

**Dietary triacylglycerol digestion and absorption and bile acid status in
neonatal piglets: A model for preterm human infants**

by

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GENERAL INTRODUCTION

Thesis Organization

This thesis is to partly fulfill the requirements of a Master of Science degree. It consists of a general literature review followed by a paper for submission to a scientific journal. The literature review introduces the topics included in the paper. The paper is complete on its own as it has an abstract and introduction, as well as materials and methods, results, discussion, and a bibliography. The paper is followed by a general summary, general conclusion, and a future research section. The paper is written in the style of the Journal of Nutrition. A reference section for references cited in the literature review, general summary, general conclusion, and future research section concludes this thesis.

Literature Review

Swine Management Practices

In the past years, farmers and researchers have been searching for the optimal balance between pig health and economics. Recently, confinement facilities have become increasingly popular. In 2002, just 3% of swine operations in the United States housed 53% of the total pigs. Confinement facilities each can house more than 5,000 pigs. While small operations of one to 99 pigs make up nearly 57% of the total number of operations, they account for only one percent of the total number of pigs (1). Confinement practices usually allow producers to grow more piglets to market weight on their farms and lead to more economic gains for farmers. Though they have been ridiculed for pollution and crowding, confinement facilities also offer a method of raising more sows to farrow in less space than do conventional methods.

Farrowing. Confinement facilities have changed the way farmers raise and farrow their sows. Currently, the common practice is for a sow to be bred and then spend approximately 110 days in a gestation crate. At that time, generally sows are moved to farrowing crates for the remaining week of gestation and for the suckling period. Farrowing crates allow piglets to suckle the sow but help eliminate the risk of crushing. The sow is confined to the center of the crate, but the piglets are allowed access to the center as well as the sides of the crate. The sides of the crate usually have heating pads so that the piglets will be comfortable in the cooler (65°F) ambient

temperature that is optimal for the sows. Usually, sows and piglets stay in farrowing crates until weaning time, which is typically 21 days.

Along with increasing public awareness of animal welfare have come new, or rather old, methods of piglet rearing. Because consumers demand a quality product that has been produced with as little negative impact on the environment as reasonably possible and in an animal-friendly manner, hoop structures (new) and open-pasture methods (old) of raising pigs have become increasingly popular. Lawmakers in Florida took the issue of animal rights and negative environmental impacts of confinement facilities one step further in the fall of 2002 when they made it illegal for farmers to house pregnant sows in gestation crates (2). Is this a foreshadowing of what is to follow in the rest of the nation? We will have to wait to see.

Weaning. If allowed to naturally suckle and consume solid-based feed during the nursing period, piglets will begin to consume mostly dry feed between the ages of 9 and 12 weeks of age (3). Swine management practices have decreased the typical weaning age down to 3-4 weeks. Some piglets are weaned even earlier, at 1-2 weeks of age, with the help of segregated weaning pens and added medications in the feed (4). At the time of weaning, piglets are separated from their dam and are usually grouped with other piglets. The piglets commonly are fed a “starter” or, in some cases, a “pre-starter” feed. Both starters and pre-starters usually are pelleted and contain high percentages of milk products. They provide a more gradual switch from sows’ milk to the normal swine feed that consists primarily of ground corn and

soybean meal. As the piglets become more accustomed to eating solid foods, they are fed grower feeds that are formulated to provide a proper balance of nutrients for increased growth rates. Growing pigs to slaughter weight faster is advantageous to farmers because a shorter feeding period results in decreased feed costs and space for additional pigs, which allows farmers to produce more pigs per year in their housing facilities.

Early weaning alternatives. Recently, some farmers have adopted early-wean rearing practices. Early weaning usually refers to weaning piglets between the ages of 10 and 14 days (5). Piglets thrive in early weaning practices as long as they are kept with their dams long enough to receive the full benefit of colostrum (6). In fact, early weaning limits the transfer of disease from the sow herd to animals in the nursery. Research shows that aberrant behaviors associated with the stress of early weaning, such as tail and sheath sucking, disappear by harvest (5). Early-weaned piglets often are fed milk pellets instead of liquid milk replacers before being fed pre-starters because of difficulties in feeding liquid feeds to large numbers of piglets. It is difficult to keep large amounts of milk replacer wholesome for long periods of time as it makes a good environment for microbes to grow. Milk pellets are a good alternative because they are composed mostly of milk-based products and contain very little plant-derived material. They provide the same nutrients as liquid feeds but are more convenient to feed to large numbers of piglets. They can be left in feeders so that piglets have ad libitum access to the feed.

Although evidence indicates that piglets at 10 d grow faster, have greater feed intakes, and have higher gain to feed ratios than do piglets weaned at 21 days (6), the practice is not widely used outside of confinement facilities because of the added cost of feeding such young piglets the proper diet and medications. Depending on the number of piglets a farmer weans and either sells or grows each year, the early weaning alternative is not always economically feasible.

Piglet Nutrition

Sow milk provides essential antibodies and nutrients to piglets for the duration of the suckling period. During the first 48 hours, sows produce colostrum, which is a watery, yellowish secretion rich in nutrients and antibodies especially important to the survival of the neonate. Colostrum contains high concentrations of immunoglobulins that are passed from the dam to the piglets and provides the piglets immunity against some pathogens (**Table 1**) (7). At 0 hours lactation, IgG concentration in sow colostrum is 95.6 mg/mL, IgM is 9.1 mg/mL, and IgA is 21.2 mg/mL. By 48 hours of lactation, the concentrations of these three specific immunoglobulins in sow milk decrease to 6.3 mg/mL, 2.7mg/mL, and 5.2 mg/mL, respectively, resulting in a decrease in total milk solids and an increase in percentage of fat and lactose (7).

Sow milk composition varies with diet and stage of lactation but on average contains about 18% total solids, 6.5% fat, 6% lactose, and 5.5% total protein (**Table 2**) (7).

Throughout lactation, milk solids remain relatively constant after cessation of

colostrum production. Total fat and lactose increase with duration of lactation, whereas total protein decreases at the same time.

Supplemental milk replacers. Recently, the effects of feeding supplemental milk replacers to piglets during the suckling period has been examined; however, the benefits of feeding supplemental milk replacer to piglets suckling their dams have not been determined. One study notes that litters supplemented with milk replacer were heavier at weaning and tended to have more pigs weaned; however, performance from weaning to harvest was unchanged (8). Many powdered milk formulas are commercially available. These formulas are based on the composition of sows' milk to assure that all of the piglets' nutritional requirements are met. Most powdered formulas are supplemented with one or two antibiotics, such as oxytetracycline or neomycin, to help prevent scours and other common illnesses.

One problem associated with liquid formula feeding is keeping it wholesome and safe for the piglets to consume after it has been reconstituted. Weaning houses for early-weaned piglets are usually kept at 83°F. These warm temperatures provide an ideal warm and moist environment for bacterial growth in the ad libitum-fed liquid formula. Once numbers of pathogenic bacteria in the formula and feeding apparatus increase, it is nearly inevitable that the piglets will become ill. For this reason, few operations use liquid formulas and those that do must flush the formula delivery system after each feeding to remove excess formula and pathogenic microbes that might be present.

Comparison of Pigs to Humans

Pigs are a monogastric species. Their entire digestive tract remarkably resembles that of humans. Pigs, like humans, have a gastric stomach, small intestine that can be separated into the ileum, jejunum, and duodenum, and a large intestine or colon. Additionally, pigs and humans are of similar size, which allows for the comparison of environmental impacts. These similarities make the pig an ideal model for human nutrition studies (9). Neonatal piglets are appropriate models for preterm human infants because of their size, nutritional requirements, and vulnerability to the environment. Neonatal piglets weigh approximately 900 g when they are born. Preterm human infants usually weigh between 500 and 2,500 g. Neonatal humans and piglets have decreased ability to absorb dietary fats as compared with that of adults. Neonates absorb only 45-80% of the dietary triacylglycerols (TAGs) absorbed by adults (10, 11). Both preterm human infants and neonatal piglets have difficulty regulating their body temperatures; so, it is sometimes necessary to keep preterm infants in incubators, and piglets are routinely kept in warm rooms.

Preterm Human Infant Nutrition

Preterm human infants have distinctive nutritional requirements. Because they are born before full growth and maturation can occur in utero, preterm infants must be supplied with the proper formulation of nutrients postnatally to support growth. The ideal growth rate for preterm infants is growth equal to that of a fetus in the womb of equal post-conceptual age (12). Reaching this goal is difficult, however, because preterm infants are often low-birth weight infants, even for gestational age, and tend

to lose weight after birth. In order to balance electrolytes and support growth, preterm infant formulas for enteral feeding must supply adequate amounts of water-soluble vitamins, minerals, and energy, have enough water available for kidney function, and supply 67-94 kcal/100 mL (12).

