Dietary Polyunsaturated Fat Decreases Coronary Artery Atherosclerosis in a Pediatric-Aged Population of African Green Monkeys

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Abstract  The hypothesis tested was that juvenile African green monkeys consuming diets enriched with n-6 polyunsaturated fat from birth until young adulthood would have significantly less coronary artery atherosclerosis than comparable animals consuming diets enriched with saturated fat. African green monkeys (Cercopithecus aethiops, n=108) of both sexes were fed atherogenic diets (0.8 mg cholesterol/kcal) throughout their lives so that death at 16, 32, or 60 months of age permitted quantification of atherosclerosis. In the coronary arteries, the average intimal area increased significantly with age (P=.02), showing increases of 28-fold and sevenfold between 32 and 60 months in the saturated fat- and polyunsaturated fat–fed groups, respectively. Young adult male animals at 60 months of age were found to have significantly more coronary artery atherosclerosis than female animals. Animals fed polyunsaturated fat had significantly (P=.03) less coronary artery atherosclerosis than female animals at 60 months of age. By 60 months of age in the animals consuming polyunsaturated fat, the average coronary artery intimal area was one fourth and the average size of the largest coronary intimal lesion was one fifth that in monkeys fed saturated fat. Low-density lipoprotein (LDL) cholesterol and LDL particle size were each found to be positively correlated with coronary artery atherosclerosis end points in both diet groups. In addition to the coronary arteries, atherosclerosis in the abdominal and thoracic aorta and carotid arteries was also evaluated; the coronary arteries were the only arterial system with significantly less atherosclerosis in the polyunsaturated fat group as measured by intimal area. However, evaluation of histological sections of abdominal aorta showed relatively more sterol clefts in the saturated fat-fed group, and more free cholesterol was measured, suggesting that lesions were more complicated in this group. These results show that dietary intervention early in life with n-6 polyunsaturated fat can be effective in decreasing the development of atherosclerosis, particularly in the coronary arteries of primates. This outcome supports the concept that dietary intervention beginning early in childhood can have beneficial effects on the coronary heart disease of later life.

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

Atherosclerosis, or development of lipid-filled lesions within the artery wall, leads to the complications of coronary heart disease (CHD). Studies on autopsied persons from different populations have defined age-related sequences of atherosclerotic lesion development and progression. The existence of coronary artery lesions early in life (before the occurrence of clinical complications) and variability in the extent and severity of raised lesions later in life combine to suggest that individual differences in atherosclerosis susceptibility, while of uncertain origin, are due to both environmental and genetic factors. Several prospective studies in adult humans report associations between CHD risk factors and atherosclerosis; however, there is limited information concerning the relation between CHD risk factors and the early stages of atherosclerosis. In young people from the Bogalusa project, associations were found between lesion extent and antemortem cholesterol concentrations that were independent of race, gender, or age.

Modifications of dietary fat intake to decrease the percentage of calories from total and saturated fat and to increase the proportion of calories from polyunsaturated fats have been recommended to the general public for improving CHD risk profiles. While it is still uncertain if this emphasis on dietary regulation will institute larger changes in diet habits, the US Department of Agriculture statistics for the period up to 1980 show that the public incorporated more vegetable fat containing n-6 polyunsaturated fatty acids into the diet without decreasing total fat intake. Hegsted et al and Keys et al carefully define the value of substituting polyunsaturated fatty acids for saturated fatty acids in the diet to lower serum cholesterol in their pioneering studies. In short-term feeding studies of adults as outpatients and inpatients, dietary polyunsaturated fat alters concentrations of lipids, lipoproteins, and apolipoproteins. Similarly, the lipid and apolipoprotein levels of infants and children are susceptible to changes induced by consumption of dietary polyunsaturated fat. Nevertheless, little is known about the effect of dietary enrichment in n-6 polyunsaturated fatty acids on coronary artery atherosclerosis and its complications.

The difficulty in maintaining well-controlled, long-term feeding studies in human beings and in subse-
quent assessing the atherosclerosis end point has led to the development of animal models for studying atherosclerosis. The adult African green monkey has served as a model for investigating the relation between dietary polyunsaturated fat-associated modifications of lipid and lipoprotein risk factors and atherosclerosis. African green monkeys are similar to human beings in their responses to the cholesterol-lowering effects of dietary polyunsaturated fat23,24 and in the morphology of their atherosclerotic lesions.25 After consuming polyunsaturated fat-enriched diets for 5 years, the extent and severity of coronary artery atherosclerosis in adult male African green monkeys are significantly lower than in animals consuming saturated fat-enriched diets.26

The fact that initiation of atherosclerosis begins early in life and silently progresses as an individual ages strongly supports the proposal for early intervention to reduce the risk of CHD in adults. Although modifications in dietary fat intake beginning in childhood have been proposed as a means for preventing atherosclerotic CHD,9,10 the long-term risk/benefit ratio from early diet intervention is difficult to assess and remains unknown. The objective of this study was to use the African green monkey to evaluate, in a pediatric-aged population, the long-term effects of dietary polyunsaturated fat consumption on coronary artery atherosclerosis. We hypothesized that a lifetime of dietary treatment with n-6 polyunsaturated fatty acids would result in a favorable alteration of CHD risk profiles and lessen development of coronary artery atherosclerosis. The effects of long-term consumption of polyunsaturated fat-enriched diets on lipoprotein risk factors are presented in separate publications.26,27 This article presents atherosclerosis measurements and their relations to CHD risk factors. The consumption of a diet enriched in polyunsaturated fat (versus saturated fat) beginning early in life can have positive effects in reducing CHD risk.27 These data are the first to establish that reduction of risk in pediatric-aged primates at high risk actually leads to less coronary artery atherosclerosis in later life.

