Coronary heart disease (CHD) is a leading cause of death in Westernized society and is more frequently found in those aged 50 years and older because age is an unavoidable risk factor for CHD (1). The plasma level of high-density lipoprotein cholesterol (HDL-C) is known to be inversely associated with the risk of CHD (2). High-density lipoprotein (HDL) exerts many beneficial effects on the maintenance of a healthy physiologic system, including antioxidant, anti-inflammatory, and antithrombotic effects (3). These activities are exerted in accordance with the composition of essential apolipoproteins and associated enzymes. Among the apolipoproteins, apoA-I is the principal protein component of HDL. An inverse relationship between apoA-I levels and CHD has been reported in a prospective study (4). Mature human apoA-I comprised a single polypeptide chain of 243 amino acids, which is characterized by 11 and 22 amino acid homologous repeats (5).

As with HDL-C, the apoA-I content in lipoprotein (Lp-A-I) is also important in maintaining the antioxidant and anti-inflammatory activities of HDL. Modification of apoA-I, such as cleavage, oxidation, nitration, and chlorination, could lead to the production of dysfunctional apoA-I and HDL (6). Truncation of apoA-I at the C-terminus, yielding apoA-I (1–192), results in decreased lipid-binding ability (7). Chlorination might occur at Tyr192, as reported by Shao and colleagues (8), because Tyr192 is a selective target for myeloperoxidase modification due to the presence of the YxxK motif.

In addition to the modification of HDL, Patsch and colleagues (9) reported that familial hyperalphalipoproteinemia is associated with longevity. Nikkila and Heikkinen (10) also showed that higher HDL levels are found among healthy elderly, 85–89 years of age, as compared with middle-aged adults. These reports raised the importance of HDL and apoA-I in protection from chronic age-related diseases and mortality.

Aging is a chronic process that involves several physical and biochemical modifications that affect HDL structure and function (11,12), including nonenzymatic glycation to produce advanced glycated end products (AGEs) via the Maillard reaction (13). Aging is accompanied by an increase in circulating proinflammatory cytokines, acute phase reactants, and C-reactive protein (14). Recently, Collins and colleagues (15) showed that age-accelerated atherosclerosis...
correlates with failure to upregulate antioxidant genes; the role of oxidative stress is important in the development of an aging vasculature. However, there have been no reports addressing age-related changes in functional and structural properties of HDL and apolipoproteins. In order to compare changes in lipoprotein metabolism with aging, we analyzed the lipid and protein compositions of individual lipoprotein fractions that were purified from elderly male participants, with young participants serving as controls.

**Materials and Methods**

**Recruitment of Participants**

Healthy male volunteers (average age, 71 ± 4 years; n = 26; elderly group), who attended the Health Promotion Program of Yeungnam University Hospital (Daegu, South Korea) for regular health examinations, were recruited. All were interviewed, and the absence of clinical evidence of coronary or other peripheral vascular diseases was confirmed by thorough history taking and physical examination and by reviewing medical history. Treadmill exercise test and/or multidetector computed tomography angiography were done at the physician’s discretion (D.-G.S.) in the presence of any suspicion. We excluded patients who had a history of diabetes, hypertension, and any other evidence of systemic diseases.

The young male volunteers (average age, 22 ± 2 years; n = 18; control group) were recruited from students who were enrolled in Yeungnam University (Gyeongsan, South Korea). In both groups, we excluded heavy alcohol consumers (>30 g EtOH/d) and those who had consumed any prescribed drugs to treat hyperlipidemia, diabetes mellitus, or hypertension. All participants had unremarkable medical records without histories of endocrinologic disorders. Informed consent was obtained from all participants prior to enrollment in the study, and the Institutional Review Board at the Yeungnam University Medical Center (Daegu, South Korea) approved the protocol.

**Plasma and Lipoprotein Analysis**

Blood was drawn in an overnight fasting state from all patients and controls. Blood was collected using a vacutainer (BD Biosciences, Franklin Lakes, NJ) containing ethylenediaminetetraacetic acid (final concentration, 1 mM). Blood parameters, lipids, and glucose concentrations were determined using an automatic blood analyzer (chemistry analyzer AU4500; Olympus, Tokyo, Japan).

