Relationship between High-density Lipoprotein-cholesterol and Malondialdehyde-modified Low-density Lipoprotein Concentrations

Akira Kondo¹, Jiangzhen Li¹, Mitsuhisa Manabe², Kazunori Saito², Takashi Kanno¹, and Masato Maekawa¹

¹Department of Laboratory Medicine, Hamamatsu University School of Medicine, Shizuoka, Japan. ²Diagnostics Research Laboratories, Daiichi Pure Chemicals Co., Ibaraki, Japan

Several reports have suggested that HDL has anti-oxidative actions. We investigated the relationship between HDL-cholesterol (HDL-C) and malondialdehyde-modified LDL (MDA-LDL) concentrations using enzyme linked immunosolvent assay. We divided our study subjects into four groups on the basis of concentrations of triglyceride (TG) and HDL-C by the following lipid profiles: serum TG ≤ 1.69 mmol/L and HDL-C ≥ 1.16 mmol/L (control group, n = 26); TG >1.69 and HDL-C ≥ 1.16 (high TG group, n = 22); TG >1.69 and HDL-C ≤ 0.91 (high TG & low HDL group, n = 67); TG ≤ 1.69 and HDL-C ≤ 0.91 (low HDL group, n = 21). MDA-LDL concentrations, MDA-LDL/apolipoprotein B (apo B) ratio, and LDL size were different between subjects in high TG & low HDL and control groups. MDA-LDL concentrations in both high TG and low HDL groups did not differ significantly from those in the control. However, MDA-LDL/apo B ratio in low HDL group was significantly higher than that in the control (P < 0.05). The MDA-LDL/apo B ratio reflects the extent of MDA modification of apo B in LDL. Therefore, our data suggest that as HDL-C concentrations fall, the extent of MDA modification per one LDL particle increases. Moreover, accompanied by high TG concentration, LDL size in subjects with lower HDL-C concentrations became smaller.  J Atheroscler Thromb, 2003; 10: 72–78.

Key words: Small dense low-density lipoprotein, Triglyceride, Apolipoprotein B, Oxidation

Introduction

High-density lipoprotein (HDL) protects against atherosclerosis by returning excess cholesterol from peripheral tissues back to the liver for reuse or excretion into the bile. There is an inverse relation between the HDL-cholesterol (HDL-C) concentration and the incidence of coronary heart disease (CHD) (1, 2).

Recently, it has been postulated that the various oxidative modifications of low-density lipoprotein (LDL) contribute to atherogenic processes by multiple mechanisms (3–5). It is noted that HDL plays a role in this oxidative theory of atherosclerosis. Several reports suggest an anti-oxidative function for HDL, which may contribute to its anti-atherogenic activity. In vitro experiments show that HDL is an effective scavenger of superoxide (6) and that it not only protects LDL from oxidative modification (7) but that it prevents lipid peroxidation by metal ions and by cells in tissue culture (8).

There are reports (9–12) that HDL may protect against the oxidation of LDL. Also, the role of HDL was investigated from the aspect of distribution of lipid-soluble antioxidants in lipoproteins (13, 14). If these mechanisms are impaired, peroxidation of lipid in LDL increases with degradation of the lipid, resulting in modification of LDL through degradation products such as malondialdehyde (MDA) and 4-hydroxynonenal.

Li et al. (15) reported that subjects with high triglyceride (TG) (> 1.69 mmol/l) and low HDL-C (≤ 0.91 mmol/l) concentrations frequently have higher concentrations of small dense LDL. Kotani et al. (16) previously showed that malondialdehyde (MDA)-modified LDL (MDA-LDL) is distributed to small dense LDL fractions by density gradient

Address for correspondence: Akira Kondo, Diagnostics Research Laboratories, Daiichi Pure Chemicals Co., 3–1 Kôyodai 3-Chome, Ryugasaki City, Ibaraki 301–0852, Japan. E-mail: kondoh@daichichem.co.jp
Received  April 26, 2002. Accepted for publication  November 8, 2002.
Relationship between HDL-C and MDA-LDL

ultracentrifugation. Therefore, it seems that a concentration of MDA-LDL is associated with small dense LDL concentrations, affected by HDL levels as well as by TG levels. A previous report showed the effect of TG concentration on MDA-LDL concentrations (17).

