Enhanced functional and structural properties of high-density lipoproteins from runners and wrestlers compared to throwers and lifters

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Plasma high-density lipoprotein cholesterol (HDL-C) levels are inversely correlated with the risk of cardiovascular disease, and are known to increase with repetitive exercise. In the current study, HDL fractions from athletes' sera were isolated and compared as a function of the type of sport (runners [n = 10], throwers [n = 10], wrestlers [n = 10], and weight lifters [n = 8]), and as an age- and gender-matched reference group (n = 14). Among athletes, HDL from runners had the strongest antioxidant activity. Immunodetection showed that runners and wrestlers had the highest levels of apoA-I and lowest levels of apoA-II in their HDL. Electron microscopy also revealed that HDL2 of runners and wrestlers were the largest in size. In conclusion, although all athlete groups had significantly better serum lipid/lipoprotein profiles than the reference group, runners and wrestlers had the most desirable lipoprotein function and structure, including antioxidant activity, HDL-associated enzyme activities and increased particle size. [BMB reports 2009; 42(9): 605-610]

INTRODUCTION

It is well known that the level of plasma high-density lipoprotein cholesterol (HDL-C) is inversely correlated with the risk of cardiovascular disease (1). HDL-C exerts many beneficial effects for maintaining a healthy physiologic system, including antioxidant, anti-inflammatory and anti-thrombotic activities (2, 3). It has been firmly established that repetitive exercise and physical training reduces the risk of atherosclerosis, coupled with an increase in serum HDL-C (4). Plasma apoA-I and HDL2-C levels are also higher in trained athletes than in sedentary age-matched controls (5). These modifications in blood lipid and lipoprotein cholesterol concentrations may be correlated to changes in lipoprotein size and composition.

Furthermore, it is possible that improvements in the lipid/lipoprotein profile might depend on the type of sport, such as aerobic vs. anaerobic exercise (6). Generally, aerobic exercise significantly improves serum lipid and lipoprotein profiles; specifically, aerobic exercise lowers the level of serum total cholesterol (TC) and triacylglycerol (TG) while increasing the level of HDL-C (7). Thus, we hypothesize there might be a difference in lipid/lipoprotein metabolism depending on the type of sport, since physical exercise is closely correlated with reverse cholesterol transport as well as the size and composition of HDL-particles (8). There have been no reports comparing the elevated levels of individual lipoproteins, especially the lipid and apolipoprotein content of LDL and HDL. HDL has two major subclasses, HDL2 and HDL3. HDL2 are less dense, larger in particle size and cholesterol- and phospholipid-enriched, whereas HDL3 are more dense, relatively smaller in size and protein-enriched. Some clinical data suggest that HDL2 and HDL3 concentrations are reduced in patients with coronary artery disease (CAD), with the reduction in HDL2 being proportionally greater than HDL3 (9). The principal objective of this study was to compare the lipid/lipoprotein profiles, HDL-associated enzyme activity, antioxidant ability and HDL particle size among national-class male athletes as a function of their primary type of sport, such as middle distance runners (1,500 m), throwers (hammer), wrestlers and weight-lifters.

RESULTS

Serum parameters

Regardless of the type of sport and BMI, athletes’ sera contained significantly lower TC and TG concentrations than the sera of the reference group, as shown in Table 1. All athletes had similar levels of TC and TG, with approximate means of 147 ± 18 mg/dl and 79 ± 50 mg/dl, respectively. In comparison, the reference values were 172 ± 22 mg/dl and 84 ± 20 mg/dl for TC and TG, respectively. HDL-C levels in runners and wrestlers were significantly higher (52 ± 5 and 58 ± 6 mg/dl, respectively), constituting 35-37% of the TC, whereas throwers and lifters had levels of approximately 31% and the reference group had levels of only 24%. The sera of all athletes
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Table 1. Serum parameters of athletes and references