Plasma was isolated by low-speed centrifugation, and each aliquot of plasma was stored at −80°C until analysis. Very low-density lipoprotein (VLDL) (d < 1.019 g/mL), low-density lipoprotein (LDL) (1.019 < d < 1.063), HDL₂ (1.063 < d < 1.125), and HDL₃ (1.125 < d < 1.225) were isolated from individual participants and control sera via sequential ultracentrifugation, with the density being appropriately adjusted by the addition of NaCl and NaBr, in accordance with standard protocols (16). Samples were centrifuged for 24 hours at 10°C at 100,000g using a Himac CP-90α (Hitachi, Tokyo, Japan) in the Instrumental Analysis Center at Yeungnam University.

For each of the lipoproteins that were purified individually, total cholesterol (TC) and triglyceride (TG) measurements were obtained using commercially available kits (cholesterol, T-CHO and TG, CleanTech TS-S; Wako Pure Chemical, Osaka, Japan). The protein concentrations of lipoproteins were determined via the Lowry protein assay, as modified by Markwell and colleagues (17) using the Bradford assay reagent (Bio-Rad, Seoul, South Korea) with bovine serum albumin as a standard. To assess the degree of oxidation of individual LDL, the concentration of oxidized species in LDL was determined by the thiobarbituric acid reactive substances method using malondialdehyde (MDA) as a standard (18). In order to compare the extent of glycation between the groups, the content of AGEs in the individual lipoproteins was determined by reading the fluorometric intensities at 370 nm (excitation) and 440 nm (emission), as described previously (19).

**Ferric-Reducing Ability of Plasma Assay**

The ferric-reducing ability (FRA) was determined using the method described by Benzie and Strain (20). Briefly, the FRA reagents were freshly prepared by mixing 25 mL of 0.2 M acetate buffer (pH 3.6), 2.5 mL of 10 mM 2,4,6-tripyridyl-s-triazine (Fluka Chemicals, Buchs, Switzerland), and 2.5 mL of 20 mM FeCl₃ 6H₂O. The antioxidant activities of HDL₃ were then estimated by measuring the increase in absorbance induced by the generated ferrous ions. The freshly prepared FRA reagent (300 μL) was mixed with equally diluted HDL₃ (2 mg/mL, 10 μL), which was diazoyed extensively against phosphate-buffered saline (PBS), after which the FRA was determined by measuring the absorbance at 593 nm every 20 seconds over a 10-minute period at 25°C using a DU 800 Spectrophotometer (Beckman Coulter, Fullerton, CA) equipped with a MultiTemp III Thermocirculator (Amersham Biosciences, Uppsala, Sweden).

**Copper-Mediated Oxidation of LDL**

To compare the susceptibility of copper-mediated LDL oxidation, 300 μg of LDL was incubated with 5 μM CuSO₄ for up to 3 hours. During the incubation, the quantity of formed conjugated dienes was monitored by following the absorbance at 234 nm (Abs234) at 37°C (21) using a Beckman DU 800 Spectrophotometer equipped with a MultiTemp III Thermocirculator.

**Paraoxonase Assay**

Paraoxonase (PON)-1 activity toward paraoxon was determined by evaluating the hydrolysis of paraoxon into...
p-nitrophenol and diethylphosphate, which was catalyzed by the enzyme (22). PON-1 activity was then determined by measuring the initial velocity of p-nitrophenol production at 37°C, as determined by measuring the absorbance at 405 nm (microplate reader, Bio-Rad model 680). To accomplish this, the same amount of equally diluted serum (20 mg/mL, 10 µL) or HDL$_3$ (2 mg/mL, 10 µL), which was dialyzed extensively against PBS, was added to 200 µL of substrate (paraaxon-ethyl, Sigma D-9286; Sigma, St. Louis, MO) in a solution consisting of 90 mM Tris–HCl, 3.6 mM NaCl, and 2 mM CaCl$_2$ (pH 8.5). The PON-1 activity of 1 U/L was defined as 1 µmol of p-nitrophenol formed per minute. The molar extinction coefficient of p-nitrophenol was 17,000/M/cm.