In this study, we investigated the relationship between concentrations of MDA-LDL and HDL-C. Especially, a possible role of HDL in protection from oxidative stress was evaluated by measuring the ratio of serum MDA-LDL to apolipoprotein B (apo B) in four groups of individuals distinguished by concentrations of TG and HDL-C. This ratio represents the extent of modification of apo B by MDA.

Materials and Methods

Subjects
On the basis of concentrations of TG and HDL-C, 155 subjects were arbitrarily selected from among individuals whose fasting serum lipids were examined at the Hamamatsu University School of Medicine Hospital. The subjects included 110 patients (48 women and 62 men; age range, 21–77 years) with elevated TG (>1.69 mmol/l (1,500 mg/l)) or low HDL-C (<0.91 mmol/l (350 mg/l)) and 26 subjects (12 women and 14 men; age range, 22–77 years) who had normal lipid profiles and were not taking lipid-lowering medications. These subjects were divided into four groups (Table 1) according to the following lipid profiles: serum TG ≤1.69 mmol/l and HDL-C ≥1.16 mmol/l (450 mg/l) (control group, n = 26); serum TG >1.69 mmol/l and HDL-C ≥1.16 mmol/l (high TG group, n = 22); serum TG >1.69 mmol/l and HDL-C ≤0.91 mmol/l (high TG & low HDL group, n = 67); serum TG ≥1.69 mmol/l and HDL-C ≤0.91 mmol/l (low HDL group, n = 21). Frequencies of diabetes mellitus (DM) in the groups were 19%, 14%, 54%, and 10%, respectively. In addition, of the 26 subjects in the control group, 3 (12%) were found to have CHD. In the other groups, the prevalence of CHD was 9%, 10%, and 10%, respectively. Furthermore, the number of patients with a history of cerebrovascular disease was 3 (14%) in the high TG group, 15 (22%) in the high TG & low HDL group, and 1 (5%) in the low HDL group. All subjects were matched for age and sex, and the relations between groups were compared. The remaining 19 of 155 subjects (8 women and 11 men; age range, 27–76 years) were also matched for age and sex and had serum HDL-C concentration of >0.91 mmol/l and <1.16 mmol/l. In this group, we found DM in 47% of patients and either CHD or cerebrovascular disease in 5% of patients.

Reagents
Monoclonal antibodies against MDA-LDL (ML25) and apo B (AB16) were obtained from Daiichi Pure Chemicals Co. (Tokyo, Japan), and 2–15% nondenaturing polyacrylamide gels were also purchased from Daiichi.

Analytical methods for lipids parameters
Serum total cholesterol (TC), TG, HDL-C, and LDL-C concentrations were determined enzymatically (Daiichi). Serum apo B and apolipoprotein A–I (apo A–I) were measured by commercial immunoturbidimetric assay (Daiichi). These assays, as well as the following electrophoresis, were carried out within 1 week after blood was drawn and serum samples were stored at 4°C.

Quantitation of MDA-LDL by enzyme linked immunosolvent assay (ELISA)
MDA-LDL was done by ELISA as previously described by Kondo et al (17). Briefly, serum samples were diluted 2400-fold in the dilution buffer. The preincubated diluted sample was added to the wells of plates coated with antibody ML25. The reaction mixture was kept at room temperature for 1h, and the plates were washed. β–Galactosidase-conjugated antibody (AB16) was then added, and the mixture was incubated for 30 min. Excess enzyme-labeled antibody was washed away, and the substrate was pipetted into the wells. The reaction was stopped after 2h. Absorbance in the individual wells was determined at 415 nm with a microplate reader. An aliquot of reference sera was applied for each assay.

Procedure for nondenaturing gradient-gel electrophoresis
The major peak LDL diameter was estimated by nondenaturing polyacrylamide gradient-gel electrophoresis according to the method reported by Williams et al. (18) but with some modification. After electrophoresis in a 2% to 15% polyacrylamide gradient gel, the gels were stained with Sudan Black B liquid stain (Daiichi), as previously described (15). For a reference standard, control serum was prepared from a healthy subject and run with each gel. Gels were scanned by a densitometer interfaced with a computer. Individual bands were compared to those of the control serum in each gel. The predominant LDL size in each sample lane was calculated by the previously reported equation (17) based on a standard curve. Serum samples were stored at –80°C after being added into 250 g/l sucrose and 1.3 mmol/l disodium EDTA for stabilizing the sample until analysis.

