

Differences in cholesterol metabolism in juvenile baboons are programmed by breast- versus formula-feeding

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Abstract We estimated the effects of breast- and formula-feeding on cholesterol and bile acid metabolism for 1.5 years after weaning in 35 newborn baboons that were breast-fed ($n = 12$) or fed one of two formulas with high ($n = 11$) or low ($n = 12$) polyunsaturated/saturated (P/S) fatty acid composition. Infants were weaned at 15 weeks to a high cholesterol, saturated fat diet. Because formula P/S ratio did not affect any variable for 1.5 years after weaning, the data were averaged for the two formula groups. After weaning, serum cholesterol and lipoprotein cholesterol concentrations among the infant diet groups were not different until after 52 weeks of age. From 70 to 97 weeks of age, serum cholesterol and high density lipoprotein-2 (HDL₂)-cholesterol (HDL₂-C) concentrations were lower ($P < 0.04$) among baboons that were breast-fed as infants compared with those fed formulas. We observed no significant postweaning differences in low density lipoprotein (LDL)-C, HDL₃-C, or serum apolipoprotein A-I, B, or E concentrations. At 97 weeks of age baboons that were breast-fed until 15 weeks compared with those formula-fed had a 25% lower total bile acid synthetic rate (36.6 vs. 48.6 $\mu\text{mol/day}$ per kg body weight, $P < 0.02$) due principally to a 29% lower cholic acid synthetic rate (23.2 vs. 32.5 $\mu\text{mol/day}$ per kg body weight, $P < 0.004$). Baboons breast-fed as infants had a 44% higher hepatic LDL-receptor mRNA concentration than those formula-fed (1.45 vs. 1.01 pg mRNA/ μg total RNA, $P < 0.003$). These results suggest that breast- versus formula-feeding in baboons imprints differences in bile acid synthesis, regulation of LDL receptor expression, and HDL-C subfraction concentrations.—Mott, G. E., E. M. Jackson, L. DeLallo, D. S. Lewis, and C. A. McMahan. Differences in cholesterol metabolism in juvenile baboons are programmed by breast- versus formula-feeding. *J. Lipid Res.* 1995. 36: 299–307.

Supplementary key words bile salts • LDL-receptor • ACAT • LCAT • apolipoproteins • lipoproteins • mRNA • infant • liver

Previous experiments with baboons have shown that breast- versus formula-feeding imprints postweaning serum lipoprotein concentrations and cholesterol metabolism until at least young adulthood (1–4). These metabolic changes were associated with differences in arterial fatty

streaks between infant diet groups (4). We also observed significant differences between breast- and formula-fed baboons during the preweaning period in lipoprotein concentrations, bile acid metabolism, the LDL receptor, and thyroid hormone concentrations (5–8). Although studies with experimental animals have demonstrated imprinting of cholesterol metabolism by neonatal diet, premature weaning or with drug and hormone treatments, most studies with adolescent and young adult humans reported no deferred effects of infant diet on serum cholesterol concentrations (see reviews, 9–14). However, infant feeding regimens including breast- versus bottle-feeding recently were reported to differentially affect serum cholesterol, lipoproteins, and atherosclerosis in elderly humans (15). In young experimental animals, stimulation of bile acid excretion and synthesis with cholestyramine persisted after weaning (16).

Dietary saturated versus unsaturated fat have significant effects on lipoprotein and apolipoprotein concentrations and cholesterol and bile acid metabolism in adult humans and nonhuman primates (17–21). Because breast milk fatty acid composition is generally more saturated than most commercial formulas, we compared the effects of formulas with high and low polyunsaturated/saturated (P/S) fatty acid ratios on postweaning lipoprotein concentrations and on measures of bile acid and cholesterol metabolism. We also compared the effects of breast-feeding with formula-feeding on these measures. The objective of the current experiment with baboons was to

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; ACAT, acyl-CoA:cholesterol acyltransferase; LDL-R, LDL-receptor; C, cholesterol.

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identify, early in the postweaning period, key metabolic pathways of cholesterol and bile acid metabolism that are influenced by these preweaning infant diets.

MATERIALS AND METHODS

Animal procedures

Thirty-five infant baboons (16 males, 19 females) derived from 3 sires and 35 dams were randomly assigned to one of three infant diet groups: breast-fed ($n = 12$), commercial infant formula (Similac, Ross Laboratories, Columbus, OH) with a high fatty acid polyunsaturated/saturated (P/S) ratio (1.69) ($n = 11$), or a formula with a low P/S ratio (0.33) ($n = 12$). The low P/S formula had a fatty acid composition similar to that of baboon breast milk. The compositions of baboon breast milk and the formulas were described previously (7). The infants were weaned at

TABLE 1. Composition of high cholesterol-saturated fat diet fed to baboons after weaning

