Effect of dietary cholesterol on cholesterol synthesis in breast-fed and formula-fed infants

William W. Wong,[1] David L. Hachey, William Insull, Antone R. Opekun, and Peter D. Klein

USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX 77030

Abstract The fractional synthesis rate (FSR) of cholesterol was measured in 6 breast-fed and 12 formula-fed infants (ages 4 to 5 months) using the ^2H_2O method. The breast-fed infants had higher cholesterol intakes (18.2 ± 4.0 vs. 3.4 ± 1.8 mg/kg per day, $P = 0.001$), plasma total cholesterol (183 ± 47 vs. 112 ± 22 mg/dl, $P = 0.013$), and plasma low density lipoprotein (LDL)-cholesterol (83 ± 26 vs. 48 ± 16 mg/day, $P = 0.023$) than the formula-fed infants (6.9 ± 2.6 vs. 2.1 ± 0.6 %/day, $P < 0.001$). Among all infants, there was a significant inverse relationship ($P = 0.002$, $r = 0.66$) between the FSR of cholesterol and dietary cholesterol intake. Our findings indicate that the greater cholesterol intake of the breast-fed infants was associated with elevated plasma LDL-cholesterol concentrations and that cholesterol synthesis in human infants may be efficiently regulated via HMG-CoA reductase when infants are challenged with high intakes of dietary cholesterol.—**Wong, W. W., D. L. Hachey, W. Insull, A. R. Opekun, and P. D. Klein.** Effect of dietary cholesterol on cholesterol synthesis in breast-fed and formula-fed infants. *J. Lipid Res.* 1993. **34:** 1403–1411.

Supplementary key words fatty acids • plasma cholesterol • LDL-cholesterol

Cholesterol is an essential component of cell membranes and is required for growth, replication, and maintenance (1). It is well documented that infants fed human milk have higher plasma cholesterol concentrations than formula-fed infants (2–5). This difference is assumed to result from the fact that the cholesterol content of human milk is greater than that of commercial formulas (150 mg/l vs. 0–50 mg/l) (3, 5–8). Results of animal studies suggest that the early postnatal ingestion of a diet high in cholesterol protects against high cholesterol challenges later in life (9–12). Among human studies, however, only one has shown lower plasma cholesterol levels in adults (68 32-year-old women) who were breast-fed rather than formula-fed for the first 5 months of infancy (13). Most longitudinal studies have revealed no statistically significant difference in plasma cholesterol levels in relation to early feeding regimens (14, 15). Thus, measurement of plasma cholesterol alone may not be sufficient to identify the effects of early postnatal cholesterol ingestion on cholesterol homeostasis later in life.

In animals, dietary cholesterol suppresses mevalonate synthesis and cholesterol synthesis (16–18) by feedback inhibition of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase activity (19). In adult humans, studies using radioactive tracers have described the regulation of HMG-CoA reductase by dietary cholesterol and the effect of dietary cholesterol on in vivo cholesterol synthesis, but the use of such tracers in healthy infants is unethical, and consequently, no such data exist for infants. The cholesterol synthesis rate can be studied in healthy infants, however, by using stable isotope tracers such as deuterium oxide (^2H_2O). The rate of incorporation of deuterium from body water into erythrocyte cholesterol (20, 21) can be determined because 22 of the 46 hydrogen atoms in cholesterol originate from body water (22). In addition, the ^2H_2O method has been used successfully to estimate the rapid response of cholesterol synthesis to dietary and drug perturbations (21, 23–26).

The purpose of the present study was to determine whether plasma cholesterol levels and cholesterol synthesis respond to the differences in cholesterol intake between breast-fed and formula-fed infants. Thus, we tested the hypothesis that cholesterol synthesis in human infants is regulated by an inhibition mechanism when infants are challenged with high intakes of dietary cholesterol.

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; TBW, total body water; FSR, fractional synthesis rate; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; apoA, apolipoprotein A-I; apoB, apolipoprotein B-100; MCSFA, medium-chain saturated fatty acids; PUFA, polyunsaturated fatty acids; LCSFA, long-chain saturated fatty acids; MUFA, monounsaturated fatty acids.

[1]To whom reprint requests should be addressed at: Children's Nutrition Research Center, 1100 Bates Street, Houston, TX 77030.

METHODS

Subjects

We studied 6 exclusively breast-fed and 12 exclusively formula-fed infants who were between 4 and 5 months of age. All infants were born after uncomplicated term pregnancies, had no complications during the neonatal period and early infancy, and were healthy at the time of study. Among the 12 formula-fed infants, 4 were fed SMA (Wyeth Laboratories Inc, Philadelphia, PA), 4 were fed Enfamil (Mead Johnson & Co, Evansville, IN), and 4 were fed Similac (Ross Laboratories, Columbus, OH). Upon admission to the Metabolic Research Unit at the Children's Nutrition Research Center, physical characteristics of the infants (body weight, length, and skinfold thicknesses at the triceps, biceps, and subscapular area) were measured by standard anthropometric techniques and total body water (TBW) was measured by the $H_2{}^{18}O$ dilution method (27, 28). The study was approved by the Institutional Review Board for Human Research at Baylor College of Medicine.

Nutrient intakes

Energy, fat, protein, lactose, and cholesterol intakes were determined from actual volumes of milk consumed and their nutrient concentrations. Human milk consumed by breast-fed infants was measured during the study by the test-weighing procedure (29). Formula consumed by the formula-fed infants was measured by weighing the bottles before and after each feed. All formula-fed infants had consumed the same formula since birth and continued to do so during the study. A 24-h milk sample was collected from each breast-feeding mother, and a bottle of each formula lot was saved for nutrient analysis. The energy, fat, protein, and lactose contents of human milk and formula were measured by standard biochemical analyses (30). Total cholesterol was determined after the addition of 3β-dihydrocholesterol (internal standard), solvent extraction, saponification, and purification by chromatography on 1-g silica solid-phase extraction columns. The sterols were acetylated with pyridine–acetic anhydride 20:3 (v/v) and analyzed by capillary gas chromatography using a Hewlett-Packard HP-5890 gas chromatograph (Hewlett-Packard Corp., Palo Alto, CA) with a 0.32 mm × 30 m DB capillary column at 250 to 330°C at 10°C/min. The fatty acid methyl esters were prepared as described earlier (31) and were analyzed with a Hewlett-Packard HP-5890A gas chromatograph (Hewlett-Packard Corp.) using a 0.25 mm × 30 m, 0.20 μm SP-2330 capillary column at 100 to 260°C at 5°C/min.

