BMB
*reports*

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

*Hwahyung Lee*[1], *Jeong-Euy Park*[2], *Inho Choi*[1] *& Kyung-Hyun Cho*[1,*]

[1]School of Biotechnology, Yeungnam University, Gyeongsan 712-749, [2]Division of Cardiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 440-746, Korea

**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 $HDL_2$ 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 $HDL_2$-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 possibile 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, $HDL_2$ and $HDL_3$. $HDL_2$ are less dense, larger in particle size and cholesterol- and phospholipid-enriched, whereas $HDL_3$ are more dense, relatively smaller in size and protein-enriched. Some clinical data suggest that $HDL_2$ and $HDL_3$ concentrations are reduced in patients with coronary artery disease (CAD), with the reduction in $HDL_2$ being proportionally greater than $HDL_3$ (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.

*Corresponding author. Tel: 82-53-810-3026; Fax: 82-53-814-3026; E-mail: chok@yu.ac.kr

**Received 31 December 2008, Accepted 24 April 2009**

**Keywords:** Antioxidant, Apolipoproteins, Cholesteryl ester transfer protein, High-density lipoproteins

## 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

**Table 1.** Serum parameters of athletes and references

| | Age (yr) | BMI ($kg/m^2$) | TC (mg/dl) | HDL-C (mg/dl) | HDL-C/TC (%) | TG (mg/dl) | LDL-C (mg/dl) | GOT (U/L) | GPT (U/L) |
|---|---|---|---|---|---|---|---|---|---|
| Running[1] (n = 10) | 24 ± 3.9 | 19.7 ± 1.2[a,3] | 149 ± 21[a] | 52 ± 5[a] | 36 ± 6[a] | 56 ± 17[a] | 88 ± 16[a] | 22 ± 4[a,b] | 13 ± 4[a] |
| Throwing[2] (n = 10) | 20 ± 0.0 | 27.6 ± 2.5[b] | 142 ± 18[a] | 44 ± 7[b] | 31 ± 6[b] | 79 ± 37[a,b] | 82 ± 17[a] | 25 ± 5[a] | 15 ± 5[a] |
| Wrestling (n = 10) | 20 ± 0.4 | 22.9 ± 1.8[a] | 155 ± 23[a] | 58 ± 6[a] | 38 ± 3[a] | 67 ± 18[a] | 84 ± 16[a] | 19 ± 3[b] | 9 ± 2[b] |
| Lifting (n = 8) | 21 ± 0.5 | 29.3 ± 3.8[b] | 141 ± 10[a] | 44 ± 13[b] | 31 ± 8[b] | 54 ± 19[a] | 79 ± 6[a] | 29 ± 10[a] | 19 ± 10[a] |
| Reference (n = 14) | 22 ± 3.5 | 21.5 ± 2.7[a] | 172 ± 22[b] | 42 ± 5[b] | 24 ± 2[c] | 84 ± 20[b] | 98 ± 22[b] | 26 ± 10[a] | 20 ± 8[a] |

C, cholesterol; GOT, glutamic oxaloacetic transaminase; GPT, gamma-glutamic pyruvic transaminase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride. [1]Middle distance (1500 m), [2]Hammer-throwing, [3]Means that are not labeled by a common letter (superscript a, b, and c) are significantly different between the each group ($P < 0.05$).

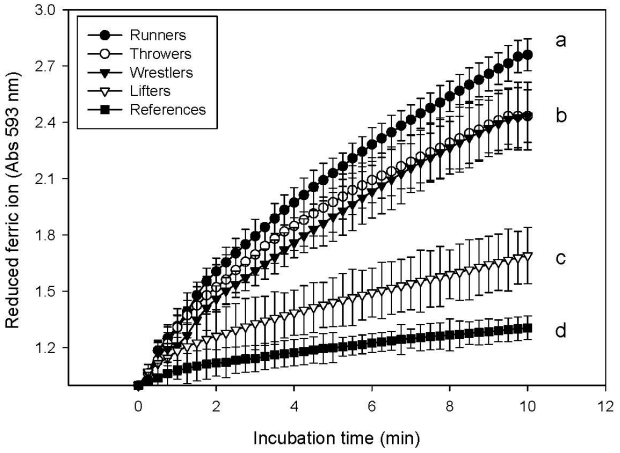


**Fig. 1.** Comparison of ferric reducing ability (FRA) of HDL from athletes and reference group. Data are expressed as the mean ± SD from three independent experiments per group with triplicate samples. Means not labeled by a common letter (a-d) are significantly different between each group ($P < 0.05$) after 10 min of incubation.

and reference group members had levels of similar serum glutamic oxaloacetic transaminase (GOT), gamma-glutamic pyruvic transaminase (GPT) and glucose, although wrestlers had significantly lower GOT and GPT values than the other groups.

