Childhood Consumption of Dietary Polyunsaturated Fat Lowers Risk for Coronary Artery Atherosclerosis in African Green Monkeys

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This study was designed to test the hypothesis that consumption of diets enriched in polyunsaturated fatty acids beginning at birth and continuing into young adulthood would lower the risk for atherosclerotic coronary heart disease early in life through their effects on plasma lipid and apolipoprotein concentrations while supporting good health and normal development. Accordingly, African green monkeys (n=140) were raised on atherogenic diets (0.8 mg cholesterol per kcal) enriched with either saturated or n-6 polyunsaturated fatty acids. Breast milk from mothers fed the polyunsaturated fat diet became enriched in polyunsaturated fatty acids relative to the saturated group; thus, the period of nursing also reflected the dietary fatty acid shift. Age, gender, and dietary fat type independently affected plasma lipid and apolipoprotein concentrations. Age effects were similar for all lipid and lipoprotein variables; the concentrations were low immediately after birth, increased dramatically during the first 4–6 months of life, and then attained levels similar to those of adult animals by 2 years of age. Significant differences by gender were found such that females maintained lower total plasma cholesterol concentrations and higher high density lipoprotein (HDL) cholesterol and apolipoprotein (apo) A-I concentrations. Dietary fat effects were age dependent. Before weaning at 5 months of age, total plasma cholesterol and apoB concentrations were lower in animals consuming polyunsaturated fat, and this pattern was maintained into young adulthood. Lower concentrations of plasma triglycerides, HDL cholesterol, and apoA-I for polyunsaturated fat–fed animals were found only in the postweaning period (6–60 months of age). Since this pattern of response to dietary polyunsaturated fat in the juvenile animals was similar to that for adult animals fed these same diets in which there was less atherosclerosis and because subsequent studies have documented less coronary artery atherosclerosis in the polyunsaturated fat–fed juveniles, we conclude that early dietary intervention was beneficial in this group for lowering the risk of coronary artery atherosclerosis. The results in this primate model support the concept that intervention to modify coronary heart disease risk that is initiated early in childhood will have beneficial effects.

Key Words: age • gender • polyunsaturated fat • nonhuman primates • risk factors • coronary artery atherosclerosis

Results from autopsy studies have provided evidence that atherosclerosis, both aortic and coronary, begins in childhood, although clinically significant childhood atherosclerosis has been reported primarily in children with familial hypercholesterolemia. Although the factors initiating atherosclerosis are not fully understood, prospective studies in adult humans have identified associations between plasma lipid concentrations and development of atherosclerotic coronary heart disease (CHD).

Russell Holman was among the first to address the question of whether the type of diet consumed during childhood and adolescence was important in the development of atherosclerosis. Dietary recommendations to the general public have included changes in eating patterns in which family members over the age of 2 years would consume less saturated fat and more unsaturated fat. In short-term dietary studies with infants and children, plasma lipid and lipoprotein concentrations have been found to be susceptible to manipulation by altering dietary fat type. However, there have been no studies for determining the benefit-to-risk ratio to atherosclerotic CHD from the long-term consumption of polyunsaturated fat–enriched diets that was initiated during childhood.

An excellent primate model for studying diet-induced modifications of lipids and lipoproteins and their relations to atherosclerosis is the African green monkey.
Adult animals of this species have similarities to human beings in the morphology of their atherosclerotic lesions and in their lipid and lipoprotein responses to diet-induced hypercholesterolemia and dietary n-6 polyunsaturated fatty acids.

With the advent of recommendations for dietary modification to include young children and because atherosclerosis begins in childhood, it is important to determine whether early dietary intervention will alter lipid and apolipoprotein concentrations and thereby reduce the risk for atherosclerosis. This study was designed to test the basic hypothesis that consumption of diets enriched in n-6 polyunsaturated fat beginning at birth and continuing into young adulthood can be beneficial in lowering the risk for atherosclerosis early in life through effects on plasma lipid and apolipoprotein concentrations. We chose to study dietary n-6 polyunsaturated fatty acids because their consumption has steadily increased in our society for several decades, and evidence in African green monkeys shows that less atherosclerosis occurs when these animals are fed high levels of these substances. This article describes age- and gender-dependent changes in plasma lipids and apolipoproteins that occurred in a pediatric-aged population of African green monkeys that consumed n-6 polyunsaturated fat–enriched diets over their lifetimes from birth through adolescence into young adulthood. Effects of dietary polyunsaturated fat feeding on high-density lipoprotein (HDL) density heterogeneity have been published. For all lipid and apolipoprotein variables measured, their concentrations rose after birth, and patterns by age and gender were similar between animals consuming saturated and polyunsaturated fat–enriched diets. The patterns of response of plasma lipids and apolipoproteins in juvenile animals appear similar to those seen in adult animals, in which polyunsaturated fat protected against atherosclerosis. These data suggest that protection by diet against atherosclerosis can begin early in life.

