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

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Abstract

In two experiments, 144 neonatal pigs were force-fed 12 ml of triglyceride containing fatty acids of even (C8, C10) or odd (C7, C9) medium-chain length (even-MCT, odd-MCT, respectively) or long- (>C16) chain length (LCT). Pigs were individually caged for measurement of urinary N excretion and/or blood metabolites over 24 h. In Exp. 1, pigs were force-fed immediately after birth and were not allowed to suckle. Supplementation with triglyceride decreased 24-h N excretion compared with control animals given 12 ml of water, suggesting decreased breakdown of body protein and improved energy status. Blood glucose increased over 24 h in all pigs (P < .05), but more in pigs given MCT (1.38 mM) than in those given LCT (.61 mM) and more in animals given even-MCT (1.87 mM) than in those given odd-MCT (1.14 mM). In Exp. 2, pigs were allowed to suckle and were force-fed at 6, 18 or 48 h of age. An apparent improvement in utilization of even-MCT was observed between 6 and 18 h, as evidenced by a twofold vs a sixfold increase in 3-OH-butyrate (BHBA) concentration 1 h after dosing and a twofold vs 12-fold increase in plasma fatty acid concentration. This was not seen in pigs given odd-MCT. The BHBA response with odd-MCT was approximately half that observed with even-MCT in pigs 18 and 48 h old, but not in pigs 6 h old. No change in BHBA concentration (P > .1) was observed in pigs after force-feeding LCT at either 6, 18 or 48 h of age. Collectively, these data suggest that MCT may be better utilized than LCT and that there may be a differences in the utilization of even-MCT vs odd-MCT, depending on the age of the neonate. This could be related to chain length effects on digestion and absorption because plasma decanoate concentration changed very little, even though it composed 25% of the even-MCT supplement.

(Key Words: Piglets, Neonates, Medium-Chain Triglycerides, Odd-Chain Fatty Acids, Ketone Bodies, Development.)

Introduction

High mortality of neonatal piglets is a sizeable problem to the swine industry. Pre-weaning mortality varies considerably across different production units, but it has been estimated to average as high as 28% (Bereskin et al., 1973). Factors contributing to the high mortality include nutritional deficiency, hypothermia, low immunity and disease resistance and crushing by the dam. The relative importance of these factors is difficult to establish from retrospective surveys because the cause of death is not always determined precisely and interactions between the factors complicate interpretation (English and Morrison, 1984).
Consequently, controlled experiments are needed to understand better the developing neonate’s nutritional, immunological and behavioral responses to its environment. The work reported herein focuses on a nutritional component of the multifactorial etiology, energy balance in the neonate.

The unique nutritional attributes of medium-chain triglycerides (MCT) have been exploited in a number of human clinical nutrition settings, including energy support of premature infants (Roy, 1981), but only a few reports have evaluated MCT in neonatal piglet nutrition (Newport et al., 1979; Benevenga et al., 1986, 1989). Therefore, the primary objective of the experiments reported herein was to compare the utilization of oral supplements of triglycerides containing fatty acids of even (C8, C10) (even-MCT) or odd (C7, C9) chain lengths (odd-MCT) or long-chain triglycerides (LCT) by newborn piglets. Hypothetically, MCT containing odd-chain (C7, C9) fatty acids are less ketogenic due to the anaplerotic potential (i.e., they can give rise to net TCA cycle carbon) of the propionyl-CoA resulting from their oxidation (Krebs, 1966). The second experiment included as assessment of developmental changes in triglyceride utilization over the first 48 h of life because changes in rate of fat oxidation have been reported during the early postnatal period in guinea pigs (Shipp et al., 1982; Stanley et al., 1983).

Materials and Methods

Seventy-two crossbred piglets (Large White × Landrace × Duroc) were used in each of two experiments. In both experiments the sows were fed standard corn-soybean meal diets. They were moved into farrowing crates after 109 d of gestation and were allowed to farrow naturally.

Experiment 1

Piglets were allotted to one of six treatments (Table 1) replicated over 12 litters according to a randomized complete block design. Aside from water that was given to control animals, the triglyceride products ranged in fatty acid composition from medium-chain length (treatments 2 to 4) to long-chain length (treatment 6). Treatment 5 contained a mixture of medium- and long-chain fatty acids esterified to the same glycerol molecule (P. J. Reilly, Capital City Products, Columbus, OH, personal communication). Of the MCT products, treatment 2 contained even-chain fatty acids (C8 and C10), whereas odd-chain acids were exclusive to treatments 3 (tri-C9) and 4 (C7 and C9).