Plasma lipids

Plasma samples collected 2–4.5 h postprandially from each infant were pooled and plasma total cholesterol, triglyceride, high density lipoprotein-cholesterol (HDL-C), apolipoprotein A-I (apoA), and apolipoprotein B-100 (apoB) were measured by the Atherosclerosis Laboratory at The Methodist Hospital (Houston, TX) using standard methods (32). The Atherosclerosis Laboratory is a CDC certified reference laboratory. Low density lipoprotein-cholesterol (LDL-C) was calculated from total plasma cholesterol and HDL-C.

Cholesterol synthesis

Cholesterol synthesis was estimated by the ^2H_2O method (21) simultaneously with the measurement of total body water (TBW). After a baseline blood sample (8 ml) was collected, each infant received an oral bolus dose of ^2H_2O (500 mg/kg body weight; Isotec Inc., Miamisburg, OH) in addition to the $H_2{}^{18}O$ (60 mg ^{18}O/kg body weight; Isotec Inc., Miamisburg, OH). To maintain a constant level of ^2H enrichment in the plasma water during the study, each infant also received two oral doses of ^2H_2O (50 mg/kg) daily for the next 2 days. Additional 8-ml blood samples were collected from each infant at 12, 36, and 60 h after the administration of the bolus dose of ^2H_2O. Erythrocytes were harvested from the blood by centrifugation. Cholesterol was extracted from the erythrocytes, purified by preparative HPLC, and converted to CO_2 and H_2O by combustion. The H_2O produced from the combustion was reduced to H_2 for ^2H abundance measurement by gas-isotope-ratio mass spectrometry (33). Similarly, plasma water was reduced to H_2 for ^2H abundance measurement.

Kinetic analysis

A single-pool model was used to compute the fractional synthesis rate (FSR) of cholesterol in units of "percent per day" from the rise in ^2H enrichment of erythrocyte cholesterol (k_H) and the mean ^2H enrichment in plasma water (TBW_H), assuming 22 of the 46 hydrogen atoms (a ratio of 0.4732) in cholesterol came from body water (22) as follows:

$$\text{FSR (\%/d)} = \frac{100 \times 24 \times k_H}{0.4732 \times TBW_H}$$

We used a single-pool model based on four time points because of the limits on the total quantity of blood and the number of blood samples that can be taken from healthy infants. Our experience with adult human (25, 26) and animal (21) studies, in which blood samples were collected frequently, and with compartmental model simulations from published models (34) indicated that the single-pool model is a good approximation of the FSR of unesterified cholesterol in the central, rapidly miscible pool defined by compartmental analysis (35). The exchange between lipoprotein compartments is much more rapid than the sample interval used here would detect.

TABLE 1. Characteristics of the study infants

Variable	Breast-Fed (n = 6)	Formula-Fed			
		All (n = 12)	SMA (n = 4)	Enfamil (n = 4)	Similac (n = 4)
Age (wk)	17.7 ± 2.0[a]	19.3 ± 1.4	18.8 ± 1.3	20.0 ± 1.8	19.0 ± 1.2
Sex (M/F)	3/3	6/6	2/2	2/2	2/2
Weight (kg)	6.4 ± 0.8	7.1 ± 0.6	6.9 ± 0.3	7.2 ± 0.9	7.1 ± 0.7
Length (cm)	63.1 ± 1.6	64.6 ± 2.3	64.5 ± 1.2	64.4 ± 1.9	65.1 ± 3.7
Skinfold thicknesses					
Biceps (mm)	6.2 ± 1.4	7.1 ± 1.4	7.5 ± 1.3	7.3 ± 1.6	6.4 ± 1.4
Triceps (mm)	8.3 ± 1.9	9.1 ± 2.0	7.7 ± 1.7	9.9 ± 1.9	9.6 ± 2.2
Subscapular (mm)	7.7 ± 0.8	9.0 ± 1.9	9.1 ± 2.5	10.2 ± 1.3	7.8 ± 1.0
Total body water (%)[b]	61.0 ± 4.8	62.0 ± 5.3	65.5 ± 2.9	60.3 ± 5.6	60.2 ± 6.3
Body fat (%)[c]	23.8 ± 6.0	22.5 ± 6.7	18.1 ± 3.7	24.6 ± 6.9	24.7 ± 7.9

[a] Mean ± SD.
[b] Percentage relative to body wt.
[c] Percentage body fat = 100 − percentage total body water/0.8, where 0.8 is the hydration constant of lean body mass for the 4- to 5-month-old infants (53).

Statistical analysis

A two-sample *t*-test (36) was used to compare the physical characteristics, total body water, nutrient intakes, plasma lipid profiles, and cholesterol synthesis rates between the breast-fed and formula-fed infants. Regression analysis (36) was used to identify the effect of dietary intakes of cholesterol, fat, and energy on cholesterol synthesis, plasma total cholesterol, and plasma LDL-C concentrations.

RESULTS

The physical characteristics of the infants are summarized in **Table 1.** No significant differences were observed in the anthropometric measurements or total body water estimates between the breast-fed and formula-fed infants.

The nutrient composition of the human milk and formulas are summarized in **Table 2.** The nutrient composition of the human milk consumed by the breast-fed infants in the study is similar to that reported by other