Other methods
Distinguishing from aforementioned groups, we randomly selected further two groups after matched for TG concentration, age and sex. Each group had normal TG concentrations (≤1.69 mmol/l) and HDL-C concentrations of ≤0.91 mmol/l (low HDL & normal TG group) or ≥1.16 mmol/l (normal HDL & normal TG group), respectively. These groups were compared to the corresponding protein and free amino group contents in LDL isolated by ultracentrifugation as described previously (17). The protein content in LDL fractions was estimated according to the method described by

Materials and Methods

Subjects
On the basis of concentrations of TG and HDL-C, 155 subjects were arbitrarily selected from among individuals whose fasting serum lipids were examined at the Hamamatsu University School of Medicine Hospital. The subjects included 110 patients (48 women and 62 men; age range, 21–77 years) with elevated TG (>1.69 mmol/l (1,500 mg/l)) or low HDL-C (<0.91 mmol/l (350 mg/l)) and 26 subjects (12 women and 14 men; age range, 22–77 years) who had normal lipid profiles and were not taking lipid-lowering medications. These subjects were divided into four groups (Table 1) according to the following lipid profiles: serum TG ≤1.69 mmol/l and HDL-C ≥1.16 mmol/l (450 mg/l) (control group, n = 26); serum TG >1.69 mmol/l and HDL-C ≥1.16 mmol/l (high TG group, n = 22); serum TG >1.69 mmol/l and HDL-C ≤0.91 mmol/l (high TG & low HDL group, n = 67); serum TG ≤1.69 mmol/l and HDL-C ≤0.91 mmol/l (low HDL group, n = 21). Frequencies of diabetes mellitus (DM) in the groups were 19%, 14%, 54%, and 10%, respectively. In addition, of the 26 subjects in the control group, 3 (12%) were found to have CHD. In the other groups, the prevalence of CHD was 9%, 10%, and 10%, respectively. Furthermore, the number of patients with a history of cerebrovascular disease was 3 (14%) in the high TG group, 15 (22%) in the high TG & low HDL group, and 1 (5%) in the low HDL group. All subjects were matched for age and sex, and the relations between groups were compared. The remaining 19 of 155 subjects (8 women and 11 men; age range, 27–76 years) were also matched for age and sex and had serum HDL-C concentration of >0.91 mmol/l and <1.16 mmol/l. In this group, we found DM in 47% of patients and either CHD or cerebrovascular disease in 5% of patients.

Reagents
Monoclonal antibodies against MDA-LDL (ML25) and apo B (AB16) were obtained from Daiichi Pure Chemicals Co. (Tokyo, Japan), and 2–15% nondenaturing polyacrylamide gels were also purchased from Daiichi.

Analytical methods for lipids parameters
Serum total cholesterol (TC), TG, HDL-C, and LDL-C concentrations were determined enzymatically (Daiichi). Serum apo B and apolipoprotein A–I (apo A–I) were measured by commercial immunoturbidimetric assay (Daiichi). These assays, as well as the following electrophoresis, were carried out within 1 week after blood was drawn and serum samples were stored at 4°C.

Quantitation of MDA-LDL by enzyme linked immunosolvent assay (ELISA)
MDA-LDL was done by ELISA as previously described by Kondo et al (17). Briefly, serum samples were diluted 2400-fold in the dilution buffer. The preincubated diluted sample was added to the wells of plates coated with antibody ML25. The reaction mixture was kept at room temperature for 1h, and the plates were washed. β–Galactosidase-conjugated antibody (AB16) was then added, and the mixture was incubated for 30 min. Excess enzyme-labeled antibody was washed away, and the substrate was pipetted into the wells. The reaction was stopped after 2h. Absorbance in the individual wells was determined at 415 nm with a microplate reader. An aliquot of reference sera was applied for each assay.

