# Walk-Run Training Improves the Anti-Inflammation Properties of High-Density Lipoprotein in Patients With Metabolic Syndrome

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**Context:** Metabolic syndrome (MetS) is a constellation of cardiovascular risk factors, including central obesity, dysglycemia, hypertension, and dyslipidemia. The anti-inflammatory properties of high density lipoprotein (HDL) can be compromised in MetS. Exercise is recognized as an important factor in the prevention and treatment of MetS.

**Objective:** This study was designed to investigate whether walk/run training without any specific diet could enhance anti-inflammation capacity of HDL from MetS patients.

**Design:** This was a case control study.

**Setting:** The study was conducted in a Zhoudian community, Taian.

**Patients:** Thirty nine patients with MetS were recruited and divided into a control group (n = 12) remaining in an untrained state and exercise group (n = 27) performing a 10-week walk/run training program.

**Main Outcome Measures:** The anti-inflammation capacities of HDL3 (HDL subfractions) from MetS patients with or without exercise were investigated by co-incubating with TNF- $\alpha$-injured endothelial cells in vitro.

**Results:** The training did not influence serum lipoprotein level in MetS patients and cholesterol efflux capacity of circulating HDL. However, walk/run training increased paraoxonase-1 (PON1) activity and decreased the levels of malondialdehyde in either serum or isolated HDL from MetS patients prominently. More importantly, HDL3 isolated from MetS patients with 10 weeks training protected endothelial cells against tumor necrosis factor-a (TNF-a) -induced injury, decreased monocyte chemotactic protein-1 levels in media and vascular cell adhesion molecule-1 expression markedly. Furthermore, HDL3 isolated from MetS patients with walk/run training inhibited the TNF-á-induced monocyte adhesion to endothelial cells and obviously increased nitric oxide production by activating endothelial nitric oxide synthase.

**Conclusion:** Walk/run training leads to a significant improvement in HDL anti-inflammation capacity in subjects with MetS without restricted diet, the mechanism underlying which at least partially is due to increased PON1 activity in HDL, NO production, and eNOS expression in endothelial cells. (***J Clin Endocrinol Metab*** **100: 870–879, 2015)**

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Abbreviations: Apo, apolipoprotein; eNOS, endothelial nitric oxide synthase; FPLC, fast protein liquid chromatography; HUVECs, human umbilical vein endothelial cells; LDL-C, low-density lipoprotein C; MDA, malondialdehyde; MCP-1, monocyte chemotactic protein-1; NO, nitric oxide; PON1, paraoxonase-1; SAA, serum amyloid A; SOD, superoxide dismutase; SR-BI, scavenger receptor B type I; TG, triglycerides; VCAM-1, vascular cell adhesion molecule-1.

Metabolic syndrome (MetS) is a constellation of cardiovascular risk factors, including central obesity, glucose intolerance, hypertension, and dyslipidemia (1). Each component of MetS predisposes people to atherosclerosis, and when these components cluster together, they promote atherosclerosis even more prominently (2, 3). High-density lipoprotein (HDL) possesses key cardioprotective function, including reverse cholesterol transport (RCT), antioxidant, anti-inflammatory, antiapoptotic, and antithrombotic properties. Prospective studies have revealed that coronary heart disease (CHD) risk is elevated by 3% in women and 2% in men for each decrement of 1 mg/dL in HDL (4). Therefore, HDL-based therapies have long been focused on increasing the plasma HDL levels (5). Nevertheless, emerging evidence suggests that HDL function is not always accurately predicted by HDL levels. In healthy individuals, HDL is anti-inflammatory. But in those with chronic illnesses that are characterized by systemic oxidative stress and inflammation, HDL is susceptible to chemical modification including oxidation, glycation or homo-cysteinylation and may lose its beneficial role against atherosclerosis, even exhibits proatherogenic effect, such as induction of endothelial cell apoptosis and proinflammatory activity (6, 7). Therefore, MetS is characterized not only by elevated cardiovascular risk and low HDL levels but also by defective HDL function (3, 8).

Exercise is recognized to play an important role in the prevention and treatment of MetS (2, 9) and also prescribed as a modality to increase HDL level. Frequent aerobic exercise has been shown to increase HDL levels by approximately 5% as early as 2 months from start of regular exercise in sedentary (2). However, it is plausible that exercise-induced improvements in HDL function may be more important than changes in HDL levels. Roberts et al found a high-fiber, low-fat diet, and three weeks of aerobic exercise improved the anti-inflammatory function of HDL in obese men (10). The previous studies showed that increasing HDL required a major amount of exercise (2, 9), which may be not suitable for all populations, such as the old or obese subjects. The data in the field are not clear that lower-intensity/duration exercise improves relevant aspects of HDL. The transition exercise between walking and running has been extensively investigated as a mode of exercise in several populations. Walk/run training is a suitable alternative and is an accepted prescription for the old or obese subjects, since they do not always adopt more intense activities. Therefore, the present study was designed to investigate whether walk/run training could enhance anti-inflammation capacity of HDL in MetS patients without diet restriction and evaluate the endothelial-protective effects of HDL from the subjects before and after training.