TABLE 2. Nutrient composition of human milk and formulas

Variable	Breast-Fed (n = 6)	Formulas				
		All (n = 12)	Significance[a]	SMA (n = 4)	Enfamil (n = 4)	Similac (n = 4)
Energy (kcal/g)	0.64 ± 0.12[b]	0.67 ± 0.02	NS	0.67 ± 0.01	0.68 ± 0.01	0.66 ± 0.03
Protein nitrogen (mg/g)	1.40 ± 0.10	1.99 ± 0.08	<0.001	1.96 ± 0.01	1.98 ± 0.08	2.04 ± 0.10
Lactose (mg/g)	66.41 ± 2.23	75.67 ± 4.89	<0.001	81.56 ± 0.51	73.61 ± 3.79	71.84 ± 1.33
Cholesterol (μg/g)	142.0 ± 32.8	22.5 ± 11.1	<0.001	35.6 ± 4.1	21.5 ± 2.5	10.4 ± 1.8
Fat (mg/g)	31.1 ± 13.0	34.8 ± 0.8	NS	34.3 ± 0.9	35.2 ± 0.9	34.8 ± 0.4
Fatty acids (%):						
C8:0	0.04 ± 0.09	1.91 ± 1.05	<0.001	1.11 ± 0.66	2.72 ± 1.03	1.90 ± 0.92
C10:0	1.56 ± 0.60	1.66 ± 0.79	NS	1.04 ± 0.51	2.31 ± 0.72	1.63 ± 0.66
C12:0	5.90 ± 3.47	14.07 ± 5.62	0.002	9.05 ± 2.83	19.47 ± 4.23	13.70 ± 4.15
C14:0	7.32 ± 3.28	6.76 ± 1.69	NS	5.26 ± 0.73	8.75 ± 0.80	6.29 ± 0.89
C16:0	20.71 ± 1.37	11.86 ± 1.32	<0.001	13.40 ± 0.17	11.82 ± 0.30	10.35 ± 0.19
C16:1	1.68 ± 0.43	0.35 ± 0.47	<0.001	0.98 ± 0.00	0.04 ± 0.04	0.03 ± 0.04
C18:0	8.48 ± 1.78	6.14 ± 1.68	0.025	8.16 ± 0.72	5.42 ± 1.02	4.84 ± 0.65
C18:1	35.96 ± 4.30	26.50 ± 13.50	0.044	44.56 ± 2.71	16.75 ± 2.15	18.26 ± 1.63
C18:2	16.65 ± 2.25	27.10 ± 10.30	0.0054	14.69 ± 1.17	29.07 ± 3.14	37.94 ± 3.77
C18:3	1.20 ± 0.32	3.26 ± 1.59	0.001	1.44 ± 0.17	3.46 ± 0.52	4.88 ± 1.02
C20:0	0.05 ± 0.09	0.22 ± 0.20	0.031	0.25 ± 0.16	0.22 ± 0.27	0.19 ± 0.22
C20:1	0.07 ± 0.12	0.02 ± 0.08	NS	0.07 ± 0.15	0.00 ± 0.00	0.00 ± 0.00
C20:3	0.15 ± 0.17	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C20:4	0.24 ± 0.27	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
P/S[c]	0.42 ± 0.09	0.74 ± 0.35	0.012	0.43 ± 0.08	0.65 ± 0.13	1.13 ± 0.28

[a] NS, not significant at the 0.05 level, breast milk vs. formulas.
[b] Mean ± SD.
[c] P/S = ratio of polyunsaturated fatty acids/saturated fatty acids.

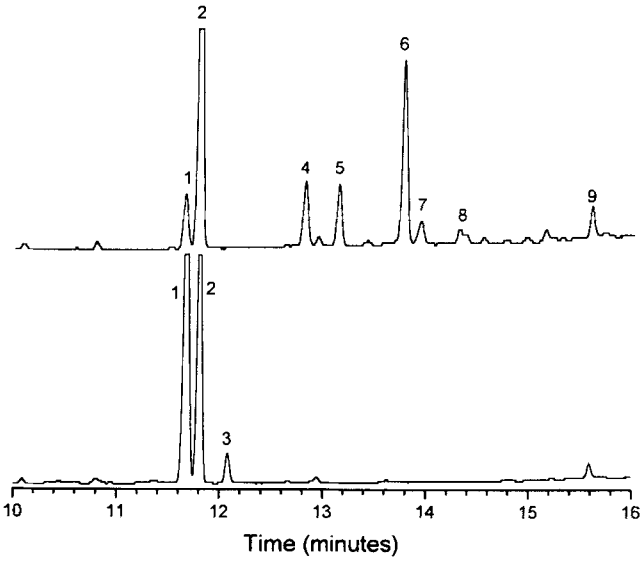

Fig. 1. Gas chromatograms of sterols in Similac (top) and in human milk (bottom). The individual peaks are (1) cholesterol; (2) β-dihydrocholesterol, internal standard; (3) desmosterol; (4) campesterol; (5) stigmasterol; and (6) β-sitosterol. The other peaks (7, 8, and 9) probably are plant sterols. However, identification of these peaks was not performed because reference spectra and standards were unavailable.

investigators (37, 38). The energy and fat contents of the human milk and formulas were similar. Significant differences between the human milk and formulas were observed, however, in their protein, lactose, cholesterol, and individual fatty acid contents. Human milk contained less protein and lactose, but approximately 6-fold more cholesterol, than the formulas. In addition, human milk contained less C8:0, C12:0, C18:2, and C18:3, but more C16:0, C16:1, C18:0, and C18:1 than the formulas. Among the three formulas, SMA had the highest concentration of cholesterol. The ratio of polyunsaturated to saturated fatty acids (P/S) in human milk was significantly different from the P/S ratio in the formulas.

The sterol contents of the formulas and the human milk are illustrated by the gas chromatograms in **Fig. 1.** The formulas contained significant amounts of plant sterols, which were not present in the human milk.

When standardized to body weight (**Table 3**), milk volume and lactose intakes did not differ significantly between feeding groups. The formula-fed infants consumed more energy, fat, and protein than the breast-fed infants. The breast-fed infants, however, consumed 5-fold more cholesterol than the formula-fed infants. Among the formula-fed infants, daily cholesterol intakes were the highest in the SMA group, intermediate in the Enfamil group, and the lowest in the Similac group. When fatty acid values were normalized to body weight, no significant difference was observed in the dietary intake of total saturated fatty acids between the two feeding groups. However, the formula-fed infants consumed more medium-chain saturated fatty acids (MCSFA) and polyunsaturated fatty acids (PUFA) but fewer long-chain saturated fatty acids (LCSFA) than the breast-fed infants.

Regression analyses indicated that significant correlations existed between plasma total cholesterol concentrations in these infants and their dietary intakes of protein ($P = 0.038$) and individual fatty acids such as C8:0 ($P = 0.014$), C12:0 ($P = 0.044$), C16:1 ($P = 0.024$), C18:2 ($P = 0.037$), C18:3 ($P = 0.008$), and C20:0 ($P = 0.051$). No significant relationship, however, existed between plasma total cholesterol concentrations in these fatty acids, MCSFA, ICSFA, LCSFA, MUFA, palmitic acid, and stearic acid. Most importantly, any significant rela-

