EFFECT OF COLOSTRUM OR MEDIUM-CHAIN TRIGLYCERIDE SUPPLEMENTATION ON THE PATTERN OF PLASMA GLUCOSE, NON-ESTERIFIED FATTY ACIDS AND SURVIVAL OF NEONATAL PIGS

Allan J. Lepine, R. Dean Boyd, Janet A. Welch and Karl R. Roneker
Cornell University
Ithaca, NY 14853-4801

ABSTRACT

A total of 48 neonatal pigs were used to determine whether intubation with colostrum or medium-chain triglyceride (MCT) would enhance glucose homeostasis and survival. Pigs were removed from the sow prior to nursing and allotted to three treatment groups. Fasted pigs received only water for 30 h, whereas those allotted to supplemented groups received either 30 ml of colostrum or 15 ml of MCT at 6 and 16 h after birth (t6 and t16). Supplementation with MCT (t6) resulted in a 2.4-fold elevation in plasma non-esterified fatty acid (NEFA) concentration compared with fasted pigs (260 vs 109 μEq/liter at t8; P < .05). This difference increased following the second MCT dose (436 vs 117 μEq/liter at t18; P < .05). Colostrum supplementation also elevated plasma NEFA (201 and 259 μEq/liter at t8 and t18, respectively); however, less triglyceride fatty acid was presented via colostrum compared with MCT. Supplementation with MCT resulted in a greater increase in plasma glucose concentration, relative to fasting levels (75 vs 56 mg/ml at t8; 76 vs 62 mg/ml at t18), than was obtained with colostrum (68 and 65 mg/ml at t8 and t18, respectively). Residual effects of supplementation to t30 were evident for both MCT and colostrum pigs in NEFA levels, but only the MCT group had a greater (P < .05) concentration of plasma glucose at t30 compared with the fasted group (63 vs 49; P < .05). This regimen of MCT supplementation was employed in an experiment with nursing pigs to determine whether MCT would improve glucose status and survival of less-competitive pigs. Supplementation of sow-reared, low birth weight pigs (< 1.14 kg) with 25 ml MCT reduced survival relative to saline-dosed controls at 30 h (P < .10), 7 d (P < .10) and 21 d of age. This disadvantage was associated with lower (P < .05) plasma glucose, perhaps due to direct or indirect effects of an excessive dose of fatty acids (25 vs 15 ml of MCT used in fasting study).

(Key Words: Neonatal Pig, Hypoglycemia, Survival, Colostrum, Medium Chain Triglycerides.)


Introduction

The problem of poor glucoregulation in the newborn pig is multifaceted and has been reviewed in a number of publications (Mersmann, 1974; Robinson et al., 1981; Lepine, 1987). Serious limitations have been observed in both metabolic (e.g., fatty acid oxidation) and endocrine (e.g., glucagon secretion) components, but the primary factor(s) responsible for poor gluconeogenesis have not yet been identified despite its importance to both the fasting and nursing pig.

Ensuring even a very limited colostrum intake improves the glycemic status of the
fasting neonatal pig (Lepine et al., 1984). Attempts to understand the potential effects on metabolism that may promote such a response have led to evaluation of medium-chain triglyceride (MCT) as a possible alternative to supplementation with colostrum. Fatty acids of MCT are absorbed from the gastrointestinal tract via the portal vein, thereby enhancing the opportunity for hepatic uptake. In addition, medium-chain fatty acids (MCFA) do not require the carnitine acyltransferase system for transport into the mitochondria for oxidation (Bremer, 1983). These characteristics result in a greater potential for hepatic uptake and oxidation of medium-chain fatty acids compared with the long-chain fatty acids (LCFA) (Frost and Wells, 1981) predominant in sow colostrum and milk. A markedly greater oxidation rate of octanoate (8 carbons) relative to LCFA was demonstrated in neonatal pigs by Duee et al. (1985) and Lepine (1987) using hepatocytes. Hence, MCT potentially is important as a means of providing a concentrated source of oxidizable substrate, which could have a marked impact on glucose homeostasis in the less-competitive pig.

This study was designed to examine the extent to which plasma non-esterified fatty acids (NEFA), glucagon and, ultimately, glucose could be elevated and persist in neonatal pigs utilizing a program of colostrum supplementation applicable in commercial practice. In addition, supplementation with a source of MCFA (8, 10 carbons) was evaluated for its effectiveness in promoting normoglycemia relative to colostrum.

