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Effects of Early Cholesterol Intake on Cholesterol Biosynthesis and Plasma Lipids Among Infants Until 18 Months of Age

Théa A. Demmers, MS, PD†*; Peter J. H. Jones, PhD*; Yanwen Wang, PhD*; Susan Krug, MS, RD‡; Vivian Creutzinger, BSN, RN‡; and James E. Heubi, MD‡

ABSTRACT. *Background.* The endogenous cholesterol fractional synthesis rate (FSR) is related inversely to infant dietary cholesterol at 4 months of age; however, it remains to be established whether this effect is permanent, possibly contributing to later hypercholesterolemia.

Objective. To determine whether levels of dietary cholesterol in infancy induced changes in FSR and plasma lipid levels that persisted at 18 months.

Methods. A prospective clinical trial was conducted with 47 infants, from their first week of life until 18 months of age, who received human milk (HM) until weaned ($n = 15$) or were randomized to receive modified cow's milk formula (MCF) with added cholesterol ($n = 15$) or cow's milk formula (CF) ($n = 17$) for 12 months. Cholesterol contents of HM, MCF, and CF were 120, 80, and 40 mg/L, respectively. FSR and plasma lipid levels were measured at 4 and 18 months.

Results. At 4 months, total cholesterol and low-density lipoprotein cholesterol levels were higher for infants fed HM and MCF, compared with CF. High-density lipoprotein cholesterol levels were higher in the MCF group than in the HM and CF groups. FSR in the HM group (0.034 ± 0.005 pools per day) was lower than that in the CF group (0.052 ± 0.005 pools per day). There was no difference between the HM and MCF (0.047 ± 0.005 pools per day) groups or between the MCF and CF groups. At 18 months, there were no differences in FSRs or plasma lipid profiles between the groups.

Conclusion. Although cholesterol intake before weaning affects FSRs and plasma lipid profiles at 4 months, these differences do not persist after weaning to an unrestricted diet at 18 months. This provides additional evidence that there is no imprinting of FSR in infancy with differing dietary levels of cholesterol. *Pediatrics* 2005;115:1594–1601; cholesterol, breastfed, formula-fed, fractional synthesis rate, deuterium.

ABBREVIATIONS. FSR, fractional synthesis rate; HM, human milk; MCF, modified cow's milk formula; CF, cow's milk formula; CCHMC, Cincinnati Children's Hospital Medical Center; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

From the *School of Dietetics and Human Nutrition, McGill University, Montreal, Québec, Canada; and ‡Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio.

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Address correspondence to Peter J. H. Jones, PhD, School of Dietetics and Human Nutrition, Faculty of Agriculture and Environmental Science, McGill University, 2111 Lakeshore Rd, Montreal, QC, Canada H9X 3V9. E-mail: peter.jones@mcgill.ca

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Early intake of cholesterol and its possible imprinting of later cholesterol metabolism have been studied to determine the influence of infant nutrition on adult cholesterol metabolism and subsequent cardiovascular disease risk.^{1–3} The cholesterol content of human milk (HM) is typically higher (90–150 mg/L) than that of regular cow's milk formulas (CFs) (10–40 mg/L), whereas soy milk-based formulas contain no cholesterol. Reiser and Sidelman⁴ first hypothesized, on the basis of their studies with rats, that the function of cholesterol in milk was to establish control of serum cholesterol homeostasis. Early exposure to dietary cholesterol appeared to “protect” against diet-induced hypercholesterolemia in adulthood, because adult male offspring exhibited an inverse relationship between serum cholesterol concentrations and the cholesterol content of their mothers' milk. Results from studies in a variety of species produced conflicting results; other rat studies supported⁵ or refuted⁶ the hypothesis, studies in pigs supported the hypothesis,⁷ and studies in baboons^{8,9} and guinea pigs¹⁰ did not support the hypothesis.