Procedure for nondenaturing gradient-gel electrophoresis
The major peak LDL diameter was estimated by nondenaturing polyacrylamide gradient-gel electrophoresis according to the method reported by Williams et al. (18) but with some modification. After electrophoresis in a 2% to 15% polyacrylamide gradient gel, the gels were stained with Sudan Black B liquid stain (Daiichi), as previously described (15). For a reference standard, control serum was prepared from a healthy subject and run with each gel. Gels were scanned by a densitometer interfaced with a computer. Individual bands were compared to those of the control serum in each gel. The predominant LDL size in each sample lane was calculated by the previously reported equation (17) based on a standard curve. Serum samples were stored at –80°C after being added into 250 g/l sucrose and 1.3 mmol/l disodium EDTA for stabilizing the sample until analysis.

Other methods
Distinguishing from aforementioned groups, we randomly selected further two groups after matched for TG concentration, age and sex. Each group had normal TG concentrations (≤1.69 mmol/l) and HDL-C concentrations of ≤0.91 mmol/l (low HDL & normal TG group) or ≥1.16 mmol/l (normal HDL & normal TG group), respectively. These groups were compared to the corresponding protein and free amino group contents in LDL isolated by ultracentrifugation as described previously (17). The protein content in LDL fractions was estimated according to the method described by
Statistical analysis

Data are shown as mean ± SD. Mean values in the four groups were compared with one-way ANOVA followed by the Bonferroni test. Linear regression analysis was performed, and Pearson correlation coefficients were obtained to examine the relation between the MDA-LDL/apo B ratio and LDL particle size or concentrations of HDL-C, TG, or LDL-C in the entire study group. The relation between the MDA-LDL/apo B ratio and predominant LDL particle size in each group was also evaluated by the same tests. Stepwise multiple regression analysis was used to assess the relation of age, sex, TG, TC, HDL-C, apo A–I, and LDL size to MDA-LDL/apo B ratio. Further comparisons between the normal HDL & normal TG and low HDL & normal TG groups were made by two-tailed unpaired t-test. All statistical analysis was performed with the Statistical Package for the Social Science (SPSS 10.0J for Windows). The level of significance was set at \( P < 0.05 \).

Results

Correlation between MDA-LDL/apo B and lipid parameters in the entire study population

We carried out a correlation study between MDA-LDL/apo B ratio and LDL size or concentrations of HDL-C, TG, or LDL-C in the entire study population (Fig. 1). There were significant associations \( (P < 0.001) \) among all of these combinations. If the MDA-LDL/apo B ratio was greater than 0.10, LDL size in subjects was widely distributed (top left, Fig. 1). When the ratio was greater than 0.10, all subjects had concentrations of HDL-C within 0.91 mmol/l (bottom left, Fig. 1). As the correlation between the MDA-LDL/apo B and HDL-C concentrations, the regression equation and Pearson correlation coefficients were

\[
y = -5.52x + 1.56
\]

and

\[
r = -0.47
\]

respectively.

Comparisons of lipid parameters between controls and patients

Table 1 shows lipid parameters in each group after adjustment for age and sex. Although the TC concentration was significantly lower \( (P < 0.05) \) in patients in the low HDL group than in the controls, LDL-C concentrations in all patient groups did not differ from those in the control group. As expected, concentrations of HDL-C and apo A–I were significantly lower \( (both \ P < 0.001) \) in both the high TG & low HDL and low HDL groups than in the control group. TG concentrations in the high TG and high TG & low HDL groups were significantly higher \( (P < 0.001) \) than that in the control group, whereas apo B concentration differed statistically only in the high TG & low HDL group \( (P < 0.05) \). LDL particle size was significantly lower in that group than in the control group, and MDA-LDL concentration was significantly higher. The high TG group had a slightly higher MDA-LDL concentration than the control group. The relationship of the mean MDA-LDL/apo B ratio between the control group and the other groups was different from the relationship of apo B, MDA-LDL, and LDL size between those groups. That is, the MDA-LDL/apo B ratio was significantly greater not only in the high TG & low HDL group \( (P < 0.001) \), but also in the low HDL group \( (P < 0.05) \) (Fig. 2). The MDA-LDL/apo B ratio in the high TG group was identical to that in the control group.

Relation between MDA-LDL/apo B and LDL size

Correlations between MDA-LDL/apo B ratio and LDL size in each group are shown in Figure 3. In the control group, all subjects had an MDA-LDL/apo B ratio within 0.10 and an LDL particle size more than 25.5 nm in diameter. Patients in high TG group had similar values to those of the control. In the high TG & low HDL group, there was a significant correlation \( (P < 0.001) \) between both parameters. All patients in the low HDL group had LDL particle size greater than 25.5 nm in diameter. Nevertheless, there were also many patients with an MDA-LDL/apo B ratio of over 0.10 in the low HDL group.

