Deferred Effects of Preweaning Diet on Atherosclerosis in Adolescent Baboons

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We examined the effects of breast and formula feeding during infancy on the serum lipoproteins and on atherosclerosis in young adult baboons. Baboons were breast-fed (n = 13) or formula-fed (n = 32) until weaning at 16 weeks of age and thereafter they were fed a diet containing 1.7 mg cholesterol/kcal and 40% of calories as lard until 5 years of age. At 12 weeks of age, breast-fed baboons had higher serum high density lipoprotein cholesterol concentration (HDL-C, 68 vs. 51 mg/dl), lower serum triglyceride concentration (37 vs. 68 mg/dl), and lower very low density plus low density lipoprotein cholesterol (VLDL + LDL-C) to HDL-C ratio (0.65 vs. 0.98) than formula-fed infants. From weaning to 92 weeks of age, breast-fed baboons had a lower serum triglyceride concentration (23 vs. 38 mg/dl) than formula-fed baboons. After weaning, the VLDL + LDL-C/HDL-C ratio increased from 0.65 to 1.0 in breast-fed baboons, but decreased from 0.98 to 0.72 in formula-fed baboons. From 92 to 246 weeks of age, the VLDL + LDL-C/HDL-C ratio was consistently higher in breast-fed baboons compared to formula-fed baboons. At 5 years of age, baboons breast-fed as infants had a greater percentage of intimal surface area involved with atherosclerosis in the abdominal aorta, the iliac-femoral artery, the aortic arch, the brachial artery, and the carotid artery, than did those formula-fed as infants. The greater prevalence of lesions in breast-fed baboons was explained mainly by the higher VLDL + LDL-C/HDL-C ratio.

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Preweaning infant diets may have a long-term influence on lipoprotein metabolism and may thus predispose individuals to atherosclerosis.1,2 The only direct evidence supporting this concept is derived from animal experiments, which tested the effects of infant cholesterol intake, premature weaning, and breast versus formula feeding on serum cholesterol and lipoprotein concentrations.3-9 The results of these and other experiments, which tested the effects of infant cholesterol intake, premature weaning, and breast versus formula feeding on serum cholesterol and lipoprotein concentrations,3-9 but the cholesterol content of mother's milk,6 premature weaning, and breast feeding compared to formula feeding did affect some of these variables.4,5,7-10 None of these reports has described the effects of premature weaning and breast and formula feeding on experimental atherosclerosis. Human studies of long-term effects of infant diet on juvenile or adult cholesterol levels11-16 have yielded conflicting results.

We recently reported that consumption of a high caloric formula during the preweaning period increased the adiposity of young adult baboons.17 In that study, infant baboons were fed one of four preweaning diets (breast milk, low, normal, or high caloric formulas) and were weaned onto a saturated fat and high cholesterol diet. This report describes the effects of breast and formula feeding on serum lipid and lipoprotein concentrations and on atherosclerosis in young adult baboons.

Methods

Experimental Design

Forty-five newborn baboons (Papio cynocephalus), the progeny of six sires, were randomly assigned to one of four preweaning feeding regimens; breast-fed, or fed one of three infant formulas (40.5, 67, or 94.5 kcal/100 g) as described previously.17 Formula-fed infants were bottle-fed four times per day at 0700, 1000, 1300, and 1600 hours. The formula (Similac, 67 kcal/100 g, Ross Laboratories, Columbus, OH) contained approximately 1 mg cholesterol per 100 ml formula and had a polyunsaturated/saturated fat (P/S) ratio of 1.6. In contrast, baboon breast milk contains approximately 30 mg/dl cholesterol and has a P/S ratio of 0.46.18 At 16 weeks of age, all baboons were weaned over a 2-week period onto a gruel prepared by mixing solid food with formula. After 18 weeks of age, all baboons were fed the same solid diet. The solid diet was prepared by mixing Monkey Chow 25-5045-6 (Ralston-Purina Company, St. Louis, MO), dried egg yolk (4.8%), lard (14.3%), sodium chloride (0.1%), retinyl acetate (0.001%), ascorbic acid (0.1%), and cholesterol (0.5%).19 The postweaning diet provided 40% of calories from fat, 39% from carbohydrate, and 21% from protein, and it contained 1.7 mg cholesterol per kcal. All animals were housed individually indoors until 6 months of age, in indoor cages with four animals per cage until 1 year of age, and thereafter in outdoor gang cages in groups of 20 animals until 5 years of age.
The experimental protocol was approved by the Animal Research Committee of the Southwest Foundation for Biomedical Research, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