Component	g/kg
Special Purina Monkey Chow ^a	814
Lard	165
NaCl	11.2
Ascorbic acid	2.13
Vitamin A acetate	0.05
Sterols	
Cholesterol	6.37
β -Sitosterol	0.48
Campesterol	0.20
Stigmasterol	0.04
Energy	
Content as fed	15.4 kJ/g
Distribution	%
Protein	20.1
Carbohydrate	39.8
Fat	40.1
Fatty acids	% of total
C12:0	0.05
C14:0	1.3
C16:0	22.5
C16:1	2.4
C18:0	17.0
C18:1	38.9
C18:2	13.8
C18:3	0.6
C20:0	0.4
C20:1	1.0
C22:1	0.1

^aSpecial Purina Monkey Chow (5045-6) constituents (wt %): ground yellow corn, 36.5; soybean meal, 30.3; ground wheat, 15.2; gluten-meal, 9.1; dry skim milk, 5.1; brewers' dried yeast, 0.6; vitamins, 0.8 (B-12, riboflavin, Ca pantothenate, niacin, choline Cl, menadione, NaHSO₃, folic acid, thiamin, cholecalciferol, vitamin E) and minerals, 3.2 (CaCO₃, Ca₃(PO₄)₂, FeCO₃, NaCl, Ca(IO₃)₂, MnO, CuSO₄, CoCO₃, ZnSO₄, ZnO).

15 weeks to a chow-based diet (Table 1) with 40% of the calories from fat (P/S ratio, 0.35) and 0.41 mg cholesterol/kJ (1.7 mg/kcal), and were housed together. The animals were offered an average of 500 g of food per day. There were no postweaning differences in body weight among the infant diet groups. At 97 weeks males were larger than females (6.59 vs. 5.37 kg, $P < 0.0002$). Results from these animals during the preweaning period were reported previously (5-8). All animal procedures were approved by the Institutional Animal Research Committee. The Southwest Foundation for Biomedical Research and the University of Texas Health Science Center are accredited by the American Association for Accreditation of Laboratory Animal Care.

Lipoprotein, apolipoprotein, and cholesterol assays

We fractionated the serum lipoproteins by density gradient ultracentrifugation (7) from blood samples obtained under ketamine immobilization (10 mg/kg body weight) at 25, 34, 52, 70, 88, and 97 weeks of age after the animals had fasted overnight. The lipoprotein subfractions were obtained at the following densities; VLDL, 1.006-1.019 kg/l, LDL, 1.020-1.040 kg/l, HDL₁, 1.041-1.069 kg/l, HDL₂, 1.070-1.125 kg/l, HDL₃, 1.126-1.21 kg/l. VLDL + LDL and HDL also were fractionated by precipitation with heparin and MnCl₂ (7). Serum cholesterol and lipoprotein cholesterol concentrations were measured enzymatically and serum apolipoprotein A-I, B, and E concentrations were measured by electroimmunoassay (7, 22). Hepatic cholesterol (total and free) concentrations were measured by an enzymatic fluorometric method (7, 23) from liver biopsies (100 mg) obtained at 34 and 97 weeks of age by laparotomy under ketamine and pentathol anesthesia.

Enzyme activities

Lecithin:cholesterol acyltransferase (LCAT) activity was assayed at 34, 52, and 70 weeks of age by incubating [³H]cholesterol substrate with EDTA-plasma (24). The results were expressed as percent [³H]cholesterol converted to cholesteryl ester and as mass of free cholesterol esterified as described (7, 25). Hepatic acyl-CoA:cholesterol acyltransferase (ACAT) activity was assayed (7) in a total volume of 0.5 ml of hepatic microsomes (~100 μ g of protein) after the addition of 20 μ l containing 50 nmol of pure cholesterol dissolved in an aqueous solution of 3.0% Tween 80 (26).

Hepatic mRNA concentrations

Approximately 300 mg of liver was biopsied as described above at 34 and 97 weeks of age, frozen quickly in liquid nitrogen, and stored at -70°C until assayed. Hepatic apolipoprotein A-I, B, and E and LDL-receptor (LDL-R) mRNA concentrations were measured by a modification (8) of the solution hybridization method of Azrolan and Breslow (27).

Bile lipid composition

Biliary cholesterol, bile acids, and phospholipids were measured by enzymatic procedures (5) with gallbladder bile obtained during laparotomy at 34 and 97 weeks of age. Bile cholesterol saturation index was calculated from the bile lipid composition with a computer program obtained from E. H. Mosbach (28) as described (29).

Bile acid kinetic methods

Bile acid kinetic measures were made by intravenous injection of ^{14}C - and ^3H -labeled bile acid mixtures as described by Jackson et al. (5) at 34 and 97 weeks of age. Bile acids in bile samples obtained at laparotomy were fractionated and quantitated by reverse phase HPLC, and radioactivity in the bile acid glycine and taurine conjugates was measured. Chenodeoxycholic and cholic acid pool sizes, fractional turnover rates, and synthetic rates were calculated as described by Vantrappen, Rutgeerts, and Ghoois (30). Bile acid hydrophobicity index was calculated from the concentrations of the individual bile acid conjugates (31) as described previously (29).

