Effect of feeding diets of varying fatty acid composition on apolipoprotein expression in newborn swine

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Wang, Heng, Felicia Hunter, and Dennis D. Black. Effect of feeding diets of varying fatty acid composition on apolipoprotein expression in newborn swine. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G645–G651, 1998.—The purpose of this study was to determine the effect of chronic (1 wk) feeding of dietary triacylglycerol (TG) of varying fatty acid composition on small intestinal and hepatic apolipoprotein expression, as well as serum lipid and apolipoprotein concentrations, in newborn swine. Two-day-old female swine were fed one of three diets by gavage with the following lipid composition: medium-chain TG (MCT; MCT oil), intermediate-chain saturated TG (ICST; coconut oil), and long-chain polyunsaturated TG (LCPUT; safflower oil) at 753 kJ·kg⁻¹·day⁻¹ with 51% of energy from fat. After 1 wk, serum lipids and apolipoprotein concentrations were measured, and jejunal apolipoprotein B (apo B) and apo A-I mass and apo B, apo A-I, apo A-IV, and apo C-III synthesis were measured. Liver was processed for determination of apo B and apo A-I mass and apo B, apo A-I, apo C-III, and β-actin mRNA abundance by slot blot hybridization. Compared with the MCT and LCPUT groups, the ICST group had higher total serum cholesterol, TG, high-density lipoprotein (HDL)-cholesterol, and apo A-I concentrations. There were no differences among the three groups for intestinal apolipoprotein mass or synthesis. In liver, apo A-I mass was highest in the ICST group. Liver apo A-I and apo C-III mRNA abundance was highest in the ICST group. Among all three groups, hepatic apo A-I mass correlated significantly with plasma HDL-cholesterol concentrations, and serum TG concentrations correlated with hepatic apo C-III mRNA abundance. In conclusion, we found that in the newborn piglet, chronic feeding of ICST increases serum total cholesterol, TG, HDL-cholesterol, and apo A-I concentrations and hepatic expression of apo A-I and apo C-III mRNA, compared with feeding of MCT or LCPUT. We speculate that increased hepatic apo A-I expression may contribute to the higher serum HDL and apo A-I concentrations in the ICST animals. Increased hepatic expression of apo C-III with ICST feeding may contribute to the higher serum TG concentrations by apo C-III-mediated inhibition of the catabolism of triacylglycerol-rich lipoproteins.

enzyme-linked immunosorbent assay; immunoprecipitation; mRNA; triacylglycerol

DIETARY LIPID IS A CRUCIAL nutrient in the neonatal mammal. Although breast milk is the ideal source of dietary fatty acids, commercial formulas are extensively used for the nutrition of human infants. The appropriate fatty acid composition of such formulas has been the subject of much debate. The effect of chronic feeding of diets containing triacylglycerols of varying chain lengths and degrees of saturation on plasma lipid profiles and intestinal and hepatic apolipoprotein expression has been investigated in adult humans and animal species, including nonhuman primates (19, 25). However, studies in the newborn mammal have been limited. It has been shown in the human infant that the quality and quantity of dietary fat intake can modulate the plasma lipid profile (26, 27, 31, 35, 36) and that dietary cholesterol intake can modulate serum cholesterol levels, as well as cholesterol synthesis (14). Generally, infants who are breast-fed have significantly higher serum total cholesterol and low-density lipoprotein (LDL)-cholesterol concentrations than formula-fed infants. A similar observation has been made in newborn swine (23). This effect has been attributed to the relatively low polyunsaturated-to-saturated fatty acid ratio and higher cholesterol content in breast milk, compared with most commercial formulas. However, detailed studies of the effects of specific classes of dietary fatty acids on intestinal and hepatic apolipoprotein expression in the neonate are lacking.

We have developed the newborn piglet as a model to test the effects of dietary lipid on intestinal and hepatic apolipoprotein expression (2–6, 33, 37). This model offers several advantages for such studies. Intestinal development in the precocial newborn swine is very similar to that of the human infant (13, 22), and there is significant homology with human apolipoprotein and lipoprotein metabolism (3, 5, 11). Dietary manipulation is relatively easy, and large amounts of sample material are readily available. We have previously defined the effects of acute (24 h) feeding of dietary lipid of varying fatty acid composition on intestinal apolipoprotein expression in newborn swine (4–6, 37). The purpose of the present study was to determine the effects of chronic (1 wk) feeding of dietary triacylglycerol of varying fatty acid composition on small intestinal and hepatic apolipoprotein B (apo B), apo A-I, apo A-IV, and apo C-III expression, as well as serum lipid profiles, in newborn swine.

