Cholesterol Metabolism in Juvenile Baboons
Influence of Infant and Juvenile Diets

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The deferred effects of infant diets and the effects of juvenile diets on cholesterol metabolism were estimated in 83 baboons (Papio sp.) at 3.5 years of age. As infants, the animals were breast-fed or fed one of three formulas containing approximately 2, 30, or 60 mg/dl cholesterol. After weaning at 14 weeks of age, the animals were fed one of four juvenile diets high or low in cholesterol with saturated (P/S = 0.37) or unsaturated (P/S = 2.1) fats. Cholesterol absorption and cholesterol turnover were measured by fecal isotopic methods, and variables of cholesterol metabolism were estimated from a two-pool model. Among juvenile animals breast-fed during infancy, the percentage of cholesterol absorption was higher, while the fluxes of cholesterol from Pool A (QAA, QA, and QAB) and the cholesterol mass of Pool B were lower, compared to those fed formulas. The level of cholesterol in formulas fed during infancy did not influence cholesterol metabolism during the juvenile period. During the juvenile period, saturated fat significantly decreased the cholesterol production rate (QA) and increased the rate constants for cholesterol flux between Pool A and Pool B (KAB and KBA) compared to unsaturated fat. High cholesterol intake increased bile acid and neutral steroid excretion, cholesterol turnover rate, the mass of Pool A, and the rate constant KAB and fluxes QA and QAA for removal of cholesterol from Pool A. However, KAB, t1/2B (half-time of Pool B), and the percentage of cholesterol absorbed were decreased. Dietary cholesterol and saturated fat had similar effects on serum cholesterol and lipoprotein concentrations in these animals, but they had opposite effects on several aspects of cholesterol metabolism.

The potential effects of infant diets on cholesterol homeostasis later in life have been the basis for a number of studies. Most manipulations of infant cholesterol intake did not affect serum cholesterol concentrations at later ages in experimental animals or in humans. However, breast feeding, as compared to formula feeding, produced delayed differences in the serum cholesterol concentration in rats, in high density lipoprotein cholesterol (HDL-C) concentrations in baboons, and in serum cholesterol or HDL-C in humans. Neither the deferred effects of infant diets nor the immediate effects of dietary cholesterol and type of fat on serum cholesterol and lipoprotein concentrations have been clearly explained in metabolic terms.

This report describes the effects of prior infant diet, and of juvenile dietary cholesterol and type of fat, on variables of cholesterol metabolism in juvenile baboons and relates these metabolic variables to the serum lipid responses to diet described previously. Genetic effects on serum lipoprotein concentrations and cholesterol metabolism in these animals have already been reported.

Methods

The 83 baboons (Papio sp.) used for this experiment were progeny of 83 dams and six sires, born and maintained at the Southwest Foundation for Biomedical Research, San Antonio, Texas. As infants, the animals were either breast-fed (baboon breast milk contains 20 to 30 mg/dl cholesterol, 0.25 to 0.37 mg/Kcal cholesterol, P/S = 0.46 and ~56% of calories from fat) or fed one of three formulas (Ross Laboratories, Columbus, Ohio) that contained ap-
approximately 2, 30, or 60 mg/dl cholesterol (0.03 mg/Kcal, 0.44 mg/Kcal, 0.87 mg/Kcal, respectively), P/S = 1.0 and ~48% of calories from fat. At 14 weeks of age, the animals were weaned to one of the four juvenile diets varying in type of fat and cholesterol content. The juvenile diets were formulated with Special Monkey Chow 25 (No. 5045-6, Ralston Purina Company, St. Louis, Missouri) and saturated (SF) or unsaturated (UF) plant fats prepared for this experiment (Proctor and Gamble Company, Cincinnati, Ohio). The saturated fat (P/S = 0.37) was a blend of palm oil, safflower oils, and partially hydrogenated soybean oil; the unsaturated oil (P/S = 2.1) was a mixture of safflower oils, cottonseed oil, and partially hydrogenated soybean oil. Fat comprised approximately 40% of the total calories. The high cholesterol diets contained approximately 1.0 mg/Kcal cholesterol, and the low cholesterol diets contained 0.01 mg/Kcal. Detailed descriptions of the infant diets were provided in the methods section. The animals were assigned at birth to one of the four infant diets and one of the four juvenile diets by a restricted random process. Infant diet, juvenile diet, and sex had a factorial arrangement. Sires were approximately balanced across diet and sex. The sire progeny groups were comprised of 8 to 19 progeny. Each dam contributed only one infant to the study.

