxidation systems is further supported by the observance that natural and synthetic ligands (ie. ligands of the peroxisome proliferator activated receptor or PPAR) which increase peroxisome proliferation and peroxisomal enzymes, also increase mitochondrial enzymes involved in fatty acid metabolsim (i.e. CPT I and HMG-CoA synthase).

3.2. Regulation of Carnitine Palmitoyltransferase I (CPT-I)

Using fed, fasted, and alloxan-diabetic rats, McGarry and Foster (1980) have reported considerable evidence establishing the allosteric control of CPT-I by malonyl-CoA. During physiological states in which lipogenesis is occurring, ACC is activated and the associated high level of malonyl-CoA serves to inhibit CPT-I and thereby prevent the simultaneous and futile oxidation of fatty acids by preventing their entry into the mitochondria. As such, regulation at CPT-I is thought to function in directing FA-CoA between esterification and oxidative fates. Beyond changes in malonyl-CoA concentration, changes in the sensitivity of

CPT-I to malonyl-CoA inhibition have also been reported in various physiological states including the perinatal period in rabbits (Prip-Buus et al., 1990). Furthermore, low levels of carnitine in tissues of colostrum-deprived neonates (Borum, 1983) could limit transport and thus oxidation. Milk, however, is high in carnitine (Kerner et al., 1984), and suckling results in elevated hepatic carnitine postnatally (Robles-Valdes et al., 1976). Medium-chain fatty acids also are a valuable probe in studying regulation at CPT-I because they can diffuse across the mitochondrial membranes and be activated by an alternative *acyl-CoA synthetase* (Figure 1, enzyme 5; EC 6.2.1.2) located in the matrix (Groot et al., 1976). Thus, medium-chain fatty acids may bypass, in part, regulation via CPT-I and be oxidized independently of carnitine. Medium-chain triglyceride utilization by neonatal pigs has been studied extensively in our laboratory has been previously reviewed (Odle, 1997, 1998).

Recent advances in regulation of CPT have accompanied cloning of the genes for CPT-I and II (McGarry and Brown, 1997 review). Two isoforms of CPT-I exist - the L-form (liver) which possesses relatively low sensitivity to malonyl-CoA inhibition and the M-form (muscle), possessing very high sensitivity to malonyl-CoA. Both forms show high interspecies homology (>80%). In rats, hepatic concentrations of CPT-I mRNA have been shown to increase markedly (up to 5-fold) within 24 h after birth, while CPT-II expression was constitutive (Thumelin et al., 1994; Asins et al., 1995). Studying hepatocytes cultured from perinatal rats, Chatelain et al., (1996) have shown that mRNA levels for CPT-I (L) respond markedly and rapidly to *in vitro* supplementation with clofibrate, linoleate and dibutyryl-cAMP, presumably through interaction with respective cis-acting elements and transcription factors (e.g., PPAR-RXR, FFAR and CREB, respectively).

3.3. Regulation of Acetyl-CoA Carboxylase (ACC)

Regulation of ACC plays a central role in controlling carbon flux through both anabolic and catabolic pathways of fatty acid metabolism. As the rate limiting step in denovo fatty acid biosynthesis, and because of the allosteric influence of the product (malonyl-CoA) of this enzyme on CPT-I (described above), its regulation takes many forms including rapid allosteric and phosphorylation/dephosporylation mechanisms as well as longer-term mechanisms at the level of gene expression (Kim, 1997 review). Hormonal stimulation (e.g. glucagon) results in increased intracellular cAMP and causes rapid inactivation of ACC via phosphorylation at multiple sites. Recent findings have suggested that different isozymes (designated α and β) of ACC, encoded for by different genes, may differentially regulate anabolic and catabolic carbon flux. Indeed, the recently cloned β form (Ha et al., 1996) has an additional 150 amino acids at the N-terminus that may direct it to insertion into the mitochondrial membrane where it may play a direct role in regulating CPT-I. This may be of particular importance in tissues (e.g., piglet liver) in which fatty acid synthesis is negligible and yet CPT-I is highly sensitive to malonyl-CoA inhibition.

3.4. Regulation of Mitochondiral HMG-CoA Synthase (mHMGCS)

Since the establishment of the CPT-I-control theory, a growing body of evidence has accumulated suggesting other possible intramitochondrial regulatory sites, particularly in neonates (Pegorier et al., 1983; Escriva et al., 1986; Decaux et al., 1988). Much of the early evidence was indirect and speculative. However, researchers at Cambridge (Lowe and Tubbs, 1985a, 1985b, 1985c; Quant et al., 1989, 1990, 1991, 1993) reported compelling evidence that control of mHMGCS activity is likely. Specifically, they have shown that its activity is

