Sialic Acid Content of LDL in Coronary Artery Disease: No Evidence of Desialylation in Subjects With Coronary Stenosis and Increased Levels in Subjects With Extensive Atherosclerosis and Acute Myocardial Infarction

Relation Between Desialylation and In Vitro Peroxidation

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Abstract—We recently showed that sialic acid content of LDL was not a marker of early cardiovascular disease (Arterioscler Thromb Vasc Biol. 1995;15:334–339). Here, we investigated this parameter in patients with advanced coronary artery disease (CAD). We first examined 100 patients having undergone coronary angiography. The distribution of LDL sialic acid values was very similar in subjects with no coronary stenosis (31.3 ± 3.7 nmol/mg LDL protein, mean ± SD) and those with ≥75% stenosis in at least one main coronary artery or ≥50% stenosis in at least two main coronary arteries (32.1 ± 5.5 nmol/mg LDL protein). In contrast, LDL sialic acid content was significantly increased in patients with both coronary stenosis and peripheral arterial atherosclerotic lesions compared with those with either no lesion or only one or the other type of lesion. We then examined LDL sialic acid content in 20 patients with acute myocardial infarction. LDL sialic acid content was significantly higher (35.9 ± 3.2 nmol/mg LDL protein) than that in the CAD(−) control group. These data suggest that LDL sialic acid content increases with the extension of atherosclerosis and its progression to acute complications. To explain the discordance with Orekhov and coworkers (Atherosclerosis. 1991;86:153–161), who showed that LDL sialic acid content in patients with advanced CAD was lower than that in healthy subjects, we studied the time courses of sialic acid, TBARS, and vitamin E levels in LDL dialyzed in different experimental conditions. A continuous decrease in both sialic acid and vitamin E levels and an increase in TBARS levels were observed in LDL samples containing less than 1 mmol/L EDTA, the intensity and rapidity of which varied with the EDTA concentration in the buffer. Our data support the idea that desialylation may result from in vitro peroxidation of LDL. (Arterioscler Thromb Vasc Biol. 1998;18:876-883.)

Key Words: low-density lipoprotein ■ sialic acid ■ coronary artery disease ■ angiography ■ peroxidation

There is a strong relation between LDL cholesterol levels and the incidence of CAD. However, LDL comprises a continuum of particles differing in their chemico-physical and biological properties, and some subfractions have been more strongly incriminated than others in the development of atherosclerosis. Sialic acid is a component of both the protein and lipid moieties of LDL. In vitro, the sialic acid content of LDL can modulate its receptor-mediated cell uptake, its avidity for arterial proteoglycans, and its susceptibility to oxidation. Several investigators have reported large interindividual differences in LDL carbohydrate content. Thereby, the important question is whether these differences in LDL sialic acid content have implications for the development of atherosclerosis. Tertov et al and Orekhov et al reported that LDL isolated from plasma of patients with angiographically assessed advanced CAD had a total sialic acid content 40% to 75% lower than that from healthy subjects. This finding was explained by a greater proportion of desialylated LDL in patients with CAD. Based on these data, the authors propose LDL sialic acid content as a marker of the cardiovascular risk. To test whether LDL sialic acid content could be used early for effective prevention of atherosclerosis, we recently examined hypercholesterolemic subjects with and without subclinical atherosclerosis. Surprisingly, we found no relation between LDL sialic acid content and the prevalence of peripheral plaques and/or coronary calcifications detected respectively by high-resolution ultrasonography and ultrafast CT. As our population consisted of asymptomatic subjects, we postulated that desialylation of LDL might occur much later in the atherosclerotic process. Only two other groups studied LDL sialic acid content in patients with and without documented CAD.

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Ruelland et al\(^\text{25}\) found a far less marked decrease (23\%) than that reported by Orekhov and coworkers. Melajärvi et al\(^\text{26}\) found no difference between patients with and without CAD, either in diabetic or nondiabetic individuals. In view of these contradictory findings, we decided to investigate again LDL sialic acid content in subjects with advanced CAD. Here, we examined angiographed patients with and without coronary stenosis on the one hand and patients with acute MI on the other hand.