MATERIALS AND METHODS

Animals. Two-day-old female domestic swine were obtained from Cargill Swine Project (Russellville, AR). The animals were suckled by the sow during the first 2 days of life. Animals were housed in groups of three in heated stalls with straw bedding. The experimental protocol was approved by the University of Arkansas for Medical Sciences Institutional Animal Care and Use Committee.

Experimental formulas consisted of a fat-free formula base (Sowena, Merrick, Madison, WI) reconstituted and blended with lipid and fed by gavage using an 8 French soft plastic
feeding tube (Biosearch Medical Products, Somerville, NJ). Animals were fed by bolus five times per day for 1 wk to provide 753 kJ·kg−1·24 h−1 with 51% of energy from fat, 27% from carbohydrate, and 22% from protein. Calculations of daily feeding volumes were based on body mass measurements made that same morning. The following three experimental groups were studied: 1) six animals received medium-chain triacylglycerol (MCT) in the form of MCT oil (Mead Johnson, Evansville, IN) (75% 8:0 and 24% 10:0 fatty acids); 2) six animals received intermediate-chain saturated triacylglycerol (ICST) in the form of coconut oil (ICN, Aurora, OH) (46% 12:0 and 19% 14:0 fatty acids); and 3) seven animals received long-chain polyunsaturated triacylglycerol (LCPUT) in the form of safflower oil (21st Century Foods, Uniondale, NY) (77% 18:2 and 13% 18:1 fatty acids). The MCT and ICST diets contained 4% of lipid energy as LCPUT to prevent essential fatty acid deficiency. Fatty acid composition of these diets as determined by capillary gas chromatography has been previously reported (37). Aliquots of the formulas were also extracted and subjected to TLC on silica gel G plates, using a mixture of petroleum ether, diethyl ether, and acetic acid (80:20:1, vol/vol/vol). Lipid bands were identified by exposure to iodine vapor and comparison to cochromatographed standards. The lipids from all three formulas consisted of ~98% triacylglycerol.

Procurement of samples for determination of intestinal apolipoprotein synthesis and mass and hepatic apolipoprotein mRNA levels and mass. At the end of the experimental feeding period, the animals were anesthetized, and a 10-cm segment of proximal jejunum was isolated 10 cm distal to the ligament of Treitz by two ligatures. Radiolabeling was performed by instilling 1.0 mCi of [1,4-3H]leucine (20Ci/mmol; Amersham, Arlington Heights, IL) into the segment. Nine minutes later, the segment was removed and prepared for immunoprecipitation as described below. This labeling time has previously been shown to be optimal in similar experiments in the adult rat, and we have used it previously in the piglet (2–5, 15, 16). Adjacent segments were removed for homogenization and processing for determination of apolipoprotein mass. Liver tissue was taken from the medial right lobe for homogenization and processing for determination of apolipoprotein mass, as well as for RNA extraction.

Preparation of mucosal cytosolic supernatants for immunoprecipitation. Radiolabeled intestinal segments were flushed with 50 ml of ice-cold PBS (50 mmol/l phosphate, and 100 mmol/l NaCl, pH 7.4) and 20 mmol/l leucine, and the mucosa was scraped and homogenized on ice in 1 ml of PBS, 1% Triton X-100, 2 mmol/l leucine, 1 mmol/l phenylmethylsulfonyl fluoride, and 1 mmol/l benzamidine, pH 7.4, as previously described (3). Aliquots of the homogenate were taken for measurement of TCA-precipitable radioactivity. The remainder was pelleted at 105,000 g for 65 min in a 50.3 Ti rotor (Beckman Instruments, Palo Alto, CA), followed by collection of the cytosolic supernatant. The nonradioabeled segments were similarly prepared for apolipoprotein mass determination. Although most intracellular apolipoprotein is membrane associated, this technique has been shown previously to result in extraction and solubilization of 84–94% of total recoverable apolipoprotein mass with no discernible effect of the state of lipid flux (16). All procedures were performed at 0–5°C, and the mucosal supernatant samples were stored at −80°C until analysis.