## Materials and Methods

### Participants

The ethics committee of Taishan Medical University approved the present protocol of the study. The diagnosis of the MetS was based on clinical evidence (1) and 39 individuals with MetS between the ages of 38 and 65 years were recruited and the criteria for inclusion, exclusion, and elimination was described in the supplemental data. According to the principle of volunteering, they were divided into two groups, 12 remaining in an untrained state (control group) and 27 performing a 10-week walk/run training program (exercise group).

### Exercise protocol

All exercise training sessions were supervised by a professional exercise physiologist. Participants were encouraged to train five times a week, with a training session consisting of a brief warm-up for 5 minutes and the following procedures: the subjects began to walk at a speed of 3.5 km/h, and the speed increased by 0.3 km/h every 30 s until the subjects felt they needed to run. The participants were asked to run as long as they could while they felt comfortable. The running speed was then reduced to 4 km/h for 2 minutes and the subjects went back to walking for 3 minutes, and began a new cycle again. The duration of each session was gradually increased from approximately 30 minutes to 60 minutes and the training intensity gradually increased to 60–70% of the maximal heart rate by the end of tenth week. During the 10 weeks, all participants ad libitum ate without control of calories.

### Pulse wave velocity

Weight and height were measured and body mass index (BMI) was calculated as weight (kg) divided by square of height ($m^2$). Waist circumference (WC) was assessed at the level of the umbilicus. After the subjects rested in a sitting position for 10 minutes, a qualified physician measured the blood pressure (BP) at the right arm twice using a standard mercury sphygmomanometer. The pressure pulse waveform was recorded by a Colin waveform analyzer. Extremity BP was measured using an oscillometric method. Right brachial-ankle pulse wave velocity (rt. baPWV: right upper arm, right ankle) and left brachial-ankle pulse wave velocity (lt. baPWV: right upper arm, left ankle) were measured.

### Biochemical measurements

The fasting blood samples were drawn at baseline and at the end of walk/run training for 10 weeks. Serum was isolated by centrifugation and aliquots of serum were stored at −70°C for later analysis. Total cholesterol (TC), HDL-C, triglycerides (TG), and blood glucose were measured using enzymatic methods, and low-density lipoprotein C (LDL-C) was calculated using the Friedewald formula. Lipoprotein profiles were obtained by fast protein liquid chromatography (FPLC) as described previously (11).

### Measurement of serum oxidative stress and inflammatory markers

Serum levels of malondialdehyde (MDA) and the activity of superoxide dismutase (SOD) were determined according to the manufacturer's instructions by commercial kits (11). The activity of paraoxonase-1 (PON1) was measured as described previously (12) and expressed in international units (U) per milliliter of serum. Concentrations of interleukin-6 (IL-6) and serum amyloid A (SAA) in serum were determined by ELISA kits according to the manufacturer's instructions.

### Isolation of HDL

HDL was isolated by sequential ultracentrifugation using solid sodium bromide for density adjustment. HDL3 ($d = 1.125$–1.210 g/mL) was separated according to the method of Havel et al (13). After ultracentrifugation, the lipoproteins were stored at 4°C and used within 10 days. Before being used in the experiments, sodium bromide was removed from the HDL solutions by exhaustive dialysis with PBS for 24 h at 4°C. PON1 activity and MDA level in HDL or LDL were determined by the methods described as detection of PON1 and MDA in serum.

### Cholesterol efflux study

Cholesterol efflux experiments were performed as described in our lab (11). In brief, RAW264.7 macrophages at 50% confluence were labeled with $^3$H-cholesterol (1 $\mu$Ci/mL) loaded with 100 $\mu$g/mL acetylated LDL for 30 minutes. Then, macrophages were washed and equilibrated for 24 h. On the following day, the medium was supplied with 200 $\mu$g/mL HDL. Twelve hours later, the radioactivity of the medium and the macrophages was measured respectively by liquid scintillation counter. Cholesterol efflux is represented as the percentage of medium radioactivity to total radioactivity (medium plus cellular radioactivity).

### Cell culture and treatment

Human umbilical vein endothelial cells (HUVECs) were purchased and cultured in DMEM supplemented with 10% (v/v) fetal bovine serum in a humid incubator (37°C, 5% $CO_2$) until subconfluent, and then incubated with serum-free medium for 12 h before HDL3 (100 $\mu$g protein/mL) was added. After coincubation with HDL3 for 18 h, the cells were stimulated with tumor necrosis factor a (TNF-a, 10 ng/mL) for another 6 h. Then the supernatants were collected for analysis, and the cells were used for protein isolation.

### 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The same quantity of HUVECs was seeded in 96-well plates and treated as above. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo-lium bromide (MTT) reduction assay. MTT was added to each well to a final concentration of 0.5 mg/mL for 4 h. Then the supernatant was removed, and 150 $\mu$L of dimethyl sulfoxide was added to each well. After the plates were vibrated for 10 minutes at room temperature, the absorbance at 490 nm was recorded with a microplate reader. Cell viability is expressed as the percentage of the optical density (OD) of the treated cells relative to that of the untreated control cells.

### Concentration of NO and MCP-1 in media

The concentration of nitric oxide (NO) released from HUVECs was measured by NO assay kits (14) and the level of monocyte chemotactic protein-1(MCP-1) in media was detected by the ELISA kits following the manufacturer's instructions.

