Reduced Proteoglycan Binding of Low Density Lipoproteins From Monkeys (Macaca fascicularis) Fed a Fish Oil Versus Lard Diet

Iris J. Edwards, Abraham K. Gebre, William D. Wagner, and John S. Parks

We examined the effect of isocaloric substitution of dietary fish oil for lard on the properties of low density lipoproteins (LDL) important in binding to arterial proteoglycans (PG). Cynomolgus monkeys (n=10) were fed atherogenic diets enriched in fish oil or lard in a crossover study consisting of two 15-week phases of atherogenic diet separated by a 6-week monkey chow “wash-out period.” LDL were isolated from plasma during each dietary phase, characterized for chemical and physical properties, and assessed for their ability to interact in vitro with arterial PG. Plasma LDL cholesterol was similar during fish oil and lard consumption (356±34 and 331±17 mg/dL, mean±SEM), but during fish-oil feeding relative to that of lard, LDL size was smaller (4.2±0.1 versus 4.9±0.1 g/mlol) and LDL particles differed in chemical composition. When animals were fed fish oil, significantly fewer (p<0.05) LDL particles bound to PG in both dietary phases: 1.00±0.17 (x10^12) versus 5.31±0.83 (x10^12) particles/µg PG in phase 1 and 3.56±0.67 (x10^12) versus 6.00±0.52 (x10^12) in phase 2 for LDL from animals fed fish oil and lard, respectively. These studies indicate that dietary fat-induced changes in LDL particles lead to altered in vitro interactions with artery wall PG and suggest a novel mechanism for the protective effect of fish oil against atherosclerosis. (Arteriosclerosis and Thrombosis 1991;11:1778–1785)

Iinterest in the antiatherogenic properties of dietary fish oil began with the observation by Dyerberg and Bang that Eskimos with a high intake of dietary fat were protected from coronary heart disease (CHD) compared with their Danish counterparts. Examination of the Eskimo diet indicated that it was lower in saturated fatty acids and higher in polyunsaturated and monounsaturated fatty acids. As a result of enhanced fish oil consumption, Eskimos ingested four times the usual intake of ω-3 polyunsaturated fatty acids. Several additional epidemiological studies have confirmed that increased fish consumption is associated with a reduced CHD risk, particularly when combined with a low dietary intake of saturated fat. Recently in a multiple risk factor intervention trial (MRFIT), a significant decline in risk of CHD was associated with increased consumption of ω-3 fatty acids when risk values were adjusted for age, race, blood pressure, plasma lipids, and cigarette smoking. In addition, fish-oil feeding has been shown to retard the development of experimental atherosclerosis in monkeys and pigs. The cardioprotective effects of ω-3 fatty acids may result through several mechanisms. Considerable interest has focused on the decreased potential for thrombotic and inflammatory events due to substitution of cell membrane arachidonic acid (in animal fat-rich diets) by eicosapentaenoic acid (in fish oil-rich diets). Prostanoids derived from eicosapentaenoic acid compared with arachidonic acid possess lower platelet aggregating activity, lower vasoconstrictive action, and lower leukocyte chemotaxis-inducing activity. A second area of interest centers around alterations in plasma lipoproteins, which may occur as a result of fish-oil feeding. Lower plasma triglyceride and very low density lipoprotein (VLDL) levels have been reported in several human studies, but the effects on low density lipoprotein (LDL) and high density lipoprotein (HDL) concentrations have varied (for a review, see Reference 14).

As a result of fish-oil feeding, the lipoprotein particles may be modified chemically or physically to a state less conducive to accumulation in the artery wall. Although specific properties of LDL and precise mechanisms of entrapment of plasma LDL in arterial tissue are still to be defined, the interaction of LDL with arterial proteoglycans (PG) is thought to
be of major importance in the retention of LDL in the tissue. Studies by Hoff et al.\textsuperscript{15} and Hollander et al.\textsuperscript{16} have demonstrated that chemically modified LDL is present in atherosclerotic arteries and that extraction of this LDL results in the coisolation of glycosaminoglycans (GAG), the carbohydrate moiety of PG. LDL with high affinity for PG in vitro has been positively associated with CHD in vivo.\textsuperscript{17-19}