Methods

Animals

Juvenile African green monkeys (Cercopithecus aethiops) used in this study were born and raised in the primate facility of our institution.27 The breeding colony consisted of 22 sires and 64 dams; 50% of the dams had progeny in both diet groups. Therefore, many of the animals in both groups had a shared genetic background, a situation that we felt could improve our chances to detect dietary effects. Juvenile animals were group-housed with their mothers until weaning, when they were group-housed with other juveniles of both sexes until sexual competence developed. Animals were then individually caged for the remaining portion of the study.

Diets

The juvenile animals consumed diets enriched in polyunsaturated or saturated fat over their lifetimes.26,27 The compositions of the diets have been published.28 Both diets contained approximately 40% of calories from fat, 40% from carbohydrate, and 20% from protein; n-6 polyunsaturated fatty acid enrichment (from 5.5% to 20% of total calories27) was at the expense of both saturated and monounsaturated fatty acids so that the ratio of the latter two fatty acid species remained constant. The mothers consumed the same diet as the progeny before and during pregnancy and lactation. The fatty acid composition of the mothers' milk reflected their diets, so that each animal effectively entered its respective experimental diet group at conception.27 To induce atherosclerosis, the diets contained cholesterol (0.8 mg/mgcal), principally from dried egg yolk.

Lipoprotein and Apolipoprotein Measurements

Periodic measurements of lipid and lipoprotein risk factors were made in each animal.26,27 Briefly, lipoproteins were isolated from whole plasma by a combination of ultracentrifugation and gel-filtration chromatography29 and were pooled into separate fractions containing very-low-density plus intermediate-sized low-density lipoprotein (VLDL+IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Cholesterol concentrations were then measured for each fraction,30 and the average LDL particle size was determined based on the elution position of the LDL peak.31 Apolipoprotein (apo) A-I and apoB concentrations in whole plasma were quantified by enzyme-linked immunosorbent assay.27,28,29

Atherosclerosis Evaluations

Some of the monkeys in this study were killed at 16 and 32 months of age; others were monitored from birth through puberty and into young adulthood and were killed at 60 months of age. A total of 108 animals was necropsied at one of three ages: 16 months (n=33); 32 months (n=29); and 60 months (n=46). The distribution by gender between the two groups was similar at all ages and reflected the overall male-to-female birth ratio of ~2:1.27 In the polyunsaturated fat–diet group, at 16 months n=10 males and n=6 females, at 32 months n=8 males and n=5 females, and at 60 months n=13 males and n=12 females; in the saturated fat–diet group, at 16 months n=12 males and n=5 females, at 32 months n=11 males and n=5 females, and at 60 months n=14 males and n=7 females.

All animals were fasted overnight before death. Animals were anesthetized with ketamine hydrochloride (25 mg kg), and a midline incision was made to expose the inferior vena cava, which was then snared with a hemostat. Animals were then killed by electrocution and infused with lactated Ringer’s solution (pH 7.0) through a cannula placed in the apex of the left ventricle of the heart until no blood appeared in the flush solution. The aorta, carotid arteries, and carotid artery bifurcations were removed, cleaned of adventitia, and opened along the anterior midline. Arteries were then flattened on cardboard and submersion-fixed in 10% neutral buffered formalin. The heart and coronary arteries were perfusion-fixed for 1 hour in a closed system with 10% neutral buffered formalin. The heart and coronary arteries were perfusion-fixed for 1 hour in a closed system with 10% neutral buffered formalin. The heart and coronary arteries were perfusion-fixed for 1 hour in a closed system with 10% neutral buffered formalin. The heart and coronary arteries were perfusion-fixed for 1 hour in a closed system with 10% neutral buffered formalin. The heart and coronary arteries were perfusion-fixed for 1 hour in a closed system with 10% neutral buffered formalin. The heart and coronary arteries were perfusion-fixed for 1 hour in a closed system with 10% neutral buffered formalin.

