Alterations of atherogenic low-density lipoproteins and serum fatty acids after 12 week moderate exercise training in sedentary Thai women

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INTRODUCTION
Death from cardiovascular disease (CVD) is now emerging as a leading community health problem in developing nations. In recent years there has been a gradual decline in CVD in several developed countries including North America and many Western European countries.¹ Increasing CVD mortality has recently become a major health problem in Asia.²,³ In Thailand, the death rate due to CVD has increased more than 3 fold within three decades. Now, CVD has become the leading cause of death from non communicable disease.⁴

CVD has been linked to elevated plasma cholesterol (C) and low-density lipoprotein cholesterol (LDL-C).⁵ More recently, it has become apparent that elevated plasma C and LDL-C are not unique characteristics for individuals who suffer from CVD. Many patients who develop CVD have a similar plasma C value to those who do not suffer from CVD.⁶ Part of the explanation is that the most of the metabolic disorders contributing to CVD are not detected by routine tests of plasma C and LDL-C.

Human LDL particles comprise two different main fractions: large, buoyant LDL (lb-LDL) and small, dense LDL (sd-LDL) particles.⁷,⁸ Evidences have shown that sd-LDL particles are an independent risk factor for CVD development.⁹,¹⁰,¹¹ Subjects with predominant sd-LDL particles have a 3-fold increased risk of developing CVD independent of age, sex, and relative weight.¹² Furthermore, LDL sizes were found to be correlated positively with plasma high-density lipoprotein cholesterol (HDL-C) levels and negatively with plasma triacylglycerol (TG) levels¹²,¹³ and thus the combination of sd-LDL, decreased HDL-C and increased TGs has been defined as the atherogenic lipoprotein phenotype.¹⁴ Such profiles may explain the increased vascular disease incidence in populations that consume diets rich in highly digestible carbohydrates and saturated fats.²,¹⁵

Physical exercise is believed to have many beneficial effects, especially in preventing CVD.¹⁶ Observations by many investigators have shown that physically active

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individual experience higher cardiorespiratory fitness and improved lipoprotein profiles. Most research on this issue has examined the effect of exercise by focusing on plasma C, LDL-C and HDL-C levels. However, in some investigations, LDL-C or HDL-C did not respond to the exercise, implying that exercise had probably failed to reduce CVD risk factor. Alternatively, LDL sub-populations, exclusively sd-LDL particles are better parameters for CVD prediction rather than LDL-C and more appropriate parameters for monitoring the effect of physical activity on CVD compared to plasma and LDL-C.

Since CVD risk is gender-influenced, it has been found that a predominant presence of sd-LDL particles in women was significantly associated with 6.6-time increased CVD risk compared to 2.7-time in men. Differently from men, CVD risks drastically increase in women after menopause, evidently due to reductions in the amount of endogenous sexual steroid hormones, especially estrogens. The present experiment was therefore performed exclusively on middle-aged sedentary women. The effect of 12 weeks aerobic exercise training on lipoproteins profiles focusing on sd-LDL was studied. Profiles of serum fatty acid were also observed, since their metabolism is also influenced by physical exercise.

MATERIALS AND METHODS

Participants
Forty sedentary Thai women aged between 40-55 years old volunteered to participate in this study. Fourteen of all subjects were menopausal. All of them were non-smokers, did not exercise regularly, did not have any contraindications for exercise, had a body mass index (BMI) (calculated as weight/height²) < 23 kg/m², did not take medication affected lipid metabolism, were non-hypertensive and did not suffer from metabolic disorders (including diabetes) and did not use lipid-lowering drugs. All subjects were classified as normolipemic with plasma C <230 mg/dL and plasma TGs <150 mg/dL. All subjects underwent physical examinations by a doctor, and liver and kidney function tests prior to participation. Twenty volunteers joined an exercise program administered by Fitness Center of Health Science Services Unit, Faculty of Allied Health Sciences, Chulalongkorn University. The control group was matched with the first group in terms of age, BMI, lipoprotein profiles and menopausal status. Written consent forms were obtained from the subjects after explanation of the purpose, nature and potential risks of this study. This study was approved by the Ethics Committee of Health Sciences, Chulalongkorn University.

Exercise training program
The exercise program included 10 minutes warm-up, 25 minutes cycling on a bicycle ergometer and 10 minutes cool-down, 3 times a week for 12 weeks at an individual intensity of 60% heart rate reserve (HRR) which was calculated from the difference between maximum and resting heart rate.