Multiple regression analysis of factors contributing to the MDA-LDL/apo B ratio

We performed a stepwise multiple regression analysis to study the determinants of the MDA-LDL/apo B ratio. The F-to-enter limit was 2.0. In the entire study population, serum HDL-C concentration was the major determi-
nant of the MDA-LDL/apo B ratio (Table 2). LDL size and the concentration of TC also appeared to be independent determinants of the MDA-LDL/apo B ratio. Concentrations of HDL-C and TC, and LDL size explained 30% of the variation in the MDA-LDL/apo B ratio.

### Table 1. Comparisons of variables between controls and patients

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>High TG</th>
<th>High TG&amp; Low HDL</th>
<th>Low HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 26)</td>
<td>(n = 22)</td>
<td>(n = 67)</td>
<td>(n = 21)</td>
</tr>
<tr>
<td>Sex</td>
<td>M/F</td>
<td>14/12</td>
<td>12/10</td>
<td>38/29</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>54.8 ± 15.8</td>
<td>56.0 ± 15.8</td>
<td>55.6 ± 11.3</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td></td>
<td>4.55 ± 0.71</td>
<td>5.15 ± 0.39</td>
<td>4.99 ± 1.44</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td></td>
<td>1.53 ± 0.37</td>
<td>1.41 ± 0.19</td>
<td>0.78 ± 0.11**</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td></td>
<td>2.78 ± 0.62</td>
<td>2.99 ± 0.54</td>
<td>3.05 ± 0.91</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td></td>
<td>0.89 ± 0.27</td>
<td>2.84 ± 0.88**</td>
<td>3.06 ± 1.68**</td>
</tr>
<tr>
<td>Apo A-I (mg/l)</td>
<td></td>
<td>1400 ± 220</td>
<td>1540 ± 150*</td>
<td>1050 ± 150**</td>
</tr>
<tr>
<td>Apo B (mg/l)</td>
<td></td>
<td>880 ± 180</td>
<td>1000 ± 160</td>
<td>1020 ± 190*</td>
</tr>
<tr>
<td>LDL size (nm)</td>
<td></td>
<td>27.0 ± 0.6</td>
<td>26.6 ± 0.6</td>
<td>25.6 ± 0.8**</td>
</tr>
<tr>
<td>MDA-LDL (units/l)</td>
<td></td>
<td>65 ± 15</td>
<td>73 ± 19</td>
<td>116 ± 30**</td>
</tr>
<tr>
<td>MDA-LDL/apo B</td>
<td></td>
<td>0.075 ± 0.013</td>
<td>0.074 ± 0.018</td>
<td>0.114 ± 0.023**</td>
</tr>
</tbody>
</table>

Abbreviations: TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein; LDL-C, LDL-cholesterol; TG, triglyceride; MDA-LDL, malondialdehyde-modified LDL; apo B, apolipoprotein B; apo A-I, apolipoprotein A-I

Control group, serum TG ≤ 1.69 mmol/l and HDL-C ≥ 1.16 mmol/l; high TG group, serum TG > 1.69 mmol/l and HDL-C ≥ 1.16 mmol/l; high TG & low HDL group, serum TG > 1.69 mmol/l and HDL-C ≤ 0.91 mmol/l; low HDL group, serum TG ≤ 1.69 mmol/l and HDL-C ≤ 0.91 mmol/l. Values are mean ± SD.

Statistical differences between controls and patients are shown by P values; *P < 0.05, **P < 0.001.

### Comparison between the low HDL & normal TG and normal HDL & normal TG groups

In the low HDL & normal TG and normal HDL & normal TG groups (HDL-C concentrations of ≤ 0.91 mmol/l and ≥ 1.16, respectively), we compared the corresponding free

Fig. 2. Comparison of MDA-LDL concentrations and MDA-LDL/apo B ratio between the control and other groups.

![Fig. 2](image1.png)

Fig. 3. Correlation studies between MDA-LDL/apo B ratio and predominant LDL particle size in each group. For high TG & low HDL group, linear regression analysis and Pearson correlation coefficients were $y = -17.7x + 27.6$ and $r = -0.48$.  