Piglets were removed from the sow at birth and were not allowed to suckle. After six piglets were born in a litter, birth weights were recorded and each was given a 12-ml intragastric dose of the assigned treatment as described by Benevenga et al. (1989). The amount of triglyceride was calculated to provide a level of gross energy (ca. 100 kcal), which approximate 50% of daily maintenance energy requirement of a newborn piglet reported by Mount (1968). Earlier work (Benevenga et al., 1989) has shown that even-MCT at this level did not inhibit colostrum consumption, whereas 24 ml did. The average time delay between birth and force-feeding was 77 min.

A 1.5-ml heparinized blood sample was obtained by jugular venipuncture prior to

<table>
<thead>
<tr>
<th>Treatment Description</th>
<th>Abbreviation</th>
<th>C7</th>
<th>C8</th>
<th>C9</th>
<th>C10</th>
<th>C18:n</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Water control</td>
<td>Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Captex 300</td>
<td>C8, C10</td>
<td>75</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Tripelargonate</td>
<td>C9</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Mixed odd-chain</td>
<td>C7, C9</td>
<td>75</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Captex 810B</td>
<td>C8, C18</td>
<td></td>
<td></td>
<td></td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Corn oil</td>
<td>C18</td>
<td></td>
<td></td>
<td></td>
<td>88</td>
<td></td>
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</tr>
</tbody>
</table>

*Composition supplied by manufacturer.

*Supplied by Capital City Products, Columbus, OH.

*Supplied by Abbott Laboratories, North Chicago, IL.
force-feeding and after 12 and 24 h. Packed cell volume was determined, and 1 ml of blood was deproteinized as described by Somogyi (1930) and stored at -20°C. The pigs were housed in metabolism cages for the 24-h period without access to food or water. Room temperature (30 ± 1°C) was maintained at 4°C below critical temperature (Stanier et al., 1984), so the day-old piglet should have had a 20% increase in metabolic rate (Mount, 1968). After 24 h the piglets were sedated with Na pentobarbitol and euthanatized by an intracardial injection of saturated KCl. Bladders were removed and contents were acidified and frozen. Body weights were recorded at each sampling time. The 24-h excreta were collected on stainless steel pans acidified with 1 N H₂SO₄. The excreta were rinsed from the pans using 0.01 N H₂SO₄, filtered through Whatman #4 filter paper and stored at -20°C.

The deproteinized whole blood samples were analyzed for glucose using the glucose oxidase assay described by Campbell and Kronfeld (1961). Urea-N concentration in the blood was measured after urease hydrolysis using the Berthelot reaction (Kerscher and Ziegenhorn, 1983). Blood ammonia-N values were negligible (<1 µM) compared with the concentration of urea N (>4 mM), so no corrections of the urea N data were performed. Total N excretion over the 24-h period was determined on composites of the acidified excreta and bladder samples using a micro-Kjeldahl procedure (Brotz and Schaefer, 1984).

The whole blood concentration of D-3-OH-butyrate (BHBA) was determined by a fluorometric adaptation of the enzyme assay described by Williamson and Mellanby (1974) using a fluorometer equipped with filters yielding excitation light of 310 to 400 nm (365 nm peak) and monitoring emitted light at 410 to 525 nm (445 nm peak). Differences in fluorescence were observed between standards diluted with distilled water and standards diluted with deproteinized blood, so the method of standard addition (Cardone, 1983) was employed. Each sample was assayed with standard additions of 0, 0.5 and 1.0 nmol D-beta hydroxybutyrate (BHBA). Fluorescence deflections were corrected for deflection observed in a blank containing no enzyme and were regressed on the level of standard added. The absolute value of the x-intercept reflected the amount of analyte in the sample.

The data were analyzed according to a randomized complete block design (Steel and Torrie, 1960), and the treatment effect was partitioned into five orthogonal contrasts. The following three preplanned contrasts were of primary interest: 1) treatment vs control (treatments 2, 3, 4, 5, 6 vs 1), 2) medium- vs long-chain triglycerides (treatments 2, 3, 4 vs 5, 6), and 3) even- vs odd-MCT (treatment 2 vs 3, 4).