regulated by a succinylation-desuccinylation mechanism. In the first step of its normal catalytic cycle, mHMGCS becomes acetylated at the active site by reaction with acetyl-CoA (its first substrate). They have shown that succinyl-CoA (at physiological concentrations) may also react leading to competitive inhibition of the enzyme (Lowe and Tubbs, 1985c). Furthermore, various *in vivo* treatments which stimulate ketogenesis (fasting, glucagon or mannoheptulose injection, alloxan diabetes, high fat feeding, etc.) all increased mHMGCS activity by decreasing its degree of succinylation (Quant et al., 1989). The enzyme was 40-50% succinylated (and inactive) in the livers of normal fed rats and could be rapidly (within minutes) activated *in vitro*. This led the authors to speculate that the succinylation-control mechanism could allow for rapid changes in ketogenic flux rate *in vivo*. More recently (Quant et al., 1991), the control of ketogenesis in the neonatal rat has been shown to be mediated, in part, by changes in the amount and activity of mHMGCS, presumably at the level of gene expression (Casals et al., 1992; see Hegardt, 1999 for review). Indeed, Thumelin et al. (1993) showed that hepatic mRNA concentrations for mHMGCS increased by about three-fold within 24 h of birth in rats, remained constant throughout suckling and then rapidly declined when animals were weaned onto a low fat diet. Furthermore, mRNA in cultured fetal hepatocytes increased by four-fold within 4 h after exposure to glucagon. Additional research (Ayte et al., 1993) has suggested that expression may be regulated in part by methylation/demethylation of the 5' flanking region of the gene and has identified CRE and C-EBP as potential *cis* regulatory elements (Brady et al., 1993; Gomez et al., 1993) which could mediate the glucagon effects. Subsequent research also identified the PPAR-RXR diad as an important regulator of expression induced by clofibrate and fatty acids (Rodriguez et al., 1994).

3.4. Hormonal support of fatty acid oxidation and ketogenesis

Regardless of the underlying biochemical regulatory mechanism(s), the major hormonal influence is most likely mediated by the insulin/glucagon ratio (McGarry and Foster, 1977). When the ratio is low, as observed during the neonatal period, fasting, or diabetes, ketogenesis is stimulated. The hormonal effect may be mediated through regulation of acetyl-CoA carboxylase (Borthwick et al., 1986), thus affecting malonyl-CoA levels and CPT-I activity and/or by decreasing succinyl-CoA levels and thereby activating mHMGCS (Quant et al., 1989) and/or by affecting levels of TCA cycle intermediates. Similarly, hormonal alteration of intracellular cAMP concentrations (Pegorier et al., 1989) may directly impact gene transcription (as described previously) via interaction with cAMP response elements. Furthermore, *in vivo* and *in vitro* exposure of rodent tissues to the peroxisome-proliferating hypolipidemic drugs (eg., clofibrate) and dehydroepiandrosterone (Brady et al., 1991) has been shown to upregulate fatty acid oxidation and/or ketogenesis by increasing transcription of CPT and/or mHMGCS genes.

3.5. Hepatic lipid metabolism in the piglet

Clearly, a fundamental understanding of the developmental aspects of lipid metabolism is essential in order to optimize postnatal fat utilization by the piglet. Putative changes in metabolic capacity within the first week of life are of greatest concern because 75% of mortality occurs during this time period (USDA, 1991). Unfortunately, relatively little research has been focused on these animals. The following review examines pig-specific studies.

3.6. Development of fatty acid oxidation in piglets

Postnatal increases in fatty acid oxidation have been reported (Wolfe et al., 1978), but must be interpreted in light of the general increase in metabolic rate which occurs after birth (Odle et al., 1991b). For example, the oxidation of U-^{14}C palmitate to CO_2 and acid soluble products (considered to represent ketone bodies and/or TCA cycle intermediates) by liver homogenates was reported to increase four fold between 0 and 7 d of age (Mersmann and Phinney, 1973); however, marked increases in mitochondrial respiration from several TCA cycle intermediates has likewise been reported (Mersmann et al., 1972) wherein oxygen consumption increased by 5-fold between 6 and 12 h postpartum. Subsequent histological work suggested rapid mitochondrial proliferation during this early neonatal period. Odle et al. (1991b) have likewise observed developmental increases in hepatic fatty acid oxidation in small and normal-birth-weight pigs during the first 48 h of life which could be largely explained by increases in oxygen consumption. Thus, increases in fatty acid oxidation postnatally may be accounted for, in part, by increased oxidative metabolism in general, and may not necessarily infer an increased reliance upon fat as a fuel (Adams et al., 1997a).

3.7. Lack of ketogenesis in piglets

While piglets appear to display a hormonal profile (low insulin/glucagon) which would support ketogenesis (Pegorier et al., 1981), and have ample substrate from the fat present in milk, they do not display hyperketonemia (Bengtsson et al., 1969; Pegorier et al., 1981), despite elevated plasma non-esterified fatty acids (Adams and Odle, 1993b). This starkly contrasts with other mammalian species (e.g., rats, rabbits, etc.) which show pronounced hyperketonemia during suckling (Foster and Bailey, 1976) as well as ruminant species which under extreme lactational stress can die from ketoacidosis. 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 hypoglycemia (Swiatek et al., 1968). Furthermore, insofar as fatty acid oxidation also is required to support active gluconeogenesis, impaired fat oxidation also could contribute indirectly to hypoglycemia (Lepine et al., 1993; Duee et al., 1996). In theory, low ketone concentrations could be due to a low production rate (i.e., ketogenesis) and/or a high rate of utilization. Using continuous-infusion isotope kinetics, we have observed limited β-OH-butyrate oxidation rates *in vivo* (Tetrick et al., 1995). Piglets were arterially infused with ^{14}C- β -OH-butyrate at rates sufficient to supply 15, 30, 45 and 60% of their estimated ATP turnover. Michaelis-Menten analysis of measured oxidation rates versus plasma concentrations showed that β -OH-butyrate could supply a maximum of 32% of the piglet's total body ATP turnover, but at physiological concentrations would supply <5% of the animal's energy need. More likely, an impaired rate of hepatic ketogenesis (Pegorier et al., 1983; Duee et al., 1994) is the cause. Comparative *in vivo* research (Adams and Odle, 1993b) showed that the relative ketogenic capacity (measured by regression of plasma β -OH-