Human infants have increased serum cholesterol concentrations in proportion to the cholesterol content of HM and study formulas.^{11–19} After weaning, however, the differences in serum cholesterol concentrations moderate, with no consistent differences seen from 1 to 16 years of age.^{11,20–24} In most adult studies, both total cholesterol and low-density lipoprotein (LDL) cholesterol levels were lower among adults who had been breastfed as infants.^{2,3,25–27} Arising out of the aforementioned observations are numerous speculations concerning the mechanisms through which neonatal dietary cholesterol may be responsible for long-lasting perturbations of cholesterol metabolism.^{28–33} It has been hypothesized that differences in plasma lipid concentrations in infancy and adulthood might be accounted for in part by changes in endogenous cholesterol fractional synthesis rates (FSRs), modulated by the quantity of dietary cholesterol.^{34–36} Theoretically, adaptations in synthesis rates related to cholesterol exposure from infancy might persist and be the definitive mechanism through which cholesterol metabolism is imprinted.³⁷ An adjustment in the cholesterol FSR, caused by either high dietary cholesterol levels (as for breastfed infants) or low intake of dietary cholesterol (typical of formula-fed infants), could potentially alter the adult metabolic response

to dietary cholesterol. Studies among infants showed that the cholesterol FSR is inversely related to cholesterol intake at 4 months of age.^{16–19} However, the formulas used different levels of phytosterols, phytoestrogens, and hormones, confounded by different fatty acid profiles and questions regarding the bioavailability of supplemental cholesterol.¹⁹ The present study attempted to address all of the aforementioned issues by feeding identical formulas that differed only in terms of cholesterol content; the modified cow's milk formula (MCF) was supplemented with a more bioavailable form of cholesterol to an intermediate level between regular CF and HM, to elucidate clearly the relationship between dietary cholesterol, plasma lipids, and FSR. An understanding of the impact of dietary cholesterol on these variables and metabolic parameters, taken with the plausibility of imprinting, could have significant ramifications for the production of infant formulas, by pointing to a benefit from supplementation with cholesterol.

The purpose of this study was to determine whether the level of dietary cholesterol in early life induced changes in cholesterol FSRs and plasma lipid profiles that persisted beyond weaning at 18 months and to assess whether an intermediate level of cholesterol supplementation to CF resulted in a corresponding intermediate FSR. It was hypothesized that, at 4 months of age, infants fed HM would have a lower FSR, compared with those fed MCF, who would have a lower FSR than those fed CF. The level of dietary cholesterol would vary inversely with endogenous cholesterol FSR. Furthermore, at 18

months, the pattern of FSR would remain similar to that of infants at 4 months of age.

METHODS

Subjects, Study Design, and Protocol

A total of 68 healthy term infants who were of appropriate size for gestational age and who had no parental history of hypercholesterolemia or hypertriglyceridemia were recruited during the first 2 weeks of life. This double-blind, partially randomized, prospective, clinical trial took place at the Cincinnati Children's Hospital Medical Center (CCHMC) and other area hospitals between January 1999 and June 2002. Fifty-two infants were monitored until 4 months of age; 47 were monitored until 18 months of life on an unrestricted diet (Fig 1). The HM group included 18 infants who were breastfed exclusively until 6 months of age, after which they received HM supplemented with intake of MCF until 12 months of age; in this way, the HM group served as a control group with continuous high cholesterol intake. Solids were introduced after 6 months by parents or physicians. The remaining infants were randomized by the study coordinator, according to a computer-generated, random-number table, to receive MCF (ready-to-serve Carnation Good Start plus 40 mg/L cholesterol; Nestlé Laboratories, Eau Claire, WI) ($n = 16$) or CF (ready-to-serve Carnation Good Start; Nestlé Laboratories) ($n = 18$). HM-fed infants could not be randomized, because breastfeeding involves an a priori decision and commitment on the part of the mother. The added cholesterol (R.W. Greeff and Co [Stamford, CT], as Cholesterol National Formulary) was solubilized with a small quantity of ethanol. The ethanol was then evaporated, and the cholesterol was distributed evenly throughout the formula by the manufacturer. MCF- and CF-fed infants received only the assigned formula, with introduction of solid foods after the age of 4 months by parents or physicians. Sample sizes at 18 months were as follows: HM, $n = 15$; MCF, $n = 15$; CF, $n = 17$. The cholesterol contents of HM, MCF, and CF were 120, 80, and 40 mg/L, respectively, as shown in Table 1.