The time delay between birth and force-feeding was recorded to the nearest minute and was evaluated along with initial body weight as a covariable. Preliminary analysis revealed treatment differences (P < .05) in blood glucose and urea concentration at the start of the trial that were correlated (P < .01) with the 12- and 24-h values. Therefore, the data were analyzed as differences between 12- and 0-h values, 24- and 12-h values, and overall between 24- and 0-h values. Inferences regarding differences between periods at 12 and 0 h vs 24 and 12 h were based on a split-plot analysis of variance in which period was the subplot.

**Experiment 2**

Three triglyceride treatments (Table 1, treatments 2, 3 and 6) and three piglet ages (6, 18 and 48 h) were studied in a 3 × 3 factorial plan arranged in eight randomized complete blocks, each consisting of nine littermates. In contrast to Exp. 1, piglets were allowed to suckle. Piglets were used at approximately 6 h (3 to 6 h), 18 h (15 to 18 h) or 48 h (45 to 48 h) of age and were fasted for 1 h prior to force-feeding 12 ml of triglyceride. A 2-ml heparinized blood sample was obtained as described for Exp. 1 at time 0, just prior to force-feeding. Piglets were used at approximately 6 h (3 to 6 h), 18 h (15 to 18 h) or 48 h (45 to 48 h) of age and were fasted for 1 h prior to force-feeding. Piglets were used at approximately 6 h (3 to 6 h), 18 h (15 to 18 h) or 48 h (45 to 48 h) of age and were fasted for 1 h prior to force-feeding. Piglets then were individually caged and additional blood samples were drawn at 1, 2, 4 and 8 h. One millimeter of blood was deproteinized for BHBA analysis as described in Exp. 1, and packed cell volume was determined on samples collected at 0, 4 and 8 h. The remaining blood was centrifuged to remove cells and plasma was stored at -20°C for medium-chain fatty acid (MCFA) analysis.

The plasma concentrations of octanoate (C8), nonanoate (C9) and decanoate (C10)
were determined in samples taken from piglets force-fed MCT. Radiolabeled octanoate (1-14C, 4.1 μCi/μmol) was added to .5 ml of each plasma sample (ca. 65,000 dpm/sample) as an internal standard. Radiochemical purity of the isotope was evaluated using HPLC. Greater than 99% of the radioactivity co-eluted with authentic octanoate. After acidification, the fatty acids were extracted into HPLC-grade methyl-t-butyl ether and subsequently were back-extracted into an alkaline (KOH) aqueous fraction yielding the K+ salts of the fatty acids. After lyophilization, the phenacyl derivatives were formed using a crown ether catalyst (Durst et al., 1975). The derivatives were separated by HPLC using a 3.9-mm x 30-cm μBondapak C18 reversed-phase column and a convex acetonitrile gradient from 55 to 99% acetonitrile and a flow rate of 1 ml/min. Peaks were detected by UV absorption at 242 nm (Figure 1), and the radioactivity present in the phenacyl-octanoate peak was determined by liquid scintillation counting with correction for background counts and counting efficiency. Quantification was based on integration of peak area and comparison to standards with correction for recovery of internal standard that ranged from 75 to 100%.

The data were analyzed first with a comprehensive statistical model, blocked by litter with a factorial whole plot (three triglycerides x three perinatal ages) and a split plot in time (five blood sampling times). Due to an significant three-way interaction (triglyceride x perinatal age x sampling time) for BHBA and fatty acid concentration, each factor was assessed at all combinations of the other factors with several analysis of variance models. The Bonferroni multiple comparison procedure was used for evaluating sampling time effects, and protected LSD tests were used for evaluating triglyceride and perinatal age effects (Steel and Torrie, 1960).

### Results

#### Experiment 1

Hematocrit levels decreased (P < .05) slightly (from 40 to 38%) over the 24-h period (data not shown), but no treatment effects were seen (P > .1). The concentrations of BHBA were at the lower detection limit of the assay (approximately 5 μM) at all time points measured regardless of treatment (data not shown). The changes in blood urea concentration were not different (P > .1, Table 2) between animals given triglyceride and those given water, nor were differences observed between MCT and LCT groups or between even- and odd-MCT groups. All treatments were associated with the same pattern of decrease during the first 12-h period followed by an increase during the second 12-h period.

