

New Insights into the Utilization of Medium-Chain Triglycerides by the Neonate: Observations from a Piglet Model^{1,2}

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ABSTRACT Because of their unique digestive and metabolic properties, medium-chain triglycerides (MCT) are used in a variety of nutritional settings, including use as a readily digestible energy source for the neonate. This review examines recent findings from our laboratory related to MCT digestion and metabolism that are drawn from a neonatal piglet model, but which may be clinically relevant to human infants. We have shown that MCT utilization improves rapidly with postnatal age (within 24 h), which is likely due to the ontogeny of pancreatic lipase. Additional data delineate the dramatic effects of emulsification and fatty acid chain length (within the medium-chain family) on utilization, with the suggestion that triacylhexanoate is utilized at the highest rate. Again, these effects are likely mediated via an increase in the kinetics of digestion rather than metabolism. Indeed, using both *in vitro* and *in vivo* radiotracer techniques, we were unable to detect metabolic differences among even-chain fatty acid homologues. However, studies with isolated hepatocytes have shown greater oxidation rates of odd-chain fatty acids compared with even-chain homologues, in part as a result of the anaplerotic potential of propionyl-CoA arising from odd-carbon fatty acid oxidation. *In vivo* radiotracer studies also showed an improvement in octanoate oxidation to CO₂, with a concomitant reduction in urinary dicarboxylic acid excretion when colostrum-deprived piglets were supplemented with L-carnitine. Further metabolic research led to the novel finding that piglets have a very limited hepatic capacity to synthesize ketone bodies, and that acetate may be a relatively important product of hepatic fatty acid oxidation in this species. *J. Nutr.* 127: 1061–1067, 1997.

KEY WORDS: • *medium-chain triglyceride* • *fatty acid metabolism* • *carnitine* • *swine*

Research examining the nutritional and metabolic properties of medium-chain triglycerides (MCT)³ spans a period of 40 y. Following early studies of the 1950s (Bloom et al. 1951), which described effects of fatty acid chain length on the portal vs. lymphatic routes of absorption, research focused on delineating the unique features of MCT digestion and metabolism in contrast to the established dogma for long-chain triacylglycerols (LCT). Since then, because of their rapid rate and extent of digestion, accelerated rate of portal absorption and obligatory oxidation (detailed below), MCT have been examined as a specialized energy source within a wide variety of clinical nutrition settings. These include treatment of pancreatic and biliary insufficiency (e.g., cystic fibrosis), gastroenteritis, chylothorax, obesity and diabetes. They also have been employed in total parenteral nutrition and preterm infant formulas. Recent development of reduced-calorie, structured lipids containing medium-chain fatty acids also represents an exciting new application (Finley et al. 1994, Webb et al. 1993).

Given this immense and heterogeneous literature base, it

is well beyond the scope of this review to provide a comprehensive survey of the MCT literature. Rather, the intent is to provide a brief overview of the unique attributes of MCT utilization to enhance the reader's appreciation for their application in neonatal nutrition and then to summarize the new findings from our laboratory, which have employed the increasingly popular neonatal piglet model (Reeds and Odle 1996).

ESTABLISHED DOGMA OF MCT DIGESTION, ABSORPTION AND METABOLISM

The following discussion provides a basic, general contrast of MCT and LCT utilization. For further detail, the interested reader can examine several review articles, symposia and textbooks (Babayán 1987, Bach and Babayan 1982, Borum 1992, Greenberger & Skillman 1969, Hamosh et al. 1991, Jensen & Jensen 1992, Kaunitz and Johnson 1968, Lima 1989, Roy et al. 1981) that review in detail the experiments supporting the basic tenets of MCT utilization.

Differences in digestion, absorption and metabolism of MCT and LCT (and their corresponding fatty acids, MCFA and LCFA, respectively) stem largely from differences in their physical chemistry. The melting point of MCFA (C8:0, 16.7°C) is considerably lower than that of LCFA (C16:0, 61.1°C). Consequently, MCFA and MCT are liquid at room temperature. Because of their smaller molecular size and because they are highly ionized at physiological pH, MCFA are

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³ Abbreviations used: LCFA, long-chain fatty acids; LCT, long-chain triglycerides; MCFA, medium-chain fatty acids; MCT, medium-chain triglycerides.

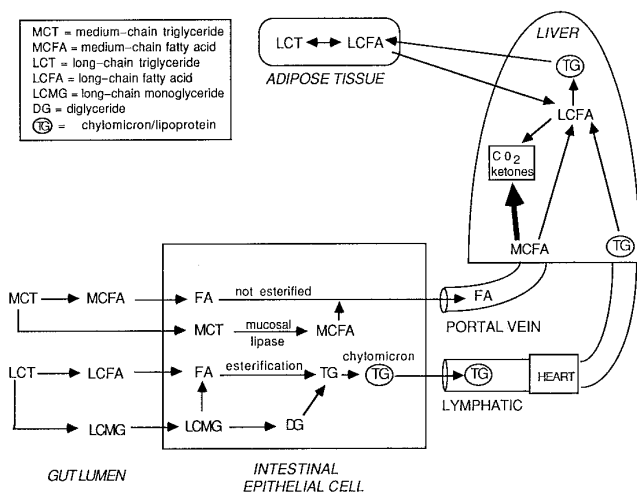


FIGURE 1 Metabolism of medium- and long-chain triglycerides and fatty acids by intestine, liver and adipose tissue. Abbreviations are defined within the figure. In contrast to LCT, MCT are more rapidly digested, and the resulting MCFA are more rapidly absorbed via portal circulation than the corresponding LCFA which are re-esterified and packaged into chylomicrons for lymphatic absorption. Furthermore, MCFA are predominantly oxidized, whereas LCFA may be directly deposited into adipose triglyceride stores. (Adapted from Greenberger and Skillman 1969.)

also much more soluble in aqueous biological fluids than are LCFA. Because many lipid-metabolizing enzymes are sensitive to the hydrophobicity of their substrate, these physical-chemical differences greatly affect both the rate and the fate of carbon metabolism from MCT relative to LCT.

