Glycosaminoglycan Fractions From Human Arteries Presenting Diverse Susceptibilities to Atherosclerosis Have Different Binding Affinities to Plasma LDL

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Abstract The topographic distribution of atherosclerotic lesions is influenced by biochemical factors intrinsic to the arterial wall. In the present work we have investigated whether the composition/chemical structure of glycosaminoglycans constitutes one of these factors. Normal human arteries were obtained at necropy, and in order of decreasing susceptibility to atherosclerosis, consisted of the abdominal and thoracic aortas and the iliac and pulmonary arteries. The results showed similar concentrations of total glycosaminoglycan and collagen. Of the glycosaminoglycans known to interact with low-density lipoprotein (LDL), dermatan sulfate was present in all arteries in comparable concentrations, but the aortas had a 30% higher content of chondroitin 4/6-sulfate, which in turn was slightly enriched in 6-sulfated disaccharide units. LDL-affinity chromatography with dermatan sulfate + chondroitin 4/6-sulfate fractions demonstrated that increasing affinity to LDL matched an increasing susceptibility to atherosclerosis. Analysis of glycosaminoglycans in the eluates indicated a positive correlation between affinity to LDL and increasing molecular weight and the existence of a fraction of glycosaminoglycans of high affinity to LDL in the aortas only. These results suggest that arterial glycosaminoglycans participate in the multifactorial mechanisms that modulate the differential localization of atherosclerotic lesions. (Arterioscler Thromb. 1994;14:115-124.)

Key Words • glycosaminoglycans • chondroitin sulfate • heparan sulfate • atherosclerosis risk factors • LDL • arterial connective tissue

The occurrence of atherosclerotic lesions is associated with a number of risk factors, such as elevated serum lipid and lipoprotein levels, hypertension, smoking, sex, and family history. However, factors intrinsic to the arterial bed and its blood-carrying functions also influence the occurrence of lesions. The primary evidence came from morphological study of necropsy material, which demonstrated that the incidence and/or severity of atherosclerotic lesions differed as a function of anatomic location. Additionally, these studies revealed that in affected arteries, lesions tended to be located at critical sites, such as the entrances of vessels and flow dividers, where the effects of fluid turbulence would be more pronounced. Experimental physiological investigations have supported these findings and have shown that the shear force due to blood circulation, chiefly at arterial pressures, is an important cause of endothelial dysfunction at these sites.

Still, arterial geometry and pressure are not sufficient to explain the localization of lesions, since major areas of high risk for atherosclerosis also occur away from the ostia and bifurcations and some arteries subjected to normal arterial blood pressure are little affected by atherosclerosis. In fact, studies with experimental animals have demonstrated that low-density lipoprotein (LDL) metabolism, endothelial permeability to LDL, the glycoprotein coating of endothelial cells, and the activity of acid cholesterol esterase vary among vessels, and this variation could be related to differences in atherosclerosis susceptibility. A further aspect of arterial constitution that has been correlated with regional differences in the incidence of atherosclerosis is the degree of folding of the internal elastic membrane in humans, which somehow reflects the amount of elastin in the vessel wall.

Taken together, the results of these mostly recent studies show that focal metabolic and biochemical factors inherent in the arterial wall modulate the initiation and differential localization of atherosclerotic lesions. Consequently, a precise understanding of the individual roles of such factors is essential for understanding the pathogenesis of atherosclerosis.

It is well established that arterial glycosaminoglycans (GAGs) proteoglycans play an important role in the early phases of atherogenesis, since these molecules are capable of binding and holding plasma LDL that insulates into the arterial wall after endothelial dysfunction. GAG-LDL complexes are in turn more easily internalized by macrophages than LDL alone, thereby enhancing the formation of foam cells. Also, GAGs induce structural alterations to LDL molecules that may potentiate their atherogenic effects. The nature of the GAG-LDL interaction has therefore been extensively studied, and it is known that certain GAG species— including particular populations of a given species— have greater affinity for LDL. Thus, should normal
variations in GAG composition occur among arteries, they might lead to variations in LDL retention/alteration capacity in these arteries. Indeed, an early and succinct overview of arterial GAGs indicates that some variations in composition do seem to exist, and similar anatomic differences have been observed in relation to the distribution of other components of the extracellular matrix, such as collagen and elastin, which intimately associate with GAGs.