Dietary manipulation of LDL particles and its effect on functional properties such as PG binding are therefore of considerable interest. In the African green monkey, we have demonstrated that isocaloric substitution of fish oil for lard resulted in the production of plasma LDL with decreased molecular sizes, decreased cholesteryl ester (CE) content, and lower CE melting temperature. From direct examination of coronary arteries, atherosclerotic lesion development was significantly lower in the animals fed fish oil compared with animals fed diets rich in saturated fat.\textsuperscript{8}

More recently, we have extended these studies to the cynomolgus macaque, a species more susceptible to diet-induced atherosclerosis. A crossover design that allowed each animal to serve as its own control was used to demonstrate that during fish-oil feeding versus lard feeding, LDL CE and phospholipids were enriched in $\omega-3$ but poor in $\omega-6$ fatty acids, CE melting temperature was lower, LDL phosphatidylcholine was decreased, and lysophosphatidylcholine and sphingomyelin were increased.\textsuperscript{20} The purpose of the present study was to determine if the alterations in the chemical and physical properties of LDL that accompany fish-oil feeding affect the binding of LDL to arterial PG. The results indicate that interaction of LDL with artery-derived PG was significantly lower during the fish-oil-feeding phase of the study.

**Methods**

**Materials**

Ultrapure guanidine hydrochloride (GdnHCl) was purchased from ICN Biomedicals, Inc., Irvine, Calif.; benzamidine hydrochloride and 8-aminoheptanoic acid from Eastman Kodak, Rochester, N.Y.; tryptamine hydrochloride and 1,10-phenanthroline from Sigma Chemical Co., St. Louis, Mo.; and Sepharose CL-4B from Pharmacia LKB Biotechnology, Inc., Piscataway, N.J. All other reagents were purchased from Fisher Scientific.

**Experimental Design**

The animals and dietary manipulations used in these studies have been previously described.\textsuperscript{20} Briefly, 10 adult male cynomolgus monkeys (Macaca fascicularis) were divided into two groups based on similar total plasma cholesterol (TPC), HDL cholesterol, and triglyceride concentrations. A crossover study consisting of two 15-week phases of experimental diet was carried out as illustrated in Figure 1. Each diet contained 40% of calories as fat with 0.26 mg cholesterol/kcal. The fat calories were derived from 50% egg yolk and 50% lard or menhaden oil. Complete details of the diets are given in our previous publications.\textsuperscript{20,21} In phase 1, group 1 monkeys consumed the lard diet and group 2, the fish oil diet. This was followed by an 8-week "wash-out period," during which time the animals were fed monkey chow to allow plasma lipids to return to baseline. The diets were reversed in phase 2, during which time group 1 animals received the fish oil and group 2, the lard. Arrows denote time of removal of blood for lipoprotein analysis.

**Lipoprotein Preparation**

Blood samples for plasma lipid measurements and LDL isolation were taken at 15 weeks into each dietary phase as indicated by arrows in Figure 1. After an 18-hour fast, animals were sedated with ketamine hydrochloride (10 mg/kg), and blood was removed from the femoral vein into tubes containing 0.1% EDTA and 0.02% NaN$_3$ (pH 7.4, final concentration). Lipoproteins were isolated from the plasma by ultracentrifugation and column chromatography on Superose 6B as previously described.\textsuperscript{20} LDL of known molecular weight was used to generate a molecular-weight calibration curve for the column. Chemical compositions of LDL were measured by standard colorimetric and enzymatic procedures.\textsuperscript{20}

**Proteoglycan Preparation**

Two adult Macaca fascicularis monkeys fed a monkey chow diet were used as a source of arterial PG for these studies. For detection of PG during purification procedures, one animal was injected with 10 mCi sulfur-35-labeled sodium sulfate 24 hours before necropsy. At necropsy, the thoracic aortas were excised, cleaned of adventitia, quick-frozen in liquid nitrogen, and stored at $-70^\circ$C. Before PG extraction, tissues were thawed, minced into approximately 2x2-mm sections, and incubated for 24 hours at 4°C in 4.0 M GdnHCl and 0.05 M sodium acetate (pH 4.5, 15 mL/g wet tissue) containing protease inhibitors.\textsuperscript{22} After removal of extracted tissues by filtration, the GdnHCl solution containing the PG was con-