The serum-specific radioactivity was measured 25 times over a 4-month period at 3.5 years of age after injecting intravenously 35 μCi of (4-14C)cholesterol (57mCi/μmol). The radioactive dose was prepared by adding 100 μl of acetone containing the tracer to 3 ml of sterile autologous serum and was mixed with magnetic stirring. Blood samples were obtained under sedation with ketamine hydrochloride (10 mg/kg body weight) (Ketaset, Parke-Davis Company, Detroit, Michigan). All animal facilities and procedures exceeded the standards established by the American Association for the Accreditation of Laboratory Animal Science. Serum cholesterol was measured by a cholesterol esterase-oxidase method with BMC Autoflo reagents (Boehringer-Mannheim, Indianapolis, Indiana). This method met the criteria of the Center for Disease Control Lipid Standardization Program. Coefficient of variation of blind duplicates was less than 2%. Radioactivity in serum was measured by liquid scintillation spectrometry. The specific radioactivity data were fitted to a sum of two exponential functions, and the parameters of cholesterol metabolism were estimated using a two-pool model (Figure 1). These parameters are listed below.

### Definitions of Two-Pool Model Parameters

- **Pool A** = rapidly exchanging compartment of cholesterol (mg/kg); includes arteries, fat, and muscles
- **Pool B** = slowly exchanging compartment of cholesterol (mg/kg); includes intestines, liver, plasma, spleen, and lungs

\[
\begin{align*}
K_A &= \text{rate constant (days}^{-1}\text{) for cholesterol excretion from Pool A} \\
K_{AB} &= \text{rate constant (days}^{-1}\text{) for cholesterol transfer from Pool A to Pool B} \\
K_{BA} &= \text{rate constant (days}^{-1}\text{) for cholesterol transfer from Pool B to Pool A} \\
K_{AA} &= K_A + K_{AB} \\
t_{1/2A} &= \text{half-time of first exponential (days)} \\
t_{1/2B} &= \text{half-time of second exponential (days)} \\
Q_A &= K_A \cdot \text{Pool A} = \text{excretion of cholesterol from Pool A (equivalent to production rate, mg/kg/day)} \\
Q_{AB} &= K_{AB} \cdot \text{Pool A} = \text{flux of cholesterol from Pool A to Pool B (mg/kg/day)} \\
Q_{AA} &= Q_A + Q_{AB} = \text{total cholesterol flux from Pool A.}
\end{align*}
\]

The assumptions of the model include excretion of cholesterol only from Pool A. A minimum estimate of the cholesterol mass of Pool B was calculated with the assumption that no cholesterol synthesis occurred in Pool B. Cholesterol turnover, that is, excretion of bile acids plus endogenous neutral steroids, was measured by the isotopic balance method of Grundy and Ahrens from two 5-day fecal collections 1 month apart, 2 to 3 months after injection of the radioactive cholesterol. Briefly, the radioactivity in a weighed portion of the feces was measured after saponification, petroleum ether extraction of neutral steroids, acidification, and extraction of bile acids with diethyl ether. The mass of neutral steroids excreted was calculated by dividing the total dpm excreted in the neutral steroid fraction per day by the serum cholesterol-specific radioactivity 1 day before the midpoint of the fecal collection.

Similarly, the bile acid excretion rate was calculated by dividing the total dpm in the fecal bile acid fraction by the serum cholesterol-specific radioactivity 2 days before the midpoint of the fecal collection. Following the cholesterol metabolism studies, the percentage of cholesterol absorbed was measured by the method of Borgstrom as applied to baboons. In brief, approximately 10 μCi of (1,2-

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### Absorption and Synthesis

**Figure 1.** Schematic drawing of two-pool model of cholesterol metabolism. See text for definitions.
Dietary Effects on Cholesterol Metabolism

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$^3$H)cholesterol and 5 $\mu$Ci (4-14C)$\beta$-sitosterol were added to 7 g of feed and given to the animal. Feces were collected for 6 days and were pooled; then the ratio of $^3$H to $^{14}$C was measured. $^3$H is lost during saponification from the 2-position of coprostanone derived from (1,2-$^3$H)cholesterol. Therefore, $^3$H counts were corrected for enolization losses by dividing the counts in the coprostanone fraction isolated from thin-layer chromatography (TLC) by (1 - 0.45), which was the fraction of $^3$H retained as described. The corrected coprostanone counts were added to the total tritium counts in cholesterol and coprostanol, which provided the total $^3$H counts not absorbed. The percentage of cholesterol absorbed was calculated as 1 minus the ratio of the corrected $^3$H/14C in a fecal aliquot to the $^3$H/14C in the dose administered times 100. Estimates of feed intake were made from three separate 5-day periods by weighing the diet before and after feeding during the cholesterol metabolism and cholesterol absorption measurements. There were no significant differences in feed intake by infant or juvenile diet groups, but male baboons ate significantly more of the juvenile diets than did females (285 g/day vs 240 g/day).