![Fig. 3](image2.png)
Table 2. Stepwise multiple regression analysis in the whole study population using the ratio of MDA-LDL/apo B as a dependent variable

<table>
<thead>
<tr>
<th></th>
<th>R²</th>
<th>F to enter</th>
<th>Standard regression Coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C</td>
<td>0.218</td>
<td>42.73</td>
<td>-0.355</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL size</td>
<td>0.263</td>
<td>9.30</td>
<td>-0.264</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC</td>
<td>0.299</td>
<td>7.59</td>
<td>-0.192</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Abbreviations: MDA-LDL, malondialdehyde modified LDL; apo B, apolipoprotein B; HDL-C, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein; TC, total cholesterol

Amino group contents in LDL isolated by ultracentrifugation (Table 3). The MDA-LDL/apo B ratio was significantly greater in the low HDL-C group (P < 0.05) compared to the ratio in the normal HDL-C group, whereas the mean difference in MDA-LDL concentrations between groups did not reach statistical significance. Furthermore, the group with the lower HDL-C concentration had slightly, but not statistically, lower free amino group content.

Discussion

In the entire study population, we first confirmed associations between MDA-LDL concentration and lipid parameters (concentrations of TG, HDL-C, or LDL-C, or LDL particle size). As described in other reports (17, 22), MDA-LDL concentration was positively correlated with TG and LDL-C concentrations (r = 0.36 and 0.42, respectively) and inversely correlated with HDL-C concentration and LDL particle size (r = -0.42 and -0.40, respectively). There were significant associations (P < 0.001) among all of these combinations.

Austin and colleagues (23) showed two distinct LDL phenotypes, pattern A and pattern B. The pattern A phenotype is characterized mainly by large LDL particles (≥ 25.5 nm in diameter), whereas the pattern B phenotype is defined by a high proportion of small dense LDL (< 25.5 nm) particles. There is evidence that the pattern B phenotype is frequent in patients with CHD (24).

Li et al. (15) reported that subjects with high TG (> 1.69 mmol/l) and low HDL-C (< 0.91 mmol/l) concentrations frequently have higher concentrations of small dense LDL, resulting in the pattern B phenotype. We also showed that only in high TG & low HDL group was LDL particle size smaller than in the control group. Furthermore, in the high TG & low HDL group, the MDA-LDL concentration was highest (Table 1), and the MDA-LDL/apo B ratio correlated with high distributions of small dense LDL. In this entire study population, many patients with DM were mainly distributed in the high TG & low HDL group. Inversely, our previous reports showed that, as compared to the control group, patients with DM had high MDA-LDL concentration as well as high TG and low HDL-C concentrations in sera (17, 25). Therefore, there is a possibility that subjects with high TG (> 1.69 mmol/l) and low HDL-C (< 0.91 mmol/l) concentrations suffer from DM. Detection of MDA-LDL by ELISA could result from quantitative and qualitative variance. That is, differences of MDA-LDL concentrations between distinct subjects may be determined from the extent of MDA modification per one LDL molecule and the mass of apo B in LDL modified by MDA. As one LDL particle has only one apo B molecule, the MDA-LDL/apo B ratio would become a convenient indicator of the extent of MDA modification per one LDL particle. The apo B molecule is also contained in other lipoproteins such as chylomicrons and VLDL. Therefore, it is possible for the MDA-LDL/apo B ratio to cause underestimation of the extent of MDA modification per one LDL particle because MDA-LDL measured by ELISA locates in LDL fractions (16). Nevertheless, the MDA-LDL/apo B ratio values; *P < 0.05, **P < 0.001.