Cardiorespiratory endurance testing
Cardiorespiratory endurance was measured in all subjects by estimation of maximal oxygen uptake (VO₂max) with a traditional Astrand-Ryhming bicycle ergometer sub-maximal exercise test protocol. Body composition in term of %Fat was assessed using a sum of skinfold method. Triceps, suprailium, and anterior thigh skinfolds were measured three times on the right side of body to the nearest 0.5 mm with a Lange clipper. A reliability criterion of 2 mm was established for triplicate measurements, and the mean of these measurements was used for analytical purposes.

Plasma lipid and lipoprotein measurements
Venous blood samples were collected from 12-hour overnight fasting subjects before entering the study (week -1) and at weeks 6 and 12 during the exercise program. Lipoproteins were isolated from EDTA plasma by sequential ultracentrifugation, using a Hitachi Microlutra centrifuge CS100 with Hitachi S100AT5 rotor (Hitachi Koki, Tokyo, Japan). Triacylglycerol-rich lipoproteins (TRLs) or combined very low-density lipoproteins (VLDLs) (d<1.006 g/mL) and intermediate-density lipoproteins (IDLs) (1.006<d<1.019 g/mL) were isolated at 500,000g ultracentrifugation for 3 h. After TRLs were separated from supernatant by tube slicing techniques (TSU2 tube slicer, Hitachi Koki, Tokyo, Japan), infranant was adjusted to density of 1.063 g/mL and centrifuged for 4 h at 500,000g in order for floating LDLs to be separated from HDLs infranant. LDL supernatant was re-separated for purifying LDL subfractions at density of 1.05 g/mL. Large, buoyant LDL (b-LDL; 1.019<d<1.05 g/mL) was then isolated from sd-LDL (1.05<d<1.063 g/mL) after 500,000g ultracentrifugation for 4 h. Cholesterol and TGs in plasma and all lipoprotein fractions were assessed by enzymatic kits (Human, Germany).

Fatty acid determination
Fatty acids in serum and lipoproteins were transesterified by acetyl chloride and determined by gas-liquid chromatography as described by Lepage and Roy. The obtained fatty acid methyl esters (FAMEs) were separated on DB-23 silica column (J&W Scientific) in a Fison 8000 gas chromatograph equipped with a flame ionization detector and auto sampler (Fison, Italy). The fatty acid compositions were expressed as individual FAME in grams per 100 g of total FAMEs.

Statistical analysis
Statistical analyses were analyzed using SPSS for Windows version 10.0. Mean values and standard error of mean (SE) are shown. Lipid and lipoprotein concentrations between exercise and control groups week -1, week 6 and week 12 were compared by two-way ANOVA. The level of significance was set at p<0.05.

RESULTS
Five of the 20 subjects in the control group were excluded: 3 dropped out while 2 increased their regular physical activity by joining a neighborhood exercise program. The results are therefore based on the remaining 35 subjects (20 from exercise and 15 from control groups) who completed this study. No serious complications or injuries were observed during the 12 weeks of exercise sessions.
Table 1. Baseline values (week -1) of body fitness, blood lipids and lipoprotein profiles compared between exercise and control groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=15)</th>
<th>Exercise (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>46.3±1.3</td>
<td>47.0±1.2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>55.5±2.6</td>
<td>58.8±2.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.5±0.8</td>
<td>23.7±1.5</td>
</tr>
<tr>
<td>% Fat</td>
<td>29.8±0.9</td>
<td>31.4±1.2</td>
</tr>
<tr>
<td>VO₂max (mL/kg/min)</td>
<td>33.3±2.0</td>
<td>31.6±1.5</td>
</tr>
<tr>
<td>Plasma C (mg/dL)</td>
<td>220±4.3</td>
<td>223±4.2</td>
</tr>
<tr>
<td>Plasma TGs (mg/dL)</td>
<td>110±7.1</td>
<td>117±5.2</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>143±8.1</td>
<td>141±4.7</td>
</tr>
<tr>
<td>lb-LDL-C (mg/dL)</td>
<td>98.7±4.6</td>
<td>100±3.1</td>
</tr>
<tr>
<td>sd-LDL-C (mg/dL)</td>
<td>26.7±4.3</td>
<td>28.4±1.1</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>57.6±3.0</td>
<td>55.3±2.4</td>
</tr>
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All parameters are in Means±SE; n, number of subjects; BMI, body mass index; VO₂max, maximal oxygen uptake; C, cholesterol; TG, triacylglycerols; LDL, low-density lipoproteins; lb-LDL, large, buoyant low-density lipoproteins; sd-LDL, small, dense low-density lipoproteins; HDL, high-density lipoproteins.

Table 1 shows the matched baseline characteristics for both groups.