Figure 1 summarizes the major differences between MCT and LCT in regard to their intraluminal hydrolysis, intestinal absorption, route of transport and metabolism by the intestine and liver. The hydrolysis of MCT is more rapid and complete than hydrolysis of LCT. This is due in part to the higher rate and extent to which they form coarse-emulsion particles and enter solution, thereby increasing the available surface area for interaction with gastrointestinal lipases. Furthermore, MCFA exert less allosteric inhibition on pancreatic lipase, and medium-chain 2-monoglycerides isomerize more rapidly than those of long-chain length, thereby facilitating rapid and complete hydrolysis (Desnuelle and Savary 1963). Preduodenal lipases (Hamosh 1990) also hydrolyze MCT preferentially, and the resulting MCFA can be absorbed in part through the stomach mucosa.

For LCT-carbon to penetrate the mucosal cells of the intestinal tract, the LCT first must be emulsified, then hydrolyzed within the gut lumen, and then the resulting LCFA and 2-monoglycerides must mix with bile salts to form mixed-micelles, which facilitate diffusion through the unstirred water layer and into the enterocytes. Therefore, in cases of pancreatic and/or bile secretion insufficiency, absorption of LCT is limited, resulting in steatorrhea. Under these conditions, MCT are better utilized than LCT. Studies in which pancreatic and bile secretions were surgically diverted from the duodenum suggest that MCT may also be absorbed (intact) into the enterocytes (Fig. 1) and subsequently hydrolyzed intracellularly by a specific microsomal lipase (Playoust and Isselbacher 1964).

Once inside the mucosal cell, the products of LCT digestion are re-assimilated into long-chain phospholipids and triglycerides in the endoplasmic reticulum and are subsequently packaged into chylomicrons, which enter the lacteal of the lymphatic system.

In contrast, the enhanced water solubility of MCFA, coupled with their diminished metabolism by CoA-activating and esterifying enzymes, results in extensive absorption via the portal vein with very little incorporation into lipoproteins. The increased flow of blood relative to lymph also contributes to more rapid entry of MCFA into the circulatory system. As a consequence, after absorption, the liver is the first organ perfused by MCFA, whereas LCFA enter systemic circulation via chylomicrons in the thoracic duct and may perfuse other tissues prior to the liver. Furthermore, because of the higher water solubility of MCFA, there is less competition with bilirubin for albumin-binding sites, which is an important consideration for infants with hyperbilirubinemia.

Figure 1 shows the general metabolic fates of MCFA and LCFA within the liver. Long-chain fatty acids may be re-assimilated into lipoproteins or oxidized to CO₂ and/or ketone bodies. The relative partitioning between these fates is dependent on the physiological state of the animal. Although there is evidence (Carnielli et al. 1994) that MCFA may be elongated to LCFA by enzymes housed in the mitochondria, the major metabolic fate of MCFA is oxidation. Very little MCFA are re-esterified. Long-chain fatty acids are activated to their CoA-thioesters by enzymes located in the endoplasmic reticulum and in the outer mitochondrial membrane (Groot et al. 1976). These enzymes have maximal activity toward LCFA (i.e., peak activity toward C12:0 and C16:0 fatty acids), but can activate fatty acids of medium-chain length. In contrast, medium-chain acyl-CoA synthetase is located within the mitochondrial matrix, suggesting that a portion of MCFA entry into the mitochondria may be independent of the carnitine acyltransferase system. This has at least three important ramifications for the neonate. First, MCFA oxidation may be less carnitine dependent. If so, this would facilitate their use by newborns that may have low carnitine levels (Baltzell et al. 1987). Second, it represents a bypass of carnitine palmitoyltransferase I, which represents an important control point in LCFA oxidation (McGarry and Foster 1980). Malonyl-CoA, an intermediate in the lipogenic pathway, allosterically inhibits carnitine palmitoyltransferase I and thereby decreases transport into the mitochondria, limiting oxidation. This control serves to limit energy loss from wasteful recycling of fatty acid carbon. Because MCFA can by-pass this control, they are extensively oxidized regardless of the physiological state of the animal. Third, the rapid and "uncontrolled" entry into the liver mitochondria results in more pronounced ketone body production from MCFA than from LCFA. Although mild hyperketonemia would not pose a problem to the neonate (Williamson 1982), higher levels could result in serious metabolic acidosis. A quantitatively less important oxidation pathway (not shown) involves oxidation at the ω -carbon, which results in the production of dicarboxylic acids (Gregersen et al. 1983).

Thus far, this review has presented in general terms the basic tenets of MCT utilization. The remainder is focused more specifically on recent advances in our knowledge of MCT digestion, absorption and metabolism as gleaned from the neonatal piglet model.

NEW INSIGHTS INTO MCT UTILIZATION FROM A PIGLET MODEL

Initial studies in development of a piglet model. Progress in understanding infant nutrition and metabolism has been greatly facilitated by the use of animal models. Since the hallmark research of Widdowson (1971), an increasing number of laboratories have used the perinatal piglet as a pediatric model for studies of nutrition, metabolism and growth biology (Reeds

and Odle 1996). In many respects, the piglet is an excellent model for pediatric research. Piglets exhibit accelerated postnatal growth and development (compared with human infants) and therefore are very sensitive to environmental variables (i.e., nutritional inputs and pharmaceutical drug testing). From a digestive physiology standpoint, they have many similarities to humans (see Moughan et al. 1992), and with birth weights ranging from 0.7 to 3.0 kg, they are of a size that permits both reduction science methodologies (e.g., muscle, liver and intestinal-tissue studies) and also whole-animal, pre-clinical investigations that involve such techniques as catheterization and multiple blood sampling. Furthermore, because they are a litter-bearing species, with natural variation in birth weight, they provide a good model for the study of intrauterine growth retardation wherein low-birth-weight piglets can be compared with their larger littermates.

Our studies with the piglet model began about 10 y ago and stemmed from our dual interests in the problem of postnatal morbidity and mortality, a problem shared by both medical and agricultural sciences. Indeed, neonates of all mammalian species face a number of similar nutritional and metabolic challenges related to the adaptations required to support independent life outside of the uterus. Our initial attention was focused on the bioenergetics of the piglet and the role that MCT might serve as a unique supplemental fuel source. Initial studies indicated that sow-suckled piglets tolerated intragastric infusions of about 12 mL of MCT oil (~24 h worth of gross energy) without impairment of colostrum consumption (Benevenga et al. 1986). Furthermore, this dose was marginally capable of sparing muscle glycogen (Benevenga et al. 1989), reducing nitrogen excretion and maintaining blood glucose in fasted piglets (Odle et al. 1989). In addition to sparing carbohydrate oxidation, fatty acid oxidation may directly support gluconeogenesis in the piglet (Lepine et al. 1989 and 1993).

