Age and Dietary Polyunsaturated Fat Alter High Density Lipoprotein Subfraction Cholesterol Concentrations in a Pediatric Population of African Green Monkeys

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African green monkeys were raised from birth to 60 months of age on diets containing cholesterol (0.8 mg/kcal) and enriched in polyunsaturated (polyunsaturated to saturated fat ratio [P:S]=2.5) or saturated (P:S=0.3) fat. Lipoproteins were isolated from plasma of a group of animals (N=123) and were separated by gel filtration chromatography at 9, 14, 26, 38, and 50 months of age, which covered a period through adolescence into young adulthood. Total plasma cholesterol (TPC) concentrations were 16% lower (p=0.01) in the polyunsaturated fat–fed group, and high density lipoprotein (HDL) cholesterol concentrations averaged 20% lower (p=0.008) in this group between 14 and 50 months of age, while plasma apolipoprotein A-I (apo A-I) averaged 7% lower (p=0.06) over this age interval in the animals. The HDL cholesterol to apo A-I ratio was found to be significantly lower (p=0.006) in the animals fed the polyunsaturated fat diet. This suggested that the HDL subtraction distribution might differ between groups. In a subset of animals (n=105, 64 male and 41 female), HDL was subfractionated by density gradient ultracentrifugation into six subfractions, HDL-I to HDL-VI, from lowest to highest density. The saturated fat–fed animals had significantly higher cholesterol concentrations in HDL-I and significantly lower cholesterol concentrations in HDL-III, HDL-IV, and HDL-V. These effects held across all ages studied; therefore, these diet effects were not age dependent. In both diet groups, the HDL subtraction pattern changed with age such that the HDL-I and HDL-II cholesterol concentrations decreased, and those of HDL-IV, HDL-V, and HDL-VI increased as the animals matured. The decrease in HDL-I with age appeared to result primarily from a decrease in HDL-I in males, while the HDL-I cholesterol concentration in females did not change with age. We conclude that diet, age, and gender all affect HDL subtraction distribution and therefore can potentially modify the relative atherogenicity of the plasma HDL populations. It remains for future studies to demonstrate the effectiveness of each subtraction in promoting or preventing the cholesterol deposition of atherosclerosis. (Arteriosclerosis and Thrombosis 1991;11:617–628)
fat–fed diets are consumed, and animals fed polyunsaturated-fat diets have also been found to have less coronary artery atherosclerosis (CAA). The paradox of lower HDL cholesterol and less CAA in adult animals consuming polynsaturated-fat–enriched diets is unexplained. This paradox requires special consideration of the influence of dietary polynsaturated fat on HDL and suggests either that HDL cholesterol concentration by itself may not provide enough information for understanding HDL's antiatherogenic potential or that the beneficial effects of polyunsaturated fat on LDL are of overriding importance.

HDL of human and nonhuman primate species has been shown by several laboratories10–16 to be a heterogeneous lipoprotein class composed of particles differing in cholesterol and apolipoprotein concentration. A previous study in adult African green monkeys fed saturated or polyunsaturated fat–enriched diets found that overall, the type of HDL heterogeneity was similar to that seen in human beings, but the subtraction distribution was different between the two groups.11 A higher concentration of intermediate density HDL was found in the polyunsaturated-fat–fed group despite lower total HDL concentrations, and the polyunsaturated-fat–fed animals had less atherosclerosis (L. Rudel et al, unpublished observations).

Atherosclerosis is believed to begin early in life and to progress as an individual ages and matures; therefore, early dietary intervention could be important in modulating lipoprotein metabolism. In cross-sectional studies, HDL cholesterol concentrations have been measured in children from several demographic regions17–20; however, there are limited data on HDL subpopulations,21,22 and we are not aware of data defining the effects of dietary fat type thereon. Given the importance of HDL to atherosclerosis and the similarity between human beings and adult African green monkeys in their response to dietary fat and cholesterol, we undertook this project. We have attempted to determine if dietary polyunsaturated fat could alter HDL and its subtraction heterogeneity during the pediatric-age years. Animals entered this study at birth and consumed the same diet for up to 6 years. This gave us the opportunity to monitor long-term effects of dietary polyunsaturated fat on HDL and HDL heterogeneity from adolescence through periods of growth and development into young adulthood. We have found that aging of the animals was associated with a redistribution of cholesterol from less dense to more dense HDL. In addition, we have identified patterns of HDL response to dietary fat in juvenile animals that were similar to those seen in adults in which polyunsaturated fat actually protected against atherosclerosis. These findings suggest that protection against atherosclerosis induced by diet can begin early in life.