**Experimental Procedure**

**Experiment 1.** Litters from eight crossbred (Yorkshire × Landrace) first-, second- and third-parity sows were used in this study. Parturition was induced by an i.m. injection of 10 mg of prostaglandin F₂₄ at 0800 on d 110 of gestation, followed approximately 24 h later by a 40 IU injection (i.m.) of oxytocin to induce parturition. This procedure facilitated timely delivery and attendance during farrowing on d 111. Six pigs per litter were removed from the sow at birth prior to nursing. They were weighed and placed in individual cages in an environmentally controlled room (32 ± 1°C). Two pigs then were allotted (sex balanced) to each of three treatment groups: fasted, colostrum-supplemented or MCT-supplemented. Fasted pigs received water ad libitum from birth for the duration of the study (30 h). Colostrum and MCT supplemented pigs were intubated at 6 and 16 h after birth (t₆ and t₁₆) with either 30 ml of colostrum or 15 ml of a commercially available MCT solution using a 14-french feeding tube attached to a 50-ml syringe. The amount of colostrum used was judged to provide a significant quantity without causing discomfort to the pig. The volume of MCT intubated relative to colostrum was not isocaloric (111 vs 33 kcal gross energy per intubation for MCT and colostrum, respectively), but was intended to represent a dose that could be utilized in a practically applicable management scheme. Intubations occurred after obtaining blood samples at respective time points. Colostrum was collected from the sow after the completion of farrowing in an amount adequate to provide for the colostrum dosings required within that litter (i.e., >120 ml). Water was available to all pigs ad libitum.

Blood samples (approximately 3 ml) were acquired via puncture of the anterior vena cava (Carle and Dewhirst, 1942) with a 22-gauge needle 6, 8, 16, 18 and 30 h after birth (t₆, t₈, t₁₆, t₁₈ and t₃₀). All pigs were returned to the sow after the t₃₀ sampling. Blood sampling times were selected to allow for observation of short-term (t₈ and t₁₈) and prolonged (t₁₆ and t₃₀) influences of colostrum or MCT supplementation. Blood was collected into an evacuated glass tube containing sodium heparin and was chilled on ice until centrifugation. Following centrifugation, 1 ml of plasma was transferred to a tube containing 500 KIU of aprotinin and frozen for subsequent analysis of insulin and glucagon. The balance of the plasma was frozen in a separate tube awaiting glucose and NEFA analysis. Plasma glucose was determined using an automated procedure based on the modification of the glucose oxidase method (Gochman and Schmitz, 1972). An enzymatic colorimetric method, effective in recovering C-8 through C-18 fatty acids and

---

1Lutalyse, The Upjohn Co., Kalamazoo, MI.
2Captex 300, Capital City Prod Co., Columbus, OH.
3Monoject, St. Louis, MO.
4Vacutainer, Becton Dickinson and Co., Rutherford, NJ.
5Sigma Chemical Co., St. Louis, MO.
6Technicon II Autoanalyzer, Technicon Instrument Corp., Tarrytown, NY.
7NEFA-C, Wako Chemicals USA, Inc., Dallas, TX.
modified for use with a smaller sample volume, was used to measure total plasma NEFA concentration (Lepine, 1987). Radioimmunoassay was used to determine plasma insulin concentration (Herbert et al., 1965) and plasma glucagon (Falcona and Unger, 1974) using purified porcine insulin and bovine-porcine glucagon. Radiolabeled glucagon and insulin were obtained commercially, as were insulin and glucagon antibody. The validation procedure for insulin and glucagon included evaluation for parallelism with the standard curve by using three different volumes of plasma.

Experiment 2. A second experiment was conducted to determine the effect of MCT supplementation on the survival of the low birth weight pig. A total of 33 neonatal pigs (≤ 1.14 kg) were selected from crossbred litters (Yorkshire × Landrace) and allotted to two treatment groups. Pigs were intubated twice within the initial 24-h period following birth (6 to 8 and 16 to 18 h) with either 25 ml of saline or 25 ml of MCT using a 14-french catheter. All pigs remained with the sow. Blood samples were acquired 30 h after birth and assayed for plasma NEFA and glucose concentration as described previously (Exp. 1). Survival to 30 h, 7 d and 21 d was determined for control and MCT-supplemented pigs.