**Cholesteryl Ester Transfer Assay**

A reconstituted high-density lipoprotein (rHDL) containing apoa-I and cholesteryl (CE) oleate was synthesized in accordance with the method described by Cho and colleagues (23) using trace amounts of [3H]-CE oleate (TRK886, 3.5 µCi of apoa-I per milligram; GE Healthcare, Uppsala, Sweden). Next, to facilitate separation from the CE acceptor, the rHDL was immobilized using a CNBr-activated Sepharose 4B resin (Amersham Biosciences) in accordance with the manufacturer’s instructions. The CE transfer reaction was allowed in 300-µL reaction mixtures that contained each of the serum samples (20 µL) as a cholesteryl ester transfer protein (CETP) source: rHDL-agarose (50 µL, 0.25 mg/mL) and human LDL (50 µL, 0.25 mg/mL) as a CE donor and CE acceptor, respectively. After incubation at 37°C, the reaction was halted via brief centrifugation (10,000g) for 3 minutes at 4°C. The supernatant, which contained the CE acceptor (150 µL), was then subjected to scintillation counting, and the percentage transfer of [3H]-CE from rHDL to LDL was calculated.

**Enzyme-Linked Immunosorbent Assay, Western Blot, and Electrophoresis**

For the sandwich enzyme-linked immunosorbent assay (ELISA), diluted primary antibody (1:2,000 or 1:4,000) was coated onto a 96-well plate (Maxisorp 439454; Nunc, Roskilde, Denmark) overnight at 4°C. As primary antibodies, three apoa-I antibodies (ab7613, ab33470, and ab52945; Abcam, Cambridge, UK) for detection of full-length apoa-I and C- and N-terminals of apoa-I, respectively, and apoc-III antibody were obtained from Chemicon (catalog number ab821; Temecula, CA). The serum amyloid A (SAA) concentration was determined via a competitive binding ELISA technique using a commercially available immunoassay kit (catalog number KNA0012; Biosource, Camarillo, CA) in accordance with the instructions of the manufacturer. Horse-radish peroxidase (HRP)–conjugated apoa-I antibody (ab20784) and HRP-apoc-III antibody (ab27624) were purchased from Abcam and used as secondary antibodies. A substrate reagent pack (DY999; R&D Systems, Minneapolis, MN) was used for color development. An equal amount of sample (20 µL) was taken from equally diluted lipoprotein fractions as follows: VLDL, 0.6 mg of protein per milliliter; LDL, 1.6 mg of protein per milliliter; HDL$_2$, 0.7 mg of protein per milliliter; and HDL$_3$, 2 mg of protein per milliliter.

**Purification of Apolipoprotein A-I**

ApoA-I was purified from human plasma using ultracentrifugation, column chromatography, and organic solvent extraction following the method described by Brewer and colleagues (24). The purified apoA-I was lyophilized at −80°C until use.

**Glycation of Apolipoprotein A-I**

Determination of apoA-I glycation was developed using the method of McPherson and colleagues (19) using apoA-I. Briefly, the purified lipid-free apoA-I (1 mg/mL) was incubated with d-fructose (final 10 mM) in 200 mM potassium phosphate/0.02% sodium azide buffer (pH 7.4) for up to 72 hours under gas with air containing 5% CO$_2$ at 37°C. The extent of AGEs in the apoA-I was determined by reading the fluorometric intensities at 370 nm (excitation) and 440 nm (emission).

**Electron Microscopy**

Transmission electron microscopy (TEM) was performed with a Hitachi electron microscope (model H-7600; Hitachi, Ibaraki, Japan) operating at 80 kV. HDL was negatively stained with 1% sodium phosphotungstate (PTA; pH 7.4), with a final apolipoprotein concentration of 0.3 mg/mL in TBS. Five microliters of the HDL suspension was blotted with filter paper and immediately replaced with a 5-µL droplet of 1% PTA. After a few seconds, the stained HDL fraction was blotted onto a Formvar carbon-coated 300-mesh copper grid and then air-dried. The shape and size of HDL$_2$ were determined by TEM photography at a magnification of ×40,000 according to our previous report (25).

**Data Analysis**

All data are expressed as the M ± SD. Two-group comparisons were carried by independent t tests using SPSS (version 14.0; SPSS, Inc., Chicago, IL). Statistical significance was defined as a p < .05.

