

# Improvement of Lipid Absorption in Young Pigs as a Model for Preterm Infants

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### Summary and Implications

Preterm infants and neonatal suckling piglets have a limited bile acid pool that may hinder absorption of dietary lipids such as fatty acids, triacylglycerols (TAGs), and other lipid-soluble nutrients. Because dietary lipids are a valuable source of energy for growth, it is important that they are efficiently absorbed. The hypothesis of this study is that oral administration of 0.2 g/kg body weight daily of cholylsarcosine, an artificial bile acid, would decrease fecal excretion of dietary fatty acids and TAGs in suckling piglets. Twelve 7-d-old piglets were housed individually and fed a commercial milk replacer with or without oral cholylsarcosine until 21 d of age. Cholylsarcosine treatment decreased fecal excretion of stearic acid (18:0) and palmitic acid (16:0) ( $P \leq 0.02$ ). Cholylsarcosine supplementation had no effect on absorption of unsaturated fatty acids of 16 or 18 carbons ( $P > 0.05$ ). Cholylsarcosine increased fecal excretion of deoxycholic acid ( $P = 0.03$ ). Apparent absorption of dietary TAGs was increased from 77% in piglets not fed cholylsarcosine to 83% in the piglets that received oral cholylsarcosine. These results support the hypothesis that cholylsarcosine increases absorption of dietary TAGs.

### Introduction

Preterm human infants have low lipid absorption during the first few weeks of life. Contributing factors include inadequate amounts of lipolytic enzymes, high percentage of long-chain and saturated fatty acids in milk, incomplete development of the digestive tract, and the small bile acid pool. Long-chain fatty acids are hydrolyzed more poorly from milk TAGs for absorption than are short- or medium-chain fatty acids, and saturated fatty acids are hydrolyzed more poorly from milk TAGs for absorption than are unsaturated fatty acids. In addition, a relatively small bile acid pool likely limits the absorption of dietary fatty acids in preterm human infants.

Neonatal piglets have been used as a model for studies of pediatric growth, nutrition, and metabolism for many years. Neonatal piglets are similar to human preterm infants in their size, organ structure, and nutritional needs. Neonatal piglets exhibit many of the same limitations of

lipid digestion and absorption as do preterm human infants. The bile acid pool in very young, suckling pigs is small on a per weight basis compared with that in weaned pigs. Additionally, lipid absorption in neonatal piglets depends on the type of dietary fatty acid and the development of the digestive tract. Like preterm human infants, researchers have noted that piglets do not efficiently absorb long-chain saturated fatty acids. Approximately 40% of fatty acids in human milk are saturated. These saturated fatty acids, palmitic acid and stearic acid in particular, are not emulsified or hydrolyzed efficiently from TAGs for absorption by piglets. As a result of these similarities, the suckling neonatal piglet is useful as a model for the study of lipid absorption in preterm human infants.

Bile acids, which are essential to the emulsification and absorption of fatty acids and other lipid-soluble nutrients from the diet, are synthesized in liver and stored in the gallbladder. Bile acid release from the gallbladder into the small intestine is stimulated in response to eating. Bile acids act as detergents to emulsify lipids. Lipids must be emulsified before they can be broken apart and absorbed. Bile acid concentration in preterm human infants and in neonatal piglets is very low compared with that of adults of the same species. This low bile acid concentration is likely to cause low dietary lipid absorption because dietary lipids are not emulsified, and, therefore, breakdown and absorption of fatty acids from dietary lipids occurs more poorly than is needed for optimal growth.

The hypothesis is that oral cholylsarcosine, a synthetically conjugated bile acid, will increase the efficiency of dietary fat absorption in neonatal piglets. Improved absorption efficiency during the suckling period is expected to result in increased energy available for growth of neonatal piglets, thereby increasing rate of gain. To test this hypothesis, fecal excretion of fatty and bile acids by suckling piglets with or without cholylsarcosine supplementation in their diet was evaluated.

### Materials and Methods

#### Experimental Design

Animal use for this study was approved by the Committee of Animal Care at Iowa State University. Twelve 5-d-old crossbred (Duroc x Hampshire x Yorkshire) female piglets were obtained from the Iowa State University Swine Research Center. Piglets were weighed and placed in metabolism cages in groups of 3 for 2 d to allow for adjustment to metabolism cages and for time for piglets to learn how to use the feeders. After 2 d, piglets were weighed and placed in individual metabolism cages. Piglets were assigned randomly to a treatment: control (formula only) or cholylsarcosine (0.2 g/kg body weight

cholylsarcosine divided equally and fed twice daily with the formula). All piglets were fed ad libitum Piglet Liquewean® milk replacement formula (Milk Specialties, Inc., Dundee, IL). The diet contained 25% protein, 13% total fat, 43% lactose, 0.15% fiber, and 9.8% ash, with the remaining percentage being composed of minerals, extra amino acids, and antibiotics on a dry-matter basis. The fatty acid composition was 3.2% 14:0, 26.6% 16:0, 3% 16:1, 13.5% 18:0, 43% 18:1, and 11% 18:2.

#### Chemical Analyses

Fecal samples were weighed, freeze-dried, and reweighed to determine dry matter content. Freeze-dried samples were ground by using a mortar and pestle and then stored in desiccators until analyzed for lipid and bile acid composition and for lipid class composition. Total lipid content of the feces as well as fatty acid composition of the TAG and of free fatty acid fractions and bile acid composition of feces were determined.