butyrate on plasma octanoate concentrations following intraperitoneal injection of octanoate) of neonatal pigs is greatly attenuated (by up to 1-2 orders of magnitude) compared to weanling or mature swine and to neonatal or mature rabbits. Furthermore, using radio-HPLC to characterize products of radiolabeled fatty acid oxidation, we have observed trivial accumulation of isotope in ketone bodies from piglet liver compared with neonatal rat liver preparations (Adams et al., 1997a). Concurrent with these findings, measurements of hepatic mHMGCS have shown 70% lower activities in neonatal pigs than in neonatal rabbits (Adams and Odle, 1993a) or adult rats (Duee et al., 1994). Following the cloning of the pig gene (Adams et al., 1997b), most recent findings confirmed the attenuated expression of this enzyme (compared with rats) during suckling, but showed that starvation led to increased mRNA concentrations. However, substantial increases in mRNA did not occur until 2-3 weeks of age, indicating a developmental lag in induction compared with the rapid postnatal rise observed in the suckling rat (Thumelin et al., 1993). Subsequent examination of the upstream regulatory region of the pig gene has failed to identify any idiosyncrasies that might impair expression in that it contains the anticipated PPAR response element. (Ortiz et al., 1999). Despite increased mRNA concentrations, enzyme activity still remained low. Current research findings indicate that pig mRNA codes for a catalytically active protein, and low synthase activity is a result of attenuated translation. Furthermore, the decrease in mRNA translation was not a result of alteration of the polyadenylate tail which can influence both mRNA stability and subsequent translation into protein (Barrero et al., 2001).

3.8. Regulation of fatty acid oxidation and ketogenesis in piglets

The degree to which the regulatory features of ketogenesis (reviewed previously) described in other species extrapolates to the neonatal pig is not known. A putative limitation of fatty acid oxidation at the level of CPT-I was suggested by data from Honeyfield and Froseth (1991) who reported a >10-fold increase in oxygen consumption when palmitoylcarnitine was compared to palmitoyl-CoA in heart and liver homogenates from newborn pigs. Likewise, using hepatocytes isolated from piglets at birth and from piglets fed or fasted 24h from birth, we (Odle et al., 1995) have obtained evidence consistent with the hypothesis that CPT-I is a potential regulatory site: 1) The molar oxidation rate of octanoate was 4-times higher than of palmitate. This is 2-fold higher than can be explained by the molar energy difference between these fatty acids, and implies a putative limitation in the oxidation of palmitate compared to octanoate, given the measured oxygen consumption rates were similar in cells incubated with each fatty acid. While differences in the relative activities of the medium- and long-chain acyl-CoA synthetases cannot be excluded, this observation could be explained by a limitation of palmitoyl-CoA transport into the mitochondrion via CPT. 2) Carnitine (a co-substrate for CPT) increased oxidation and decreased esterification of palmitate, but had no effect on octanoate metabolism. 3) TDGA (an irreversible inhibitor of CPT-I) reduced oxidation and increased esterification of palmitate, but again, had no effect on octanoate metabolism.

Developmental changes in liver and muscle CPT activity during the neonatal period have been reported (Bieber et al., 1973; Lin and Odle, 1995; Schmidt and Herpin, 1998). The activity doubled in the liver of pigs between 0 and 1 d of age but then plateaued to equal the activity in mitochondria from 24-d-old animals. Interestingly, palmitoyl-CoA oxidation continued to increase, doubling between 1 and 2 d postpartum. This suggests that something other than CPT activity was limiting oxidation as the animals aged. Perhaps decreases in the sensitivity of CPT to malonyl-CoA (Duee et al., 1994; Lin and Odle, 1995; Schmidt and