Though the required dietary lipid concentration is limited only by meeting the requirements of the essential fatty acids, linoleic and α -linoleic acids, it is common for infant formulas to be comprised of approximately 50 percent of energy from lipid. This mixture closely matches the composition of human breast milk in total lipid content. Commercial formulas, however, typically have higher concentrations of medium-chain triacylglycerols (MCTs) than does human breast milk. Composition of human milk is highly dependent on diet composition and does not contain high proportions of TAGs rich in medium-chain fatty acids (**Table 3, Table 4 (14)**) (13). In commercial formulas for infants, MCTs account for up to 50 percent of the total lipid. It is still unknown whether or not this difference accounts for any digestive or absorptive differences in preterm infants fed formula instead of human breast milk. When preterm infants were fed human milk, there was no correlation between duodenal bile acid concentration and lipid absorption; however, when preterm infants were fed a milk replacement formula, there was a significant positive correlation between total duodenal bile acid concentration and lipid absorption ($r=0.630$, $p<0.001$) (15). This finding supports the idea that low total bile acid concentration is a limiting factor for the digestion and absorption of MCTs but not of long-chain fatty acids in TAGs found in human breast milk. The preceding statement

is counterintuitive. Logic would lead one to believe that if the long-chain TAGs present in human milk are efficiently hydrolyzed and absorbed in preterm infants, the MCTs should be more easily hydrolyzed and absorbed because bile acids and lipase are not required for the absorption of all MCTs (16). Because MCTs are slightly soluble in aqueous solutions, some are absorbed directly into the mucosal cells where they are hydrolyzed intracellularly and the medium-chain fatty acids are transported to the liver via the portal vein (16).

Bile Acids

Functions. The primary function of bile acids is to emulsify dietary lipids in the small intestine to accommodate the absorption of dietary TAGs into the mucosa for chylomicron assembly (17). Bile acids also promote the absorption of fat-soluble dietary components such as fat-soluble vitamins and drugs. Bile acids interact with dietary lipids to form lipid micelles in the lumen of the small intestine. The lipids in these micelles are arranged so that their hydrophobic regions are in the center of the micelle and their more hydrophilic regions interact with the aqueous contents of the small intestine. While the TAGs are in micellar solution, they interact with pancreatic lipase, which hydrolyzes the fatty acids from the glycerol backbone. Typically, the fatty acids in the 1 and 3 positions are hydrolyzed more readily, resulting in free fatty acids and 2-monoacylglycerols for absorption into the mucosa. Once in the enterocytes of the small intestine, fatty acids are reassembled into TAGs. These TAGs, in conjunction with cholesterol, cholesteryl esters, phospholipids, and apolipoproteins, form chylomicrons that subsequently are transferred into lymph and

transported to the general circulation. The type of TAG and the size of the chylomicrons are dependent on the TAG content of the diet (18). The TAG composition of chylomicrons closely matches that of the diet, and the more lipids a diet contains, the larger and more numerous the chylomicrons will be in the lymph and plasma.

Cholesterol metabolism. Chylomicrons are lipoproteins that contain about 84% TAG, 8% cholesterol, 7% phospholipid, and 2% protein (19). While in the general circulation, most of the TAGs in the chylomicron are cleaved by action of lipoprotein lipase (LPL), leaving chylomicron remnants, which contain very little TAG, for delivery to the liver. Therefore, TAGs carried by the chylomicrons ultimately may be redistributed to very low-density lipoproteins (VLDLs) in the liver. Once the VLDLs are packaged, they are secreted from liver and circulate through the blood. As VLDLs circulate, LPL on the surface of capillary cells, primarily in skeletal muscle and adipose tissue, stimulates the release of TAGs from the lipoprotein as monoacylglycerols and fatty acids that are “shuttled” to the cells, effectively shrinking the size of the VLDL. As VLDL deliver their lipid to organs, the ratio of protein to lipid is increased and the smaller VLDL are called either intermediate-density lipoproteins (IDL) or low-density lipoprotein (LDL), depending on protein:TAG. Low-density lipoproteins continue to circulate through the body and deliver lipid and cholesterol to tissues as needed via LDL receptor-mediated endocytosis.

The liver also produces nascent high-density lipoproteins (HDLs). High-density lipoproteins contain the lowest lipid to protein ratio of all the lipoproteins. High-density lipoproteins circulate through the blood and “pick up” cholesterol from extrahepatic tissues by action of lecithin:cholesterol acyltransferase activity within the HDLs. High-density lipoproteins then return the lipids and cholesterol to the liver for recycling. At this point, cholesterol may be used for synthesis of bile acids or redistributed throughout the body in lipoproteins by the liver.

Bile acid synthesis. A high percentage, 50-80%, of cholesterol returning to the liver is used for bile acid synthesis (20). The extent of bile acid synthesis has a role in plasma cholesterol homeostasis. Production of insufficient amounts of bile acids will result in hypercholesterolemia because the cholesterol that returns to the liver will be redistributed throughout the body via the blood rather than being secreted as cholesterol or bile acids into the gallbladder for storage and eventual excretion to the small intestine. The enzyme responsible for regulating the rate of bile acid synthesis from cholesterol is cholesterol 7 α -hydroxylase (21). Cholesterol undergoes a series of hydroxylation, oxidation, and reduction reactions that finally result in the synthesis of the primary bile acids. The two principal primary bile acids in humans are cholic acid and chenodeoxycholic acid. In pigs, the major primary bile acids are chenodeoxycholic acid and hyocholic acid (**Figure 1**). Hyocholic acid can be found in trace amounts in humans, and cholic acid can be found in trace amounts in pigs. Primary bile acids are conjugated with either taurine or glycine in the peroxisomes of

the liver (22) and then stored in the gallbladder until their release is stimulated by the release of cholecystinin in response to digesta in the duodenum.

Secondary bile acids. Once in the small intestine, primary bile acids can be converted to secondary bile acids by microbes that inhabit the gut. Microbes are responsible for additional hydroxylation, and/or reduction steps that result in the production of up to five secondary bile acids (**Figure 2**). Secondary bile acids include deoxycholic acid, lithocholic acid, ursocholic acid, ursodeoxycholic acid, and hyodeoxycholic acid. Secondary bile acids, as well as unaltered primary bile acids, in the small intestine may be reabsorbed and transferred to the liver where they are resecreted via bile and stored in the gallbladder in a process called recycling. Those bile acids that are not recycled are excreted with the feces.

Bile acid status. Low interduodenal concentrations of bile acids are a factor in the decreased absorption of dietary fatty acids in preterm human infants and in neonatal piglets (**23, 24**). Improving bile acid concentration in the small intestine to the minimum micellar concentration has been shown to improve dietary fatty acid utilization in neonatal piglets (**25, 26, 27, 28**). By increasing bile acid concentration in the duodenum of preterm human infants or neonatal piglets, their absorption of dietary TAGs might be improved.

Choly sarcosine. Recently, investigators have introduced an artificially conjugated bile acid, choly sarcosine, which is produced by conjugating cholic acid with

sarcosine (29) (**Figure 3**). Orally administered cholylsarcosine generally is well tolerated and does not cause changes in biliary lipid composition or liver function (29). Cholylsarcosine increases the digestion and absorption of fatty acids from the diet in humans with compromised intestinal function (30, 31), but, unlike glycine and taurine conjugates of bile acids, is resistant to bacterial deconjugation and dehydroxylation in the intestine (29). These characteristics make cholylsarcosine an ideal choice as a feed additive for piglets to determine if it increases the absorption of dietary lipid.

Table 1.*Concentration of IgG, IgM, and IgA in sow's milk during lactation^a*

<i>Stage of Lactation</i>	<i>IgG</i>		<i>IgM</i>		<i>IgA</i>	
	<i>Mean^b</i>	<i>CV^c</i>	<i>Mean</i>	<i>CV</i>	<i>Mean</i>	<i>CV</i>
0 h	95.6	34	9.1	49	21.2	42
12 h	32.1	51	4.2	58	10.1	60
24 h	14.2	72	2.7	46	6.3	72
48 h	6.3	47	2.7	39	5.2	55
7 d	1.5	41	1.8	44	4.8	45
14 d	1.0	37	1.5	47	4.8	36
21 d	0.9	28	1.4	49	5.3	31

^a n = 25 sows.^b mg/ml^c Coefficient of variance.

(Klobasa et al., 1987)

Table 2.*Composition of sow milk throughout lactation^a*

Stage of Lactation	Total Solids		Fat		Lactose		Total Protein ^b		Whey Protein ^b		NPN ^c	
	Mean ^d	CV ^e	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
0 h	25.6	13	5.0	23	3.1	13	15.7	15	14.3	14	0.11	33
12 h	18.4	14	4.9	19	4.1	12	8.8	26	7.0	29	0.09	33
24 h	17.3	9	5.6	21	4.6	8	6.4	21	4.6	30	0.10	28
48 h	18.6	10	6.5	24	4.8	7	6.4	11	3.9	17	0.12	34
7 d	18.4	7	6.5	18	5.5	6	5.5	5	3.2	11	0.13	22
14 d	18.2	6	6.4	15	5.9	5	5.1	10	2.7	11	0.13	28
21 d	18.7	9	6.6	15	5.8	8	5.2	8	2.8	13	0.14	23

^a n = 25 sows.^b Percentage protein = percentage nitrogen multiplied by 6.37.^c NPN = non-protein nitrogen expressed as a percentage.^d Percent.^e CV = Coefficient of variation.

(Klobasa et al. 1987)

Table 3

Estimates of the concentrations of nutrients in mature human milk

<i>Nutrient</i>	<i>Concentration in Human Milk</i>
Lactose (g/L)	72.0 ± 2.5 ^a
Protein (g/L)	10.5 ± 2.0
Fat (g/L)	39.0 ± 4.0
Calcium (mg/L)	280 ± 26

^aMean ± standard deviation.

(National Academy of Sciences Website, 2000).