### Methods

#### Angiographed Subjects

The study was conducted in accordance with the Helsinki Declaration of 1975. Coronary angiography was always carried out for diagnostic purposes. Subjects had either a cardiac history or symptoms sufficient to warrant angiography. The different indications were cardiomyopathy, mitral or aortic valve disease, cardiac arrhythmias, history of MI, clinical evidence of angina pectoris, and suspect chest pain. Cardiac catheterization was also undertaken as a presurgical evaluation of heart disease in patients with peripheral arterial atherosclerosis. After excluding patients with acute or recent MI, subjects were selected for this study according to angiography data as follows: Control patients, designated CAD\(^1\), are defined as those with strictly normal angiograms; subjects with significant stenosis on the one hand and patients with acute MI on the other hand.

**Blood Sampling**

In angiographed patients, blood was drawn after an overnight fast at the time of catheterization, prior to the injection of heparin or fluids. In patients with acute MI, blood was collected once during the acute phase (within 24 hours after the onset of symptoms) and once again on the sixth day after the ischemic injury.

Blood for lipid analyses was drawn into a tube with no anticoagulant. Blood aimed to LDL preparation was drawn into tubes (Vacutainer) containing disodium EDTA. Plasma and serum were separated by low-speed centrifugation (20 minutes at +15°C).

#### Analytical Methods

Serum was routinely analyzed for total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and Lp(a) as previously described. Total LDL (d=1.019 to 1.063 g/mL) was isolated by sequential ultracentrifugation in a Beckman L90 ultracentrifuge (Beckman Instruments Inc) according to a procedure previously described in detail.\(^\text{27}\) Collected LDL was dialyzed for 24 hours at +4°C against four changes of 100 vol 10 mmol/L Tris-HCl buffer, pH 7.4, containing 1 mmol/L EDTA, and was stored at +4°C in the dark. In vitro studies, LDL was isolated by preparative ultracentrifugation from normal lipopidemic plasma pools according to a procedure previously described in detail\(^\text{28}\) and then dialyzed as indicated above. The absence of contaminating lipoproteins (VLDL and HDL) in LDL preparations was checked by agarose gel electrophoresis.

Total LDL sialic acid content was determined as previously described. Briefly, bound sialic acid was released from sialoglycoconjugates by mild hydrolysis (15 minutes at 80°C in 0.05 mol/L H\(_2\)SO\(_4\)). Warren’s periodate-TBA assay was used. A standard curve was constructed using N-acetylneuraminic acid (No. A2388, Sigma Chemical Company) treated in the same conditions. Values are means of duplicate assays, and the within-assay coefficient of variation was below 5%. Aldehydes, which also react with TBA to form a chromophore with an absorption maximum at 532 nm, interfere with the conventional absorbance measurement of sialic acid at 549 nm (spectra overlap). To avoid this, second-derivative spectrophotometry, which separates peaks, was also used after colorimetric reaction with TBA. The second-derivative spectrum of LDL samples from study subjects was very close to that of the sialic acid standard (single peak at 549 nm). When aldehydes were present (in vitro studies), calibration was carried out by constructing a standard curve for the second derivative at 549 nm. LDL sialic acid content was also determined by means of a chromatographic method with fluorometric detection, and the results obtained by the two methods correlated well (r=0.85). TBARS were measured by using a kit from Sobioba in a modified version of Yagi’s assay with fluorometric detection. Vitamin E was determined by using high-performance liquid chromatography as previously described with \(\alpha\)-tocopherol acetate (Sigma) as internal standard and \(\delta\)-tocopherol (Sigma) as reference standard. Total LDL protein was measured by using Peterson’s method\(^\text{29}\) with bovine serum albumin as standard.