Cholesterol absorption

Cholesterol absorption was measured by the dual isotope method of Zilversmit (32) and Corey and Hayes (33). Briefly, 2 μCi (74 kBq) of $[1,2\text{-}^3\text{H}]$ cholesterol dissolved in 50 μl acetone was mixed with 1.5 ml baboon serum, stirred overnight at 4°C , and filtered through a sterile 0.45- μm syringe filter. One hour after an oral dose of 2 μCi (74 kBq) of $[4\text{-}^{14}\text{C}]$ cholesterol mixed with 20 g feed was fed to the animal, the dose of $[^3\text{H}]$ cholesterol was injected under ketamine immobilization (10 mg/kg body weight). Blood samples were drawn 72 and 96 h after the isotopes were administered and the $^{14}\text{C}/^3\text{H}$ ratios were measured in 0.5–1.0 ml serum by liquid scintillation counting. The mean $^{14}\text{C}/^3\text{H}$ ratios in serum at the two time points and in the administered doses were used to calculate the percent cholesterol absorption. The data from several animals that refused to eat the oral dose or vomited were excluded from the analysis.

Statistical methods

The data were log-transformed and analyzed by ANOVA. The linear model included the effects of infant diet, sex, and sire, and all two-factor interactions. The parameters of the linear model were estimated and the tests of hypotheses were performed as described (3). We used $P < 0.05$ as the significance level, but also reported other P values to supplement the discussion of other differences among variables. We estimated the partial correlation coefficients among variables after adjusting for the effects of infant diet, sire, and sex.

RESULTS

Serum lipids and lipoproteins

We observed no significant differences in serum cholesterol or lipoprotein concentrations between the high and low P/S formula-fed groups (results not shown). Therefore, we averaged the two formula-fed groups for comparison with the breast-fed group. The metabolic measures at the end of the preweaning period at 14 weeks reported previously for these same animals (5, 7–8) also are included in the figures for comparison with the postweaning measures. Breast-fed infants had significantly lower postweaning serum cholesterol concentrations from 70–97 weeks of age ($P < 0.04$, Fig. 1A). Difference in LDL-C contributed minimally to the difference in serum cholesterol. We observed a slight difference in LDL-C only at 97 weeks (breast-fed, mean = 1.51 mmol/l, formula-fed, mean 1.89 mmol/l, $P = 0.093$, Fig. 1A). Instead, the infant diet effect on postweaning serum cholesterol concentration was primarily due to lower HDL₂-C ($P < 0.03$) and HDL₁-C ($P = 0.12$) (Fig. 1B) concentrations among breast-fed versus formula-fed baboons. Similarly, total HDL-C measured by heparin- Mn^{2+} precipitation was significantly lower among breast-fed (mean, 2.32 mmol/l, 95% confidence interval (C. I.) = 2.01–2.67) compared with formula-fed baboons (2.87 mmol/l, 95% C. I. = 2.56–3.23) at 70–97 weeks of age ($P < 0.03$). There were no significant effects of the infant diets on HDL₃-C concentrations after weaning. The mean HDL₃ concentrations from 34–97 weeks of age were 0.41 mmol/l for breast-fed baboons and 0.44 mmol/l for those fed formulas ($P = 0.23$). VLDL-C concentrations at 34 weeks were higher among breast-fed baboons compared with those fed formulas (0.29 vs. 0.16 mmol/l, $P < 0.03$), but were not different at other ages (results not shown). The calculated VLDL+LDL-C concentration was not affected in the postweaning period by infant diet except that at 97 weeks the concentration was slightly lower among breast-fed baboons compared with those fed formulas (breast-fed, mean, 2.35 mmol/l; formula-fed, mean, 2.87 mmol/l, $P < 0.06$). The VLDL+LDL/HDL-C ratio from 70–97 weeks of age was not significantly affected by prior infant diet, although the ratio for the breast-fed group was about 10% higher than for the combined formula-fed groups (1.05 vs. 0.93, $P = 0.25$).

Serum triglyceride concentrations were significantly lower at 34 weeks among baboons previously breast-fed versus those fed the high P/S formula (0.504 vs. 0.709 mmol/l, $P < 0.04$) and at 43 weeks (0.402 vs. 0.780 mmol/l, $P < 0.04$), but these differences disappeared after 43 weeks (results not shown). There were no significant effects of infant diets on postweaning serum