<table>
<thead>
<tr>
<th></th>
<th>Age (yr)</th>
<th>BMI (kg/m²)</th>
<th>TC (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>HDL-C/TC (%)</th>
<th>TG (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>GOT (U/L)</th>
<th>GPT (U/L)</th>
</tr>
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<tbody>
<tr>
<td>Running¹ (n = 10)</td>
<td>24 ± 3.9</td>
<td>19.7 ± 1.2a</td>
<td>149 ± 21a</td>
<td>52 ± 5a</td>
<td>36 ± 6a</td>
<td>56 ± 17a</td>
<td>88 ± 16a</td>
<td>22 ± 4b</td>
<td>13 ± 4a</td>
</tr>
<tr>
<td>Throwing² (n = 10)</td>
<td>20 ± 0.0</td>
<td>27.6 ± 2.5b</td>
<td>142 ± 18a</td>
<td>44 ± 7b</td>
<td>31 ± 6b</td>
<td>79 ± 37b</td>
<td>82 ± 17a</td>
<td>25 ± 5b</td>
<td>15 ± 5b</td>
</tr>
<tr>
<td>Wrestling (n = 10)</td>
<td>20 ± 0.4</td>
<td>22.9 ± 1.8a</td>
<td>155 ± 23a</td>
<td>58 ± 6b</td>
<td>38 ± 3a</td>
<td>67 ± 18a</td>
<td>84 ± 16a</td>
<td>19 ± 3b</td>
<td>9 ± 2b</td>
</tr>
<tr>
<td>Lifting (n = 8)</td>
<td>21 ± 0.5</td>
<td>29.3 ± 3.8b</td>
<td>141 ± 10a</td>
<td>44 ± 13b</td>
<td>31 ± 8b</td>
<td>54 ± 19a</td>
<td>79 ± 6a</td>
<td>29 ± 10a</td>
<td>19 ± 10a</td>
</tr>
<tr>
<td>Reference (n = 14)</td>
<td>22 ± 3.5</td>
<td>21.5 ± 2.7a</td>
<td>172 ± 22b</td>
<td>42 ± 5b</td>
<td>24 ± 2c</td>
<td>84 ± 20b</td>
<td>98 ± 22b</td>
<td>26 ± 10a</td>
<td>20 ± 8a</td>
</tr>
</tbody>
</table>

C, cholesterol; GOT, glutamic oxaloacetic transaminase; GPT, gamma-glutamic pyruvic transaminase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride. Middle distance (1500 m), Hammer-throwing. Means that are not labeled by a common letter (superscript a, b, and c) are significantly different between the each group (P < 0.05).

Wrestlers and throwers had the second highest activity, with a 243% increase in absorbance. Lifters showed the weakest antioxidant activity (a 169% increase), whereas the reference group exhibited only a 130% increase. Taken together, these results indicate that the antioxidant activity of HDL₃ does not depend on the body mass index (BMI), as evidenced by throwers having higher antioxidant activity than lifters.

**LCAT activity and protein expression in HDL₃**
Although HDL₃ from athletes had higher lecithin : cholesterol acyltransferase (LCAT) activity than the reference group (14.5 ± 1.0% cholesteryl ester [CE]-conversion), it was runners and wrestlers who demonstrated the most potent activities with 25 ± 3.5 and 24 ± 1.5% CE-conversions, respectively. These increased activities were comparable to those of the throwers and lifters (Suppl. Fig. 1), which had 18 ± 2.5% and 16 ± 1.5% CE-conversions, respectively, based on an identical quantity of HDL₃ (100 μg of total protein). Western blotting with HDL₃ (6 μg per lane) showed that HDL₃ expression of all athletes was 20-40% higher than the reference group members (bottom photo of Suppl. Fig. 1).

**Paraoxonase (PON) activity and protein expression**
HDL₃ obtained from athletes demonstrated higher PON activity than that of reference HDL₃, indicating superior antioxidant activity. Wrestlers and runners had the highest PON activities, 14.5 ± 3.5 U/L and 9.6 ± 2.0 U/L, respectively, which were 20-fold greater than the reference group (Fig. 2A). However, the PON activities of the throwers and lifters were only 4.2 ± 0.9 and 2.7 ± 0.9 U/L, respectively. PON-1 protein expression was the highest in runners (BI = 3.0), closely followed by wrestlers (BI = 2.8). However, in general, athletes had higher PON activity than did the reference group (BI = 1.0, Fig. 2A). This closely correlates with the results generated by the FRA assay that found the antioxidant activity of HDL₃ is highly de-
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Fig. 2. HDL-associated enzyme activity assay. Data are expressed as the mean ± SD from three independent experiments per group with triplicate samples. Means not labeled by a common letter (a-c) are significantly different between each group (P < 0.05). (A) Paraoxonase activity was determined using equally diluted HDL3. The bottom photo shows the immunodetected band of PON from HDL3 (6 μg per lane). (B) CE-transfer assay. The same amount of HDL1 (100 μg of protein) was utilized as a CETP source. The bottom photo shows the immunodetected band of CETP from HDL1 (6 μg per lane).