Therefore, the purpose of the present study was to determine whether an eventual anatomic heterogeneity of arterial GAGs might be a factor contributing to the differential topographic distribution of atherosclerotic lesions. To carry out this investigation, our experimental approach consisted of the following: (1) a more detailed characterization of GAG composition in normal human arteries presenting diverse susceptibilities to atherosclerotic lesions; (2) an analysis of the binding affinities of GAG fractions from these arteries to plasma LDL; and (3) further characterization of the GAG samples once their binding affinities had been established.

Methods

Normal human arteries were obtained at necropsy. The cases consisted of young adults aged 25 to 35 years who had died accidentally and, so far as could be verified, with no history of cardiovascular or metabolic diseases. The vessels comprised the thoracic and abdominal aortas and the iliac and pulmonary arteries. The iliac artery samples consisted of the distal segment of the common iliac artery and the proximal portions of the external and internal iliac arteries. Arterial segments without macroscopically visible lesions were then excised, fixed in acetone, and kept at 4°C. After their adventitial layer was stripped off, these segments, which therefore consisted of the intimal and medial layers, were finely homogenized in acetone and subjected to two changes of 50 volumes of this solvent for 24 hours each. The final defatted powder was obtained by drying this material at 60°C. Standard chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, and twocystealized papain (15 U/mg protein) were purchased from Sigma Chemical Co, St Louis, Mo, and Yangen bro-mide-activated Sepharose 4B was from Pharmacia, Uppsala, Sweden. Chondroitin AC lyase (Chondroitinase AC-II) from Arthrobacter aurescens and chondroitin ABC lyase from Proteus vulgaris were from Miles Laboratory Co, Elkhart, Ind.

All methods employed for the purification, characterization, and quantitation of GAGs, as well as the assay for total arterial collagen, have been described in detail elsewhere. Estimations of the concentration of total arterial GAGs and collagen were made separately for each individual. Subsequent analyses were made with pooled extracts for each artery.

Isolation of Total Arterial GAGs

One hundred milligrams of the acetone-treated powder was rehydrated for 24 hours at 4°C in 3.7 mL of 0.1 mol/L sodium acetate buffer, pH 5.0, containing 5 mmol/L cysteine and 5 mmol/L EDTA. Papain (0.7 mg) was then added to the mixture, which was incubated at 60°C for 24 hours with mild agitation. The incubation mixture was centrifuged (2000g, 20 minutes at room temperature), the supernatant was retained, and the pellet was resuspended in 3.7 mL of distilled water and centrifuged. A 10% cetylpyridinium chloride (CPC) solution was added to the combined supernatants to a final concentration of 0.5%, and the mixture was left to stand at room temperature for 24 hours. Next, the solution was centrifuged and the pellet washed with 15.0 mL of a 0.05% CPC solution. This pellet, a GAG-CPC complex, was then dissolved with 3.7 mL of 2-mol/L NaCl/absolute ethanol (100:15, vol/vol) solution, and the GAGs were precipitated with the addition of 7.4 mL absolute ethanol. After 24 hours at 4°C, the precipitates were collected by centrifugation and washed twice with 15.0 mL 80% ethanol and once with the same volume of absolute ethanol. The final pellet, which constituted the total tissue GAG preparation, was dried at 60°C for 30 minutes and dissolved in 1.1 mL distilled water. Papain digestion under these conditions completely solubilized all arterial samples, and the subsequent CPC and ethanol precipitations had a recovery >90% with regard to a solution containing known amounts of GAGs.