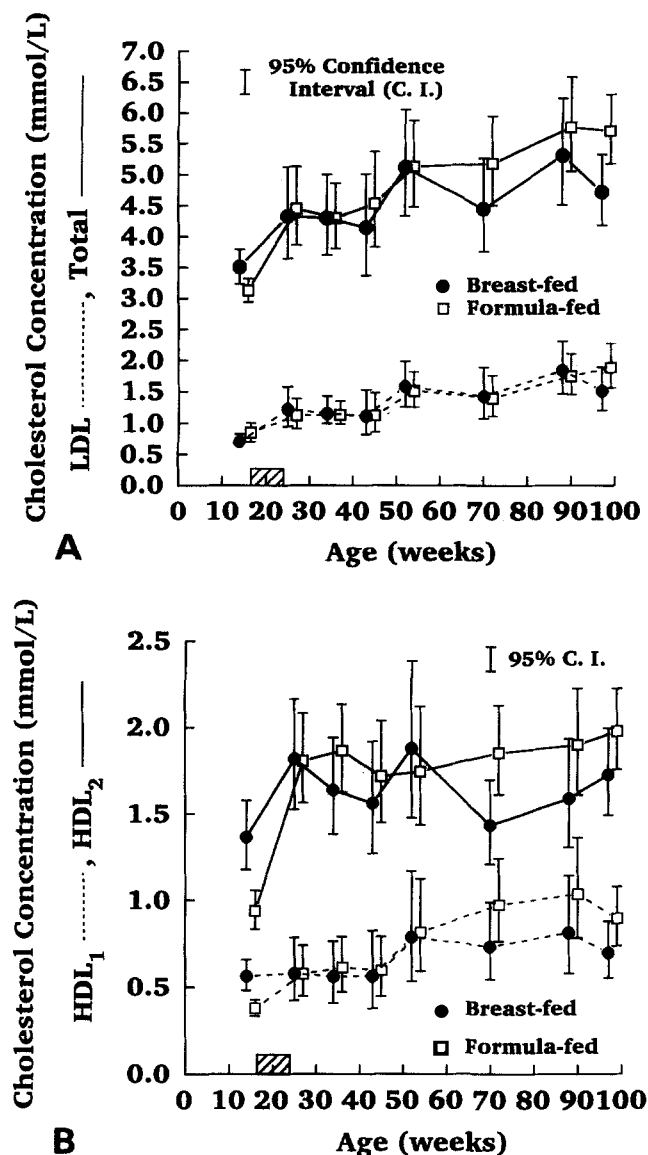


Fig. 1. A: Serum cholesterol and LDL-C by breast and formula feeding from 14 to 97 weeks of age. From 70 to 97 weeks the breast-fed group had lower serum cholesterol concentrations than the combined formula-fed groups ($P < 0.04$). Box with hatched lines represents age at weaning ± 1 SD. Data at 14 weeks were derived from reference 7. B: HDL₁-C and HDL₂-C by breast and formula feeding from 14 to 97 weeks of age. From 70 to 97 weeks the breast-fed group had lower HDL₁-C ($P = 0.12$) and HDL₂-C ($P < 0.03$) than the combined formula-fed groups. Box with hatched lines represents age at weaning ± 1 SD. Data at 14 weeks were derived from reference 7.

apolipoprotein concentrations. The overall means from 34–97 weeks were 1.52 g/l for apoA-I, 0.38 g/l for apoB, and 0.08 g/l for apoE.

We did not observe significant differences between the sexes for lipoprotein or apolipoprotein concentrations (results not shown). The preweaning lipid, lipoprotein, or apolipoprotein values were not predictive of the postweaning measurements.

Cholesterol esterifying enzymes

Plasma LCAT activities were not significantly different among the infant diet groups at 34, 52, and 70 weeks of age (results not shown). The mean activities at 70 weeks expressed as percent and by mass of cholesterol esterified were 7.91%/h and 108 $\mu\text{mol/h}$ per l of EDTA plasma, respectively. In contrast to adult baboons (25) no differences in LCAT activity were observed between male and female prepubertal baboons at 34, 52, or 70 weeks of age.

We observed 68% higher ($P < 0.05$) hepatic ACAT activities among breast-fed compared with formula-fed baboons at 34 weeks, but at 97 weeks the 18% difference between those diet groups was not statistically significant (Fig. 2). Formula P/S ratio did not affect ACAT activities at 34 or 97 weeks (data not shown). Females had higher ACAT activity compared with males at both 34 and 97 weeks, but the difference was statistically significant only at 97 weeks (1315 pmol/min per mg microsomal protein vs. 939 pmol/min per mg, $P < 0.04$).

Hepatic cholesterol concentrations

At 34 weeks of age breast-fed baboons compared with those fed formulas had higher hepatic total cholesterol; 8.29 $\mu\text{mol/g}$ tissue, 95% C. I. = 7.23–9.50 versus 6.89 $\mu\text{mol/g}$, 95% C. I. = 6.17–7.70, ($P < 0.05$) and higher cholesteryl ester concentrations, 2.29 $\mu\text{mol/g}$, 95% C. I. = 1.76–2.99 versus 1.70 $\mu\text{mol/g}$, 95% C. I. = 1.37–2.11 ($P < 0.08$). At 34 weeks hepatic unesterified (free) cholesterol concentrations were not significantly different between breast- and formula-fed baboons (5.75 $\mu\text{mol/g}$ vs. 5.16 $\mu\text{mol/g}$, $P = 0.18$). Hepatic microsomal total cholesterol, unesterified and ester concentrations also