Blood glucose concentration (Table 2) increased over both 12-h periods in all treatment groups except those given corn oil (treatment 6). The average increase was .28 mM during the first 12-h period and .72 mM during the second. The increase in blood glucose was not different between piglets given water and piglets given the triglyceride products (P > .1); however, this was due to the low response to LCT relative to MCT, as demonstrated by the difference observed (P < .01) in the LCT vs MCT contrast over the 24 h. This difference was due primarily to the low response to corn oil (treatment 6), which was less (P < .05) than that observed for piglets given the structured triglyceride (treatment 5). Furthermore, even-MCT supported larger increases in glucose concentration than did odd-MCT (P < .05) over the 24-h period.

Piglets lost more weight (P < .01) during the first 12-h period (80 g; pooled SEM = 3.5) than during the second (55 g). Control animals lost less weight (P < .01) during the second 12-h period and over the entire 24 h than did
TABLE 2. EFFECT OF FORCE-FEEDING TRIGLYCERIDES WITH DIFFERENT FATTY ACID COMPOSITION ON CHANGES IN BLOOD UREA AND GLUCOSE CONCENTRATIONS, BODY WEIGHT LOSS AND NITROGEN EXCRETION IN NEWBORN PIGLETS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product force-fed</strong></td>
<td><strong>Water</strong></td>
<td><strong>C8, C10</strong></td>
<td><strong>C9</strong></td>
<td><strong>C7, C9</strong></td>
<td><strong>C8, C18</strong></td>
<td><strong>C18</strong></td>
</tr>
<tr>
<td><strong>Urea, mM</strong></td>
<td><strong>Time 0</strong></td>
<td>3.3</td>
<td>3.5</td>
<td>3.0</td>
<td>4.1</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>Diff 12-0</strong></td>
<td>-.75</td>
<td>-.93</td>
<td>-.85</td>
<td>-1.53</td>
<td>-.93</td>
<td>-.95</td>
</tr>
<tr>
<td><strong>Diff 24-12</strong></td>
<td>.29</td>
<td>.19</td>
<td>.82</td>
<td>.01</td>
<td>.50</td>
<td>.05</td>
</tr>
<tr>
<td><strong>Diff 24-0</strong></td>
<td>-.46</td>
<td>-.60</td>
<td>-.03</td>
<td>-1.45</td>
<td>-.46</td>
<td>-.90</td>
</tr>
<tr>
<td><strong>Glucose, mM</strong></td>
<td><strong>Time 0</strong></td>
<td>2.5</td>
<td>2.2</td>
<td>2.2</td>
<td>2.4</td>
<td>2.2</td>
</tr>
<tr>
<td><strong>Diff 12-0</strong></td>
<td>.19</td>
<td>.63</td>
<td>.35</td>
<td>.27</td>
<td>.59</td>
<td>-.34</td>
</tr>
<tr>
<td><strong>Diff 24-12</strong></td>
<td>.66</td>
<td>1.11</td>
<td>.65</td>
<td>.88</td>
<td>.47</td>
<td>.52</td>
</tr>
<tr>
<td><strong>Diff 24-0</strong></td>
<td>.84</td>
<td>1.90</td>
<td>1.00</td>
<td>1.26</td>
<td>1.08</td>
<td>.18</td>
</tr>
<tr>
<td><strong>Body Weight, g</strong></td>
<td><strong>Time 0</strong></td>
<td>1,492</td>
<td>1,492</td>
<td>1,492</td>
<td>1,492</td>
<td>1,492</td>
</tr>
<tr>
<td><strong>Diff 12-0</strong></td>
<td>-67</td>
<td>-99</td>
<td>-83</td>
<td>-73</td>
<td>-85</td>
<td>-74</td>
</tr>
<tr>
<td><strong>Diff 24-12</strong></td>
<td>-41</td>
<td>-65</td>
<td>-73</td>
<td>-42</td>
<td>-61</td>
<td>-49</td>
</tr>
<tr>
<td><strong>Diff 24-0</strong></td>
<td>-109</td>
<td>-167</td>
<td>-157</td>
<td>-115</td>
<td>-149</td>
<td>-125</td>
</tr>
<tr>
<td><strong>N excretion, mg/(kg-d)</strong></td>
<td>147</td>
<td>117</td>
<td>121</td>
<td>126</td>
<td>125</td>
<td>128</td>
</tr>
</tbody>
</table>