Methods

Animal Colony

African green monkeys (Cercopithecus aethiops) born in our primate facility over a 5-year period were studied. The breeding colony consisted of 22 sires and 64 dams, all of whom were feral animals. Females were fed one of two experimental diets, and the progeny entered the diet group of the mother. Until weaning (5 months of age), juvenile animals consumed breast milk and had access to the experimental diet. At the time that a female had finished nursing an infant, her diet was switched, and if she had another infant, it was raised on the other experimental diet. The pregnancy rate was approximately 50-60% annually. Fifty percent of the females had progeny in both diet groups. Over a 5-year period a total of 140 animals (47 males and 24 females), and 60 months of age (saturated fat group, n=14 males and n=7 females; polyunsaturated fat group, n=13 males and n=12 females).

Diets

Both semipurified diets were designed to mimic the consumption of dietary fat by North Americans, with approximately 40% of calories from fat, 40% of calories from carbohydrate, and 20% of calories from protein. The substitution of safflower oil for lard in the polyunsaturated fat diet resulted in diets with differing fatty acid patterns and a polyunsaturated-to-saturated fatty acid ratio of 0.3 for saturated fat and 2.5 for polyunsaturated fat–enriched diets. The relatively high level of dietary cholesterol (0.8 mg/kcal) was selected to put the animals in the range of hypercholesterolemia (300–400 mg/dL) typical of humans at high risk for premature CHD. All diets were stored frozen. For feeding, diets were thawed under refrigeration and animals were fed twice daily. Enough food was made available to provide a daily intake of 150 kcal/kg body wt.

Breast Milk Lipid Composition

Random breast milk samples from lactating mothers were analyzed for fatty acid composition and triglyceride and cholesterol concentrations. Breast milk was expressed manually from mothers after an intramuscular injection of oxytocin. Breast milk samples were stored frozen at −70°C until they were analyzed. Breast milk samples were thawed and remixed by sonication for 20 minutes in a water bath sonicator and were mixed by vortexing. Aliquots of 50–300 μL of breast milk were diluted to 500 μL with 0.9% saline, and lipid was extracted with chloroform/methanol by using the method of Foleh et al. In some instances tritiated oleic acid (NEN, Boston) was added as an internal standard. Briefly, freshly prepared 2:1 (vol/vol) chloroform/methanol (high-performance liquid chromatography [HPLC] grade, Fisher Scientific) was placed into acid-washed, glass-stoppered conical tubes, and the breast milk sample was injected forcefully through a 26-gauge needle into the solvent. After 1 hour at room temperature, 0.05% H2SO4 was added to each tube and the contents were mixed by inversion. Phases were separated by low-speed centrifugation, and the upper phase was aspirated and discarded. The lower phase was then washed three times with “Folch upper phase” (chloroform/methanol/0.05% H2SO4, 3:48:47, vol/vol). The lower phase was dried under nitrogen and reconstituted in a standard volume of chloroform.

Aliquots of the chloroform were analyzed in triplicate for triglycerides. Additional aliquots were taken for cholesterol quantification and for total fatty acid composition. These aliquots were dried under nitrogen and redissolved in 3 mL of redistilled ethanol. Heptadecanoic acid (17:0) was added as an internal standard for fatty acid analysis, and 0.3 mL of redistilled water and 10 mL of hexane were then added. Tubes were shaken vigorously for 1 minute and then aliquots of hexane were taken for cholesterol quantification. The hexane was removed, the lower layer was then acidified, and fatty acids were then extracted in another 10 mL of hexane. For analysis, the fatty acid extract of each milk sample was methyl-
Polyunsaturated Fat Lowers CAA Risk in Pediatric-Aged Monkeys

Wolfe et al

Plasma Lipid and Apolipoprotein Measurements

Measurements of total plasma cholesterol (TC), HDL cholesterol, and apolipoproteins A-I and B (apoA-I and apoB, respectively) were made by using blood plasma collected at 0, 2, 4, and 6 months of age and at 3-month intervals thereafter beginning at 12 months of age. For blood collection, animals were sedated with ketamine hydrochloride (15 mg/kg body wt i.m. injection, USP, Parke-Davis) or, for animals ≤4 months of age, were physically restrained. Blood samples were collected into a cocktail containing 1 mg/mL Na3 EDTA (final concentration), 1 mg/mL NaN3 (final concentration), and 0.4 mg/mL dithiobisnitrobenzoic acid (final concentration). After collection, blood samples were immediately cooled and maintained for up to 2 hours on ice. Plasma and cells were then separated by low-speed centrifugation at 4°C, 2,500 rpm for 30 minutes, and plasma was removed by pipetting. HDL was separated from other lipoproteins within 24 h of blood collection by heparin–MnCl2 precipitation.57 Because only small volumes of blood were available from animals <6 months of age, the procedure was modified so that 50-μL plasma samples could be used. Separate plasma aliquots for cholesterol, triglyceride, and apo-

lipoprotein analyses were stored frozen (−20°C for lipids and −70°C for apolipoproteins).