**Lipoprotein Cholesterol and Triglyceride Analyses**

Venous blood samples were obtained at birth and every 12 weeks from each baboon after an overnight fast. Baboons were immobilized during this procedure with ketamine hydrochloride (10 mg/kg of Vetalar, Parke-Davis and Company, Detroit, MI).

Serum cholesterol was measured on all blood samples by an enzymatic method using an ABA 100 Bichromatic Analyzer (Abbott Laboratories, South Pasadena, CA). High density lipoprotein cholesterol (HDL-C) in the serum supernatant was measured after dextran sulfate- 
CaCl$_2$ precipitation at 12, 48, and 92 weeks of age, and after heparin-manganese precipitation at 136, 180, 224, and 246 weeks of age. A comparison of 13 samples analyzed by both methods yielded correlation coefficients of 0.99 between the two very low density lipoprotein plus low density lipoprotein cholesterol (VLDL + LDL-C) measurements and 0.96 between the two HDL-C measurements. HDL-C values measured by heparin-manganese precipitation averaged 7.1 mg/dl lower than HDL-C measured by dextran sulfate precipitation. All comparisons of lipoprotein cholesterol concentrations were made with values measured by the same method. Serum triglyceride concentrations were measured at birth, 12, 48, 92, 136, 224, and 246 weeks by an enzymatic procedure that corrected for free glycerol. Apolipoproteins A-I (apo A-I) and B (apo B) were measured in serum obtained at 246 weeks of age by the electroimmunoassay procedure of Laurell as described by Mott et al. Purified LDL and HDL were used as the primary standards for apo B and apo A-I measurements, respectively.

The quality control of serum cholesterol, triglyceride, and lipoprotein concentration measurements was assured by participation in Part III of the Centers for Disease Control Lipid Standardization Program. Internal quality control was maintained by analysis of baboon serum pools and commercial human sera.

**Necropsy**

At 5 years of age, the baboons were immobilized with ketamine hydrochloride (10 mg/kg), anesthetized with Pentothal, and exsanguinated. The aorta and the coronary, iliac-femoral, brachial, and carotid arteries were opened longitudinally, fixed with the adventitia adherent to chipboard in 10% buffered formalin, stained with Sudan IV, and packaged in plastic bags.

**Grading Atherosclerosis**

Fatty streaks were defined as Sudan IV-stained intimal areas that were elevated slightly or not at all above the surrounding intimal surface. Fibrous plaques were defined as firm, distinctly elevated areas regardless of whether their intimal area was stained for lipid. These definitions are identical to those used in grading human lesions. Most of the lesions observed were fatty streaks. The percentage of intimal surface area involved with lesions was evaluated independently by two pathologists (H. C. McGill, Jr. and J. P. Strong, New Orleans, Louisiana). Intraclass correlation coefficients between the gradings of the two pathologists ranged from 0.50 in the innominate artery to 0.94 in the abdominal aorta. The means of the two observations were used in the statistical analyses. The percentage surface area involvement of the coronary arteries was small, and lesions were evaluated as present or absent. Arteries were positive if either pathologist scored the artery as having lesions. There were no significant differences between pathologists in grading prevalence. Group values also were expressed as prevalence (percentage of baboons with lesions).