The data were analyzed by analysis of variance. The linear model included terms for the overall mean and effects of infant diet, juvenile dietary cholesterol, juvenile dietary fat, sex, and sire, and the two-factor interactions: infant diet by cholesterol, infant diet by fat, infant diet by sex, cholesterol by fat, cholesterol by sex, cholesterol by sire, fat by sex, fat by sire, and sex by sire. All effects were assumed to be fixed. The variables of the linear model were estimated by robust M-estimates, and tests of hypotheses were made by robust likelihood ratio type tests. The M-estimator gives less weight to observations far from the values predicted from the model, and thus is less sensitive to outliers in the data than the classical methods. The measurements were logarithmically transformed before statistical analysis to better satisfy the assumptions of the statistical model.

Results

Deferred Effects of the Infant Diet Regimen

Table 1 shows the serum cholesterol concentration and the variables of cholesterol metabolism by infant diet groups. In 3.5-year-old baboons, there were statistically significant differences in several metabolic variables between the group breast-fed and those fed formula for 14 weeks during infancy, although there were no differences in serum cholesterol concentrations. The percentage of cholesterol absorbed was higher in animals previously breast-fed than in those fed formula ($p < 0.05$). The effect of breast feeding compared to formula feeding on cholesterol absorption (1.13) means that animals breast-fed as infants had a 13% higher cholesterol absorption rate at 3.5 years than those previously fed formula. In the breast-fed group compared to those fed formulas, the values of all cholesterol fluxes from Pool A were lower; by 12% for $Q_M$ ($p < 0.005$), by 8% for $Q_*$ ($p = 0.05$), and by 16% for $Q_\beta$ ($p < 0.05$). The minimum estimate of the cholesterol mass of Pool B was 10% lower in the breast-fed group ($p < 0.005$).

There also were several interactions of the infant diet with dietary cholesterol during the juvenile period (not shown in Table 1). In juveniles, the high cholesterol diets increased bile acid excretion by 38% among animals fed formula as infants, but only by...
4% among those that were breast-fed (interaction, \( p = 0.089 \)) (Figure 2). Also, the high cholesterol diets increased cholesterol turnover by 61% in the groups fed formulas as infants and by 26% in those fed breast milk (interaction, \( p = 0.062 \)) (Figure 3). Thus, cholesterol feeding to animals fed formula as infants increased cholesterol turnover and bile acid excretion to a large extent, but dietary cholesterol increased these variables much less in breast-fed animals. Among the three groups fed low, medium, or high cholesterol formulas during infancy, there were no statistically significant differences in serum cholesterol concentration or in any measure of cholesterol metabolism at 3.5 years of age.

### Effects of Juvenile Dietary Cholesterol and Type of Fat

The serum cholesterol concentration and variables of cholesterol metabolism are shown by juvenile diet group in Table 2. Significant (\( p < 0.05 \)) main effects of dietary cholesterol were found for all variables except \( t_{VA} \), the cholesterol mass of Pool B, \( K_{AA} \), \( K_{AB} \), and \( Q_{AB} \). In the juveniles fed high cholesterol diets, compared to those fed low cholesterol diets, the percentage of dietary cholesterol absorbed, the half-time of the second exponential (\( t_{VB} \)), and \( K_{AB} \) were decreased (\( p < 0.005 \)); but serum cholesterol concentration, cholesterol turnover rate, bile acid