### Ferric reducing ability as antioxidant activity

The ferric reducing ability (FRA) assay is a rapid and reproducible method for determining the molar concentrations of existing antioxidants (10). We performed the FRA assay separately for each group using the same concentration of $HDL_3$ (2 mg/ml) to compare antioxidant effects. As shown in Fig. 1, $HDL_3$ from runners had the highest antioxidant activity, demonstrated by a 276% increase in absorbance at 593 nm ($A_{593}$). 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 $HDL_3$ 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 $HDL_3$

Although $HDL_3$ 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 $HDL_3$ (100 μg of total protein). Western blotting with $HDL_3$ (6 μg per lane) showed that $HDL_3$ 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

$HDL_3$ obtained from athletes demonstrated higher PON activity than that of reference $HDL_3$, 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 $HDL_3$ is highly de-

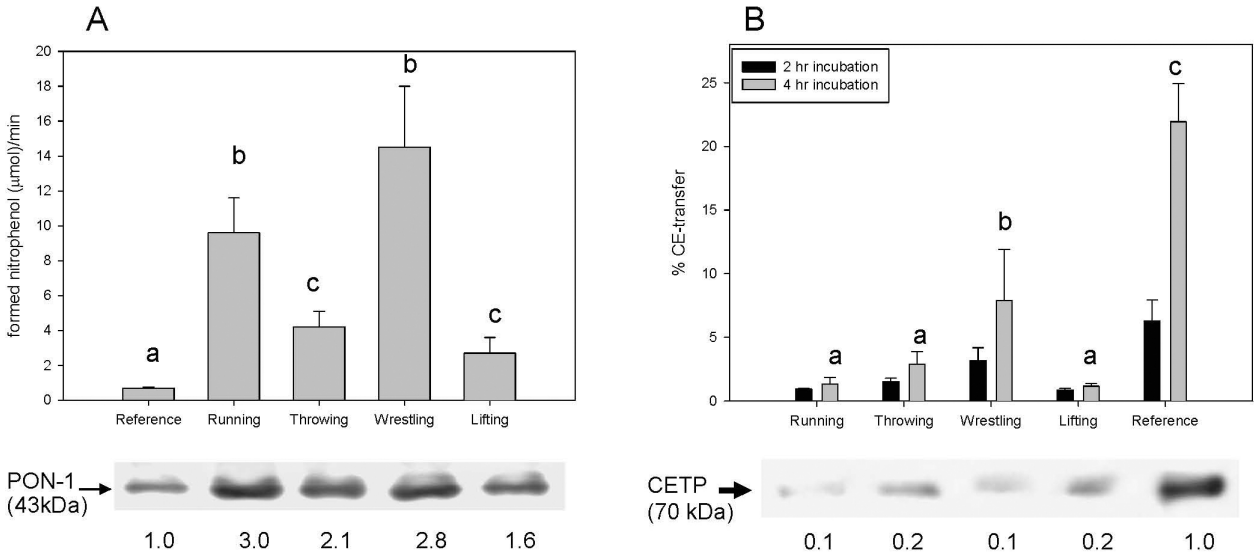


**Fig. 2.** HDL-associated enzyme activity assay. Data are expressed as the mean ± SD from three independent experiments per group with triplicate samples. Means not labeled by a common letter (a-c) are significantly different between each group ($P < 0.05$). (A) Paraoxonase activity was determined using equally diluted $HDL_3$. The bottom photo shows the immunodetected band of PON from $HDL_3$ (6 μg per lane). (B) CE-transfer assay. The same amount of $HDL_3$ (100 μg of protein) was utilized as a CETP source. The bottom photo shows the immunodetected band of CETP from $HDL_3$ (6 μg per lane).

pendent on the HDL-associated enzymes LCAT and PON, with respect to activity and protein expression.

### Cholesteryl ester transfer protein (CETP) activity and protein expression

After 2 and 4 hours of incubation, the CETP activity of $HDL_3$ 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 $HDL_3$ (50 μl [2 mg/ml of protein]) as the CETP source. In addition, immunodetection with $HDL_3$ 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 $HDL_2$ 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 $HDL_3$, 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 $HDL_2$ and $HDL_3$ fractions, respectively.