**Statistical Analyses**

Percentage intimal surface area involved with lesions and body weight, VLDL + LDL-C, HDL-C concentrations, VLDL + LDL-C/HDL-C ratio, and triglyceride concentrations were analyzed separately by analysis of variance (ANOVA). When there were multiple measurements during a defined age interval, we fitted linear regression lines to the observations from each animal. The means and slopes of the linear regression lines were analyzed for the effects of infant diet by ANOVA. Logarithmic transformations of the serum lipid concentrations were used to meet the distributional assumptions for the ANOVA. Examination of the residuals indicated no violations of the statistical assumptions. The ANOVA, means, and confidence intervals were based on robust M-estimates to lessen the influence of values in the extreme tails of each distribution. The prevalence of lesions was analyzed using multiple logistic regression. Predictor variables included preweaning diet (breast or formula feeding), VLDL + LDL-C/HDL-C ratio, sex, and sire.

**Results**

**Effect of Preweaning Feeding on Serum Lipids**

Serum lipid concentrations were compared among infant diet groups at birth and at 12 weeks of age (preweaning), from after weaning to 92 weeks of age (period of greatest increase in serum lipids), and from after 92 to 246 weeks of age (period of relatively little change in serum lipid concentrations). The serum concentrations of VLDL + LDL-C, HDL-C, and triglycerides of baboons fed low (mean energy intake from birth to 16 weeks of age = 14,960 kcal), normal (24,749 kcal) and high (32,540 kcal) caloric formulas are shown in Table 1. During preweaning, body weight was significantly ($p < 0.05$) influenced by caloric intake in female and male baboons, but after weaning, only females that were overfed had a significant ($p < 0.05$) increase (29%) in body weight by 5 years of age. At no age were significant differences in serum triglyceride and lipoprotein cholesterol levels related to the caloric intake, and values for all the formula-fed baboons were combined for comparison with those of breast-fed baboons. At 12 weeks of age, there was no significant difference in serum cholesterol levels between breast-fed and formula-
Table 1. Serum Lipoprotein Cholesterol and Triglyceride Concentrations by Age and Infant Formula

<table>
<thead>
<tr>
<th>Age (wks)</th>
<th>Caloric density of infant formula</th>
<th>Number of animals</th>
<th>Lipoprotein cholesterol VLDL + LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>Low</td>
<td>8</td>
<td>40 (27 to 60)</td>
<td>45 (39 to 52)</td>
<td>56 (23 to 133)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>12</td>
<td>39 (29 to 53)</td>
<td>46 (41 to 52)</td>
<td>41 (20 to 84)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>12</td>
<td>42 (31 to 58)</td>
<td>46 (41 to 52)</td>
<td>40 (23 to 71)</td>
</tr>
<tr>
<td>12</td>
<td>Low</td>
<td>8</td>
<td>56 (45 to 68)</td>
<td>55 (48 to 64)</td>
<td>51 (54 to 122)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>12</td>
<td>48 (41 to 56)</td>
<td>47 (42 to 53)</td>
<td>63 (46 to 87)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>12</td>
<td>49 (42 to 58)</td>
<td>51 (45 to 57)</td>
<td>62 (45 to 85)</td>
</tr>
<tr>
<td>48 to 92</td>
<td>Low</td>
<td>8</td>
<td>59 (44 to 79)</td>
<td>98 (85 to 113)</td>
<td>36 (25 to 53)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>12</td>
<td>76 (61 to 96)</td>
<td>90 (81 to 101)</td>
<td>34 (25 to 47)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>12</td>
<td>68 (54 to 85)</td>
<td>93 (83 to 104)</td>
<td>44 (32 to 59)</td>
</tr>
<tr>
<td>136 to 246</td>
<td>Low</td>
<td>8</td>
<td>84 (62 to 114)</td>
<td>115 (101 to 131)</td>
<td>41 (31 to 53)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>12</td>
<td>84 (66 to 107)</td>
<td>104 (94 to 115)</td>
<td>48 (39 to 59)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>12</td>
<td>91 (72 to 116)</td>
<td>103 (94 to 114)</td>
<td>46 (38 to 57)</td>
</tr>
</tbody>
</table>

Values are the means in mg/dl, with 95% confidence levels in parentheses.