Following the initial descriptive studies, we conducted a series of progressive experiments that utilized a variety of in vitro and in vivo procedures to examine both digestive and metabolic aspects of MCT utilization in the neonatal piglet. Our findings are outlined in detail below.

Factors affecting MCT digestion and absorption

Effects of fatty acid chain length. As discussed previously, a plethora of studies have identified the stark differences in digestion and absorption of medium- and long-chain triacylglycerols (e.g., Chiang et al. 1990). In contrast, few studies have examined differences within the medium-chain family. We therefore established a simple in vivo screening protocol (Odle et al. 1991a) to examine the relative rates of utilization of MCT of varying fatty acid chain length. The protocol involved gastric intubation of piglets with various nonemulsified MCT oils, followed by serial sampling of peripheral blood for analysis of MCFA by HPLC. **Figure 2A** shows the profound effect of fatty acid chain length within the medium-chain family. The peak systemic fatty acid concentration at 1 h after feeding was 50-fold higher in piglets fed tri-C7:0 than in those fed tri-C10:0, with C8:0 and C9:0 concentrations being intermediate. These data suggested that although the route of absorption may be similar for all MCFA, the rate of digestion and absorption might differ considerably. Using a similar protocol, several follow-up studies (Fig. 2B) consistently indicated that tri-C6:0 yielded the highest blood fatty acid profile (Wieland et al. 1993a and 1993b). To extend these collective observations, radiolabeled triglycerides were synthesized from ^{14}C -hexanoate and ^{14}C -octanoate and fed to piglets housed in respira-

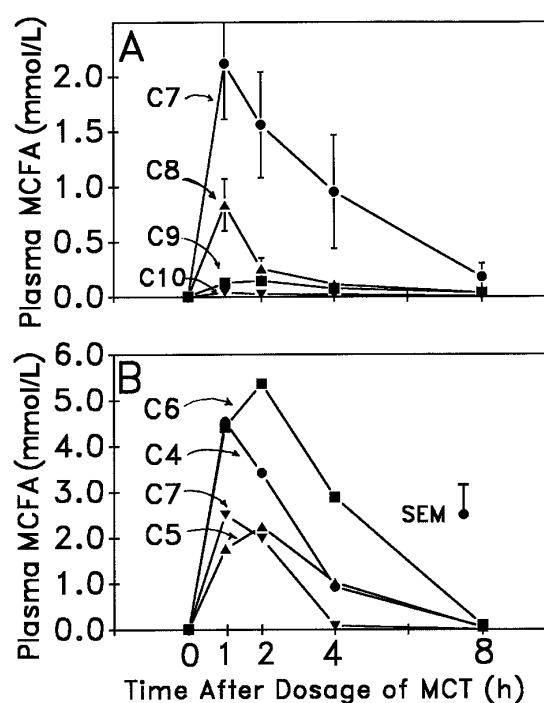


FIGURE 2 Effect of fatty acid composition of medium-chain triglycerides (MCT) on plasma medium-chain fatty acid (MCFA) concentrations in piglets. One-d-old piglets were gastrically intubated at time zero with nonemulsified MCT oil containing tri-C7:0 to tri-C10:0 (Panel A, $n = 7$ pigs/treatment, error bars show \pm SEM) or tri-C5:0 to tri-C7:0 (Panel B, $n = 6$ pigs/treatment; pooled SEM applies to all points). (Adapted from Odle et al. 1991 and Wieland et al. 1993b, respectively.)

tion chambers capable of trapping expired $^{14}\text{CO}_2$ (Odle et al. 1994). Total collections of CO_2 at 20-min intervals allowed for detailed kinetic analysis of relative utilization rates (Fig. 3A). The peak rate of $^{14}\text{CO}_2$ expiration occurred 3.5 h after feeding, and the corresponding maximal utilization rate of tri-C6:0 exceeded tri-C8:0 by 37%. However, total utilization over the 24-h collection was not significantly affected by chain length ($P < 0.05$). In complementary studies (Odle et al. 1992) involving intravenous infusion of radiolabeled fatty acids, no differences in whole-animal oxidation of fatty acids ranging in chain length from C7:0 to C10:0 were detected, thus suggesting that differences in the kinetics of digestion and absorption were likely responsible for the previously observed effects of chain length. Indeed, when we subsequently examined chain-length specificity of pancreatic lipase in developing piglets (unpublished observations), we observed greater rates of hydrolysis of tri-C6:0 when presented as an equimolar composite mixture with other MCT (containing C4:0, C6:0, C8:0 and C10:0 fatty acids). Collectively, based on these data, we contend that tri-C6:0 may offer additional clinical utility beyond that afforded by MCT composed of tri-C8:0/C10:0 under cases of severe pancreatic/biliary insufficiency and steatorrhea.

Effects of emulsification. Given the putative constraint on digestion and absorption inferred from our previous studies, we expected that emulsification would enhance utilization of the MCT oils. Thus, we prepared 30% oil-in-water emulsions using various emulsifying agents (Wieland et al. 1993a). When piglets were gavaged with a 30% (v/v) Tween-80 emulsion, peak circulating MCFA were elevated as much as 14-fold (Fig. 3B) over concentrations observed in controls fed equimolar, nonemulsified MCT. Similarly, when piglets in respiration chambers were fed emulsified ^{14}C -MCT (Odle et al. 1994),