The study was composed of 2 test periods, the first from recruitment to 4 months of age and the second from 4 months of age

Fig 1. Study design and protocol. Plasma lipids include total cholesterol, LDL cholesterol, HDL cholesterol, very low-density lipoprotein cholesterol, and triglycerides. Diet histories were analyzed for average energy intake per day and average cholesterol per day.

TABLE 1. Composition of Types of HM and Formulas Used

	HM*	MCF	CF
Energy, kJ/L	2847	2792	2792
Protein, g/L	10.5	15.1	15.1
Carbohydrates, g/L	72.0	74.7	74.7
Fat, g/L	39.0	34.2	34.2
Polyunsaturated/saturated ratio	0.3	0.46	0.46
Cholesterol, mg/L	120.0	80.0	40.0
Linoleic acid, mg/L	3971	5695	5695

* The composition of HM was determined from literature reports.^{17–19} HM varies with stage of lactation and among mothers. The compositions of formulas were based on the manufacturer's estimates.

to the end of the study at 18 months of age. Period 1 evaluated the effects of cholesterol supplementation of infant formulas on cholesterol FSR at 4 months of age; at this age, infants receive exclusively HM or formula, thus eliminating the potential for interference from other dietary components. At 18 months, typically infants have been introduced to solid foods. Therefore, the purpose of period 2 was to evaluate the imprinting hypothesis. The outcome measures of FSR, serum lipid profiles, and weight were recorded at 4 and 18 months (Fig 1).

The cholesterol content was the only difference in composition between MCF and CF. The stability and solubility of the cholesterol concentration in the formula were tested by the manufacturer at different time points, to assess the composition and ensure bioavailability. All infants began receiving formula within the first 3 to 7 days of life. Formula was provided to the subjects at no cost for the entire duration of the study, to improve compliance. Breast milk cholesterol content was analyzed for each mother of a breast-feeding infant at 4, 8, and 12 months. Mothers from the 3 groups were required to keep bimonthly, 3-day, diet diaries recording either the volume of formula intake per day (MCF and CF groups) or the frequency of breastfeeding and the volume of supplemental MCF per day after the infants were 6 months of age (HM group). During period 2, the 3-day, diet diary recordings were obtained every 3 months. The food records were analyzed by a registered dietitian with the Food Processor (version 7.4; ESHA Research, Salem, OR), to determine whether any significant deviations in food intake occurred that might confound the outcome variable of FSR. Total energy intakes, as well as fat and cholesterol levels, were compared among the diet groups. The study protocol was reviewed and approved by the institutional review board at the CCHMC, and informed consent was obtained from parents before enrollment of the infants.

Plasma Lipid Analyses

Plasma total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, and LDL cholesterol levels were determined with enzymatic techniques validated by the National Institutes of Health Lipid Research Clinics and used previously for infants.^{16–19}

Cholesterol Biosynthesis Measurements

During the 2-day FSR study period, 2 blood specimens were obtained by CCHMC General Clinical Research Center nurses, placed on ice, and centrifuged within 30 minutes. On day 1, 8 mL of blood were obtained to determine baseline body water and erythrocyte membrane cholesterol deuterium enrichment. Infants were then given 500 mg/kg body weight deuterium oxide (99.96% deuterium; Isotec, Miamisburg, OH) orally. On day 2, 8 mL of blood were obtained to determine excess deuterium enrichment. All blood samples were obtained between 9:00 AM and 12:00 noon. For each day, the sample was fractionated and frozen at –80°C until analysis. The plasma was used for determination of lipid concentrations and the red blood cell fraction was used for cholesterol FSR determination.