### Western blot analysis

Equal amounts of protein were subjected to 8–12% SDS-PAGE and transferred by electroblotting onto PVDF membranes. The membranes were incubated with primary antibodies against vascular cell adhesion molecule-1 (VCAM-1) and endothelial nitric oxide synthase (eNOS). Primary antibody binding was detected using horseradish peroxidase-conjugated secondary antibodies and visualized by an enhanced chemiluminescence reagent. Apolipoprotein (Apo) A-1 in equal volume serum was also detected by western blot analysis.

### Endothelial monocyte adhesion

Human monocytes were separated and resuspended in RPMI 1640 containing 10% serum and labeled with 2,7-bis(2-carboxyethyl) –5(6)-carboxyfluorescein acethoxymethyl ester (BCECF/AM, 10 $\mu$g/mL). HUVECs, cultured in 24-well plate, were incubated with HDL3 (100 $\mu$g protein/mL) from different subjects for 18 h. Then TNF-a (10 ng/mL) was added to the wells and incubated for another 6 h. Afterwards, culture media was changed, and HUVECs were co-incubated with BCECF/AM-labeled monocytes (50 000/well) for 1 h at 37°C. Unbound cells were removed by washing wells with PBS, and adherent monocytes labeled with BCECF/AM were counted on four randomly selected high-power fields using a fluorescence microscope.

### Statistical analysis

All data are presented as means ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Student-Newmann-Keuls multiple comparison tests. Pre-intervention and postintervention values were compared using matched-pair t-tests. Probability values less than 0.05 were considered statistically significant. All analyses were performed with GraphPad Prism software.

## Results

### Characteristics of the study subjects

According to the voluntary principle, 27 patients preferred to take part in walk/run training, while the others kept their original lifestyle. There were no differences in all anthropometric characteristics between the control group and the exercise group at baseline (Table 1). No significant changes were observed between baseline values and the end of the tenth week in the control patients (Table 1). Walk/run training for 10 weeks did not reduce body weight, BMI, WC, SBP, TG, blood glucose, and PWV and did not increase HDL-C concentrations (Table 1) significantly. Serum lipoprotein profile by FPLC showed an increase tendency in serum HDL-C levels in the exercise group compared with that before training (Figure 1).

**Table 1.** Clinical Characteristics of the Participants in Control and Exercise Groups at Baseline and the End of the Tenth Week

| | Control | | Exercise | |
|---|---|---|---|---|
| | **Baseline** | **10 Weeks** | **Baseline** | **10 Weeks** |
| Age (y) | 60.55 ± 1.38 | | 59.08 ± 0.81 | |
| Gender (male/female) | 4/8 | | 9/18 | |
| Body weight (kg) | 72.00 ± 2.66 | 73.45 ± 10.34 | 71.35 ± 1.65 | 70.2 ± 8.28 |
| Body mass index (kg/m$^2$) | 28.32 ± 0.87 | 28.88 ± 3.45 | 28.59 ± 0.58 | 28.09 ± 2.44 |
| Waist (cm) | 104.4 ± 3.17 | 104.5 ± 11.25 | 101.8 ± 2.92 | 98.1 ± 6.55 |
| Systolic blood pressure (mmHg) | 140.2 ± 5.17 | 140.5 ± 19.89 | 143.6 ± 3.52 | 138.8 ± 17.90 |
| Diastolic blood pressure (mmHg) | 84.82 ± 2.37 | 83.45 ± 9.73 | 86.09 ± 1.48 | 84.04 ± 9.67 |
| Total cholesterol (mmol/L) | 5.17 ± 1.06 | 5.22 ± 1.19 | 5.04 ± 1.07 | 5.11 ± 0.97 |
| Triglycerides (mmol/L) | 2.56 ± 1.82 | 2.82 ± 1.53 | 2.39 ± 1.30 | 2.24 ± 1.02 |
| HDL-C (mmol/L) | 1.27 ± 0.12 | 1.22 ± 0.32 | 1.25 ± 0.27 | 1.35 ± 0.33 |
| LDL-C (mmol/L) | 2.74 ± 0.28 | 2.73 ± 0.80 | 2.80 ± 0.83 | 2.68 ± 0.53 |
| Glucose (mmol/L) | 7.55 ± 0.77 | 8.59 ± 3.36 | 8.18 ± 2.48 | 7.76 ± 2.46 |
| RbaPWV | 1590 ± 62.2 | 1571 ± 141.9 | 1603 ± 67.9 | 1559 ± 103.3 |
| LbaPWV | 1547 ± 70.5 | 1593 ± 130.3 | 1641 ± 66.3 | 1609 ± 91.7 |

### Effects of walk/run training on antioxidative and inflammatory markers

As shown in Figure 2, A and B, the activity of PON-1 increased in both serum and HDL3 fractions from MetS patients, while serum MDA decreased by 42.68% after 10-week training (Figure 2C). Notably, the protein-bound MDA content also decreased in HDL or LDL from MetS patients after walk/run training (Figure 2, D and E). However, the activity of serum SOD did not alter significantly after training (Figure 2F). Consistent with the previous studies (15), no significant changes in SAA and IL-6 were observed between the control group and exercise group (Figure 2, G and H).