Methods

Animals

African green monkeys were raised from birth in the primate facility of our institution. The animals in this study consumed diets enriched in polyunsaturated or saturated fat over their lifetimes. The animals' mothers consumed the diets during pregnancy and lactation, so that the animals entered their respective experimental group at birth. The animals were weaned from their mothers into social groups at 5 months of age. This report is from a larger project investigating the long-term effects of diets enriched in polyunsaturated fat on CHD risk factors and atherosclerosis. HDL was isolated from blood samples of 123 animals at ages 9, 14, 26, 38, and 50 months. This postweaning age period spanned from adolescence into early adulthood in this species. In a subset of these animals (n=105, 64 male and 41 female), HDL was subfractionated by density gradient ultracentrifugation at the same ages.

Diet

Semipurified diets11 were prepared in the diet kitchen of our center, were stored frozen, and then were thawed under refrigeration for feeding to the animals twice daily (approximately 150 kcal/kg body wt). The diets were calorically balanced and differed only in fatty acid composition. Both diets provided approximately 40% kcal from fat, 20% kcal from protein, and 40% kcal from carbohydrate. The fatty acid extract of each diet sample was methylated,23 and the fatty acids beginning with myristate (14:0) were separated on a Hewlett-Packard Model 5890A gas–liquid chromatograph (Palo Alto, Calif.) on a DB-225 column (30 mx0.25 mm, J&W Scientific, Folsom, Calif.) with a flame ionization detector. The fatty acid composition of the diets is shown in Table 1. The diet enriched in saturated fat was designed to mimic the dietary fatty acid consumption pattern of the typical North American and had a polyunsaturated to saturated fat (P:S) ratio of 0.3. The diet enriched in polyunsaturated fat substituted safflower oil for lard and had a P:S ratio of 2.5. To induce atherosclerosis and to achieve total plasma cholesterol (TPC) concentrations equivalent to those in human beings at increased risk for CHD, the animals consumed dietary cholesterol, principally from dried egg yolk (4.1±0.1 mg/g dry wt [n=14] and 4.0±0.1 mg/g dry wt [n=105]).

### Table 1. Percentage Fatty Acid Composition of Experimental Diets

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Saturated (%)</th>
<th>Polyunsaturated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid, 14:0</td>
<td>1.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Palmitic acid, 16:0</td>
<td>27.1</td>
<td>15.7</td>
</tr>
<tr>
<td>Stearic acid, 18:0</td>
<td>13.0</td>
<td>5.1</td>
</tr>
<tr>
<td>Palmitoleic acid, 16:1 ω7</td>
<td>2.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Oleic acid, 18:1 ω9</td>
<td>40.9</td>
<td>24.2</td>
</tr>
<tr>
<td>Linoleic acid, 18:2 ω6</td>
<td>13.1</td>
<td>52.1</td>
</tr>
<tr>
<td>Other</td>
<td>2.0</td>
<td>1.3</td>
</tr>
<tr>
<td>P:M:S</td>
<td>1/1.5/1.1</td>
<td>1/0.5/0.4</td>
</tr>
<tr>
<td>P:S</td>
<td>0.3</td>
<td>2.5</td>
</tr>
</tbody>
</table>

were defined by the following density intervals: HDL-I (d<1.09 g/ml), HDL-II (1.09-1.10 g/ml), HDL-I
(1.10-1.11 g/ml), HDL-IV (d=1.11-1.12 g/ml), HDL-V (d=1.12-1.13 g/ml), and HDL-VI (d>1.13 g/ml).
HDL subfractions. HDL cholesterol was added to 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 sequentially
nonsignificant interaction terms were removed from the model. When all possible comparisons by age and
dietary fat were made, the significance level for the multiple comparisons was corrected for multiple
comparisons with the Bonferroni technique. When a significant effect by age was found, the differences
in the variable between different ages were tested by Duncan's multiple range test.