Apolipoprotein immunoprecipitation. Intestinal cytosolic supernatant fractions were subjected to specific immunoprecipitation of apo B, apo A-I, apo A-IV, and apo C-III under conditions of antibody excess as described previously (4, 6). For apo B, apo A-I, and apo A-IV, aliquots of cytosolic supernatants were preincubated with washed IgG-SORB (Enzyme Center, Malden, MA) and subsequently reacted with excess rabbit polyclonal anti-swine apolipoprotein antiserum for 18 h at 4°C. For apo C-III immunoprecipitation, sheep anti-swine apo C-III antiserum (provided by Dr. J. Rapacz, University of Wisconsin, Madison, WI) was used with protein G-Sepharose (Pharmacia, Piscataway, NJ), rather than IgG-SORB. After a second addition of IgG-SORB or protein G-Sepharose and extensive washing, the liberated immunocomplex was applied to either 5.6% (apo A-I, apo A-IV), 4% (apo B), or 10% (apo C-III) SDS-polyacrylamide tube gels. After electrophoresis, gels were sliced into 2-mm slices and solubilized in Solvable (DuPont, Boston, MA) at 50°C for 3 h followed by the addition of Ultima Gold scintillation fluid (Packard, Meriden, CT) and incubation overnight at room temperature. Liquid scintillation counting was performed in a Packard model 2000 liquid scintillation counter (Downers Grove, IL). Apolipoprotein species were identified by comparison to stained coelectrophoresed apolipoproteins. Apolipoprotein synthesis rates were expressed as the percentage of specific immunoprecipitated apolipoprotein counts compared with total protein TCA-precipitable counts. Apolipoprotein synthesis was thereby expressed as a percentage of total protein synthesis.

Apolipoprotein quantitation by ELISA. ELISA assays for swine apo B and apo A-I were performed as described previously (4). Briefly, 96-well microtiter plates (Falcon; Becton-Dickinson, Oxnard, CA) were coated with either purified swine plasma LDL (apo B assay) or high-density lipoprotein (HDL) (apo A-I assay) at 100 ng protein/well. Competition assays were performed using serial dilutions of serum samples and cytosolic supernatants prepared from the intestine and liver tissue homogenates as the competing antigen. After incubation with biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) followed by avidin-biotinylated peroxidase complex (Vectastain, Vector Laboratories), color was developed by addition of o-phenylenediamine. Color development was stopped by the addition of 2 mol/l sulfuric acid. Standard antigens consisted of swine plasma LDL (apo B) and HDL (apo A-I) applied to the microtiter plate wells in serial dilutions. Assay plates were read at 492 nm by a Bio-Rad microtiter plate reader (Richmond, CA). All samples were run in triplicate in the same assay. Intra-assay variability was <5%, and interassay variability was <10%.

Determination of hepatic apolipoprotein mRNA levels by slot blot hybridization. Total RNA was extracted from snap-frozen liver samples by the method of Chomczynski and Sacchi (12). Intactness of each RNA preparation was verified by agarose gel electrophoresis and visualization of ribosomal RNA subunits. We applied 10 μg of RNA to nitrocellulose filters using a slot blot apparatus (Hoefer, San Francisco, CA). Filters were serially hybridized with swine apo B cDNA (32), swine apo A-I cDNA (33), swine apo C-III cDNA (33), and murine β-actin cDNA (provided by Dr. L. Kedes, Stanford University). Hepatic samples were not analyzed for apo A-IV mRNA levels, since apo A-IV is not significantly expressed in liver in swine. Autoradiographs were scanned, digitized, and analyzed using NIH Image software. Apolipoprotein mRNA abundance was expressed as apolipoprotein mRNA to-β-actin absorbance ratio.

Protein and serum lipid measurement. Protein was measured using the Bradford technique (7). Serum total cholesterol, triglyceride, and HDL-cholesterol concentrations were measured using enzymatic assays (Sigma Chemical, St. Louis, MO).

Statistical analysis. Data in experimental groups were analyzed by one-way ANOVA, followed by the Fisher least
significant difference test to compare specific groups. Correlations among variables were analyzed by linear regression. The null hypothesis was rejected at $P < 0.05$.

RESULTS

Weight gain and serum lipid and apolipoprotein concentrations. Figure 1 shows weight gain for the three groups of animals during the 7 days of study. As shown, weight gain was equivalent in all three experimental groups. Weight gain was comparable to that reported for sow-nursed piglets (17). Figure 2A shows serum total cholesterol, triacylglycerol, and HDL-cholesterol concentrations. Serum apo B and apo A-I concentrations are shown in Fig. 2B. Animals receiving the ICST diet had significantly higher serum total cholesterol, triacylglycerol, HDL-cholesterol, and apo A-I concentrations compared with the MCT and LCPUT groups. Compared with the other two groups, approximately one-half of the increased total serum cholesterol in the ICST group may be accounted for by the increased HDL-cholesterol in this group. Serum apo B concentrations were not different among the three groups.