*Three animals died (treatments 1, 4 and 5) after the 12-h sampling, so values requiring 24-h data reflect 11 observations for treatments 1, 4 and 5, whereas n = 12 otherwise. Differences between 12-0 h and 24-12 h periods noted in the text are inferred from a split-plot analysis of variance using the residual error term for tests of significance. Period × treatment interactions were not significant. Notations of significance are based on two-way analysis of variance on each period separately.

bSee Table 1 for abbreviations.

cTreatment differences at time 0 and significant correlations between 0-, 12- and 24-h values precluded direct analysis of blood concentrations of urea and glucose at 12 and 24 h. Therefore, the data were analyzed as differences between 12- and 0-h data, 24- and 12-h data and 24- and 0-h data.

dDifference between odd medium-chain triglycerides (P < .05; 3 vs 4).

eDifference between medium-chain and long-chain triglycerides (P < .05; 2, 3, 4 vs 5, 6).

fDifference between even and odd medium-chain triglycerides (P < .05; 2 vs 3, 4).

gDifference between treatment and control (P < .05; 2, 3, 4, 5, 6 vs 1).

bDifference between long-chain triglycerides (P < .05; 5 vs 6).

hDifference between medium-chain triglycerides (P < .05; 3 vs 4).

iInitial body weight was retained as a significant covariable in the analysis of body weight data; reported data represent covariate adjusted means.

animals receiving triglyceride supplementation. There were no differences in body weight change in piglets receiving LCT vs MCT (P > .1), but animals given even-MCT lost more weight during the first 12-h period (P < .05) and over the entire 24 h (P < .01) than did animals given odd-MCT.

Nitrogen excretion was greater in control animals than in those given triglyceride (P < .05). Animals given even-MCT excreted the least amount of N; however, no significant differences were detected among the various triglyceride treatments.

**Experiment 2**

In agreement with Exp. 1, there was no detectable effect (P > .1) of triglyceride treatment on hematocrit levels (data not shown). Packed cell volume declined (P < .05) from 29 to 27% as the piglets aged from 6 to 48 h. At each age, a further decline (P < .05) in packed-cell volume was associated with blood sampling (total of 10 ml drawn per pig over 8 h). This suggests that the intensive blood sampling exacerbated the normal decline in packed cell volume with age, but was not overly severe because the packed cell volume of 6-h-old pigs after all blood samples were taken (27.5%) was equivalent to the time-0 value in 48-h-old piglets (27%).

Due to a three-way interaction (triglyceride × perinatal age × sampling time, P < .05) when tested against the residual error term, the transient changes in BHBA and medium-chain fatty acid (MCFA) concentration after an oral
triglyceride load are shown graphically in Figure 2 for each age period. There was no detectable rise in BHBA concentration in animals force-fed corn oil (LCT) regardless of age (P > .1). In contrast, a significant rise in BHBA concentration was observed 1 to 2 h after force-feeding piglets even- or odd-MCT. The BHBA concentrations plateaued at 20 μM in 6-h-old animals but peaked higher (30 to 50 μM) and fell within 2 to 4 h in 18- and 48-h-old piglets. Furthermore, the ketogenic response to even-MCT was about twice that observed in 18- and 48-h-old piglets given odd-MCT (P < .05).

The lower panels of Figure 2 show the changes in plasma octanoate (C8), nonanoate (C9) and decanoate (C10) concentrations. It should be noted that the two dashed lines in the lower three panels represent C8 and C10 concentrations observed in plasma of piglets force-fed even MCT, which contained both fatty acids (Table 1, treatment 2). The MCFA concentrations were not measured in piglets force-fed corn oil (LCT) because no MCFA was expected. Plasma concentrations of C8 and C9 closely paralleled the circulating concentration of BHBA when even- and odd-MCT were given, an observation consistent with a substrate-product relationship. Even though C10 composed 25% of the fatty acid content of the even-MCT product (Table 1, verified by HPLC analysis of the saponified triglyceride), less than .03 mM was detected in systemic circulation as the free fatty acid, and the concentration did not change over the 8-h observation period.