Cholesterol FSRs were determined as the rate of incorporation of the stable isotopic compound deuterium oxide from body water into red blood cell membrane cholesterol, which serves as an index of hepatic cholesterol synthesis rates. The analytical procedure for FSR has been described previously.^{18,19}

Statistical Analyses

Variables were tested for normality and, in cases of nonnormality, variables were natural-logarithmically transformed. Percent changes were calculated as the average of the differences between outcome variables at 18 and 4 months divided by the value at 4 months. One-way analysis of variance was used to test the effect of infant diet on outcome measures and percent changes (SAS software, version 8; SAS Institute, Cary, NC), with 4-month outcome variables included as covariates for testing the effect of infant diet at 18 months. The Tukey-Kramer test was used to adjust for multiple comparisons, to determine differences between pairs of groups. With a SD of 1.11 for the FSR observed for CF-fed infants at 4 months in previous studies,¹⁹ a sample size of 14 in each group was predicted to yield a power of 80% in detecting a 20% difference in FSR means between groups with an α value of .05 for a 2-sided test. The analysis at 18 months included all participants for whom data at 4 months existed. Statistical significance was considered for $P < .05$. Results are presented as means $\pm$ SEMs and, where applicable, as geometric means.

RESULTS

Body weights are summarized in Table 2. There were no significant differences in weight between the diet groups at any time during the study. Sixty-eight infants were recruited, 52 infants completed the first test period at 4 months, and 47 continued and completed the second test period at 18 months. During the first period, dropping out was largely attributable to parent preference ($n = 12$) or families lost to follow-up monitoring ($n = 2$). In the HM group, additional reasons for dropping out included introduction of formula before 4 months ($n = 1$) and unwillingness to participate in the blood drawing ($n = 1$). Reasons for dropping out during the second test period included introduction of formula and weaning from HM before 1 year of age in the HM group ($n = 3$) and families lost to follow-up monitoring, because of moving, in the MCF and CF groups ($n = 2$). No adverse effects in response to the formulas used were reported. There was insufficient blood drawn to determine FSR at 4 months for 1 infant in the MCF group; this was not a problem at 18 months. The average caloric and cholesterol intakes at 4 and 18 months of age are shown in Table 3. Results at 18 months are shown with adjustment for the corresponding 4-month outcome variables, which were included as covariates in the statistical analysis. The infants' gender was included in the statistical model, with no significant effect on FSRs or plasma lipid profiles. During the course of the study, CF-fed infants had the largest percent change in dietary cholesterol levels from 4 to 18 months, and HM-fed infants had the largest percent change in caloric intake.

At 4 months, plasma total cholesterol concentra-

TABLE 2. Effect of Infant Diet on Body Weights

Age, mo	Body Weight, kg			<i>P</i>
	HM	MCF	CF	
0	3.5 $\pm$ 0.1	3.3 $\pm$ 0.1	3.5 $\pm$ 0.1	.0649
4	6.8 $\pm$ 0.2	6.6 $\pm$ 0.2	7.0 $\pm$ 0.2	.3358
18	11.1 $\pm$ 0.3	11.7 $\pm$ 0.4	11.7 $\pm$ 0.3	.4025

Values are mean $\pm$ SEM. Sample sizes were as follows. HM: birth, $n = 18$; 4 months, $n = 18$; 18 months, $n = 15$; MCF: birth, $n = 16$; 4 months, $n = 16$; 18 months, $n = 15$; CF: birth, $n = 18$; 4 months, $n = 18$; 18 months, $n = 17$.

TABLE 3. Average Dietary Cholesterol and Energy Intakes

	HM	MCF	CF	P
Average dietary cholesterol, mg				
4 mo	124.2 ± 2.2	126.0 ± 5.5	59.6 ± 3.0*	<.0001
18 mo	119.1 ± 14.3	182.8 ± 24.0	156.5 ± 16.2	.0685
Change, %†	−4	45	163‡	<.0001
Average energy, kJ				
4 mo	2593.3 ± 46.4§	3631.6 ± 159.9	3746.8 ± 185.9	<.0001
18 mo	4507.1 ± 259.6	5213.8 ± 255.0	4919.5 ± 219.0	.5474
Change, %†	74§	44	31	.0045

Values are mean ± SEM. Sample sizes were as follows. HM: 4 months, *n* = 18; 18 months, *n* = 15; MCF: 4 months, *n* = 16; 18 months, *n* = 15; CF: 4 months, *n* = 18; 18 months, *n* = 17.

* CF versus HM, CF versus MCF, *P* < .0001.

† Presented as arithmetic means, calculated as the difference between FSR group means divided by the 4-month FSR group mean and multiplied by 100.