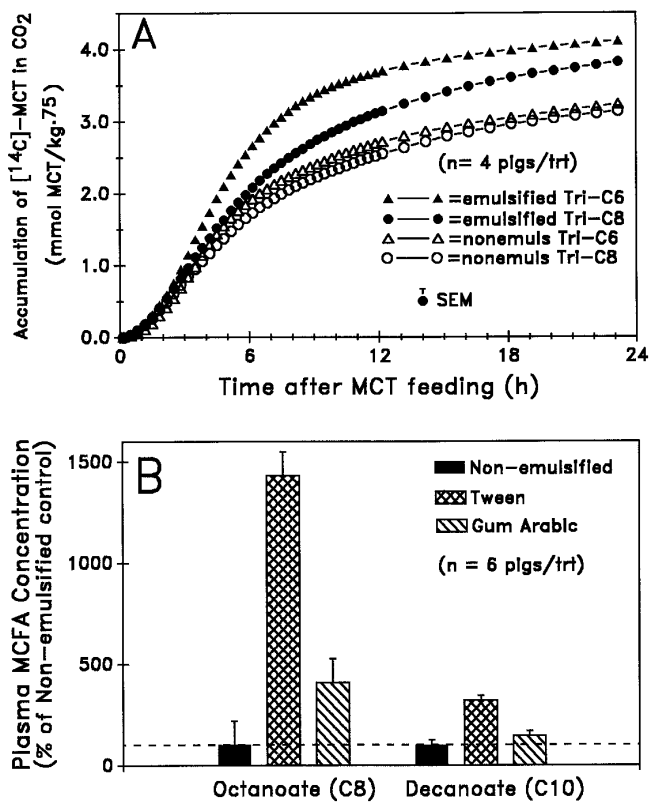


FIGURE 3 Emulsification and fatty acid chain length affect the utilization of medium-chain triglycerides by 1-d-old piglets. *Panel A:* Accumulative oxidation of [¹⁴C]-tri-C6:0 or tri-C8:0 fed to piglets (at time 0) in nonemulsified form or as 30% (v/v) oil-in-water emulsions ($n = 4$ pigs/treatment; pooled SEM applies to all data points). *Panel B:* Plasma C8:0 and C10:0 concentrations in piglets ($n = 4$ per treatment) 1 h after feeding medium-chain triglyceride emulsions prepared using various emulsifying agents. Concentrations are expressed as a percentage of those observed in piglets fed nonemulsified triglycerides. Error bars indicate SEM. (Adapted from Odle et al. 1994 and Wieland et al. 1993a, respectively.)

both the maximal rate and extent of utilization were enhanced by 20% (Fig. 3A). Additional experiments indicated that utilization of MCT of all chain lengths was enhanced to a similar extent by emulsification (Wieland et al. 1993b). Notably, toxicity was observed when too much readily digestible emulsified MCT was administered. Circulating MCFA concentrations in excess of 15 mmol/L were observed in some piglets. Even when concentrations exceeded 3 mmol/L, piglets lapsed into a transient narcoleptic state, but would recover as the absorbed MCFA were metabolized. This prompted further dose-response studies to establish safe intake levels (i.e., < 6.5 mmol MCT/kg^{0.75} per dose) for piglets (Wieland et al. 1993a). The narcoleptic properties of MCFA have long been recognized (Samson et al. 1956) and therefore require caution when MCT are used in various clinical applications. Collectively, these studies support the use of emulsified MCT to maximize the rate and extent of utilization, with the provision that appropriate doses be given to avoid toxicity.

Effects of piglet age. When considering the practical application of these research findings in production agriculture, it was important to examine the ontogenic aspects of MCT utilization. Because the majority of postnatal piglet death losses occur during the first 3 d following parturition (USDA 1991), our attention has necessarily focused on utilization within the first 48 h of life. In an initial experiment, piglets were gavaged

with MCT at 6, 18 or 48 h of age, after which the circulating MCFA concentrations were measured. Peak concentrations were up to fivefold higher in piglets gavaged at 18 h compared with those gavaged at 6 h, but no further increase occurred between 18 and 48 h. Subsequent research (Dicklin et al. 1995) also showed that emulsification enhanced circulating MCFA concentrations in both 5- and 29-h-old piglets. Examination of lingual, gastric and pancreatic lipase activities revealed that the last mentioned has primary importance in the piglet. Interestingly, lipase activity per gram wet weight of pancreas decreased steadily from birth to 18 h of age, likely because the rate of enzymic discharge by the pancreas exceeded the rate of synthesis. Activity in 48-h-old piglets rebounded, and levels exceeded those at birth when expressed on a whole-organ basis.

Overall, at a practical level, our studies would suggest maximal efficacy of emulsified (30%, v/v) trihexanoate administered to piglets from birth to 48 h of age and at a dosage not to exceed 6.5 mmol triglyceride/kg body weight^{0.75}. Inclusion of L-carnitine may also be beneficial (discussed later), but has not been tested under practical conditions. Previous field studies (Lee and Chiang 1994, Lepine et al. 1989) using nonemulsified C8/C10 MCT given at multiple and higher doses have not demonstrated improved piglet survival.

Factors affecting metabolism of medium-chain fatty acids

Effects of fatty acid chain length. Given the dramatic effect of chain length on the rate of digestion/absorption, one might expect some effect on the rate of metabolism as well. To examine this, day-old piglets were surgically fitted with indwelling catheters (via umbilical arteries) and were infused systemically with increasing amounts of ¹⁴C-medium-chain fatty acids ranging in chain length from C7:0 to C10:0 (Odle et al. 1992), thereby eliminating effects mediated via differences in rate of digestion/absorption. Rates of oxidation to ¹⁴CO₂ were proportional to infusion rates, but were unaffected by fatty acid chain length (Fig. 4A). Interestingly, only 60% of infused radiolabel could be recovered in expired CO₂. Although the metabolic fate of the remaining 40% is unknown, Carnielli et al. (1994) have documented significant incorporation of octanoate-carbon into long-chain fatty acids in preterm infants.