Herpin, 1998) are responsible as has been reported in rabbits (Prip-Buus et al., 1990). Indeed, Pegorier et al. (1983) challenged the idea of control mediated via CPT-I. In contrast to our findings (Odle et al., 1991a, 1991b; Odle et al., 1995; Lin et al., 1996), they reported low oxidation rates for octanoate (vs oleate). In addition, they were unable to increase oleate oxidation by isolated piglet hepatocytes incubated with glucagon (which should have decreased malonyl-CoA levels). This may not be surprising because the liver is not a major site of lipogenesis in the pig (Mersmann et al., 1973). In general, malonyl-CoA concentrations are higher in lipogenic tissues as the synthesis of malonyl-CoA is the first committed step of lipogenesis. The role of malonyl-CoA in non-lipogenic tissues, such as skeletal muscle, is regulation of CPT I activity. Thus, in non-lipogenic tissues the concentration of malonyl-CoA is much less than what is observed in lipogenic tissues and CPT I exhibits greater sensitivity to malonyl-CoA. Recent cloning of the pig M- and L-CPT I isoforms has revealed that L-CPT I is a natural chimera of rat M- and L-CPT I isoforms (Nicot et al., 2001). Pig L-CPT I shows similar kinetics towards carnitine as rat L-CPT I, however, IC_{50} for malonyl-CoA is similar to rat M-CPT I. Thus, pig L-CPT I has a greater sensitivity to malonyl-CoA than rat L-CPT I.

In addition to the potential control by CPT I, regulation may also shift to an intramitochondrial site (e.g., mHMGCS). Research reported by Duee et al. (1994) verified the low rate of ketogenesis in mitochondria from 2 d old piglets, and implicated an intramitochondrial constraint. Namely, when the mitochondria were treated with malonate (a blocker of succinate dehydrogenase) to decrease TCA cycle flux, the major end product of palmitoyl-carnitine oxidation was presumed to be acetoacetate. In this case, the rate of palmitoyl-carnitine utilization was only 2.5 nmol/min/mg protein. However, when malate was added the end product was citrate and the rate of palmitoyl-carnitine utilization increased by 3 fold.

3.9. Peroxisomal β -oxidation in piglets

We have characterized the postnatal development and tissue distribution of peroxisomal fatty acid oxidation in piglet (Yu et al., 1997a, 1997b, 1998). In general, activity measured in liver, kidney and heart as either antimycin/rotenone-insensitive palmitate oxidation or palmitoyl-CoA dependent KCN-insensitive reduction of NAD, was high (i.e., 40-50% of total fat β -oxidation) compared with published rat values (e.g., 25%) and developed rapidly after birth. Hepatic activity of peroxisomal oxidation was increased when suckling-aged piglets were fasted, presumably due to elevated plasma free fatty acids and/or glucagon. Most recent data from our laboratory (Yu et al., 2001) showed that hepatic activities of fatty acid oxidase (an enzyme unique to peroxisomes) as well as CPT were dramatically induced in piglets fed milk replacer containing clofibrate. Clofibrate is a hypolipidemic drug which is known to induce peroxisome (and possibly mitochondrial) biogenesis in rodents (Brady et al., 1991), through interaction with the peroxisome-proliferator activated receptor (PPAR). We hypothesize that the extra thermogenesis associated with peroxisomal β-oxidation may be important in the suckling piglet's maintenance of homeothermy.

4. FUTURE PERSPECTIVES

Research findings to date indicate impairment in ketone body synthesis for the neonatal pig when compared to other species. This reduction in ketone bodies during a period when fatty acids are the main source of energy implies that the young pig is inefficient in utilizing

dietary fat, and thus may impact piglet survival and performance. In order to optimize nutrition for the neonatal pig an understanding of the underlying mechanisms of lipid metabolism must be elucidated. Further insight into the regulation of key lipid enzymes, including rate of synthesis and degradation of mRNA and protein, and subsequent protein activity is required. Recent research on CPT I and HMG-CoA synthase has shown key differences between these enzymes in piglets when compared to the rat, a popular model in lipid research. These differences highlight the need for more research in the neonatal pig; only then will the mechanisms responsible for lipid oxidation be defined so that improvements in lipid metabolism can be made at a cellular level.

Research in the arena of molecular mechanisms of lipid oxidation will not provide overnight insight into improving current practices for piglet survival and performance. However, as swine production becomes increasingly specialized the demand for the formulation of milk replacers which optimize early growth will increase also. The use of such milk replacers will allow dietary manipulation which cannot be achieved through nutritional management of the dam. Control over the piglets diet would allow for manipulation of the relative percent of energy contributed by carbohydrates versus fat and could have a profound impact on piglet morbidity and mortality. The specific fatty acids added to the diet also could be altered to increase the amount of MCT (for example) which serve as obligatory fuels. Furthermore, current research in the use of peroxisome proliferators indicate that their supplementation into the diet may provide a useful tool in improving fatty acid utilization by the pig. Further research in these areas will hopefully assist in development of strategies to enhance fatty acid oxidation and ultimately improve piglet survival and growth.

REFERENCES

Adams, S.H., Odle., J., 1993a. Does HMG-CoA synthase play a role in limitation of ketogenesis in neonatal pigs? FASEB J. 7, A379.

Adams, S.H., Odle, J., 1993b. Plasma β-hydroxybutyrate after octanoate challenge: attenuated ketogenic capacity in neonatal swine. Amer. J. Physiol. 265, R761-R765.

Adams, S.H., Lin, X., Yu, X.X., Odle, J., Drackley, J.K., 1997a. Hepatic fatty acid metabolism in pigs and rats: Major Differences in end-products, O_2 uptake and β-oxidation. Amer. J. Physiol. 272, R1641-1646.