Data for Exp. 1 were analyzed by analysis of variance procedures appropriate for the split-plot design with repeated measures (SAS, 1982). Dietary treatments were compared (fasted vs colostrum, fasted vs MCT and colostrum vs MCT) using a single degree of freedom contrast at each time point. The coefficient of variation was calculated using the error mean square. Preliminary evaluation for sex, litter and birth weight differences was conducted by one-way analysis of variance. No differences were observed. Data for Exp. 2 were analyzed using analysis of variance procedures for plasma glucose and NEFA; the chi-square test was used to compare survival rates.

Results and Discussion

The effects of colostrum and MCT supplementation on plasma NEFA concentration are shown in Figure 1. Fasted pigs exhibited a relatively unresponsive pattern throughout the study. Supplementation with colostrum or MCT, 6 h after birth, affected increases in plasma NEFA within 2 h (t8); response to colostrum was not different (P > .05) from the fasting group. Persistence of elevated plasma NEFA to t16 was evident only for pigs receiving MCT (202 μEq/liter vs 138 for control and 146 for colostrum). Hence, MCT resulted in an increase in NEFA initially, with a potentially important increase (+46%) for at least 10 h. The second dose of MCT (t16) resulted in plasma NEFA concentrations approximately 3.7- and 1.7-fold greater (P < .05) than observed for either fasting or colostrum-supplemented pigs, respectively (t18). Intubation with colostrum (t16) elevated plasma NEFA relative to pigs in the fasting group at t18 (259 vs 117 μEq/liter; P < .05). In contrast to the initial dose, the second administration of colostrum (t16) resulted in an increased (P < .05) plasma NEFA level relative to fasting pigs at t18 and tended to maintain this response to t30. Differences between MCT and colostrum in plasma NEFA response appears to be a function of the quantity of triglyceride fatty acids provided by a single dose, which favors MCT.

Plasma glucose concentrations were similar for treatment groups prior to intubation (t6). Following the initial dose (t8), plasma glucose increased (P < .05) for both the colostrum and MCT groups relative to fasted pigs (68 and 75 vs 56 mg/dl, respectively). Although not different (P > .05), supplementation with MCT resulted in an 11% greater rise in plasma glucose than was observed for colostrum-fed pigs. These elevated concentrations of plasma glucose, for either group of supplemented pigs, did not persist following the initial dose (t6 to t16). The concentration of plasma glucose declined for the MCT group to a level equivalent to that of fasted pigs (64 mg/dl); an even more dramatic decline was observed for the colostrum group (53 vs 64 mg/dl; P < .05). A second dose of colostrum (t16) increased plasma glucose at t18 to a concentration similar to that observed for the fasting group (65 vs 61 mg/dl, respectively). Concentrations at t30 were remarkably similar and low for both control and colostrum pigs (49 vs 52 mg/
Figure 1. Effect of medium-chain triglyceride (MCT) on the pattern of plasma glucose and non-esterified fatty acid (NEFA) concentration in neonatal pigs. Pigs were intubated with either 15 ml of MCT (solid bars) or 30 ml of colostrum (shaded bars) at 6 and 16 h after birth or were fasted (hatched bars) from birth. Means within time period with different letters differ (P < .05). The coefficient of variation is 21.9% and 87.7% for glucose and NEFA, respectively.

We are unable to compare the present results with other results on colostrum or MCT supplementation because few data are available in the literature. Steinman and Benevenga (1985) administered a single dose of MCT (12 ml) to fasting neonatal pigs and observed no effect on plasma glucose levels at 12 or 24 h of age. The time of MCT administration relative to the plasma glucose determinations was not specified, however, making interpretation of the data difficult and direct comparison to the present study impossible.

The effect of supplementation regimen on the concentration of key glucoregulatory hormones is shown in Figure 2. Plasma insulin concentration did not differ among the three treatment groups prior to the initial dose (t6); however, 2 h after the first and second intubations with colostrum, insulin levels were approximately threefold and fourfold greater, respectively, than those observed in fasting pigs (P < .05). The influence of MCT on plasma insulin was less pronounced than was apparent for colostrum. An insulin response was anticipated and is important for uptake and metabolism of absorbed nutrients. The
increase resulting from colostrum or MCT supplementation did not persist to t16 or t30. Administration of MCT resulted in an elevation \((P < .05)\) in plasma insulin at t18 but not at t8. The markedly greater insulin secretory response to the initial colostrum intubation may explain the fall of plasma glucose to levels below that of fasted pigs at t16.