Repeated-measures analysis of covariance was used for analyzing cholesterol concentrations of
HDL subfractions. HDL cholesterol was added to the statistical model as the covariate. Repeated-measures
analysis of covariance was also used in correlation analysis for determining relations between
HDL cholesterol and HDL density subfractions by age and dietary fat treatment. The correlation
between variables was estimated by modeling polynomial relations separately across time. The
consistent of the model across ages and between dietary fat groups was examined. All interaction terms of
age, dietary fat, and HDL cholesterol were found to be nonsignificant, and the slope of the line for each
correlation within a dietary fat group did not change significantly over time.
Results

Lipoprotein A-I Concentrations

Lipoprotein measurements for studying HDL were made at ages 9, 14, 26, 38, and 50 months. The measurements of cholesterol and apolipoproteins presented in this article covered a period of adolescence into young adulthood. The ages of 9, 14, and 26 months were before sexual maturation, 38 months was peripubertal, and 50 months of age was after sexual maturity.

Mean TPC concentrations from the two dietary fat groups for the five ages are shown in Figure 1. There was a significant effect of age (\(p=0.0001\)) on TPC concentrations in the two groups. TPC concentrations rose significantly in both groups between 9 and 26 months of age (\(p<0.05\)). As the animals matured and aged beyond 26 months, TPC declined in both groups; however, the TPC concentrations at the older ages were not significantly different from the values at 26 months of age (\(p>0.05\)).

The effect of age on TPC was accompanied by a significant effect of dietary fat (\(p=0.01\)) (Figure 1). The mean differences in TPC between the two groups were largest at the younger ages. Animals fed dietary polyunsaturated fat had mean TPC concentrations that averaged 16% lower overall than their saturated-fat–fed counterparts.

Lipoproteins were subfractionated by agarose column chromatography at 9, 14, 26, 38, and 50 months of age. Mean HDL cholesterol concentrations are shown for the two dietary fat groups in Figure 2A. The effect of dietary fat on HDL cholesterol was not equivalent for all ages, as shown by the significant interaction term of diet x age (\(p=0.008\)). As shown in Figure 2, HDL cholesterol concentrations were similar at 9 months of age in the two groups. At 14 months of age, the average HDL cholesterol concentration was higher in the saturated-fat group, and it remained relatively constant at this higher level thereafter. In contrast, in the animals fed polyunsaturated fat, mean HDL cholesterol concentrations were progressively lower between 9 and 38 months of age and somewhat higher at 50 months. Between 14 and 50 months of age, HDL cholesterol concentrations averaged 126 mg/dl in the saturated-fat group and 96 mg/dl in the polyunsaturated-fat group. A significant difference in mean HDL cholesterol concentrations at individual ages between the two dietary fat groups was only found at 26 months (126±12 versus 93±8 mg/dl for saturated and polyunsaturated, respectively, \(p=0.03\)). The 18% (\(p=0.2\)) and 32% (\(p=0.09\)) lower HDL cholesterol levels in the polyunsaturated-fat group at 14 and 38 months of age, respectively, failed to achieve statistical significance at the 95% confidence interval after correction of the significance level for multiple comparisons by the Bonferroni technique, although these differences appeared reproducible.

Whole-plasma apo A-I concentrations were also measured at the individual ages by ELISA, and the mean concentrations for the two groups are shown in Figure 2B. Pilot studies have shown that essentially all of the plasma apo A-I is associated with the HDL fraction. The types of differences in apo A-I concentrations were similar to those of HDL cholesterol, although differences due to diet were less marked. There was an overall effect of age on apo A-I concentration (\(p=0.02\)); however, differences in apo A-I concentration between individual ages did not achieve statistical significance at the 95% confidence interval. Apo A-I concentrations were similar between the two groups at 9 months of age and afterward averaged 7% lower in the polyunsaturated-fat group (\(p=0.06\)).