### Table 1. Cholesterol Metabolism Variables by Infant Diet Group in 3.5-Year-Old Baboons

<table>
<thead>
<tr>
<th>Variables</th>
<th>Breast</th>
<th>Formula 2</th>
<th>Formula 30</th>
<th>Formula 60</th>
<th>Effect†</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>22</td>
<td>22</td>
<td>21</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>118.4</td>
<td>124.3</td>
<td>124.7</td>
<td>125.9</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>(106.8–131.2)</td>
<td>(112.1–137.8)</td>
<td>(112.7–137.9)</td>
<td>(112.4–141.1)</td>
<td></td>
</tr>
<tr>
<td>Cholesterol absorption (%)</td>
<td>46.6</td>
<td>42.9</td>
<td>40.9</td>
<td>40.1</td>
<td>1.13‡</td>
</tr>
<tr>
<td></td>
<td>(43.2–50.3)</td>
<td>(39.8–46.3)</td>
<td>(37.9–44.1)</td>
<td>(36.9–43.6)</td>
<td></td>
</tr>
<tr>
<td>Cholesterol turnover (mg/kg/day)</td>
<td>32.0</td>
<td>33.4</td>
<td>34.3</td>
<td>34.5</td>
<td>0.94§</td>
</tr>
<tr>
<td></td>
<td>(28.7–35.7)</td>
<td>(30.0–37.3)</td>
<td>(30.8–38.2)</td>
<td>(30.6–38.9)</td>
<td></td>
</tr>
<tr>
<td>Bile acid excretion (mg/kg/day)</td>
<td>17.7</td>
<td>18.9</td>
<td>18.7</td>
<td>20.2</td>
<td>0.92§</td>
</tr>
<tr>
<td></td>
<td>(15.5–20.2)</td>
<td>(16.5–21.6)</td>
<td>(16.4–21.3)</td>
<td>(17.5–23.5)</td>
<td></td>
</tr>
<tr>
<td>Neutral sterol excretion (mg/kg/day)</td>
<td>13.8</td>
<td>14.1</td>
<td>15.1</td>
<td>14.0</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>(12.4–15.3)</td>
<td>(12.7–15.7)</td>
<td>(13.6–16.8)</td>
<td>(12.5–15.7)</td>
<td></td>
</tr>
<tr>
<td>tVA (days)</td>
<td>3.42</td>
<td>3.19</td>
<td>2.98</td>
<td>3.13</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>(3.12–3.74)</td>
<td>(2.91–3.45)</td>
<td>(2.72–3.25)</td>
<td>(2.83–3.45)</td>
<td></td>
</tr>
<tr>
<td>tVB (days)</td>
<td>23.1</td>
<td>22.8</td>
<td>22.8</td>
<td>22.4</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>(22.0–24.2)</td>
<td>(21.8–23.9)</td>
<td>(21.7–23.8)</td>
<td>(21.3–23.6)</td>
<td></td>
</tr>
<tr>
<td>Mass of pool A (mg/kg)</td>
<td>322</td>
<td>324</td>
<td>328</td>
<td>326</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>(300–346)</td>
<td>(301–348)</td>
<td>(306–352)</td>
<td>(301–352)</td>
<td></td>
</tr>
<tr>
<td>Mass of pool B (mg/kg)</td>
<td>374</td>
<td>403</td>
<td>432</td>
<td>417</td>
<td>0.90‡</td>
</tr>
<tr>
<td>( K_{AA} \times 10^1 ) (days⁻¹)</td>
<td>1.73</td>
<td>1.87</td>
<td>1.99</td>
<td>1.89</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>(1.59–1.89)</td>
<td>(1.71–2.05)</td>
<td>(1.82–2.17)</td>
<td>(1.72–2.09)</td>
<td></td>
</tr>
<tr>
<td>( K_{A} \times 10^1 ) (days⁻¹)</td>
<td>1.03</td>
<td>1.10</td>
<td>1.10</td>
<td>1.08</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>(0.95–1.11)</td>
<td>(1.02–1.19)</td>
<td>(1.02–1.19)</td>
<td>(0.99–1.18)</td>
<td></td>
</tr>
<tr>
<td>( K_{AB} \times 10^2 ) (days⁻¹)</td>
<td>6.89</td>
<td>7.50</td>
<td>8.50</td>
<td>7.99</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>(5.98–7.93)</td>
<td>(6.52–8.64)</td>
<td>(7.41–9.76)</td>
<td>(6.84–9.34)</td>
<td></td>
</tr>
<tr>
<td>( K_{SA} \times 10^2 ) (days⁻¹)</td>
<td>5.90</td>
<td>6.03</td>
<td>6.44</td>
<td>6.46</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>(5.38–6.48)</td>
<td>(5.49–6.62)</td>
<td>(5.86–7.06)</td>
<td>(5.83–7.16)</td>
<td></td>
</tr>
<tr>
<td>( Q_{AA} ) (mg/kg/day)</td>
<td>55.9</td>
<td>61.0</td>
<td>65.0</td>
<td>63.9</td>
<td>0.88‡</td>
</tr>
<tr>
<td></td>
<td>(52.0–60.1)</td>
<td>(56.7–65.6)</td>
<td>(60.5–69.8)</td>
<td>(58.9–69.3)</td>
<td></td>
</tr>
<tr>
<td>( Q_{A} ) (mg/kg/day)</td>
<td>33.0</td>
<td>35.7</td>
<td>36.0</td>
<td>36.2</td>
<td>0.92‡</td>
</tr>
<tr>
<td></td>
<td>(31.2–34.9)</td>
<td>(33.7–37.8)</td>
<td>(34.1–38.1)</td>
<td>(34.0–38.5)</td>
<td></td>
</tr>
<tr>
<td>( Q_{AB} ) (mg/kg/day)</td>
<td>22.2</td>
<td>24.5</td>
<td>27.8</td>
<td>27.1</td>
<td>0.84‡</td>
</tr>
<tr>
<td></td>
<td>(19.6–25.1)</td>
<td>(21.7–27.8)</td>
<td>(24.6–31.4)</td>
<td>(23.6–31.0)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means, with 95% confidence intervals in parentheses.