#### Statistical Analysis

The statistical analysis was carried out on a computer (Apple Macintosh) with Statview II (Abacus Concepts, Inc) software. Comparisons between groups were performed with one-way ANOVA and, when appropriate, with Student’s unpaired two-tailed t test. Comparisons of categorical data (proportions of subjects) were made by using analysis of frequencies. Student’s paired two-tailed t test was used to compare day 0 and day 5 samples in patients with acute MI and samples collected before and after injection of heparin in angiographed subjects. Correlations were analyzed by calculating Pearson coefficients (normal variables) and Spearman’s rank order coefficients (nonnormal variables). Logarithmic transformations were reviewed independently by two cardiologists. The number, location, and severity of lesions on each arterial segment were recorded. All patients received intravenous heparin (50 IU/kg body weight) after puncturing the artery and inserting the catheter.

### Patients With Acute MI

Patients were admitted to the intensive care unit in the hospital within 24 hours after the onset of symptoms. The diagnosis of first MI included enzyme assays and electrocardiographic examination. Of the 20 patients selected for the study, 3 were treated with heparin only and 2 by prehospital thrombolysis; the other 15 underwent only and 2 by prehospital thrombolysis; the other 15 underwent direct angioplasty. Information on smoking, medication, medical history were obtained during interviews with a physician. Blood pressure, serum lipid parameters, and BMI (weight/height\(^2\)) were determined. Hypertension was defined by a systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg. Cardiac catheterization was performed through the femoral approach and images were stored on 35-mm cinefilm. Coronary angiograms were reviewed independently by two cardiologists. The number, location, and severity of lesions on each arterial segment were recorded. All patients received intravenous heparin (50 IU/kg body weight) after puncturing the artery and inserting the catheter.

### Coronary Investigations

Coronary angiography was performed through the femoral approach with Judkins 6F catheters. The left main, left anterior descending, left circumflex, and right coronary arteries were carefully examined, and images were stored on 35-mm cinefilm. Coronary angiograms were reviewed independently by two cardiologists. The number, location, and severity of lesions on each arterial segment were recorded. All patients received intravenous heparin (50 IU/kg body weight) after puncturing the artery and inserting the catheter.
were used when appropriate. Probability values of <0.05 were considered significant.

**Results**

The first study population included 100 angiographed patients who were classified into two groups according to the degree of coronary stenosis: 58 had significant coronary stenosis and formed the CAD(+) group, and the other 42 had strictly normal angiograms and constituted the CAD(−) control group. This population did not include patients with acute or recent MI. The characteristics of the groups are detailed in Table 1. They were homogeneous regarding age and sex and comprised statistically equivalent proportions of hypertensive subjects, smokers, and patients on the different categories of medication. No difference in the lipid parameters was observed between the groups. Only the BMI in the CAD(+) group was significantly higher than that in the CAD(−) group. The distribution of patients in each group was studied according to their medical history: the control CAD(−) group included a majority of subjects with nonischemic heart disease, such as cardiomyopathy and mitral or aortic valve disease; 28% of subjects in this group were referred to our cardiology center with suspected angina pectoris, which turned out to be unrelated to coronary stenosis. In contrast, the CAD(+) group included a large proportion of subjects with known or documented angina pectoris and only 5% of subjects with cardiomyopathy or valve failure. Finally, both groups contained about 30% of subjects with peripheral arterial atherosclerotic lesions.

Figure 1 compares LDL sialic acid content in the two groups. The frequency distribution of LDL sialic acid content ranged from 23.8 to 42.2 nmol/mg LDL protein in the CAD(−) group and from 19.4 to 44.8 nmol/mg LDL protein in the CAD(+) group, with mean values of 31.3±3.7 and 32.1±5.5 nmol/mg LDL protein, respectively. No significant difference was found between the groups (P=0.43).

We also compared LDL sialic acid content in four groups of subjects defined as follows by the combined results of clinical investigations: coronary stenosis (absence or presence) with or without extracoronary atherosclerotic lesions (Table 2). LDL sialic acid content in patients with both coronary stenosis and peripheral arterial atherosclerotic lesions was significantly higher than that in the other subgroups (P<0.01).

We then examined LDL sialic acid content in 20 patients with acute MI. Lipid determination and LDL isolation were performed when appropriate. Probability values of <0.05 were considered significant.