Colostrum supplementation is primarily responsible for the treatment effect observed for plasma glucagon concentration \((P < .05; \text{Figure 2})\). Plasma glucagon of colostrum-dosed pigs increased to a level 59% greater than fasting levels by t8 \((P < .05)\) and continued to increase it by an additional 18% prior to the second colostrum administration \((t16, P < .05)\). Glucagon concentration increased further in response to the second colostrum intubation \((816 \text{ vs } 498 \text{ pg/ml at t18, } P < .05)\) and remained 1.2-fold higher than fasted pigs at t30, although this difference was not significant \((P > .05)\). Results are in agreement with previous observations of a poor glucagon secretory response in the fasting neonatal pig and the requirement for colostrum intake to stimulate this response (Pegorier et al., 1981; Kasser et al., 1982; Lepine et al., 1984).

Dosing with MCT was less effective than with colostrum in increasing plasma glucagon concentration because no differences were apparent between the MCT and fasted groups at any time. There was, however, a clear upward trend in glucagon concentrations with MCT supplementation.

The insulin/glucagon molar ratios primarily reflect changes in plasma insulin (Figure 2). From this it can be concluded that recipients of colostrum exhibit increased concentrations of plasma glucagon but have a higher insulin/glucagon molar ratio following intubation \((t8, t18)\). Increases in plasma glucagon coincident with elevated NEFA were shown previously to be important to overall glucoregulatory status in vivo (Boyd et al., 1985).

The effectiveness of the colostrum supplementation scheme on elevating plasma NEFA and glucagon is readily apparent. Unfortunately, a more stable plasma glucose pattern did not result. This does not exclude colostrum supplementation as a useful management tool, but it suggests that more frequent dosing may be required to increase plasma glucose. Further, these results were obtained using normal and lower birth weight pigs. Effects might be more pronounced if applied strictly to lower birth weight, less-competitive pigs in which energy reserves at birth are lower and an increased surface area to body weight ratio increases body heat loss. Although not reporting effects on plasma glucose, Moody et al. (1966) observed a 200% decrease in mortality of low birth weight (1.0 kg) neonatal pigs allowed to nurse by supplementing with 15 ml of milk once to twice a day for 7 d after birth. This dosing schedule is not practical for incorporation into a neonatal pig management scheme, but it indicates the effectiveness of colostrum supplementation for the smaller pigs in the litter.

Supplementation with MCT markedly increased plasma NEFA and plasma glucose concentration relative to fasting controls. The mechanism by which MCT improved plasma glucose concentration cannot be determined from the present study. Several possibilities exist. Plasma glucose concentration is the balance between glucose supply (gluconeogenesis, glycogenolysis and intestinal absorption) and the rate of glucose metabolism or oxidation. Glucose oxidation by peripheral tissues can be reduced by providing fatty acids as an alternative energy source. The dramatic elevation in plasma NEFA resulting from MCT supplementation may be of particular relevance. Unlike the LCFA of colostrum, the MCFA of MCT are not dependent on the carnitine acyltransferase system for uptake into the mitochondria, prerequisite to oxidation (Bremer, 1983). This allows more rapid hepatic uptake and oxidation of MCFA than of LCFA (Frost and Wells, 1981). Data generated by the use of isolated hepatocytes, acquired from the fasted neonatal but “normal” birth weight pig, demonstrated that oxidation rates were at least 17- to 20-fold greater for MCFA (octanoate) than for LCFA. Further, MCT-derived fatty acids result in greater generation of ketones in vivo. These serve as alternative energy substrates to glucose-dependent tissues (e.g., brain) and serve to metabolically regulate a decrease in glucose use as a fuel source by peripheral tissues (Robinson and Williamson, 1980). Therefore, providing a more highly oxidizable fatty acid source may conserve glucose through direct use of fatty acids for oxidative purposes and indirectly through metabolic regulation (ketones). In contrast, infusion of LCFA (oleic acid) in vivo led to a depression in plasma glucose by fasting 1-d-old pigs unless glucagon was co-infused (Boyd
Figure 2. Effect of medium-chain triglyceride (MCT) or colostrum on the pattern of plasma glucagon, insulin and the insulin/glucagon molar ratio in neonatal pigs. Pigs were intubated with either 15 ml of MCT (solid bars) or 30 ml of colostrum (shaded bars) at 6 and 16 h after birth or were fasted (hatched bars) from birth. Means within time period with different letters differ (P < .05). The coefficient of variation is 128.3%, 42.6% and 115.1% for plasma insulin, glucagon and the I/G molar ratio, respectively.
et al., 1985). This latter observation, in concert with the marked difference in oxidation rate between MCFA and LCFA, argues against mass of fatty acid alone accounting for the differences in plasma glucose responses.