### Cellular cholesterol efflux capacity of HDL

The ability of the isolated HDL particles to elicit cholesterol efflux from cholesterol-loaded macrophages was tested. As shown in Figure 3A, there was no significant difference in the macrophage cholesterol efflux capacity of circulating HDL from patients with MetS before and after walk/run training. We did not also find walk/run training for 10 weeks changed ApoA-1 level in serum (Figure 3B).

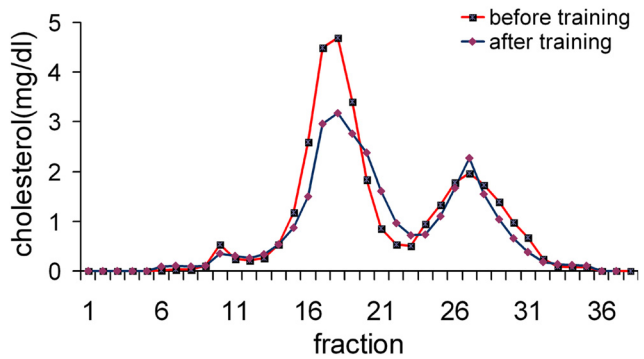


**Figure 1.** The FPLC cholesterol profiles. Cholesterol content of serum lipoprotein fractions in pooled serum samples of 2 to 3 persons per group were showed by FPLC assay.

### Anti-inflammatory properties of HDL

The anti-inflammatory ability of HDL was tested by observing the protective effects of HDL3 isolated from patients with MetS on the TNF-α-injured HUVECs. In order to evaluate the anti-inflammatory ability of HDL3 particles from the patients, we isolated HDL3 particles from a healthy group (n = 10) as the positive control and tested the protective effects on injured HUVECs after the stimulation with TNF-a.

First, the effect of HDL3 on cell viability was observed by MTT assay. As shown in Figure 4A, the HDL3 from health subjects increased cell viability compared with TNF-α-injured HUVECs. When HUVECs were incubated with HDL3 from MetS patients before training or without training for 10 weeks, the TNF-α-induced decrease in cell viability of HUVECs was not significantly ameliorated. However, the HUVECs viability increased significantly ($P < .05$) after 10-week training, which was close to the viability of the cells treated with HDL3 from the healthy.

MCP-1 level was low in the media of the unstimulated HUVECs. TNF-α induced a marked increase in MCP-1 level and pretreatment with HDL3 from MetS patients before training or without training did not effectively inhibit the TNF-α-induced MCP-1 elevation. However, pretreatment with HDL3 from MetS patients with 10-week training led to a significant reduction in MCP-1 level compared with pretreatment with HDL3 from MetS patients before training ($P < .05$) (Figure 4B). Next we explored whether HDL3 could inhibit the TNF-α-induced expression of VCAM-1 in HUVECs. We found that in the presence of HDL3 from MetS patients with 10-week training