#### Statistical Analyses

To determine differences between treatment groups and ages of piglets, all data on the excretion of fatty and bile acids were analyzed by using the Proc Mixed procedure in SAS version 8.0 (The SAS Institute, Cary, NC) to accurately analyze the repeated measures in this experiment. The LSMeans procedure also was used to obtain means and standard errors for fatty and bile acids as well as percentage enrichments by stable isotopes. Because two of the piglets became ill,  $n$  equaled 10. Significance was declared when  $P < 0.05$ . When  $P < 0.10$ , a tendency for groups to differ was declared.

#### Results

On average, all piglets consumed approximately 1100 kcal digestible energy daily, which closely matches the NRC recommendation. Piglets receiving cholylsarcosine were expected to have an increased rate of gain when compared with those that did not receive cholylsarcosine because cholylsarcosine should increase available dietary energy for growth. Contrary to predictions, rate of gain in piglets fed cholylsarcosine was similar to that of controls ( $P > 0.05$ ) (Table 1). Likewise, there was no effect of cholylsarcosine on feed intake ( $P > 0.05$ ).

To determine whether bile acid status of piglets limited fatty acid absorption from dietary TAG, the effect of supplemental cholylsarcosine on fecal excretion of fatty acids was determined. Apparent absorption of dietary TAG in suckling piglets supplemented with cholylsarcosine was approximately 83%. In suckling piglets fed formula alone (control), apparent absorption was approximately 77%. Fecal output was similar for the two groups. Cholylsarcosine, however, did influence absorption of specific dietary fatty acids. Fecal excretion of palmitic and stearic acids was decreased by oral administration of cholylsarcosine ( $P \leq 0.02$ ) (Table 2). Excretion of

unsaturated fatty acids of 16 and 18 carbons showed little change as expected as they are efficiently hydrolyzed and absorbed in nearly all neonates. Excretion of all 16 carbon fatty acids and stearic acid decreased as the age of the suckling piglets increased ( $P < 0.05$ ). Excretion of unsaturated 18 carbon fatty acids tended to decrease ( $P = 0.07$ ) as age of the suckling piglets increased. There was no interaction between age and treatment effects.

#### Discussion

Cholylsarcosine increases the apparent absorption of dietary fatty acids in neonatal piglets by about 6%; however, this increase did not translate to increased gain or gain:feed. The maximal increase in growth that can be expected from a 6% increase in lipid absorption is 2.3 g body weight per day given the formula these piglets were fed. Considering this fact, it is not surprising that the difference in growth over the 14-d period was not significant. Fecal excretion of saturated fatty acids was decreased by treatment with oral cholylsarcosine (Table 2), which is in accordance with other studies that show that, when emulsifiers were added to the diet or when vegetable oils or short-chain fatty acids were fed to suckling or early-weaned piglets, lipid digestibility was improved. The decrease in fatty acid excretion caused by supplementation with dietary cholylsarcosine also agrees with studies that show cholylsarcosine decreases fatty acid excretion in humans with compromised bowel function. Evidently, the supplemented cholylsarcosine enhanced the emulsification of dietary TAGs, thereby facilitating the absorption of greater amounts of dietary TAGs. It is possible that, by feeding a diet with a higher concentration of saturated fatty acids, greater differences in absorption might have become evident, resulting in an improvement in growth data.

#### Conclusion

Bile acids probably are one of the limiting factors in lipid absorption in neonatal piglets. Results from this study indicate that the addition of an orally administered model bile acid improves saturated fatty acid absorption. No change in growth, however, was observed. A longer-term study or a study with more piglets seems necessary to determine if cholylsarcosine increases the growth rate of neonatal piglets. Our results indicate that it seems feasible to supplement preterm human infants with cholylsarcosine to improve absorption of dietary TAGs and lipid-soluble nutrients. At least, the findings support a clinical trial with humans to test the effects of cholylsarcosine on fatty acid excretion in preterm infants.

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**Table 1. Daily formula intake, growth, and fecal excretion of suckling piglets from 7 to 21 days of age.**

	<i>Cholylsarcosine</i>	<i>Control</i>	<i>P-value</i>
Total Gain (kg) <sup>b</sup>	4.31	4.21	NS <sup>a</sup>
Average Daily Gain (g/d) <sup>b</sup>	308	301	NS
Intake (L/d)	1.41	1.42	NS
Feed Efficiency <sup>c</sup>	1.22	1.18	NS
Fecal Output (g)	65.02	72.42	NS

<sup>a</sup> Not significant.

<sup>b</sup> Growth over 14 d.

<sup>c</sup> Average daily gain (g)/average daily intake on dry matter basis (g).

n = 10

**Table 2. Effect of cholylsarcosine and increasing age on the excretion of fatty acids by suckling piglets.**

Fatty Acid	Cholylsarcosine	Control	Average Daily Change	<i>P-Value</i>	
				Treatment	Age
16:1	0.937 ± 0.17 <sup>a</sup>	1.004 ± 0.19	-2.826 <sup>b</sup>	0.71	0.05
16:0	46.757 ± 2.14	55.534 ± 2.41	-0.149	0.02	0.0001
18:1,2,3	10.833 ± 0.94	10.602 ± 1.06	-0.643	0.85	0.07
18:0	51.330 ± 1.94	65.296 ± 4.24	-0.869	0.003	0.02

<sup>a</sup> Mean ± standard error expressed as μmoles fatty acid/g dry feces.

<sup>b</sup> Average daily change was calculated by the difference between the concentrations of bile acids excreted on consecutive days for pigs in both groups. Values are expressed as μmoles/g • d dry feces.

*P*-value indicates differences in fatty acid excretion: Treatment: Between cholylsarcosine and control groups. Age: As a function of increasing age of piglets.

n = 10