![FIGURE 1. Diagram of the experimental design of diet administration. Two 15-week phases of experimental diets were administered in a crossover design separated by an 8-week "wash-out period," during which time animals were fed monkey chow. Experimental diets contained 40% of calories as fat, of which 50% were derived from either lard or menhaden oil. In phase 1, group 1 animals received the lard and group 2, the fish oil diet; in phase 2, group 1 animals received the fish oil and group 2, the lard. Arrows denote time of removal of blood for lipoprotein analysis.](image-url)
Table 1. Plasma Lipoprotein Cholesterol Distribution in Macaca fascicularis Monkeys Fed Lard or Fish Oil Diets

<table>
<thead>
<tr>
<th>Cholesterol</th>
<th>Lard</th>
<th>Fish oil</th>
<th>Fish oil</th>
<th>Lard</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC</td>
<td>419±21</td>
<td>405±46</td>
<td>411±58</td>
<td>413±21</td>
</tr>
<tr>
<td>V+I</td>
<td>25±6</td>
<td>30±6</td>
<td>17±5</td>
<td>32±9</td>
</tr>
<tr>
<td>LDL</td>
<td>342±27</td>
<td>346±43</td>
<td>366±57</td>
<td>320±24</td>
</tr>
<tr>
<td>HDL</td>
<td>52±11</td>
<td>29±4*</td>
<td>28±3*</td>
<td>61±14</td>
</tr>
</tbody>
</table>

Values are plasma cholesterol in milligrams per deciliter and represent mean±SEM.
TPC, total plasma cholesterol; V+I, very low and intermediate density lipoproteins; LDL, low density lipoprotein; HDL, high density lipoprotein. V+I, LDL, and HDL were fractionated from d<1.25 g/ml lipoproteins by size-exclusion chromatography on Superose 6B. Group 1 (n=5) received the lard diet first followed by the fish oil diet; group 2 (n=5) received the diets in opposite order.

* p<0.05 by paired t test.

centrinated in an Amicon ultrafiltration cell fitted with a PM 30 membrane and chromatographed on Sepharose CL-4B with 4.0 M GdnHCl and 0.05 M sodium acetate (pH 5.8) as the elution buffer. As previously observed with samples of human23 and pigeon24 arteries, two major PG peaks were separated, one eluting near the column void volume, containing predominantly chondroitin sulfate (CS) PG, and a second eluting at a Kav of 0.4, containing predominantly dermatan sulfate PG. The CSPG fractions eluting near the column void volume were pooled and further purified by isopycnic gradient centrifugation in cesium chloride (p=1.40 g/ml) as previously described.23 The bottom two fifths of the gradient (d>1.45 g/ml), which contained 64% of the [35S]sulfate, was isolated; measured for uronic acid,25 protein,26 and hexosamine27; and stored at −70°C. The PG preparation contained 130 µg/ml uronic acid, 149 µg/ml protein, 112 µg/ml hexosamine, and, based on uronic acid analysis, comprised approximately 50% of the total extracted arterial PG.

Proteoglycan–Low Density Lipoprotein Interactions

Previous publications have described the in vitro assay system used to assess PG–LDL interaction, the specificity of the interaction, the importance of intact PG structure, and the involvement of divalent cations in the formation of particulate PG–LDL complexes.24,28,29 Before binding with LDL, the PG was dialyzed against a binding buffer (5 mM tris(hydroxymethyl)aminomethane, 6 mM KCl, and 1 mM MgSO4 [pH 7.2]) for 18 hours at 4°C. The LDL samples were dialyzed against the same buffer for 2 hours at 4°C. PG–LDL particulate complex formation was assessed by incubating known amounts of PG (as hexuronic acid) and LDL (as cholesterol) in binding buffer containing varying concentrations of calcium chloride. When the optimal calcium chloride level was determined, that concentration was used for all further studies. In some experiments, samples of LDL in binding buffer with calcium chloride were used as controls. After a 30-minute incubation at 24°C, complex formation was assessed either by measurement of percent light transmittance at 600 nm (i.e., light scatter) or, after centrifugation at 1,500g for 30 minutes and removal of the supernate, by assay of the pelleted LDL–PG complex for cholesterol content.

Data Analysis

Values are presented as mean±SEM. Either a t test or a paired t test was used to determine significant differences between means.