The percentage of surface covered with atherosclerotic lesions in the abdominal and thoracic aorta was measured, and morphometric analyses of the three major coronary arteries, the thoracic and abdominal aorta, the left and right common carotid arteries, and the carotid artery bifurcations were performed on histological sections.32 Three types of atherosclerotic lesions were considered: fatty streaks were defined as areas of Sudan staining that were not raised; fatty plaques were defined as raised sudanophilic regions; and fibrous plaques were defined as raised, nonlipid-staining regions. Histological preparations were stained with Verhoeff–Van Gieson’s stain. Intimal area, defined as the area between the internal elastic lamina and the lumen, was measured, and the average intimal area for 15 serial sections of coronary arteries

Average intimal area for 15 serial sections of coronary arteries...
was calculated. Average intimal areas for five equidistant sections of both the thoracic and abdominal aortas, for three sections of each of the common carotid arteries, and for each of the two carotid artery bifurcations were determined.34 Maximal intimal thickness was determined by identifying and measuring the thickness of the largest lesion from among the histological sections for each arterial bed.

**Cholesterol Quantification of Abdominal Aorta**

Free and esterified cholesterol content of the abdominal aorta was determined for 60-month-old male animals, whose average intimal area was ≥1 mm². After sections were taken for histological analyses, the remaining abdominal aorta was cleaned of adventitia and weighed; a portion was then used for lipid extraction with chloroform/methanol, 2:1.35 Approximately 0.1 g of abdominal aorta was minced with scissors and transferred into a capped, acid-washed tube. Five milliliters of freshly prepared chloroform/methanol was added, the contents were mixed by vortexing, and the tubes were placed in a 45°C heating block for 1 hour. After cooling for at least 3 hours at room temperature, the lipid extract was filtered and made to a known volume with chloroform/methanol. Sulfuric acid (0.05%) was added to each tube, and the contents were mixed by inversion. Phase separation was completed by low-speed centrifugation, and the upper phase was aspirated and discarded. After filtration of the lipid extract the protein pellet was dried at 60°C for 5 hours. Protein concentration was measured in the presence of sodium dodecyl sulfate by using the Lowry procedure.36

Aliquots of the lipid extract were used for separating free and esterified cholesterol by thin-layer chromatography.37 Bands containing free and esterified cholesterol were scraped, and lipid was extracted from the silica gel with chloroform.

5α-Cholestane (NuChek Prep, Inc) was added to each tube as an internal standard. Samples for total, free, and esterified cholesterol were saponified and extracted into hexane.38 Cholesterol was quantified by using a Hewlett Packard 5890A gas chromatograph with a capillary-on-column injector on a DB1 column (15 m x 0.533 mm; J&W Scientific Products) with a 1-μm film thickness. A 1-m precolumn (0.533-mm internal diameter) was used with a head pressure at 10 psi and helium as the carrier and make-up gas.

**Statistical Analysis**

Data in this report are presented as mean±SEM. Statistical analyses of atherosclerosis data were done with SAS (Statistical Analysis System). Atherosclerosis measurements were analyzed by using the nonparametric rank transformation method by performing two-way ANOVA on the ranks of the atherosclerosis end points.38 The statistical model used main effects and they were natural logarithmically transformed before analysis. The significance level was adjusted for multiple pairwise comparisons by dietary fat type or gender by using the Bonferroni technique.39 Differences in disease incidence were determined by χ² analysis.39

Statistical analyses of abdominal aorta free and esterified cholesterol concentrations were done with the Mann-Whitney U nonparametric test using STATVIEW SE+GRAPHICS software (Abacus Concepts, Inc) developed for the Macintosh microcomputer. Pearson's product-moment correlation analysis was used to determine possible relations of lipoprotein and apolipoprotein variables with atherosclerosis measurements of extent (intimal area) and severity (maximal intimal thickness). A constant (.01) was added to the atherosclerosis variables, and they were natural logarithmically transformed before correlation analysis. Lipoprotein and apolipoprotein variables (measurements at five individual ages and means across the five different ages) were correlated with atherosclerosis measurements. In all instances the correlations using the mean lipoprotein and apolipoprotein data were similar to those using data at individual ages, so only the comparisons using mean data are presented in this report.

**Results**

**Effects of Age and Dietary Polyunsaturated Fat on Coronary Artery Atherosclerosis**

The effects of age and dietary fat on coronary artery atherosclerosis extent and severity are shown in Fig 1. The extent of atherosclerosis (estimated as the average intimal area of the coronary arteries; panel A) was small, especially at 16 and 32 months of age. However, there was a significant (P=.02) age-related increase, most notably between 32 and 60 months, when mean intimal area increased 28-fold and sevenfold in the saturated fat– and polyunsaturated fat–fed groups, respectively. Atherosclerosis severity (defined as maximal intimal thickness; panel B) was determined by measuring the thickness of the largest lesion. Maximal intimal thickness increased significantly (P=.007) with age in both groups in a manner similar to intimal area, with the largest increase occurring between 32 and 60 months of age (sixfold and threefold for the saturated and polyunsaturated fat groups, respectively).
The extent and severity of coronary artery atherosclerosis were also affected by dietary fat type. As shown in Fig 1, there was significantly less intimal area \((P=.01)\) and maximal intimal thickness \((P=.007)\) in the monkeys that consumed the polyunsaturated fat–enriched diet. The largest difference between the groups occurred at 60 months of age, when mean intimal area in the polyunsaturated fat group was one fifth \((P=.02)\) that in the saturated fat group. Animals fed polyunsaturated fat also had significantly \((P=.007)\) smaller lesions as measured by maximal intimal thickness. This effect was present by 16 months of age, and by 60 months of age mean maximal intimal thickness was one fourth \((P=.01)\) that in the saturated fat–fed group.