Beyond the effect of carbon chain length per se, we also hypothesized that carbon parity (i.e., odd vs. even carbon) would alter metabolism because of the propionyl-CoA produced upon complete β -oxidation of odd-carbon fatty acids (Guisard et al. 1973). Theoretically, this effect might be greatest in hepatic tissue because the gluconeogenic potential of propionyl-CoA could increase carbohydrate flux, and its anaplerotic potential (i.e., ability to increase tricarboxylic-acid-cycle carbon) could diminish ketogenic flux. In support of this, Linseisen and Wolfram (1993a, 1993b and 1993 c) reported elevated plasma glucose, lactate, pyruvate and hepatic glycogen, but reduced ketone bodies in rabbits maintained on total parenteral nutrition containing tri-C9:0. Using the piglet model, we did not observe the anticipated improvement in blood glucose concentrations in piglets given odd- vs. even-chain MCT orally (Odle et al. 1989). Circulating ketone body concentrations were surprisingly low in all piglets, but were higher in piglets given MCT containing a mixture (3:1, mol/mol) of C8:0 and C10:0 than in piglets fed MCT containing C9:0 (Odle et al. 1989). To examine effects on hepatic metabolism directly, hepatocytes were isolated from newborn piglets and incubated with ¹⁴C-fatty acids ranging from C7:0 to C10:0 (Odle et al. 1991a). Accumulation of fatty acid carbon in both

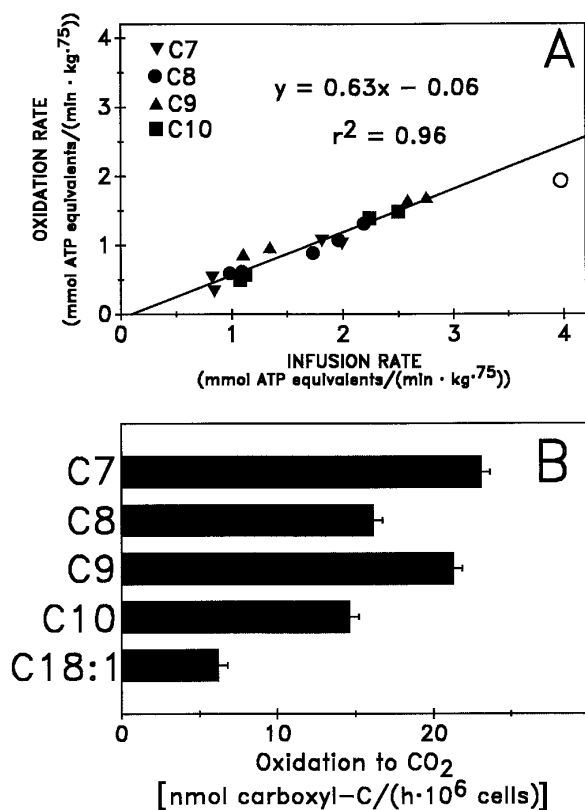


FIGURE 4 Comparative oxidation rates of odd- and even-carbon medium-chain fatty acids of varying chain length in piglets. *Panel A:* In vivo oxidation rates of various [1-¹⁴C]-fatty acids parenterally infused into neonatal pigs at increasing rates. *Panel B:* In vitro oxidation of [1-¹⁴C]-fatty acids by hepatocytes isolated from neonatal pigs ($n = 4$ per treatment; error bars represent SEM). (Adapted from Odle et al. 1992 and Odle et al. 1991a, respectively.)

CO₂ (Fig. 4B) and acid-soluble products showed a “zig-zag” pattern, which was higher for odd-carbon than for even-carbon fatty acids. Rates of oxygen consumption followed a similar pattern. Further examination of the acid-soluble products by radio-HPLC (Lin et al. 1996) showed much greater accumulation of ¹⁴C in various tricarboxylic-acid-cycle intermediates when hepatocytes were incubated with radiolabeled C7:0 compared with C8:0. Collectively, these data indicate that odd-chain MCT may be a promising alternative fuel for use in enteral and parenteral nutrition.

Effects of birth weight and postnatal age. Because domestic swine are a litter-bearing species, the natural incidence of intrauterine growth retardation is fairly high. As such, within a litter, it is not uncommon for one or two piglets to be born small for gestational age, whereas other littermates are of a size appropriate for gestational age. We used this natural variability to examine the effects of prenatal and postnatal development on hepatic fatty acid metabolism by the neonate (Odle et al. 1991b). These studies showed minor effects of birth weight, but major effects of postnatal age (6 vs. 48 h of age) on fatty acid oxidation. The effects were congruent with developmental increases in metabolic rate. Further study of low-birth-weight piglets (Odle et al. 1995) provided evidence consistent with normal regulation of fatty acid oxidation via carnitine palmitoyltransferase I, despite low rates of ketogenesis. The attenuated ketogenic capacity of neonatal piglets appears to stem from limited expression of mitochondrial HMG-CoA synthase (Duée et al. 1994). Although capacity appears to

develop somewhat with age (Adams and Odle 1993), it remains far below other species such as rats and rabbits. We have estimated that at maximum concentrations observed physiologically, β -hydroxybutyrate contributes no more than 3–4% of the piglets’ basal energy needs (Tetrick et al. 1995). Most recent radio-HPLC evidence (Fig. 5) suggests that acetate, rather than ketone bodies, may represent a major product of fatty acid oxidation in piglet hepatocytes (Lin et al. 1996, Odle et al. 1995). Of the fatty acid carboxyl-carbon accumulating in acid-soluble products, 40–70% was found in acetate. The degree to which acetogenesis replaces ketogenesis and the subcellular origin (mitochondrial vs. peroxisomal) of acetogenesis in the porcine species remain to be elucidated.

Effects of supplemental L-carnitine. When viewed from the prescribed dogma (discussed previously), MCFA oxidation should be in part independent of carnitine, because of the ability of MCFA to freely diffuse across the mitochondrial