Cholesterol measurements for animals ≤2 months of age were done by the method of Rudel and Morris.34 Triglycerides were not measured at ≤2 months of age. Cholesterol and triglyceride measurements for animals ≥4 months of age were determined by the Lipid Analytic Laboratory of our institution, which is standardized by the Lipid Research Clinics.28 For samples analyzed before the autumn of 1986, the laboratory used the procedures designed for the Technicon Instrument II. Beginning in the autumn of 1986, the laboratory measured both cholesterol and triglycerides enzymatically using the Technicon RA-500.

Plasma concentrations of apoA-I and apoB were measured by enzyme-linked immunosorbent assay (ELISA).29,30 The primary standard for the apoA-I ELISA was purified African green monkey apoA-I and for the apoB ELISA, African green monkey low-density lipoprotein of d=1.026–1.052 g/mL. The secondary standard for both ELISAs was a plasma pool from animals consuming the polyunsaturated fat–enriched diet, and the control plasma was a pool from animals consuming the saturated fat–enriched diet. The ELISA interassay coefficients of variation were 4.6% (n=70) for apoA-I and 9.2% (n=75) for apoB, as determined on four concentrations of the control plasma run in duplicate.

Statistical Analysis

Statistical analyses of triglyceride and cholesterol concentrations of breast milk were done by two-way analysis of variance using BMDP statistical software.31 Percent fatty acid composition of breast milk was compared by dietary fat type using Student’s t test,32 with corrections of the significance level for multiple comparisons by the Bonferroni technique.33 All statistical analyses of longitudinal data in this study were done using the SAS software package (Statistical Analysis System, Raleigh, N.C.). Longitudinal measurements of lipids and apolipoproteins were analyzed by univariate repeated-measures analysis of variance. Data in tables and figures are presented as mean±SEM. The statistical model considered various outcome variables measured at several ages as the repeated measures and dietary fat as the treatment effect. All interaction terms were considered in the statistical model and were included in the model only if they were significant at p≤0.05.

The statistical analysis was a hierarchical procedure in which the full model containing main effects (dietary fat and age and, in some cases, gender) and all interactions were tested, and then nonsignificant terms were removed sequentially from the model. When all possible comparisons by age and dietary fat type were made, the significance level for multiple comparisons was corrected by the Bonferroni technique.33 When a significant effect by age was found, mean differences among ages were tested for significance using Duncan’s multie range test.34 For purposes of statistical analyses for lipid and apolipoprotein variables, data were segregated into preweaning and postweaning periods. This was due to the fact that the animals within each treatment group were exposed to two different dietary regimens during
their lifetimes (breast milk plus experimental diet for 0–5 months of age and experimental diet for 0–60 months of age).

This study was designed to evaluate atherosclerosis in animals at 16, 32, and 60 months of age; therefore, after 6 months of age the number of animals in each dietary fat group decreased with increasing age. Animals were chosen randomly for atherosclerosis evaluations at the three ages. The repeated-measures statistical analysis was designed to accommodate missing data and removal of animals from the study. To determine that statistical outcomes for effects of age and dietary fat on lipid and apolipoprotein measurements were not the result of specific animals' being removed from the study, statistical analyses in the postweaning period were repeated for shorter age intervals of 6–16 and 6–32 months of age. In all instances the statistical outcomes did not change when these shorter age intervals were analyzed.

Repeated-measures analysis of covariance was used for determining relations between lipid and apolipoproteins by modeling polynomial relations separately across time. The consistency of the model across ages and between dietary fat groups was examined. When the relation was linear, Pearson's product-moment correlation coefficient was calculated. "Tracking" of lipid and apolipoprotein measurements was analyzed by calculation of Spearman rank-order correlation coefficients at yearly intervals from 12 to 60 months of age. Because the magnitudes of the correlations and their changes with age were similar between the two dietary fat groups, the data were combined.

Results

Because animals were monitored from birth into young adulthood, measurements were made of growth, development, and general health status as a method for determining the efficacy of consumption of diets enriched in polyunsaturated fat. Our results showed no evidence that polyunsaturated fat feeding was associated with maleficent effects on growth or general well-being of the animals (M.S. Wolfe et al, unpublished observations). Age-related changes in body weight are shown by gender and dietary fat type in Figure 1. Male and female animals of both diet groups grew steadily at similar rates until the animals were about 28–30 months of age. Around these ages, body weights of the sexes diverged, and while mean weight gains in females plateaued after about 36 months of age, male animals continued to gain weight steadily until about 48 months of age. By 4 years of age male animals weighed approximately 25% more than female animals. Similar patterns were observed for body mass and skeletal growth. In addition, hormonal changes and secondary sexual development in male animals were observed to occur during the interval from 24 to about 54 months of age. Therefore, we classified development by age as prepubertal, 0–28 months; peripubertal, beginning around 28 months of age; and young adulthood, after 36 months of age in females and 48 months of age in males.