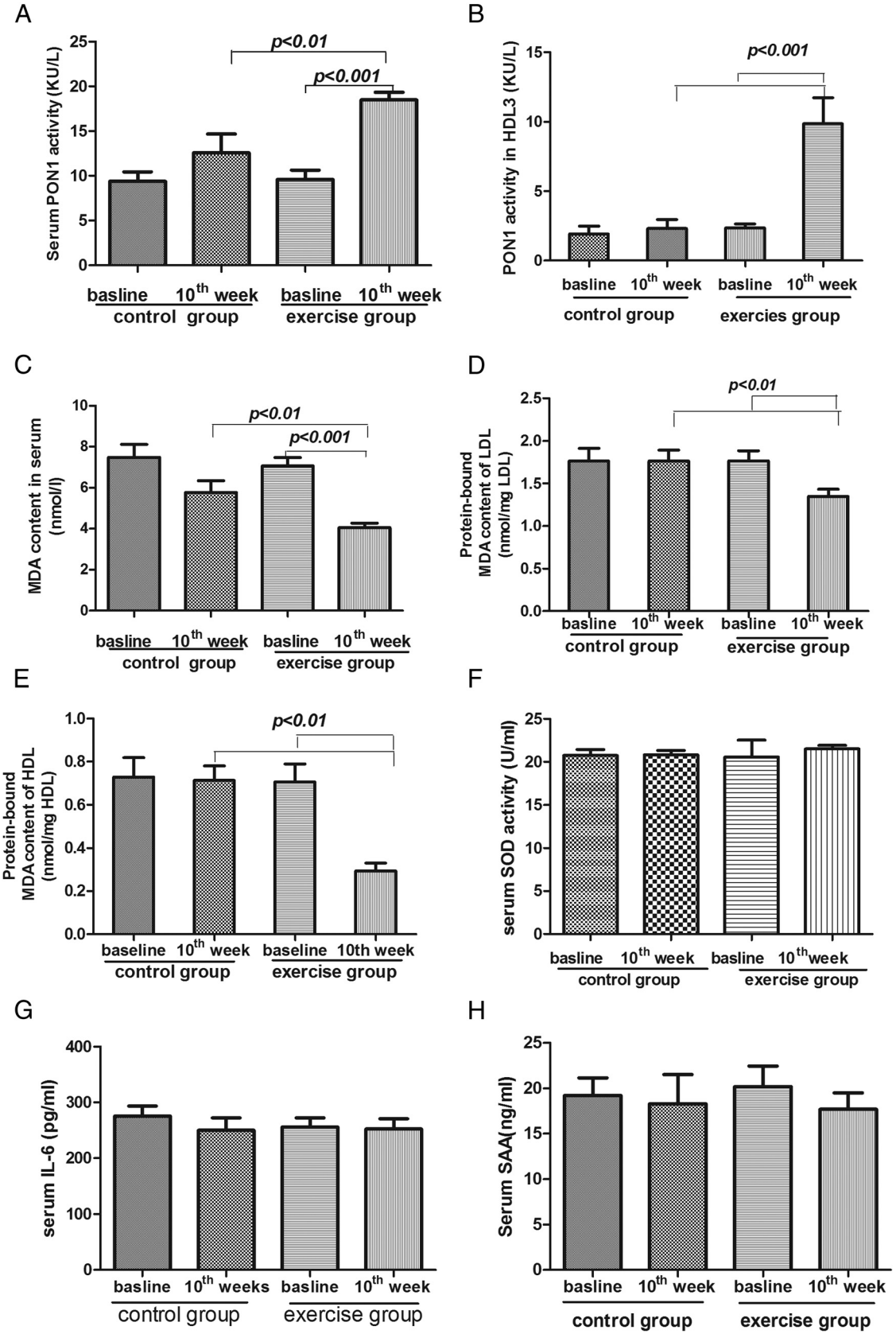


**Figure 2.** Effects of walk/run training on the serum antioxidant and inflammatory biomarkers of patients with metabolic syndrome. (A) Serum PON-1 activity in MetS patients of control or exercise group, (B) PON-1 activity in equal volume HDL3, (C) serum MDA, (D) Protein-bound MDA in equal quality LDL, (E) Protein-bound MDA in equal quality HDL, (F) serum SOD activity, (G) serum IL-6, (H) serum SAA. n = 12 in control group, n = 27 in exercise group.

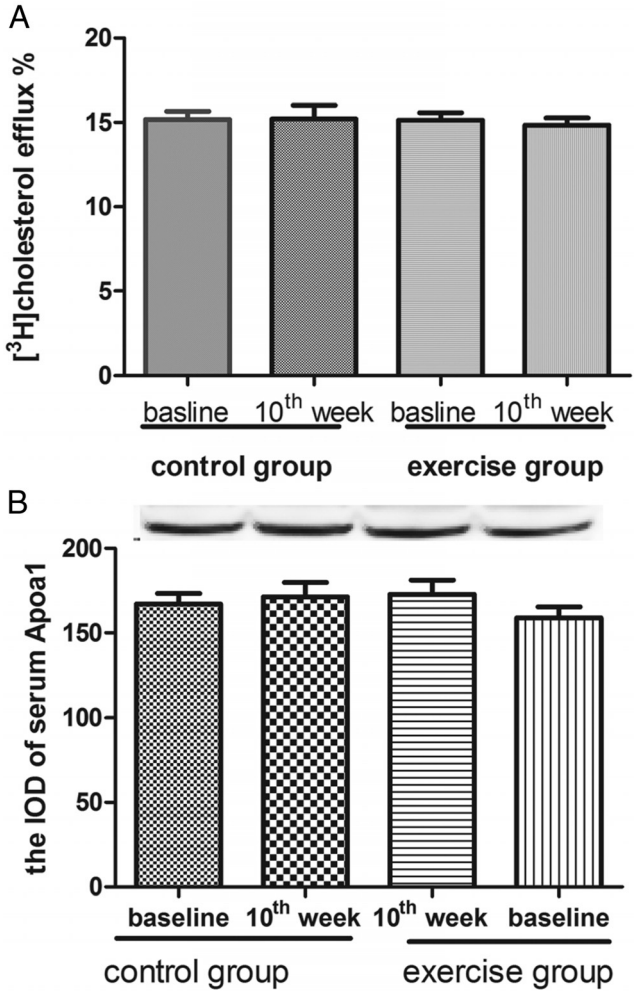


**Figure 3.** Effects of HDL on cholesterol efflux from macrophages. (A) RAW264.7 macrophages were labeled with $^3$H-cholesterol (1 $\mu$Ci/mL) loaded with 100 $\mu$g/mL acetylated LDL for 30 minutes. Then, macrophages were washed and equilibrated for 24 h. On the following day, the medium was supplied with 200 $\mu$g/mL HDL from MetS patients with or without exercise for 12 h. The radioactivity of the medium and the macrophages was measured respectively by liquid scintillation counter. Cholesterol efflux is represented as the percentage of medium cpm to total cpm (medium plus cellular cpm). B, Apolipoprotein (Apo) A-1 content in equal volume serum was detected by western blot analysis.

or from the healthy, the VCAM-1 expression displayed by western blot assay decreased significantly compared with that in the presence of TNF-$\alpha$ alone (Figure 4C).

To further investigate the anti-inflammatory property of HDL3, the adhesion of monocytes to the vascular endothelium was observed. A small number of monocytes adhered to the unstimulated HUVECs, while monocytes adhesion to HUVECs increased significantly in the presence of TNF-$\alpha$ alone. However, the monocytes adhesion to HUVECs markedly reduced when cells were pretreated with HDL3 from both healthy subjects ($P < .001$) and MetS patients with the 10-week training ($P < .01$) (Figure 4D).