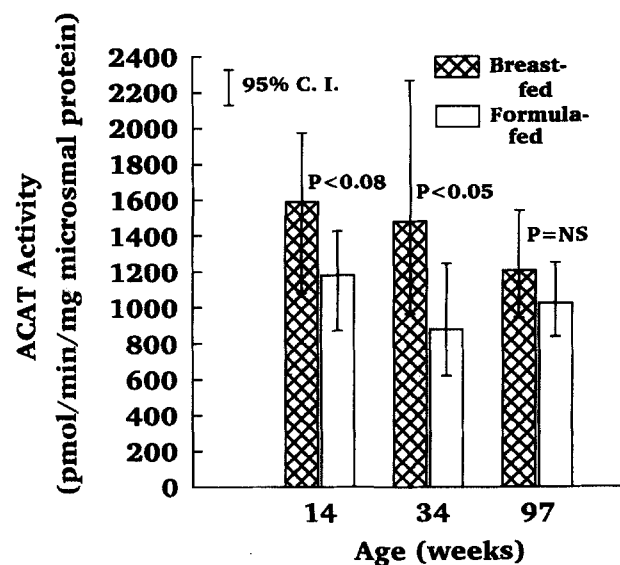


Fig. 2. Hepatic microsomal ACAT activity by breast and formula feeding at 14, 34, and 97 weeks of age. Data at 14 weeks were derived from reference 7.

were not affected by infant diet at 34 weeks (results not shown). By 97 weeks of age, differences in hepatic cholesterol (total and ester) concentrations between breast- and formula-fed infants disappeared, but the hepatic microsomal cholesteryl ester concentration was higher among breast-fed compared with formula-fed baboons (9.60 nmol/mg microsomal protein vs. 6.83 nmol/mg protein, $P = 0.07$).

Hepatic mRNA concentrations

Hepatic LDL-R mRNA concentration was 44% higher at 97 weeks among breast-fed infants compared to the combined formula-fed groups (1.45 vs. 1.01 pg/ μ g RNA, $P < 0.003$, Fig. 3). No differences in LDL-R mRNA were observed at 34 weeks among the infant diet groups (Fig. 3) or between the sexes (results not shown). There was a significant positive association (partial correlation coefficient, $r = 0.57$, $P < 0.0009$) between the hepatic ACAT activity and the LDL-receptor mRNA concentration measured at 97 weeks of age (Fig. 4). We did not observe significant infant diet, sire, or sex effects at 34 weeks for hepatic apolipoprotein A-I mRNA (overall mean = 94 pg/ μ g total RNA), apoB mRNA (mean = 1088 pg/ μ g RNA), or apoE mRNA (mean = 133 pg/ μ g RNA), or at 97 weeks for apoB mRNA (mean = 1152 pg/ μ g RNA) and apoA-I mRNA (mean = 88 pg/ μ g RNA).

Bile acid kinetics

At 34 weeks total bile acid synthetic rates among breast-fed infants were 27% lower ($P < 0.03$) and at 97 weeks, 25% lower ($P < 0.02$) compared with formula-fed infants (Fig. 5). The difference in total bile acid synthetic rate between breast-fed compared with formula-fed

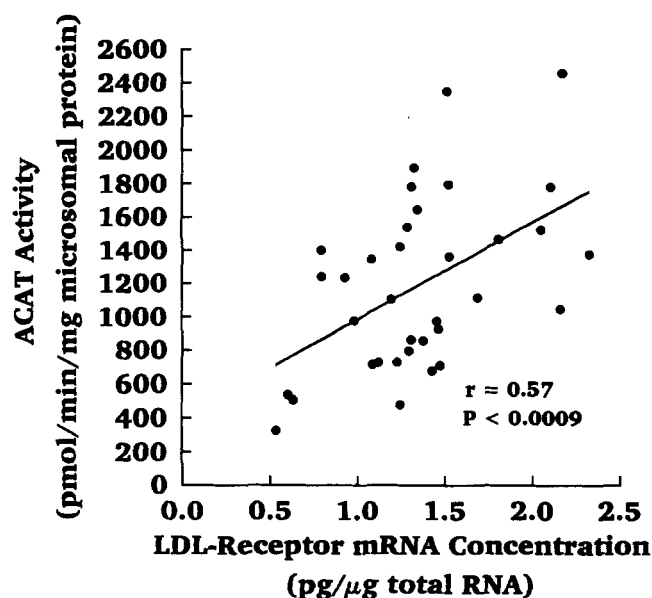


Fig. 4. Relationship of hepatic ACAT activity with LDL-R mRNA concentration. Data were adjusted for effects of infant diet, sire, and sex.

animals was reflected in lower cholic and chenodeoxycholic acid synthetic rates at 34 and 97 weeks (Table 2). The similar effect on both cholic and chenodeoxycholic acid synthetic rates is consistent with a strong positive correlation between these two variables at 97 weeks even after adjustment for the effects of infant diet, sire, and sex ($r = +0.88$, $P < 0.0001$). At 34 weeks chenodeoxycholic acid synthetic rate among breast-fed baboons was 40% lower ($P < 0.005$) and at 97 weeks cholic acid synthetic rate was 29% lower ($P < 0.004$) compared with those fed