Cholesteryl ester transfer protein (CETP) activity and protein expression
After 2 and 4 hours of incubation, the CETP activity of HDL3 obtained from the athletes was severely reduced, as shown in Fig. 2B. In addition, with the exception of wrestlers (8% CE-transfer), the CE-transfer activity of all athletes was <3% after 4 hours of incubation. However, the CE-transfer activity of the reference group was 22%, using an identical quantity of HDL1 (50 μl [2 mg/ml of protein]) as the CETP source. In addition, immunodetection with HDL3 revealed that the CETP band produced from the athletes’ sera was almost 10-fold lower in intensity (BI = 0.1-0.2) than that of the reference sera (BI = 1.0).

Levels of expression of apoA-I, A-II, and B-48
As shown in Fig. 3, immunodetection revealed that the HDL2 of the wrestler group had the highest level of apoA-I (BI = 2.6). However, all athletes had higher levels of expression than the reference group. In the case of HDL3, runners had the highest levels of apoA-I (BI = 3.2) compared to the other athletes and reference group (BI = 1.0). The wrestler group showed the highest and 2nd highest apoA-I levels in the HDL2 and HDL3 fractions, respectively.

However, the apoA-II band in HDL3 obtained from athletes was less intense than that of the reference group (BI = 1.0). Among all athletes, runners and wrestlers had the lowest level of apoA-II expression in HDL3 (BI = 0.4 and 0.2, respectively), compared to lifters who had the highest level of apoA-II. Similarly, all athletes had a lower level of apoB-48 expression, with 60% less band intensity than the reference group. These
results indicate that runners and wrestlers had the highest levels of apoA-I expression and the lowest levels of apoA-II expression in the HDL-C species.

Runners and wrestlers had a larger HDL₂ particle size
HDL₂ from all the athletes and reference groups had the shape as shown in Suppl. Fig. 2. Specifically, HDL₂ from wrestlers were the largest in size (46 ± 5 nm in length and 26 ± 4 nm in width), followed by the runners (45 ± 6 nm in length and 25 ± 3 nm in width). Among athletes, the lifters had the smallest HDL₂ particles (33 ± 5 nm in length and 17 ± 10 nm in width), although all of the athletes contained larger HDL₂ particles than the reference group (28 ± 4 nm in length and 23 ± 4 nm in width). It is interesting to note that HDL₂ obtained from the runners and wrestlers were enriched with well-developed particles. This result indicates that repetitive aerobic exercise can increase HDL particle size, apoA-I concentration, HDL-C and antioxidant activity regardless of the type of sport or BMI.

DISCUSSION
In the current study, all athletes demonstrated improved lipid and lipoprotein profiles, reduced serum TC and TG levels and increased HDL-C and HDL particle sizes compared to the reference group. These beneficial changes in lipoprotein parameters were fairly consistent with a study conducted by Williams et al. (11) that revealed prominent increases in the HDL-C levels of long-distance runners.

Since HDL₁ is a protein-enriched lipoprotein, it possesses a high level of enzymatic activity. For example, HDL₁ is the principal source of LCAT activity. According to Jonas group (12), HDL₁ has 1.3% reactivity while HDL₂ has 16% reactivity.

PON is an important enzyme that contributes to the antioxidant function of HDL. Runners and wrestlers contained HDL₁ with significantly higher enzyme activity and expression than did throwers and lifters. Indeed, lifters exhibited the lowest enzyme activity and expression of PON among all athletes, although both factors were higher than those observed in the reference group. These weak LCAT and PON activities in the lifter group may have contributed to the lower FRA ability measured in the athletes, as shown in Fig. 2 and Suppl. Fig. 1.