Intestinal apolipoprotein synthesis and mass. Figure 3A shows jejunal apo B, apo A-I, apo A-IV, and apo C-III synthesis for the three experimental groups. Among the four apolipoproteins, apo A-I demonstrated the highest synthesis and apo C-III the lowest among all three groups. However, there were no significant differences among the three experimental groups for jejunal synthesis of any of the apolipoproteins studied. Jejunal mucosal apo B and apo A-I mass was measured, which may be posttranslationally regulated (18, 34). Figure 3B shows that neither apo B nor apo A-I jejunal mass was significantly different among the three dietary groups.

Hepatic apolipoprotein mRNA abundance and mass. Hepatic apo B, apo A-I, and apo C-III mRNA abundance as determined by slot blot hybridization is shown in Fig. 4A. There were no differences in hepatic apo B mRNA abundance among the three experimental groups using $\beta$-actin mRNA as a control for RNA loading. However, liver mRNA abundance for both apo A-I and apo C-III was significantly higher in the ICST group compared with the MCT and LCPUT groups. Liver apo B and A-I mass is shown in Fig. 4B. There were no differences among the three groups for apo B mass. However, both the ICST and LCPUT groups had a higher hepatic apo A-I mass than the MCT group.

Correlations. Figure 5A demonstrates a significant linear correlation of the serum HDL-cholesterol concentration with liver apo A-I mass across all three experimental groups. Also, Fig. 5B shows a significant correlation of serum triacylglycerol concentrations with liver apo C-III mRNA abundance across all three groups.

DISCUSSION

In the present study, newborn swine were fed diets differing greatly in their fatty acid composition for 7 days. Experimental formulas contained either medium-chain-length saturated fatty acids (predominantly 8:0 and 10:0 fatty acids; MCT group), intermediate-chain-length saturated fatty acids (mainly 12:0 and 14:0 fatty acids; ICST group), or long-chain-length unsaturated fatty acids (mainly 18:1 and 18:2 fatty acids; LCPUT...
The diets were well tolerated, and weight gain was comparable in all three groups and similar to that reported for sow-fed piglets (17). Because the animals were fed by gavage, intake of comparable amounts of formula by all three groups of animals was assured. The results of this study demonstrate that serum lipid and apolipoprotein concentrations can be regulated by dietary fatty acids in the newborn piglet independent of cholesterol content. Serum total cholesterol, triacylglycerols, HDL-cholesterol, and apo A-I concentrations were higher in the ICST group after 1 wk, compared with the other two groups. Approximately one-half of the increase in the serum total cholesterol concentration in the ICST group, compared with the other two groups, was accounted for by the increase in the HDL-cholesterol fraction. Although serum lipoprotein and apolipoprotein concentrations are the net result of both entry (synthesis/secretion) and exit (uptake/degradation) from the plasma compartment, patterns of regulation of apolipoprotein intestinal synthesis and hepatic mRNA levels in the present study suggest a major role for the liver in determining the differences in serum lipid and apolipoprotein concentrations observed with feeding the various diets for 1 wk.

Studies in adult humans and animals (including nonhuman primates) have demonstrated an increase in serum LDL-cholesterol, apo B, HDL-cholesterol, and apo A-I concentrations after feeding diets enriched in saturated fatty acids relative to mono- and polyunsaturated fatty acids (19, 21, 24). These effects of dietary saturated fatty acids appear to be due primarily to decreased clearance of lipoproteins and associated apolipoproteins, rather than increased production (9, 19, 25, 28–30). The results of the present study in the newborn piglet show that the ICST diet resulted in higher serum total cholesterol, HDL-cholesterol, and apo A-I concentrations without significant change in the serum apo B concentration, compared with the LCPUT and MCT diets. Furthermore, increased hepatic expression of apo A-I mRNA in the ICST animals suggests that at least part of the increased serum apo A-I concentration may be due to increased hepatic synthesis and secretion. The strong direct linear corre-

Fig. 3. A: jejunal apolipoprotein synthesis (given as % total protein synthesis) in the 3 groups of animals. B: jejunal apolipoprotein mass (in ng apolipoprotein/µg total protein) in the 3 groups of animals. Bars represent means ± SE. There were no differences in synthesis or mass among the 3 groups by ANOVA.

Fig. 4. A: hepatic apolipoprotein mRNA abundance (expressed as apolipoprotein-to-β-actin absorbance ratios) in the 3 groups of animals. B: hepatic apolipoprotein mass (in ng apolipoprotein/µg total protein) in the 3 groups of animals. Bars represent means ± SE. Group means were analyzed by ANOVA, followed by the Fisher least significant squares test. Bars with different superscripts are significantly different at P < 0.05.
lation of liver apo A-I mass with serum HDL-cholesterol concentration across all three experimental groups further supports this hypothesis. No significant differences in the jejunal synthesis of any of the apolipoproteins were noted among the three experimental groups, suggesting that intestinal expression of apo A-I does not contribute to the observed differences in serum apo A-I concentrations. Because apo B and apo A-I may be regulated at the posttranslational level (18), jejunal mass of these two apolipoproteins was also measured and likewise was not different among the three groups.