**TABLE 1. Characteristics of the Study Groups of Angiographed Subjects**

<table>
<thead>
<tr>
<th>Study Variables</th>
<th>CAD(−)</th>
<th>CAD(+)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>42</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Gender, % male</td>
<td>81</td>
<td>88</td>
<td>0.332</td>
</tr>
<tr>
<td>Age, y</td>
<td>60±10 (35–87)</td>
<td>62±9 (39–81)</td>
<td>0.432</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.6±2.7 (20.2–32.3)</td>
<td>26.2±3.5 (19.7–35.8)</td>
<td>0.021</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>148±21 (120–220)</td>
<td>145±22 (110–200)</td>
<td>0.476</td>
</tr>
<tr>
<td>Diastolic</td>
<td>81±11 (60–110)</td>
<td>82±10 (60–100)</td>
<td>0.962</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>32</td>
<td>31</td>
<td>0.992</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>55</td>
<td>38</td>
<td>0.091</td>
</tr>
<tr>
<td>Serum lipids, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.82±1.06 (3.70–7.90)</td>
<td>5.79±1.06 (3.20–8.55)</td>
<td>0.886</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.78±1.06 (0.76–5.31)</td>
<td>1.45±0.90 (0.49–6.14)</td>
<td>0.098</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.18±0.34 (0.64–2.14)</td>
<td>1.19±0.34 (0.64–2.30)</td>
<td>0.889</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.88±1.00 (2.16–5.76)</td>
<td>3.93±0.86 (1.76–6.52)</td>
<td>0.776</td>
</tr>
<tr>
<td>Lp(a), g/L</td>
<td>0.29±0.24 (0.01–1.14)</td>
<td>0.39±0.45 (0.001–1.72)</td>
<td>0.223</td>
</tr>
<tr>
<td>Medical history, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angina pectoris (known or suspected)</td>
<td>28</td>
<td>54</td>
<td>0.010</td>
</tr>
<tr>
<td>Cardiomyopathy, heart valve failure</td>
<td>41</td>
<td>5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other arterial lesions of atherosclerosis</td>
<td>31</td>
<td>34</td>
<td>0.756</td>
</tr>
<tr>
<td>Old MI</td>
<td>0</td>
<td>15</td>
<td>0.010</td>
</tr>
<tr>
<td>Therapeutics, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-blockers</td>
<td>24</td>
<td>36</td>
<td>0.200</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>33</td>
<td>42</td>
<td>0.362</td>
</tr>
<tr>
<td>Nitrates</td>
<td>29</td>
<td>40</td>
<td>0.254</td>
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<tr>
<td>Angiotensin-converting enzyme inhibitors</td>
<td>21</td>
<td>17</td>
<td>0.617</td>
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<tr>
<td>Diuretics</td>
<td>19</td>
<td>14</td>
<td>0.503</td>
</tr>
<tr>
<td>None</td>
<td>28</td>
<td>16</td>
<td>0.147</td>
</tr>
</tbody>
</table>

Values are mean±SD (range) or percentage of total subjects in each group. P indicates significance levels between groups (Student’s unpaired two-tailed t test).
performed once within the first 24 hours (day 0 sample) and
again 6 days after the ischemic injury (day 5 sample). The
characteristics of the MI group are detailed in Table 3. A
significant decrease in total and LDL cholesterol and a
significant increase in triglycerides were observed in sera
collected on day 5, relative to those collected on day 0. Figure
2 shows LDL sialic acid content in MI patients. LDL sialic
acid content ranged from 31.6 to 43.6 nmol/mg LDL protein
in samples collected on day 0 and from 31.4 to 46.9 nmol/mg
LDL protein in samples collected on day 5, with mean values
of 35.9±3.2 and 37.2±3.4, respectively. No significant
difference was found between day 0 and day 5 samples
\((P<0.08)\). In contrast, LDL sialic acid content in MI patients
was significantly higher than that in both the CAD(−) control
group and the CAD(+) group of angiographed subjects
\((P<0.01)\).