*Infant formulas contained approximately 2, 30, or 60 mg/dl cholesterol.

†Multiplicative main effect, breast/formula (22 animals/61 animals).

‡Statistically significant effect (\( p < 0.05 \)).

§Interaction with juvenile dietary cholesterol intake (\( p < 0.10 \)), see Figures 2 and 3.
and endogenous neutral steroid excretion, mass of Pool A, K₄A, Q₄, and K₄ were increased (p < 0.005).

In animals fed saturated fat compared to those fed unsaturated fat, the cholesterol production rate (Q₄) (p < 0.01) was lower, and serum cholesterol (p < 0.05), K₂₂ (p < 0.05) and K₄ (p < 0.01) were higher.

The effect of dietary cholesterol on serum cholesterol (1.38) indicates that feeding the high cholesterol diet (1 mg/Kcal cholesterol) resulted in serum cholesterol concentrations that were 38% higher than those observed for the low cholesterol diet (0.01 mg/Kcal cholesterol). Also, saturated fat increased serum cholesterol concentrations by 15% compared to unsaturated fat.

### Interactions of Juvenile Dietary Cholesterol by Type of Fat

Several of the main effects of juvenile dietary cholesterol and type of fat must be interpreted cautiously because of significant cholesterol by fat interactions. For example, compared to saturated fat, unsaturated fat increased neutral steroid excretion when fed with the high cholesterol diet, but this effect of fat was reversed with low cholesterol intake (interaction, (p < 0.05). Also, the mass of Pool A was greater on the low cholesterol-unsaturated fat diet than on the low cholesterol-saturated fat diet, but little difference in this variable due to type of fat was observed on the