The exercise training improved the cardiorespiratory fitness by increasing VO₂max by 11% (p<0.01) after 6 weeks and was sustained after 12 weeks without alteration in body weight, BMI, and %fat. No change in any variables was seen in the control group (Table 2). Liver (aspartate transaminase, alanine aminotransferase and alkaline phosphatase) and kidney function tests (blood urea nitrogen and creatinine) of both groups were also investigated during the participation. There were no significant changes in any parameters of both exercise and control groups (data not shown).

Changes in lipoprotein profiles from week -1, week 6 and week 12 after exercise intervention are shown in Figure 1. Figure 1A shows that 12-week aerobic exercise training resulted in 9.0% decrease in plasma TGs (117±5.2 v 106±4.7 mg/dL, for week -1 v week 12, p<0.05) and 8.0% decrease in TRL-TGs (72.3±3.9 v 65.9±3.5 mg/dL, for week -1 v week 12, p<0.01), whereas LDL-C was not affected by exercise. When LDL fractions were considered, sd-LDL-C reduced 17.0% (28.4±1.1 v 23.2±1.1 mg/dL, for week -1 v week 12, p<0.01), without changes in lb-LDL-C, plasma C and HDL-C (Figure 1B). No change was seen in the control group (Figure 1C and 1D). Figure 2 shows lipid and lipoprotein profiles compared between exercise and control groups at week 12. Plasma TGs, VLDL-TGs and sd-LDL-C after exercise decreased significantly (p<0.05). No correlation was found between changes in VO₂max and changes in lipid and lipoprotein profiles (data not shown).

When sd-LDL-C to lb-LDL-C (S/L) ratio was calculated as shown in Figure 3, it was found that moderate exercise training decreased S/L ratio significantly (0.27±0.01 v 0.22±0.01 for week -1 v week 12, p<0.01). Regarding fatty acid profiles, no significant change was observed in all serum fatty acids of exercise and control groups. No change was found when considered as either groups of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) of both the omega-3 and omega-6 types (data not shown).

DISCUSSION

This is the first study performed in sedentary Thai women demonstrating that moderate exercise training is able to reduce atherogenic sd-LDL-C. The results also revealed that 12 weeks of moderate exercise at 60% heart rate reserve (HRR), 25 min, 3 times a week, significantly decreased plasma TGs, TRL-TGs and sd-LDL-C, decreased S/L ratio without altering concentrations of C in plasma, LDL and HDL.

Regular physical activity is inversely related to the prevalence of CVD. In order to develop and maintain cardiorespiratory fitness, The American College of Sports Medicine (ACSM) recommends a healthy adult undertake regular exercise, including 20 to 60 minutes of physical activity at intensity 50 to 85% HRR, 3 to 5 times a week. Since cycling is one form of exercise which is not only low-risk and low-cost intervention but also easy to introduce to the vast majority of the public, cycling was used as the form of exercise in our experiment, namely 25 minutes of cycling at 60% of HRR, 3 times a week for 12 weeks (as recommended by ACSM). Subsequently, it was found that such an exercise program benefited cardiorespiratory fitness by improving VO₂max up to 11% by the end of sixth week of the program. This increment is sufficient evidence that moderate exercise is able to stimulate cardiorespiratory adaptation to training. No
correlation of changes between VO2max and profiles of neither lipids nor lipoprotein was found in this study similarly to results as observed by Katzmarzyk and colleagues.18

Normalization of lipid and lipoprotein levels, i.e. plasma C and TGs, HDL-C and LDL-C, for primarily preventing CVD begins with a multifaceted lifestyle approach including physical activity.27,28 The insignificant decreases in plasma C and LDL-C with slight increase in HDL-C observed in exercise group are in agreement with several studies previously reported.18,20,27 Plasma C and LDL-C are yet insensitive indicators for reflecting benefit of exercise. Many investigations finally focused on LDL-C found no reduction amongst the exercise subjects.29,30 Some authors have concluded that moderate exercise training is unable to ameliorate LDL-C concentrations.18,19,20 Therefore, in order to determine the advantage of physical activity on CVD, finely monitoring the distribution of LDL subpopulation is strongly suggested.

In the present investigation, the moderate exercise training undertaken by subjects was found to have no effect on plasma C, LDL-C and HDL-C as previously described by aforementioned investigations. However, a significant decrease of sd-LDL-C level was finally found (Figure 1B). These findings agree with the report of Halle and colleagues that moderate exercise reduced sd-LDL

Figure 1. The mean changes in concentrations of triacylglycerols and cholesterol in plasma and lipoprotein fractions of exercise (n=20) (Panels 1A and 1B) and control groups (n=15) (Panels 1C and 1D). All values are expressed as means±SE of concentration changes from week -1: at week 6 (open bar) and at week 12 (solid bar).