As shown in Figure 5A, TNF-$\alpha$ decreased NO production of HUVECs, and treatment with HDL3 from MetS patients without training did not induce more NO production yet. However, pretreatment with HDL3 from healthy subjects and MetS patients after 10-week training attenuated the inhibitory effect of TNF-$\alpha$ on NO production. Furthermore, we compared the effects of HDL3 from healthy subjects and MetS patients with or without training on eNOS protein expression. As shown in Figure 5B, HDL3 from healthy subjects or MetS patients after 10-week training increased eNOS expression in TNF-$\alpha$-stimulated endothelial cells. However, HDL3 from patients with MetS without training had no similar effect. These findings suggest that training is a potential pathway contributing to the recovery capacity of HDL from MetS patients to stimulate endothelial NO production.

## Discussion

The major finding of the present study is that walk/run training leads to a significant improvement in HDL anti-inflammation capacity in subjects with MetS without a restricted diet, and the mechanism underlying which at least partially is due to the increased PON1 activity in HDL, NO production and eNOS expression in the endothelial cell.

Low HDL levels and defective HDL function were associated with MetS (2, 3). Recent studies have shown that lifestyle modifications can change HDL levels and function. Intake of moderate amounts of alcohol (30–40 g per day) or smoking cessation increases HDL levels (16). So we recruited MetS patients who were nonsmokers and abstainers or only moderate alcohol consumers ($< 20$ g/d). Exercise is a key strategy for treatment of patients with MetS. A meta-analysis of randomized and clinical controlled trials indicates that endurance training has a favorable effect on most of the cardiovascular risk factors associated with the MetS (17). Walk/run training is quite an acceptable exercise prescription, and all participants completed the training. Walk/run training for 10 weeks resulted in a reduced tendency in body weight, BMI, WC, SBP, TG, and blood glucose as well as an increased tendency in HDL levels, although these did not achieve statistical significance probably because of the unlimited dietary and the lower-intensity/duration exercise. These results demonstrate that walk/run training may have the potential role in decreasing some cardiovascular risk factors in the MetS patients.

Accumulating evidence shows that hypertension, hypercholesterolaemia and diabetes are associated with increased arterial stiffness and PWV increase in subjects with MetS (18, 19). PWV is now considered the gold standard of noninvasive measurement of arterial stiffness and