Results

TPC concentrations for the two groups of animals increased rapidly after initiation of the experimental diets, returned to baseline during the wash-out phase, and increased again during experimental phase 2. Plasma lipoprotein cholesterol distribution for groups of animals consuming the lard versus fish oil diets is shown in Table 1. The values for TPC, VLDL and intermediate density lipoproteins, and LDL cholesterol were similar between diets for both groups of animals. At no time during the study were these values significantly affected by the type of dietary fat.20 However, HDL cholesterol was significantly lower when the animals were fed the fish oil diet (p<0.01). The effect of the type of dietary fat on LDL particle size and chemical composition was investigated. During fish-oil feeding, LDL molecular weights were smaller for both groups of animals (Table 2). Since plasma LDL concentrations were similar, this size difference resulted in a significantly higher LDL particle concentration when animals were consuming fish oil versus lard (2.3±0.2 versus 1.8±0.1 µM; p<0.0004). Chemical analysis of the LDL particles demonstrated lower phospholipid as the only significant difference resulting from fish-oil feeding in both phases of the diet. Differences in free cholesterol and CE in either phase 1 or phase 2 were significant when the data from both dietary phases were combined.20

A previously described in vitro assay system24 was used to measure complex formation between the purified LDL and the artery-derived PG. Based on a preliminary experiment to determine optimum binding conditions for monkey PG and LDL (data not shown), the calcium chloride concentration of the buffer was 15 mM. Saturation curves for the assay

Downloaded from http://atvb.ahajournals.org/ by guest on October 30, 2017
TABLE 2. Low Density Lipoprotein Molecular Weight and Chemical Composition in Macaca fascicularis Fed Lard or Fish Oil Diets

<table>
<thead>
<tr>
<th>Weight/Component</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lard (n=5)</td>
<td>Fish oil (n=5)</td>
</tr>
<tr>
<td>LDL MW</td>
<td>4.86±0.19</td>
<td>4.37±0.14</td>
</tr>
<tr>
<td>Amino acids</td>
<td>8,674±180</td>
<td>8,026±170*</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>1,261±152</td>
<td>1,092±42*</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>1,301±90</td>
<td>656±28*</td>
</tr>
<tr>
<td>Cholesteryl ester</td>
<td>3,571±155</td>
<td>3,501±174</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>50±5</td>
<td>54±9</td>
</tr>
</tbody>
</table>

Low density lipoprotein molecular weight (LDL MW) is grams per micromole. Values represent mean±SEM. All chemical compositions are molecules per particle, and values represent mean±SEM. Amino acids were calculated from the equation ([% protein/100%]x[MWx10])/110.

*p<0.05 by paired t test.

were generated by holding LDL constant and varying PG concentration (Figure 2). Complex formation by the LDL isolated from lard-fed animals resulted in a highly turbid assay solution, which increased light scattering and reduced transmittance at 600 nm to ~20% (Figure 2A, closed circles). Saturation occurred at approximately 1 μg PG. By contrast, little turbidity was observed when a similar concentration of LDL from a fish oil-fed animal was allowed to complex to the PG (Figure 2A, open circles). When the complexes were pelleted by centrifugation and assayed for total cholesterol, the marked difference in PG binding characteristics of LDL from animals fed a lard versus fish oil diet was confirmed (Figure 2B). At saturation, only 12% of the LDL cholesterol was recovered in a pelleted complex with LDL from an animal fed fish oil compared with 65% recovery of LDL from an animal fed lard. In addition, less PG (0.5 μg versus >1.0 μg) was required to saturate the LDL from the animal fed fish oil. When PG was not added to the assay tubes (0 μg PG, control) LDL did not precipitate, indicating that PG was necessary for complex formation and that the presence of calcium chloride in the incubation mixture was not sufficient to precipitate LDL in the absence of PG.

The binding curves for LDL with a constant amount of PG are illustrated in Figure 3. Over a range of LDL concentrations from 100 to 1,000 μg (as cholesterol), lower PG-LDL complex formation was measured with LDL from fish oil-fed compared with lard-fed animals. The differences appeared to widen with increasing LDL concentration.