TABLE 3. Milk and nutrient intakes of the breast-fed and formula-fed infants

Variable	Breast-Fed (n = 6)	Formula-Fed				
		All (n = 12)	Significance[a]	SMA (n = 4)	Enfamil (n = 4)	Similac (n = 4)
Milk (g/kg/day)	132.2 ± 38.6[b]	148.1 ± 16.7	NS	157.3 ± 18.0	140.3 ± 8.8	146.8 ± 20.4
Energy (kcal/kg/day)	81.8 ± 16.1	98.7 ± 10.2	0.05	105.0 ± 11.6	94.7 ± 6.2	96.4 ± 11.3
Fat (g/kg/day)	3.9 ± 0.9	5.1 ± 0.6	0.021	5.4 ± 0.6	4.9 ± 0.3	5.1 ± 0.7
Cholesterol (mg/kg/day)	18.2 ± 4.0	3.4 ± 1.8	0.0001	5.6 ± 0.8	3.0 ± 0.4	1.5 ± 0.2
Protein (g/kg/day)	1.2 ± 0.4	1.8 ± 0.2	0.0084	1.9 ± 0.2	1.7 ± 0.1	1.9 ± 0.3
Lactose (g/kg/day)	8.8 ± 2.6	11.2 ± 1.6	NS	12.8 ± 1.5	10.3 ± 0.7	10.5 ± 1.3
Fatty acid[c] (g/kg/day)						
MCSFA	0.05 ± 0.03	0.10 ± 0.03	0.0099	0.07 ± 0.01	0.13 ± 0.01	0.12 ± 0.02
ICSFA	0.44 ± 0.31	0.64 ± 0.18	NS	0.47 ± 0.11	0.76 ± 0.21	0.69 ± 0.09
LCSFA	0.92 ± 0.21	0.61 ± 0.26	0.017	0.76 ± 0.31	0.51 ± 0.25	0.56 ± 0.21
Total SFA	1.41 ± 0.47	1.35 ± 0.36	NS	1.30 ± 0.41	1.40 ± 0.48	1.36 ± 0.27
MUFA	1.19 ± 0.30	0.93 ± 0.66	NS	1.62 ± 0.69	0.50 ± 0.28	0.68 ± 0.29
PUFA	0.57 ± 0.12	1.04 ± 0.63	0.026	0.58 ± 0.26	0.96 ± 0.50	1.59 ± 0.66

[a] NS, not significant at the 0.05 level, breast-fed vs. formula-fed infants.
[b] Mean ± SD.
[c] MCSFA, medium-chain saturated fatty acids (C8:0, C10:0); ICSFA, intermediate-chain saturated fatty acids (C12:0, C14:0); LCSFA, long-chain saturated fatty acids (C16:0, C18:0); MUFA, monounsaturated fatty acids (C16:1, C18:1, C20:1); and PUFA, polyunsaturated fatty acids (C18:2, C18:3, C20:3, C20:4).

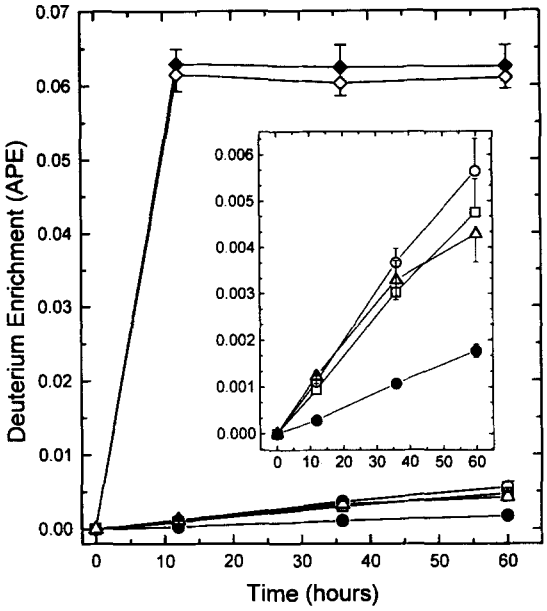

Fig. 2. The ^2H enrichments of the plasma water (breast-fed infants, ♦; formula-fed infants, ◊) and erythrocyte cholesterol (breast-fed infants, ●; formula-fed infants: SMA, □; Similac, ○; Enfamil, △).

tionship that existed between plasma cholesterol concentrations in these infants and their dietary intakes became insignificant when dietary cholesterol intake was included as a covariate in the regression analyses.

The ^2H enrichments of the plasma water and erythrocyte cholesterol of the infants following administration of the bolus and maintenance doses of ^2H_2O are illustrated in **Fig. 2.** The plasma water of both feeding groups reached an average plateau ^2H value of approximately 0.06 atom % excess in 12 h and remained fairly constant during the study. By 60 h (Fig. 2, insert), the erythrocyte cholesterol of the formula-fed infants reached ^2H values between 0.004 and 0.006 atom % excess. The erythrocyte cholesterol of the breast-fed infants, however, never reached ^2H values above 0.002 atom % excess.

Table 4 summarizes the FSR of cholesterol and the plasma lipid profiles of the infants. The mean FSR of the breast-fed infants was 3-fold less than that of the formula-fed infants. Plasma total cholesterol and LDL-C concentrations were higher in breast-fed than in formula-fed infants. There were no statistically significant differences in concentrations of plasma triglyceride, HDL-C, apoA, and apoB between the two feeding groups. However, the plasma apoB concentrations of the breast-fed infants were apparently elevated when compared to those of the formula-fed infants. One of the breast-fed infants had significantly higher plasma concentrations of cholesterol (268 mg/dl), triglyceride (689 mg/dl), HDL-C (113 mg/dl), LDL-C (117 mg/dl), and apoB (138 mg/dl) than the other breast-fed infants. When the data from this infant were excluded, the mean cholesterol FSR of the breast-fed infants remained 3-fold less than that of the formula-fed infants (2.0 ± 0.6 vs. 6.9 ± 2.6 %/day; $P < 0.001$). Although excluding these data reduced the variability of the plasma lipid and lipoprotein measurements among the breast-fed infants, concentrations of plasma total cholesterol (167 ± 23 vs. 112 ± 22 mg/dl; $P = 0.002$) and LDL-C (76 ± 23 vs. 48 ± 16 mg/dl; $P = 0.048$) remained significantly higher than those of the formula-fed infants.

Among all 18 infants, the FSR of cholesterol correlated inversely with cholesterol intake (**Fig. 3,** $P = 0.002$; $r = 0.66$). As shown in **Fig. 4,** plasma cholesterol ($P < 0.001$; $r = 0.77$) and LDL-C ($P = 0.002$; $r = 0.647$) were significantly correlated with dietary cholesterol intake. The FSR of cholesterol was also significantly correlated with dietary intakes of energy ($P = 0.032$), protein ($P = 0.005$), fat ($P = 0.015$), and individual fatty acids such as C8:0 ($P = 0.005$) and C16:1 ($P = 0.047$). However, when dietary cholesterol intake was included as a covariate in the regression analyses, dietary cholesterol intake became the sole factor significantly affecting the FSR in these infants.