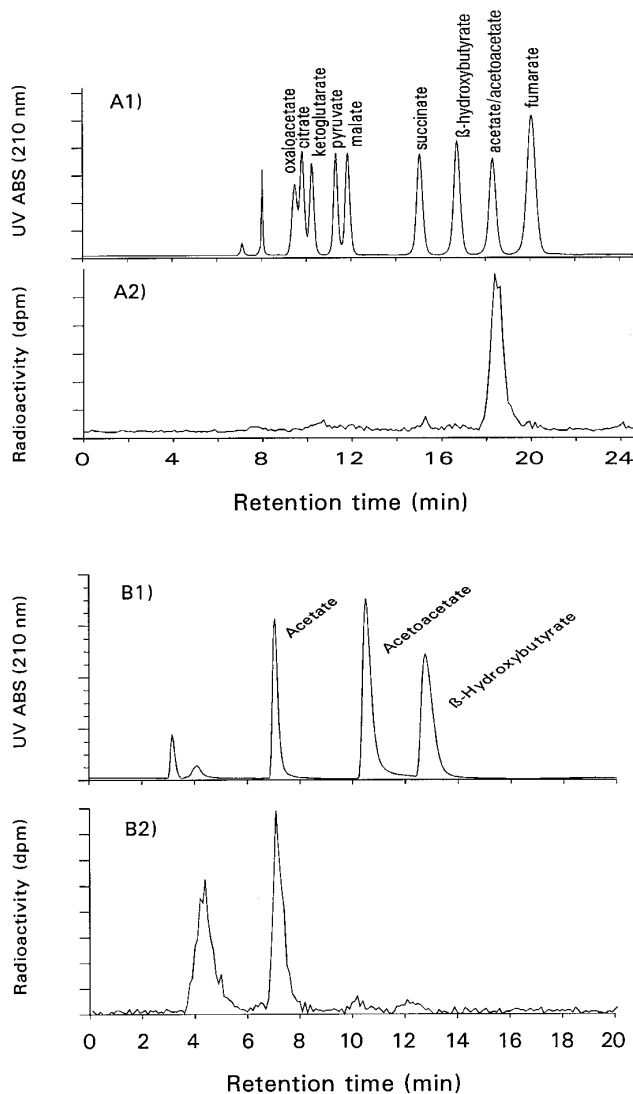


FIGURE 5 Radio-HPLC profiles of [¹⁴C]-metabolites resulting from the oxidation of [1-¹⁴C]-medium-chain fatty acids by isolated hepatocytes from neonatal pigs. *Panels A1 and A2:* Normal phase separation of standards (A1) compared with a radiolabeled sample (A2). *Panels B1 and B2:* Reversed-phase separation of standards (B1) and a radiolabeled sample (B2), presumptively identifying acetate as a major oxidation product. (Adapted from Odle et al. 1995 and Lin et al. 1996, respectively.)

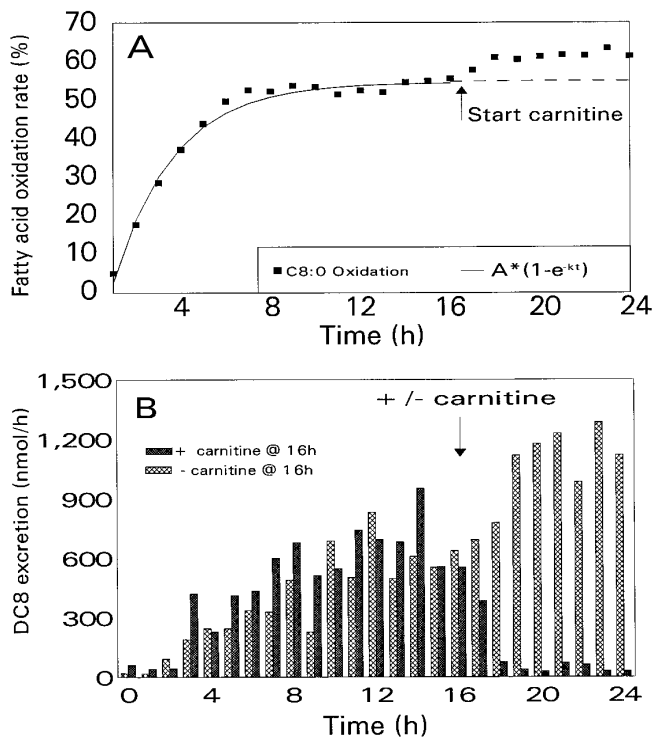


FIGURE 6 Example data showing the effect of L-carnitine infusion on the oxidation of [1-¹⁴C]-octanoate to CO₂ (Panel A) and on the rate of urinary dicarboxylic acid excretion (Panel B) in colostrum-deprived piglets, parenterally infused with octanoate for 16 h. (Adapted from Kempen and Odle 1995.)

membranes (thus bypassing carnitine palmitoyltransferase I) and to be activated to CoA esters via medium-chain CoA synthetases located within the mitochondrial matrix. This is likely true for hepatic tissue, because when isolated piglet hepatocytes were incubated with supplemental L-carnitine, octanoate oxidation was unaffected (Odle et al. 1991 and 1995), whereas oleate oxidation was increased and esterification was reduced (Odle et al. 1995). However, other *in vitro* investigations (Groot and Hulsmann 1973) have reported carnitine stimulation of MCFA oxidation by muscle tissue. More recently, *in vivo* studies (Kempen and Odle 1992, Rebouche et al. 1990, Rössle et al. 1990) have documented alterations in plasma and urinary concentrations of carnitine esters following ingestion of MCT. These studies have suggested that carnitine status might influence MCFA metabolism (and *visa versa*), especially in the colostrum-deprived neonate whose carnitine status is marginal (Baltzell et al. 1987). To test this hypothesis, we parenterally infused varying levels of 1-¹⁴C-octanoate into colostrum-deprived piglets (Kempen and Odle 1993 and 1995). After a steady-state oxidation rate was attained (at ~16 h), piglets received a primed continuous co-infusion of L-carnitine, and the effect on octanoate β -oxidation to CO₂ and ω -oxidation to dicarboxylic acids was monitored (Fig. 6A, B). The rate of octanoate oxidation to CO₂ was increased by 5–20% when carnitine was supplemented, and the stimulation was proportional to the octanoate infusion rates beyond 50% of the piglets' metabolic rate. A concurrent 8–48% reduction in substrate (i.e., 8-carbon dicarboxylic acid) excretion in the urine also was observed. The mechanism(s) by which carnitine affects MCFA oxidation remains unclear, but it may be related to buffering (lowering) of the acyl-CoA to free CoA ratio, which could be particularly important in a species limited in ketogenic capacity.

In conclusion, medium-chain triglycerides possess many unique nutritional and metabolic characteristics that are useful in a variety of clinical nutrition settings. This review has presented a focused summary of research from our laboratory which has utilized the neonatal piglet model to further examine MCT and MCFA metabolism by the neonate. Our findings suggest that among the MCFA homologues, hexanoate (C6:0) may be preferentially utilized, that odd-chain MCFA should be further explored as a supplemental fuel, that L-carnitine may prove beneficial for MCFA metabolism, and that the potential toxicity of MCT should not be ignored. Although the piglet possesses many digestive and metabolic similarities to humans (Moughan et al. 1992, Reeds and Odle 1996), the extrapolation of findings across species with known idiosyncrasies (such as the deficit of ketogenesis in piglets) should be made with caution. As illustrated in our studies, the piglet model can provide invasive, *in vitro* data of interest, but is also amiable to whole-animal studies which can closely simulate the clinical environment of the preterm infant. Clearly, no single animal model is perfect, and thus care must be used when relating findings from these models to human clinical situations. Breadth in our understanding of several animal models coupled with clinical observations on infants will afford the best balance toward improving clinical treatments.