The net effect of CETP on HDL-C is the depletion of CE and enrichment of TG, which causes an overall reduction in the size of the HDL-C particle (13). Therefore, it has been hypothesized that inhibition of CETP increases the level of HDL-C. However, evidence pertaining to the relationship between exercise and CETP with respect to mass and activity is debatable. Ochiwaha et al. previously reported no differences in either CETP mass or activity between athletes and reference group members (14). The results of the current study indicate that the activity and mass of CETP in HDL₁ are significantly decreased in athletes compared to the reference group (Fig. 2B). Although there may have been differences between the Ochiwaha et al. study (14) and the current one with respect to blood sampling method, the type of sport (primarily triathletes vs. runners, throwers, wrestlers and lifters) and the average age (33.6 ± 1.1 years vs. 21.3 ± 2.3 years) showed that CETP activity was significantly reduced in all groups of athletes. These results provide evidence that CETP activity can be reduced by chronic, repetitive exercise regardless of BMI or age. This finding is consistent with the conclusions made by Seip et al. (15), who showed that exercise reduced plasma CETP in a sedentary senior group (60-72 years of age) when compared to pre-training levels.

Since the function of HDL is greatly influenced by the relative composition of apoA-I : apoA-II, ratios of apoA-I : apoA-II in HDL₁ and HDL₃ were compared by immunodetection, as shown in Fig. 3. Interestingly, apoA-II was not detected in HDL₁ obtained from runners, while HDL₂ from lifters showed the highest level of expression. These results are in good agreement with other functional studies regarding antioxidant and anti-atherosclerotic activities (Fig. 1 and 2). Several coincidences were observed regarding the expression pattern of apolipoprotein in terms of HDL and particle size. For example, regarding runners and wrestlers, apoA-I levels were significantly increased while apoA-II levels were reduced in HDL-C, compared to the other athletes and reference group (Fig. 3). Regarding throwers and lifters, the relative elevation of apoA-II in HDL₁ and/or HDL₃ could have caused reductions in PON and antioxidant activities, as apoA-II enrichment displaces PON from HDL-C and impairs its antioxidant properties (16). In addition, a recent report from our research group found that an obese Caucasian patient with very low blood TC and apoA-I levels possessed HDL₂ of increased size and no evidence of atherosclerosis (17). Taken together, it appears that a reduction of apoA-II in HDL-C exerts beneficial effects in the form of reverse cholesterol transport, and enhances antioxidant activity by stimulating LCAT and PON activity.

These results suggest that athletes engaging in aerobic and dynamic exercises, namely runners, exhibited the most desirable lipid/lipoprotein and HDL profiles, i.e., enhanced activities and expression of LCAT and PON, decreased CETP activity, increased apoA-I levels and larger HDL particle size. These results show that exhausting the oxygen supply during exercise may improve the function and quality of HDL, an event possibly associated with increased anti-atherogenic potential. Interestingly, wrestlers showed similar results to runners despite the fact that wrestling is classified as a static exercise. Unlike lifters and throwers, wrestlers engage in aerobic exercise for training and weight control, as reflected by the general physiologic profiles of successful wrestlers which include both high anaerobic capacity and sufficient aerobic power (18). Indeed, treadmill testing for average VO₂max values found that wrestlers have enhanced VO₂max values between 52 and 63 ml/kg/min (18), whereas elite middle distance runners score between 68 ml/kg/min and 77 ml/kg/min (19). This difference may contribute to the physiologic differences between

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wrestlers and lifters. Physiological differences between wrestlers and lifters may be due to differences in their BMI. Further research is necessary to elucidate the underlying physiological mechanism inherent in the serum HDL of wrestlers when compared with other aerobic sports, including swimmers and long-distance runners.

In conclusion, athletes engaging in aerobic and dynamic exercises (runners and wrestlers) exhibit the most desirable lipid/lipoprotein and HDL-C profiles, i.e., enhanced LCAT and PON enzyme activities and expression, increased apoA-I levels and larger HDL-particle size.