Table 4.
Fatty acid composition of total lipids in human colostrum and milk

Fatty Acids	Colostrum ^a	Milk ^b 3 months	Fatty Acids	Colostrum	Milk 3 months
14:0	4.55 ± 0.29 ^c	5.48 ± 0.41	18:2 n-6	11.24 ± 0.43	12.71 ± 0.60
16:0	26.79 ± 0.41	23.98 ± 0.57	18:3	0.06 ± 0.01	0.17 ± 0.05
18:0	7.65 ± 0.42	9.52 ± 0.80	20:2	0.90 ± 0.06	0.30 ± 0.04
20:0	0.29 ± 0.03	0.22 ± 0.01	20:3	0.66 ± 0.04	0.40 ± 0.02
22:0	0.15 ± 0.01	0.13 ± 0.02	20:4	0.95 ± 0.04	0.50 ± 0.02
24:0	0.21 ± 0.03	0.08 ± 0.01	22:4	0.47 ± 0.05	0.09 ± 0.01
			22:5	0.12 ± 0.01	0.05 ± 0.00
16:1	2.26 ± 0.18	2.32 ± 0.18			
18:1	37.89 ± 0.91	38.85 ± 1.38	18:3 n-3	0.66 ± 0.05	0.71 ± 1.16
18:1 n-7	2.64 ± 0.12	3.25 ± 0.50	20:5	0.06 ± 0.01	0.06 ± 0.01
20:1	1.01 ± 0.05	0.49 ± 0.03	22:5	0.29 ± 0.03	0.15 ± 0.02
22:1	0.21 ± 0.02	0.08 ± 0.01	22:6	0.58 ± 0.04	0.35 ± 0.06
24:1	0.36 ± 0.02	0.12 ± 0.01			

^a Collected at 1 d after parturition.

^b Collected at 3 months after parturition.

^c Mean ± standard error.

(Marangoni et al., 2002)

Figure 1. Primary bile acids in humans and pigs

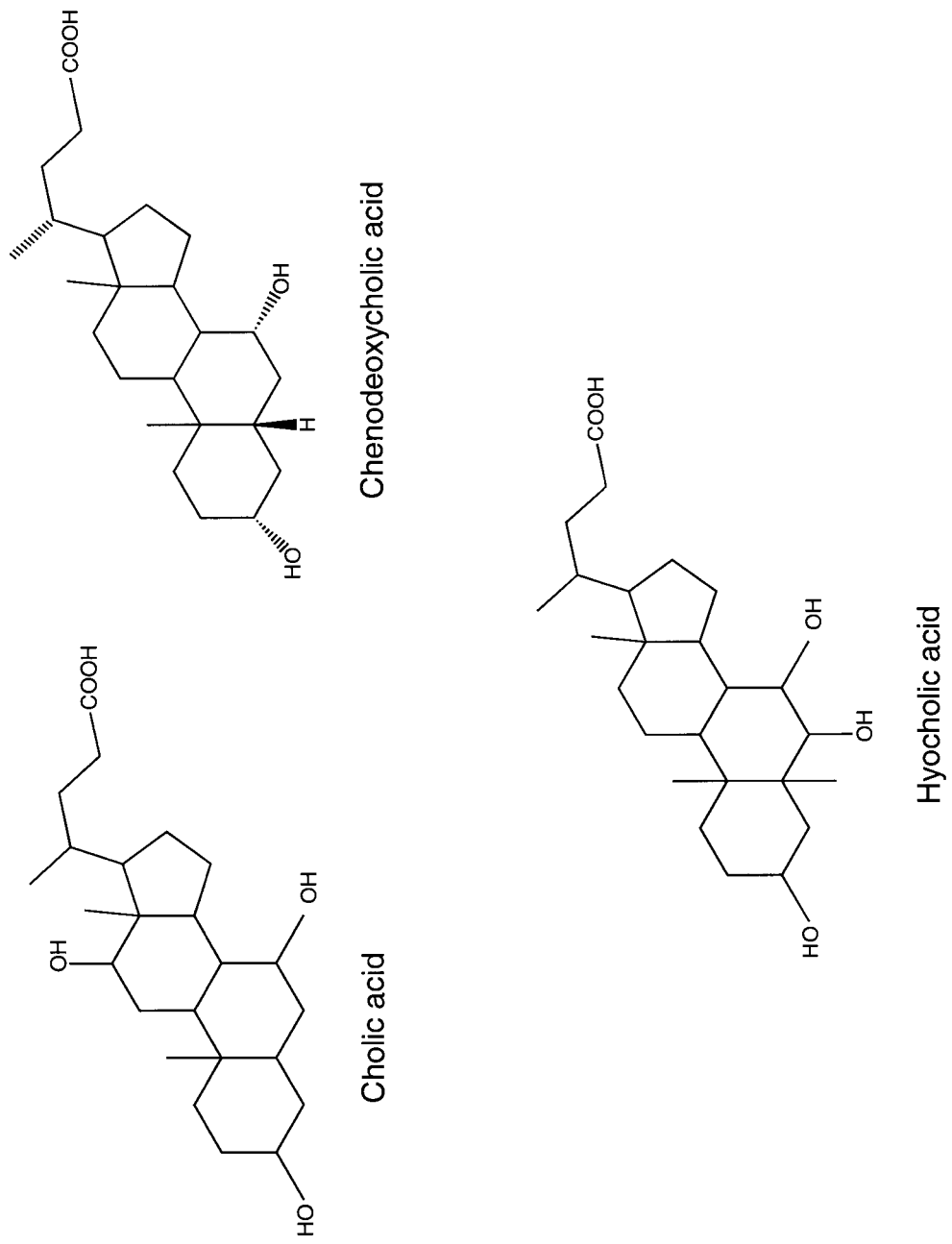


Figure 2. Secondary bile acids in humans and pigs

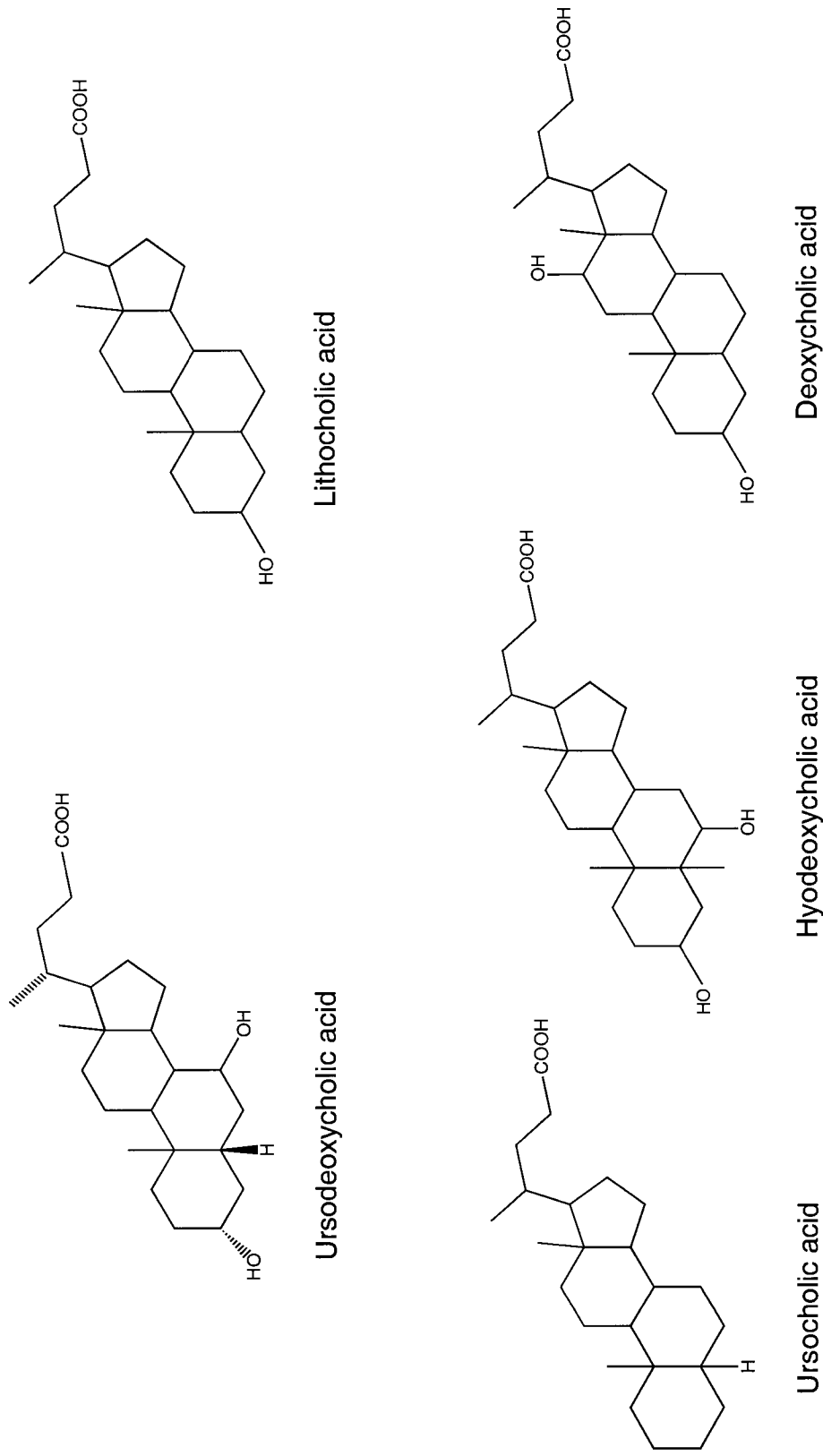
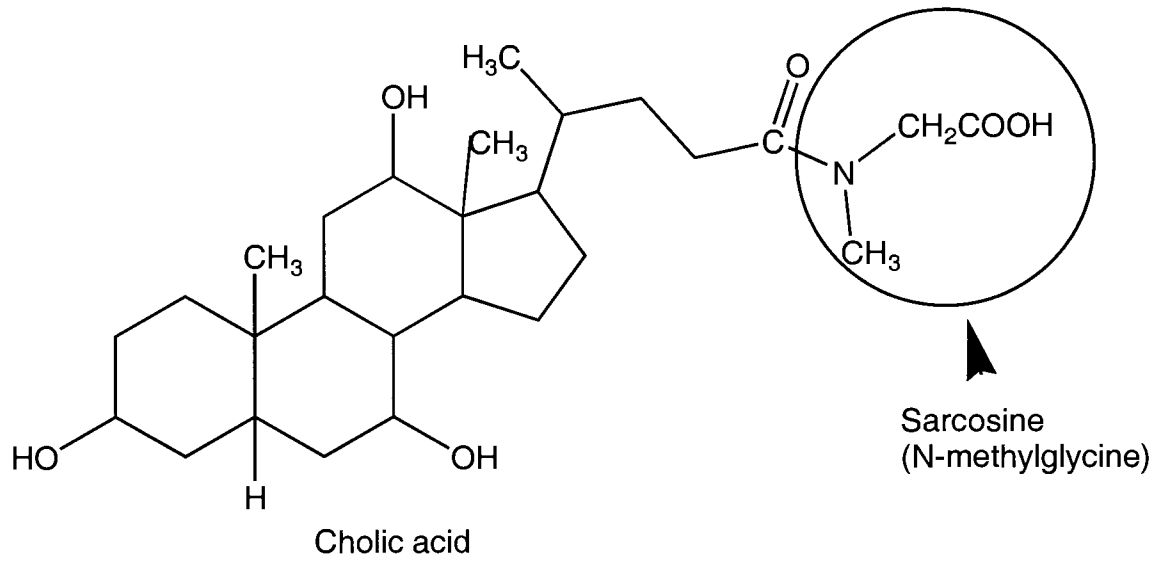