In the whole angiographed study population, as in MI
patients, the relation between LDL sialic acid content and
clinical and biological parameters was examined. LDL sialic
acid content was not related to sex, age, BMI, blood pressure,
smoking status, therapeutic status, or the different lipid
parameters. We paid particular attention to Lp(a) because it is
highly sialylated and has a density range that overlaps that of
LDL. In our previous study,24 we observed no correlation
between LDL sialic acid content and serum Lp(a) when the
latter was below 2 g/L. The present study confirms the
absence of interference of Lp(a) with the determination of
LDL sialic acid content in these conditions, as shown by the
linear regression analysis after logarithmic transformation of
serum Lp(a) (Figure 3) and the Spearman’s rank test
\((r=0.07, P=0.48, n=115)\).

Because heparin is systematically administered during
angiography procedure, we examined its effect on LDL sialic
acid content. Ten patients undergoing angiography were
sampled once at the time of catheterization, prior to the
intravenous injection of a heparin bolus (50 IU/kg body
weight), and again 60 minutes after administration of heparin,
at the end of the angiography procedure. Mean sialic acid
values in LDL isolated before and after injection of heparin
were 32.9±2.6 and 32.2±2.2 nmol/mg LDL protein, respec-
tively. Paired comparison of LDL sialic acid values showed
no significant difference \((P=0.37)\).

Given the discordance with data obtained by Tertov et
al14,21 and Orekhov et al,16,17,22 we examined LDL sialic acid
content during LDL dialysis and storage in different experi-

### Table 2. Comparison of LDL Sialic Acid Content of Subjects
With and Without Extracoronary Atherosclerotic Lesions in
Groups With and Without Coronary Stenosis

<table>
<thead>
<tr>
<th>Coronary Stenosis</th>
<th>Extracoronary Atherosclerotic Lesions</th>
<th>LDL Sialic Acid Content, nmol/mg LDL protein</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Absent</td>
<td>31.4±3.8</td>
<td>29</td>
</tr>
<tr>
<td>Absent</td>
<td>Present</td>
<td>31.2±3.4</td>
<td>13</td>
</tr>
<tr>
<td>Present</td>
<td>Absent</td>
<td>30.3±4.8</td>
<td>38</td>
</tr>
<tr>
<td>Present</td>
<td>Present</td>
<td>35.5±5.1*</td>
<td>20</td>
</tr>
</tbody>
</table>

Values of LDL sialic acid content are mean±SD.

*\(P=0.01\) versus the other subgroups.
mental conditions chosen according to those described by these authors for the isolation of desialylated LDL. LDL prepared from plasma pools was dialyzed against either PBS or Tris-HCl buffer (10 mmol/L) containing, in each case, various EDTA concentrations (0, 10, and 100 mmol/L and 1 mmol/L). LDL dialyzed against Tris buffer containing 1 mmol/L EDTA was representative of our own experimental conditions. At various times up to 7 days, each LDL solution was assayed for sialic acid, TBARS, and vitamin E contents. Figure 4 shows the concomitant time courses of sialic acid, vitamin E, and TBARS levels in LDL in the different experimental conditions. No change with time was observed in these three parameters, in either PBS or Tris buffer containing 1 mmol/L EDTA. As regards LDL samples dialyzed against PBS, a large and continuous decrease in both sialic acid and vitamin E levels was observed in LDL samples containing 0 and 10 mmol/L EDTA. In these dialysis conditions, sialic acid and vitamin E contents of LDL were respectively reduced by 20% to 32% and 33% to 43% within the first 24 hours and by 62% to 71% and 90% to 95% after 7 days of dialysis. TBARS levels increased after the third day, corresponding to a loss of 70% vitamin E in LDL, and were fourfold to fivefold higher on the 7th day compared with LDL samples containing 1 mmol/L EDTA. Our data show that the intensity of LDL peroxidation is inversely proportional to the EDTA concentration in buffers and suggest greater protective power of Tris buffer than PBS against oxidation.

### Discussion

Our data clearly show that patients in whom significant coronary stenosis has been assessed by angiography have an LDL sialic acid content within the range of values in subjects with normal coronary arteries. They are also in accordance with findings more recently reported by Melajärvi et al., who found no difference in LDL sialic acid content between patients with and without CAD, either in diabetic or nondiabetic individuals. In contrast, our data showed that LDL sialic acid content was increased in patients with both coronary stenosis and peripheral atherosclerotic lesions, relative to those with either no lesion or only one or the other type of lesion, and in patients with acute MI.