MATERIALS AND METHODS

Blood sampling
National class male athletes were recruited from the following representative sports: running (1,500 m middle distance, n = 10), throwing (hammer, n = 10), wrestling (n = 10), and weightlifting (n = 8). All athletes in this study were enrolled at the Korea National Sport University (Seoul, Korea) at the time of the study and had been training in their respective sports for at least 6 years, exercising at least 8 hours per day. Age- and gender-matched sedentary reference subjects (n = 14) were recruited from healthy volunteers who visited the Health Center of Samsung Medical Center (Seoul, South Korea) for regular health examinations. Although they had been doing regular exercise with moderate intensity less than 1 hr per week, they had unremarkable medical records without a history of endocrinologic disorders.

All athletes and reference individuals were healthy, with unremarkable medical records. None of the individuals enrolled in this study had a history of taking lipid-lowering medications, excessive alcohol consumption or smoking. Subjects fasted for 12 hours prior to blood sampling. Informed consent was obtained from all of the athletes and reference individuals, and the protocol of this study was approved by the Institutional Review Board of Samsung Medical Center.

Isolation of lipoproteins
Very low-density lipoproteins (VLDL, d ≤ 1.019 g/ml), low-density lipoproteins (LDL, 1.019 ≤ d ≤ 1.063) and high-density lipoproteins (HDL2, 1.063 ≤ d ≤ 1.125; and HDL3, 1.125 ≤ d ≤ 1.225) were isolated from sera via sequential ultracentrifugation as previously described (20) using Himac CP-90tx (Hitachi, Tokyo, Japan) at the Instrumental Analysis Center at Yeungnam University.

Determination of serum lipids and proteins
Serum parameters, lipids and glucose concentrations were measured with an automatic blood analyzer (Fuji DRI-CHEM, FDC-3000; Tokyo, Japan). Protein concentrations were determined using Lowry protein assays, as modified by Markwell et al. (21), and the Bradford assay reagent (BioRad, Hercules, CA, USA), using bovine serum albumin as a standard.

CE conversion assay
CE conversion was conducted via LCAT assays, as previously described (22). Fifty μl of HDL2 (2 mg/ml of protein) was utilized as the enzyme source. Discoidal rHDL was prepared via sodium cholate dialysis using the initial molar ratios of palmitoyloleoyl phosphatidylcholine (POPC)-cholesterol-apoA-I-sodium cholate at 95 : 5 : 150 (wt/wt/wt) (23).

CETP assay
An rHDL containing apoA-I and cholesteryl oleate was synthesized according to previously described methods (24) with trace amounts of (1H)-cholesteryl oleate (TRK886, 3.5 μCi/mg of apoA-I; GE Healthcare).

PON assay
PON-1 activity was measured after paraoxon hydrolysis into p-nitrophenol and diethylphosphate, as catalyzed by the enzyme associated with HDL-C (25).

Electrophoresis and western blot
The apolipoprotein/lipoprotein compositions were compared via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with identical quantities of pooled aliquot from each group used as protein loading (6 μg of total protein per lane). The levels of apolipoprotein expression were analyzed by immunodetection. Anti-human apoA-I antibody (AB820) and anti-apo-B antibody (AB742) were purchased from Chemicon (Temecula, CA, USA). Anti-human apoA-I antibody, CETP antibody, LCAT antibody and anti-paraoxonase antibody were purchased from Abcam (Cambridge, UK). The relative band intensity (BI) was compared via band scanning with a Gel Doc XR (Bio-Rad) using Quantity One software, version 4.5.2.

Ferric reducing ability of serum assay
The ferric reducing ability (FRA) was determined as previously described by Benzie and Strain (10).

Electron microscopy
Transmission electron microscopy (TEM) was performed using a Hitachi electron microscope (H-7600 model; Ibaraki, Japan) operating at 80 kV. HDL2 and HDL3 were negatively stained with 1% sodium phosphotungstate (PTA; pH 7.4) with a final apolipoprotein concentration of 0.3 mg/ml in TBS as previously described (17).

Statistics
All data are expressed as the mean ± S.D. The data were evaluated via two-way variance analysis (ANOVA) using an SPSS program (version 14.0; SPSS, Inc. Chicago, IL, USA). The differences between the means were assessed using Duncan’s multiple range test. Statistical significance was defined as a P < 0.05.
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REFERENCES


