Effects of aerobic exercise training on lipoprotein metabolism and antioxidant status in sedentary but otherwise healthy young women

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Purpose: To examine the effects of aerobic exercise training on lipoprotein metabolism and antioxidant status in sedentary but otherwise healthy young women.

Methods: A total of 10 women (age 20–25 years) were randomly assigned to either the exercise group (EG, n = 5) or the control group (CG, n = 5). The EG performed aerobic exercise training (3 days per week, 30 min per session, 12 weeks) while the CG maintained their usual lifestyle. Lipid profiles, including high density lipoprotein cholesterol (HDL-C), triglycerides (TG), and antioxidant status (vitamin C and E) were assessed before and after the intervention.

Results: There were no significant differences in baseline lipid profiles between the groups. After the intervention, the EG showed significant decreases in TG and increases in HDL-C compared to the CG. Antioxidant status also improved in the EG, as indicated by a significant increase in vitamin C levels.

Conclusion: The results suggest that aerobic exercise training is effective in improving lipoprotein metabolism and antioxidant status in sedentary young women.

Introduction

Atherogenesis can be viewed as a 'postprandial phenomenon', since accumulating evidence shows the positive relationship between postprandial dyslipidemia and the pathogenesis of atherosclerosis leading to coronary heart disease. Prolonged elevation of plasma triglycerides (TG) concentrations during the postprandial period may result in several atherogenic changes: elevation in plasma chylomicrons (CMs) and very low density lipoprotein (VLDL) as well as their remnants, a decrease in high density lipoprotein (HDL) cholesterol (C), and the presence of small, dense particles of low density lipoprotein (LDL). Several studies have shown that, after a fatty meal, plasma TG levels are higher in patients with coronary heart disease, which may explain at least in part why some individuals develop atherosclerotic disease despite normal fasting lipid values. TG-rich lipoproteins are involved in atherosclerosis and thrombosis, and TG, remnant-like particle (RLP) ~C and RLP-TG levels increase after fat loading and could contribute to atherothrombosis. Although
the pathophysiology of postprandial lipoprotein metabolism is not entirely clear, a link with insulin resistance has been pointed out. Postprandial dyslipidemia has also been reported to be associated with obesity (particularly visceral obesity), metabolic syndrome, and glucose intolerance/diabetes mellitus.

We previously reported the influence on postprandial lipoprotein responses of aging and menopause in women and obesity in young men \(^1\). Then we examined the acute effects of immediately pre- versus postprandial aerobic exercise on postprandial lipoprotein metabolism in sedentary but otherwise healthy young women and showed that postprandial but not immediately preprandial exercise reduces the number of chylomicrons and chylomicron remnants and improves the exogenous lipoprotein metabolism, suggesting that postprandial exercise may be more effective at improving the postprandial lipoprotein metabolism than immediately preprandial exercise \(^2\).

Physical inactivity can lead to an increased incidence of a variety of diseases. It has been reported that less than 20% of young Japanese women have regular exercise habits \(^1\). It has been shown that although a single bout of exercise increases the oxidative challenge to the body and excessive exercise and overtraining lead to damaging oxidative stress, regular moderate exercise overcomes it \(^1\). Indeed, regular moderate physical exercise or activity provides systemic beneficial effects, including improved physiological function such as carbohydrate and lipid metabolism, decreased incidence of disease, and a higher quality of life. Regular exercise of moderate intensity and duration has been reported to have a wide range of beneficial health effects.

Most health-promoting guidelines recommend regular aerobic exercise at moderate intensity. Physical activity recommendations advise that adults should engage in moderate-intensity physical activity for a minimum of 30 min on 5 or more days of the week \(^1\). Accordingly, in the present study we employed an exercise protocol of moderate intensity and relatively short duration (30 min), compatible with the range recommended for maintaining cardiopulmonary fitness and promoting health, in order to make the findings of the present study applicable to sedentary but otherwise healthy young women. Understanding how this kind of exercise influences postprandial lipoprotein metabolism has widespread relevance.

**Aim**

The purpose of this study was to examine the effects of moderate aerobic exercise training on lipoprotein metabolism and antioxidant status in sedentary but otherwise healthy young Japanese women.

**Materials & Methods**

**Subjects**

Eight young healthy Japanese women with sedentary lifestyle, normal weight (18.5 \(\leq\) BMI < 25), normal menstrual cycle, and apolipoprotein (apo) E phenotype 3/3 participated in this study. We chose apoE phenotype to 3/3 in order to minimize the individual variability. Subjects were non-smokers, were not suffering from any apparent acute or chronic illness, and were not taking any medications or dietary supplements. This study was approved by the Institutional Review Board of the Sugiyama Jogakuen University School of Life Studies, and each subject gave written informed consent.