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LITERATURE CITED

- Adams, S. H. & Odle, J. (1993) Plasma β -hydroxybutyrate after octanoate challenge: in support of attenuated ketogenic capacity in neonatal swine. *Am. J. Physiol.* 265: R761–R765.
- Babayan, V. K. (1987) Medium chain triglycerides and structured lipids. *Lipids* 22: 417–420.
- Bach, A. C., & Babayan, V. K. (1982) Medium-chain triglycerides: an update. *Am. J. Clin. Nutr.* 36: 950–962.
- Baltzell, J. K., Bazer, F. W., Miguel, S. G. & Borum, P. R. (1987) The neonatal piglet as a model for human neonatal carnitine metabolism. *J. Nutr.* 117: 754–757.
- Benevenga, N. J., Steinman, J. K. & Crenshaw, T. D. (1986) Medium chain triglycerides—a source of energy for the newborn piglet. *Georgia Nutr. Conf.* pp. 13–18.
- Benevenga, N. J., Steinman-Goldsworthy, J. K., Crenshaw, T. D. & Odle, J. (1989) Utilization of medium-chain triglycerides by neonatal piglets I: Effects on milk consumption and body fuel utilization. *J. Anim. Sci.* 67: 3331–3339.
- Bloom, B., Chaikoff, I. L. & Reinhardt, W. O. (1951) Intestinal lymph as pathway for transport of absorbed fatty acids of different chain lengths. *Am. J. Physiol.* 166: 451–455.
- Borum, P. R. (1992) Medium-chain triglycerides in formula for preterm neonates: implications for hepatic and extrahepatic metabolism. *J. Pediatr.* 120: S139–S145.
- Carnielli, V. P., Sulkers, E. J., Moretti, C., Wattimena, J.L.D., van Goudoever, J. B., Degenhart, H. J., Zaccchello, F. & Sauer, P.J.J. (1994) Conversion of octanoic acid into long-chain saturated fatty acids in premature infants fed a formula containing medium-chain triglycerides. *Metabolism* 43: 1287–1292.
- Chiang, S. H., Pettigrew, J. E., Clarke, S. D. & Corneliussen, S. G. (1990) Limits of medium-chain and long-chain triacylglycerol utilization by neonatal piglets. *J. Anim. Sci.* 68: 1632–1638.
- Desnuelle, P. & Savary, P. (1963) Specificity of lipases. *J. Lipid Res.* 4:369–384.
- Dicklin, M. E., Zijlstra, R. T. & Odle, J. (1995) Ontogeny of gastro-intestinal lipase activity in neonatal piglets. *FASEB J.* 9: A753 (abs.).
- Duée, P. H., Pégrier, 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.
- Finley, J. W., Leveille, G. A., Dixon, R. M., Walchak, C. G., Sourby, J. C., Smith, R. E., Francis, K. D. & Otterburn, M. S. (1994) Clinical assessment of a reduced-calorie triacylglycerol. *J. Agric. Food Chem.* 42: 581–596.
- Greenberger, N. J. & Skillman, T. G. (1969) Medium-chain triglycerides. Physiologic considerations and clinical implications. *N. Engl. J. Med.* 280: 1045.