Because coronary artery atherosclerosis was not found in all of the animals, we investigated the incidence of disease to determine if there was an effect of dietary fat. The number of animals affected with coronary artery atherosclerosis divided by the number of animals in each dietary fat–age group is given in parentheses. Coronary arteries from one animal fed saturated fat were not available for analysis. Data were analyzed by \(\chi^2\) analysis as described in “Methods.” \(P>.05\) at 16 months; \(P>.05\) at 32 months; and \(*P=.009\) at 60 months.

The extent and severity of coronary artery atherosclerosis were also affected by dietary fat type. As shown in Fig 2, there was significantly less intimal area \((P=.01)\) and maximal intimal thickness \((P=.007)\) in the monkeys that consumed the polyunsaturated fat–enriched diet. The largest difference between the groups occurred at 60 months of age, when mean intimal area in the polyunsaturated fat group was one fifth \((P=.02)\) that in the saturated fat group. Animals fed polyunsaturated fat also had significantly \((P=.007)\) smaller lesions as measured by maximal intimal thickness. This effect was present by 16 months of age, and by 60 months of age mean maximal intimal thickness was one fourth \((P=.01)\) that in the saturated fat–fed group.

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The only apparent gender-related differences in coronary artery atherosclerosis occurred at 60 months of age (Fig 3). The difference between male and female animals in intimal area (panel A) was significant \((P=.03)\), as noted by the higher intimal area in males regardless of the type of dietary fat. In the saturated fat–fed group intimal area was sixfold higher in male than female animals, and in the polyunsaturated fat–fed group the intimal area of female animals was below detectable limits. A significant \((P=.03)\) difference by gender in maximal intimal thickness (panel B) was also found at 60 months of age. The mean thickness of the largest lesion of saturated fat–fed male animals was approximately threefold greater than for females. In addition to these measurements of coronary artery atherosclerosis, incidence at 60 months was also affected by gender, with a higher proportion of males having measurable coronary lesions (saturated fat, 64% versus 43% and polyunsaturated fat, 38% versus 0%, male versus female, respectively).

Relations of Coronary Heart Disease Risk Factors to Coronary Artery Atherosclerosis

Several lipoprotein and apolipoprotein CHD risk factors were evaluated in each of the animals at five ages (9, 14, 26, 38, and 50 months) covering the period from adolescence into young adulthood. Average values for each end point are shown in Table 1 for animals who consumed experimental diets for 60 months in an attempt to reflect the entire period of artery exposure to these risk factors. For most of the variables the apparent differences by dietary fat type and gender were similar to that reported for the larger group \((n=140)\), which was followed longitudinally from 0 through 60 months of age,\(^2\) although for the smaller group \((n=46)\) some of the differences failed to achieve statistical significance at the 95% confidence interval. For 60-month-old animals total plasma cholesterol concentrations averaged 12% to 15% lower in female and male animals consuming n-6 polyunsaturated fat–enriched diets compared
TABLE 1. Average Lipoprotein and Apolipoprotein Concentrations and LDL Molecular Size in Animals That Consumed Saturated Fat- or n-6 Polyunsaturated Fat-Enriched Diets for 60 Months

<table>
<thead>
<tr>
<th>Diet Group/Sex</th>
<th>n</th>
<th>TPC</th>
<th>VLDL+IDLD Cholesterol</th>
<th>LDL Cholesterol</th>
<th>HDL Cholesterol</th>
<th>ApoB</th>
<th>ApoA-I</th>
<th>LDL Molecular Size, g/μmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated fat/male</td>
<td>14</td>
<td>336±23</td>
<td>12±1</td>
<td>208±25</td>
<td>121±7</td>
<td>151±12</td>
<td>287±13</td>
<td>3.05±0.07</td>
</tr>
<tr>
<td>Saturated fat/female</td>
<td>7</td>
<td>295±35</td>
<td>12±2</td>
<td>166±36</td>
<td>117±6</td>
<td>125±13</td>
<td>265±26</td>
<td>2.93±0.09</td>
</tr>
<tr>
<td>Polyunsaturated fat/male</td>
<td>13</td>
<td>295±20</td>
<td>14±2</td>
<td>192±19</td>
<td>89±5</td>
<td>145±10</td>
<td>229±12</td>
<td>3.13±0.05</td>
</tr>
<tr>
<td>Polyunsaturated fat/female</td>
<td>12</td>
<td>250±21</td>
<td>11±1</td>
<td>136±15</td>
<td>103±7</td>
<td>118±8</td>
<td>243±21</td>
<td>2.94±0.05</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; TPC, total plasma cholesterol; VLDL, very-low-density lipoprotein; IDLD, intermediate-sliced LDL; HDL, high-density lipoprotein, and Apo, apolipoprotein. For each variable, measurements made during an animal's lifetime at 9, 14, 26, 38, and 50 months of age were analyzed for effects of dietary fat and gender by univariate repeated-measures ANOVA as described in "Methods." There were no significant interaction terms. For each variable the average were then averaged per animal across the five ages. Overall mean±SEM for each dietary fat/gender group is shown. For each lipoprotein and apolipoprotein variable, values with symbols are significantly different. Dietary fat effect, * versus †, P=.0005; gender effect, ‡ versus §, P=.01; gender effect, † versus ‡, P=.01.