Experimental Diets

The study was designed to model lifetime consumption of either saturated or polyunsaturated fat–enriched diets. Before and during both pregnancy and lactation, each mother consumed the diet for her progeny's experimental group. Samples of breast milk from representative animals of each experimental group were analyzed for lipid concentration and fatty acid composition. Triglyceride and cholesterol concentrations of the breast milk are shown in Table 1. The triglyceride concentration of the milk did not vary as a result of the type of dietary fat being consumed or the length of lactation (3.4±0.4 g/100 mL and 4.1±0.4 g/100 mL for 0–5 months for saturated and polyunsaturated fat groups, respectively; mean±SEM). The triglyceride content of the breast milk was similar to that reported for lactating rhesus monkeys (3.9 g/100 mL). With the assumption that breast milk caloric distribution is similar for the two nonhuman primate species, the nursing infants consumed approximately 50% of kilocalories from dietary fat. This is slightly higher than the 42% of kilocalories as fat in the experimental diets.
Triglyceride and cholesterol concentrations of breast milk were measured and data were analyzed as described in "Methods." Values are mean±SEM.

Although the triglyceride content of breast milk was not different, the cholesterol concentration of breast milk of polyunsaturated fat-fed mothers was approximately twice as high as that of saturated fat-fed mothers (12.7±1.2 versus 21.6±2.1, p=0.0002 for 0–5 months of saturated versus polyunsaturated fat groups, respectively). Analysis of breast milk from both groups showed that there were no differences in plant sterol or \( \beta \)-sitosterol content. In both diet groups approximately 93% of the cholesterol in milk was unesterified. The cholesterol concentration of milk also varied significantly with length of lactation in both groups (\( p=0.03 \)), being highest in the colostrum and increasing slightly between 1 and 5 months of lactation. On the basis of the caloric content of rhesus monkey breast milk, the cholesterol intake of the nursing animals averaged 0.2 and 0.3 mg/kcal for saturated and polyunsaturated fat groups, respectively. In both diet groups the cholesterol intake from breast milk was lower than that consumed in experimental diets (0.8 mg/kcal). The fatty acid composition of breast milk was found to reflect dietary fatty acid composition. Like the experimental diets, breast milk from polyunsaturated fat–fed mothers had significantly \( p<0.05 \) lower percentages by weight of palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1), and oleic acid (18:1) than the saturated fat–fed group; linoleic acid (18:2) was higher. The polyunsaturated-to-monounsaturated-to-saturated fatty acid ratios of breast milk and the experimental diet were 1/4.0/3.5 and 1/3.3/3.1, respectively, for the saturated fat group and 1/0.7/0.7 and 1/0.5/0.4, respectively, for the polyunsaturated fat group. In both experimental diets (and milks), the ratio of monounsaturated to saturated fatty acids was constant, between 1.0 and 1.2, and the substitution of n-6 polyunsaturated fatty acids into the polyunsaturated fat diet was at the expense of both saturated and monounsaturated fatty acids. Therefore, the major difference in dietary fatty acid intake between the two diet groups was the four-fold higher consumption of n-6 polyunsaturated fatty acids by animals in the polyunsaturated fat group; saturated and monounsaturated fatty acid intake was decreased in this group by about twofold.
Effects of Age and Dietary Fat Type on Plasma Triglycerides

Plasma triglycerides were measured periodically throughout each animal’s lifetime, beginning at 4 months of age. The longitudinal plot of mean triglyceride concentrations beginning at 4 months of age for the two dietary fat groups is shown in Figure 3. The large variability in triglyceride values at 4 months of age most likely resulted from the fact that the animals were not fasted before blood sampling. Plasma triglyceride concentration was low across all ages but was higher in the 4–6-month age period. Mean concentrations of both groups then declined and approached values observed in adult animals fed these same diets.21

Dietary polyunsaturated fat feeding was associated with significantly (p=0.0006) lower triglyceride concentrations in the postweaning 6–60-month-age period, averaging 25% lower in the polyunsaturated fat group (18±1 versus 24±2 mg/dL for polyunsaturated versus saturated fat groups, respectively).

Effects of Age on TPC and HDL Cholesterol

TPC concentrations (mean±SEM) measured longitudinally in the animals of both dietary fat groups are shown in Figure 4. TPC levels immediately after birth were lower than for any other age. After birth TPC increased linearly up to 4 months of age, reaching concentrations that were significantly higher than at 0 (p=0.0003) and 2 (p=0.01) months of age. After weaning at 5 months of age, TPC levels continued to increase until 24 months of age, but at a slower rate. By 2 years of age TPC concentrations in the animals of both dietary fat groups had plateaued at levels that were maintained into young adulthood.

Since mean TPC concentrations were reasonably constant in the postweaning period, correlations between repeated TPC measurements for animals at yearly intervals were determined. Spearman rank-order correlation coefficients for TPC are shown in Table 2. Correlations were highest between TPC values that differed by only 1 year. Prepubertal TPC concentrations at 12 months of age were less well correlated with other TPC measurements than were values at 24 months of age; the latter were highly correlated with TPC concentrations in young adulthood at 48 and 60 months of age.