‡ CF versus HM, *P* < .0001; CF versus MCF, *P* = .002.

§ HM versus MCF, HM versus CF, *P* < .0001; HM versus MCF, *P* = .0420; HM versus CF, *P* = .0114.

tions of infants fed HM (4.07 ± 0.15 mmol/L) or MCF (3.85 ± 0.16 mmol/L) did not differ statistically, but both were higher (*P* < .02) than concentrations for infants fed CF (3.28 ± 0.15 mmol/L) (Table 4). HDL cholesterol levels were higher (*P* = .005) in the MCF group (1.43 ± 0.07 mmol/L) than in the HM (1.09 ± 0.06 mmol/L) and CF (1.15 ± 0.06 mmol/L) groups. LDL cholesterol levels were higher in the HM group (2.08 ± 0.11 mmol/L) than in the MCF (1.56 ± 0.12 mmol/L, *P* < .003) and CF (1.20 ± 0.11 mmol/L, *P* < .0001) groups; the mean LDL cholesterol level in the MCF group was also higher (*P* = .0321) than that in the CF group. There were no statistically significant differences among groups in very low-density lipoprotein cholesterol and triglyceride levels (data not shown). The total cholesterol/HDL cholesterol ratio was lower in the MCF (2.76 ± 0.17 , *P* < .0001) and CF (2.96 ± 0.16 , *P* = .004) groups than in the HM group (3.81 ± 0.16) (Table 4). At 18 months, there was a nonsignificant trend toward lower plasma total cholesterol, HDL cholesterol, and LDL cholesterol levels in the HM group, compared with the MCF and CF groups (Table 4).

FSR at 4 months was affected by infant feeding (*P* < .05) among the 3 groups (Fig 2). The FSR in the HM group (0.034 ± 0.005 pools per day) was lower (*P* < .02) than that in the CF group (0.052 ± 0.005

pools per day); however, there was no difference between the HM and MCF (0.047 ± 0.005 pools per day) groups or between the MCF and CF groups, as shown in Table 5. At 18 months, there was no significant effect of earlier infant feeding or any differences in FSR among the 3 dietary groups. The percent change between the FSR at 4 months and that at 18 months is presented in Table 5; the HM group exhibited the largest absolute percent change in FSR, whereas the FSRs for the formula groups showed smaller differences in the 2 test periods (Table 5).

DISCUSSION

This is the first study to evaluate the potential imprinting of FSR beyond 1 year of age. We demonstrated decreased cholesterol synthesis and increasing circulating plasma cholesterol concentrations at 4 months of age as the level of dietary cholesterol increased among CF-, MCF-, and HM-fed infants. However, the differences seen as a result of infant feeding were not reflected at 18 months of age, which suggests that there is no imprinting of cholesterol biosynthesis or lasting differences in plasma lipid profiles at early life cycle stages attributable to the level of cholesterol intake before weaning.

The positive relationship between dietary cholesterol intake and serum lipid levels in the neonatal

TABLE 4. Effect of Infant Diet on Plasma Lipid Concentrations

	HM	MCF	CF	P
Total cholesterol, mmol/L				
4 mo	4.07 ± 0.15	3.85 ± 0.16	3.28 ± 0.15*	.0013
18 mo	3.58 ± 0.16	3.80 ± 0.15	4.12 ± 0.16	.0931
HDL cholesterol, mmol/L				
4 mo	1.09 ± 0.06	1.43 ± 0.07†	1.15 ± 0.06	.0018
18 mo	0.98 ± 0.04	1.10 ± 0.04	1.06 ± 0.04	.1496
LDL cholesterol, mmol/L				
4 mo	2.08 ± 0.11‡	1.56 ± 0.12§	1.20 ± 0.11	<.0001
18 mo	1.87 ± 0.17	2.19 ± 0.14	2.46 ± 0.15	.0736
Total cholesterol/HDL cholesterol				
4 mo	3.81 ± 0.16	2.76 ± 0.17	2.96 ± 0.16	<.0001
18 mo	3.67 ± 0.20	3.69 ± 0.18	4.02 ± 0.16	.2293

Values are mean ± SEM. Sample sizes were as follows. HM: 4 months, *n* = 18; 18 months, *n* = 15; MCF: 4 months, *n* = 16; 18 months, *n* = 15; CF: 4 months, *n* = 18; 18 months, *n* = 17.