with the levels in animals given the saturated fat diets. HDL cholesterol levels were significantly lower in the polyunsaturated fat group. In both diet groups LDL cholesterol, plasma apoB, and average LDL particle size were lower in females than in males.

The correlations between intimal area and lipoprotein and apolipoprotein variables are shown in Table 2. Higher correlations between intimal area and risk factors occurred in animals fed the saturated fat diet. In the saturated fat–fed males, total plasma cholesterol and LDL-related parameters such as LDL cholesterol, LDL particle size, and plasma apoB were all highly correlated with the atherosclerosis end point. Measurements associated with HDL, including HDL cholesterol and plasma apoA-I, were negatively associated with coronary artery atherosclerosis in this group, especially in females. Cholesterol concentrations of individual HDL density subfractions56 showed no significant relation with coronary artery atherosclerosis for the dietary fat group (M.S.W. and L.L.R., unpublished data, 1993). Only LDL particle size was correlated significantly with intimal area in males fed polyunsaturated fat. The lack of variability in intimal area in females fed polyunsaturated fat eliminated the possibility to observe correlations. For the polyunsaturated fat–fed group, LDL particle size and LDL cholesterol showed the strongest correlations with coronary artery intimal area. The outcomes for these same types of statistical comparisons using maximal intimal thickness of the coronary arteries showed the same results as for average coronary artery intimal area.

When data for average coronary artery intimal area for the diet groups were combined, multiple regression analysis showed that LDL cholesterol concentration accounted for 52% (P=.0001) of the variability in the atherosclerosis end point. The only additional lipoprotein or apolipoprotein variable that made a significant contribution to the regression model was VLDL+IDLD cholesterol (P=.005); VLDL+IDLD and LDL cholesterol together accounted for 60% of the variability in intimal area. These results from the correlation analyses indicated that lipoprotein and apolipoprotein variables were associated significantly with intimal area overall and after controlling for diet.

Effects of Age and Dietary Fat on Abdominal Aorta Atherosclerosis

Morphometric evaluations of the abdominal aorta were also used to assess the extent (mean intimal area) and severity (maximal intimal thickness) of aortic atherosclerosis. The mean±SEM of average intimal area (panel A) and maximal intimal thickness (panel B) are shown in Fig 4. Abdominal aorta atherosclerosis, measured as either intimal area or maximal intimal thickness, was minimal in the young animals of both diet groups at 16 and 32 months of age and then increased dramatically between 32 and 60 months of age (P=.0001) in a manner similar to that seen for coronary artery atherosclerosis. However, unlike the situation in coronary arteries, the difference between the two dietary fat groups in these end points in the abdominal aorta was not statistically significant (P=.2). Gender differences in atherosclerosis similar to that described for coronary arteries were also found in the abdominal aorta. For animals that had consumed diets for 60 months, both average intimal area (P=.0003) and maximal intimal thickness (P=.005) were significantly greater in male than female animals (intimal area: 1.5±0.5 versus 0.2±0.1 mm² for male versus female animals fed saturated fat and 1.9±0.5 versus 0.1±0.1 mm² for male versus female animals fed polyunsaturated fat, respectively; maximal intimal thickness: 0.38±0.05 versus 0.15±0.07 mm for male versus female animals fed saturated fat and 0.38±0.05 versus 0.07±0.06 mm for male versus female animals fed polyunsaturated fat, respectively).

The type of dietary fat was found not to affect the incidence of lesions in the abdominal aorta at any age. By 32 months of age more animals had lesions in the abdominal aorta than in the coronary arteries, resulting in a significantly higher incidence of atherosclerosis (32 months: 17/29 versus 8/29, P=.01, and 60 months: 38/46 versus 17/46, P<.001, for abdominal aorta versus coronary arteries, respectively).

Atherosclerosis in the abdominal aorta was also quantified by measuring the percentage of surface area occupied by fatty streaks, fatty plaques, and fibrous plaques (Fig 5). The lesions at all three ages in both diet groups were predominantly fatty streaks. By 60 months of age, the surface percentage involved with fatty streaks was highest, increasing proportions of the more complicated fatty plaques were present in both diet groups, and the most complicated lesion, fibrous plaque, was beginning to appear. Fibrous plaques were found in 6 of 27 (22%) 60-month-old male animals (4 were fed saturated fat and 2, polyunsaturated fat). The average
percentage of surface involved with lesions of any type was about 25% greater in the aortas of the animals fed saturated fat versus polyunsaturated fat for 60 months, although this difference did not achieve statistical significance.