Longitudinal changes in HDL cholesterol concentrations for the two dietary fat groups are shown in Figure 5. Shortly after birth there was a significant (p=0.03) increase in HDL cholesterol. As was found for TPC, HDL cholesterol concentrations at 2 and 4 months of age were significantly higher than at 0 months of age (p=0.007 and p=0.003 for 2 and 4 months of age, respectively).

During the postweaning period HDL cholesterol concentrations varied significantly (p=0.0001) with age. HDL cholesterol concentrations were highest at 4 and 6 months of age in both saturated and polyunsaturated fat groups and declined to a low at about 36 months of age. During the preweaning period approximately 50% of TPC was found in HDL cholesterol; however, with the decline in HDL cholesterol after 6 months of age and the increase in TPC (Figure 4), the percentage of plasma cholesterol in HDL decreased to a low average of 27% at 24 months of age.

Spearman rank-order correlations for repeated HDL cholesterol measurements in the postweaning period are shown in Table 3. Correlation coefficients for HDL cholesterol were similar for measurements made after
36 months of age but were lower than for repeated TPC values. As was found for TPC, HDL cholesterol concentrations at 12 and 24 months of age were correlated significantly (p<0.04) with HDL cholesterol levels when animals were young adults (48 and 60 months of age).

**Effect of Dietary Fat Type on TPC and HDL Cholesterol**

When the data for TPC (Figure 4) were analyzed for effects of dietary fat type, TPC concentrations were found to respond early in life to differences in dietary fatty acid composition. TPC was significantly lower in the polyunsaturated fat–fed animals in both preweaning (p=0.01) and postweaning (p=0.003) periods. In the preweaning period when the animals were consuming primarily breast milk, TPC averaged 15% lower in the polyunsaturated fat–fed group. These lower TPC concentrations in animals fed polyunsaturated fat were maintained into the postweaning period. For the 20–60-month plateau, TPC averaged 18% lower in the polyunsaturated fat–fed animals (261 versus 321 mg/dL for polyunsaturated versus saturated groups, respectively).

Dietary fat type also was found to affect HDL cholesterol concentrations as shown in Figure 5. Mean HDL cholesterol concentrations averaged 19% lower (p=0.02) in the polyunsaturated fat group in the preweaning period. This effect of polyunsaturated fat feeding to lower HDL cholesterol was maintained throughout the postweaning period, even though HDL cholesterol concentrations were changing with age. For the age interval of 24–60 months, HDL cholesterol averaged 13% lower in the polyunsaturated fat group (76 versus 87 mg/dL for polyunsaturated and saturated fat groups, respectively). HDL cholesterol concentrations after about 2 years of age were found to be similar to those observed in adult animals fed these same diets.

Despite the effects of polyunsaturated fat in lowering TPC and HDL cholesterol, there was no significant effect of dietary fat on the TPC-to-HDL cholesterol ratio in either the preweaning (p=0.5) or postweaning (p=0.4) periods. This median ratio in the preweaning period was 2.4 (2.10–2.74, 95% confidence interval [CI]) versus 2.28 (2.00–2.59, 95% CI) and in the postweaning period was 3.4 (2.94–3.92, 95% CI) versus 3.7 (3.14–4.35, 95% CI) for polyunsaturated versus saturated fat–fed groups, respectively.

In the postweaning period TPC and HDL cholesterol concentrations were found to have a polynomial relation (r²=0.11, p=0.002), similar to that seen in adults. For all ages in the postweaning period, TPC concentrations <306 mg/dL were correlated positively with HDL cholesterol, and TPC concentrations >306 mg/dL were correlated negatively with HDL cholesterol. Dietary fat had a significant (p=0.02) effect on the correlation of TPC with HDL cholesterol so that for individual TPC concentrations, HDL cholesterol was lower in the polyunsaturated fat–fed group.

**FIGURE 5.** Line plot of age and dietary fat effects on high-density lipoprotein (HDL) cholesterol concentrations in juvenile animals. Data are presented as mean±SEM. Data were analyzed as described in the legend to Figure 3 and "Methods." Preweaning age effect, p=0.0001; 0 months versus 2 months, p=0.007, and 0 months versus 4 months, p=0.003; dietary fat effect, p=0.02. Postweaning age effect, p<0.0001; and dietary fat effect, p=0.02.
TABLE 3. Rank-Order Correlations for HDL Cholesterol

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HDL, high-density lipoprotein; *r*, Spearman rank-order correlation coefficient.