**Anthropometric and body composition measurement**

Body weight, and waist and hip circumferences were measured by standard methods. The waist circumference was assessed as the abdominal girth at the level of the umbilicus, and the hip was measured at the level of the greater trochanters. The waist-to-hip ratio (W/H) was calculated. Body composition including visceral fat area (VFA) was analyzed by an eight-polar bioelectrical impedance method, using InBody720 (BioSpace, Tokyo, Japan).

**Exercise training**

An incremental exercise protocol was designed to increase walking speed, aiming at the intensity of ca 50% \(\text{Vo}\cdot\text{max}\) using a treadmill. Following a 3–5 min warm-up, the initial speed was set at 3.7 km/h; the speed was increased by 0.3 km/h every 3 min for the first 15 min, and then by 0.2 km/h every 3 min for the latter 15 min. The aerobic exercise was totally 30 min and the final speed was 5.9 km/h. Heart rates were monitored every 3 min with a cardiotachometer (HR<40, Japan Precision Instruments, Shibukawa, Japan), and blood pressures were recorded before and after exercise. \(HR_{\text{max}}, \%HR_{\text{max}}, \text{ and } \%\text{Vo}_{\text{max}}\) were calculated using following formulas:

\[
HR_{\text{max}} = \frac{220 - \text{age (y)}}{100}
\]

\[
\%HR_{\text{max}} = \frac{\text{mean HR (beats/min)}}{HR_{\text{max}} (\text{beats/min})} \times 100
\]

\[
\%\text{Vo}_{\text{max}} = 1.35 \times \%HR_{\text{max}} - 35.0
\]

Subjects were instructed to maintain their sedentary lifestyle and eating habits during the training period.

**Fat loading test**

Oral fat tolerance test (OFTT) cream (Jomo, Takasaki, Japan) was used as described previously \(^1, 4\). This cream
contains essentially no carbohydrates, and has been reported not to significantly affect plasma insulin levels. The oral fat tolerance test was performed at pre-training and post-training (ca 24 h after the last day's exercise) stages. The subjects fasted 12 h overnight, and were given OFTT cream (1 g/kg body weight, 0.35 g/kg as fat). During the 12-h overnight fast, subjects abstained from caffeine and alcohol intake. Venous blood samples were taken before (0 h) and 1, 2, 4 and 6 h after the fat loading. All the blood samples were taken in the supine position. The subjects were allowed to drink water ad libitum and were restricted from exercise during the test period.

Biochemical analysis

Plasma and serum samples were immediately frozen and kept at –80°C until analysis. The concentrations of serum total cholesterol (TC) were measured enzymatically (Sysmex, Hyogo, Japan). HDL-C was measured by a direct method (Fujirebio, Tokyo, Japan). LDL-C was calculated by the Friedewald formula. These were measured only in the fasting state. The concentrations of serum TG (Sekisui Medical, Tokyo, Japan), lactate (Kyowa Medex, Tokyo, Japan), and free fatty acid (FFA) (Eiken Chemical, Tokyo, Japan) were measured enzymatically. Remnant-like particle (RLP) -cholesterol (C) and RLP-TG were measured by an immunosorbent assay (Otsuka Pharmaceutical, Tokyo, Japan). We measured both RLP-TG and RLP-C; however, because some of the RLP-C concentrations were less than the lower limit of detection, we did not include those data in this article. Lipoprotein (a) (Lp (a)) was measured by a latex agglutination method (Sekisui Medical, Tokyo, Japan). The concentration of apoB48 was measured by CLEIA (Fujirebio, Tokyo, Japan). LPL mass was measured by ELISA (Daichii Pure Chemicals, Tokyo, Japan). The apoE phenotype was measured using the isometric electrophoresis method (Phenotyping ApoE IEF System, Joko, Tokyo, Japan). The serum concentration of glucose was measured by a mutarotase GOD method (Wako, Osaka, Japan). The concentration of serum insulin was measured by chemiluminescent enzyme immunoassay (Fujirebio, Tokyo, Japan). Insulin resistance was evaluated by homeostasis model assessment-insulin resistance (HOMA-IR).

Serum biological antioxidant potential (BAP) and derivatives of reactive oxygen metabolites (dROMs) were measured using FRAS 4 (Wismerrll, Tokyo, Japan). Plasma 8-epi-prostaglandin F2α, (8-epiPGF2α) was measured by 8-epiPGF2α, EIA kit (Cayman Chemical, Ann Arbor, USA).