TABLE 4. Fractional synthesis rates of cholesterol (FSR) and plasma lipid profiles of the breast-fed and formula-fed infants

Variable	Breast-Fed (n = 6)	Formula-Fed				
		All (n = 12)	Significance[a]	SMA (n = 4)	Enfamil (n = 4)	Similac (n = 4)
FSR (%/day)	2.1 ± 0.6[b]	6.9 ± 2.6	<0.001	7.4 ± 3.6	5.5 ± 2.0	7.8 ± 2.2
Cholesterol (mg/dl)	183 ± 47	112 ± 22	0.013	109 ± 5	134 ± 7	95 ± 25
Triglyceride (mg/dl)	318 ± 209	112 ± 57	NS	118 ± 83	137 ± 51	81 ± 11
HDL-cholesterol (mg/dl)	53 ± 32	42 ± 13	NS	36 ± 10	47 ± 11	43 ± 19
LDL-cholesterol (mg/dl)	83 ± 26	48 ± 16	0.023	49 ± 15	59 ± 7	35 ± 15
ApoA (mg/dl)	90 ± 35	110 ± 32	NS	98 ± 25	137 ± 36	97 ± 20
ApoB (mg/dl)	77 ± 36	51 ± 12	NS	54 ± 17	57 ± 4	42 ± 9

[a] NS, not significant at the 0.05 level, breast-fed vs. formula-fed.
[b] Mean ± SD.

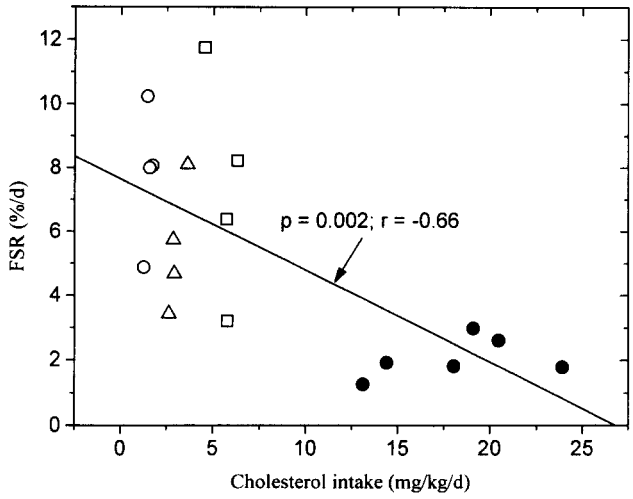

Fig. 3. The inverse relationship between FSR and dietary cholesterol intake (breast-fed infants, ●; formula-fed infants: SMA, □; Similac, ○; Enfamil, △).

DISCUSSION

Our data (Table 4) support previous observations (2–5) that breast-fed infants have higher plasma cholesterol levels than formula-fed infants, presumably because the cholesterol content of human milk (142 ± 33 mg/l) is 6-fold greater than that of the standard infant formulas based on bovine milk (23 ± 11 mg/l).

In adult studies (39–41), increased intake of dietary cholesterol, saturated fatty acids, such as palmitic acid (C16:0), and total energy has been shown to raise plasma total cholesterol and LDL-C concentrations. However, increased intake of stearic acid (C18:0), monounsaturated fatty acids (MUFA: C16:1, C18:1, C20:1), and PUFA (C18:2, C18:3, C20:3, C20:4) have been shown to decrease plasma total cholesterol levels in humans.

As shown in Table 3, our breast-fed infants consumed approximately 5-fold more cholesterol than the formula-fed infants. As illustrated in Fig. 4, an increase in the dietary cholesterol intake of these 4-month-old infants led to simultaneous increases in the plasma levels of total cholesterol and LDL-C but no significant increases in plasma HDL-C and apoB (Table 4). Fomon and Bartels (3) have also shown that when infant dietary cholesterol intake is manipulated, plasma cholesterol levels are altered, mainly because LDL-C levels change and HDL-C levels do not (3).

Cholesterol synthesis is especially difficult to measure in the human for several reasons. Its synthesis rate is low (< 5%/day), it is distributed into several kinetically distinct compartments, it has a complex transport system, and its synthesis is regulated by feedback inhibition of a single rate-limiting enzyme, which has a circadian oscillation (1, 12, 22, 34). Although radioactive tracer kinetic measurements have been made in adults, similar studies have not been undertaken in infants because of radiation exposure, frequent blood collections, and the likelihood that growing infants might not be in a steady-state over the duration of the study. The deuterium oxide method for estimating cholesterol synthesis, however, is ethically acceptable in infants, because deuterium oxide is nonradioactive, and the method requires only four blood samples with a total volume of less than 32 ml. The use of erythrocyte cholesterol for estimation of cholesterol synthesis also enabled us to use the plasma for plasma lipid profile measurements.

Erythrocyte free cholesterol has been shown to be in rapid exchange and to reach isotopic equilibrium with plasma free cholesterol within 24 h (42–44). Our first blood collection did not begin until 12 h after the administration of the bolus dose of ^2H_2O. Subsequent blood samples were collected at 36 and 60 h postdose. Therefore ^2H abundances of the erythrocyte free cholesterol and the FSR of cholesterol calculated from them would have to reflect the ^2H abundances and the FSR of cholesterol in the plasma free cholesterol. The paucity of samples, however, did limit the options available for a compartmental analysis of the data.

Our kinetics results reported here are based on the initial rate of incorporation of ^2H from body water into cholesterol. Our kinetic simulations using rate constants published by Goodman et al. (35) for adults suggested that their computations provide a close approximation to the true kinetic rate constant, if the ^2H enrichment is below 10% of the maximum attainable level. Because 4-month-old infants grow very slowly (~0.4%/day), they are in a de facto steady-state during the 3-day study.

In animals, dietary cholesterol inhibits cholesterol synthesis, as measured by both the ^2H_2O and [^{14}C]acetate methods (16–18). In rats, dietary cholesterol has been

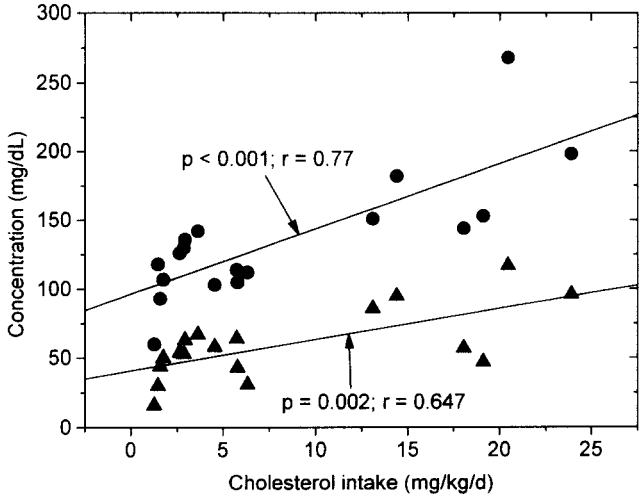

Fig. 4. The relationships between plasma total cholesterol (●) and LDL-C (▲) with dietary cholesterol intake.