### Table 3. Characteristics of Subjects With Acute Myocardial Infarction

<table>
<thead>
<tr>
<th>Study Variables</th>
<th>Study Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject, n</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Gender, % male</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>65±13 (36–85)</td>
<td></td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.6±3.8 (21.3–33.9)</td>
<td></td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Smokers, %</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Serum lipids, mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.10±1.09 (2.91–8.80)</td>
<td>4.94±1.12 (2.28–7.32)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.06±0.55 (0.35–2.09)</td>
<td>1.65±0.80 (0.80–3.37)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.96±0.29 (0.64–1.52)</td>
<td>1.03±0.28 (0.58–1.57)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.38±0.91 (1.77–6.01)</td>
<td>3.15±0.90 (1.23–5.65)</td>
</tr>
<tr>
<td>Lp(a), g/L</td>
<td>0.28±0.33 (0.02–1.24)</td>
<td>0.31±0.35 (0.02–1.45)</td>
</tr>
<tr>
<td>Therapeutics, %</td>
<td></td>
<td></td>
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<tr>
<td>β-blockers</td>
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<td>Calcium channel blockers</td>
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<td>Angiotensin-converting enzyme inhibitors</td>
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<td>Nitrates</td>
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<td></td>
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<td>Diuretics</td>
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<td></td>
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<tr>
<td>Antiplatelet agents</td>
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<td>Hypolipidemic agents</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>30</td>
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</table>

Values are mean±SD (range) or percentage of total subjects. P indicates significance levels derived from comparison of day 5 samples versus day 0 samples (Student’s paired two-tailed t test).
relative to both the CAD(−) control group and the CAD(+) group of angiographed subjects. This finding suggests that LDL sialic acid content increases with the extension of atherosclerosis and its progression to acute complications. The reasons for this increase remain to be discovered. It appears unrelated to serum lipid levels, as LDL sialic acid content did not vary between days 0 and 5 in patients with MI, in spite of the lipid changes that occurred during this period. It is also unrelated to the presence of aldehydes interfering in sialic acid determination. Indeed, we measured TBARS in LDL of MI patients because malondialdehyde-conjugated LDL had been described in these patients.35 TBARS levels were always below 1 nmol/mg LDL protein, ie, within the range of values in control subjects (data not shown). Interestingly, Meljärv et al26 also found an increase in LDL sialic acid content in diabetics relative to nondiabetics, irrespective of CAD. Taken together, these observations and our data suggest a possible relation between LDL sialic acid content and the inflammatory process, as already described for sialic acid in serum.36

The present study confirms the heterogeneous distribution of LDL sialic acid values among individuals, as observed by other groups13,14 and ourselves.24 Our data showed no relation between LDL sialic acid content and the different clinical and biological parameters in the study populations, in keeping with the recent report by Meljärv et al.26 According to Senn et al12,37 gangliosides (sialic acid–containing glycosphingolipids) are excreted by the liver into the circulation along with apolipoprotein B–containing lipoproteins, and these sialylated lipids are then distributed among the different lipoprotein classes, especially LDL. La Belle and Krauss13 have also reported that the heterogeneity in LDL sialic acid content is directly related to the total lipid content of LDL. Only a minor portion of LDL-associated gangliosides are truly anchored in the LDL outer monolayer, whereas the bulk is absorbed to the lipoprotein surface.38 Gangliosides are also released by cells into the circulation, and the equilibrium between bound and unbound gangliosides can be shifted in some pathological conditions.36,38 Accordingly, it may be that differences between individuals related to the synthesis and intravascular processing of LDL precursors on the one hand and to pathological circumstances on the other hand contribute to the formation of various LDL subspecies that differ in their sialic acid content.