Divalent cations, particularly calcium, have been shown to play an important role in PG-LDL complex formation by stabilizing complexes and presumably by cross-linking small "nonparticulate" complexes to form large particulate ones. To determine whether calcium requirements for the formation of measurable complexes in our assay were different for LDL from animals fed fish oil versus lard, the optimum calcium concentration from each type of LDL was assessed (Figure 4). LDL from the lard-fed animal showed maximum complex formation with PG in the presence of 15 mM CaCl2, and LDL from the fish oil-fed animal required 20 mM CaCl2 for optimum binding. At no concentration of calcium was complex formation equivalent for both LDL types. Based on the calcium curves illustrated in Figure 4, it was
FIGURE 3. Binding curves for low density lipoprotein (LDL) and chondroitin sulfate proteoglycan (CSPG) interaction with PG held constant. CSPG (1 μg as hexuronate) was incubated with 100 μg–1 mg LDL (as cholesterol) selected at random from each dietary group in phase 2 of the study. Binding buffer was 5 mM tris(hydroxymethyl)aminomethane, 6 mM KCl, 15 mM CaCl₂, and 1.5 mM MgSO₄ (pH 7.2), and the formation of PG–LDL complexes was measured as micrograms cholesterol in a 1,500g pellet. ●, Lard diet; ○, fish oil diet.

The binding properties of LDL from all experimental animals in both dietary phases are shown in Figure 5. Because of differences in molecular weights and percent composition of LDL particles from animals consuming lard versus fish oil diets, these data are presented normalized to the number of LDL particles bound per microgram PG. In both dietary phases of the study, significant differences (p<0.05) in the number of LDL particles complexed to 1 μg PG were observed between LDL from animals fed fish oil versus lard. In phase 1, the mean number of LDL particles bound per microgram PG was 5.31±0.83 (×10^{12}; mean±SEM) for LDL from animals fed the lard diet compared with 1.00±0.27 (×10^{12}) for LDL from animals consuming the fish oil diet. When the experimental diets were reversed in phase 2 of the study, PG binding properties of the LDL were also reversed. The mean number of LDL particles bound per microgram PG was 6.00±0.52 (×10^{12}) and 3.56±0.67 (×10^{12}) for LDL from animals fed the lard and fish oil diets, respectively. Figure 6 illustrates the change in LDL binding to PG for each animal in the study. LDL binding to PG to form particulate complexes was lower in eight of 10 animals during fish oil consumption and was, on average, 41% of that observed during the lard phase of the diet (2.3±0.6 versus 5.7±0.5 (×10^{12}) particles per microgram PG; mean±SEM for all 10 animals). These data taken together demonstrate that LDL from animals fed the
fish oil diet were less avidly bound to PG regardless of the order of diet administration.

**Discussion**

In this study, we have demonstrated that dietary fat–induced chemical changes in LDL particles lead to altered interactions with artery wall PG in vitro. This is the first study to examine PG–LDL interactions in relation to dietary fat manipulation and provides a novel hypothetical mechanism for the protective effect of fish oil against atherosclerosis.

The association of PG and LDL occurs through ionic interactions between negatively charged sulfate groups of the GAG and positively charged amino groups of the LDL apolipoprotein (apo) B. Both the charge density and the iduronic acid content of the GAG have been shown to be important factors in LDL binding. Camejo et al. have investigated the properties of LDL that may be important for interaction with PG. Compared with LDL from normal individuals, LDL from hypercholesterolemic patients had a higher affinity for PG as a result of an LDL subclass characterized by a lower sialic acid content, higher isoelectric point, and increased ratio of CE to triglyceride. A decreased ratio of surface polar lipid to core nonpolar components also may be a discriminator for increased PG binding potential.

In African green monkeys, fish oil compared with lard consumption led to the production of significantly smaller LDL particles that contained less CE and an altered CE fatty acid ratio. In addition, the CE melting temperature was lower in fish oil–fed compared with lard-fed animals. Both the CE fatty acid ratio (saturated and monounsaturated/polysaturated species) and CE melting temperature were significantly associated with increased aortic atherosclerosis in these animals. In the cynomolgus macaques of the present study, similar effects on LDL were observed and have been previously reported. LDL particle size was smaller during fish-oil feeding (4.2±0.1 versus 4.9±0.1 g/μmol), and LDL CE was relatively poor in 18:1 and 18:2 but enriched in ω-3 fatty acids. LDL CE melting temperature was 11°C lower during fish oil consumption. LDL phospholipid changes were identified as decreased phosphatidylcholine and increased lysophosphatidylcholine and sphingomyelin during fish oil compared with lard feeding.

It is not known how changes in the LDL molecules affect their binding capacity for artery-derived PG. The phospholipid differences merit further investigation, as it has been reported that phospholipase treatment of LDL before interaction with PG inhibits complex formation. Several investigators have proposed that the role of calcium in the formation of insoluble PG–LDL complexes involves electrostatic bridging between the sulfate groups of the PG and the phospholipids of the LDL. Changes within choline species were the only differences in LDL phospholipid distribution during fish-oil compared with lard feeding for the animals in the present study, and whether polar head group–induced electrostatic differences are responsible for the different interactions with PG remains to be defined. A more likely probability is that phospholipid changes may alter the conformation of apo B on the LDL particles, and this may affect the exposure of PG binding sites.