shown to diminish HMG-CoA reductase activity in the liver (19). In addition, hepatic HMG-CoA reductase activity in 5-day-old, formula-fed pigs is greater than that of naturally fed pigs (45). Using the ^2H_2O method, we have demonstrated that lovastatin almost completely inhibits cholesterol synthesis in hypercholesterolemic adults following acute administration of the drug (25). The inhibition of cholesterol synthesis is obvious within 15 h of lovastatin therapy and precedes significant changes in plasma lipid levels. We also have demonstrated a 17-fold decrease in the FSR of cholesterol in obese pigs fed a diet containing 0.5% cholesterol versus pigs fed a diet containing no cholesterol (21).

The cholesterol synthesis measurements in this study may not reflect the true cholesterol synthesis rate for many reasons. However, the data probably portray with good fidelity the relative degree of synthesis in breast-fed and formula-fed infants. Implicit in this approach is the assumption that the mechanisms for deuterium incorporation from NADPH and body water into cholesterol are identical and that acetyl-CoA is not labeled during biosynthesis. These assumptions are reasonable for intergroup comparisons in subjects that were closely matched for age and nutrient intakes. Moreover, these assumptions were implicit in many earlier studies that have used this approach even though they are difficult to verify experimentally. In addition, the decreased cholesterol fractional synthesis rates seen in the breast-fed infants are likely to contribute to the apparent decrease in FSR. However, the relative contributions of each cannot be determined without measurements of absorption and pool size. Data from such studies would need to be analyzed with a comprehensive, steady-state compartmental model that described independent entry of cholesterol from diet and synthesis, and also the losses by catabolism to bile acids and cholesterol distribution to peripheral tissues. We have developed such a model for studies in adult subjects in whom multiple tracers are permitted and from whom frequent blood samples of sufficient volume can be collected to permit reliable analytical measurements. Such a complex protocol, however would be too invasive for studies in infants.

Although the absolute magnitude of the central pool is unknown in these infants, a 1.6-fold expansion in the plasma (and hepatic ?) cholesterol components of the central pool is insufficient to completely explain the 3.3-fold decrease in FSR. Only about one-half of the difference in FSR between the two feeding groups can be explained by an expanded central pool. The remainder is most likely due to down-regulation of HMG-CoA reductase and cholesterol synthesis. The expansion of the central pool is probably due to the increased absorption of dietary cholesterol in the breast-fed infants coupled with a down-regulation of LDL-receptor activity in the liver (46, 47). In this scenario, dietary cholesterol is absorbed by the intestine and enters the hepatocytes where it fulfills the metabolic needs of the cells. Consequently, the hepatocytes down-regulate both synthesis and receptor-mediated clearance of LDL-C via mechanisms that are poorly understood. Since intestinal absorption is not actively controlled by a feed-back inhibition system, the continual absorption of dietary cholesterol supplied at each nursing overwhelms the hepatic control systems and results in elevated plasma cholesterol.

Animal studies have shown the beneficial effect of feeding cholesterol in early postnatal life on cholesterol homeostasis in later life (9, 10). Studies of plasma cholesterol levels in children, however, have failed to document a protective effect of breast feeding as the children mature (13, 14). After infants have been weaned, their plasma cholesterol levels are indistinguishable, whether formerly breast-fed or formula-fed (4, 6, 7). Studies in juvenile and adult baboons that were breast-fed as infants show that the animals had lower cholesterol synthesis rates than the baboons that were formula-fed (11, 12). Using the ^2H_2O incorporation method, we found that the greater cholesterol intake of breast-fed infants resulted in a 3-fold suppression of cholesterol synthesis with concomitant increases in plasma total cholesterol and LDL-C concentrations (see Figs. 3 and 4, Tables 2 and 3).

Plant sterols are assumed to be hypocholesterolemic because they inhibit dietary cholesterol absorption (48). Fat in SMA, Enfamil, and Similac came predominately (15–45%) from soy. As shown in Fig. 1, the formulas consumed by our infants contained a significant amount of plant sterols, which were not present in the human milk. These plant sterols might have interfered with the intestinal cholesterol absorption in formula-fed infants, further minimizing the rise in the plasma cholesterol levels and stimulating the FSR of cholesterol.

The breast-fed infants consumed approximately 18 mg cholesterol/kg body weight per day, an amount almost 5-fold higher than the recommended cholesterol intake for an adult (4 mg/kg per day). If we assume an average growth rate of 20 g/day for a 4-month-old infant (49) and an average tissue cholesterol content of 1.8–3.2 mg/g of wet tissue (50), the cholesterol requirement for growth is between 36 and 64 mg/day, excluding brain, nervous tissues, and skin. Therefore, the cholesterol intake of the breast-fed infants exceeds the amount required for growth. The fate of the remainder of the dietary cholesterol in breast-fed infants is unknown, but some of it may be converted to bile acids. One report, in fact, has demonstrated an increased production of bile acids in low birth weight infants who were fed human milk (51).

In summary, our results indicated that dietary cholesterol is the predominating factor that affects plasma total cholesterol concentrations and cholesterol synthesis in infants. These observations are further supported by data in a separate study indicating that addition of

cholesterol to a soy formula led to increase in plasma total cholesterol concentrations with reciprocal down-regulation of cholesterol synthesis in six soy formula-fed infants (52). It is therefore possible that cholesterol synthesis might be down-regulated in children and adults who were breast-fed during infancy. However, a better understanding of the long-term effect of early postnatal ingestion of a large quantity of cholesterol will require a variety of experimental determinations on cholesterol absorption, synthesis, and excretion.

We are indebted to L. L. Clarke and M. Llaurador for performing the preparative HPLC purification of the erythrocyte free cholesterol and the gas-isotope-ratio mass spectrometric measurements; the MRU staff for their assistance in carrying out the protocol; M. Thotathuchery and N. F. Butte for the macronutrient analyses; L. B. Booth for the dietary cholesterol measurements; J. F. Gray for the fatty acid measurements; W. C. Heird, for his critical review of the manuscript; E. O. Smith for statistical assistance; E. R. Klein for editorial review; and C. Fedrick for preparation of the manuscript; we especially thank the parents and their infants who volunteered for the study. J. F. Gray was the recipient of a National Institutes of Health Minority High School Science Teacher Research Apprentice Program. We also thank Ross Laboratories, Mead Johnson & Co, and Wyeth-Ayerst Laboratories for providing the infant formulas used in this study. This work is a publication of the USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX. This project has been funded in part with federal funds from the US Department of Agriculture, Agricultural Research Service under Cooperative Agreement number 58-6250-1-003. The contents of this publication do not necessarily reflect the views or policies of the US Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Manuscript received 20 October 1992 and in revised form 1 March 1993.