Because larger average differences in concentrations of HDL cholesterol than apo A-I were observed between the animals fed saturated and polyunsaturated-fat–enriched diets, the HDL cholesterol to plasma apo A-I ratio was calculated and examined for effects of age and dietary fat. The average ratios for the two groups at each age are shown in Figure 2C. At 9 months of age, the ratio appeared higher in the polyunsaturated-fat group, which reflected the patterns of HDL cholesterol and apo A-I (panels A and B) at this age. The statistically significant effect of the type of dietary fat on the ratio (\(p=0.006\)) was most evident at 14–50 months of age, when the average concentration of HDL cholesterol relative to apo A-I was higher in the saturated-fat–fed group. After correction for multiple comparisons, a statistically significant difference in the ratio at individual ages was
only found at 26 months of age (0.48±0.03 versus 0.38±0.02, p=0.03 for saturated- versus polyunsaturated-fat groups, respectively). The ratio also averaged approximately 20% lower in the polyunsaturated-fat group at 38 and 50 months, but these differences failed to achieve statistical significance at the 95% confidence interval (p=0.09 and p=0.6, respectively).

**High Density Lipoprotein Density Subfractions**

Since HDL cholesterol was lowered more than plasma apo A-I by dietary polyunsaturated fat at most ages, it seemed likely that HDL subfraction distribution was affected. To investigate HDL heterogeneity, HDL isolated by agarose column chromatography was subfractionated by density gradient ultracentrifugation. Figure 3 shows density gradient profiles of HDL measured at 280 nm from two representative animals at 26 months of age. Although the total amount of HDL protein placed into the gradient was the same for the two animals, the HDL gradient profiles were obviously different. Panel A shows data from a saturated fat–fed animal, and panel B shows data from a polyunsaturated fat–fed animal. The saturated fat–fed animal possessed more lower-density HDL subfractions than the polyunsaturated fat–fed animal, whose major HDL subfraction was of a higher density.

For individual animals, the HDL was pooled into six subfractions as described previously: HDL-I, HDL-II, HDL-III, HDL-IV, HDL-V, and HDL-VI. In a previous report by Babiak et al,11 HDL heterogeneity within individual density fractions has been analyzed by analytical ultracentrifugation and nondenaturing gradient gel electrophoresis. HDL density subfractions I and II contained predominantly HDLα material (94% and 64%, respectively, by analytical ultracentrifugation), and HDLβ was the predominant species in subfraction III. By analytical ultracentrifugation, HDLγ was the only constituent of HDL-VI and was the major constituent of HDL-IV and HDL-V. Further characterization of fractions IV–VI by nondenaturing gradient gel electrophoresis found peaks in HDL-IV and HDL-V corresponding to HDLα and HDLβ, respectively, while HDL-VI contained a peak of HDLγ size along with some larger HDLδ-size material.
The measurements of HDL cholesterol concentrations in the young animals had shown a lack of equivalence between the two groups and an age effect within each dietary fat group (Figure 2A). Therefore, differences in HDL subfraction cholesterol concentrations between the diet groups were harder to interpret. To determine if the dietary fat effect on HDL density subfraction cholesterol concentration was dependent on the HDL cholesterol level within an animal, the relation between HDL cholesterol and concentrations of cholesterol in individual subfractions was modeled for the two dietary fat groups at each age interval. The cholesterol concentration of each HDL density subfraction (except HDL-VI) was found to be significantly correlated with HDL cholesterol. The statistical model showed a constancy by age and dietary fat in the relation between HDL cholesterol and the cholesterol concentration of density subfractions I–V. Increases in HDL cholesterol concentrations were reflected in linear increases in the cholesterol concentration of each density subfraction.

Effects of Dietary Polyunsaturated Fat on High Density Lipoprotein Density Subfraction Cholesterol Concentrations

The age period studied in these animals represented a time of rapid growth and development in these animals. Feeding polyunsaturated fat to the animals during this age interval induced an effect on HDL subfraction cholesterol concentrations similar to that observed in adult animals. Overall mean differences showed significantly lower (37%, \( p=0.0001 \)) HDL-I cholesterol concentrations and significantly higher cholesterol concentrations of the intermediate density subfractions HDL-IV (14%, \( p=0.01 \)) and HDL-V (10%, \( p=0.02 \)) in animals fed the polyunsaturated fat–enriched diet compared with the saturated fat–enriched diet.