Discussion

The early postnatal period poses a stark challenge to the energy balance of the mammalian biological system. In utero, the fetus is provided with a continuous supply of energy from maternal circulation, primarily in the form of glucose, lactate and amino acids (Battaglia and Meschia, 1978). Nourishment is ended abruptly at parturition, and the newborn must elicit the behavioral responses necessary to acquire milk from the dam. Even though the neonate possesses the basic suckling instinct at birth, greater than 30 min may lapse before the first milk is consumed by average-sized piglets, and even longer for those of low birth weight (Rohde Parfet and Gonyou, 1988). It is considerably longer before positive energy balance is regained. Furthermore, the metabolism of the newborn must adapt to utilize fat, the major source of calories in milk.

In the swine production environment, the period of negative energy balance is exacer-
bated by a harsh thermal environment immediately postpartum, which demands an increase in metabolic rate of two- to three-fold. In utero, the metabolic rate of the fetus is similar to that of the sow and increases at birth in accordance to its increased metabolic body size (Kleiber, 1975). This amounts to an increase in heat production from about 35 kcal/d in a 1.5-kg fetus to 100 kcal/d in the newborn at thermoneutrality (Curtis, 1974). The piglet must generate additional heat to dry off and attempt homeothermy in an environmental temperature that often is below its critical temperature of 34°C (Stanier et al., 1984). Whether the piglet can survive this transition depends on its ability to utilize the endogenous energy stores of carbohydrate, fat and protein that were established in utero. The present line of research focused on a method to supplement the endogenous fuels during the period of negative energy balance immediately postpartum. A proper source and quantity of supplemental energy we hope will improve the chance of piglet survival.

The selection of fat as the oral supplemental energy source is justified because of its high caloric density and low osmotic effects in the gastrointestinal tract. Furthermore, it is a major source of calories (60%) in the piglet's natural diet of milk (Ferre et al., 1986). Interest in MCT (C6 to C12 fatty acids) stems from their use in human clinical nutrition as an energy source for premature infants (Roy, 1981). The shorter chain length has marked effects on their physiochemical properties (Greenberger and Skillman, 1969), which greatly affect their metabolism relative to LCT (>C16 fatty acids). In addition to a more rapid rate of gastrointestinal hydrolysis (Desnuelle and Savary, 1963; Greenberger et al., 1966) of MCT and more rapid rate of portal absorption of the component medium-chain fatty acids (Bloom et al., 1951; Greenberger et al., 1966), mammalian metabolism is such that MCFA are more extensively oxidized and less extensively esterified than are long-chain fatty acids (LCFA) (Frost and Wells, 1981). Collectively, these features suggest that MCT should be an effective fuel source for the newborn piglet.

**Experiment 1**

This experiment was designed to evaluate the ability of several triglycerides of varying fatty acid composition (Table 1) to improve the clinical energy status of the piglet immediately postpartum. In addition to comparing MCT to LCT, one of the triglycerides (treatment 5) was composed of a mixture of MCFA and LCFA. This special class of lipid, referred to as structured triglyceride, has received recent attention in human clinical nutrition (Babayan, 1987). The LCFA provides a source of essential fatty acid and may influence the rate of MCFA absorption. Two MCT treatments also were included that contained MCFA of odd-chain length (treatments 3 and 4). Odd-MCT is of interest because of the higher ketone body production from MCT than from LCT (Allee et al., 1972; Bach et al., 1977; Frost and Wells, 1981), probably a consequence of rampant uncontrolled oxidation of the MCFA. Because MCFA are thought to be activated to their CoA thioesters within the mitochondrion (Aas, 1971), entry into the mitochondrion presumably is independent of the carnitine acyltransferase/translocase system, which is reported as a major site for control of fatty acid oxidation (Bremer, 1977). This uncontrolled entry into liver mitochondria may increase carbon flux through the beta-oxidation pathway and exceed the capacity of the TCA cycle to combust the acetyl-CoA produced, therefore, ketone body production rises concomitantly (Krebs, 1966; Bach, 1978). We hypothesized that if the capacity of the TCA cycle could be enhanced by supply of anaplerotic carbon from propionyl-CoA arising from the last three carbons of odd-MCFA, ketone body production would be diminished relative to that seen when even-MCT was used.