Quantification of postprandial metabolism

Postprandial metabolism was quantified by calculating the incremental area under the curve (IAUC), which was estimated as the difference between the area defined below the baseline concentration and the area under the serum curve between 0 h and 6 h, representing the increase in area after fat loading compared to fasting concentrations.

Statistics

All data are expressed as means ± SEM. Statistical analyses were performed using StatView ver. 5.0 (SAS Institute, Cary, USA). Differences in the time-course changes from the initial state were analyzed using repeated measure one-way ANOVA, followed by the post-hoc test of Fisher’s PLSD. p < 0.05 was considered to be significant in all analyses.

Results

The average heart rate during exercise was 119.2 ± 3.6 beats/min at the pre-training (first exercise) and 112.6 ± 3.0 beats/min at the post-training (last exercise). %HR-max and %V̇o2 max were calculated as 60.2 ± 3.6% and 46.2 ± 3.6% at the pre-training, and 56.8 ± 3.0% and 41.7 ± 3.0% at the post-training, respectively. None of the subjects asked to be withdrawn due to poor physical condition during the experiments, and their blood pressure remained within the normal range before and after the exercise.

Physical characteristics and fasting blood chemical data of the subjects before and after training are shown in Table 1. Body weight, BMI, waist, W/H, and VFA showed no differences between pre-training and post-training. Likewise, serum glucose, insulin, TG, RLP-TG, and FFA showed no differences. The mean values of LDL-C concentration and LPL mass were higher, and those of HDL-C, apoB48, and hsCRP were lower at the post-training compared to the pre-training, but without significance. Plasma 8-epiPGF2α concentration was significantly lower at the post-training compared to the pre-training. The mean value of BAP was higher, and, that of dROMs was lower at the post-training compared to the pre-training, but without significance. In the oral fat tolerance test, serum TG concentrations increased following fat loading in both trials, and reached a peak at 2 h (Table 2). The mean values of serum TG concentrations were lower, but not significantly, at all post-training times compared with the pre-training. IAUC-TG was significantly lower at the post-training compared with the pre-training (Table 3). The mean values of RLP-TG concentrations were slightly

116 (44)
lower, but not significantly, at all post-training times compared with the pre-training (Table 2). The mean value of IAUC–RLP–TG was lower but not significantly at the post-training compared with the pre-training (Table 3). The mean values of serum apoB48 concentrations, a marker of extrinsic lipoprotein particle numbers, were lower, but not significantly, at all post-training times compared with the pre-training (Table 2). Noticeably, at both the pre- and post-training, although serum TG and RLP–TG concentrations returned to fasting levels (0 h) at the end of the

Table 1 Anthropometric and clinical characteristics at pre- and posttraining.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-training</th>
<th>Post-training</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>21.8 ± 0.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157.1 ± 2.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>49.7 ± 1.3</td>
<td>49.3 ± 1.2</td>
<td>ns</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.1 ± 0.4</td>
<td>20.0 ± 0.4</td>
<td>ns</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>25.5 ± 1.4</td>
<td>24.5 ± 1.5</td>
<td>ns</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>69.8 ± 0.8</td>
<td>67.6 ± 1.1</td>
<td>ns</td>
</tr>
<tr>
<td>W/H</td>
<td>0.80 ± 0.01</td>
<td>0.80 ± 0.01</td>
<td>ns</td>
</tr>
<tr>
<td>VFA (cm³)</td>
<td>26.9 ± 4.0</td>
<td>24.4 ± 4.1</td>
<td>ns</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>186.3 ± 8.5</td>
<td>177.3 ± 9.2</td>
<td>ns</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>113.7 ± 8.6</td>
<td>101.9 ± 8.3</td>
<td>ns</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>62.7 ± 3.6</td>
<td>65.7 ± 3.3</td>
<td>ns</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>49.5 ± 5.6</td>
<td>48.1 ± 3.9</td>
<td>ns</td>
</tr>
<tr>
<td>RLP–TG (mg/dL)</td>
<td>7.8 ± 1.4</td>
<td>7.6 ± 0.6</td>
<td>ns</td>
</tr>
<tr>
<td>Lp (a) (mg/dL)</td>
<td>12.2 ± 2.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>FFA (µmol/L)</td>
<td>518 ± 75</td>
<td>489 ± 45</td>
<td>ns</td>
</tr>
<tr>
<td>apoB48 (mg/L)</td>
<td>2.4 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>ns</td>
</tr>
<tr>
<td>LPL mass (ng/mL)</td>
<td>55.8 ± 8.8</td>
<td>64.0 ± 8.7</td>
<td>ns</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>85.8 ± 1.7</td>
<td>85.3 ± 2.0</td>
<td>ns</td>
</tr>
<tr>
<td>Insulin (µIU/mL)</td>
<td>6.2 ± 0.7</td>
<td>6.0 ± 0.5</td>
<td>ns</td>
</tr>
<tr>
<td>BAP (mmol/L)</td>
<td>3119 ± 90</td>
<td>3380 ± 110</td>
<td>ns</td>
</tr>
<tr>
<td>dROMs (U.Carr)</td>
<td>283.5 ± 10.6</td>
<td>247.7 ± 13.2</td>
<td>ns</td>
</tr>
<tr>
<td>8–epiPGF₁α (pg/dL)</td>
<td>14.1 ± 0.6</td>
<td>11.9 ± 0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>hsCRP (ng/dL)</td>
<td>204.0 ± 61.9</td>
<td>101.0 ± 11.1</td>
<td>ns</td>
</tr>
</tbody>
</table>