DEAE-Cellulose Chromatography

Arterial GAGs (100 to 200 mg) were applied to a DEAE-cellulose column (2×11 cm, 20-mL bed) equilibrated with 50 mmol/L sodium acetate buffer, pH 6.0. The column was then washed with 100 mL of this same buffer and subjected to a linear gradient of 0 to 1.0 mol/L NaCl at a flow rate of 12 mL/h, and fractions of 2 mL were collected. These were assayed by the carbazole reaction, and their salt concentrations were estimated by conductivity. Fractions containing GAGs were pooled and precipitated with three volumes of absolute ethanol, and the yield was ~85% with regard to the applied material.

Agarose Gel Electrophoresis

GAGs (10 µg) were applied to a 0.5% agarose gel in 0.05 mol/L 1,3-diaminopropane/acetate buffer, pH 9.0. After electrophoresis (120 V for 1 hour), the GAGs in the gel were fixed with 0.1% N-cetyl-N,N,N-trimethylammonium bromide in water, stained with 0.1% toluidine blue in acetic acid/ethanol/water (0.1:5:5, vol/vol/vol), and washed for about 30 minutes in acetic acid/ethanol/water (0.1:5:5, vol/vol/vol). The GAGs in the agarose gel electrophoresis were quantitated by densitometry using a Quick Scan densitometer (Helena Laborato ries, Beaumont, Tex).

Polyacrylamide Gel Electrophoresis (PAGE)

The molecular weights of the GAGs were estimated by PAGE. GAG samples (10 µg) were applied to a 6%, 1-mm-thick polyacrylamide gel slab and after electrophoresis (100 V for 30 minutes), the gel was stained with 0.1% toluidine blue in 1% acetic acid. After staining, the gel was washed for about 4 hours in 1% acetic acid. The molecular-weight markers were the same as those used previously.

Enzymatic Degradations

GAGs (100 µg) were incubated with 0.01 U chondroitin AC lyase, 0.01 U chondroitin ABC lyase, 23 or 10 µL heparitinases in 0.05 mol/L ethylenediamine/acetate buffer, pH 8.0, in a final volume of 50 µL. After incubation at 37°C (30°C for heparitinases) for 12 hours, the mixtures were spotted on Whatman No. 1 papers and chromatographed on isobutyric acid/1 mol/L NH₄OH (5:3, vol/vol) for 24 hours. The products were located by silver nitrate staining and quantitated by densitometry.

Isolation of LDL From Plasma

LDL (d=1.020 to 1.050 g/mL) was purified by the method of Havel et al, with human plasma obtained from healthy donors. After sequential ultracentrifugations in potassium bromide, the LDL preparation was dialyzed exhaustively at 4°C against 0.9% NaCl containing 0.01% EDTA and stored at 4°C. Purity of LDL in this preparation was assessed by agarose gel electrophoresis in barbital buffer, which showed a single band. The concentration of LDL in these preparations was typically 1.8 mg/mL as protein.

LDL-GAG Interaction Experiments

Experiments on the interaction between human plasma LDL and arterial GAGs were performed essentially as de-
was obtained by papain digestion and cetylpyridinium chloride precipitation, and ion-exchange chromatography.

Table 1. Total Hexuronic Acid and Hydroxyproline Contents in Different Arteries

<table>
<thead>
<tr>
<th>Artery</th>
<th>Hexuronic Acid</th>
<th>Hydroxyproline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal Aorta</td>
<td>8.1±0.71</td>
<td>50.7±3.82</td>
</tr>
<tr>
<td>Thoracic Aorta</td>
<td>8.2±0.71</td>
<td>54.7±4.91</td>
</tr>
<tr>
<td>Iliac Artery</td>
<td>7.7±1.36</td>
<td>76.0±11.51</td>
</tr>
<tr>
<td>Pulmonary Artery</td>
<td>7.2±1.32</td>
<td>69.5±6.84</td>
</tr>
</tbody>
</table>

Contents are given in micrograms per milligram of dry, defatted tissue. Hexuronic acid was measured on total glycosaminoglycan extracts from tissue samples by a carbozale method. For the hydroxyproline assay, tissue samples were hydrolyzed in 6 mol/L HCl and evaporated to dryness, and the residues, redissolved in water, were assayed according to a chloramine T,dimethylaminobenzaldehyde method. n indicates number of individuals.