Effect of Age and Dietary Fat Type on Plasma Apolipoproteins A-I and B

Longitudinal data for plasma apoA-I concentrations are shown in Figure 6. The value at 0 months of age is only an estimate of apoA-I because only one animal per diet group was measured. By 2 months of age, apoA-I concentrations were apparently higher and showed only marginal changes during the remainder of the first year of life. In the postweaning period there was a significant (*p*=0.0001) effect of age on apoA-I. In a manner similar to that for HDL cholesterol (Figure 5), apoA-I concentration decreased between 6 and 24 months of age. From 24 months on, differences in apoA-I concentration by age were not significant (*p*>0.05).

The correlation between HDL cholesterol and apoA-I concentrations was examined in the postweaning period, and the two variables were found to be positively correlated (*r*=0.59, *p*=0.0001).

Spearman rank-order correlations for repeated plasma apoA-I values were also measured (data not shown). The correlation pattern for apoA-I was similar to that for HDL cholesterol. ApoA-I concentrations at 12 months of age often were not significantly correlated with values at older ages. At 24 and 36 months of age apoA-I values were correlated similarly with young-adult apoA-I values (48 and 60 months of age). ApoA-I concentrations at 48 and 60 months of age had the highest correlation (*r*=0.71, *p*=0.0001).

Dietary fat type had a significant (*p*=0.002) effect on plasma apoA-I concentrations in the postweaning period; however, preweaning plasma apoA-I concentrations were found not to differ. For ages 6–60 months, apoA-I concentrations were 12% lower in the polyunsaturated fat-fed animals (262±9 versus 296±9 mg/dL for polyunsaturated versus saturated groups, respectively, mean±SEM).

Plasma apoB concentrations for the two dietary fat groups are shown by age in Figure 7. The age-related changes in both dietary groups were similar to those found for TPC. Immediately after birth apoB concentrations were low (<100 mg/dL) and increased linearly with age (*p*=0.001), being nearly twofold higher at 4 months of age (*p*=0.003). ApoB concentrations continued to show an effect of age (*p*=0.0001) in the postweaning period, although after 12 months of age mean apoB concentrations were not significantly different among older ages.

Rank-order correlations between repeated apoB measurements in the postweaning period were determined (data not shown). Individual apoB concentrations were generally not highly correlated when comparing values at 12 and 24 months of age with values at the older ages, although the value at 24 months of age was highly correlated to apoB concentration at 60 months of age (*r*=0.63, *p*=0.0006). The highest correlation was between apoB concentrations at 48 and 60 months of age (*r*=0.65, *p*=0.0001).

ApoB concentrations in the preweaning period were significantly lower (37%, *p*=0.0005) in animals fed polyunsaturated fat. At 4 months of age apoB concen-

**Figure 6.** Line plot of effects of age and dietary fat saturation on plasma apolipoprotein (apo) A-I concentrations in juvenile animals. Data are presented as mean±SEM. Data were analyzed as described in the legend to Figure 3 and "Methods." Preweaning age effect, *p*=0.001, and dietary fat effect, *p*=0.2. Postweaning age effect, *p*=0.0001, and dietary fat effect, *p*=0.005.
trations were approximately 30% lower (p=0.004) in the polyunsaturated fat group. Although at some ages in the postweaning period the differences in mean apoB concentrations between dietary fat groups were minimal, overall apoB averaged 12% lower (p=0.03) in the polyunsaturated fat-fed group. For the 12–60-month age interval, apoB averaged 9% lower in the polyunsaturated fat group (139 versus 153 mg/dL for polyunsaturated versus saturated fat groups, respectively).

Effect of Gender on Lipids and Apolipoproteins

Figure 1 shows that there were age- and gender-related differences in body weight in both dietary fat groups. In addition, we have reported on gender differences in HDL density heterogeneity in this group of animals.20 We wanted to determine whether gender differences also existed in the longitudinal measurements of lipids and apolipoproteins during the postweaning period. There was a significant (p=0.048) interaction of age with gender on TPC in the postweaning period. As shown in Figure 8, mean TPC concentrations were higher in the male animals of both dietary fat groups at all ages. The difference by gender in TPC concentration was greater between male and female animals in the saturated fat group (20%; 273±32 versus 343±30 mg/dL for female versus male animals, respectively, mean±SEM) than in the polyunsaturated fat group (15%; 243±23 versus 286±21 mg/dL for female versus male animals, respectively). In addition, during this age interval polyunsaturated fat-fed females had significantly (p=0.02) lower TPC concentrations than saturated fat-fed males.

Mean HDL cholesterol concentrations for individual gender/dietary fat groups are shown in Figure 9. There was a significant (p=0.0007) interaction of gender with age, which resulted in gender-specific changes in HDL cholesterol at different ages. During the prepubertal age interval, mean HDL cholesterol concentrations in male and female animals within each diet group were similar and showed age-related declines in concentration. In the peripubertal and young-adulthood age intervals, mean HDL cholesterol levels were consistently lower in...
male animals of both diet groups, although differences at individual ages were not statistically significant. For female animals HDL cholesterol concentrations appeared to remain reasonably constant beyond 24 months of age, while for male animals, HDL cholesterol concentrations continued to decline and, compared with values in females, averaged 17% and 12% lower in males for polyunsaturated and saturated fat-fed groups, respectively.