Adams, S.H., Alho, S.C., Asins, G., Hegardt, F.G., Marrero, P.F., 1997b. Gene expression of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase in a poorly ketogenic mammal: effect of starvation during the neonatal period of the piglet. Biochem. J. 65-73.

Asins, G., Serra, D., Arias, G., Hegardt, F.G., 1995. Developmental changes in carnitine palmitoyltransferases I and II gene expression in intestine and liver of suckling rats. Biochem. J. 379-384.

Ayte J., Gomez, G.G., Hegardt, F.G., 1993. Structural characterization of the 3' noncoding region of the gene encoding rat mitochondrial 3-hydroxy-3-methylglutaryl coenzyme A synthase. Gene 123, 267-270.

Bach, A.C., Babayan, V.K., 1982. Medium-chain triglycerides: an update. Amer. J. Clin. Nutr. 36, 950-962.

Barrero, M.J., Alho, C.S., Oritz , J.A., Hegardt, F.G., Haro, D., Marrero, P.F., 2001. Low activity of mitochondrial HMG-CoA synthase in liver of starved piglets is due to low levels of protein despite high mRNA levels. Arch. Biochem. Biophys. 385, 364-371.

Battaglia, F.C., Meschia, G., 1978. Principle substrates of fetal metabolism. Physiol. Rev. 58, 499.

Bengtsson, N.J., Gentz, J., Hakkarainen, J., Hellstrom, R., Persson, B., 1969. Plasma levels of FFA, glycerol, B-Hydroxybutyrate and blood glucose during the postnatal development of the pig. J. Nutr. 97, 311-315.

Bieber, L.L., Markwell, M.A.K., Blair, M., Helmrath, T.A., 1973. Studies on the development of carnitine palmitoyltransferase and fatty acid oxidation in liver mitochondria of neonatal pigs. Biochim. Biophys. Acta 326, 145-154.

Brady, L.J., Ramsay, R.R., Brady, P.S., 1991. Regulation of carnitine acyltransferase synthesis in lean and obese zucker rats by dehydroepiandrosterone and clofibrate. J. Nutr. 121, 525-531.

Brady, P.S., Barke, R.A., Brady, L.J., 1993. Regulation of the 68-kDa hepatic carnitine palmitoyltransferase. Ch. 14. In: Nutrition and Gene Expression. CRC Press. pp. 319-334.

Borthwick, A.C., Edgell, N.J., Denton, R.M., 1986. Mechanisms involved in the short-term regulation of acetyl-CoA carboxylase by insulin. Biochem. Soc. Trans. 14, 563-565.

Borum, P.R., 1983. Carnitine. Ann. Rev. Nutr. 3, 233-259.

Casals, N., Roca, N., Guerrero, M., Gil-Gomez, G., Ayte, J., Ciudad, C.J. Hegardt, F.G., 1992. Regulation of the expression of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene. Its role in the control of ketogenesis. Biochem. J. 283, 261-264.

Chatelain, F., Kohl, C., Esser, V., McGarry, J.D., Girard, J., Pegorier, J.P., 1996. Cyclic AMP and fatty acids increase carnitine palmitoyltransferase I gene transcription in cultured fetal rat hepatocytes. Eur. J. Biochem. 789-798.

Coleman, R.A., Lewin, T.M., Muoio, D.M., 2000. Physiological and nutritional regulation of enzymes of triacylglycerol synthesis. Ann. Rev. Nutr. 20, 77-103.

Duee, P.-H., Pegorier, J.P., Quant, P.A., Herbin, C., Kohl, C., Girard, J., 1994. Hepatic ketogenesis in newborn pigs is limited by low mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase activity. Biochem. J. 298, 207-212.

Duee, P.-H., Pegorier, J.P., Darcy-Vrillon, B., Girard, J., 1996. Glucose and fatty acid metabolism in the newborn pig. In: Tumbleson, M.E., Schook, L.B. (Eds.), Advances in Swine in Biomedical Research, New York. Plenum Press, p. 865-884.

Decaux, J.F., Robin, D., Robin, P., Ferre, P., Girard, J., 1988. Intramitochondrial factors controlling hepatic fatty acid oxidation at weaning in the rat. FEBS Lett. 156-158.

Escriva, F., Ferre, P., Robin, D., Decaux, J.F., Girard, J., 1986. Evidence that the development of hepatic fatty acid oxidation at birth in the rat is concomitant with an increased intramitochondrial CoA concentration. Eur. J. Biochem. 156, 603-607.

Ferre, P., Decaux, J.F., Issad, T., Girard, J., 1986. Changes in energy metabolism during the suckling and weaning period in the newborn. Reprod. Nutr. Develop. 26, 619-631.

Ferre, P., Satabin, P., Decaux, J.F., Escriva, F., Girard, J., 1983. Development and regulation of ketogenesis in hepatocytes isolated from newborn rats. Biochem. J. 214, 937-942.

Foster, P.C., Bailey, E., 1976. Changes in hepatic fatty acid degradation and blood lipid and ketone body content during development in the rat. Enzyme 21, 397-407.