Figure 3. Cholylsarcosine structure

EFFECTS OF ORAL SUPPLEMENTATION WITH CHOLYLSARCOSINE ON THE DIGESTION AND ABSORPTION OF TRIACYLGLYCEROLS AND ON BILE ACID STATUS IN NEONATAL PIGLETS

A paper to be submitted to the Journal of Nutrition

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Abstract

Preterm infants and neonatal suckling piglets have a limited bile acid pool that may hinder absorption of dietary fatty acids, triacylglycerols (TAGs), and other lipid-soluble nutrients. Because dietary lipids are a valuable source of energy for growth, it is important for that TAGs be efficiently absorbed and utilized. 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 in metabolism cages and fed a commercial milk replacer with or without oral cholylsarcosine until 21 d of age. At 14 d of age, ¹³C hexadecanoic acid and tri-(D₃₁ hexadecanoic acid) were administered orally to quantify absorption of dietary fatty acids and TAG.

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 ($P > 0.05$). Oral supplementation with cholylsarcosine increased fecal excretion of deoxycholic acid ($P = 0.03$). Cholylsarcosine tended to stimulate greater incorporation of palmitic acid from dietary TAG into the free fatty acids in blood lipids ($P = 0.135$). Cholylsarcosine did not change incorporation of palmitic acid into plasma TAGs when fed as free palmitic acid or as palmitic acid in TAG. Apparent absorption of dietary TAGs was increased from 77% in piglets not fed cholylsarcosine to 83% in the piglets that did receive oral cholylsarcosine. Together, 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 (1). Several factors contribute to this poor fat absorption including the presence of inadequate amounts of lipolytic enzymes, the high percentage of long-chain fatty acids in milk, the high percentage of saturated fatty acids, the inadequate development of the digestive tract, and the small bile acid pool (1, 2). 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 (2).

Neonatal piglets have been used as a model for studies of pediatric growth, nutrition, and metabolism for many years (3). Neonatal piglets are similar to human preterm infants in their size, organ structure, and nutritional needs (4). 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 (5). 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, piglets do not efficiently absorb long-chain saturated fatty acids (3). Approximately 40% of fatty acids in human milk are saturated (6, 7). These saturated fatty acids, palmitic acid and stearic acid in

particular, are not effectively emulsified or hydrolyzed 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. Cholecystokinin stimulates bile acid release from the gallbladder into the small intestine in response to eating. Bile acids act as detergents to emulsify lipids. Bile acid concentration in preterm human infants and in neonatal piglets is very low compared to 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, hydrolyzation and absorption of fatty acids from dietary TAG cannot occur.

The hypothesis is that oral cholylsarcosine, a synthetically conjugated bile acid, will increase the efficiency of dietary fatty acid 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 and incorporation of dietary fatty acids and TAG into plasma lipids by suckling piglets with or without cholylsarcosine supplementation in their diet was evaluated. Additionally, the percentage enrichment by stable isotope labeled dietary TAG and free fatty acid into

the plasma lipids of suckling and weaned piglets with or without cholylsarcosine in their diet was measured.

Materials and Methods

Experimental Design

Twelve standard pig metabolism cages were modified to house young piglets. Cages were equipped with a removable feeding bottle with a sipper tube attached to allow liquid milk replacement formula to be fed and its intake to be measured. Stainless steel screens were placed under the flooring of each cage to allow fecal collections. The ambient temperature in the room was maintained at 83°F. The room was maintained with a cycle of 12 h (7:00 AM -7:00 PM) of light and 12 h (7:00 PM -7:00 AM) of dark.

Animal use for this study was approved by the Committee of Animal Care at Iowa State University. All regulations were followed. Twenty-four 5-d-old crossbred (Duroc x Hampshire x Yorkshire) female piglets were obtained from the Iowa State University Swine Research Center. Piglets were obtained in 2 groups of 12. 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 choly sarcosine (0.2 g/kg body weight choly sarcosine 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. Formula was mixed with water to be 179 g of dry powder per liter of formula according to package directions. The formula contained two antibiotics, oxytetracycline and neomycin. The inclusion of these antibiotics might possibly have an effect on the fatty acid composition of feces, either increase or decrease depending on the change in microflora of the small intestine, however that effect is probably not significant in the scope of this project.

Treatments began when the piglets were 7 d old. Piglets were weighed each day at the evening feeding. That weight was used to calculate the cholylsarcosine dose for the next day for animals in the cholylsarcosine treatment group. Fecal samples from morning and evening collections were combined for each piglet and stored at -20°C until analysis. When the piglets were 14 d old, 25 mg/kg body weight of each a free fatty acid and a TAG were administered orally by gelatin capsule. The free fatty acid was 1, 2, 3, 4- ^{13}C hexadecanoic acid, and the TAG was glycerol tri-(D_{31} hexadecanoic acid). Blood samples were drawn just before administration of the labeled lipids and again 4, 8, 12, and 24 h later. An additional blood sample was drawn 1 wk later. Blood was collected by using Monoject tubes (Tyco Healthcare Group, Mansfield, MA) with 15% EDTA as the anticoagulant. Blood samples were stored immediately in ice before centrifugation at 4°C for 20 min at $15,000 \times g$ to separate plasma. Plasma was stored in Cryovials (Fisher Scientific, Pittsburgh, PA) at -80°C until analysis. When 21 d of age, piglets were weighed, placed in one large

pen, and weaned with ad libitum access to pelleted feed. When piglets were 54 d old, they were weighed and put back into metabolism cages. Then, the stable isotope experiment was repeated by using the procedures already outlined. Fecal samples were collected twice per day as previously outlined. Pigs were killed by using excess ketamine (Sigma-Aldrich, St. Louis, MO) injection when they were 58 d old.

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 of feces and plasma were determined. Composition of fatty acids and bile acids in feces were determined by gas chromatography according to the method of Batta et al. (8) with a few modifications. All standards and samples were treated identically. Fatty acid external standards of palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and arachidonic acids were used for peak identification. Bile acid external standards included ursodeoxycholic,ursocholic, chenodeoxycholic, hyocholic, lithocholic, and deoxycholic acids. Cholic acid and heptadecanoic acid were the internal standards. Equal molar concentrations (2.447 μM) of all standards, both internal and external, were used. By following the procedure of Batta et al. (8), esterification creates butyl esters of fatty acids and bile acids and trimethylsylether derivatives of bile acids. Samples

and standards were stored under nitrogen at -20°C until analysis with a gas chromatograph (GC).

Quantification by gas chromatography was performed on a Hewlett Packard 6890 (Hewlett Packard, now Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector and autosampler by using a Varian Chrompak CP-Sil 5 CB Low Bleed/MS fused silica capillary column 25 m x 0.25 mm ID x 0.25 μm film thickness (Varian Instruments, Walnut Creek, CA). The carrier gas was helium at a flow rate of 1.5 mL/min. The injector temperature was 260°C , and the detector temperature was 290°C . The oven temperature gradient was as follows: Initial temperature was 100°C , which was held for 2 min; then, temperature was increased at a rate of $35^{\circ}\text{C}/\text{min}$ to a final temperature of 278°C , which was held for 22 min. One microliter of each sample was injected onto the column and split 1:10 so the column would not be overloaded.