Because of differences in HDL cholesterol concentrations between groups, the effects of age and dietary fat type on subfraction cholesterol concentrations were evaluated after covarying for HDL cholesterol concentration in the statistical model. Table 2 shows the mean cholesterol concentrations of HDL density subfractions I–VI at five ages. Of the six subfractions, the highest cholesterol concentration was found within the HDL-I subfraction, and the least cholesterol was found in the most dense HDL subfraction, HDL-VI.

HDL density subfraction cholesterol concentrations were affected both by age and dietary fat. As shown in Table 2, at each age HDL-I cholesterol was consistently higher in the saturated-fat group \( (p=0.0008) \). This difference in HDL-I cholesterol for individual HDL-I cholesterol levels was translated into higher mean HDL-I cholesterol concentrations in the saturated-fat group across all ages. From 9–38 months of age, mean HDL-I cholesterol concentrations in the polyunsaturated fat–fed animals averaged 20–30% lower than in the saturated fat–fed animals. The difference between the two dietary fat groups in HDL-I cholesterol was diminished at 50 months. Unlike HDL-I cholesterol, HDL-II cholesterol concentrations were not affected significantly by the dietary fat group of the animals \( (p=0.3) \).

For intermediate HDL density subfractions III–V, an opposite effect of the type of dietary fat on cholesterol concentration than for HDL-I was found. Significantly higher mean concentrations of HDL subfractions III–V \( (HDL-III, \ p=0.0004; HDL-IV, \ p=0.0001; HDL-V, \ p=0.004) \) were present in the polyunsaturated fat–fed animals. The statistically significant effect of dietary fat on HDL-III cholesterol was found only after controlling for HDL cholesterol levels within the animals by covariate analysis. Animals fed dietary polyunsaturated fat had mean HDL-III cholesterol concentrations that averaged 12–20% higher than the saturated-fat–fed animals across all five ages. Likewise, dietary polyunsaturated-fat feeding was associated with HDL-IV cholesterol concentrations that averaged 10–25% higher than in the saturated-fat–fed group. Effects of dietary polyunsat-
### TABLE 2. Effect of Dietary Fat on Cholesterol Concentrations of High Density Lipoprotein Density Subfractions

<table>
<thead>
<tr>
<th>Age group</th>
<th>HDL-I</th>
<th>HDL-II</th>
<th>HDL-III</th>
<th>HDL-IV</th>
<th>HDL-V</th>
<th>HDL-VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sat'd</td>
<td>56±4</td>
<td>29±2</td>
<td>12±1*</td>
<td>7±1</td>
<td>4±1</td>
<td>3±1</td>
</tr>
<tr>
<td>Poly</td>
<td>43±4</td>
<td>33±2</td>
<td>15±1*</td>
<td>10±1</td>
<td>6±1</td>
<td>3±1</td>
</tr>
<tr>
<td>14 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sat'd</td>
<td>48±5</td>
<td>29±2</td>
<td>14±1</td>
<td>9±1</td>
<td>6±1</td>
<td>4±1</td>
</tr>
<tr>
<td>Poly</td>
<td>39±4</td>
<td>32±2</td>
<td>16±1</td>
<td>12±1</td>
<td>7±1</td>
<td>4±1</td>
</tr>
<tr>
<td>26 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sat'd</td>
<td>48±4</td>
<td>26±2</td>
<td>13±1†</td>
<td>10±1‡</td>
<td>7±1‡</td>
<td>5±1</td>
</tr>
<tr>
<td>Poly</td>
<td>36±5</td>
<td>25±2</td>
<td>17±1†</td>
<td>15±1‡</td>
<td>11±1‡</td>
<td>5±1</td>
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<td>38 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sat'd</td>
<td>45±6</td>
<td>24±3</td>
<td>15±1‖</td>
<td>11±1‖</td>
<td>9±1</td>
<td>7±1</td>
</tr>
<tr>
<td>Poly</td>
<td>33±8</td>
<td>27±4</td>
<td>21±2‖</td>
<td>17±2‖</td>
<td>11±1‖</td>
<td>6±1</td>
</tr>
<tr>
<td>50 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sat'd</td>
<td>33±8</td>
<td>17±3</td>
<td>17±2</td>
<td>17±2</td>
<td>15±1</td>
<td>7±1</td>
</tr>
<tr>
<td>Poly</td>
<td>32±17</td>
<td>18±8</td>
<td>20±4</td>
<td>19±4</td>
<td>15±3</td>
<td>7±3</td>
</tr>
<tr>
<td>Dietary fat effect</td>
<td>p=0.0008</td>
<td>p=0.3</td>
<td>p=0.0004</td>
<td>p=0.0001</td>
<td>p=0.004</td>
<td>p=0.9</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Data were analyzed as described in “Methods” by repeated-measures analysis of covariance, where HDL cholesterol was the covariate. Significance level is given for main effects of dietary fat across all ages. Within individual HDL density subfractions for specific ages, cholesterol concentrations with the same symbol are significantly different.