* CF versus MCF, *P* = .0104; CF versus HM, *P* = .0004, at 4 months.

† MCF versus HM, *P* = .0008; MCF versus CF, *P* = .0046, at 4 months.

‡ HM versus MCF, *P* = .0027; HM versus CF, *P* < .0001, at 4 months.

§ MCF versus CF, *P* = .0321, at 4 months.

|| HM versus MCF, *P* < .0001; HM versus CF, *P* = .004, at 4 months.

Fig 2. Effect of infant diet on FSR of endogenous cholesterol at 4 months (white bars) and 18 months (patterned bars). *The FSR for the HM group was lower ($P = .0171$) at 4 months, compared with the CF group. At 18 months, there were no significant differences between groups. The differences in percent change within each group were not statistically significant. Sample sizes were as follows: HM: 4 months, $n = 18$; 18 months, $n = 15$; MCF: 4 months, $n = 16$; 18 months, $n = 15$; CF: 4 months, $n = 18$; 18 months, $n = 17$.

TABLE 5. Effect of Infant Diet on FSR of Endogenous Cholesterol

	FSR, Pools per Day			<i>P</i>
	HM	MCF	CF	
4 mo	0.034 ± 0.005*	0.047 ± 0.006	0.052 ± 0.005	.0479
18 mo	0.052 ± 0.007	0.053 ± 0.007	0.047 ± 0.006	.8232
Change, %†	53	13	-10	.4790

Values are mean ± SEM. Sample sizes were as follows. HM: 4 months, $n = 18$; 18 months, $n = 15$; MCF: 4 months, $n = 16$; 18 months, $n = 15$; CF: 4 months, $n = 18$; 18 months, $n = 17$.

* HM versus CF, $P = .0171$.

† Calculated as the difference between FSR group means divided by the 4-month FSR group mean and multiplied by 100.

period is well established in both animal⁴⁻¹⁰ and infant¹¹⁻¹⁹ studies. The higher HDL cholesterol levels exhibited by the MCF-fed infants (Table 4), compared with HM- and CF-fed infants, might be attributable in part to differences in the form of cholesterol in HM and MCF. HM cholesterol contains both free cholesterol (85%) and esterified cholesterol (15%) as components of the total cholesterol content,³⁸ whereas the MCF contained 100% free cholesterol. Other differences in formulas that could affect serum lipids include a larger proportion of medium-chain fatty acids, a smaller proportion of longer-chain polyunsaturated fatty acids, and higher phytosterol and galactose concentrations.³⁹ The present study confirms and reinforces the concept that dietary cholesterol in infancy elevates plasma total cholesterol levels through a direct mechanism and that the effect persists only until weaning. At 18 months, plasma total cholesterol, HDL cholesterol, and LDL cholesterol levels tended to be lower among the HM-fed infants, compared with MCF- and CF-fed infants; however the differences were not significant. A larger sample size and better control of dietary cholesterol might have resulted in significant differences; however, the study sample size was determined for differences in FSR and not plasma lipid levels. Previous human infant studies showed a significant inverse relationship between dietary cholesterol and FSR at 4 months.¹⁶⁻¹⁹ With deuterium incorporation methods, previous investigators estimated that endogenous cholesterol FSR ranged from 0.02 pools per day among breastfed infants to 0.11 pools per day among infants fed formulas with varying concentrations of cholesterol.