Results

Comparative Analysis of GAGs From Different Human Arteries

The four arteries under study were not significantly different in their total GAG and collagen contents as measured by hexuronic acid and hydroxyproline, respectively (Table 1). A purified extract of total arterial GAGs was obtained by papain digestion and cetylpyridinium chloride precipitation, and ion-exchange chromatography on DEAE-cellulose resolved this extract into three peaks, identified as hyaluronic acid, heparan sulfates, and dermatan+chondroitin 4/6-sulfate (Fig 1). These data, combined with those from the degradation of glycosaminoglycans (dermatan sulfate+chondroitin 4/6-sulfate) with specific GAG lyases (Table 2), permitted estimation of the concentration of several GAG species (Table 3). It can be seen that whereas there are no important differences in the contents of dermatan sulfate, the aortas have on average 31% more chondroitin 4/6-sulfate than other arteries when absolute values are considered. Furthermore, the pulmonary artery has approximately twice as much hyaluronic acid as the other arteries, and the iliac artery has a somewhat higher concentration of heparan sulfate. These two arteries also gave different elution profiles on DEAE-cellulose chromatography compared with the aortas (Fig 1A through 1D), indicative of possible structural dissimilarities in the GAG chains.

The proportions of disaccharide units making up the glycosaminoglycans obtained from DEAE-cellulose chromatography were determined by chondroitin AC and ABC lyase degradation, and the results are shown in Table 2. Although the figures do not vary much, if arteries are ordered according to decreasing 6-sulfated disaccharide (glucuronic acid and 6-sulfated N-acetyl-D-galactosamine) contents, the series abdominal aorta = thoracic aorta > iliac artery = pulmonary artery is obtained. If, on the other hand, ordering is based on the glucuronic acid–bearing, 4-sulfated disaccharide (glucuronic acid and 4-sulfated N-acetyl-D-galactosamine), approximately the same sequence is obtained but in reverse order. The aortas have therefore the highest glucuronic acid–6-sulfated N-acetyl-D-galactosamine content, whereas the pulmonary and iliac arteries have the smallest. With respect to the iduronic acid–bearing disaccharide, a similar pattern is less evident.

A comparable disaccharide analysis was performed with the heparan sulfate fraction recovered from the DEAE column (Table 4). Here, though, the overall proportions of disaccharides making up this GAG are quite similar.
Fig. 1. Fractionation by DEAE-cellulose chromatography (A through D) and identification of the resulting fractions by agarose gel electrophoresis (E) of the total glycosaminoglycans (GAGs) from the abdominal aorta (A), thoracic aorta (B and E), iliac artery (C), and pulmonary artery (D). Recovered materials were identified as hyaluronic acid (HA), heparan sulfate (HS), and dermatan sulfate + chondroitin 4/6-sulfate (DS/CS). Identifications were carried out as follows. HA is not detected on toluidine blue-stained agarose gels and yields exclusively an unsaturated, nonsulfated disaccharide (Δglucuronic acid-Δacetyl-β-glucosamine) after chondroitin AC lyase digestion (not shown). HS comigrates with the corresponding standard on agarose gel electrophoresis (E) and resists chondroitin AC or ABC lyase digestion (not shown) but is degraded by heparitinases, forming unsaturated disaccharides shown in Table 4. The third peak from the DEAE column contains both DS and C4/6-S as evidenced by a combination of agarose gel electrophoresis (E) with the following procedures: incubation of this fraction with chondroitin AC lyase only degrades the GAG, which shows electrophoretic migration similar to that of standard C4/6-S, forms the unsaturated disaccharides described in Table 2, and leaves a peak that comigrates with the standard of dermatan sulfate (E); finally, treatment with chondroitin ABC lyase degrades the GAGs with electrophoretic migration similar to that of C4/6-S and DS standards (E). All arteries gave similar analytic results. Profiles in E were obtained by densitometry of toluidine blue-stained agarose gels; broken lines represent the electrophoretic mobilities of standards of HS, DS, and C4-S.
Interaction Between GAGs From Various Arteries and Plasma LDL