Table 2. Serum Lipoprotein Cholesterol and Triglyceride Concentrations In Breast-fed and Formula-fed Baboons

<table>
<thead>
<tr>
<th>Age (wks)</th>
<th>Preweaning diet</th>
<th>Number of animals</th>
<th>Lipoprotein cholesterol VLDL + LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>Breast-fed</td>
<td>13</td>
<td>44 (32 to 60)</td>
<td>46 (41 to 52)</td>
<td>47 (26 to 83)</td>
</tr>
<tr>
<td></td>
<td>Formula-fed</td>
<td>32</td>
<td>40 (33 to 50)</td>
<td>46 (42 to 49)</td>
<td>45 (29 to 70)</td>
</tr>
<tr>
<td>12</td>
<td>Breast-fed</td>
<td>13</td>
<td>43 (36 to 51)</td>
<td>68* (60 to 77)</td>
<td>37* (27 to 50)</td>
</tr>
<tr>
<td></td>
<td>Formula-fed</td>
<td>32</td>
<td>51 (46 to 56)</td>
<td>51 (47 to 55)</td>
<td>68 (56 to 84)</td>
</tr>
<tr>
<td>48 to 92</td>
<td>Breast-fed</td>
<td>13</td>
<td>84 (66 to 105)</td>
<td>87 (87 to 108)</td>
<td>23* (17 to 32)</td>
</tr>
<tr>
<td></td>
<td>Formula-fed</td>
<td>32</td>
<td>67 (58 to 78)</td>
<td>94 (87 to 100)</td>
<td>36 (31 to 48)</td>
</tr>
<tr>
<td>136 to 246</td>
<td>Breast-fed</td>
<td>13</td>
<td>97 (76 to 123)</td>
<td>99 (90 to 110)</td>
<td>36 (29 to 45)</td>
</tr>
<tr>
<td></td>
<td>Formula-fed</td>
<td>32</td>
<td>86 (74 to 101)</td>
<td>107 (101 to 114)</td>
<td>45 (39 to 51)</td>
</tr>
</tbody>
</table>

Values are the means in mg/dl, with 95% confidence intervals in parentheses.

*Significantly different from formula-fed (p < 0.01).

Serum triglyceride and lipoprotein cholesterol levels of breast- and formula-fed baboons from birth to 246 weeks of age are shown in Table 2. Breast-fed baboons had lower serum triglyceride levels at 12 weeks (p = 0.002), from weaning to 92 weeks (p = 0.009), and from 136 to 246 weeks of age (p = 0.086) than formula-fed baboons. The breast-fed infants had higher HDL-C (p = 0.0003) than formula-fed infants only at 12 weeks of age. The rate of increase in VLDL + LDL-C concentrations from 12 weeks to 92 weeks of age was higher (p = 0.003) in breast-fed baboons than in formula-fed baboons. Between 48 and 246 weeks of age, breast-fed baboons had higher VLDL + LDL-C levels than formula-fed baboons, but the difference was not significant.

The mean VLDL + LDL-C/HDL-C ratios of the breast- and formula-fed baboons from birth to 246 weeks of age are shown in Figure 1. There was no significant difference between diet groups at birth. At 12 weeks of age, formula-fed infants had a higher VLDL + LDL-C/HDL-C ratio than did breast-fed infants (p = 0.0001). After weaning, the values are the means in mg/dl, with 95% confidence intervals in parentheses. Asterisks denote statistically significant difference (p < 0.03).

Figure 1. Mean VLDL + LDL-C/HDL-C ratio in baboons breast-fed and formula-fed as infants. Breast-fed infants are indicated by the solid line; formula-fed infants are indicated by the dashed line. Vertical bars represent 95% confidence intervals. Asterisks denote statistically significant difference (p < 0.03).
VLDL + LDL-C/HDL-C ratio decreased in the formula-fed infants but increased in the breast-fed infants until 92 weeks, when the breast-fed baboons had a higher VLDL + LDL-C/HDL-C ratio than formula-fed baboons (p = 0.006). From 136 to 246 weeks of age, the VLDL + LDL-C/HDL-C ratio remained relatively constant, and the baboons that had been breast-fed as infants had a consistently higher VLDL + LDL-C/HDL-C ratio than did baboons that had been formula-fed (p = 0.09).