Girard, J., Ferre, P., Pegorier, J.P., Duee, P.H., 1992. Adaptations of glucose and fatty acid metabolism during the perinatal period and suckling-weaning transition. Physiol. Rev. 72, 507-562.

Gomez, G.G., Ayte, J., Hegardt, F.G., 1993. The rat mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme-A-synthase gene contains elements that mediate its multihormonal regulation and tissue specificity. Eur. J. Biochem. 213, 773-779.

Groot, P.H.E., Scholte, H.R., Hulsmann, W.C., 1976. Fatty acid activation: specificity, localization and function. Adv. Lipid Res. 14, 75-126.

Ha, J., Lee, J.K., Kim, D.S., Witters, L.A., Kim, K.H., 1996. Cloning of human acetyl-CoA carboxylase-ß and its unique features. Proc. Natl. Acad. Sci. USA 93, 11466-70.

Hegardt, F.G., 1999. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase: a control enzyme in ketogenesis. Biochem. J. 338, 569-582.
Hettema, E.H., Tabak, H.F., 2000. Transport of fatty acids and metabolite across the peroxisomal membrane. Biochim. Biophys. Acta 1486, 18-27.
Honeyfield, D.C., Froseth, J.A., 1991. Evaluation of energy sources with and without carnitine in newborn pig heart and liver. J. Nutr. 121, 1117-1122.
Kim, K.H., 1997. Regulation of mammalian acetyl-coenzyme A carboxylase. Ann. Rev. Nutr. 17, 77-99.
Kerner, J., Froseth, J.A., Miller, E.R., Bieber, L.L., 1984. A study of the acylcarnitine content of sows' colostrum, milk and serum and newborn piglet tissues: Demonstration of high amounts of isovaleryl-carnitine in colostrum and milk. J. Nutr. 114, 854-861.
Kondrup, J., Lazarow, P.B., 1985. Flux of palmitate through the peroxisomal and mitochondrial ß-oxidation systems in isolated rat hepatocytes. Biochim. Biophys. Acta 835, 147-153.
Latruffe, N., Cherkaoui Malki, M., Nicolas-Frances, V., Jannin, B., Clemencet, M.-C., Hansmannel, F. Passilly-Degrace, P., Berlot, J.-P., 2001. Peroxisome-proliferator-activated receptors as physiological sensors of fatty acid metabolism: molecular regulation in peroxisomes. Bio. Soc. Trans. 29, 305-309.
Lazo, O., Contreras, M., Singh, I., 1990. Topographical localization of peroxisomal acyl-CoA ligases: differential localization of palmitoyl-CoA and lignoceroyl-CoA ligases. Biochem. 29, 3981-3986.
Lewin, T.M., Kim, J.H., Granger, D.A., Vance, J.E., Coleman, R.A., 2001. Acyl-CoA synthetase isoforms 1, 4, and 5 are present in different subcellular membranes in rat liver and can be inhibited independently. J. Biol. Chem. 276, 24674-79.
Lepine, A.J., Watford, M., Boyd, R.D., Ross, D.A., Whitehead, D.M., 1993. Relationship between hepatic fatty acid oxidation and gluconeogenesis in the fasting neonatal pig. Brit. J. Nutr. 70, 81-91.
Lin, X., Odle, J., 1995. Regulation of fatty acid oxidation by hepatic mitochondria from neonatal pigs via carnitine palmitoyltransferase I. J. Anim. Sci. 73 (Suppl 1), 77.
Lin, X., Adams, S.H., Odle, J., 1996. Acetate represents a major product of heptanoate and octanoate ß-oxidation in hepatocytes isolated from neonatal piglets. Biochem. J. 318, 235-240.
Lowe, D.M., Tubbs, P.K., 1985a. 3-Hydroxy-3-methylglutaryl-coenzyme A synthase from ox liver. Purification, molecular and catalytic properties. Biochem. J. 227, 591-599.
Lowe, D.M., Tubbs, P.K., 1985b. 3-Hydroxy-3-methylglutaryl-coenzyme A synthase from ox liver. Properties of its acetyl derivative. Biochem. J. 227, 601-607.
Lowe, D.M., Tubbs, P.K., 1985c. Succinylation and inactivation of 3-hydroxy-3-methylglutaryl-CoA synthase by succinyl-CoA and its possible relevance to the control of ketogenesis. Biochem. J. 232, 37-42.
McGarry, J.D., Brown, N.F., 1997. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur. J. Biochem. 244, 1-14.
McGarry, J.D., Foster, D.W., 1977. Hormonal control of ketogenesis. Arch. Int. Med. 137, 495-501.
McGarry, J.D., Foster, D.W., 1980. Regulation of hepatic fatty acid oxidation and ketone body production. Ann. Rev. Biochem. 49, 395-420.
Mersmann, H.J., Goodman, J., Houk, J.M., Anderson, S., 1972. Studies on the biochemistry of mitochondrial and cell morphology in the neonatal swine hepatocyte. J. Cell. Biol. 53d, 335-347.

Mersmann, H.J., Phinney, G., 1973. In vitro fatty acid oxidation in liver and heart from neonatal swine (Sus Domesticus). Comp. Biochem. Physiol. 44B, 219-223.