**RESULTS**

**Serum Profiles**

Although the elderly group had slightly high body weight (64 ± 5 kg) than the young control group (59 ± 5 kg), the elderly group had a body mass index in the normal range,
Reduced Antioxidant Activity in the Elderly Group

The FRA assay is known to be a rapid and reproducible method for the determination of molar concentrations of existing antioxidants. The FRA assay revealed that HDL3 in the elderly group had a 23% increase in $A_{993}$ during a 10-minute incubation, whereas HDL3 in the control group had a 219% increase, as shown in Figure 1A. This result indicates that HDL3 from the elderly group (E-HDL3) had weaker antioxidant activity than the control group.

LDL from the elderly group (E-LDL) was more susceptible to copper-mediated oxidation, as shown in Figure 1B. E-LDL showed faster electromobility than LDL from the control group (C-LDL). More oxidized LDL is prone to move faster due to an increased charge with a smaller particle size.

In addition, without cupric ion treatment, the content of oxidized species was elevated in E-LDL (3.5 ± 0.5 nM of MDA) compared with C-LDL (2.1 ± 0.3 nM of MDA) using the same amount of LDL (30 μg of protein). These results suggest that the serum antioxidant activity was weakened (Figure 1A) in the elderly group with increased susceptibility of LDL oxidation (Figure 1B) and elevated oxidized species in LDL (Figure 1C).

PON Activity Was Reduced in the Elderly Group

PON activity was reduced in the elderly group, especially in serum and the HDL3 level, as shown in Figure 2. When equally diluted serum (20 mg of total protein per milliliter) was used, the PON activity in the elderly group was 78 ± 15 U/L compared with 119 ± 29 U/L in the control group. Under the same amount of protein in HDL3, control group showed significantly higher PON activity (88 ± 5 U/L) than elderly group (55 ± 3 U/L). However, no significant difference was detected in the HDL2 and lipoprotein-deficient serum (LPDS) fractions between the groups, indicating that the major PON activity was associated with HDL3.

CE Transfer Activity Was Increased in the Elderly Participants

Serum CE transfer activity was not significantly different between the elderly and control participants (28 ± 7% and 24 ± 3%, respectively). However, the CETP activity of the VLDL and HDL3 fractions was almost twofold increased in the elderly group, as shown in Figure 3. E-HDL3 revealed 10 ± 1.7% CE transfer, whereas the control group had 5 ± 0.3% transfer. This result correlated well with our recent report (25) of an increase in TG in the lipoprotein fraction (Table 2) because elevated CETP activity is strongly associated with production of TG-enriched lipoprotein. Immunodetection showed that the elderly group had an elevated CETP level in HDL3 (bottom photo of Figure 3).