This was first analyzed by in vitro formation of insoluble GAG-LDL complexes, whereby greater binding affinity was translated into greater turbidity of the reaction mixture. GAG fractions from DEAE-cellulose chromatography of several arteries were added to lipoprotein preparations purified from human plasma, and the results are shown in Fig 2. Of the three GAG fractions, only dermatan sulfate+chondroitin 4/6-sulfate interacted appreciably with LDL, although a clear distinction between the different arteries could not be made. Thus, affinity chromatography, a method enabling greater resolution in the analysis of GAG-LDL interaction, was subsequently employed.

A column for affinity chromatography was prepared by coupling human LDL to Sepharose 4B, and to this column the dermatan sulfate+chondroitin 4/6-sulfate fractions were applied (Fig 3). The proportions of GAGs contained in each fraction into which the eluates were divided are shown in Table 5. The elution profiles have irregular contours and span a relatively wide range of ionic strength, probably reflecting the heterogeneous composition of the dermatan sulfate+chondroitin 4/6-sulfate fraction. But most importantly this figure reveals marked differences among the elution profiles of the four arteries, with the abdominal aorta having the greatest binding affinity for LDL, since it contains the highest proportion (25%) of GAGs in the fraction that eluted at the highest ionic strength, together with lesser amounts of material in the fractions of lower ionic strength. This is followed in order of decreasing binding affinity by the thoracic aorta, the iliac artery, and the pulmonary artery. The latter two arteries have very similar elution profiles, and their GAG contents eluted in their entirety in fractions 1 and 2 (Fig 3, horizontal brackets), which indicates that as a whole, these GAGs have weaker affinities for LDL compared with those from the aorta. A very pronounced difference in binding affinity, for example, can be observed at 0.2 mol/L NaCl. At this ionic strength, most or all of the applied GAGs from the iliac and pulmonary arteries have already eluted, whereas elution of GAGs from the abdominal aorta is at its peak. The proportions of GAGs contained in the fractions from the eluates (Table 5) also show this difference and highlight the absence of fraction 3 from the iliac and pulmonary arteries.

The three fractions in the eluates from LDL-affinity chromatography (Fig 3, horizontal brackets) were analyzed for molecular weight and relative contents of dermatan sulfate and chondroitin 4/6-sulfate. The first analysis is shown in Fig 4, which depicts PAGE slabs (Fig 4A) and their densitometric profiles (Fig 4B through 4D). For each fraction, the molecular weight of its constituent GAGs do not vary much among arteries. However, a progressive increase in molecular weight toward fraction 3 is clearly noticeable, which means that the dermatan sulfate+chondroitin 4/6-sulfate fraction from all arteries is polydisperse and that in these dermatan sulfate+chondroitin 4/6-sulfate fractions, longer GAG chains have greater binding affinities for LDL.

Last, the relative contents of dermatan sulfate and chondroitin 4/6-sulfate in the fractions from LDL-affinity chromatography were determined. The results are shown in Table 6, from which the following observations can be made: (1) chondroitin 4/6-sulfate is the predominant GAG in all fractions from all arteries; (2) dermatan sulfate is constituted mostly of polysaccharide

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**Table 2. Proportions of Disaccharide Constituents of Arterial Glycosaminoglycans**

<table>
<thead>
<tr>
<th>Disaccharide Units</th>
<th>Abdominal Aorta</th>
<th>Thoracic Aorta</th>
<th>Iliac Artery</th>
<th>Pulmonary Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>GlcUA-GalNAc4S</td>
<td>13</td>
<td>17</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>GlcUA-GalNAc6S</td>
<td>50</td>
<td>50</td>
<td>45</td>
<td>43</td>
</tr>
<tr>
<td>IdUA-GalNAc4S</td>
<td>37</td>
<td>33</td>
<td>35</td>
<td>39</td>
</tr>
</tbody>
</table>