Serum apo A-I and apo B concentrations at 246 weeks were not different between baboons that were breast- and formula-fed as infants (mean, 95% confidence interval): apo A-I, 149 mg/dl (132 to 167) for breast-fed vs. 153 mg/dl (143 to 165) for formula-fed; apo B, 49 mg/dl (37 to 65) for breast-fed vs. 41 mg/dl (35 to 49) for formula-fed. At 246 weeks of age, there was no significant difference (p = 0.39) in the apo B/apo A-I ratio between breast-fed baboons (0.32) and formula-fed baboons (0.27).

**Effect of Sex and Sire on Serum Lipids**

There were few significant sex and sire effects on serum lipids. From weaning until 92 weeks of age, only HDL-C (p = 0.006) was significantly different among sire groups (results not shown). From weaning to 246 weeks, males had higher levels of VLDL + LDL-C/HDL-C [81 mg/dl (68 to 96) vs. 63 mg/dl (52 to 76), p = 0.003] and serum cholesterol [179 mg/dl (164 to 195) vs. 155 mg/dl (141 to 169), p = 0.030] than females.

**Association of Adiposity with Serum Lipids**

We previously reported that baboons fed the high caloric formula in infancy had greater body weights and fat depot masses at 5 years of age than did those fed the low and normal caloric formulas. As an index of adiposity, we divided the sum of the fat mass in 10 different depots (omentum, mesenteric, perirenal, popliteal, axillary, pericardial, groin, pelvic, and anterior and posterior flanks) by the body mass. The fat depot mass as a percentage of body mass ranged from 0.18% to 13.9%, with the higher values in the overfed females. There was no difference in the fat depot mass of the breast-fed baboons compared to the average of all the formula-fed baboons (results not shown). The partial correlation coefficient (adjusting for breast or formula feeding, sex, and sire) between adiposity and the VLDL + LDL-C/HDL-C ratio at 5 years of age was 0.092 (not significant).

**Effect of Preweaning Diet, Adiposity, Sex, and Sire on Arterial Lesions**

Breast-fed baboons had more extensive lesions in the thoracic aorta, abdominal aorta, and aortic arch and in the iliac-femoral and brachial arteries than did the formula-fed baboons (Table 3). No differences among the three formula-fed groups were observed (results not shown).

Because there were many zero scores for lesions in the formula-fed group, we also compared breast- and formula-fed baboons for prevalence of lesions. There were no effects of caloric density of infant formula on prevalence, nor was adiposity associated with prevalence (results not shown). Baboons that were breast-fed as infants had a greater prevalence of lesions in the thoracic aorta, abdominal aorta, and aortic arch, and in the iliac-femoral and carotid arteries, than did formula-fed baboons (Table 4).

There were no significant effects of sire or sex on prevalence of lesions or on the percentage surface area involved with lesions (results not shown).

**Regression Analysis of Lesions**

To evaluate whether infant diet exerted its observed effect (Tables 3 and 4) through serum lipoprotein concentrations, we analyzed the prevalence of lesions using multiple logistic regression analysis. The VLDL + LDL-C/HDL-C ratio made a significant contribution to predicting the prevalence of lesions even after accounting for the effect of the preweaning diet, sex, and sire for the aortic arch (p = 0.0008), thoracic aorta (p = 0.001), abdominal aorta (p = 0.01), right coronary artery (p = 0.005), left anterior descending coronary artery (p = 0.052), left circumflex coronary artery (p = 0.018), iliac-femoral arteries (p = 0.011), innominate artery (p = 0.0001), brachial arteries (p = 0.0009), and carotid arteries (p = 0.004).

After accounting for sex, sire, and the VLDL + LDL-C/HDL-C ratio, the preweaning diet had a significant effect only in the thoracic aorta (p = 0.008) and the iliac-femoral arteries (p = 0.043). In all other arteries, the additional contribution of the preweaning diet was not significant.