Table 1. Serum Profiles of Elderly and Controls

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 18)</th>
<th>Elderly (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong> (years)</td>
<td>22 ± 2</td>
<td>71 ± 4**</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>22 ± 2</td>
<td>24 ± 3</td>
</tr>
<tr>
<td><strong>Blood pressure (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Systolic</strong></td>
<td>125 ± 15</td>
<td>128 ± 7</td>
</tr>
<tr>
<td><strong>Diastolic</strong></td>
<td>67 ± 5</td>
<td>72 ± 8</td>
</tr>
<tr>
<td><strong>TC (mg/dL)</strong></td>
<td>162 ± 24</td>
<td>196 ± 31*</td>
</tr>
<tr>
<td><strong>Triglyceride (mg/dL)</strong></td>
<td>74 ± 26</td>
<td>112 ± 51*</td>
</tr>
<tr>
<td><strong>HDL-C (mg/dL)</strong></td>
<td>58 ± 5</td>
<td>53 ± 11</td>
</tr>
<tr>
<td>% HDL-C</td>
<td>37 ± 3</td>
<td>27 ± 8*</td>
</tr>
<tr>
<td><strong>LDL-C</strong></td>
<td>95 ± 20</td>
<td>131 ± 28*</td>
</tr>
<tr>
<td><strong>TC:HDL ratio</strong></td>
<td>2.7 ± 0.8</td>
<td>3.6 ± 1.1*</td>
</tr>
<tr>
<td><strong>TG:HDL ratio</strong></td>
<td>1.2 ± 0.4</td>
<td>2.1 ± 1.2*</td>
</tr>
<tr>
<td><strong>T-bilirubin</strong></td>
<td>0.9 ± 0.3</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td><strong>GPT (U/L)</strong></td>
<td>14 ± 6</td>
<td>25 ± 13*</td>
</tr>
<tr>
<td><strong>GOT (U/L)</strong></td>
<td>17 ± 2</td>
<td>24 ± 8*</td>
</tr>
<tr>
<td><strong>Uric acid (mg/dL)</strong></td>
<td>0.14±0.012 (n = 10)</td>
<td>0.13±0.06* (n = 10)</td>
</tr>
<tr>
<td><strong>WBC (K/μL)</strong></td>
<td>6.8 ± 2.3</td>
<td>7.6 ± 2.4</td>
</tr>
<tr>
<td><strong>RBC (M/μL)</strong></td>
<td>5.2 ± 0.2</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td><strong>Platelet (K/μL)</strong></td>
<td>273 ± 52</td>
<td>269 ± 62</td>
</tr>
<tr>
<td><strong>Glucose (mg/dL)</strong></td>
<td>88 ± 12</td>
<td>105 ± 23</td>
</tr>
<tr>
<td><strong>IL-6 (pg/mL)</strong></td>
<td>89 ± 44 (n = 10)</td>
<td>71 ± 24 (n = 10)</td>
</tr>
<tr>
<td><strong>ApoC-III (mg/dL)</strong></td>
<td>9.4 ± 4</td>
<td>11 ± 5</td>
</tr>
<tr>
<td><strong>ApoA-I (mg/dL)</strong></td>
<td>152 ± 31</td>
<td>195 ± 37*</td>
</tr>
<tr>
<td><strong>Uric acid (mg/dL)</strong></td>
<td>2.8 ± 1.6</td>
<td>6.2 ± 1.6**</td>
</tr>
</tbody>
</table>

*Note: BMI = body mass index; GPT = glutamic oxaloacetic transaminase; hscRP = high-sensitivity C-reactive protein; HDL = high-density lipoprotein; HDL-C = high-density lipoprotein cholesterol; IL = interleukin; LDL-C = low-density lipoprotein cholesterol; RBC = red blood cell; TC = total cholesterol; TG = triglyceride; WBC = white blood cell.

*p < .05 versus control, **p < .01 versus control.
Expression of ApoA-I in HDL and LPDS

The elderly group showed a significantly elevated level of expression of apoA-I in LPDS, although there was no significant difference in apoA-I in HDL\textsubscript{2}, as shown in Figure 4a. However, the elderly group (9 ± 3 µg/mL) had much less apoA-I content in HDL\textsubscript{2} than the control group (29 ± 4 µg/mL). These different levels of expression were confirmed by Western blot analysis, as shown in Figure 4B. Interestingly, the apoA-I band in the HDL\textsubscript{2} fraction was much decreased in the elderly group (representative participants), whereas the apoA-I band intensity in HDL\textsubscript{3} was similar between the two groups (Figure 4B) when detected by apoA-I antibody, which was raised by full-length apoA-I (ab7613; Abcam). The elderly group showed a much darker band intensity of apoA-I (28 kDa) in the LPDS fraction than the control group when detected by the same antibody. In addition, the elderly group had more distinct two fragmented apoA-I bands (12 and 17 kDa from sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE]), as indicated by the arrow (Figure 4B).

The Elderly Group Showed Truncation of the C-terminal of ApoA-I in HDL\textsubscript{3}

The apoA-I band intensities were remarkably decreased in E-HDL\textsubscript{2} when detected by N- and C-terminal-specific apoA-I antibody (ab52945 and ab33470, respectively; Abcam), whereas the control group showed distinct band intensities (Figure 4C). This result correlates well with less detection of apoA-I in E-HDL\textsubscript{2} (Figure 4a). Although both groups had a similar level of expression of apoA-I in HDL\textsubscript{3} based on the ELISA determination, Western blot analysis with various apoA-I antibodies revealed that the C-terminal of apoA-I was detected less in E-HDL\textsubscript{3} (Figure 4C). Both groups showed similar band intensities when detected by apoA-I antibody, which was directed against either full length or the N-terminal of apoA-I.