The consistent rise in blood glucose over the 24-h period was not expected based on prior reports (Swiatek et al., 1968; Bengtsson et al., 1969; Gentz et al., 1970; Pegorier et al., 1981). Increases between birth and 6 h were common, but declines by as much as 1 mM were observed by 24 h in the earlier work. The greater increase in glucose concentration of animals supplemented with even-MCT compared with those supplemented with odd-MCT and LCT may reflect better utilization of even-MCT. Duee et al. (1985) have proposed that LCFA oxidation supports hepatic gluconeogenesis by generating ATP and reducing equivalents and also by providing acetyl-CoA, an obligatory cofactor for pyruvate carboxylase (Pegorier et al., 1985). The extent to which this is true for MCFA (Turlan et al., 1983; Pegorier...
et al., 1983b) is not certain, because short-term infusions of both even- and odd-MCT in dogs have been associated with transient hypoglycemia (Guisard et al., 1973), presumably mediated by an insulin response.

Supplementation with triglyceride accelerated weight loss of the animals compared with the water-dosed controls. This is interpreted as a specific dynamic effect of fat (Kleiber, 1975), resulting in an increased metabolic rate and thus increased weight loss as water. If so, this provides further evidence for oral triglyceride utilization. Others have reported enhanced in vivo (Seaton et al., 1986) and in vitro (Berry et al., 1983) thermogenesis from even-MCT and MCFA, respectively, and suggestions have been made that the oxidation of MCFA may occur concomitantly with lipogenesis (Croizer, 1988) and may be coupled less tightly to hepatic ATP production due to greater carbon flux through beta-oxidation (Berry et al., 1983, 1985).

Finally, the reduction in total N excretion associated with triglyceride supplementation further suggests their utilization by the newborn piglet. It can be inferred from these data that the fasted animals oxidized more body protein to meet their energy needs. Assuming all of the urinary N (147 · kg⁻¹·d⁻¹) came from body protein (at 16% N), it would equate to the catabolism of .9 kg of body protein/kg. A further assumption that 4.8 kcal were released per gram of body protein catabolized yields 4.4 kcal, which is 4.4% of the piglet’s daily energy requirement.

When considered collectively, these clinical measurements provide reasonable indirect evidence for the utilization of the orally administered triglycerides and suggest that even-MCT may be utilized somewhat better than odd-MCT or LCT.

**Experiment 2**

This experiment was designed to provide more direct evidence for digestion, absorption and oxidation of MCT by monitoring blood levels of BHBA and MCFA at several time points between 0 and 8 h after force-feeding. In addition, numerous reports have documented improvements in ability to oxidize fatty acids with increasing postnatal age in rats (Augenfeld and Fritz, 1970; Lockwood and Bailey, 1970; Warshaw, 1972; Foster and Bailey, 1976; Ferre et al., 1983) and piglets (Bieber et al., 1973; Mersmann and Phinney, 1973; Wolfe et al., 1978). However, few have focused on development within the first 2 d of life (Mersmann et al., 1972), which is the critical time period for the neonate because

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**Figure 3.** Correlation of whole blood 3-OH-butyrate (BHBA, µM) and plasma octanoate (C8) or nonanoate (C9, mM) from 18- and 48-h-old piglets force-fed even- (--) or odd-MCT (−), respectively. Data are from the four right panels of Figure 2. The least squares fitted equations are as follows (± SE): C8: y = 50.8 (±4.4) − 43.3 (±4.5) · e(−0.0058 ±0.0014)· x; C9: y = 137.0 (±235.8) − 129.2 (±234.8)· e(−0.0013 ±0.0026)· x.
most preweaning mortality occurs prior to 3 d of age (Fahmy and Bernard, 1971). For this reason, piglets were studied at approximately 6, 18 and 48 h postpartum.

The lack of ketogenic response from LCT was not surprising because its entry into the body is via a lipoprotein-lymphatic route and because LCFA oxidation is regulated by allosteric control of carnitine acyltransferase (McGarry and Foster, 1980). Similarly, the greater ketogenic response from MCT than from LCT has been shown repeatedly (Allee et al., 1972; Bach et al., 1977; Frost and Wells, 1981) and was expected for reasons given earlier. However, the relative degree of hyperketonemia observed (20 to 50 μM BHBA at peak) is very low compared to other species in which it may exceed 4 mM BHBA (Yeh and Zee, 1976), as, for example, in the rat. The relatively low concentration of BHBA observed in piglets may be due to a low rate of ketogenesis and(or) a high rate of ketone body utilization in the piglet. Work with isolated hepatocytes (Pegorier et al., 1983a) and liver mitochondria (Duee et al., 1986) has suggested low rates of ketogenesis. This also is suggested by Figure 3, which is a combined plot of the data presented in Figure 2. Figure 3 illustrates a substrate-product correlation and suggests that ketone body synthesis may be maximal at relatively low MCFA concentration (< .4 mM). Although ketone body utilization has not evaluated in the piglet, ketone bodies are considered to be an important fuel source and lipogenic precursor for the developing neonatal rat (Williamson, 1982).