Plasma apoA-I showed a significant ($p=0.05$) interaction of gender with age, resulting in a pattern of age-specific changes in apoA-I concentration similar to that for HDL cholesterol. ApoB concentrations showed no significant ($p=0.6$) gender-specific differences in the postweaning age period.

**Discussion**

In a colony of juvenile African green monkeys, we studied risk factors that influence premature CHD and have documented the effects of long-term dietary n-6 polyunsaturated fatty acid consumption thereon. Age, gender, and dietary fat type all had independent effects on plasma lipids and apolipoproteins, which could alter the relative degree of atherogenicity. Age-related increases in cholesterol concentrations of all plasma lipoproteins began immediately after birth and were similar in the two dietary fat groups. TPC and apoB concentrations rose until about 12-18 months of age and then were maintained at a similar level until 5 years of age. These values remained significantly higher throughout this period in animals fed saturated fat than for those fed polyunsaturated fat. After plateauing at 6 months of age, HDL cholesterol concentrations then fell until adult levels were achieved at between 2 and 3 years of age. Females in both dietary fat groups were observed to have generally lower TPC, HDL cholesterol, and apoA-I concentrations in plasma than males, with the difference in the latter two end points beginning after about 2 years of life. Overall, these young primates showed a pattern of age-related changes in lipids and apolipoproteins quite similar to that reported for human infants and children. By early adulthood, the juvenile monkeys had attained plasma concentrations similar to those reported for adults of this species fed the same diets. Therefore, the usefulness of this primate model for studying the effects of dietary modifications on coronary artery atherosclerosis in juveniles seems high.

A major goal of this study was to determine whether beneficial effects on CHD risk from life-long consumption of a diet high in n-6 polyunsaturated fatty acids would occur in the absence of detrimental effects on health and growth. This is an important question, since the reason most often given for not supporting higher levels of n-6 polyunsaturated fatty acids in diets of human beings is not based on experimental evidence that these fatty acids are harmful to humans but rather that "no population is known that naturally consumes such a diet." Dietary intervention with polyunsaturated fat beneficially influenced some positive CHD risk factors for atherosclerosis during the period from birth through adolescence into young adulthood in the monkeys of this study. Dietary polyunsaturated fat lowered TPC and plasma apoB during the period before weaning, and differences were maintained throughout periods of growth and development into young adulthood. HDL cholesterol and plasma apoA-I concentrations were also lower in animals fed polyunsaturated fat, a finding consistent with what has been previously reported when high levels of polyunsaturated fatty acids

![FIGURE 9. Mean HDL cholesterol concentrations by gender for animals fed either saturated (panel A) or polyunsaturated (panel B) fat-enriched diets. The data were analyzed by repeated measures analysis of variance as described in "Methods." Gender x age effect, p=0.0007. Diet effect, p=0.017.](http://atvb.ahajournals.org/content/pdf/)
were fed. A shift in HDL distribution to enrich the proportion of intermediate density HDL subfractions was also associated with polyunsaturated fat feeding, a shift that may be reflective of more antiatherogenic HDL particles. These dietary polyunsaturated fat–related changes in the risk profiles of young animals were consistent overall with a decreased risk for atherosclerotic CHD because they mirrored those in adult animals fed these same diets in which less atherosclerosis was found. When atherosclerosis evaluations were made in the young animals of this study, less extensive and severe coronary artery atherosclerosis was found in the animals fed polyunsaturated fat. Therefore, the results in this primate model show a benefit of early intervention with dietary polyunsaturated fat against coronary artery atherosclerosis development. This outcome suggests that potential benefits could also occur in human beings and supports the idea that dietary treatment of coronary artery atherosclerosis in high-risk individuals at very early ages may prove beneficial.

A complete lack of detrimental effects of dietary polyunsaturated fat is difficult to establish. We evaluated 19 different clinical and hematological indices and found no effect of dietary fat type, and the number of clinical incidents was not different for the two groups (data not shown). The animals fed polyunsaturated fat were found to gain weight at rates similar to those of the saturated fat group, and birth weights were the same (Figure 1). Biliary lipid composition was similar by dietary fat type, and there was a similar incidence of gallstone disease for young primates consuming diets enriched in saturated and polyunsaturated fat. By all of these criteria, health and development seemed to be unaffected by dietary fat type, in which case any beneficial effect of protection against coronary artery atherosclerosis becomes more important. Furthermore, if there is a real benefit to behavior and development from diets containing fat and cholesterol, a polyunsaturated fatty acid–enriched diet may prove useful.

The diets used in this study were designed to mimic those of North Americans in fat content and calorie distribution. The amount of dietary saturated and n-6 polyunsaturated fatty acid was set at near-achievable extremes (for humans) to facilitate detection of differences where they may occur. Dietary cholesterol was fed at a high level (0.8 mg/kcal) relative to human consumption because, for this animal model, the higher level is necessary to achieve TPC concentrations in the range of 250–350 mg/dL, i.e., that typical of human beings who are at increased risk for atherosclerotic CHD. Such TPC elevations were needed to assure that any effect on atherosclerosis per se could be evaluated in the period of time available for study.