Lipid extracts of fecal and plasma samples were analyzed for fatty acid composition of the TAG and of the free fatty acid fractions as outlined by Li et al. (9). Following this procedure, total lipid in plasma or feces are separated into TAG, free fatty acid, cholesteryl ester, and phospholipid fractions by solid phase extraction, and esterification results in pentafluorobenzyl derivatives of fatty acids in each lipid class. Samples obtained were analyzed by gas chromatography:mass spectrometry to

determine the percentage enrichment by the stable isotope labeled hexadecanoic acid and glycerol tri-hexadecanoic acid in plasma and fecal lipids.

The mass spectrometer used for analysis of these samples was either a Fisons single quadrupole instrument equipped with a Fisons 8035 GC (Thermoquest, San Jose, CA) or a Micromass GCT (Waters Corporation, Milford, MA) time of flight spectrometer equipped with a Hewlett Packard 6890 GC. The inlet program differed for the two instruments because different columns were used in the GCs. For the quadrupole spectrometer, the program was as follows: Initial temperature was 150° C, which immediately was increased at a rate of 5° C per minute to 275° C and then held for 5 minutes. One microliter of sample was injected onto the column with a split ratio of 1:25. The column was a Supelco Omegawax fused silica capillary column (Sigma-Aldrich Corp., St. Louis, MO). It was 30 m x 0.32 mm ID x 0.25 µm film thickness. The carrier gas was helium, and the flow rate was constant at 1.0 mL per minute. The total run time for each sample was 30 minutes.

On the time of flight spectrometer, the column was a J&W DB-5MS (Scientific Instrument Services, Inc., Ringoes, NJ), which had these dimensions: 30 m x 0.25 mm ID x 0.25 µm film thickness. The temperature program for this machine was as follows: Initial oven temperature of 120° C was held for one minute. Oven temperature was increased at a rate of 10° C per minute to a final temperature of 280° C, which was held for 23 minutes. The carrier gas was helium, and the flow rate was held constant. Fifty microliters of each sample was injected onto the

column at a split ratio of 1:50. Both mass spectrometers were run in the negative chemical ionization mode and by using methane as the ionization gas. The time of flight spectrometer was used because of technical problems with the quadrupole instrument. The percentage enrichment values obtained from both machines were analyzed together as one data set.

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 in the first group and most of the piglets in the second group became ill, n equaled 10 for most analyses. When only animals in the cholylsarcosine group were analyzed, n equaled 5. 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 DE/d. This value closely matches the NRC prediction of 1010 kcal DE/d (10). 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 (11). 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 significant difference in feed intake.

Excretion of Dietary Triacylglycerols

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 did not change between the 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 (12). The unsaturated 18 carbon fatty acids were compiled as a group because they co-eluted from the column when a program that eluted both fatty acids and bile acids

was used. Excretion of all 16 carbon fatty acids and 18:0 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.

Excretion of Bile Acids

To determine the effect of dietary cholylsarcosine on bile acid status, the fecal excretion of individual primary and secondary bile acids was determined in piglets with and without dietary cholylsarcosine. Total bile acid concentration in the feces of piglets fed cholylsarcosine was approximately twice that in feces from piglets fed formula only (**Table 3**). Among the individual bile acids in feces, the concentration of deoxycholic acid was increased by dietary cholylsarcosine ($P < 0.05$).

Concentrations of the other bile acids in feces were not changed by feeding cholylsarcosine ($P > 0.05$)

Excretion of all bile acids except deoxycholic acid decreased with increasing age of the piglets (**Table 3**). Only excretion of hyodeoxycholic acid decreased significantly as age of the piglets increased ($P < 0.01$). A trend to decrease excretion of chenodeoxycholic acid in feces as age of piglets increased was also present ($P = 0.06$).

Excretion of chenodeoxycholic acid was numerically higher in piglets fed only formula when compared with the excretion of piglets that received cholylsarcosine with formula (0.05 vs. 0.014 $\mu\text{moles/g}$ dry feces respectively).

Absorption of Dietary Lipids

To measure the incorporation of the stable isotope-labeled lipids into the fatty acid and TAGs of plasma and fecal lipids, percent enrichment by dietary 4- ^{13}C hexadecanoic acid and glycerol tri-(D_{31} hexadecanoic acid) in plasma and feces was calculated (Appendix A). No difference in the enrichment of either TAG or free fatty acids of piglet plasma by dietary lipids was evident between the control or cholylsarcosine-treated groups (**Table 4**). Evidently, cholylsarcosine did not influence the absorption of hexadecanoic acid when administered as either the free acid or the TAG form to an extent great enough to be seen within the limits of this experiment. The percentage of enrichment of TAGs in the plasma by the dietary 4- ^{13}C hexadecanoate is approximately 6 times greater (0.24 vs. 1.46%) in the weaned pigs compared with the enrichment in the TAG fraction of suckling piglets regardless of cholylsarcosine supplementation ($P \leq 0.001$) (**Table 4**), suggesting that weaned pigs are much more efficient at absorbing TAGs than are suckling piglets. Fecal samples from the weaned pigs were not analyzed for stable isotope content; therefore, no data on TAG absorption for pigs weaned at 21 d are available.

There tends ($P = 0.13$) to be decreased excretion of dietary TAGs as free fatty acids in piglets treated with cholylsarcosine, which indicates that cholylsarcosine

stimulates absorption of tri-hexadecanoic acid in suckling piglets (**Table 5**). No differences of excretion of either labeled dietary TAG or labeled free fatty acid in the TAG fraction of the feces between cholylsarcosine-treated and control animals were observed, indicating that cholylsarcosine does not influence TAG excretion.

An analysis was performed to determine the time at which the greatest enrichment of free fatty acids plus TAG occurs in plasma because of oral administration of labeled fatty acid or labeled TAG (**Figure 1**). Enrichment of plasma free fatty acids and TAG four hours after administration of labeled free acid was higher ($P < 0.05$) than enrichment at any other time. That enrichment returns to baseline by 8 h after administration of the labeled free acid and does not change for the remainder of the experiment. Enrichment of plasma free fatty acids and TAG by orally administered labeled tri-hexadecanoic acid shows a slight, nonsignificant increase at 4 h. Enrichment of plasma lipids by dietary TAG decreases at 8 h but then increases again at 8 and 12 h ($P < 0.05$). One week later, enrichment is not different from that at time 0 h.

Discussion

Excretion of Dietary Triacylglycerols

Cholylsarcosine does increase 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 maximum 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 differences in growth over the 14 d period were not significant. Fecal excretion of saturated fatty acids was decreased significantly 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 (13, 14, 15, 16, 17). 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 (18, 19). 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 been apparent resulting in a change in growth data.

Excretion of Bile Acids

The total bile acid concentration in feces of piglets supplemented with dietary cholylsarcosine is almost twice that in feces of piglets fed formula alone. Because cholylsarcosine is not deconjugated or dehydroxylated in the lumen of the small intestine (20), it must have been recycled via enterohepatic circulation like it is in humans (20) as it was not detected in the feces of piglets treated with cholylsarcosine during the current experiment.

Cholylsarcosine increased the concentration of deoxycholic acid in the feces of suckling piglets. A likely cause of this effect is that when cholylsarcosine is recycled, sarcosine is replaced by either taurine or glycine. Once reconjugated, the molecule that exists is tauro- or glycocholic acid. Either of these bile acids can be modified in the small intestine by microbes to produce deoxycholic acid and other secondary bile acids (21). An increase in deoxycholic acid because of cholylsarcosine supplementation is reasonable, noting that cholic acid is its primary bile acid precursor and supplemental cholylsarcosine, increased cholic acid in the intestines.

Excretion of chenodeoxycholic acid is numerically lower in piglets treated with cholylsarcosine than in control piglets (**Table 3**). In humans, primary bile acids, as well as cholylsarcosine, are inhibitors of cholesterol 7 α -hydroxylase, which is the enzyme responsible for committing cholesterol to bile acid synthesis (22). The same inhibitory action is probably present in neonatal piglets because chenodeoxycholic acid, a primary bile acid, is decreased by cholylsarcosine supplementation.

The average daily change in bile acid excretion with increasing age of piglets was and small for all bile acids and negative for all bile acids except deoxycholic acid (**Table 3**) indicating that the bile acid recycling pathway becomes more efficient with increasing age. If piglets produce the same amount of bile acids each day but are more efficient at recycling them, a decrease in fecal output of bile acids would be expected.

Absorption of Dietary Lipids

Enrichment of plasma hexadecanoic acid and tri-hexadecanoic acid by labeled free acid as well as TAG was increased in weaned pigs when compared with the enrichment in suckling piglets regardless of treatment (**Table 4**), indicating that older weaned pigs more efficiently hydrolyze and absorb dietary lipids than do suckling piglets. This finding agrees with previous findings (15, 23, 24, 25) that suckling piglets have lesser ability to utilize dietary lipids than do weaned pigs.

Enrichment of fecal hexadecanoic acid and tri-hexadecanoic acid by labeled free acid as well as TAG was not different between piglets treated with cholylsarcosine and those that received formula alone (**Table 5**). However, cholylsarcosine tended to decrease the enrichment by labeled TAG in the free fatty acid fraction of the feces of suckling piglets ($P = 0.135$). This increase in free fatty acids from dietary TAGs in the feces of control animals suggests that cholylsarcosine might increase the rate of emulsification, hydrolysis, and subsequent absorption and thus allow utilization of

dietary lipids before the digesta moves to the colon and is excreted. These findings support those of Verkade et al. (26) in which it was found that most of the dietary lipids detected in the feces of preterm infants were in the free fatty acids.