**Table 3. Percentage Intimal Surface Area Involved with Lesions by Artery and Preweaning Diet**

<table>
<thead>
<tr>
<th>Artery</th>
<th>Breast (n = 13)</th>
<th>Formula (n = 32)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic arch</td>
<td>4.6 (2.9 to 6.3)</td>
<td>2.0 (0.9 to 3.1)</td>
<td>0.015</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>13.7 (7.9 to 19.5)</td>
<td>7.2 (3.4 to 11.1)</td>
<td>0.085</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>15.0 (9.6 to 20.5)</td>
<td>7.4 (3.9 to 11.0)</td>
<td>0.021</td>
</tr>
<tr>
<td>Iliac-femoral arteries</td>
<td>4.2 (2.8 to 5.6)</td>
<td>1.7 (0.8 to 2.6)</td>
<td>0.004</td>
</tr>
<tr>
<td>Innominate artery</td>
<td>2.7 (0.8 to 4.6)</td>
<td>2.3 (1.0 to 3.5)</td>
<td>0.674</td>
</tr>
<tr>
<td>Brachial arteries</td>
<td>3.9 (2.2 to 5.7)</td>
<td>1.8 (0.7 to 3.0)</td>
<td>0.049</td>
</tr>
<tr>
<td>Carotid arteries</td>
<td>2.6 (1.0 to 4.1)</td>
<td>1.8 (0.8 to 2.8)</td>
<td>0.406</td>
</tr>
</tbody>
</table>

Values are mean percentages, with 95% confidence intervals in parentheses.

**Table 4. Percentage of Animals Having Lesions by Artery and Preweaning Diet**

<table>
<thead>
<tr>
<th>Artery</th>
<th>Breast (n = 13)</th>
<th>Formula (n = 32)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>9.1</td>
<td>3.3</td>
<td>0.397</td>
</tr>
<tr>
<td>Left anterior descending</td>
<td>30.8</td>
<td>12.5</td>
<td>0.133</td>
</tr>
<tr>
<td>Left circumflex</td>
<td>15.4</td>
<td>3.3</td>
<td>0.653</td>
</tr>
</tbody>
</table>
Discussion

Immediate Effects of Preweaning Diet Regimen

Serum cholesterol was higher in the breast-fed infants during the preweaning period, but the difference was not statistically significant. In a previous study, infant baboons fed breast milk had higher serum cholesterol levels than did infants fed low cholesterol formula. This difference was attributed mainly to the difference in cholesterol content between baboon breast milk (averaged 30 mg/dl) and formula (approximately 1 mg/dl). Breast-fed human infants also had higher serum cholesterol concentrations than those fed formula.14, 15, 16, 30, 31

The higher serum HDL-C in breast-fed-baboon infants could be due to a lower polyunsaturated/saturated fat ratio (P/S) in baboon breast milk compared to Similac (0.46 vs. 1.6). The 4- to 6-month-old human infants fed breast milk (P/S = 0.4) or a low P/S formula (P/S = 0.3) had higher plasma HDL-C than did babies fed formula with a high P/S ratio (1.6).30 Not all studies show differences in HDL-C between breast- and formula-fed infants, but negative studies are limited to infants younger than 40 days old.2, 32, 33 The higher HDL-C and lower triglyceride concentrations in breast-fed baboons compared to infants fed formula are consistent with the well-known inverse relationship between serum triglycerides and HDL.

VLDL + LDL-C is lower in formula-fed human infants than in those breast-fed,30 but there was no difference in VLDL + LDL-C between breast- and formula-fed baboon infants. Both infant and adult baboons have lower VLDL + LDL-C than humans, possibly because baboons metabolize VLDL and LDL faster than humans.34, 35 The differences in cholesterol content and P/S ratio between breast milk and formula may not have been great enough to lead to a difference in VLDL + LDL-C.