In assessing the relative risk versus benefit for increased dietary polyunsaturated fat consumption, we presumed that, practically speaking, effective dietary modification would affect all members of a household. While it has been recommended that dietary modification for reducing saturated fat and increasing polyunsaturated fat be initiated for children after age 2, dietary modification in a family would likely affect even younger children and would also affect the mother throughout pregnancies that occur after the diet modification was initiated. The present study helps evaluate whether risk or benefit to the mother and her family may result from dietary fatty acid modification, since the mothers in this study conceived, carried to term, and nursed the infants while consuming the experimental diet of their progeny. We did not see any difference in pregnancy outcome for mothers in the polyunsaturated fat compared with saturated fat diet groups (data not shown), and the birth weight, growth rate, and apparent health of the progeny for both diet groups were the same as cited above. This outcome would predict that detrimental effects to the mothers and their families related to the ingestion of diets high in polyunsaturated versus saturated fatty acids are limited, if present at all; on the other hand, an apparent benefit for lowering premature coronary artery atherosclerosis is appreciable.

Breast milk from nursing mothers of the polyunsaturated fat group was significantly more enriched in polyunsaturated fatty acids than that from the saturated fat group (Figure 2). A similar finding has been reported for human infants, in whom consumption of such milk was associated with lower TPC concentrations. We found a higher cholesterol content in the more polyunsaturated milk, which has not been reported before. This is an interesting difference, but it was not enough to overcome the effect of polyunsaturated fatty acids in maintaining lower plasma cholesterol and apoB concentrations in nursing infants. On the other hand, it may have contributed to a lessening in the extent of the difference, although we have no data to support this possibility. It has been reported that the cholesterol content of human breast milk does not vary with increased dietary polyunsaturated fatty acids. We have no direct evidence for the reason of the increased cholesterol concentration of breast milk in the polyunsaturated fat group; however, the differences in dietary polyunsaturated fatty acid content were greater in this study. We speculate that in the mammary gland of polyunsaturated fat–fed animals, the cholesterol-to-phospholipid ratio is higher in the membranes from which fat globules are derived. In a study that examined dietary fat type and intestinal lipoprotein formation in African green monkeys, infusion of a polyunsaturated fat diet was associated with larger chylomicra containing more cholesterol per particle than those formed from a more saturated fat diet. A similar phenomenon could be involved during milk globule formation.

In making multiple measurements of lipid and apolipoprotein concentrations throughout the lifetime of each animal, we wanted to determine whether early measurements were predictive of concentrations later in life. We examined tracking, i.e., the persistence of rank orders of lipid and apolipoprotein measurements, by using Spearman rank-order correlations at yearly intervals from adolescence into young adulthood. Correlations between lipid and apolipoprotein concentrations by age showed that concentrations of apolipoproteins were less consistent than those for cholesterol with, in either case, the highest correlations occurring between observations made in young adulthood. The extent of agreement between repeated measurements in these animals was similar to that observed for school-aged children who were studied repeatedly at 2–6-year intervals. From quintile ranking for TPC concentrations, we found that many of the animals that had extremes of
TPC concentration, i.e., in the highest and lowest quintiles, had gained their respective ranks by 12 months of age. Approximately 80% of the animals that initially were in the highest and lowest quintiles for TPC at 12 months of age were in the upper two quintiles and lowest quintile, respectively, at 48 months of age (data not shown). This tendency for animals to remain in similar ranks for TPC as they matured and grew suggests that animals displaying a predisposition to hypercholesterolemia in adolescence would likely enter adulthood with increased risk for coronary artery atherosclerosis.

In our study HDL cholesterol and plasma apoA-I showed patterns for differences by gender, beginning after 24 months of age. In human children HDL cholesterol57-38 and plasma apoA-I59 have been observed to show similar differences by gender; however, because of unequal developmental rates among adolescents, it has been difficult to separate the contributions of age versus sexual maturation in explaining the observed changes in these variables. We have very limited data for the timing of puberty and sexual maturation in these primates. On the basis of measurements of body weight (Figure 1) and skeletal maturation,59 we believe that the patterns by gender in HDL cholesterol and apoA-I that resulted in lower levels in male animals coincided with the period of sexual maturation in the animals. In a separate report from this study,20 differences by gender were also demonstrated in HDL subfraction cholesterol heterogeneity. Age-related decreases in HDL cholesterol in males were associated with decreases in less dense HDL (d<1.09 g/mL) and increases in intermediate-density HDL (d=1.10–1.13 g/mL), while in females cholesterol concentrations within these subfractions were unchanged.

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Wolfe et al  Polyunsaturated Fat Lowers CAA Risk in Pediatric-Aged Monkeys 875


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