Mersmann, H.J., Phinney, G., Sanguinetti, M.C., Houk, J.M., 1973. Lipogenic capacity of liver from perinatal swine (sus domesticus). Comp. Biochem. Physiol. 46B, 493-497.

Nicot, C., Hegardt, F.G., Woldegiorgis, G., Haro, D., Marrero, P.F., 2001. Pig liver palmitoyltransferase I, with low Km for carnitine and high sensitivity to malonyl-CoA inhibition, is a natural chimera of rat liver and muscle enzymes. Biochem. 40, 2260-2266.

Odle, J., 1997. New insights into medium-chain triglyceride utilization by the neonate: Observations from a piglet model. J. Nutr. 127, 1061-1067.

Odle, J., 1998. Medium-chain triglycerides: A unique energy source for neonatal pigs. Pig News and Information 20, 25-32.

Odle, J., Benevenga, N.J., Crenshaw, T.D., 1991a. Utilization of medium-chain triglycerides by neonatal piglets: Chain length of even- and odd-carbon fatty acids and apparent digestion/absorption and hepatic metabolism. J. Nutr. 121, 605-614.

Odle, J., Benevenga, N.J., Crenshaw, T.D., 1991b. Postnatal age and the metabolism of medium- and long-chain fatty acids by isolated hepatocytes from small-for-gestational-age and appropriate-for-gestational-age piglets. J. Nutr. 121, 615-621.

Odle, J., Lin, X., van Kempen, T.A.T.G., Drackley, J.K., Adams, S.H., 1995. Carnitine palmitoyltransferase modulation of hepatic fatty acid metabolism and radio-HPLC evidence for low ketogenesis in neonatal pigs. J. Nutr. 125, 2541-2549.

Oikawa, E., Iijima, H., Suzuki, T., Sasano, H., Sato, H., Kamataki, A., Nagura, H., Kang, M., Fujino, T., Suzuki, H., Yamamoto, T.T., 1998. A novel acyl-CoA synthetase, ACS5, expressed in intestinal epithelial cells and proliferating preadipocytes. J. Biochem. 124, 679-685.

Ortiz, J.A., Mallolas, J., Nicot, C., Bofarull, J., Rodriguez, J.C., Hegardt, F.G., Haro, D., Marrero, P.F., 1999. Isolation of pig mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene promoter: characterization of a peroxisome proliferator-responsive element. Biochem. J. 337, 329-335.

Pegorier, J.P., Duee, P.H., Peret, J., Girard, J., 1981. Changes in circulating fuels, pancreatic hormones and liver glycogen concentration in fasting or suckling newborn pigs. J. Dev. Physiol. 3, 203-217.

Pegorier, J.P., Duee, P.H., Girard, J., Peret, J., 1983. Metabolic fate of non-esterified fatty acids in isolated hepatocytes from newborn and young pigs. Evidence for a limited capacity for oxidation and increased capacity for esterification. Biochem. J. 212, 93-97.

Pegorier, J.P., Garcia-Garcia, M-V., Prip-Buus, C., Duee, P-H., Kohl, C., Girard., J., 1989. Induction of ketogenesis and fatty acid oxidation by glucagon and cyclic AMP in cultured hepatocytes from rabbit fetuses. Biochem. J. 264, 93-100.

Prip-Buus, C., Pegorier, J., Duee, P., Kohl, C., Girard, J., 1990. Edivence that the sensitivity of carnitine palmitoyltransferase I to inhibition by malonyl-CoA is an important site of regulation of hepatic fatty acid oxidation in the fetal and newborn rabbit. Biochem. J. 269, 409-415.

Quant, P.A., Tubbs, P.K., Brand, M.D., 1989. Treatment of rats with glucagon or mannoheptulose increases mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase activity and decreases succinyl-CoA content in liver. Biochem. J. 262, 159-164.

Quant, P.A., Tubbs, P.K., Brand, M.D., 1990. Glucagon activates mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase in vivo by decreasing the extent of succinylation of the enzyme. Eur. J. Biochem. 187, 169-174.

Quant, P.A., Robin, D., Robin, P., Ferre, P., Brand, M.D., Girard, J., 1991. Control of hepatic mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase during the foetal/neonatal