### Table 2. Cholesterol Metabolism Variables by Juvenile Diet Group in 3.5-Year-Old Baboons

<table>
<thead>
<tr>
<th>Variables</th>
<th>LC-UF</th>
<th>LC-SF</th>
<th>HC-UF</th>
<th>HC-SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>24</td>
<td>21</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>101.5 (92.5–111.4)</td>
<td>108.4 (97.8–120.2)</td>
<td>130.3 (116.5–145.6)</td>
<td>161.1 (142.6–182.0)</td>
</tr>
<tr>
<td>Cholesterol absorption (%)</td>
<td>53.4 (49.9–57.3)</td>
<td>51.6 (47.8–55.8)</td>
<td>34.2 (31.5–37.2)</td>
<td>34.8 (31.9–38.1)</td>
</tr>
<tr>
<td>Cholesterol turnover (mg/kg/day)</td>
<td>27.3 (24.7–30.1)</td>
<td>27.2 (24.4–30.4)</td>
<td>44.3 (38.9–50.4)</td>
<td>38.4 (33.9–43.7)</td>
</tr>
<tr>
<td>Bile acid excretion (mg/kg/day)</td>
<td>17.3 (15.3–19.5)</td>
<td>16.0 (14.0–18.3)</td>
<td>23.5 (20.3–27.1)</td>
<td>19.5 (16.6–22.8)</td>
</tr>
<tr>
<td>Neutral steroid excretion (mg/kg/day)</td>
<td>9.8 (8.9–10.8)</td>
<td>11.1 (10.0–12.4)</td>
<td>20.3 (18.1–22.8)</td>
<td>16.5 (16.3–21.0)</td>
</tr>
<tr>
<td>tₓA (days)</td>
<td>3.4 (3.1–3.7)</td>
<td>3.0 (2.7–3.2)</td>
<td>3.2 (2.9–3.5)</td>
<td>3.2 (2.9–3.5)</td>
</tr>
<tr>
<td>tₓB (days)</td>
<td>24.8 (23.7–25.8)</td>
<td>24.1 (23.0–25.3)</td>
<td>21.5 (20.4–22.6)</td>
<td>20.9 (19.8–22.1)</td>
</tr>
<tr>
<td>Mass of pool A (mg/kg)</td>
<td>319 (299–340)</td>
<td>286 (266–307)</td>
<td>346 (320–373)</td>
<td>353 (325–384)</td>
</tr>
<tr>
<td>Mass of pool B (mg/kg)</td>
<td>413 (390–437)</td>
<td>405 (380–431)</td>
<td>411 (384–440)</td>
<td>395 (367–426)</td>
</tr>
<tr>
<td>K₄A × 10⁵ (days⁻¹)</td>
<td>1.75 (1.61–1.89)</td>
<td>1.97 (1.81–2.16)</td>
<td>1.91 (1.73–2.10)</td>
<td>1.85 (1.67–2.06)</td>
</tr>
<tr>
<td>K₄ × 10⁴ (days⁻¹)</td>
<td>1.00 (0.94–1.07)</td>
<td>1.03 (0.95–1.11)</td>
<td>1.19 (1.10–1.29)</td>
<td>1.10 (1.00–1.20)</td>
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<tr>
<td>K₄B × 10² (days⁻¹)</td>
<td>7.35 (6.47–8.35)</td>
<td>9.39 (8.15–10.8)</td>
<td>6.91 (5.93–8.04)</td>
<td>7.37 (6.24–8.70)</td>
</tr>
<tr>
<td>Q₄A (mg/kg/day)</td>
<td>5.71 (5.25–6.21)</td>
<td>6.69 (6.09–7.34)</td>
<td>5.89 (5.33–6.51)</td>
<td>6.58 (5.90–7.35)</td>
</tr>
<tr>
<td>Q₄ (mg/kg/day)</td>
<td>56.1 (52.5–59.9)</td>
<td>57.2 (53.2–61.5)</td>
<td>67.6 (62.5–73.1)</td>
<td>65.4 (60.0–71.2)</td>
</tr>
<tr>
<td>Q₄B (mg/kg/day)</td>
<td>32.0 (30.4–33.6)</td>
<td>29.5 (27.9–31.2)</td>
<td>42.2 (39.7–44.8)</td>
<td>38.6 (36.1–41.3)</td>
</tr>
<tr>
<td>Q₄B (mg/kg/day)</td>
<td>23.6 (21.1–26.4)</td>
<td>27.7 (24.4–31.3)</td>
<td>24.1 (21.1–27.6)</td>
<td>26.0 (22.4–30.1)</td>
</tr>
</tbody>
</table>

Values are means, with 95% confidence intervals in parentheses.

*Multiplicative main effects; high cholesterol (HC)/low cholesterol diets (LC) (38 animals/45 animals); saturated (SF)/unsaturated fat (UF) diets (44 animals/39 animals).

†Statistically significant effect (p < 0.05).

‡Interaction with infant diet (p < 0.10), see Figures 2 and 3.

§Interaction of dietary cholesterol with type of fat (p < 0.10).
were breast-fed as infants and later fed diets high in unsaturated fat had higher HDL-C concentrations (HDL-C). Juvenile animals which consumed diets high in unsaturated fat had higher HDL-C concentrations (HDL-C) than those fed saturated fat. In contrast, animals fed formulas and then weaned to diets high in unsaturated fat had lower HDL-C than those fed saturated fat. In the group originally breast fed, the higher HDL-C observed in the animals fed unsaturated compared to saturated fat may be a result of greater cholesterol absorption and lower fluxes from Pool A as compared to formula-fed groups (Table 1).

The data of Kris-Etherton et al. from rats suggest that breast feeding had a deferred effect on cholesterol metabolism. The serum cholesterol concentration of rats previously breast fed was not elevated by a dietary cholesterol challenge 1 month after the infant period, but the serum cholesterol concentration of animals fed formula in the infant period, with or without cholesterol, was increased by dietary cholesterol after the infant period. When the rats were returned to a low cholesterol chow diet after the dietary challenge period, the serum cholesterol concentration of the breast-fed group remained lower than that of both formula-fed groups. Reiser et al. also reported that breast-fed rats, but not swine, had a lower serum cholesterol level later in life compared to those fed formulas. These experiments with rats and ours with baboons support the hypothesis that breast feeding compared to formula feeding produces differences in cholesterol metabolism later in life. However, an effect on cholesterol homeostasis later in life by early cholesterol feeding as proposed by Reiser and Fomon was not observed by Kris-Etherton et al. in rats nor in the present experiment in baboons. Differences in serum cholesterol concentrations among adult humans who were breast-or formula-fed as infants are small and not consistent, although those studies in humans frequently were confounded by genetic and dietary effects (reviewed by Wissler et al.)