All biochemical values were obtained in the fasting state. All values are presented as means ± SEM. ns: not significant.

Table 2 Parameters of lipoprotein metabolism before and after fat loading at pre-training (Pre) and post-training (Post) trials.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>49.5 ± 5.6</td>
<td>51.5 ± 5.8</td>
<td>69.4 ± 7.6***</td>
<td>63.7 ± 9.0***</td>
<td>45.2 ± 5.0</td>
</tr>
<tr>
<td>Post</td>
<td>48.1 ± 3.9</td>
<td>50.4 ± 4.1</td>
<td>65.9 ± 4.8***</td>
<td>56.2 ± 5.0**</td>
<td>41.9 ± 3.9*</td>
</tr>
<tr>
<td>RLP–TG (mg/dL)</td>
<td>7.8 ± 1.4</td>
<td>10.2 ± 1.5</td>
<td>20.1 ± 3.3***</td>
<td>15.3 ± 2.6***</td>
<td>8.9 ± 1.2</td>
</tr>
<tr>
<td>Pre</td>
<td>7.6 ± 0.6</td>
<td>8.5 ± 0.8</td>
<td>17.5 ± 1.7***</td>
<td>11.8 ± 1.9**</td>
<td>7.1 ± 0.9</td>
</tr>
<tr>
<td>Post</td>
<td>2.4 ± 0.2</td>
<td>2.7 ± 0.4</td>
<td>4.7 ± 0.5***</td>
<td>3.7 ± 0.3***</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>apoB48 (mg/L)</td>
<td>2.1 ± 0.2</td>
<td>2.6 ± 0.3</td>
<td>4.0 ± 0.3***</td>
<td>3.3 ± 0.3***</td>
<td>2.9 ± 0.3*</td>
</tr>
<tr>
<td>Pre</td>
<td>518 ± 75</td>
<td>417 ± 58</td>
<td>614 ± 90</td>
<td>667 ± 82*</td>
<td>807 ± 119***</td>
</tr>
<tr>
<td>Post</td>
<td>489 ± 45</td>
<td>401 ± 34</td>
<td>477 ± 36</td>
<td>680 ± 74*</td>
<td>851 ± 125***</td>
</tr>
</tbody>
</table>

All values are presented as means ± SE.

* p < 0.05, ** p < 0.01, *** p < 0.001 compared with fasting value.
test (6 h), serum apoB48 concentrations at the end of the test (6 h) did not return to the fasting level (0 h). The mean value of IAUC-apoB48 was smaller but not significantly at the post-training compared with the pre-training (Table 3). The concentrations of FFA decreased slightly but not significantly at 1 h, and then significantly increased at 4 and 6 h in both trials, and there was no difference between the two trials (Table 2).

Discussion

The major finding of this study is that 4-week moderate (ca 50%Vo: max, 30 min) aerobic exercise training may improve antioxidant status and lipoprotein metabolism, particularly postprandial metabolism, in young sedentary but otherwise healthy women, with the main effects being decreased AUC-TG and 8-epiPGF 2α levels.

In the present study, we examined the effect of exercise training on postprandial lipemia after moderate fat ingestion (0.35 g/kg body weight). The fat content of the loaded cream was about 35% of the fat ingested by an average Japanese adult woman in a day. Although the vast majority of fat tolerance studies have employed 1-1.5 g fat/kg body weight, such high amounts of fat limit the applicability of the results to everyday conditions for Japanese people. Therefore, we have been utilizing a fat cream (OFTT cream) of moderate fat content.