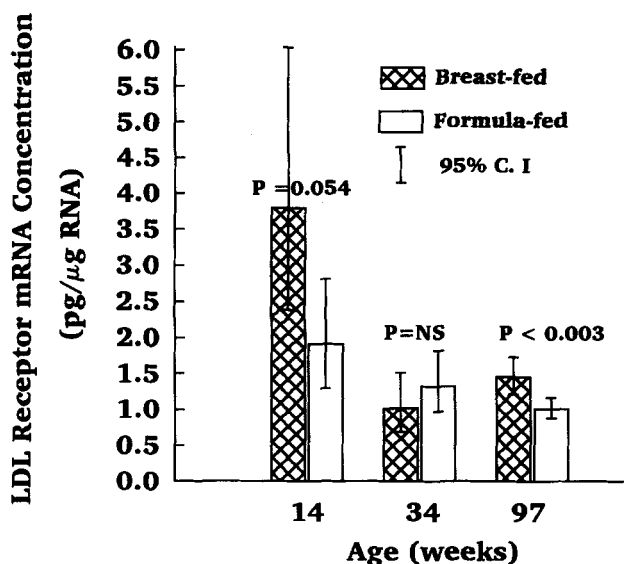


Fig. 3. Hepatic LDL receptor mRNA concentrations by breast and formula feeding at 14, 34, and 97 weeks of age. Data at 14 weeks were derived from reference 8.

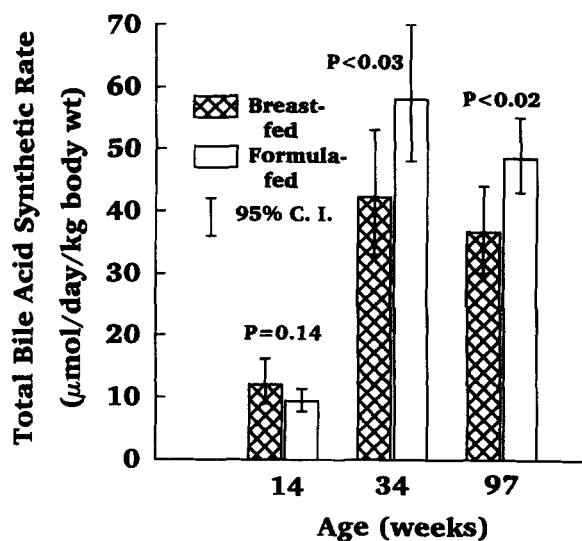


Fig. 5. Total bile acid synthetic rate by breast and formula feeding at 14, 34, and 97 weeks of age. Data at 14 weeks were derived from reference 5.

TABLE 2. Postweaning bile acid kinetic parameters by infant diet^a

Infant Diet	n ^b	34 Weeks					97 Weeks				
		Cholic Acid		Chenodeoxycholic Acid			Cholic Acid		Chenodeoxycholic Acid		
		Pool Size	FTR	Synthetic Rate	Pool Size	FTR	Pool Size	FTR	Synthetic Rate	Pool Size	FTR
		$\mu\text{mol/kg}$	days^{-1}	$\mu\text{mol/kg/d}$	$\mu\text{mol/kg}$	days^{-1}	$\mu\text{mol/kg}$	days^{-1}	$\mu\text{mol/kg/d}$	$\mu\text{mol/kg}$	days^{-1}
Breast-fed	12	147 (123-175)	0.21 (0.15-0.29)	30.6 (23.7-39.3)	33.7 (26.3-43.2)	0.34 (0.28-0.43)	155 (116-206)	0.15 (0.11-0.20)	23.2 (19.1-28.3)	48.4 (40.6-57.6)	0.28 (0.21-0.36)
Formulas	23	163 (141-188)	0.24 (0.18-0.31)	38.6 (31.4-47.4)	42.0 (34.3-51.5)	0.43 (0.36-0.51)	130 (107-158)	0.24 (0.20-0.30)	32.5 (28.4-37.1)	42.2 (37.5-47.5)	0.38 (0.32-0.45)
Infant diet effects ^c		0.90	0.88	0.79	0.80	0.80	1.19	0.61	0.71	1.15	0.73
Significance, P		0.320	0.500	0.133	0.138	0.094	0.235	0.005	0.004	0.122	0.014

^aValues are means, 95% confidence interval in parentheses.

^bn, Number of animals.

^cEffects expressed as ratio of breast-fed to formula-fed.

formulas (Table 2). We did not observe significant differences in bile acid pool sizes among the infant diet groups (Table 2). No consistent difference in any variable of bile acid metabolism was observed among the sire groups or between the sexes.

Biliary lipid concentrations

The molar proportion of biliary lipids as bile acids was lower and of cholesterol higher among breast-fed compared with formula-fed baboons at 34 weeks (Table 3). These compositional differences were not observed at 97 weeks (Table 3). P/S ratio of the formulas and sire group did not affect the bile lipid composition at 34 or 97 weeks. Breast- versus formula-feeding did not significantly affect the bile cholesterol saturation index at 34 weeks, but at 97 weeks the breast-fed group had approximately a 10% higher cholesterol saturation index than those fed formulas (71.3% vs. 63.7%, $P = 0.15$) (Table 3). Bile from males had a higher cholesterol mole percentage compared to females only at 97 weeks (3.83 vs. 2.87%, $P = 0.02$).