HDL, high density lipoprotein; Sat'd, saturated fat-fed group; Poly, polyunsaturated fat-fed group.

At 9 months, *p=0.04.
At 26 months, †p=0.05; ‡p=0.02; §p=0.03.
At 38 months, ‖p=0.04; ‖p=0.03.

### Effects of Age on High Density Lipoprotein Density Subfraction Cholesterol Concentrations

The age effects on HDL subfraction cholesterol concentrations were equivalent for the two dietary fat groups; therefore, for simplicity in illustrating the effect of age, the data were combined for the two dietary fat groups, and the mean concentrations by age for the individual subfractions are shown in Figure 4. The effect of age on HDL density subfraction cholesterol concentrations could be divided into subfractions where cholesterol decreased, did not change, or increased as the animals aged. In the fraction of least density, HDL-I, there was a significant (p=0.03) downward trend in mean cholesterol concentration with age (panel A), and as a result, more cholesterol was present in this fraction at 9 than at 50 months of age (p<0.05). HDL-II cholesterol showed a similar significant (p=0.0001) downward trend in concentration. In contrast to HDL-I cholesterol and HDL-II cholesterol, changes in HDL-III with age were not significant (p=0.1); therefore, cholesterol concentrations were comparable at all ages for HDL-III cholesterol.

### Figure 4

Bar graphs of effect of age (months, mo) on high density lipoprotein (HDL) subfraction cholesterol concentrations (mg/dl) in animals combined from the two diet groups. Data were analyzed as described in Figure 4 and “Methods.” Age effect for HDL-I, p=0.03; for HDL-II, p=0.0001; for HDL-III, p=0.1; for HDL-IV, p=0.0001; for HDL-V, p=0.0001; and for HDL-VI, p=0.003.
The effects of age on mean cholesterol concentrations of HDL-IV, HDL-V, and HDL-VI are shown in panel B. In contrast to the decreases in cholesterol in subfractions I and II as the animals aged, HDL-IV, HDL-V, and HDL-VI all showed significant increases in cholesterol concentration with age (HDL-IV, p=0.0001; HDL-V, p=0.0001; HDL-VI, p=0.003).

Effects of Type of Dietary Fat and Age on Cholesterol Distribution Among High Density Lipoprotein Density Subfractions

The previous data presented evidence for changes in HDL density subfraction cholesterol concentrations as the animals aged. The data also showed that the type of dietary fat can modify the concentration of cholesterol among the six subfractions independent of changes in total HDL cholesterol. These changes in absolute concentration with age and dietary fat group resulted in a redistribution of cholesterol among the six subfractions. The mean percentage distribution for dietary fat groups by age are shown in Figures 5A and 5B. There were significant effects (p<0.0002) of dietary fat saturation on the percentage distribution of cholesterol for HDL subfractions I, III, IV, and V. These are the same subfractions that showed significant effects of dietary fat saturation on mean cholesterol concentrations.