REFERENCES

1. Myant, N. B. 1991. Cholesterol Metabolism, LDL, and the LDL Receptor. Academic Press Inc., San Diego, CA.
2. Woodruff, C., and J. G. Coniglio. 1959. Studies on lipids in infancy: maternal-infant differences and the effects of human and cow's milk feeding. *J. Dis. Child.* **98:** 658–659.
3. Fomon, S. J., and D. J. Bartels. 1960. Concentrations of cholesterol in serum of infants in relation to diet. *J. Dis. Child.* **99:** 27–30.
4. Darmady, J. M., A. S. Fosbrooke, and J. K. Lloyd. 1972. Prospective study of serum cholesterol levels during first year of life. *Br. Med. J.* **2:** 685–688.
5. Ginsburg, B. E., and R. Zetterstrom. 1980. Serum cholesterol concentrations in early infancy. *Acta Paediatr. Scand.* **69:** 581–585.
6. Friedman, G., and S. J. Goldberg. 1975. Concurrent and subsequent serum cholesterols of breast- and formula-fed infants. *Am. J. Clin. Nutr.* **28:** 42–45.
7. Huttunen, J. K., U. M. Saarinen, E. Kostiainen, and M. A. Siimes. 1983. Fat composition of the infant diet does not influence subsequent serum lipid levels in man. *Atherosclerosis.* **46:** 87–94.
8. Mattson, F. H., B. A. Erickson, and A. M. Kligman. 1972. Effect of dietary cholesterol on serum cholesterol in man. *Am. J. Clin. Nutr.* **25:** 589–594.
9. Reiser, R., and Z. Sidelman. 1972. Control of serum cholesterol homeostasis by cholesterol in the milk of the suckling rat. *J. Nutr.* **102:** 1009–1016.
10. Reiser, R., B. C. O'Brian, G. R. Henderson, and R. W. Moore. 1979. Studies on a possible function for cholesterol in milk. *Nutr. Rep. Int.* **19:** 835–849.
11. Mott, G. E., E. M. Jackson, C. A. McMahan, C. M. Farley, and H. C. McGill, Jr. 1985. Cholesterol metabolism in juvenile baboons. Influence of infant and juvenile diets. *Arteriosclerosis,* **5:** 347–354.
12. Mott, G. E., E. M. Jackson, C. A. McMahan, and H. C. McGill, Jr. 1990. Cholesterol metabolism in adult baboons is influenced by infant diet. *J. Nutr.* **120:** 243–251.
13. Marmot, M. G., C. M. Page, E. Atkins, and J. W. B. Douglas. 1980. Effect of breast-feeding on plasma cholesterol and weight in young adults. *J. Epidemiol. Community Health.* **34:** 164–167.
14. Glueck, C. J., R. C. Tsang, W. Balistreri, and R. Fallat. 1972. Plasma and dietary cholesterol in infancy: effects of early low or moderate dietary cholesterol intake on subsequent response to increased dietary cholesterol. *Metabolism.* **21:** 1181–1192.
15. Jooste, P. L., L. J. Rossouw, H. J. Steenkamp, J. E. Rossouw, A. S. P. Swanepoel, and D. O. Charlton. 1991. Effect of breast feeding on the plasma cholesterol and growth of infants. *J. Pediatr. Gastroenterol. Nutr.* **13:** 139–142.
16. Gould, R. G., and C. B. Taylor. 1950. Effect of dietary cholesterol on hepatic cholesterol synthesis. *Fed. Proc.* **9:** 179a.
17. Siperstein, M. D., and V. M. Fagan. 1966. Feedback control of mevalonate synthesis by dietary cholesterol. *J. Biol. Chem.* **241:** 602–609.
18. Peng, S. K., K. J. Ho, B. Mikkelson, and C. B. Taylor. 1973. Studies on cholesterol metabolism in rats by application of D_2O and mass spectrometry. *Atherosclerosis.* **18:** 197–213.
19. Ness, G. C., R. K. Keller, and L. C. Pendleton. 1991. Feedback regulation of hepatic 3-hydroxy-3-methylglutaryl-CoA reductase activity by dietary cholesterol is not due to altered MRNA levels. *J. Biol. Chem.* **266:** 14854–14857.
20. Rittenberg, D., and R. Schoenheimer. 1937. Deuterium as an indicator in the study of intermediary metabolism. XI. Further studies on the biological uptake of deuterium into organic substance, with special reference to fat and cholesterol formation. *J. Biol. Chem.* **121:** 235–253.
21. Wong, W. W., D. L. Hachey, A. Feste, J. Leggitt, L. L. Clarke, W. G. Pond, and P. D. Klein. 1991. Measurement of in vivo cholesterol synthesis from ^2H_2O: a rapid procedure for the isolation, combustion, and isotopic assay of erythrocyte cholesterol. *J. Lipid Res.* **32:** 1049–1056.
22. Dietschy, J. M., and D. K. Spady. 1984. Measurement of rates of cholesterol synthesis using tritiated water. *J. Lipid Res.* **25:** 1469–1476.
23. Jones, P. J. H., A. M. Scanu, and D. A. Schoeller. 1988. Plasma cholesterol synthesis using deuterated water in humans: effect of short-term food restriction. *J. Lab. Clin. Med.* **111:** 627–633.
24. Jones, P. J. H. 1990. Use of deuterated water for measurement of short-term cholesterol synthesis in humans. *Can. J. Physiol. Pharmacol.* **68:** 955–959.