However, the apoA-II band in $HDL_3$ 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

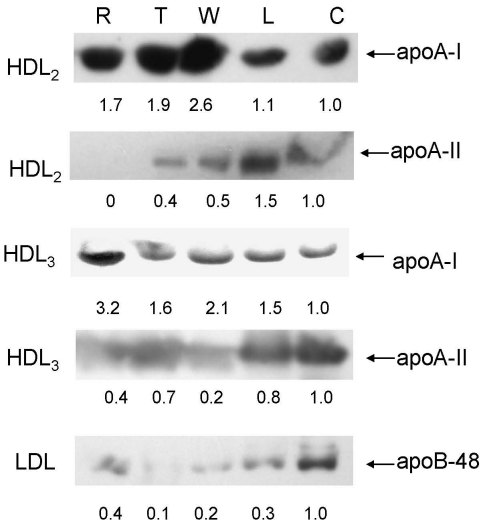


**Fig. 3.** Change in apolipoprotein A-I and A-II expression levels by immunodetection. Equally pooled and diluted HDL protein (6 μg of protein/lane) and LDL protein (4 μg of protein/lane) were loaded onto 12% and 8% polyacrylamide gels, respectively. The lower numbers indicate the relative intensity of the detected band as compared with control sera.

of apoA-II expression in $HDL_3$ (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 $HDL_2$ particle size**

$HDL_2$ from all the athletes and reference groups had the shape as shown in Suppl. Fig. 2. Specifically, $HDL_2$ 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 $HDL_2$ particles (33 ± 5 nm in length and 17 ± 10 nm in width), although all of the athletes contained larger $HDL_2$ particles than the reference group (28 ± 4 nm in length and 23 ± 4 nm in width). It is interesting to note that $HDL_2$ 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 (11) that revealed prominent increases in the HDL-C levels of long-distance runners.

Since $HDL_3$ is a protein-enriched lipoprotein, it possesses a high level of enzymatic activity. For example, $HDL_3$ is the principal source of LCAT activity. According to Jonas group (12), $HDL_2$ has 1.3% reactivity while $HDL_3$ has 16% reactivity.

PON is an important enzyme that contributes to the antioxidant function of HDL. Runners and wrestlers contained $HDL_3$ 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. Olchawa *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 $HDL_3$ are significantly decreased in athletes compared to the reference group (Fig. 2B). Although there may have been differences between the Olchawa *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 pretraining 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 $HDL_2$ and $HDL_3$ were compared by immunodetection, as shown in Fig. 3. Interestingly, apoA-II was not detected in $HDL_2$ obtained from runners, while $HDL_2$ 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 $HDL_2$ and/or $HDL_3$ 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-II levels possessed $HDL_2$ 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 $VO_{2max}$ values found that wrestlers have enhanced $VO_{2max}$ 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

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 endocronologic 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, $d \leq 1.019$ g/ml), low-density lipoproteins (LDL, $1.019 \leq d \leq 1.063$) and high-density lipoproteins ($HDL_2$, $1.063 \leq d \leq 1.125$; and $HDL_3$, $1.125 \leq d \leq 1.225$) were isolated from sera via sequential ultracentrifugation as previously described (20) using Himac CP-90α (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 $HDL_3$ (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 : 1 : 150 (wt/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 ($^3$H)-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-polyacylamide 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-II 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. $HDL_2$ and $HDL_3$ 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 $P < 0.05$.