Distinct apo B sequences that bind heparin and CSPG have been identified. Similar heparin-binding sites were reported on apo E. In the current studies, we have found that a decrease in the apo E to apo B molar ratio of the d=1.015–1.025 g/ml subfraction of LDL from animals fed the fish oil versus lard diet was related to decreased binding of these subfractions to CSPG. Therefore, the alterations in LDL phospholipid distribution or in LDL core CE composition may directly or indirectly influence PG binding by inducing changes in the particle content of apo E or in the conformation of apo B and/or apo E on the surface of LDL particles.

The molecular size differences of LDL between fish oil–fed and lard-fed animals are probably not a major factor in the different binding properties. In pigeons, LDL molecular weight increases as a result of cholesterol feeding were inversely correlated with PG binding capacity, but this occurred only when LDL molecular weights exceeded 5 g/μmol. In animals with LDL between 3.6 and 4.8 g/μmol, considerable variability in PG binding capacity existed, indicating the importance of factors other than molecular size. In the present study, since LDL particles were smaller in fish oil–fed animals, if size were an important factor in binding to PG, then these particles should show greater binding.

Several studies have indicated that in vitro interaction of plasma LDL with PG potentiates LDL uptake and accumulation in macrophages. Particulate PG–LDL complexes have been shown to enhance CE accumulation in mouse peritoneal macrophages. In addition, transient in vitro interaction of LDL with PG has been shown to modify the
conformation of apo B. This led to increased LDL internalization and stimulation of cholesteryl esterification in human monocyte–derived macrophages.\textsuperscript{46} It has been proposed, therefore, that interaction with PG may result in alterations in the LDL particles, which allows their recognition and uptake by the cells. The process of recognition and involvement of a specific macrophage receptor is at present unclear.

Also unclear is the physiological relevance of “soluble” versus particulate PG-LDL complexes to lipid accumulation in the artery wall. In vitro studies have indicated that conversion of soluble to particulate complexes is calcium dependent.\textsuperscript{47,48} Soluble complexes formed at 2 mM Ca\textsuperscript{2+} were converted to precipitable complexes in the presence of 5–25 mM Ca\textsuperscript{2+}.\textsuperscript{47} In the environment of the artery wall, the calcium concentration may therefore be a key factor in determining the result of the interaction of LDL with PG. The present studies were designed to assess only particulate complexes, and we used the optimum calcium concentration for the formation of particulate complexes with PG for LDL from both of the diet groups (Figure 4). However, at all levels of calcium studied, particulate complex formation was higher with LDL from lard-fed compared with fish oil-fed animals.

In summary, the studies indicate that when cynomolgus monkeys receive dietary fat from fish oil versus lard, their plasma LDL has a reduced capacity for in vitro binding to arterial PG. Further studies are ongoing to determine the specific physical and chemical changes in the LDL particles that impart these differences. These data suggest that in an animal consuming fish oil, LDL retention in the artery wall as a result of interaction with arterial PG will be reduced and emphasize that changes in LDL particles may be more important in atherosclerosis than changes in the amount of plasma LDL. This presents a novel hypothesis for the cardioprotective effects of fish oil and emphasizes the need for further studies of the effects of dietary manipulations on functional properties of lipoprotein particles.

Acknowledgments

The authors thank D.L. Cutter and R.W. St. Clair for their critical reviews of the manuscript and Lonnie Ellis for secretarial assistance with the manuscript preparation.

References


**KEY WORDS** • proteoglycans • low density lipoproteins • fish oil • lard • atherosclerosis • ω-3 fatty acids • monkeys
Reduced proteoglycan binding of low density lipoproteins from monkeys (Macaca fascicularis) fed a fish oil versus lard diet.
I J Edwards, A K Gebre, W D Wagner and J S Parks

*Arterioscler Thromb Vasc Biol.* 1991;11:1778-1785
doi: 10.1161/01.ATV.11.6.1778

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
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:
http://atvb.ahajournals.org/content/11/6/1778

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
http://atvb.ahajournals.org//subscriptions/