The present study demonstrated an effect of infant diet on FSR at 4 months, with CF-fed infants having an up-regulated rate of endogenous cholesterol production, compared with HM-fed infants. The infants

receiving MCF had a FSR that was intermediate between those of the HM- and CF-fed infants, which suggests that the cholesterol supplementation brought the FSR of the MCF-fed infants closer to the physiologic range seen among breastfed infants. These results demonstrate that adaptive regulatory mechanisms in early infancy enable human infants to respond to differences in cholesterol intakes. Theoretically, these homeostatic mechanisms could prevent excess cholesterol accumulation during high cholesterol intakes or, conversely, provide for an increase in cholesterol availability during instances of low or negligible intake. With the assumption that HM is the standard for infant nutrition and values for FSR among HM-fed infants are considered to be "normal," then these data indicated that CF-fed infants had a 53% increase in FSR, compared with HM-fed infants, during the first 4 months of life. This is indicative of the need for cholesterol in early infancy, a period of rapid growth. The results seen for MCF-fed infants are in contrast to those seen in the study by Bayley et al,¹⁸ in which infants fed cholesterol-supplemented formula had FSRs similar to those of infants fed regular formula at 4 months. No differences in FSR were seen before or after cholesterol challenge at 11 and 12 months of age¹⁹; however, the bioavailability of the cholesterol in the study formula was suspect. In the present study, there were no differences in FSRs among the dietary groups at 18 months; the HM- and MCF-fed infants demonstrated increases in endogenous cholesterol production, whereas the CF-fed infants had a slight decrease in FSR. Small sample sizes and individual variability in FSR with time precluded the observation of a statistically significant difference in percent changes among the groups from 4 to 18 months. At 18 months, it appears that FSR responds to the level of dietary cholesterol. The HM-fed infants had a 4%

decrease in average cholesterol intake per day, and the increase in FSR would compensate for this. Despite dramatic increases in cholesterol intake, the absence of changes in 18-month FSRs for the MCF- and CF-fed infants suggests a form of up-regulation; its permanency is an issue that deserves additional research.

Breastfeeding is associated with lower cholesterol production rates in baboons⁹ and pigs⁷ in the neonatal period. Increases in hepatic hydroxymethylglutaryl-coenzyme A reductase activity in formula-fed neonatal pigs and rats^{7,28} confirmed the presence of feedback inhibition between dietary cholesterol and endogenous cholesterol production. Breastfed baboons as infants had higher levels of hepatic acyl-coenzyme A-cholesterol acyltransferase activity, higher concentrations of hepatic cholesterol esters, and lower plasma lecithin-cholesterol acyltransferase activity, compared with formula-fed baboons; breastfed baboons metabolized exogenous cholesterol, whereas formula-fed baboons relied on *de novo* cholesterol synthesis as their principal source of cholesterol.³⁰ Breastfeeding in baboons also led to increases in LDL receptor mRNA levels of 44% to 99%, compared with formula feeding, and the increases persisted into adolescence.^{31,32} This suggests that long-term cholesterol homeostasis could be affected by the level of dietary cholesterol in the infant diet. However, these effects were not seen in guinea pigs¹⁰ and, among human fetuses, increases in hepatic LDL receptor activity were associated positively with gestational age and were correlated inversely with serum total cholesterol and LDL cholesterol levels.⁴⁰ The current study confirms feedback inhibition of cholesterol synthesis among human infants, dependent on exposure to dietary cholesterol.

It has been postulated that approximately one half of the difference in FSR between the HM- and formula-fed groups at 4 months can be explained by an expanded cholesterol central pool.¹⁶ The remainder is most likely attributable to down-regulation of hydroxymethylglutaryl-coenzyme A reductase and cholesterol synthesis. The expansion of the central pool may be attributable to increased absorption of dietary cholesterol among breastfed infants, coupled with modulation of LDL receptor activity in the liver. The exact mechanisms and the physiologic health implications deserve additional research to determine the effects of early dietary cholesterol on absorption and LDL receptor expression and activity, as well as whether such effects persist. However, such studies might be difficult to conduct in an infant population, because of the large amount of blood needed and the high costs associated with long-term cohort monitoring. To date, the present study is unique in examining prospectively the effects of early cholesterol on FSR beyond 1 year of age. Assessment at 18 months of age may be too early to see an effect, because differences in plasma lipid profiles between subjects breastfed or formula fed as infants generally have not been seen until >17 years of age.^{2,3,24-27}

The function of the higher cholesterol content in

HM has been the subject of debate for several decades, especially because infant formula composition has stood in stark contrast in this regard. The advancement of knowledge in this area has been encumbered by the difficulty of separating the metabolic effects of dietary cholesterol from those of dietary fatty acids, because HM differs from most formulas in this domain as well. Individual fatty acids can affect cholesterol metabolism, affecting serum lipoprotein concentrations independent of the intake of dietary cholesterol.^{12-14,41} Because the fatty acid profile and content of HM are modified by maternal diet^{15,42,43} and vary throughout the feeding period, the fatty acid composition cannot be mirrored by formulas. No direct inferences with respect to the effects of fatty acids on cholesterol metabolism can be made in this study, and a potential confounding effect of fatty acid composition cannot be ruled out.