Long-term Effects of Preweaning Diet on Lipoprotein Cholesterol Concentrations

After weaning, the deferred effects of breast and formula feeding on the VLDL + LDL-C/HDL-C ratio were the opposite of those before weaning. This finding suggests that metabolic processes regulating the distribution of cholesterol among lipoprotein fractions were influenced by the prior breast and formula feeding before weaning. The idea that the infant diet imprints or programs lipoprotein metabolism in juvenile and young adult baboons is supported by another experiment. Mott et al.9 examined the effect of breast and formula feeding on serum lipoproteins in 4- to 6-year-old baboons after weaning to diets that varied in cholesterol content and type of fat. They described a relationship between preweaning diet and type of postweaning dietary fat in their effects on HDL-C and apo A-I concentrations: breast-fed baboons had lower HDL-C than did formula-fed baboons when both were fed saturated fat. Evidence from our current study combined with that reported by Mott et al. (which together involve a total of 128 baboons) suggests that some aspect of breast and formula feeding affects lipoprotein metabolism in young adulthood and that the expression of this effect probably depends on consumption of a diet high in cholesterol and saturated fat during juvenile and adult life. The mechanism underlying these long-term effects is not known, but increased cholesterol absorption and decreased cholesterol flux from tissues in breast-fed baboons compared to formula-fed baboons36 may be the metabolic basis of the effects of the infant diet on lipoprotein metabolism.

Observations on humans regarding long-term differences in serum cholesterol or lipoprotein levels among children or adults who were breast- or formula-fed as infants are conflicting. Hodgson et al.11 found that 7- to 12-year-old children who had been breast-fed had higher serum cholesterol levels than did children fed formulas as infants. In another study, adult women who had been breast-fed had significantly lower plasma cholesterol concentrations than did those who were bottle-fed, but there were no differences in HDL-C concentrations. Other investigators13-18 have reported no differences in serum cholesterol concentrations related to infant feeding. However, most of these studies either measured only serum cholesterol11, 12, 16 or followed infants only for a short time after weaning.14, 15 Furthermore, the duration and composition of the postweaning diet were not controlled in these human studies.

Preweaning Diet and Atherosclerosis

Atherosclerosis was measured at the middle of adolescence (3 to 7 years of age) in baboons.37 The lesions in the 5-year-old baboons were almost exclusively fatty streaks, similar to those observed in human children and adolescents.38 The higher average percentage intimal surface area involvement with atherosclerotic lesions and the higher prevalence of lesions in breast-fed baboons compared to those fed formula were explained by the effect of preweaning diet on the distribution of cholesterol among major serum lipoproteins. The predictive ability of the VLDL + LDL-C/HDL-C ratio for atherosclerosis is similar to that found previously in older adult baboons19 and also in human children and adolescents.30 If the difference in the VLDL + LDL-C/HDL-C ratio between breast- and formula-fed baboons persists beyond 5 years of age, we would expect the difference in atherothrombotic lesions between breast-fed baboons and formula-fed baboons to become even greater, since age and puberty may be important factors in the development of atherosclerosis in nonhuman primates.39 There are no comparable results from other animal experiments or human studies on the relationship of infant preweaning feeding to atherosclerosis.

Adiposity and Atherosclerosis

We previously reported that, at 5 years of age, the females that had been overfed as infants had greater body weight, fat depot masses, and abdominal fat, and the males overfed as infants had greater abdominal fat, when compared to baboons of corresponding gender that were normally or underfed as infants.17 However, neither the degree of adiposity nor the amount of preweaning food intake was associated with serum lipoprotein concentrations or atherosclerotic lesions. These results are not consistent with observations that obesity in children40, 41 and a central abdominal fat pattern in human adults42-48 are as-
PREWEANING DIET AND ATHEROSCLEROSIS Lewis et al.

associated with higher plasma cholesterol and LDL-C levels and with lower HDL-C levels. They are consistent with those of Newman et al., 38 who found no association of the ponderal index (weight/height²) with aortic atherosclerosis in human adolescents. No differences in weight and probably no differences in body fat between overfed and normally fed baboons occurred until after they were 3 years of age,17 and the duration of obesity may not have been sufficient to affect serum lipoproteins or atherosclerosis.

Conclusions

Breast-fed baboon infants, compared to baboon infants fed infant formula, had a higher serum VLDL + LDL-C/HDL-C ratio after both were weaned onto a high cholesterol-saturated fat diet. At 5 years of age, the baboons that were breast-fed as infants had more extensive and more prevalent atherosclerosis than did baboons fed formula as infants. These results provide direct evidence that adult lipoprotein cholesterol concentrations and adult experimental atherosclerosis are influenced by the type of preweaning feeding.

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