Relative proportions of disaccharide units were determined by degradation with chondroitin AC and ABC lyases of the dermatan sulfate+chondroitin 4(4S)/6(6S)-sulfate peak from DEAE chromatography. The resulting degradation products were separated by paper chromatography on isobutyric acid/NH₄OH, followed by silver staining and quantitation by densitometry. See "Methods" for experimental details. GlcUA indicates glucuronic acid; GalNac, N-acetyl-β-galactosamine; and IdUA, iduronic acid.

**Table 3. Relative and Absolute Contents of Various Glycosaminoglycans in Different Arteries**

<table>
<thead>
<tr>
<th>Artery</th>
<th>Hyaluronic Acid</th>
<th>Heparan Sulfate</th>
<th>Dermatan Sulfate</th>
<th>Chondroitin 4/6-Sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal aorta</td>
<td>6.1 (0.5)</td>
<td>31.8 (2.6)</td>
<td>23.2 (1.9)</td>
<td>38.9 (3.2)</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>4.8 (0.4)</td>
<td>30.5 (2.5)</td>
<td>21.3 (1.7)</td>
<td>43.2 (3.5)</td>
</tr>
<tr>
<td>Iliac artery</td>
<td>4.0 (0.3)</td>
<td>46.4 (3.6)</td>
<td>17.3 (1.3)</td>
<td>32.3 (2.5)</td>
</tr>
<tr>
<td>Pulmonary artery</td>
<td>9.6 (0.7)</td>
<td>29.7 (2.2)</td>
<td>23.9 (1.7)</td>
<td>38.6 (2.6)</td>
</tr>
</tbody>
</table>

Relative values are percent of total, whereas absolute values (figures in parentheses) are in micrograms of hexuronic acid per milligram dry tissue. Proportions of hyaluronic acid, heparan sulfate, and dermatan sulfate+chondroitin 4/6-sulfate were obtained by ion-exchange chromatography on DEAE-cellulose (Fig 1A through 1D). Proportions of dermatan sulfate and chondroitin 4/6-sulfate were obtained by analysis of unsaturated disaccharides formed by chondroitin AC and ABC lyases. Absolute values were calculated from the mean total hexuronic acid concentrations (Table 1).
TABLE 4. Proportions of Disaccharide Constituents of Arterial Heparan Sulfates

<table>
<thead>
<tr>
<th>Heparitinase Products</th>
<th>Abdominal Aorta</th>
<th>Thoracic Aorta</th>
<th>Iliac Artery</th>
<th>Pulmonary Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔUA-GlcNS, 6S</td>
<td>17</td>
<td>15</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>ΔUA-GlcNS</td>
<td>26</td>
<td>25</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>ΔUA-GlcNAc6S</td>
<td>17</td>
<td>16</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>ΔUA-GlcNAc</td>
<td>40</td>
<td>45</td>
<td>36</td>
<td>40</td>
</tr>
</tbody>
</table>

Same methods were used as in Table 2, but with the heparan sulfate peak and heparitinases from *Flavobacterium heparinum*. Δ indicates unsaturated; UA, uronic acid; GlcNS, N-sulfated glucosamine; 6S, 6-sulfated; and GlcNAc, N-acetyl-α-glucosamine.