The enrichment pattern of plasma of suckling piglets after oral administration of labeled free hexadecanoic acid and tri-hexadecanoic acid is shown in Figure 1 shows the dynamics of lipid homeostasis. At time 4 h, enrichment by orally administered free hexadecanoic acid was increased in both the free fatty acids and TAGs of plasma, indicating that neonatal piglets are capable of absorbing free fatty acids and esterifying them into plasma TAGs. Orally administered TAG does not appear in either plasma free fatty acids or TAGs in quantities as high as those of oral free acid at 4 h, indicating that piglets do not absorb dietary TAGs as quickly as they absorb dietary free acids. By 8 h, all enrichment values of both plasma lipid fractions by both dietary lipids have returned to baseline. However, at 12 h, the enrichment of plasma free fatty acids by dietary labeled TAG begins to increase. By 24 hours this increase in enrichment reaches significance ($P < 0.05$). This increase in enrichment by dietary TAG 24 h after administration is probably meal related. When animals are fed, they generally store energy in adipose tissue for use by the body when food is not available. When an animal fasts between meals, fatty acids and glycerol are hydrolyzed from stored TAGs for use as energy (27). Piglets are diurnal in that they generally eat and are active during the light hours. Because the lights were turned off at 12 hours, piglets probably did not eat. During this fast, piglets mobilized TAG stores from adipose tissue that had been stored earlier in the

day, TAG stores that were labeled with stable isotopes. The result is an increase in the enrichment of plasma lipids by stable isotope-labeled TAGs that were mobilized from adipose stores. One week later, the labeled TAG and free fatty acids in the plasma have been oxidized and cleared from the body as carbon dioxide and the enrichment was decreased and is back to baseline. The same effect is not seen in the orally administered free fatty acids probably because they were oxidized and cleared from the body much earlier.

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 bile acid improves saturated fatty acid absorption. No change in growth, however, was observed. A longer-term study or more piglets seems necessary to determine if cholylsarcosine increases the growth rate of neonatal piglets. If a longer-term study using piglets as a model for preterm infants does show an increase in growth because of supplementation with cholylsarcosine, it may be feasible to supplement preterm human infants with cholylsarcosine to improve absorption of dietary TAGs and lipid-soluble nutrients. At least, the findings might 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 and growth of suckling piglets from 7 to 21 days of age

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

^a Not significant.

^b Growth over 14 d.

^c 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	P-Value	
				Treatment	Age
16:1	0.937 ± 0.17 ^a	1.004 ± 0.19	-2.826 ^b	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

^a Mean ± standard error expressed as μmoles fatty acid/g dry feces.

^b 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

Table 3.
Effect of cholylsarcosine and increasing age on the fecal excretion of bile acids by suckling piglets

Bile Acid	P-Value		
	Cholylsarcosine	Control	Average Daily Change
Ursocholic Acid	$7.8 \times 10^{-3} \pm 2.9 \times 10^{-3}$ ^b	$5.0 \times 10^{-3} \pm 3.3 \times 10^{-3}$	-1.5×10^{-3} ^a
Lithocholic Acid	0.135 ± 0.02	0.136 ± 0.02	-7.9×10^{-3}
Deoxycholic Acid	0.170 ± 0.03	0.026 ± 0.03	2.2×10^{-2}
Ursodeoxycholic Acid	$5.83 \times 10^{-3} \pm 2.7 \times 10^{-3}$	$5.25 \times 10^{-5} \pm 3.14 \times 10^{-3}$	-1.4×10^{-4}
Hyochoolic Acid ^a	0.046 ± 0.01	0.017 ± 0.01	-8.2×10^{-3}
Hyochoxycholic Acid	0.060 ± 0.1	0.049 ± 0.14	-5.5×10^{-4}
Chenodeoxycholic Acid ^a	0.014 ± 0.03	0.050 ± 0.04	-8.1×10^{-3}
Total Bile Acids	0.439 ± 0.06	0.289 ± 0.07	-4.1×10^{-3}
		Treatment	Age
		0.56	0.90
		0.74	0.16
		0.03	0.67
		0.89	0.49
		0.17	0.40
		0.80	0.002
		0.34	0.06
		0.10	0.08

^a Primary bile acid.

^b Mean \pm standard error expressed as μ moles bile acid / g dry feces.

^c 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 μ moles/ g dry feces \bullet d. P-value indicates differences in bile acid excretion: Treatment: Between cholylsarcosine and control groups during the suckling period. Age: As a function of increasing age of piglets.
 n = 10

Table 4.

Effect of cholylsarcosine on the enrichment of triacylglycerols and free fatty acids in plasma of suckling and weaned piglets by orally administered $^{13}\text{C}_4$ hexadecanoic acid and glycerol tri-(D₃₁ hexadecanoic acid)

Dietary Lipid	Plasma Triacylglycerols ^a				Plasma Free Fatty Acids ^b			
	Suckling		Weaned ^d		Suckling		Weaned	
	Cholylsarcosine	Control	Cholylsarcosine	Control	Cholylsarcosine	Control	Cholylsarcosine	Control
1,2,3,4- ^{13}C Hexadecanoate	0.24 ± 0.23 ^c	0.23 ± 0.20	1.46 ± 0.22*	0.36 ± 0.27	0.30 ± 0.28	1.70 ± 0.27*	0.38 ± 0.13	0.45 ± 0.13
Glycerol tri-(D ₃₁ Hexadecanoate)	0.40 ± 0.07	0.35 ± 0.07	0.25 ± 0.07	0.59 ± 0.13	0.45 ± 0.13	0.38 ± 0.13	0.38 ± 0.13	0.45 ± 0.13

^a Plasma TAGs derived from dietary lipids.

^b Plasma free fatty acids derived from dietary lipids.

^c Mean of all time points ± standard error expressed as percentage enrichment.

^d Cholylsarcosine and control pigs at 55 d old. Cholylsarcosine treatment was stopped at 21 d when piglets were weaned. Difference is from each suckling group, cholylsarcosine-treated or control, individually.

* $P < 0.001$

n = 10

Table 5.

Effect of cholylsarcosine on the enrichment of triacylglycerols and free fatty acids in feces of suckling piglets by orally administered ¹³C₄ hexadecanoic acid and glycerol tri-(D₃₁ hexadecanoic acid)

Dietary Lipid	Fecal Triacylglycerols ^a			Fecal Free Fatty Acids ^b		
	Cholylsarcosine	Control	P-value	Cholylsarcosine	Control	P-value
1,2,3,4- ¹³ C Hexadecanoate	2.25 ± 1.66 ^c	3.86 ± 1.85	0.521	1.14 ± 0.55	0.89 ± 0.63	0.768
Glycerol tri-(D ₃₁ Hexadecanoate)	2.27 ± 1.72	4.07 ± 1.93	0.491	3.26 ± 3.83	12.18 ± 4.83	0.135

^a Fecal TAGs derived from dietary lipids.

^b Fecal free fatty acids derived from dietary lipids.

^c Mean of all time points ± standard error expressed as percentage enrichment.
n = 10

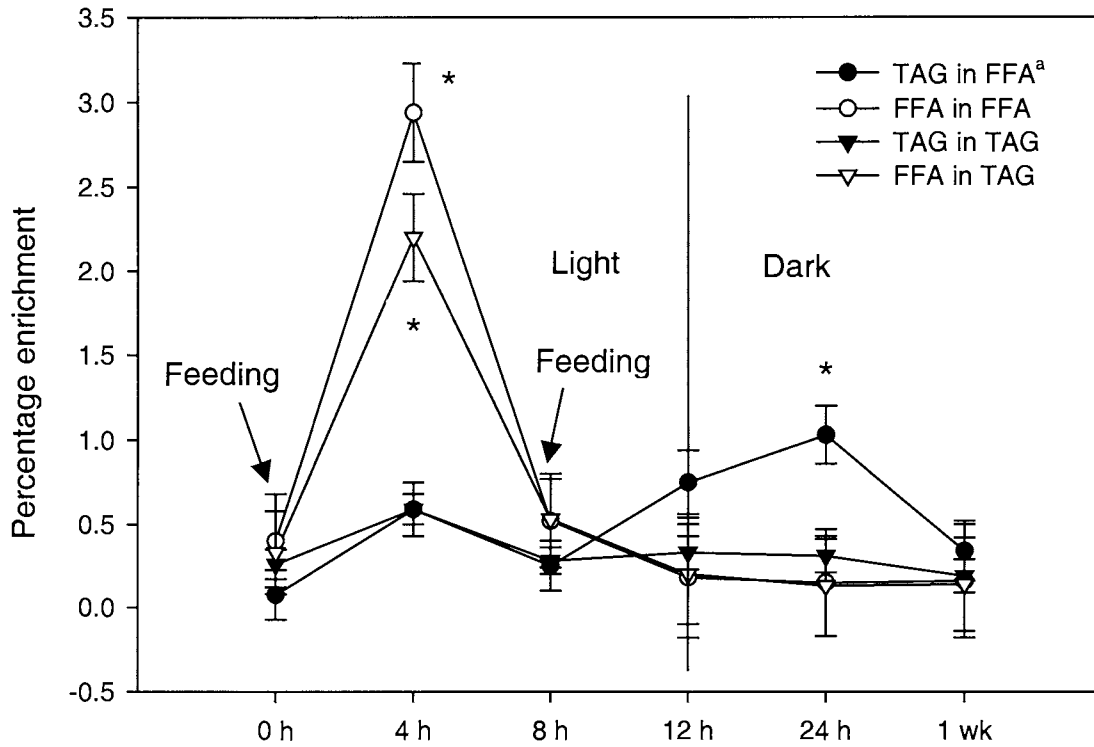


Figure 1. Transfer of dietary hexadecanoic acid or glycerol tri-hexadecanoic acid to free fatty acids and triacylglycerols in plasma in suckling piglets.