Table 3. Comparisons of variables between the normal HDL & normal TG and low HDL & normal TG groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal HDL &amp; normal TG</th>
<th>Low HDL &amp; normal TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M/F</td>
<td>6/3</td>
</tr>
<tr>
<td>Age</td>
<td>years</td>
<td>60.2 ± 15.1</td>
</tr>
<tr>
<td>TC</td>
<td>mmol/l</td>
<td>4.94 ± 1.32</td>
</tr>
<tr>
<td>HDL-C</td>
<td>mmol/l</td>
<td>1.47 ± 0.28</td>
</tr>
<tr>
<td>LDL-C</td>
<td>mmol/l</td>
<td>2.90 ± 1.19</td>
</tr>
<tr>
<td>TG</td>
<td>mmol/l</td>
<td>1.17 ± 0.30</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>mg/l</td>
<td>1350 ± 160</td>
</tr>
<tr>
<td>Apo B</td>
<td>mg/l</td>
<td>910 ± 360</td>
</tr>
<tr>
<td>MDA-LDL</td>
<td>units/l</td>
<td>66 ± 22</td>
</tr>
<tr>
<td>MDA-LDL/apo B</td>
<td>0.074 ± 0.016</td>
<td>0.101 ± 0.031*</td>
</tr>
<tr>
<td>Free amino group/ mg protein in LDL</td>
<td>719 ± 58</td>
<td>686 ± 67</td>
</tr>
</tbody>
</table>

Abbreviations: TC, total cholesterol; HDL, high-density lipoprotein; LDL-C, high-density lipoprotein-cholesterol; TG, triglyceride; MDA-LDL, malondialdehyde-modified LDL; apo B, apolipoprotein B; apo A-I, apolipoprotein A-I. Values are mean ± SD. Statistical differences between the normal HDL & normal TG and low HDL & normal TG groups are shown by P values; *P < 0.05, **P < 0.001.
LDL (≥ 25.5 nm) in the low HDL group. We confirmed this phenomenon by measuring the free amino group content of LDL. If the decreasing number of free amino residues in LDL reflects an increase in a modification by MDA of one LDL particle, the detectable absorbance in the same number of LDL particle may also increase the ELISA value. The free amino group content of LDL in subjects with low HDL-C concentrations (low HDL & normal TG group) had a tendency to lower values (not significantly), compared with values in the normal HDL & normal TG group (Table 3).

In the high TG group, the MDA-LDL concentration and MDA-LDL/apo B ratio showed no differences from those in the control group. This suggested to us that high concentrations of TG but not low HDL-C concentrations have almost no effect on MDA-LDL concentration and MDA-LDL/apo B ratio, and also on LDL particle size.

The subjects with low HDL-C concentration were divided into two types. One type had the same MDA-LDL/apo B ratio as the controls (≤ 0.10). The other type had an MDA-LDL/apo B ratio over 0.10. Despite having a low HDL-C concentration, the LDL particles in subjects of the first type were well protected from oxidation, but those in subjects of the second type were not. The antioxidant activity of HDL is largely due to the paraoxonase-1 (PON1) located on HDL (28). PON1 has 2 amino acid polymorphisms, one at position 55 (methionine/leucine, M/L) and the other at position 192 (arginine/glutamine, R/Q). Numerous studies have shown that individuals with the PON1 192 R allele are more prone to CHD than those with the Q allele (29). Therefore, when HDL-C concentration decreases, it is possible that the variance in the polymorphisms of PON1 on HDL influences the different protective effects HDL offers to LDL against oxidation. Also, the another possible mechanism is considered that, in the low HDL group, subjects with high MDA-LDL/apo B ratio (> 0.10) had significantly lower LDL-C concentrations (P < 0.01) than ones with that low ratio (≤ 0.10). LDL-C concentrations (mean ± SD) in the former (n = 7) were 1.29 ± 0.48 mmol/l, and those in the latter (n = 14) were 2.60 ± 0.83 mmol/l. As shown in Figure 1, LDL-C concentrations were inversely correlated with MDA-LDL/apo B ratio. Therefore, in subjects with low HDL-C concentrations, if low LDL-C concentrations exhibit a low number of LDL particles in sera, it is possible that the oxidative modification was concentrated on a limited number of LDL particles.

In conclusion, lower HDL-C concentration could be associated with an increase in the extent of MDA modification per one LDL molecule. Moreover, accompanied by high TG concentration, LDL particle size in subjects with lower HDL-C concentration becomes smaller.

Acknowledgment: This work was supported in part by Grants-in-Aid for Scientific Research (13557225) from the Ministry of Education, Science, Sports, Culture and Technology, Japan.

References
(13) Bowry VW, Stanley KK, and Stocker R: High density lipoprotein is the major carrier of lipid hydroperox-


Habeeb AFSA: Determination of free amino groups in proteins by trinitrobenzenesulfonic acid. Anal Biochem, 14: 328–336, 1966