Three possible mechanisms could contribute to the reduction in postprandial TG concentrations evident after moderate-intensity exercise: reduced chylomicron-TG levels, increased lipoprotein-TG clearance and reduced hepatic VLDL secretion. In this study, peak values of serum TG were lower and IAUC-TG significantly decreased after the training. The mean values of RLP-TG and apoB48, a specific marker of extrinsic lipoprotein particle levels, non-significantly decreased after the training. Accordingly, reduced chylomicron-TG levels and/or increased lipoprotein-TG clearance may participate, at least partly, in the training effect. However, no conclusion can be drawn regarding hepatic VLDL secretions from the present results.

Noticeably, in the present study, although serum TG and RLP-TG concentrations returned to the fasting level at the end of the test (6 h), serum apoB48 concentrations at the end of the test (6 h) did not return to the fasting level (0 h) in either pre- or post-training. These results suggest that chylomicron remnants with scarce or minimal TG load still remain in the circulation 6 h after only moderate fat loading, even in healthy young women.

There is clear evidence from a number of cross-sectional studies comparing endurance-trained men with untrained controls that regular exercise is associated with lower levels of postprandial lipemia and enhanced rates of TG clearance (ref.13 for review). However, interpretation of these findings is difficult because exercise could theoretically have both chronic (i.e. long-term training adaptations) and acute (i.e. short-term effects of recent exercise) influences on TG metabolism. In studies where post-training assessment of fat tolerance were made more than 48 h post-exercise, no significant effects of training on postprandial lipemia or TG clearance were observed. In addition, de-training studies provided evidence that exercise training may not markedly influence TG metabolism in the absence of recent exercise. Taken together, the evidence suggests that, while endurance-trained people have efficient TG metabolism, as evidenced by low levels of postprandial lipemia and rapid TG clearance, this favorable condition is rapidly reversed in the absence of recent exercise. Because it has been reported that post-exercise changes to LPL protein mass and LPL activity are evident after a several-hour delay, with the maximal response occurring more than 8 h post-exercise, the effect in this study may at least partly be dependent on the enhancement of TG clearance by LPL. Because most of the evidence suggests that postprandial lipemia increases rapidly when training is interrupted, only frequent exercise will maintain a low level of postprandial lipemia. In contrast, the effect on postprandial insulinemia may persist longer.

In the present study, although serum dROMs decreased non-significantly and serum BAP non-significantly increased, plasma 8-epiPGF2α significantly decreased after training. Levels of dROMs mainly indicate serum hy-
droperoxide levels whereas BAP indicates the total antioxidant potential including urate, ascorbate, and \(\alpha\)-tocopherol. \(8-epiPGF_{2\alpha}\), a product of arachidonic acid peroxidation, is a specific biomarker of lipid peroxide produced by oxidative stress and has been reported to be associated with obesity and insulin resistance\(^{20}\). Reactive oxygen species (ROS) -mediated adaptation could be one way in which exercise decreases the incidence of ROS-associated diseases, including coronary heart disease, stroke, Alzheimer disease, and certain types of cancer (ref. 6 for review). Because ROS act as signals in exercise, and these signals result in up-regulation of antioxidant enzymes, exercise itself can be considered to be an antioxidant, and interfere with free radical metabolism by exogenous antioxidants may hamper useful adaptations to training\(^{21}\). The present results suggest that 4 weeks of moderate aerobic exercise training may improve antioxidant status in young, sedentary but otherwise healthy women.

It is clear that postprandial hyperlipemia or dyslipidemia is an important risk factor for atherosclerotic diseases. In fact, a substantial part of the modern life in Japan and other developed countries is spent in the postprandial state. Accordingly, for young apparently healthy women, it will be essential to ensure a healthier lifestyle such as regular exercise and adequate nutrition/diet as early as possible and to maintain suitable postprandial lipid metabolism and prevent postprandial hyperlipemia. A systematic review of clinical trials suggested that at least 10 METs-h/week in aerobic exercise, such as brisk walking, light jogging or stationary ergometer usage, is required for visceral fat reduction, and that there is a dose-response relationship between aerobic exercise and visceral fat reduction in obese subjects without metabolic-related disorders\(^{22}\).

**Conclusion**

Our results demonstrate that 4 weeks of moderate (ca 50% \(V_{O_2}\max\) for 30 min) aerobic exercise training may improve lipoprotein metabolism and antioxidant status in sedentary but otherwise healthy young women, mainly decreasing serum IAUC-TG and \(8-epiPGF_{2\alpha}\) levels. Although further studies are needed, moderate aerobic exercise may be useful in preventing postprandial hyperlipemia and oxidative stress and avoiding future metabolic syndrome for sedentary but otherwise healthy young women.

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**References**