We also measured the free and esterified cholesterol concentrations in the abdominal aortas from 60-month-old male animals. The aortas chosen for analysis were those from animals in each diet group that had the more extensive atherosclerosis (intimal area ≥1 mm²). The intimal area of the abdominal aortas of these male animals was not significantly different by dietary fat type (P=.2). Fig 6 shows the free (left) and esterified (right) cholesterol concentrations of the abdominal aortas for the animal fed saturated fat, the mean intimal area of which was the same as for the aorta in panel A. Although the intimal thickness was similar, there were more numerous sterol clefts and some mineralization in the lesion from the saturated fat-fed animal. The intima was thinned, but there was an absence of sterol clefts and mineralization. Panel B is from the abdominal aorta of an animal fed saturated fat, the mean intimal area of which was the same as for the aorta in panel A. Although the intimal thickness was similar, there were more numerous sterol clefts and some mineralization in the lesion from the saturated fat group.

Figs 7 and 8 show photomicrographs of Verhoeff-van Gieson's-stained cross sections of abdominal aorta from 60-month-old male animals of both diet groups at moderate and severe levels of atherosclerosis. Fig 7, panel A, shows a section of abdominal aorta from an animal fed polyunsaturated fat. The intima was thickened, but there was an absence of sterol clefts and mineralization. Panel B is from the abdominal aorta of an animal fed saturated fat, the mean intimal area of which was the same as for the aorta in panel A. Although the intimal thickness was similar, there were more numerous sterol clefts and some mineralization in the lesion from the saturated fat group. Fig 8 shows cross sections from animals from either diet group with abdominal aortas having similar mean intimal areas; these represent more severe atherosclerosis. The abdominal aorta for the animal fed polyunsaturated fat (panel A) and the one fed saturated fat (panel B) were both thickened; in addition, underlying medial thinning was present in the lesion from the saturated fat-fed group. Both lesions had numerous sterol clefts, while the lesion from the saturated fat-fed animal had extensive necrosis and mineralization at the base of the lesion.

In general, our subjective impression from the histological data was that the atherosclerosis in the abdominal aortas of the animals fed saturated fat instead of polyunsaturated fat was more complicated, with more
Fig 4. Bar graphs showing effects of dietary fat and age on extent and severity of abdominal aorta atherosclerosis as measured by intimal area (A) and maximal intimal thickness (B). Data were analyzed as described in Fig 1 and are presented as mean±SEM. A, Age effect, \( P = .001 \) and dietary fat effect, \( P = .2 \). B, Age effect, \( P = .0001 \) and dietary fat effect, \( P = .2 \).

necrosis, sterol clefts, and mineralization. This finding complements the biochemical data that indicated the presence of more free cholesterol, presumably cholesterol monohydrate crystals, in the aorta of saturated fat–fed animals as well as the surface data that showed a redistribution of surface lesions to contain a greater percentage of more complicated plaques and a smaller percentage of fatty streaks (Fig 5).

Age and Dietary Fat Effects on Atherosclerosis in Other Arteries

The extent of atherosclerosis as measured by average intimal area in the thoracic aorta, carotid artery bifurcations, and common carotid arteries is shown for the two diet groups at three ages in Fig 9. As was found for the abdominal aorta and coronary arteries, atherosclerosis progressed as the animals aged and intimal area increased. There was little measurable intimal area in any of these arteries until the animals had consumed the diets for 60 months. There were no significant differences in intimal areas between saturated fat– and polyunsaturated fat–fed animals for any of these arteries (thoracic aorta, \( P = .4 \); carotid artery bifurcations, \( P = .7 \); and common carotid arteries, \( P = .7 \)). Similarly, maximal intimal thickness for these arteries was not affected by the type of dietary fat (data not shown). As was found for coronary arteries and abdominal aorta, 60-month-old male animals had significantly \( (P = .04) \) more atherosclerosis in each of these arterial beds as measured by intimal area than female animals.

Discussion

The hypothesis behind these studies was that dietary polyunsaturated fat compared with saturated fat would decrease coronary artery atherosclerosis in juvenile African green monkeys when fed throughout the developmental period from birth until adulthood. A decrease in coronary artery atherosclerosis induced by higher dietary polyunsaturated fat is found in adult males of this species, and was predicted by the dietary effects on lipoprotein and lipid risk factors. Consistent and statistically significant effects of polyunsaturated fat to lower coronary artery atherosclerosis were seen in the young adults of the present study, but significant age and gender effects were also noted. The majority of the effects would have been missed if the animals studied had not included males that had reached 60 months of age (young adults). The coronary artery atherosclerosis that developed was highly correlated with LDL particle size and plasma LDL cholesterol and apoB concentrations. This outcome indicates that the atherosclerosis that developed in these juveniles had characteristics similar to that seen in adult male African green monkeys, which is primarily associated with elevated LDL concentrations and modified LDL particle compositions. An inverse relation of coronary artery atherosclerosis with HDL cholesterol concentrations was not strongly apparent in the data of the present study; this outcome differs from that in the adult males of our previous study. In that study, the inverse relation of coronary artery atherosclerosis with HDL was not as strong as the positive relation with LDL, although it was stronger than found here. Perhaps this weaker relation is age related, since HDL cholesterol concentration changed throughout a significant portion of the early period of diet exposure in the juveniles.
factors that affect atherosclerosis per se in juveniles of any primate species. The demonstration of dietary protection in a juvenile primate model is particularly valuable, since documentation of such effects in human beings is among the more difficult research questions to approach. In other words, the data from these juvenile African green monkeys showing protection by dietary polyunsaturated fat against atherosclerosis in the coronary arteries per se has practical significance and becomes even more interesting when one considers that protection in other arterial beds was not as great.