Discussion

To assess the involvement of GAGs in the regional distribution of atherosclerotic lesions, the four arteries used in the present study were selected on the basis of their known susceptibilities to develop such lesions, so that vessels at high to low risk of lesion development would be represented. Additionally, to minimize the effects of aging, which alters arterial extracellular matrix and increases the severity and modifies the distribution of atherosclerotic lesions, only arteries from young adults were used. Thus, for the age group from which the material was obtained, the abdominal aorta is the artery where lesions occur most frequently, and it is followed not too distantly by the thoracic aorta.
TABLE 5. Relative Contents of Glycosaminoglycans in Fractions of Eluates From LDL-Affinity Chromatography

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Abdominal Aorta</th>
<th>Thoracic Aorta</th>
<th>Iliac Artery</th>
<th>Pulmonary Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (10-50)</td>
<td>38.8</td>
<td>41.4</td>
<td>44.2</td>
<td>42.1</td>
</tr>
<tr>
<td>2 (75-150)</td>
<td>36.1</td>
<td>44.7</td>
<td>52.7</td>
<td>57.9</td>
</tr>
<tr>
<td>3 (200-250)</td>
<td>25.4</td>
<td>13.9</td>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

The dermatan sulfate + chondroitin 4/6-sulfate fractions from ion-exchange chromatography were applied to low-density lipoprotein (LDL)-affinity columns and then subjected to stepwise elution with solutions of increasing sodium chloride concentration. Obtained elution fractions were pooled as fractions 1, 2, and 3 (see Fig 3), which represent ranges of sodium chloride concentration in millimoles per liter (figures in parentheses). Values for arteries are percentage of total eluted glycosaminoglycan.

The common iliac arteries are close to the thoracic aorta, whereas the internal and external iliac arteries are similar to the pulmonary artery, in that they are among the least susceptible. In terms of risk for atherosclerosis, the iliac artery samples in the present study should therefore be situated between the thoracic aorta and the pulmonary artery.

While the concentrations of total collagen and GAG were approximately the same in the material studied (Table 1), analysis of the contents of individual GAGs revealed some differences (Table 3). The higher concentration of hyaluronic acid in the pulmonary artery confirms previously published results. The aortas have similar contents of dermatan sulfate but higher contents of chondroitin 4/6-sulfate, and these GAGs have been reported to have significant interactions with LDL.

The values for dermatan sulfate are at variance with those of Murata and Yokoyama, who detected lesser amounts of this GAG in the pulmonary artery compared with the aorta. The iliac and pulmonary arteries may have sulfated GAGs with different charge densities from those of the aortas, as demonstrated by variations in the elution profiles from ion-exchange chromatography (Fig 1A through 1D). Still, with the exception of the analysis of unsaturated disaccharides in the dermatan sulfate + chondroitin 4/6-sulfate and heparan sulfate chains (Tables 2 and 4), which does not imply variations in degrees of sulfation, this has not been investigated further.

Steric factors play a role in the interaction between GAGs and LDL; as has been demonstrated, of the isomeric forms of chondroitin 4/6-sulfate, it is the 6-sulfated form that binds strongly to LDL, whereas the 4-sulfated isomer shows little or no interaction. In this respect, it is worth mentioning that both the incidence and severity of atherosclerosis and the aortic concentration of chondroitin 6-sulfate increase with age. Thus, these isomers may also influence the location of arterial lesions, since the ratio of chondroitin 6-sulfate to chondroitin 4-sulfate decreases in an order corresponding to decreasing risk for atherosclerosis (Table 2).