**Effects of Type of Fat on Cholesterol Metabolism**

Several reports conclude that no single metabolic process accounts for the differences among individuals in their serum cholesterol response to type of fat. The proposal that the reduction in serum cholesterol concentrations by unsaturated fat is caused partially by a redistribution of cholesterol from plasma to tissue in humans is supported by the observation that feeding corn oil to rats increased the free cholesterol content of a number of tissues. However, Frantz and Carey, reviewed by Jackson et al., reported lower hepatic cholesterol content of humans fed corn oil compared to those fed hydrogenated coconut oil. In the present experiment, we observed a significant dietary cholesterol by fat interaction for the cholesterol mass of Pool A. That is, animals fed the low cholesterol-unsaturated fat diet had higher cholesterol masses of Pool A (Table 2) than the group fed low cholesterol-saturated fat. There was no significant effect of type of fat on the size of Pool A with high cholesterol intake. The

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**Sire and Sex Effects**

The significant differences among sire groups for cholesterol turnover rate, cholesterol production rates, t_A, t_B, K_A, K_B and the mass of Pool A were described previously.

The only significant difference between male and female baboons for any variable was for Q_A (p < 0.01). The Q_A for females was 36.7 mg/kg/day and for males, 33.8 mg/kg/day.

**Deferred Effects of Infant Diet Regimen**

This study showed that animals which were breast-fed as infants had a greater percentage of cholesterol absorbed and lower cholesterol production rates (Q_A) as juveniles, compared to those fed formula, and that there were no significant differences in feed intake as juveniles among the groups fed different diets during infancy. These observations lead us to believe that juveniles breast-fed during infancy absorbed a greater mass of cholesterol than the groups fed formulas, and that total body cholesterol synthesis, which can be estimated as the cholesterol production rate minus the cholesterol mass absorbed, was greatly reduced in animals breast-fed as infants compared to those fed formulas. The effect of breast vs formula feeding on cholesterol turnover rate was consistent with the effect observed for cholesterol production rate (0.94 vs 0.92), although the effect was not statistically significant for turnover rate. Since there were no differences in cholesterol metabolism at 3.5 years of age among the three groups fed 2, 30, or 60 mg/dl of cholesterol in formula during infancy, aspects of breast feeding other than cholesterol content are likely responsible for the differences in cholesterol metabolism of breast- vs formula-fed animals observed at 3.5 years of age.

What effect these differences in cholesterol metabolism have on our previously reported differences in serum lipid concentrations are not clear. No significant differences in serum cholesterol concentrations were observed among juvenile animals breast- or formula-fed during infancy (Table 1). However, we observed an interaction of prior infant diet (breast vs formulas) with type of fat (saturated vs unsaturated fat) on high density lipoprotein cholesterol concentration (HDL-C). Juvenile animals which were breast-fed as infants and later fed diets high in unsaturated fat had higher HDL-C concentrations than those breast-fed and later fed saturated fat. In contrast, animals fed formulas and then weaned to diets high in unsaturated fat had lower HDL-C than those fed saturated fat. In the group originally breast fed, the higher HDL-C observed in the animals fed unsaturated compared to saturated fat may be a result of greater cholesterol absorption and lower fluxes from Pool A as compared to formula-fed groups (Table 1).

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bined effects of polyunsaturated fat in lowering serum cholesterol but increasing the cholesterol mass of Pool A in the low cholesterol diet group suggest that the cholesterol mass of tissues of Pool A also would be increased. Since the rate constant ($K_{pA}$) for cholesterol transfer from Pool A to Pool B decreases with unsaturated fat feeding, cholesterol accumulation may not occur in tissues of Pool B. These observations suggest that cholesterol transfer to tissues of Pool B is not increased by unsaturated fat, but that the cholesterol content of the tissues of Pool A is increased at least on the low cholesterol diets.

Diet high in unsaturated fats usually increase steroid excretion in normocholesterolemic humans or in experimental animals (reviewed by Grundy and by Paul et al.

We found a significant dietary cholesterol by fat interaction affecting neutral steroid excretion in baboons. In baboons on the high cholesterol diet, neutral steroid excretion was $9\%$ greater with unsaturated than saturated fat; however, in the low cholesterol diet groups, neutral steroid excretion was $13\%$ lower in the presence of unsaturated fat compared to saturated fat. In a study of normocholesterolemic men, cholesterol turnover was enhanced by unsaturated fat feeding; but the effect was greater on a high cholesterol diet. In hyperlipidemic humans and in several species of monkeys, unsaturated fat does not always increase cholesterol turnover.