25. Insull, W., D. L. Hachey, W. W. Wong, J. Jiang, B. W. Patterson, L. Clarke, and P. D. Klein. 1989. Acute effects of lovastatin on whole body cholesterol synthesis (WBCS) in man measured by deuterium incorporation. *Arteriosclerosis.* **9:** 741a.
26. Klein, P. D., J. Jiang, W. W. Wong, D. L. Hachey, and W. Insull, Jr. 1990. In vivo demonstration of cholesterol synthesis inhibition by cholesterol feeding in man. *Am. J. Clin. Nutr.* **51:** 516a.
27. Wong, W. W., W. J. Cochran, W. J. Klish, E. O. Smith, L. S. Lee, and P. D. Klein. 1988. In vivo isotope-fractionation factors and the measurement of deuterium- and oxygen-18-dilution spaces from plasma, urine, saliva, respiratory water vapor, and carbon dioxide. *Am. J. Clin. Nutr.* **47:** 1–6.
28. Prentice, A., A. Coward, T. Cole, D. Schoeller, and P. Haggarty. 1990. Practical recommendations and worked examples. *In* The Doubly-Labelled Water Method for Measuring Energy Expenditure. Technical Recommendations for Use in Humans. A. M. Prentice, editor. IAEA, Vienna, Austria. 212–247.
29. Jensen, R. G., and M. C. Neville. 1984. Human lactation. *In* Human Lactation: Milk Components and Methodologies. R. G. Jensen, and M. C. Neville, editors. Plenum Press, New York, NY. 5–21.
30. Butte, N. F., W. W. Wong, L. Ferlic, E. O. Smith, P. D. Klein, and C. Garza. 1990. Energy expenditure and deposition of breast-fed and formula-fed infants during early infancy. *Pediatr. Res.* **28:** 631–640.
31. Hachey, D. L., G. H. Silber, W. W. Wong, and C. Garza. 1989. Human lactation. II. Endogenous fatty acid synthesis by the mammary gland. *Pediatr. Res.* **25:** 63–8.
32. Manual 8. 1987. Lipid and lipoprotein determinations. *In* Atherosclerosis Risk in Communities Study. The University of North Carolina, Chapel Hill, NC.
33. Wong, W. W., L. S. Lee, and P. D. Klein. 1987. Deuterium and oxygen-18 measurements on microliter samples of urine, plasma, saliva, and human milk. *Am. J. Clin. Nutr.* **45:** 905–913.
34. Schwartz, C. C., L. A. Zech, J. M. Vandenbroek, and P. S. Cooper. 1989. Reverse cholesterol transport measured in vivo in man: the central roles of HDL. *In* High Density Lipoproteins and Atherosclerosis. II. N. E. Miller, editor. Elsevier Science Publishers B. V. (Biomedical Division), Amsterdam. 321–329.
35. Goodman, D. S., F. R. Smith, A. H. Seplowitz, R. Ramakrishnan, and R. B. Dell. 1980. Prediction of the parameters of whole body cholesterol metabolism in humans. *J. Lipid Res.* **21:** 699–713.
36. Glantz, S. A. 1981. Primer of Biostatistics. McGraw-Hill, New York, NY.
37. Van Biervliet, J. P., N. Vinaimont, R. Vercaremst, and M. Rosseneu. 1992. Serum cholesterol, cholesteryl ester, and high-density lipoprotein development in newborn infants: response to formulas supplemented with cholesterol and γ-linolenic acid. *J. Pediatr.* **120:** S101–108.
38. Hayes, K. C., A. Pronczuk, R. A. Wood, and D. G. Guy. 1992. Modulation of infant formula fat profile alters the low-density lipoprotein/high-density lipoprotein ratio and plasma fatty acid distribution relative to those with breast-feeding. *J. Pediatr.* **120:** S109–116.
39. Keys, A., J. T. Anderson, and F. Grande. 1965. Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. *Metabolism.* **14:** 776–787.
40. Hegsted, D. M., R. B. McGandy, M. L. Myers, and F. J. Stare. 1965. Quantitative effects of dietary fat on serum cholesterol in man. *Am. J. Clin. Nutr.* **17:** 281–295.
41. Grundy, S. M., and M. A. Denke. 1990. Dietary influences on serum lipids and lipoproteins. *J. Lipid Res.* **31:** 1149–1172.
42. Hagerman, J. S., and R. G. Gould. 1951. The in vitro interchange of cholesterol between plasma and red cells. *Proc. Soc. Exp. Biol. Med.* **78:** 329–332.
43. London, I. M., and H. Schwarz. 1953. Erythrocyte metabolism. The metabolic behavior of the cholesterol of human erythrocytes. *J. Clin. Invest.* **32:** 1248–1252.
44. Eckles, N. E., C. B. Taylor, D. J. Campbell, and R. G. Gould. 1955. The origin of plasma cholesterol and the rates of equilibration of liver, plasma, and erythrocyte cholesterol. *J. Lab. Clin. Med.* **46:** 359–371.
45. Jones, P. J. H., N. Hrboticky, P. Hahn, and S. M. Innis. 1990. Comparison of breast-feeding and formula feeding on intestinal and hepatic cholesterol metabolism in neonatal pigs. *Am. J. Clin. Nutr.* **51:** 979–984.
46. Cai, H-J., C-L. Xie, Q. Chen, X-Y. Chen, and Y-H. Chen. 1991. The relationship between hepatic low-density lipoprotein receptor activity and serum cholesterol level in the human fetus. *Hepatology.* **13:** 852–857.
47. Uauy, R., C. Mize, and S. Grundy. 1989. Effect of early diet on plasma lipoproteins (L) and LDL receptor activity at 1 year of age. *Pediatr. Res.* **25:** 126A.
48. Miettinen, T. A., and Y. A. Kesäniemi. 1989. Cholesterol absorption: regulation of cholesterol synthesis and elimination and within-population variations of serum cholesterol levels. *Am. J. Clin. Nutr.* **49:** 629–635.
49. Butte, N. F., W. W. Wong, L. Ferlic, E. O. Smith, P. D. Klein, and C. Garza. 1990. Energy expenditure and deposition of breast-fed and formula-fed infants during early infancy. *Pediatr. Res.* **28:** 631–640.
50. Kalen, A., E. L. Appelkvist, and G. Daliner. 1989. Age-related changes in the lipid compositions of rat and human tissues. *Lipids.* **24:** 579–584.
51. Watkins, J. B., A-L. Jarvenpää, P. Szczepanik-Van Leeuwen, P. D. Klein, D. K. Rassin, G. Gaull, and N. C. R. Räihä. 1983. Feeding the low-birth weight infant. V. Effects of taurine, cholesterol, and human milk on bile acid kinetics. *Gastroenterology.* **85:** 793–800.
52. Cruz, M. L., W. W. Wong, F. Mimouni, D. L. Hachey, P. D. Klein, and R. C. Tsang. 1992. Effect of dietary cholesterol on cholesterol synthesis rates in infants. *Pediatr. Res.* **31:** 106A.
53. Fomon, S. J., F. Haschke, E. E. Ziegler, and S. E. Nelson. 1982. Body composition of reference children from birth to age 10 years. *Am. J. Clin. Nutr.* **35:** 1169–1175.