Our data differ largely from those obtained by Tertov et al14,21 and Orekhov et al.16,17,22 This disagreement is not related to ethnic origin, because in one of their reports,17 these authors compared LDL isolated from subjects in Moscow and subjects in Houston and concluded that the properties of the LDL were strictly similar. The fact that we chose the same degree of coronary stenosis as the inclusion criterion for the CAD(+) group and a comparable number of subjects, together with the fact that we used the same method (Warren’s TBA assay) to determine LDL sialic acid content, rules out a methodological explanation for this discordance.

Only the disease-free control subjects differed between the two studies: in ours, all subjects were angiographed, and the control group consisted of subjects with no coronary artery
stenosis and not of apparently healthy subjects with no evidence of CAD based on clinical examination and medical history. Accordingly, one possible explanation was the fact that the discordance was inherent in the angiographic conditions. A first concern was the potential effect of heparin on LDL sialic acid content, because some studies have suggested that the release of lipases after an intravenous bolus injection of heparin influences the metabolic pathways of triglyceride-rich lipoproteins. Our data clearly showed that LDL sialic acid content was not modified after heparin injection. Another concern was the clinical circumstances of the coronary angiography, because there is abundant evidence that lipid levels change substantially in patients hospitalized for acute MI. As mentioned earlier, we found not a decrease but rather an increase in LDL sialic acid content in these patients.

The lack of an apparent explanation for the discordance led us to consider possible artifactual desialylation of LDL related to the experimental conditions used for isolation, handling, and storage of LDL. This is why we focused on a possible link between desialylation and in vitro peroxidation of LDL and examined the concomitant time courses of sialic acid, TBARS, and vitamin E levels in LDL dialyzed against PBS or Tris buffer with various EDTA concentrations. Our data clearly showed that only LDL in buffers containing 1 mmol/L EDTA exhibited unchanged contents of sialic acid, vitamin E, and TBARS, even after 7 days’ dialysis. In contrast, exposure of LDL to dialysis buffers containing lower EDTA concentrations for various times resulted in partial oxidation of LDL, as assessed by the decrease in vitamin E and the increase in TBARS levels, the intensity and rapidity of which varied with the EDTA concentration in the buffer. This autoxidation of LDL is slower than that previously reported by Esterbauer et al. This finding may be explained by the fact that the buffers were not continuously gassed with oxygen in our experimental conditions. As regards TBARS levels, only slight increases were observed. This observation may be explained by the fact that LDL still contained vitamin E and that free MDA (the main component of TBARS) can be released from the LDL particle during dialysis. The most important information given by these experiments is that the oxidative changes coincided with a marked decrease in LDL sialic acid content. The loss of sialic acid might be related to a subsequent alteration of lipoprotein integrity.

Some of the experimental conditions described above were used by Tertov et al and Orekhov et al for handling and storage of LDL. By contrast with these authors, we always used 1 mmol/L EDTA in our buffers. This approach supports the idea that their observations may result from an artifactual modification of LDL related to in vitro peroxidation, an hypothesis that could explain some of the characteristics of the desialylated LDL described by this group, evocative of peroxidatively modified LDL, such as higher levels of lysophosphatidylcholine and oxysterols, lower contents of vitamin E and free lysine amino groups, and greater electrophoretic mobility than sialylated LDL. This latter property is at the basis of the recent report by Tertov et al showing a strong similarity between desialylated LDL and the oxidized LDL fraction, named LDL(−), isolated from human plasma by Cazzolato et al by using ion-exchange chromatography. LDL peroxidation could explain the paradoxical increase in negative charge of desialylated LDL. Indeed, sialic acid is a negatively charged component and supports the idea that their observations may result from artifactual modification of LDL related to in vitro peroxidation.

In conclusion, the present work confirms the heterogeneous distribution of LDL sialic acid content among individuals and shows that this parameter increases with the extension of atherosclerosis and the occurrence of acute coronary complications.

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Sialic Acid Content of LDL in Coronary Artery Disease: No Evidence of Desialylation in Subjects With Coronary Stenosis and Increased Levels in Subjects With Extensive Atherosclerosis and Acute Myocardial Infarction: Relation Between Desialylation and In Vitro Peroxidation

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