A rather permissive level of dietary control during the second year of life was part of the study design. An absence of regulations governing solid food intake allowed for more normal variations and a more "real-life" test of the imprinting hypothesis, with 3-day diet records being used to assess the variation. Differences in infant diets related to characteristics of mothers who chose to breastfeed, compared with those who formula fed, would also be detected with 3-day diet records, which have been an acceptable reliable method used to estimate variations in dietary intake among infants.¹⁷⁻¹⁹ However, additional work is needed to test thoroughly the hypothesis by Reiser and Sidelman⁴ that the level of early cholesterol intake may protect subjects against later hypercholesterolemia. The current study design did not incorporate a cholesterol challenge, and the possibility of monitoring the cohort and incorporating a challenge, while controlling for other environmental factors, needs to be considered.

In this study, as in previous infant studies,¹⁶⁻¹⁹ limited blood sample size did not allow more comprehensive analysis of cholesterol metabolism. Because FSR was the main outcome variable in this study, fecal cholesterol excretion was not measured; complete collection of stools during infancy would have been difficult because of the stool consistency.

Methods for determination of endogenous cholesterol synthesis on the basis of deuterium incorporation have been well established^{16,44,45} and are advantageous because they are direct, short-term, noninvasive methods, compared with intake balance methods⁴⁶⁻⁴⁸ and isotopic kinetic decay analyses.⁴⁹⁻⁵¹ The efficacy of using erythrocyte cholesterol deuterium enrichment to study human lipid metabolism has been described previously.^{16-19,45} Measurement of cholesterol synthesis through deuterium incorporation has been validated against sterol balance analysis,⁵² mass isotopomer distribution analysis,⁵³ and sterol precursor analysis.⁵⁴

CONCLUSIONS

We examined for the first time endogenous cholesterol FSRs among human infants >1 year of age, determining that, although early intake of cholesterol

affects FSR and plasma lipid levels at 4 months, the differences observed do not persist at 18 months. This indicates that there is no imprinting of cholesterol biosynthesis at early life cycle stages with differing dietary levels of cholesterol in infancy.

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LIFE SUPPORT: HOSPITALS ON THE BRINK

“The hospital business in New York, one of the largest and most prized sectors of the region’s economy, is deep in financial trouble, which is forcing it into a sharp, swift contraction. Across the city and state, public and industry officials agree, hospital doors are likely to begin swinging shut over the next year, and thousands of jobs could be lost. Twelve New York hospitals have closed in the last 27 months, and others have shut wings, wards and clinics. The industry as a whole has lost money five years in a row in New York, while turning a profit nationally each year. Even some of New York’s biggest, most sophisticated teaching hospitals, like Mount Sinai and St. Vincent’s in Manhattan, have been hemorrhaging money. Just last week, country officials scrambled to assemble a cash infusion for Westchester Medical Center. Though it has lost 20,000 beds in the last 15 years, New York still has almost 20,000 more hospital beds than it needs, according to Dennis P. Whalen, the state’s executive deputy health commissioner—nearly one third of the current total, equivalent to dozens of hospitals and tens of thousands of jobs. Hospital officials call that figure exaggerated, but they concede that whatever the true number, it points toward a wave of closings still to come.”

Pérez-Peña R. *New York Times*. April 11, 2005

Noted by JFL, MD

Effects of Early Cholesterol Intake on Cholesterol Biosynthesis and Plasma Lipids Among Infants Until 18 Months of Age

Théa A. Demmers, Peter J. H. Jones, Yanwen Wang, Susan Krug, Vivian Creutzinger and James E. Heubi

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