transition, suckling and weaning in the rat. Eur. J. Biochem. 195, 449-454.
Quant, P.A., Robin, D., Robin, P., Girard J., Brand, M.D., 1993. A top-down control analysis in isolated rate liver mitochondria: can the 3-hydroxy-3-methylglutaryl-CoA pathway be rate-controlling for ketogenesis? Biochem. Biophys. Acta. 1156, 135-143.
Reddy, J. K., Mannaerts, G.P., 1994. Peroxisomal lipid metabolism. Annu. Rev. Nutr. 14, 343-370.
Robles-Valdes, C., McGarry, J.D., Foster, D.W., 1976. Maternal-fetal carnitine relationships and neonatal ketosis in the rat. J. Biol. Chem. 251, 6007-6012.
Rodríguez, J.C., Gomez, G.G., Hegardt, F.G., Haro, D., 1994. Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. J. Biol. Chem. 269, 18767-18772.
Rohde Parfet, K.A., Gonyou, H.W., 1988. Effect of creep partitions on teat-seeking behavior of newborn piglets. J. Anim. Sci. 66, 2165.
Schmidt, I., Herpin, P., 1998. Carnitine palmitoyltransferase I (CPTI) activity and its regulation by malonyl-CoA are modulated by age and cold exposure in skeletal muscle mitochondria from newborn pigs. J. Nutr. 128, 886-893.
Stanier, M.W., Mount, L.E., Bligh, J., 1984. In: Energy Balance and Temperature Regulation. Cambridge University Press, NY.
Swiatek, K.R., Kipnis, D.M., Mason, G., Chao, K., Cornblath, M., 1968. Starvation hypoglycemia in newborn pigs. Amer. J. Physiol. 214, 400-405.
Tetrick, M.A., Adams, S.H., Odle, J., Benevenga, N.J., 1995. Contribution (D)-(-)-3-hydroxybutyrate to the energy expenditure of neonatal pigs. J. Nutr. 125, 264-272.
Thumelin, S., Esser, V., Charvy, D., Kolodziej, M.P., Zammit,V.A., McGarry, J.D., Girard, J., Pegorier, J.P., 1994. Expression of liver carnitine palmitoyltransferase I and II genes during development in the rat. Biochem. J. 583-587.
Thumelin, S., Forestier, M., Girard, J., Pegorier, J.P., 1993. Developmental changes in mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene expression in rat liver, intestine and kidney. Biochem. J. 292, 493-496.
USDA. 1991. National swine survey: morbidity/mortality and health management of swine in the U.S. National Animal Health Monitoring System, APHIS: United States Department of Agriculture, Washington D.C.
USDA, 2002. Part III. Reference of swine health and environmental management in the United States, 2000. United States Department of Agriculture: APHIS: VS: CEAH, National, Fort Collins, CO. #N361.0902.
Van Veldhoven, P.P., Casteels, M., Mannaerts, G.P., Baes, M., 2001. Further insights into peroxisomal lipid breakdown via α- and β-oxidation. Biochem. Soc. Trans. 29, 292-297.
Verhoeven, N.M., Roe, D.S., Kok, R.M., Wanders, R.J., Jakobs, C., Roe., C.R., 1998. Phytanic acid and pristanic acid are oxidized by sequential peroxisomal and mitochondrial reactions in cultured fibroblasts. J. Lipid Res. 39, 66-74.
Wanders, R.J., Tager, J.M., 1998. Lipid metabolism in peroxisomes in relation to human disease. Mol. Aspects Med. 19, 69-154.
Wanders, R.J.A., Vreken, P., Ferdinandusse, S., Jansen, G.A., Waterham, H.R., van Roermund, C.W.T., Van Grunsven, E.G., 2001. Peroxisomal fatty acid α- and β-oxidation in humans: enzymology, peroxisomal metabolite transporters and peroxisomal diseases. Biochem. Soc. Trans. 29, 250-267.
Williamson, D.H., Bates, M.W., Krebs, H.A., 1968. Activity and intracellular distribution of enzymes of ketone-body metabolism in rat liver. Biochem. J. 108, 353-361.
Wolfe, R.G., Maxwell, C.V., Nelson, E.C., 1978. Effect of age and dietary fat level on fatty

acid oxidation in the neonatal pig. J. Nutr. 108, 1621-1634.
Yu, X.X., Drackley, J.K., Odle, J., Lin, X., 1997a. Response of hepatic mitochondrial and peroxisomal ß-oxidation to increasing palmitate concentrations in piglets. Biol. Neonate 72, 284-292.
Yu, X.X., Drackley, J.K., Odle, J., 1997b. Rates of mitochondrial and peroxisomal $-oxidation of palmitate change during postnatal development and feed deprivation in liver, kidney and heart of pigs. J. Nutr. 127, 1814-1821.
Yu, X.X., Drackley, J.K., Odle, J., 1998. Food deprivation changes peroxisomal ß-oxidation activity but not catalase activity during postnatal development in pig tissues. J. Nutr. 128, 1114-1121.
Yu, X.X., Drackley, J.K., Odle, J., 2001. Differential induction of peroxixomal ß-oxidation enzymes by clofibric acid and aspirin in piglet tissues. Am. J. Physiol. Regul. Integr. Comp. Physiol. 281, R1553-1561.

Figure 1. Pathways of hepatic lipid metabolism with emphasis on oxidative metabolism. Enzymes/pathwasy are numbered as follows: 1) long-chain acyl-CoA synthetase, 2) acetyl-CoA carboxylase, 3) various acyl-CoA transferases, 4) carnitine shuttle consistin of CPT 1, translocase, and CPT II, 5) medium-chain acyl-CoA synthetase, 6) mitochondrial hydroxymethylglutaryl-CoA synthse, 7) acyl-CoA dehydrogenase, 8) long0chain acyl-CoA synthetase, 9) very lon-chain acyl-CoA synthetase, 10) acyl-CoA oxidase, 11) carnitine octanoyltransferase.