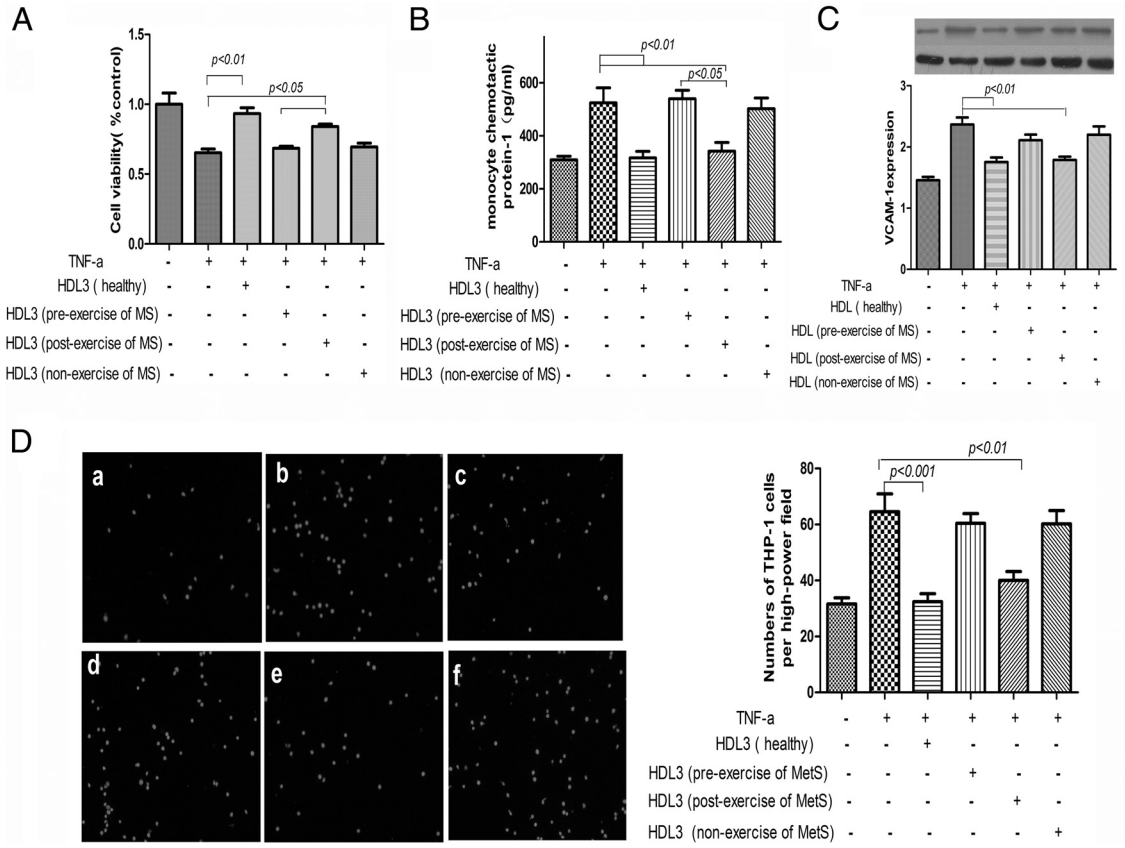


**Figure 4.** Effects of HDL3 on endothelial cells injured by TNF-$\alpha$. The serum of every 3 to 4 healthy subjects and MetS patients was pooled and HDL3 was isolated by ultracentrifugation. HUVECs were pretreated with HDL3 (100 $\mu$g protein/mL) from healthy subjects or patients with MetS with or without exercise for 18 h, then exposed to TNF-$\alpha$ (10 ng/mL) for another 6 h. A, Effects of HDL3 on endothelial cell viability were determied by MTT analysis. B, Effects of HDL3 on the level of monocyte chemoattraction protein-1 in the media were measured by ELISA kits. C, Effects of HDL3 on the protein expression of VCAM-1 were evaluated by western blot and normalized to $\beta$-actin level. D, Effects of HDL3 on endothelial monocyte adhesion. HUVECs were stimulated with HDL3 (100 $\mu$g protein/ml) for 18 h, then exposed to TNF-$\alpha$ (10 ng/mL) for another 6 h. Finally, monocytes labeled with a fluorescent dye BCECF-AM were added to HUVECs. The number of monocytes adhering to endothelial cells were recorded under high-power fields using a fluorescence microscope. a, treated with PBS; b, treated with TNF-$\alpha$; c, pretreated with HDL3 from the healthy and then injured with TNF-$\alpha$; d, pretreated with HDL3 from MetS before training and then injured with TNF-$\alpha$; e, pretreated with HDL3 from MetS after training and then injured with TNF-$\alpha$; f, pretreated with HDL3 from MetS without training and then injured with TNF-$\alpha$. Data are presented as the mean $\pm$ SD of six independent experiments.

baPWV has been widely used in clinical research because of its relative simplicity. Regular aerobic exercise is known to be efficacious for preventing and reversing arterial stiffening in middle-aged sedentary men (20). However, short-term exercise did not alter PWV in obesity (19). The present study also found baPWV was high in MetS patients and 10-week walk/run training did not change baPWV remarkably.

Inflammatory always goes with MetS. HDL inhibits the expression of MCP-1 and adhesion molecules (21), and reduces leukocyte homing to arterial endothelium (22). However, in those with chronic illnesses that are characterized by systemic oxidative stress and inflammation, HDL may present attenuated beneficial role and even demonstrate a direct pro-inflammatory effect because of chemical modification (6, 7, 23). In MetS, plasma levels of large HDL decrease in parallel with HDL-C, whereas the levels of small HDL do not, and HDL3 is a more potent protector of LDL from oxidation in vitro compared with HDL2 (3). HDL3 has been reported to be superior to HDL2 in terms of its capacity to inhibit VCAM-1 expression in endothelial cells (3). Therefore, the anti-inflammatory properties of HDL3 were investigated in the present study. We found that HDL3 from patients with MetS had no significant effect on the TNF-$\alpha$-induced inflammatory factor expression in endothelial cells. However, HDL3 from patients with MetS after walk/run training decreased the expression of MCP-1 and VCAM-1 in parallel with the reduced number of monocytes adhesion to HUVECs. Our study suggests that the anti-inflammatory activity of HDL is impaired in patients of MetS, whereas walk/run training can reverse HDL function of MetS from pro-inflammatory to anti-inflammatory to some extent.

PON1 could inhibit MCP-1 induction in endothelial cells (24) and HDL from PON1-knockout mice lost antioxidant and anti-inflammatory activities (25), suggesting