* indicates difference ($P < 0.05$) within dietary lipid and plasma lipid.

^a Labels are dietary lipid in plasma lipid fraction.

n = 10

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GENERAL SUMMARY

Preterm human infants and neonatal piglets have decreased ability to utilize dietary lipids compared with that of adults of the same specie. Malabsorption of dietary lipids leads to inefficient use of calories and nutrients available in the formula.

Increasing the absorption of dietary lipids would increase the energy available for growth of the neonatal piglet or preterm human infant.

Cholylsarcosine was tested as an agent that might increase the absorption of dietary lipids, mainly TAGs, by improving the emulsification process in the small intestine.

Cholylsarcosine was supplemented orally to 7 to 21-d-old piglets at a rate of 0.2 g/d•kg of body wt. Piglets were used as a model for preterm infants. Intake and fecal output were measured. Fatty acid and bile acid concentrations in the feces were determined by analysis by gas chromatography. A stable isotope-labeled TAG and free fatty acid were administered when piglets were 14- and 55-d-old to compare incorporation of dietary lipids into plasma lipids in piglets treated with cholylsarcosine and those that received formula alone and in weaned piglets. Incorporation of stable isotopes was measured by using GC:MS.

Results show a decrease in saturated fatty acid and some change in bile acid excretion in response to treatment with cholylsarcosine. Excretion of all fatty acids and most bile acids decreased with increasing age of the piglets, indicating that, as piglets become older, the mechanism for fatty acid absorption and for bile acid

recycling become more efficient. Enrichment of plasma lipids by stable isotope labeled dietary lipids was greater in weaned pigs than in either group of piglets, indicating that weaned piglets more efficiently utilize dietary lipids than do suckling piglets.

GENERAL 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 bile acid improves saturated fatty acid absorption. No change in growth, however, was observed. A longer-term study or more piglets seems necessary to determine if cholylsarcosine increases the growth rate of neonatal piglets. If a longer-term study using piglets as a model for preterm infants does show an increase in growth because of supplementation with cholylsarcosine, it may be feasible to supplement preterm human infants with cholylsarcosine to improve absorption of dietary TAGs and lipid-soluble nutrients. At least, the findings might support a clinical trial with humans to test the effects of cholylsarcosine on fatty acid excretion in preterm infants.

FUTURE RESEARCH

One interesting thought is the possibility that researchers are overlooking the optimal formula for feeding preterm human infants. Human breast milk is accepted widely as the optimal food for term infants (14), but is it the best food for preterm human infants? The composition of some other mammalian milks might give insight to the optimal food in this particular case. Rabbits, opossums, and kangaroos, for instance, are born as virtual fetuses, which is similar to a preterm human infant. The milk of these animal species tends to be richer in short- and medium-chain TAGs as opposed to the long-chain TAGs that make up the majority of lipid in human and other more precocious mammalian milks (**Table 1** (32)). Up to 50% of the calories in infant formulas currently on the market come from medium-chain fatty acids, but commercial formulas have no short-chain TAGs (33). Octanoate (8:0), the shortest-chain fatty acid that is present in commercial formulas is very well absorbed (100%) by infants; however, it accounts for only 0.5 to 12 % of the total fatty acids (33). Moya et al. (33) also found that, when 16:0 was present in the sn-2 position of the dietary TAG, it was much more efficiently utilized than the 16:0 in the sn-1 or sn-3 positions. Mathews et al. (34) tested the effect of source of long-chain fatty acid on the plasma docosahexanoic acid concentrations in neonatal piglets and found that algal or fungal sources of TAG were absorbed more efficiently than were the same long-chain fatty acids from egg phospholipid. According to the findings of Mathews et al. (34), use of egg phospholipids as the source of long-chain fatty acids in preterm human infant formulas should be minimized because they are not as

absorbable as algal or fungal sources. By using algal or fungal sources of TAGs, digestion and absorption of nutrients would be improved.

Maybe the ideal preterm infant formula would be one that combines the best of human breast milk with the advantages of the short- and medium-chain TAGs present in some other mammalian milk. Medium-chain TAGs currently are used in commercial formulas; however, short-chain TAGs are never included. By including more medium- and short-chain fatty acids and components of human breast milk such as linoleic acid, protein, and lactose, the resulting formula might be a more perfect food for preterm human infants. In the future, it would be interesting to formulate a preterm human infant formula that would take into consideration the absorption of different lipids and contain short- and medium-chain fatty acids as well as the essential long-chain fatty acids from the most absorbable sources. That formula then should be fed to very young neonatal piglets as a model for preterm human infants to determine their ability to utilize the TAGs present in the new formula. One would hypothesize that the short- and medium-chain TAGs would be almost completely absorbed and utilized, which would allow the neonate to take full advantage of the calorie-containing lipids supplied in the formula.

Table 1.
Fatty acid composition of milk fats of various animal species

Animal	<i>Fatty acids (mol %)</i>													
	4:0	6:0	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	>18:3	
Seal	-	-	-	-	-	5	23	21	2	35	1	1	13	
Guinea pig	-	-	-	T	T	2	26	2	4	30	22	3	-	
Human	-	-	-	2	4	6	21	6	5	42	12	1	-	
Mouse	-	-	T	6	9	13	33	5	2	29	9	-	-	
Monkey	-	7	9	8	2	2	20	1	5	27	18	-	-	
Rat	-	4	4	14	12	15	30	2	2	13	18	-	-	
Elephant	-	-	13	67	15	1	2	T	-	2	T	T	-	
Rabbit	-	1	45	23	1	1	9	1	3	8	9	1	-	
Goat	9	6	5	11	4	10	21	2	12	21	2	T	-	
Cow	12	4	2	4	4	10	24	2	12	24	2	1	-	
Sheep	13	6	4	12	6	11	21	3	9	15	2	1	-	

T = trace

- = not detected
(Dils, 1986)

APPENDIX A

Calculations

Molecular ion of hexadecanoic acid: 255

Molecular ion of hexadecanoic acid enriched with two ^{13}C atoms: 257

Natural abundance of molecular ion 257: 1.98%

Molecular ion of $^{13}\text{C}_4$ hexadecanoic acid: 259

Molecular ion of D_{31} hexadecanoic acid: 286

In samples, hexadecanoic acid (255) overloaded the column when enough sample was injected to elute stable isotope peaks, so, the hexadecanoic (257) peak was measured. The area of the 255 peak was obtained by calculation from natural abundance.

$$\frac{1.98}{100} = \frac{\text{Area of 257}}{\text{Area of 255}}$$

To calculate percentage enrichment of 259:

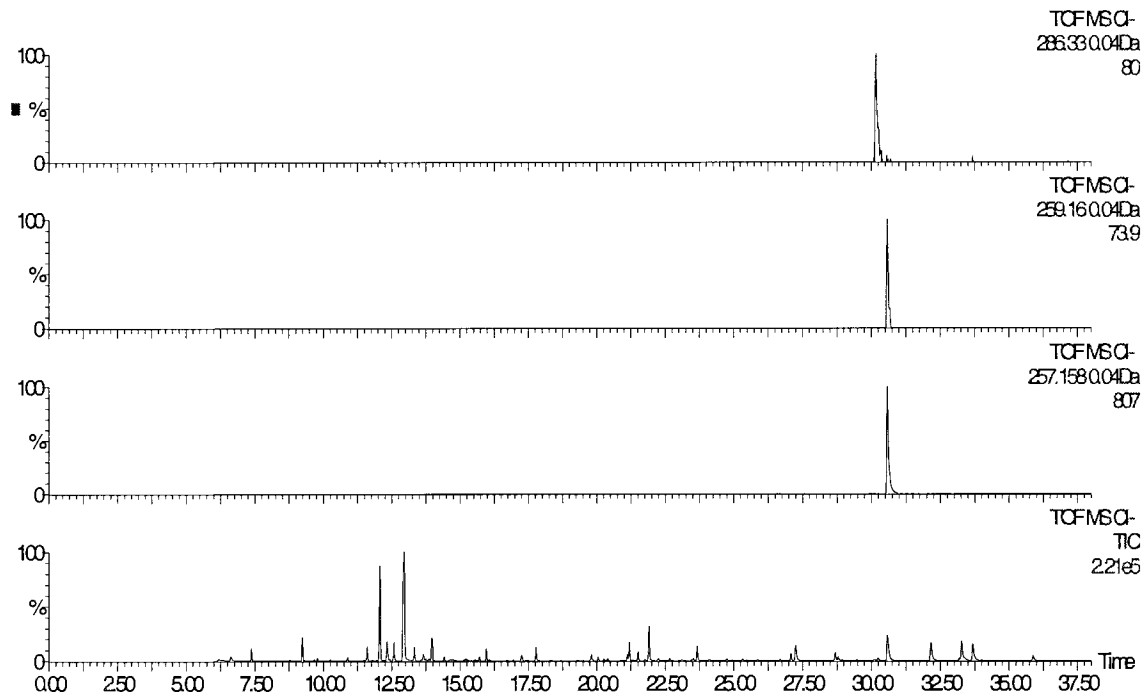
$$\left[\frac{\text{Area 259}}{\text{Area 255} + \text{Area 259}} \right] \times 100$$

To calculate percentage enrichment of 286:

$$\left[\frac{\text{Area 286}}{\text{Area 255} + \text{Area 286}} \right] \times 100$$

APPENDIX B

Typical GC trace from the time of flight mass spectrometer



TIC = Total Ion Current.