We believe this is the first study to document atherosclerosis progression in young, developing primates under controlled dietary conditions that show the effect of dietary polyunsaturated fat. Although there was a wide range of variability among animals, progression of atherosclerosis in all arteries initially was slow as the animals grew and matured. Early lesions were consistently found to be fatty streaks. More advanced fibrous plaques, when present, occurred only in the older male animals. Furthermore, the extent of atherosclerosis at any age was different among arteries. Progression of atherosclerosis in the aorta generally was found to be more extensive than in the coronary arteries. In a similar manner, autopsy studies in human beings show that atherosclerosis is an age-related, progressive disease that develops more extensively in the aorta before its appearance in the coronary arteries.14 Diet effects on atherosclerosis during the developmental years in human beings have not been directly demonstrated. The animal model data are likely all that will be available for some time to come.

The time-related pattern of development of atherosclerosis in the young animals of this study was fewer lesions in the coronary arteries until the animals were older. Therefore, the importance of diet-related protection against atherosclerosis during the early periods of growth and development (ie, in utero and during the first 32 months of life) is uncertain. However, a pattern of progression of coronary artery atherosclerosis similar to that seen in the present study can be shown in adult male African green monkeys (M.S.W. and L.L.R., unpublished data, 1993). Although adults fed the saturated fat diet for 16 or 32 months had only small amounts of coronary artery intimal area, that area nonetheless doubled between 16 and 32 months. On the other hand, the average coronary artery intimal area of adults fed the saturated fat diet for 60 months was fivefold to 10-fold greater than that seen at 32 months. At all times of diet exposure, adults had more atherosclerosis than juveniles. Thus, in pediatric-aged animals fed polyunsaturated fat instead of saturated fat, protection against atherosclerosis is believed to occur during the earliest years, and indeed, throughout the period of diet exposure.

The diets used in this study were designed to mimic the dietary fat patterns of Western society, with 40% of calories as fat. The fatty acid mixture in the saturated fat-fed group is fairly typical of the luxuri-
Atherosclerosis were achieved with polyunsaturated fat–enriched diets as well as data showing that substitution of polyunsaturated fatty acids from vegetable oils for saturated fatty acids has been a long-standing pattern in our society. The data derived, including the longitudinal measurements of lipoproteins and apolipoproteins, demonstrate that the juveniles in the present study achieved lipid-lowering effects of dietary polyunsaturated fat similar in magnitude to those that have been observed for human adults, children, and infants. Therefore, the reduction in coronary artery atherosclerosis induced by dietary polyunsaturated fat in the juvenile African green monkey appears to have direct implications for diet-induced reductions in the risk of CHD in children.

Gender differences in coronary artery, aortic, and carotid artery atherosclerosis were found at 60 months of age, after both males and females had reached sexual maturity. Gender differences in coronary artery atherosclerosis in human populations have been noted and it has been hypothesized that sex hormones are primarily responsible. It is possible that the changes in hormones associated with puberty are partially responsible for modifications in lipoprotein metabolism and/or in the artery wall itself, which could affect coronary

Fig. 8. Photomicrographs of Verhoeff-van Gieson's–stained cross sections of abdominal aortas with similar mean intimal area from 60-month-old male animals consuming (A) polyunsaturated fat– and (B) saturated fat–enriched diets. These lesions represent the more severe atherosclerosis of this study. The lesion from the polyunsaturated fat–fed animal showed a thick fibrous plaque with numerous sterol clefts but little mineralization or medial thinning. The lesion from the animal fed saturated fat was more severe. There was extensive necrosis and mineralization at the base of the lesion, and the media underlying the lesion was thinned.

Fig. 9. Bar graphs showing effects of dietary fat and age on mean±SEM intimal area of thoracic aorta (A), carotid artery bifurcations (B), and common carotid arteries (C). A, Age effect, $P=.0001$ and dietary fat effect, $P=.004$; B, age effect, $P=.0001$ and dietary fat effect, $P=.07$; C, age effect, $P=.0002$ and dietary fat effect, $P=.7$.