Biliary bile acid concentrations

Breast-fed baboons had a significantly lower percentage of the total bile acids as chenodeoxycholic acid (14.9% vs. 19.5%, $P < 0.04$) at 34 weeks compared with those fed formulas. At 97 weeks the percentage of cholic acid was higher among the breast-fed group (68.5% vs. 63.6%, $P < 0.05$). These effects resulted in a higher biliary ratio of cholic/chenodeoxycholic among breast-fed compared with formula-fed baboons at 34 weeks (4.27 vs. 3.09, $P < 0.05$) and at 97 weeks (3.68 vs. 3.09, $P = 0.062$). At 34 weeks, but not at 97 weeks, bile from breast-fed baboons had a lower bile acid glycine/taurine ratio (1.79 vs. 3.71, $P < 0.04$). The bile acid hydrophobicity index was about 8% lower for the breast-fed group at 34 and 97 weeks, but the differences were not statistically significant, $P > 0.2$.

Cholesterol absorption

No significant differences in cholesterol absorption were observed among the infant diet groups at 34 and 97 weeks. The mean percent dietary cholesterol absorbed was 21.1% at 34 weeks and 21.5% at 97 weeks.

The magnitude of the effects of breast/formula feeding on postweaning lipoprotein concentrations and metabolic variables are summarized in Fig. 6.

DISCUSSION

This study shows that breast- versus formula-fed baboons differ in bile acid synthetic rate, hepatic LDL-R mRNA concentrations, and HDL-C (principally HDL₂) more than 1.5 years after weaning. We did not observe differences in postweaning lipoprotein cholesterol concentrations until after 52 weeks of age which is consistent

TABLE 3. Postweaning bile lipid composition by infant diet^a

Infant Diet	n ^b	34 Weeks				97 Weeks			
		Bile Acids	Phospholipids	Cholesterol	Cholesterol Saturation Index	Bile Acids	Phospholipids	Cholesterol	Cholesterol Saturation Index
		mol/100 mol			%	mol/100 mol			%
Breast-fed	12	82.7 (81.2-84.3)	13.6 (12.5-14.7)	3.49 (2.69-4.52)	63.0 (49.6-80.1)	84.8 (82.8-86.9)	11.4 (10.0-13.0)	3.46 (2.92-4.11)	71.3 (62.3-81.7)
Formulas	23	84.4 (83.1-85.7)	12.6 (11.8-13.5)	2.58 (2.09-3.18)	55.4 (45.4-67.7)	84.4 (82.8-86.1)	11.7 (10.5-13.0)	3.18 (2.76-3.66)	63.7 (57.0-71.2)
Infant diet effects ^c		0.98	1.07	1.35	1.14	1.00	0.98	1.09	1.12
Significance, <i>P</i>		0.069	0.135	0.048	0.366	0.718	0.784	0.400	0.153

^aValues are means, 95% confidence interval in parentheses.^bn, Number of animals.^cEffects expressed as ratio of breast-fed to formula-fed.

with a previous experiment (4). An intriguing feature of the effects of breast- and formula-feeding is the difference in the infant diet effect on HDL concentrations from the pre- to the postweaning periods. For example, HDL₁-C and HDL₂-C cholesterol concentrations were higher among these same breast-fed baboons at 14 weeks (ref. 7 and Fig. 1B), but lower at 70-97 weeks compared with those fed formulas (Fig. 1B). This inverse relationship of pre- to postweaning measurements between breast- and formula-fed baboons also was observed for the VLDL+LDL/HDL ratio in a previous experiment with baboons fed a high cholesterol, egg yolk-containing diet (4). The type and duration of infant diet also influenced the plasma cholesterol and lipoprotein concentrations in adult humans (15). Elderly men who, as infants, were both breast-fed and fed a cow's milk-based formula and those breast-fed and weaned before 1 year of age had significantly lower serum cholesterol and LDL-C concentrations compared with those breast-fed for more than 1

year. Although that report with more than 5000 adult humans is based on feeding records from the 1920-30s and the formula probably differed considerably from modern infant formulas, the study is the largest with the longest follow-up to date that demonstrates the relationships of early feeding practices with subsequent lipoprotein concentrations. Another study of children also showed that those weaned early had lower serum cholesterol and LDL-C compared with those weaned later (34). Not all studies have shown differences in serum cholesterol among infant diet groups after weaning (see review, ref. 35).

In contrast to humans, HDL-C in these baboons accounts for greater than 50% of the total serum cholesterol and is affected by infant diet to a greater extent in the pre- and postweaning periods than is LDL-C. Because HDL₁ and HDL₂ are apoE-rich and probably bind the LDL-R more readily than LDL, differences in LDL-R mRNA may affect HDL concentrations before affecting LDL in baboons. Hepatic LDL-R mRNA concentrations among