Although we did measure serum HDL-cholesterol concentrations in the present study, we did not quantify serum LDL- and very low density lipoprotein (VLDL)-cholesterol concentrations, since these animals were not fasted at the time of death. Although approximately one-half of the increase in the serum total cholesterol concentration in the ICST animals, relative to the other two groups, may be accounted for by increased HDL-cholesterol concentrations, the source of the remaining cholesterol increase in the ICST group is not known. Because there were no differences in serum apo B concentrations among the three groups, an increased number of circulating LDL and/or VLDL particles would be unlikely.

Caprylic (8:0) and capric (10:0) acids, the major components of the MCT diet in the present study, have been thought to be equivalent to carbohydrate in their cholesterolemic effect in humans (19, 20), although at least one human feeding study has challenged this concept (10). Cater et al. (10) found an MCT oil diet to have one-half the potency of palmitic acid for raising plasma total cholesterol and LDL-cholesterol concentrations in adult humans. Also, dietary medium-chain fatty acids have been shown to result in high serum triglyceride concentrations in adult humans relative to long-chain saturated and unsaturated fatty acids (10, 19). In the present study in newborn piglets, feeding the MCT diet produced neither high serum cholesterol nor high serum triglyceride concentrations relative to the ICST and LCPUT diets, suggesting a different metabolic response in the newborn mammal.

Previous feeding studies in adult cynomolgus monkeys (8) and golden Syrian hamsters (1) have demonstrated lower hepatic apo C-III mRNA abundance in animals fed a polyunsaturated fatty acid diet relative to those fed a diet enriched in saturated fatty acids. Our results in the present study in newborn swine are in agreement with these adult studies (1, 8). The ICST group had significantly higher hepatic apo C-III mRNA abundance compared with the other two groups. We also found a significant direct linear correlation of hepatic apo C-III mRNA abundance with serum triglyceride concentrations across all three experimental groups. This finding suggests that the increased serum triglyceride concentrations in the ICST group may be due, at least in part, to apo C-III-mediated inhibition of triglyceride-rich lipoprotein catabolism.

After 1 wk of feeding, there is no differential regulation of jejunal apolipoprotein synthesis induced by the three experimental diets. In contrast, in previous studies (37) in 2-day-old piglets fed these same diets by intraduodenal infusion for 24 h, we found that the ICST diet resulted in significantly lower jejunal apo B synthesis compared with the other two diets. Furthermore, in the earlier study (37), the LCPUT diet resulted in significantly higher jejunal apo A-I synthesis compared
with the other two groups. All three experimental diets were equally effective in upregulating apo A-IV and apo C-III expression at the pretranslational level in acute feeding studies (6), as observed in the present study. However, jejunal apo A-IV synthesis in the three groups after 1 wk of feeding is approximately one-half that observed after just 24 h of feeding in the previous studies (6). Figure 6 summarizes jejunal apolipoprotein synthesis data from the previous acute feeding studies (6, 37) and compares these data with values from the present chronic feeding study. This comparison suggests that developmental changes in jejunal apolipoprotein synthesis and responsiveness to induction by lipid absorption in newborn swine are superimposed on regulation by the type of absorbed lipid in the case of apo B, apo A-I, and apo A-IV.

We conclude from the present study that the fatty acid composition of the diet may have major effects on serum lipoprotein concentrations and metabolism in the neonatal swine, a model similar in many respects to the human neonate. These effects are different from those reported in adult human and animal studies and suggest that the responses of the neonatal intestine and liver to dietary fatty acids are unique and developmentally regulated by dietary ICST by direct effects on hepatic and liver to dietary fatty acids are unique and developmentally regulated by dietary ICST by direct effects on hepatic apolipoprotein mRNA abundance and liver lipoprotein concentrations and metabolism in the neonate. These effects are different from those reported in adult human and animal studies and suggest that the responses of the neonatal intestine and liver to dietary fatty acids are unique and developmentally regulated by dietary ICST by direct effects on hepatic apo A-I expression. Also, dietary ICST may result in higher serum triglyceride levels in part by increasing hepatic apo C-III expression, which may inhibit triglyceride-rich lipoprotein catabolism. Although higher HDL-cholesterol and higher triglyceride serum levels are generally viewed to be beneficial and detrimental, respectively, in the adult, their significance in infancy is not known. Nevertheless, these effects should be considered in determining the optimal lipid composition of infant formula.

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