Effects of the type of dietary fat did not necessarily reflect similar effects of age on cholesterol distribution. Significant effects (p<0.01) of age on percentage cholesterol distribution affected all HDL subfractions, with the mean percentage decreasing in HDL-I–II and increasing in HDL-III–VI as the animals grew older. At 9 months of age, the greatest percentage of cholesterol for both dietary fat groups was found in HDL-I, and there was a stepwise decrease to HDL-VI. As young adolescents, these animals had the majority of their HDL cholesterol localized within the least-dense HDL subfractions I and II (76% saturated- and 69% polyunsaturated-fat groups, panels A and B). As the animals entered a period of maturation and development (26–38 months), the pattern of declining cholesterol in HDL-I and HDL-II continued. By the time the animals reached young adulthood at 50 months of age, HDL-I and HDL-II had decreased 30–50%, such that these subfractions together contained no more than one half of all HDL cholesterol (50% saturated- and 39% polyunsaturated-fat groups). Aging and maturing of the animals was associated with increasing concentrations of the remaining four subfractions (HDL-III–VI, Figure 5), resulting in increases in the percentage of cholesterol within these subfractions. At 50 months of age, HDL-I still had the highest mean percentage of HDL cholesterol, while the percentages found in HDL-III–V were similar within both dietary fat groups.

Effects of Gender on High Density Lipoprotein Density Subfractions

Since the animals were studied during an interval before puberty and were followed into young adulthood, we wanted to determine if sexual maturation might influence HDL subfraction cholesterol concentrations. There was no significant overall effect of gender on total HDL cholesterol (p=0.9), plasma apo
A-I ($p=0.7$), or the HDL cholesterol to apo A-I ratio ($p=0.4$); however, both HDL cholesterol and apo A-I mean concentrations were consistently 10–20% lower in male animals at 26 months of age and older. There was a pattern of age-related change by gender in HDL density subfraction cholesterol concentrations that was not different for the two diet groups. The cholesterol concentrations within HDL subfractions I, IV, and V changed significantly with age, depending on the sex of the animals (age×sex: HDL-I, $p=0.0008$; HDL-IV, $p=0.02$; HDL-V, $p=0.002$). This effect of gender was not different for the two diet groups and therefore is illustrated for combined groups in Figures 6A, 6B, and 6C. As the male animals matured and aged, cholesterol concentration of less-dense HDL-I (panel A) was found to decrease, while the cholesterol concentrations of intermediate density, HDL-IV and HDL-V, increased (panels B and C, respectively). In females, mean concentrations of these subfractions showed much smaller changes with age, and the sum of cholesterol in the intermediate fractions IV and V (panels B and C, respectively) was the same at each of five ages. The male and female animals showed a crossover of higher mean HDL-I cholesterol beginning at 26 months of age in females (panel A) due to the continued decreases in cholesterol concentrations of this subfraction between 9 and 50 months in the male animals.

**Discussion**

In the present study, animals were subjected to lifelong consumption of atherogenic diets enriched in either saturated fat or polyunsaturated fat to study the long-term effects of such diets on lipoprotein metabolism and atherogenesis. In this article, we
African green monkey is an animal that maintains maturation in young boys along with changes in HDL cholesterol concentrations decrease during fat lowers HDL cholesterol concentrations and that HDL subfraction patterns, we believe that these mechanisms for such effects. Studies in African green monkeys document a valuable animal model with which to further investigate all of these relationships. The first study to indicate this degree of complexity in HDL metabolism is not possible to explain the metabolic basis of these effects with our current information about the factors controlling HDL metabolism. However, what has been documented in studies of humans showing that polyunsaturated fat lowers HDL cholesterol concentrations and that HDL cholesterol concentrations decrease during maturation in young boys along with changes in HDL subfraction patterns we believe that these studies in African green monkeys document a valuable animal model with which to further investigate mechanisms for such effects.

At the same time, we must point out that the African green monkey is an animal that maintains very low plasma triglyceride concentrations, even though diets with 40% kcal as fat were fed. This is important since others have noted a close relation between HDL subfraction concentration and the metabolism of the triglyceride-rich lipoproteins. We did not document the various aspects of lipoprotein lipase and hepatic lipase levels and of postprandial lipemia in the animals of these studies. However, the uniformly low plasma triglyceride concentrations have been documented (L. Rudel et al, unpublished observation), and studies of many of these variables of triglyceride metabolism have been done in the past in adult monkeys, demonstrating little correlation with HDL subfraction distribution. It seems fair to say that other factors are contributing to the pattern of HDL subfraction distribution seen in these animals. While we can only speculate on what the nature of the factors involved might be, we have indications that apo A-I production is high in African green monkeys and that polyunsaturated fat affects apo A-I production, and we know that levels of cholesterol ester transfer activity are high (Reference 36 and L. Rudel et al, unpublished observation). Each of these are potential factors that could influence the concentration and composition of HDL subfractions although as yet in an undefined manner.