Ours North American diet, and the isocaloric substitution of polyunsaturated fatty acids into the diet for essentially equal amounts of saturated and monounsaturated fatty acids represents a controlled diet modification to a high level of dietary polyunsaturated fatty acids. Dietary cholesterol, principally from dried egg yolk, was added to the diets at 0.8 mg/kcal to induce atherosclerosis. This level of intake is approximately four times that of Western society; however, the elevated dietary cholesterol level was required to produce a quantifiable atherosclerosis end point. African green monkeys are relatively insensitive to diet-induced hypercholesterolemia compared with other primate species such as those of the genus Macaca, including th esus and cynomolgus monkeys. However, at the selected level of dietary cholesterol, moderate hypercholesterolemia can be achieved in adult African green monkeys. Attempting to demonstrate beneficial effects of early dietary intervention with polyunsaturated fat was an experimental design based on studies in adult green monkeys in which reductions in plasma cholesterol concentrations and coronary artery...
artery atherosclerosis development. However, the experimental design of this study does not allow such associations to be firmly established.

Based on population studies relating saturated fat intake and CHD incidence and prospective studies in which dietary fat modifications have been implemented, nutritional recommendations to the general public have highlighted reducing intake of dietary saturated fat and substituting some polyunsaturated fat. Although infants and children have been shown to be responsive to lipid-lowering effects of dietary polyunsaturated fat, there has been limited endorsement for extending the dietary recommendations to them. This reluctance is due to the lack of sufficient data documenting that coronary artery atherosclerosis will be decreased in the absence of adverse effects on healthy growth and development. We were unable to detect any maleficent effects of polyunsaturated fat, even though we maintained a high level of exposure (about 20% of calories) to polyunsaturated fatty acids throughout the entire period of fetal, infant, and juvenile development. The animals in both groups grew and gained weight at the same rate, had identical serum chemistries, had a similar incidence of cholesterol gallstones, and did not have any different pathology at necropsy. These data demonstrate that polyunsaturated fatty acids are not inherently unsafe and suggest that at least some polyunsaturated fat can be advantageously substituted into the diet of children predisposed to premature CHD without inducing any appreciable risk of maleficent side effects. However, consumption of dietary polyunsaturated fat could affect risk for diseases that we did not encounter and/or diseases that might not be manifested until later in adulthood.

Strong positive correlations were found between CHD risk factors of LDL cholesterol, LDL particle size, and plasma apoB and coronary artery atherosclerosis. Thus, dietary fat–induced modifications of LDL appear to directly contribute to the coronary artery atherosclerosis reductions. This is particularly true when considering that dietary polyunsaturated fat reduced LDL cholesterol concentrations while at the same time increasing the coronary artery atherosclerosis. VLDL concentrations are extremely low in these animals, and approximately 90% of plasma apoB is found within LDL (L.L.R., unpublished data, 1990), so that plasma apoB measurements approximate LDL particle concentrations in the two groups. Likewise, enlargement of LDL has been associated with increased LDL particle cholesteryl ester content in adult animals. The positive correlations of LDL particle number and particle size with coronary artery atherosclerosis observed in this and previous studies suggest that dietary polyunsaturated fat–associated effects on both LDL composition and concentration are potentially antiatherogenic. Cholesteryl ester composition reflects dietary fat type, with an enrichment of cholesteryl oleate in the LDL of saturated fat–fed animals and of cholesteryl linoleate in the LDL of polyunsaturated fat–fed animals, a difference associated with an altered physical state of the cholesteryl ester core (Reference 50 and J.S. Parks and L.L. Rudel, unpublished data, 1990). As a consequence, the diet-induced difference in cholesteryl ester composition of LDL could affect the relative atherogenicity. We have noted a strong association between LDL cholesteryl oleate content and coronary artery atherosclerosis. It is important to note that the changes in LDL composition induced by dietary polyunsaturated fat increase the particle content of linoleic acid and thus increase the potential for LDL oxidation. However, the animals with this LDL also had less atherosclerosis, suggesting that the increase in LDL oxidation potential is more than compensated for by beneficial effects.

Finally, it should be reemphasized that the atherosclerotic lesions identified in the animals of the present studies had become complicated by the end of the study, as indicated by the accumulation of free cholesterol. In fact, there was significantly more free than esterified cholesterol in the lesions of the abdominal aorta (Fig 6). The pathological significance of free cholesterol accumulation in atherosclerotic lesions is not well understood, although it has been a topic of interest in human atherosclerosis for several years. Similarly, we do not understand why free cholesterol accumulates in the atherosclerotic lesions of African green monkeys. However, we found a high level of free cholesterol present in the abdominal aorta of the animals with atherosclerosis, as had been suggested by the appearance of cholesterol clefs in histological sections, and significantly more free cholesterol accumulated in the aortas of male animals fed saturated fat than in those fed polyunsaturated fat. This would appear to be another similarity between atherogenesis in this experimental primate model and human beings that suggests that the etiology of free cholesterol accumulation can be studied in this animal model. As Wagner and Clarkson show, the utility of the African green monkey in this regard is unique among primate models of atherosclerosis.

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