**Acknowledgements**
This research was supported by Yeungnam University research grants in 2007.

## REFERENCES

1. Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B. and Dawber, T. R. (1977) High density lipoprotein as a protective factor against coronary heart disease. *Am. J. Med.* **62**, 707-714.
2. Lewis, G.F. and Rader, D.J. (2005) New insights into the regulation of HDL metabolism and reverse cholesterol transport. *Circ. Res.* **96**, 1221-1232.
3. Barter, P. J., Nicholls, S., Rye, K. A., Anantharamaiah, G. M., Naval, M. and Fogelman, A. M. (2004) Antiinflammatory properties of HDL. *Circ. Res.* **95**, 764-772.
4. Kraus, W. E., Houmard, J. A., Duscha, B. D., Knetzger, K. J., Wharton, M. B., McCartney, J. S., Bales, C. W., Henes, S., Samsa, G. P., Otvos, J. D., Kulkarni, K. R. and Slentz, C. A. (2002) Effects of the amount and intensity of exercise on plasma lipoproteins. *N. Engl. J. Med.* **347**, 1483-1492.
5. Sasaki, J., Tanabe, Y., Tanaka, H., Saku, K., Shindo, M. and Arakawa, K. (1988) Elevated levels of HDL2-cholesterol and apoA-I in national class Japanese male marathon runners. *Atherosclerosis* **70**, 175-177.
6. Despres, J. P., Lamarche, B., Bergeron, J., Gagnon, J., Leon, A. S., Rao, D. C., Skinner, J. S., Wilmore, J. H. and Bouchard, C. (2001) Effects of endurance exercise training on plasma HDL cholesterol levels depend on levels of triglycerides: evidence from men of the health, risk factors, exercise training and genetics (HERITAGE) family study. *Arterioscler. Thromb. Vasc. Biol.* **21**, 1226-1232.
7. Kelley, G. A. and Kelley, K. S. (2006) Aerobic exercise and lipids and lipoproteins in men: a meta-analysis of randomized controlled trials. *J. Mens Health Gend.* **3**, 61-70.
8. Leaf, D. A. (2003) The effect of physical exercise on reverse cholesterol transport. *Metabolism* **52**, 950-957.
9. Avogaro, P., Cazzolato, G., Bittolo Bon, G. and Belussi, F. (1979) Levels and composition of $HDL_2$ and $HDL_3$ and other major lipoprotein classes in survivors of myocardial infarction. *Artery* **5**, 495-503.
10. Benzie, I. F. and Strain, J. J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Anal Biochem.* **239**, 70-76.
11. Williams, P. T. (1996) High-density lipoprotein cholesterol and other risk factors for coronary heart disease in female runners. *N. Engl. J. Med.* **334**, 1298-1303.
12. Kosek, A. B., Durbin, D. and Jonas, A. (1999) Binding affinity and reactivity of lecithin cholesterol acyltransferase with native lipoproteins. *Biochem. Biophys. Res. Commun.* **258**, 548-551.
13. Morton, R. E. (1999) Cholesteryl ester transfer protein and its plasma regulator: lipid transfer inhibitor protein. *Curr. Opin. Lipidol.* **10**, 321-327.
14. Olchawa, B., Kingwell, B. A., Hoang, A., Schneider, L., Miyazaki, O., Nestel, P. and Sviridov, D. (2004) Physical fitness and reverse cholesterol transport. *Arterioscler Thromb. Vasc. Biol.* **24**, 1087-1091.
15. Seip, R. L., Moulin, P., Cocke, T., Tall, A., Kohrt, W. M., Mankowitz, K., Semenkovich, C. F., Ostlund, R. and Schonfeld, G. (1993) Exercise training decreases plasma cholesteryl ester transfer protein. *Arterioscler Thromb.* **13**, 1359-1367.
16. Ribas, V., Sanchez-Quesada, J. L., Anton, R., Camacho, M., Julve, J., Escola-Gil, J. C., Vila, L., Ordonez-Llanos, J. and Blanco-Vaca, F. (2004) Human apolipoprotein A-II enrichment displaces paraoxonase from HDL and impairs its antioxidant properties: a new mechanism linking HDL protein composition and antiatherogenic potential. *Circ. Res.* **95**, 789-797.
17. Park, S. H., Kim, J. R., Park, J. E. and Cho, K. H. (2007) A Caucasian male with very low blood cholesterol and low apoA-II without evidence of atherosclerosis. *Eur. J. Clin. Invest.* **37**, 249-256.
18. Horswill, C. A. (1992) Applied physiology of amateur wrestling. *Sports Med.* **14**, 114-143
19. Boileau, R. A., Mayhew, J. L., Riner, W. F. and Lussier, L. (1982) Physiological characteristics of elite middle and long distance runners. *Can. J. Appl. Sports Sci.* **7**, 167-172
20. Havel, R. J., Eder, H. A. and Bragdon, J. H. (1955) The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34**, 1345-1353
21. Markwell, M. A., Haas, S. M., Bieber, L. L. and Tolbert, N. E. (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem.* **87**, 206-210.
22. Matz, C. E. and Jonas, A. (1982) Micellar complexes of human apolipoprotein A-I with phosphatidylcholines and cholesterol prepared from cholate-lipid dispersions. *J. Biol. Chem.* **257**, 4535-4540.
23. Han, J. M., Jeong, T. S., Lee, W. S., Choi, I. and Cho, K. H. (2005) Structural and functional properties of V156K and A158E mutants of apolipoprotein A-I in the lipid-free and lipid-bound states. *J. Lipid Res.* **46**, 589-596.
24. Cho, K. H., Lee, J. Y., Choi, M. S., Cho, J. M., Lim, J. S. and Park, Y. B. (1998) A peptide from hog plasma that inhibits human cholesteryl ester transfer protein. *Biochim. Biophys. Acta.* **1391**, 133-144.
25. Eckerson, H. W., Wyte, C. M. and La Du, B. N. (1983) The human serum paraoxonase/arylesterase polymorphism. *Am. J. Hum. Genet* **35**, 1126-1138.