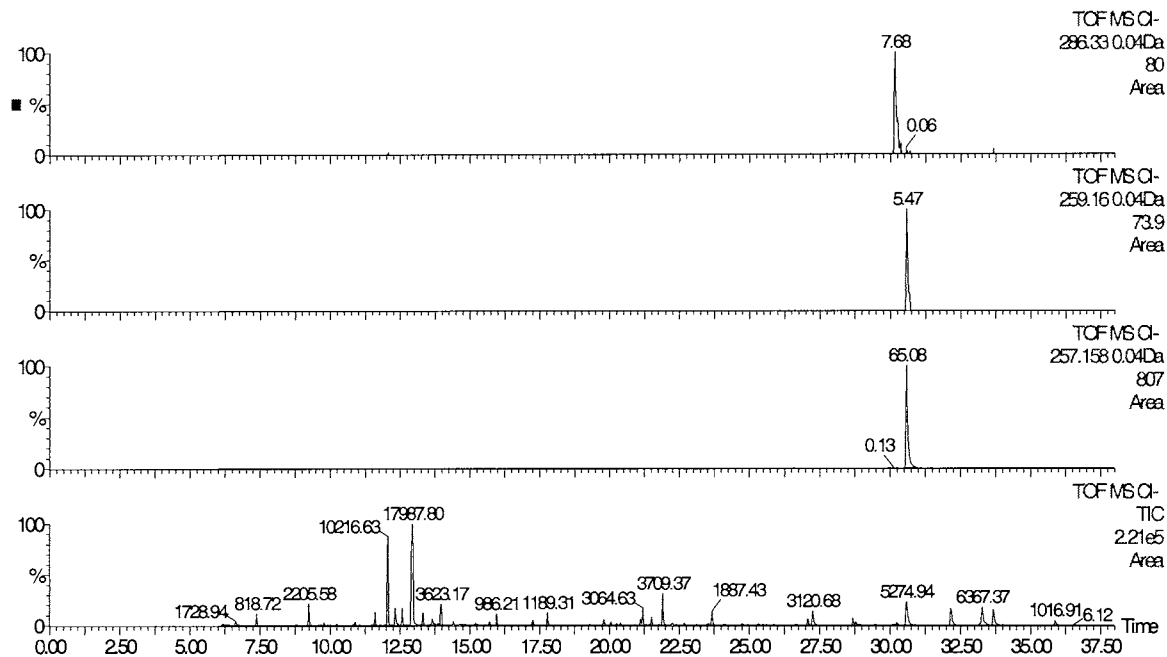
257.158 = C 16:0 naturally enriched with 2 ^{13}C atoms.

259.160 = C 16:0 enriched with 4 ^{13}C atoms.

286.330 = C 16:0 enriched with 31 D atoms.

APPENDIX C

Typical GC trace from the time of flight mass spectrometer showing area of peaks



TIC = Total Ion Current.

257.158 = C 16:0 naturally enriched with 2 ^{13}C atoms.

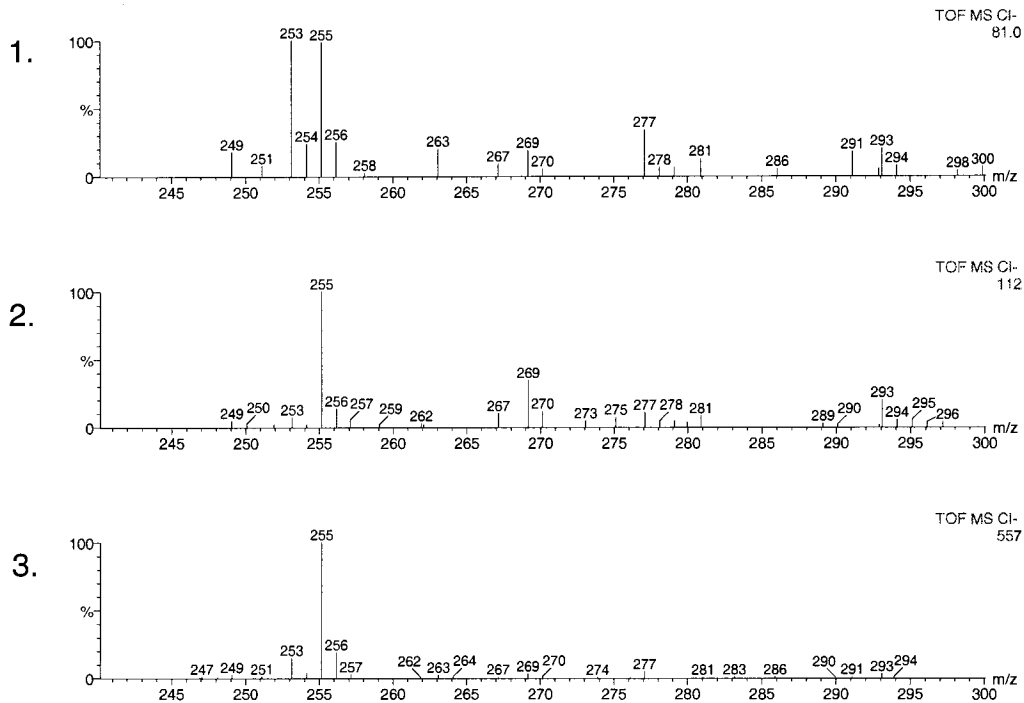
259.160 = C 16:0 enriched with 4 ^{13}C atoms.

286.330 = C 16:0 enriched with 31 D atoms.

Values over peaks are area measures.

APPENDIX D

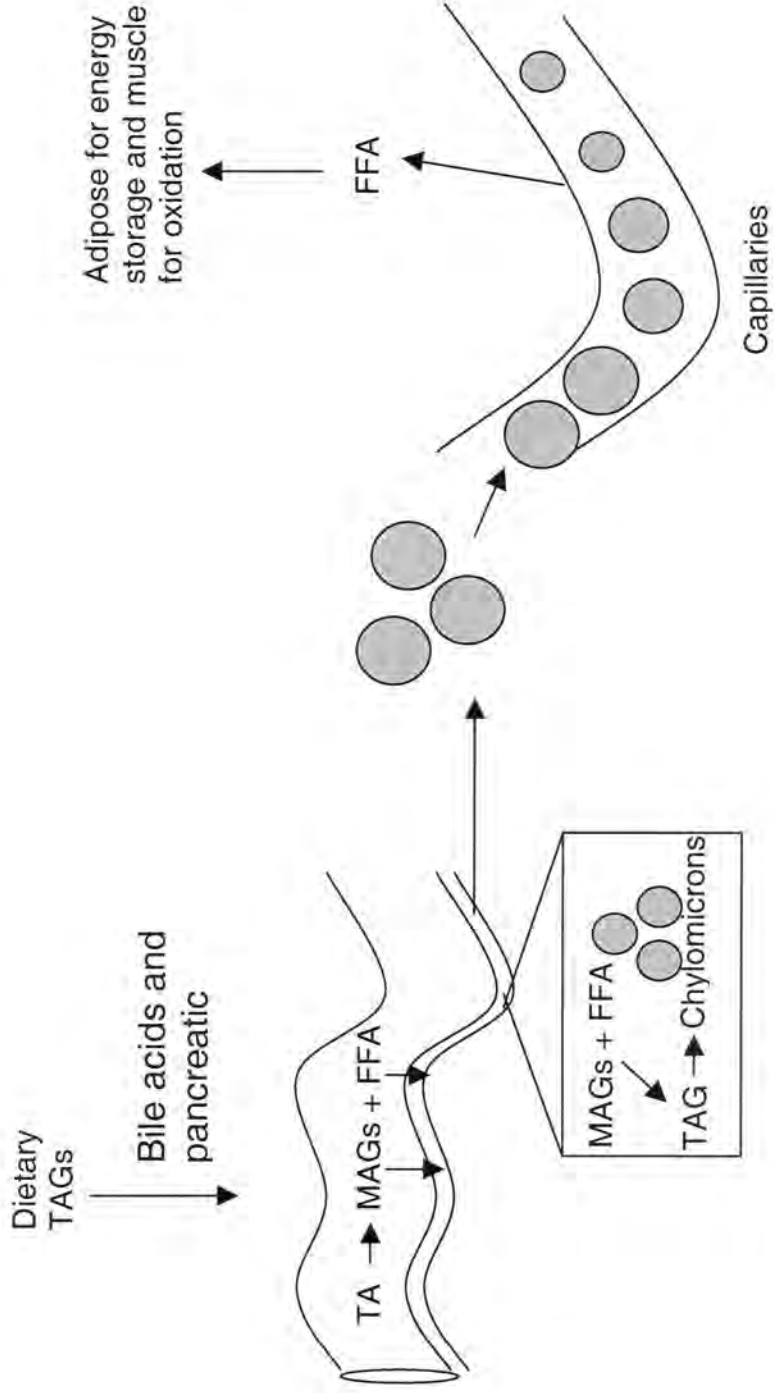
Typical mass spectrometer spectra from the time of flight GC:MS



1. Mass spectrum of 286.330 (C 16:0 enriched with 31 D atoms).
2. Mass spectrum of 259.160 (C 16:0 enriched with 4 ^{13}C atoms).
3. Mass spectrum of 257.158 (C 16:0 naturally enriched with 2 ^{13}C atoms).

APPENDIX E

Dietary Triacylglycerol Metabolism



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