Table 2. Lipid and Protein profiles of Lipoproteins

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>TP (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 18)</td>
<td>32 ± 15</td>
<td>65 ± 15</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>Elderly (n = 22)</td>
<td>36 ± 13</td>
<td>37 ± 19*</td>
<td>21 ± 14</td>
</tr>
</tbody>
</table>

Notes: HDL = high-density lipoprotein; LDL = low-density lipoprotein; TC = total cholesterol; TG = triglyceride; TP = total protein; VLDL = very low-density lipoprotein.

*p < .05 versus control.

Figure 1. Comparison of the antioxidant activity based on the ferric-reducing ability of HDL\textsubscript{3} (A) and copper-mediated LDL oxidation (B). (A) Rate of increase in absorbance at 593 nm. The HDL\textsubscript{3} of elderly group showed much weaker reducing ability than the HDL\textsubscript{3} of control group during 10 minutes of incubation. Error bars indicate the standard deviation from three independent experiments with duplicate samples. (B) Electromobility of LDL from the elderly and controls with or without treatment of copper (final 5 µM). (C) The native LDL from each group was analyzed by TBARS assay to determine the content of oxidized species. Malondialdehyde (MDA) was used as a standard, and the extent of oxidation was expressed as the MDA concentration (nanomole of MDA/30 mg of protein in LDL). Notes: C-LDL = LDL from the control group; E-LDL = LDL from the elderly group; HDL = high-density lipoprotein; LDL = low-density lipoprotein; PBS = phosphate-buffered saline; TBARS = thiobarbituric acid reactive substances.
MODIFICATION OF APOA-I BY AGING

Furthermore, the immunodetected apoa-I band intensities in the LPDS fraction were not different between the elderly and control groups. The putative truncation of apoa-I at the C-terminal in HDL	extsubscript{3} might contribute to the elevation of apoa-I in serum and LPDS, which was determined by ELISA using apoa-I antibody against full-length apoa-I (ab7613; Abcam).

**The Elderly Group Showed Increased Glycated Levels of Apoa-I in HDL	extsubscript{3}**

Nonenzymatic glycation of apoa-I via the Maillard reaction produced more multimerized apoa-I bands (Figure 5A) and fluorescence products (Figure 5B) in a fructose concentration–dependent manner after 72 hours of incubation under 5% CO	extsubscript{2} at 37°C. The multimerization is a well-known characteristic of the fructation process as a modification of proteins. SDS-PAGE (Figure 5C) and Western blot analysis (Figure 5D) confirmed that the multimerized apoa-I band was greater in E-HDL	extsubscript{3} than in HDL	extsubscript{3} from the control group (C-HDL	extsubscript{3}). In addition, the apoa-I fragment (12 kDa) was also detected in E-HDL	extsubscript{3}, as indicated by the arrowhead (Figure 5D), whereas the apoa-I fragment was not detected in C-HDL	extsubscript{3}.

Fluorescence detection revealed that all lipoproteins from the elderly group showed elevated levels of AGEs, especially in the HDL	extsubscript{2} and HDL	extsubscript{3} fractions; a twofold and threefold increase of AGEs in the HDL	extsubscript{2} and HDL	extsubscript{3} fractions was found in the elderly, respectively (Figure 5E). This result correlates well with our current finding of reduced detection of apoa-I (Figure 4C), and other reports that accumulated...
Maillard products can undergo oxidative cleavage, resulting in the formation of AGEs (26) and multimerization of serum proteins (27).

**HDL<sub>2</sub> Particle Size Was Decreased in the Elderly**

As shown in Figure 6, electron microscopic observations revealed that the elderly group had slightly decreased particle number and size of HDL<sub>2</sub> and HDL<sub>3</sub> compared with the control group. E-HDL<sub>2</sub> (n = 22) had a width of approximately 20.7 ± 3.2 nm and a length of 18.3 ± 2.8 nm, whereas the C-HDL<sub>2</sub> (n = 18) had a width of 23.2 ± 3.9 nm and a length of 21.6 ± 2.6 nm. E-HDL<sub>3</sub> (n = 22) had a width and length of 8.4–11.7 nm, whereas C-HDL<sub>3</sub> (n = 18) had a width and length of 11.8–15.3 nm.