The elevated ketogenic response to even-MCT relative to odd-MCT is consistent with the hypothesis that propionyl-CoA resulting from oxidation of odd-chain MCFA could boost TCA cycle intermediates and result in more complete combustion of acetyl-CoA units of CO₂ with less acetyl-CoA flux into ketone bodies. These results are in agreement with data from Guy and Tuley (1981), who measured BHBA levels in rats 2 h after consumption of a meal containing 68% of the calories from even- or odd-MCT. The BHBA concentration was five- to eight-fold higher in animals given even-MCT. In contrast, Guisard et al. (1973) reported no difference in ketogenic response when dogs were infused with even- or odd-MCT emulsions. No explanation for this discrepancy is offered.

An alternative hypothesis that is equally plausible is suggested by the plasma fatty acid data. Specifically, the lower ketogenic response from odd-MCT could be attributed to a lower rate of digestion and entry into circulation because the time-profile of nonanoate (C9) concentration (Figure 2) was significantly less than that observed for octanoate (C8). Neither hypothesis can be proved or dismissed based on the data reported because flux cannot necessarily be inferred from concentration. Nonetheless, the possibility of a difference in digestion and(or) absorption rate, indicated by the low levels of decanoate (C10) observed in the plasma, is given additional credence. Although it composed 25% of the fatty acids in the even-MCT product, the circulating concentration of C10 was dramatically less than that observed for C8 (Figure 2). Consequently, one may infer slower kinetics of entry into circulation as chain length increases from C8 to C9 to C10. This also would be consistent with the generally accepted belief that the rate of metabolism of fatty acids varies in proportion to their water solubility (Lloyd and Crampton, 1957; Kirschner and Harris, 1961; Senterre, 1985; Emmison and Agius, 1988) and is interesting considering that MCFA shorter than C14 enter circulation predominantly via the portal route (Bloom et al., 1951). These data have caused us to question whether rate of entry into circulation varies even though the route remains the same.

More certain is the conclusion that development in either digestion and absorption and(or) oxidation occurred between approximately 6 and 18 h of age for even-MCT. It should be emphasized that the piglets in this study were allowed to suckle in accordance with natural rearing conditions. Consequently, the apparent development may or may not be dependent on colostrum intake.

Very few studies have focused intensively on changes in fat digestion and metabolism within the first 48 h of life. Based on measurements of pancreatic lipase and amylase activities, Kitts et al. (1956) concluded that fat digestion in the piglet was relatively well developed at birth. This is consistent with data from Sheffy et al. (1951), who reported no difference in apparent digestion of lard between newborns and piglets 2 d of age and Frobish et al. (1967) reporting mean digestibility coefficients for fat in sow’s milk of 95.1% in 2-d-old pigs. Whether these observations are applicable to digestion of nonemulsified MCT is not certain and warrants further investigation.
It is quite possible that the apparent development between 6 and 18 h is in part related to changes in the ability to oxidize fatty acids. Mersmann et al. (1972) reported dramatic increases in oxidation of various TCA cycle intermediates in liver tissue from piglets between 6 and 12 h postpartum, which they attributed to rampant mitochondrial proliferation. Shipp et al. (1982) reported a doubling in concentration of MCFA and BHBA after force-feeding MCT were very transient, and 6 and 12 h of age. This development was attributed to increases in activity of fatty acid-activating enzymes.

Implications

The experiments reported herein provide evidence that MCT can be utilized by newborn piglets and that this utilization may improve over the 1st d of life. The evaluation in concentration of MCFA and BHBA after force-feeding MCT were very transient, and the amount of metabolic fuel obtained from a single 12-ml dose is unknown. This work also has raised the notion that changes in fatty acid chain length of triglycerides, even within the medium-chain family (C6 to C12), may have profound effects on their rate and extent of utilization by the neonatal piglet. These inferences must be limited to the piglets of average birth weight. In this work, future research should focus on the metabolic competency of piglets of low birth weight.

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