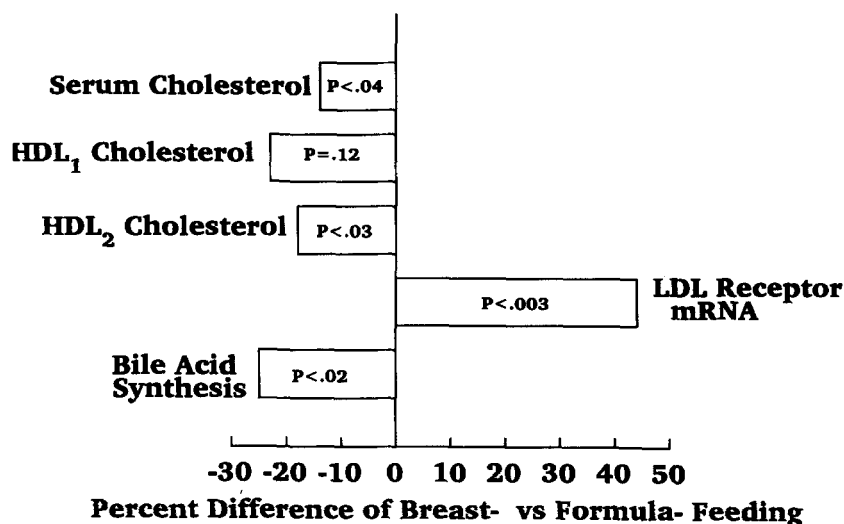


Fig. 6. Summary of long term effects of breast- vs. formula-feeding.

the breast-fed animals were higher at 97 weeks, but not at 34 weeks, which may be the reason the infant diet affected serum cholesterol and HDL₂ concentrations only after 52 weeks. Differences in LDL-R among infant diet groups do not significantly affect the relatively low LDL-C concentrations in these baboons (Fig. 1A). Large differences in LDL receptor activity may not affect LDL-C concentrations if LDL production rates are low (36).

Higher hepatic ACAT activity and microsomal cholesteryl ester concentrations among breast-fed baboons at 97 weeks may result indirectly from higher hepatic LDL-R mRNA concentrations. Supporting that suggestion is the positive correlation we observed between hepatic ACAT activity and LDL-R mRNA concentration at 97 weeks even after adjusting for the effects of infant diet (Fig. 4). A similar positive relationship between LDL binding capacity and cholesterol esterification was reported for primary hepatocytes (37). However, at 34 weeks we observed higher ACAT activity and higher hepatic cholesteryl ester concentrations, but the hepatic LDL-R mRNA concentrations of the breast- versus formula-fed groups were not significantly different. Thus, the relationships of hepatic LDL-R, ACAT, and cholesteryl ester concentrations with lipoprotein concentrations change with postweaning age and reflect differential adaptation of breast- and formula-fed baboons to the high cholesterol, saturated fat postweaning diet.

We did not observe significant postweaning effects of P/S ratio in the formulas on serum lipoprotein concentrations and measures of cholesterol metabolism. This finding is similar to the absence of differences in serum cholesterol or HDL-C concentrations among 5-year-old children who as infants were fed either a low P/S cow's milk formula or a high P/S proprietary formula (38). Because the P/S ratio of the low P/S formula in our study was similar to that of baboon breast milk, we conclude that the postweaning differences between breast- and formula-feeding are not due to differences in P/S ratio, assuming that there are no interaction effects of dietary cholesterol with P/S ratio.

Breast- versus formula-fed baboons had lower bile acid (cholic and chenodeoxycholic acids) synthetic rates at 34 and 97 weeks of age (Table 2). The lower bile acid turnover rate (equivalent to synthetic rate) among adult baboons at 7–8 years of age that were breast-fed as infants (3) suggests that the early postweaning effects persist throughout life. The lower bile acid synthetic rate among breast-fed baboons could result from a lower precursor cholesterol pool in the liver. A decreased hepatic free cholesterol pool could be mediated at 34 weeks by the higher ACAT activities and at 97 weeks by lower concentrations of apoE-rich HDL₁-C and HDL₂-C that are known to be the preferred precursors of bile acid synthesis in cultured hepatocytes (39). Although the free cholesterol concentrations of liver and hepatic microsomes were not

significantly affected by breast- versus formula-feeding, the size and location of the actual bile acid precursor pool is not known. Altered bile acid synthesis rates probably are regulated by the activities of the sterol 7 α -hydroxylases.

The age-related differences in bile acid kinetics were also dramatic. Total bile acid synthetic rates expressed per kg body weight increased by factors of 5–10 from 14 to 34 weeks of age. The percentage of total bile acids synthesized as cholic acid was approximately 30% at 14 weeks (5) and about 70% at 34 weeks. Conversely, the proportion of the total bile acids synthesized as chenodeoxycholic acid decreased from 14 to 34 weeks, although the absolute amount of chenodeoxycholic synthesized increased. These differences are consistent with the shift in the biliary cholic/chenodeoxycholic ratio from about 0.3–0.5 at 14 weeks (5) to a ratio greater than 3 at 34 and 97 weeks.

We conclude that imprinting of bile acid synthetic rate by breast- versus formula-feeding in baboons is observed soon after weaning, but the infant diet effects on postweaning HDL-C concentrations are not apparent until after about 1 year of age. A previous study shows that these effects persist into adulthood until at least 7–8 years of age (3). These results strongly suggest that factors associated with breast- and formula-feeding, other than fatty acid saturation, program the regulation of the hepatic LDL-R and of bile acid synthesis. ■

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