**The Level of Expression of SAA and ApoC-III**

SAA was more detectable in the serum and LPDS fraction, as shown in Figure 7A; the elderly group had a 1.6-fold and 1.3-fold higher level in the serum and LPDS, respectively, than the control group. The SAA level in lipoproteins was similar, with the exception of HDL<sub>2</sub>; the elderly group had an almost twofold increase in SAA content in HDL<sub>2</sub> compared with HDL<sub>3</sub>.

The elderly group had an elevated level of apoC-III in the serum, LPDS, and HDL<sub>2</sub>, as shown in Figure 7B. Although apoC-III in the elderly group was slightly increased in the serum and HDL<sub>2</sub>, it was almost twofold increased in LPDS compared with the control group. This result suggests that lipid-free apoC-III increased by aging.

**Discussion**

It has been reported that there is an age-related susceptibility of HDL to copper-mediated oxidation (11). Susceptibility of LDL to lipid peroxidation is increased by aging, with a concomitant decrease in dehydroepiandrosterone concentration. Similarly, our results support that the antioxidant ability of E-HDL<sub>3</sub> was much weakened (Figure 1A).
and the E-LDL contained more oxidized species more susceptible to copper-mediated oxidation (Figure 1B and C). It is well known that oxidation of lipoproteins plays an important role in the pathogenesis of atherosclerosis. Recently, our research group showed that myocardial infarction patients have more severely oxidized LDL compared with patients with angina pectoris without cupric ion treatment (28).

With an increase in the extent of LDL oxidation, the elderly group showed significantly reduced Pon activity in the serum and HDL\textsubscript{3} fraction (Figure 2). Pon is principally associated with HDL\textsubscript{3} in human serum and its activity is usually decreased during an acute phase response (29) and in acute kidney failure due to hemorrhagic fever with renal syndrome (HFRS) (25). In an apparently healthy population, serum PON-1 activity is decreased with senescence, although the level of PON-1 expression is not decreased (12). This result indicates that normal PON-1 activity might be hampered by an unknown factor in lipoprotein or serum levels, possibly by deprivation of an activating factor for PON with aging. PON activity is also reduced in cases of neurodegenerative disorders, such as Alzheimer’s and Parkinson’s diseases (30). Taken together, PON activity and the concentration in serum have been strongly associated

![Figure 5. Glycation of purified apoA-I and characterization of the extent of glycation in lipoprotein from the participants.](https://academic.oup.com/biomedgerontology/article-abstract/65A/6/600/618084)
with the incidence of a variety of diseases, indicating that PON is involved in the strengthening of systemic defense mechanisms via antioxidant activity.

On the other hand, CETP activity in VLDL and HDL was significantly increased with aging (Figure 3), even though the PON-1 activity of HDL from the elderly was decreased (Figure 2). Although there was no difference in CETP activity in HDL-2 between the groups, E-HDL-3 had a much increased CE transfer activity, indicating that CE transfer activity might be lipoprotein specific. Zeller and colleagues (31) reported that serum CETP activity was elevated in myocardial infarction patients despite decreased HDL-C levels. Our research group also reported that CETP activity and mass were increased in serum and LDL and HDL-3 fractions in Korean myocardial infarction patients despite decreased HDL-C levels. Our research group also reported that CETP activity and mass were increased in serum and LDL and HDL-3 fractions in Korean myocardial infarction patients (28). In addition, we also reported that an increase in apoC-III content in rHDL containing apoA-I did not reduce CETP activity (32). Furthermore, there are three methionine (86th, 112th, and 148th amino acid) in the mature human apoA-I. It is also possible to speculate that the elevated oxidation of apoA-I in E-HDL-3 might cause production of methionine sulfoxide in apoA-I, which increased CETP activity in the delipidated HDL particle, as suggested previously (33). Taken together, these reports and the current results demonstrate that CETP activity in HDL-3 is increased by aging, despite a loss of antioxidant activity, PON activity, and C-terminal truncation of apoA-I. An increase in oxidized LDL in the elderly coexisted with a decrease in PON activity and an increase in CETP activity. These results suggest that oxidation sensitivity is correlated with development of dysfunctional HDL and greater systemic impairment of antioxidant potential with senescence.