The mechanism by which glucose utilization is reduced is described by Newsholme and Leech (1985). The acetyl-CoA/CoA ratio increases as muscle B-oxidation increases, resulting in decreased pyruvate dehydrogenase activity. Elevated citrate concentrations also potentiate ATP inhibition of phosphofructokinase. The resulting “slowdown” of glycolysis increases the level of glucose-6-P, thereby inhibiting hexokinase and the commitment of glucose to oxidation. This also may account for the greater hepatic glycogen content of 24-h fasted neonatal pigs receiving a single oral dose of MCT (Steinman and Benevenga, 1985).

We are not aware of a similar comparison between fatty acid chain length and rate of oxidation for muscle tissue. Campion et al. (1986) did not observe any effect of palmitate on glucose oxidation rate in skeletal muscle isolated from fasted (24 h) neonatal pigs; however, palmitate may not have been the most appropriate fatty acid to test given clear differences between LCFA (Boyd et al., 1982) and between medium and long chain in hepatocytes (Lepine, 1987). The relative response of neonatal pigs to MCT and the potential benefits in survival warrant a definitive study relative to glucose kinetics.

We anticipate that increased glucose entry, via gluconeogenesis, may not be part of the mechanism for increased plasma glucose. This is based on the observation that increased oxidation of octanoate by hepatocytes did not enhance glucose synthesis from lactate or pyruvate (Lepine, 1987). This relationship between fatty acid oxidation and gluconeogenesis has been demonstrated for the neonatal rat (Ferre et al., 1978a,b) but has not held for the neonatal pig. Hence, the primary factor limiting hepatic glucose synthesis does not appear to be fatty acid oxidation.

It is clear that MCT supplementation promotes improved glucose status relative to the fasting pig. A preliminary attempt was made to determine whether the effect on glucose status was sufficient to enhance survival of the smaller, less-competitive neonatal pig. Results of the experiment comparing the effects of dosing low birth weight pigs (≤ 1.14 kg) with either 25 ml of saline or 25 ml of MCT twice within the initial 24 h postpartum is shown in Table 1. Surprisingly, plasma glucose concentration (P < .10) and the percentage survival at 30 h (P < .08), 7 (P < .10) and 21 d postpartum were lower for the MCT-dosed neonates compared with the saline-dosed controls. This may have resulted from an excessive volume of MCT, which promoted a degree of satiety that interfered with the pattern of nursing and the quantity of nutrients consumed (glucose, triglyceride fatty acids). Recipients of MCT in the nursing group appeared lethargic and less active. Nursing frequency or volume of colostrum consumed were not measured. However, MCT-derived fatty acids may either directly or indirectly cause satiety and depress activity. This has been demonstrated with cholecystokinin when given as a bolus (Baldwin et al., 1982). Cholecystokinin has been determined in many species, including swine, to produce satiety (Anika et al., 1980). Certain amino acids and fatty acids cause release of cholecystokinin from duodenal mucosa. This adverse

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Mean Birth wt, kg</th>
<th>Mean Plasma glucose, mg/dl</th>
<th>Mean Plasma NEFA μEq/liter</th>
<th>30 h Survival, %</th>
<th>7 d Survival, %</th>
<th>21 d Survival, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (16)</td>
<td>.92 ± .10</td>
<td>66c</td>
<td>326c</td>
<td>100c</td>
<td>47c</td>
<td>18c</td>
</tr>
<tr>
<td>MCT (17)</td>
<td>.94 ± .12</td>
<td>57d</td>
<td>288c</td>
<td>75d</td>
<td>31d</td>
<td>6c</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate total pigs per treatment.

bSamples obtained at 30 h following birth.

cdMeans within a column with different superscripts differ. (P < .10).
effect of MCT on glucose status and survival may not have been exhibited if a smaller volume of MCT were utilized (e.g., 10 ml). Although MCT can effectively improve the glucose status of the fasting pig when given MCT, these results illustrate that the optimum dose of MCT for low birth weight pigs allowed to nurse the sow needs to be defined.

Literature Cited