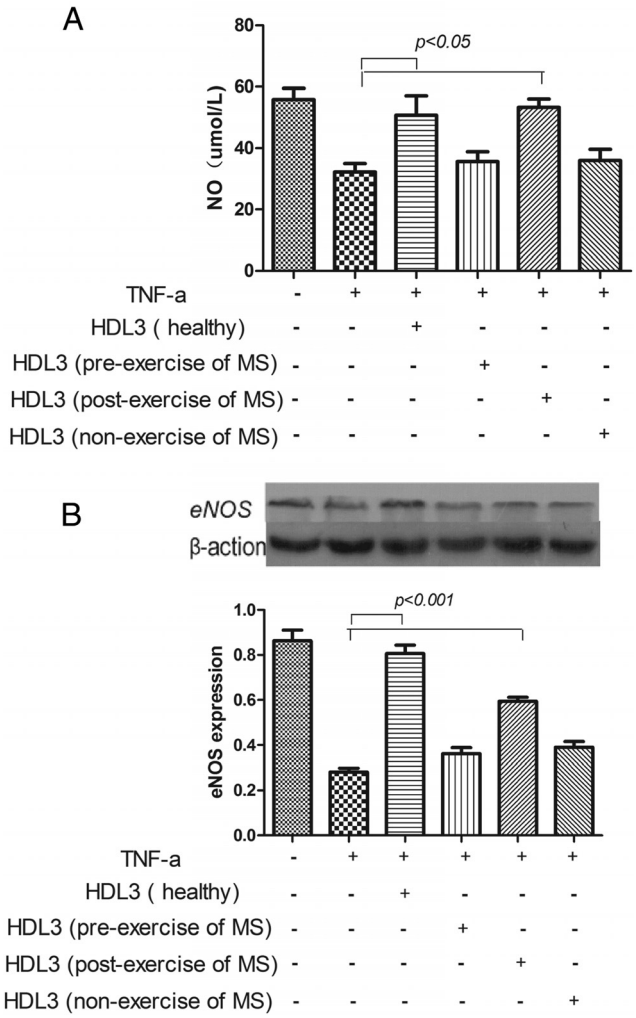


**Figure 5.** Effects of HDL3 on NO and eNOS expression in TNF-$\alpha$ -injured HUVECs. HUVECs were pretreated with HDL3 (100 $\mu$g protein/mL) from healthy subjects or patients of MetS with or without training for 18 h, then exposed to TNF-$\alpha$ (10 ng/mL) for another 6 h. A, Effects of HDL3 on the level of NO in the media were detected by the ELISA kits. B, Effects of HDL3 on the protein expression of eNOS were evaluated by western blot and normalized to $\beta$-actin level. Data are presented as the mean $\pm$ SD of six independent experiments.

the antioxidant and anti-inflammatory properties of HDL are attributed partly to the role of PON1. Exercise increased PON activity in some studies (26, 27), and our results also found regular exercise increased PON1 activity. However, the reasons for the increase in PON1 activity have not been elucidated clearly. Marta et al (26) found the effect of exercise on PON1 activity depended on the training status and the PON1 polymorphisms. We postulated that regular exercise could increase HDL and more HDL-bound form of PON1 protein would be present thus causing higher enzyme activity. SAA is an acute-phase protein and mediates pro-inflammatory cellular responses. HDL is a major carrier of SAA in human. Circulating SAA is elevated in patients with type 2 diabetes compared with healthy subjects (28). Higher SAA levels may impair HDL anti-inflammatory properties. SAA can induce production of MCP-1 in human monocytes (29), and lead to monocytes migration and tissue infiltration in atherosclerotic plaques (30). In the present work, we found that walk/run training increased serum PON1 activity but it did not decrease serum SAA or IL-6 level. Taken together, these findings suggest that walk/run training can enhance serum PON1 activity to improve the HDL anti-inflammatory capacity.

Stimulation of endothelial NO production is important for HDL to inhibit the inflammatory response of endothelial cells such as VCAM-1 expression and monocytes adhesion (31). PON1 in HDL is required to activate eNOS and stimulate NO production in endothelial cells, as HDL from PON1-deficient mice failed to stimulate endothelial NO production (31, 32). Our findings indicated that the walk/run training for 10 weeks increased PON1 activity in HDL3, improved eNOS-dependent NO production and attenuated the proinflammatory effects of HDL from patients with MetS. So we speculated HDL-associated PON1 activity may be involved in the effects of HDL on eNOS-derived NO production.

In the study we did not observe a significant difference in the macrophage cholesterol efflux capacity of HDL from MetS patients before and after training. Apart from the changes in HDL level, the components of HDL can also influence the ability of HDL to induce cholesterol efflux from cells. ApoAI is thought to play a central role in cholesterol transport from macrophages to the liver. In the present study, we investigated the ApoAI content in equal volume serum and did not find significant change in MetS patients after training. SAA is significantly elevated in metabolic syndrome and can displace apoAI from the phospholipid surface of HDL, thus altering the structures and functions of HDL. Lipid-free SAA can inhibit the binding of HDL to scavenger receptor B type I (SR-BI), while SR-BI-mediated cholesterol efflux from cells to HDL requires the binding of HDL to SR-BI (33). In addition, SAA has an inhibitory effect on LCAT activity (34), which may also lead to impairment in cholesterol efflux to HDL (35). In the present study, walk/run training did not increase apoAI and decrease SAA level in patients with MetS, which may be key reasons for not observing the significant difference in the macrophage cholesterol efflux capacity of HDL from patients with MetS before and after training.

Although our study indicates a marked improvement in HDL anti-inflammation capacity in MetS subjects with walk/run training, the design was a small sample size and the results only were responsible for a small part of MetS patients, so further studies to enlarge the sample size are necessary. Additional studies are needed to compare the effects of walk/run training on the variables in obese and

nonobese men and women for different age groups. It is also essential to define the appropriate frequency, duration, and intensity of exercise in modulating HDL function of MetS patients. The HDL proteome has been found to be highly malleable to different diseases, therefore a comprehensive analysis of the changes of HDL proteome before and after walk/run training will need to be explored further in future studies.

In summary, our observations suggest that walk/run training strategies exert beneficial effects by reversing the anti-inflammation function of HDL in MetS subjects without the requirement for dietary modification. All findings indicate that the nonpharmacologic interventions on HDL metabolism and function go beyond the plasma HDL cholesterol levels, and highlight the importance of evaluating the functional aspects of the lipoproteins in addition to their plasma levels.

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