**Effects of Dietary Cholesterol on Cholesterol Metabolism**

As with unsaturated fat, a principal metabolic response to cholesterol feeding in most studies with humans or experimental animals is an increased steroid excretion rate. In our experiment, neutral steroid and, to a lesser extent, bile acid excretion rates were increased in the juvenile baboons fed high dietary cholesterol. Eggen reported a similar increase in adult baboons. In humans also, endogenous neutral steroid excretion is dramatically increased in response to high cholesterol intake, but in rats and dogs, the principal increase is in bile acid excretion. Some of the differences among animal species may be due to significant interactions of dietary cholesterol with other factors. For example, in the present experiment the effect of dietary cholesterol on bile acid excretion and cholesterol turnover rate was dramatically influenced by the type of infant diet (Figures 2 and 3). In our experiment, the halftime of the first exponential ($t_1/2A$) was not affected by cholesterol feeding, but the cholesterol mass of Pool A was increased, which is consistent with an expansion of the serum cholesterol pool. The half-time of the second exponential ($t_2/2B$) was lowered by cholesterol feeding, but the mass of Pool B was not affected. The absence of an effect on the mass of Pool B also is consistent with reports that cholesterol content of most peripheral tissues does not increase with cholesterol feeding. Also, dietary cholesterol did not significantly increase cholesterol flux from Pool A to Pool B ($Q_{AB}$). These results suggest that cholesterol feeding does not cause cholesterol accumulation in the tissues of Pool B, although we cannot exclude the possibility that expansion of the cholesterol pool may occur in a small subcompartment of Pool B.

The effects on serum cholesterol and cholesterol metabolism of the types of dietary fat used in this experiment are smaller than the effects of the two levels of dietary cholesterol (1.0 vs 0.01 mg/Kcal) (Table 2). In another experiment with baboons, the effects of type of fat (coconut oil vs corn oil) on serum cholesterol, HDL-C, and apolipoprotein B concentrations were greater than the effects of dietary cholesterol fed at 1.7 mg/Kcal compared to 0.01 mg/Kcal. Thus, in baboons as in humans, there are large differences in the responses of serum lipoproteins and cholesterol metabolism to various types of fat and significant interactions of type of fat with dietary cholesterol.

These results indicate that breast feeding, as compared with formula feeding, affects cholesterol metabolism more than 3 years later. However, the level of cholesterol intake in the infant formula does not have a significant deferred effect on cholesterol metabolism. Therefore, the effect of breast vs formula feeding probably is not due to differences in the cholesterol content. Cholesterol metabolism in the juvenile period is affected not only by the deferred effects of breast vs formula feeding in infancy, but also by interactions of the infant diet with the level of dietary cholesterol intake during the juvenile period.

We also conclude that, in the absence of dietary cholesterol, unsaturated compared to saturated fat increases the cholesterol content of tissues of Pool A. This observation supports the proposal that lowering the serum cholesterol concentration by unsaturated fat may be mediated partially by a redistribution of cholesterol from the serum compartment to tissues. A second mechanism for the hypocholesterolemic effect of unsaturated fat is an enhanced cholesterol production rate which is likely due to increased bile acid excretion.

The primary metabolic responses to high cholesterol intake are a decreased percentage of cholesterol absorbed, and increased neutral steroid and bile acid excretion rates. However, these responses do not completely compensate and increased serum cholesterol concentrations result.

**Acknowledgments**

We thank Don Smith, Merle Taylor, and Steve Ingram for excellent technical assistance, Thomas C. Cartwright for assistance in designing the experiment, and K. Dee Carey for care and maintenance of the animals. Douglas Eggen provided advice in planning this experiment and supplied the computer program for the two-pool model of cholesterol metabolism. Bryan Flow assisted with the statistical analysis. We also thank Fred Mattson and the Procotor and Gamble Company for providing the fats for the diets. Herman Wigodsley offered many helpful suggestions during the manuscript preparation.
References


Index Terms: breast feeding • infant formulas • neonatal nutrition • 2-pooll model • cholesterol turnover • deferred effects of infant diet • dietary cholesterol • saturated fat • unsaturated fat
Cholesterol metabolism in juvenile baboons. Influence of infant and juvenile diets.
G E Mott, E M Jackson, C A McMahan, C M Farley and H C McGill, Jr

doi: 10.1161/01.ATV.5.4.347
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Ave., Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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