- Gregersen, N., Mortensen, P. B. & Kolvraa, S. (1983) On the biologic origin of C₆-C₁₀-dicarboxylic and C₆-C₁₀- ω -1-hydroxy monocarboxylic acids in human and rat with acyl-CoA dehydrogenation deficiencies: in vitro studies on the ω - and ω -1-oxidation of medium-chain (C₆-C₁₂) fatty acids in human and rat liver. *Pediatr. Res.* 17: 828–834.
- Groot, P.H.E. & Hulsmann, W. C. (1973) The activation and oxidation of octanoate and palmitate by rat skeletal muscle mitochondria. *Biochim. Biophys. Acta* 316: 124–135.
- Groot, P.H.E., Scholte, H. R. & Hulsmann, W. C. (1976) Fatty acid activation: specificity, localization and function. *Adv. Lipid Res.* 14: 75–126.
- Guisard, D., Bach, A., Debry, G. & Métais, P. (1973) Comparison of the metabolic effects of short and medium even-chain fatty acids (C8 and C10) triglycerides and odd-chain fatty acid (C9) triglycerides in dogs. *Horm. Metab. Res.* 5: 191–195.
- Hamosh, M. (1990) In: *Lingual and Gastric Lipases: Their Role in Fat Digestion*. CRC Press, Boca Raton, FL.
- Hamosh, M., Spear, M. L., Bitman, J., Mehta, N. R., Wood, D. L. & Hamosh, P. (1991) Medium chain triglycerides: advantages and possible drawbacks. In: *Inborn Errors of Metabolism* (Schaub, J., Van Hoof, F. & Vis, H. L. eds.), pp. 81–92. Vevey/Raven Press, Ltd., New York, NY.
- Jensen, G. L. & Jensen, R. G. (1992) Specialty lipids for infant nutrition. II. Concern, new developments, and future applications. *J. Pediatr. Gastroenterol. Nutr.* 15: 382–394.
- Kaunitz, H. & Johnson, R. E. (1968) Nutritional properties of medium-chain triglycerides. *J. Am. Oil Chem. Soc.* 45: 19–22.
- Kempen, T.A.T.G. van & Odle, J. (1992) Quantification of carnitine esters by high-performance liquid chromatography. Effect of feeding medium-chain triglycerides on the plasma carnitine ester profile. *J. Chromatogr.* 584: 157–165.
- Kempen, T.A.T.G. van & Odle, J. (1993) Medium-chain fatty acid oxidation in colostrum-deprived newborn piglets: stimulative effect of L-carnitine supplementation. *J. Nutr.* 123: 1531–1537.
- Kempen, T.A.T.G. van & Odle, J. (1995) Carnitine affects octanoate oxidation to carbon dioxide and dicarboxylic acids in colostrum-deprived piglets: in vivo analysis of mechanisms involved based on CoA- and carnitine-ester profiles. *J. Nutr.* 125: 238–250.
- Lee, H. F. & Chiang, S. H. (1994) Energy value of medium-chain triglycerides and their efficacy in improving survival of neonatal pigs. *J. Anim. Sci.* 72: 133–138.
- Lepine, A. J., Boyd, R. D., Welch, J. A. & Roneker, K. R. (1989) Effect of colostrum or medium-chain triglyceride supplementation on the pattern of plasma glucose, non-esterified fatty acids and survival of neonatal pigs. *J. Anim. Sci.* 67: 983–990.
- 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. *Br. J. Nutr.* 70: 81–91.
- Lima, L.A.M. (1989) Neonatal parenteral nutrition with medium-chain triglycerides: rationale for research. *J. Parent. Enteral Nutr.* 13: 312–317.
- Lin, X., Adams, S. H. & Odle, J. (1996) Acetate represents a major product of heptanoate and octanoate β -oxidation in hepatocytes isolated from neonatal piglets. *Biochem. J.* 318: 235–240.
- Linseisen, J. & Wolfram, G. (1993a) Odd-numbered medium-chain triglycerides (trionanoin) in total parenteral nutrition—effects on parameters of fat metabolism in rabbits. *J. Parent. Enteral Nutr.* 17: 522–528.
- Linseisen, J. & Wolfram, G. (1993b) Odd-numbered medium-chain triglycerides (trionanoin) in total parenteral nutrition—parameters of carbohydrate and protein metabolism. *Ann. Nutr. & Metab.* 37: 320–327.
- Linseisen, J. & Wolfram, G. (1993c) Organ changes after intravenous trionanoin administration in rabbits. *Ann. Nutr. & Metab.* 37: 328–334.
- McGarry, J. D. & Foster, D.W. (1980) Regulation of hepatic fatty acid oxidation and ketone body production. *Annu. Rev. Biochem.* 49: 395–420.
- Moughan, P. J., Birtles, M. J., Cranwell, P. D., Smith, W. C. & Pedraza, M. (1992) The piglet as a model animal for studying aspects of digestion and absorption in milk-fed human infants. In: *Nutritional Triggers for Health and in Disease* (Simopoulos, A. P., ed.), vol. 67, pp. 40–113. Karger, Basel, Switzerland.
- Odle, J., Benevenga, N. J. & Crenshaw, T. D. (1989) Utilization of medium-chain triglycerides by neonatal piglets II: Effects of even- and odd-chain triglyceride consumption over the first 2 days of life on blood metabolites and urinary nitrogen excretion. *J. Anim. Sci.* 67: 3340–3351.
- 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., Benevenga, N. J. & Crenshaw, T. D. (1992) Evaluation of [¹⁴C]-medium-chain fatty acid oxidation by neonatal piglets using continuous-infusion radiotracer kinetic methodology. *J. Nutr.* 122: 2183–2189.
- 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.
- Odle, J., Lin X., Wieland, T. M. & van Kempen, T.A.T.G. (1994) Emulsification and fatty acid chain length affect the kinetics of [¹⁴C]-medium-chain triacylglycerol utilization by neonatal piglets. *J. Nutr.* 124: 84–93.
- Playoust, M. R. & Isselbacher, K. J. (1964) Studies on the intestinal absorption and intramucosal lipolysis of a medium-chain triglyceride. *J. Clin. Invest.* 43: 878–885.
- Rebouche, C. J., Panagides, D. D. & Nelson, S. E. (1990) Role of carnitine in utilization of dietary medium-chain triglycerides by term infants. *Am. J. Clin. Nutr.* 52: 820–824.
- Reeds, P. J. & Odle, J. (1996) Pigs as models for nutrient functional interaction. In: *Swine in Biomedical Research* (Tumbleson, M. E. & Schook, L. B., eds.), pp. 709–710. Plenum Press, New York, NY.
- Rössle, C., Carpentier, Y. A., Richelle, M., Dahlan, W., D'Attellis, N. P., Furst, P. & Elwyn, D. H. (1990) Medium-chain triglycerides induce alterations in carnitine metabolism. *Am. J. Physiol.* 258: E944–E947.
- Roy, C. C. (1981) Use of medium-chain triglycerides in infant foods. American Academy of Pediatrics Committee on Nutrition, Report to Food and Drug Administration. pp. 1–20.
- Samson, F. E., Dahl, N. & Dahl, D. R. (1956) A study on the narcotic action of the short chain fatty acids. *J. Clin. Invest.* 35: 1291–1298.
- Tetric, M. A., Adams S. H., Odle, J. & Benevenga, N. J. (1995) Contribution of (D)-(-)-3- hydroxybutyrate to the energy expenditure of neonatal pigs. *J. Nutr.* 125: 264–272.
- U.S. Department of Agriculture (1991) National Animal Health Monitoring System, Animal and Plant Health Inspection Service, Veterinary Services. National Swine Survey: Morbidity/mortality and health management of swine in the U.S. Washington, DC.
- Webb, D. R., Wood, F. E., Bertram, T. A. & Fortier, N. E. (1993) A 91-day feeding study in rats with caprenin. *Food Chem. Toxicol.* 31: 935–946.
- Widdowson, E. M. (1971) Intra-uterine growth retardation in the pig. *Biol. Neonate* 19: 329–340.
- Wieland, T. M., Lin, X. & Odle, J. (1993a) Utilization of medium-chain triglycerides by neonatal pigs: effects of emulsification and dose delivered. *J. Anim. Sci.* 71: 1863–1868.
- Wieland, T. M., Lin, X. & Odle, J. (1993b) Emulsification and fatty-acid chain length affect the utilization of medium-chain triglycerides by neonatal pigs. *J. Anim. Sci.* 71: 1869–1874.
- Williamson, D. H. (1982) The production and utilization of ketone bodies in the neonate. In: *The Biochemical Development of the Fetus and Neonate* (Jones, C. T., ed.), pp. 622–650. Elsevier Biomedical Press, New York, NY.