More elucidating results, however, were obtained in the GAG-LDL interaction experiments performed with the GAG fractions from ion-exchange chromatography. Formation of insoluble GAG-LDL complexes (Fig 2), a less discriminating technique, only confirmed the known fact that arterial heparan sulfate and hyaluronic acid do not bind LDL, without disclosing significant differences among the chondroitin 4/6-sulfate + dermatan sulfate fractions from the four arteries. LDL-affinity chromatography with these latter GAG fractions, on the other hand, revealed that the four arteries differed markedly (Fig 3 and Table 5), and their ordering
according to decreasing binding affinity matched the ordering according to decreasing susceptibility to atherosclerosis. Other important differences were detected in the subsequent analyses carried out on three fractions obtained from affinity chromatography (Fig 3, horizontal brackets). PAGE revealed a positive correlation between affinity for LDL and increasing molecular weight, which was observed in all of four arteries (Fig 4). These data confirm as well as extend those of a previous study that has demonstrated that chondroitin 4/6-sulfate and dermatan sulfate fractions of higher molecular weight from human thoracic aortas bind more efficiently to LDL. However, the present results show that for a given affinity-chromatography fraction, variations in molecular weight are minimal, so the effects of this parameter as a determinant of atherosclerosis risk should be more limited. For example, the abdominal aorta is at higher risk than the thoracic aorta, and this is reflected in the elution profiles from LDL-affinity chromatography. Yet analysis of molecular weight in the eluates did not show appreciable differences between these two arteries (Fig 4). Other aspects of GAG composition that affect interaction with LDL may thus be involved, and the degree of sulfation is an important one. Alternatively or in addition, the greater propensity to atherosclerosis of the abdominal aorta in relation to the thoracic aorta may be due to the fact that, so far as GAGs are concerned, the former has nearly twice as much eluted material in fraction 3 (Table 5). This fraction has been shown herein to be composed of GAG chains of higher molecular weight (Fig 4) and to be enriched in dermatan sulfate (Table 6) compared with the other two fractions. Earlier work has shown that higher-molecular-weight GAGs as well as dermatan sulfate bind more avidly to LDL than lower-molecular-weight GAGs and either chondroitin 6- or 4-sulfate, respectively. These two aspects of the interaction between GAGs and LDL thus explain the greater efficiency of GAG-LDL binding in fraction 3. Since the two aortas have very similar contents of chondroitin 4/6-sulfate and dermatan sulfate (Table 3), the abdominal aorta has nearly two times more GAGs of higher affinity to LDL than the thoracic aorta. In contrast, the pulmonary and iliac arteries either totally lack or have but a small amount of fraction 3.

Therefore, the abdominal aorta and to a lesser extent the thoracic aorta possess a particular GAG composition that potentially confers greater LDL binding capacity to these arteries compared with the iliac and pulmonary arteries.

Reasons for these localized differences in biochemical composition and metabolism along the arterial bed are still unclear. In a study with an atherosclerosis-susceptible pigeon breed, Park et al have demonstrated that normal arteries from young animals already have increased amounts of extracellular matrix proteins. That study also showed that the biosynthetic activity for extracellular matrix components was normal in other tissues, indicating that in the atherosclerosis-susceptible breed, cells with altered phenotypes are specifically located in arterial tissue. A similar conclusion was reached in investigations on GAGs from this same breed, where alterations of synthesis/molecular structure were detected. In different experiments, an in vitro-elicited change of rabbit aortic smooth muscle cells from a contractile to a less-contractile phenotype was accompanied by an increase in the synthesis of sulfated GAGs, and subsets of arterial smooth muscle cells expressing different cytoskeletal phenotypes were indeed demonstrated in atherosclerotic plaques and adjacent areas. Of the complex array of mediators taking part in atherogenesis, growth factors have a salient role, being present in lesions since their earliest phases. Moreover, growth factors are capable not only of inducing phenotypic changes in arterial smooth muscle cells but also of increasing the synthesis of chondroitin sulfate proteoglycan by these cells. This proteoglycan also has longer GAG chains and a higher content of the 6-sulfated isomer. In all of these cases, modifications in cells and/or extracellular matrixes might favor the development of atherosclerosis, and it is noteworthy that growth factor-induced alterations in chondroitin sulfate proteoglycan correlate with our results for arteries at higher risk for atherosclerosis. It may be speculated, therefore, that lesion-prone areas in arteries are populated with cells of analogous, potentially atherogenic phenotypes, whose synthetic activities are or may become distinct from those of cells in less susceptible areas.

In conclusion, the results of the present work demonstrated that GAGs from different human arteries vary both in composition and in binding affinity to plasma LDL. The consistency of these findings with known data on the differential incidence of atherosclerotic lesions among arteries suggests that this heterogeneity of GAG composition participates in the multifac-
torial mechanisms that determine the susceptibility of a given artery to atherosclerosis.

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