The animals were raised on semipurified diets with a nutrient composition similar to that of Western society. The fatty acid composition of the saturated fat diet was also representative of that of Western society, while the fatty acid composition of the polyunsaturated-fat diet essentially represents an extreme that could be achieved by humans. Since the purpose of this study was to demonstrate differences where they occur, the selection of fatty acid compositions that were markedly different was an advantage. The cholesterol level of the diets was chosen to induce a modest hypercholesterolemia in the young animals, which was necessary for atherosclerosis induction.

The animals achieved TPC levels during their pediatric-age years similar to those of human beings at increased risk for CHD, which allowed us, in separate studies, to monitor long-term effects of hypercholesterolemia on atherosclerosis development in a pediatric population in addition to its effects on plasma lipids and apolipoproteins.

While the data on atherosclerosis progression will be presented in a separate publication, it is of note that less atherosclerosis developed in the polyunsaturated-fat group and less atherosclerosis developed in the pediatric-age animals compared with fully adult animals fed the same diets for the same lengths of time. Therefore, the changes in plasma lipids and lipoproteins observed in the pediatric-age animals are likely associated with decreased atherogenicity in the African green monkey.

The specific lipid and lipoprotein factors associated with the decreased rate of atherosclerosis progression remain unknown. It should be of note that a decreased amount of atherosclerosis occurred in the polyunsaturated-fat group despite consistently lower HDL cholesterol and apo A-I concentrations. In this case, it seems useful to consider the possibility that the modification in HDL subfractions toward increased concentra...
centrations of intermediate density HDL subfractions may actually be beneficial for protection against ath-
erosclerosis. The pathway of reverse cholesterol trans-
port postulates the movement of cholesterol from peripheral tissues to HDL with direct removal from plasma or with transfer of the cholesteryl ester to triglyceride-rich lipoproteins for uptake and excretion by the liver. The higher concentrations of intermedi-
ate subfractions may be reflective of more efficient transport of cholesterol than for larger HDL. Possibly, intermediate-density HDLs are better than are larger HDLs in promoting cholesteryl efflux from arteries due to their lower cholesterol to phospholipid ratio.11

In this way, intermediate-density HDL would aid in reducing buildup of cholesteryl in arteries, thereby decreasing atherosclerosis.

In addition, the modification of HDL subfraction pattern by dietary polyunsaturated fat may be an indication of a difference in plasma cholesteryl ester transfer. Smaller HDL subfractions may promote a higher rate of cholesteryl ester transfer than larger HDL since the smaller particle would provide more surface area per unit mass. The decreased concent-
tration of HDL-I cholesteryl may also indicate an increased transfer via cholesteryl ester transfer protein from intermediate-density HDL into apo B–containing lipoprotein instead of into HDL. Under conditions in vivo where cholesteryl ester transfer protein activity has been inhibited in rabbits, choles-
terol esterification was maintained; however, HDL cholesteryl ester concentration was increased and the initial rates of HDL and plasma cholesteryl ester clearance were reduced.30

The buildup of larger, cholesteryl-enriched HDL in the circulation of the saturated fat–fed group may in fact represent an impairment of reverse cholesterol transport, possibly from inefficient cholesteryl ester transfer into triglyc-
eride-rich particles.

Acknowledgments

The authors acknowledge the technical assistance of Beverly Sonbert, Joy Martin, and Susan Pelkey as well as the skilful manuscript preparation of Linda Odham.

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**KEY WORDS** • high density lipoproteins • cholesterol • age • polyunsaturated fats • nonhuman primates
Age and dietary polyunsaturated fat alter high density lipoprotein subfraction cholesterol concentrations in a pediatric population of African green monkeys. M S Wolfe, J S Parks, T M Morgan and L L Rudel

*Arterioscler Thromb Vasc Biol.* 1991;11:617-628
doi: 10.1161/01.ATV.11.3.617

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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