The serum apoA-I level was increased in the elderly group by ELISA, which was carried out with primary antibody raised against full-length apoA-I (Table 1). Although the amount of apoA-II in HDL-3 was not different between the groups, apoA-I in HDL-2 was remarkably decreased (Figure 4A), as based on ELISA determination. Western blot analysis with the same antibody revealed a darker band intensity and fragmented apoA-I band in the LPDS of elderly group (Figure 4B). Detection of the C-terminal of apoA-I in HDL-3 revealed much weaker band intensity, whereas detection of the N-terminal revealed almost equal band intensity. These results indicate that putative proteolysis might occur in the apoA-I of HDL-3 in the elderly group, as indicated by the arrowhead in Figure 5D, and the fragment might be released to plasma. Consequently, mature HDL-2 particles of the elderly group had less content of apoA-I than the control group, although the apoA-I content in the LPDS fraction was similar between the groups. These
results allow us to postulate that the cleaved apoa-I fragment was released to serum as a lipid-free apoa-I fragment, detected as 17- and 12-kDa bands, as shown in Figure 4B. The truncation of apoa-I in HDL₃ (Figure 5D) may have influenced the reductions in PON and antioxidant activities, causing the displacement of PON from HDL-C and impairing its antioxidant properties (34). Similarly, Eberini and colleagues (35) reported that macrophage matrix metalloproteinase degrades HDL-associated apoa-I at both the N- and the C-terminal ends and the proteolyzed apoa-I may be detached to serum by the proteolytic attack. Moreover, the cleavage of apoa-I is induced by thrombolyis in coronary patients (36).

Furthermore, more multimerized apoa-I bands, proteolytic fragments (Figure 5C and D), and glycated fluorescence products (Figure 5E) were found in the E-HDL similar to the glycated apoa-I multimerization and increased fluorescence (Figure 5A and B) as our recent report (37). Nonenzymatic glycation of protein is a chronic process with aging. The glycation of lipoproteins with accumulated Maillard reaction products can undergo oxidative cleavage, resulting in the formation of AGEs (26).

These results allow us to postulate that the reduced particle size of E-HDL (Figure 6) correlated well with the elevation of CETP activity and cleavage of apoa-I in HDL. Rye and colleagues (38) reported that CETP-mediated reduction of HDL particle size and dissociation of lipid-free apoa-I from HDL are linked to a reduction in particle size (39).

The Saa level was elevated in the serum, and LPDS and HDL₂ fractions from the elderly group, although no difference in the level of expression was found in HDL₃, VLDL, and LDL between the groups. This result is comparable with the previous report that SAA-enriched HDL from the myocardial infarction patients contained increased TG content (40). Although the SAA-enriched HDL had a density comparable with that of HDL₃ from normolipidemic participants, results from the current study and the other study (25) indicate that a coincrease of TG and SAA is associated with chronic inflammation. It is known that the hallmark of acute phase HDL from infection and inflammation is an increase in apoSAA and a reduction in apoa-I content (41). In addition, our research group has reported that acutely increased SAA in plasma is mainly located in HDL₃ from a patient in the oliguric phase of HF (25). As distinct from the acute inflammatory phase, the elderly group showed an increased level of HDL₂-bound SAA and lipid-free SAA in LPDS (Figure 7A). This result indicates that there might be a different mechanism for displacement of apoa-I; specifically, the age-related increase in SAA is bound to HDL₂ rather than to HDL₃. Moreover, this correlates well with the decrease in apoa-I in HDL₂ (Figure 4B and C). An increase in apoC-III was observed in HDL₂ and LPDS, although no significance was detected in the increase of apoC-III in the serum and HDL₃. Coincidentally, SAA and apoC-III were increased in HDL₂ (Figure 7A and B), with a decrease in apoa-I content and particle size reduction of HDL.

In conclusion, there was a loss in serum antioxidant activity, cleavage of apoa-I, an increase in CETP activity, reduction of HDL particle size, and an increase in SAA and apoC-III content in lipoprotein associated with aging, as well as an undesirable change in the serum lipid profile. These alterations may deteriorate beneficial functions of HDL, which are involved in antiatherogenic and antiaging properties in the circulation.

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References