Figure 2. Concentrations of triacylglycerol (Panel 2A) and cholesterol (Panel 2B) in plasma and lipoprotein fractions of control (open bar) and exercise groups (solid bar) at the end of the study (week 12). All values are expressed as means±SE.
The effect of moderate exercise training on ratios of small, dense LDL-C to large, buoyant LDL-C (S/L ratio) in exercise and control groups.

In this study, although the potential mechanism of sd-LDL generation was not under investigation, it is likely that a decrease in cholesteryl ester transfer protein (CETP) activity induced by exercise training prevents the generation of TG-rich LDL particles. Reduction of CETP activity delays the core lipid transfer between VLDL and LDL particles which finally retards sd-LDL formation. Maintaining LDLs in the lb-LDL fraction together with preventing the shift of lb-LDL to sd-LDL particles benefited by moderate exercise, as observed in this study, is particularly important for the prevention of CVD. Another mechanism which prevents sd-LDL generation is the reduction of plasma TGs. High fasting and postprandial TG concentrations is independent risk marker for CVD, especially as evidenced during the postprandial period. In our experiment, plasma TGs decreased at the end of exercise concurrently with the reduction of TRL-TGs (Figure 1A). One explanation for this is that exercise increases lipoprotein lipase (LpL) activity, leading to an improvement of the capacity for TG hydrolysis. This confirms that exercisers have better clearance of incoming TGs than sedentary individuals. Therefore, exercise leads to shorten TRL residences in plasma.

The effect of regular moderate-intensity exercise on the fatty acid compositions in serum has been studied previously [36-38]. During exercise, fatty acids are hydrolyzed from TG storages in adipose tissues. According to the reports of Mougiou and colleagues, acute exercise increases unsaturated fatty acids (UFAs), especially MUFA, consequent to increase in UFAs / SFAs (U/S) ratio. After 24 hour of exercise, the percentage of fatty acids is adjusted toward baseline values, suggesting that changes in profiles of plasma fatty acid are merely temporary. Chronic exercise generally show no changes in the fatty acid profile, as confirmed in this study (Figure 4).

In conclusion, the study demonstrates that moderate exercise training has a beneficial effect on LDL subpopulations. The effect of an exercise on the generation of sd-LDL has never been studied in sedentary Thai women before. This effect is particularly important, as sd-LDL particles have been shown to be closely related to CVD. Moderate exercise training at 60% HRR, 3 days a week for 12 weeks induces changes in LDL metabolism. The reduction in sd-LDL-C, concomitant with an increase (but not significant) in lb-LDL-C implies the delay of lb-LDL modification toward sd-LDL generation. These factors monitored in Thai middle-aged sedentary women will be introduced to the public as an incentive for the adoption of exercise in order to prevent CVD.

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AUTHOR DISCLOSURES

Raveen Sittiwicheanwong, Tipayanate Ariyapitipun, Somnuk Gulsatitporr, Vanida Nonponpunth, Mahinda Abeywardena and Winai Dahl, no conflicts of interest.

REFERENCES


Original Article

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泰國久坐型態女性 12 星期中度運動訓練後致動脈粥化低密度脂蛋白和血清脂肪酸之變化

有氧運動在心血管疾病(CVD)的潛在益處，被認為是透過增加高密度脂蛋白(HDLs)。然而，運動對低密度脂蛋白(LDLs)及其亞群的影響在泰國仍不清楚的。20位久坐型態的泰國女性接受 12 週的運動訓練計畫(保留 60%心率)，包括每週 3 次，每次 10 分鐘暖身緩和運動，接著騎 25 分鐘腳踏車，另有 20 位久坐型態對婦女為控制組。分析血漿脂蛋白中三酸甘油酯(TGs)和膽固醇(C)包括富含三酸甘油脂的脂蛋白TRLs)、大且有浮力的 LDL(lb-LDL)、小且稠密的 LDL(sb-LDL)和 HDL，及血清脂肪酸組成。在 12 週運動後，發現血漿中 TGs、TRL-TGs、sd-LDL-C 和 sd-LDL-C/lb-LDL-C (S/L) 比率比一開始時顯著下降，分別為-9%, -8%, -17% 和-19%(p<0.05)；血清脂肪酸沒有改變。沒有運動的控制組中沒有任何數據的改變。這些結果顯示中度運動，即使 HDL 沒有改變，仍能阻止 lb-LDL 轉換成更多能致動脈粥化的 sd-LDL，因此可能可以預防健康及久坐型態的泰國女性的心血管疾病。

關鍵字：小且稠密低密度脂蛋白、中度運動、靜態活動婦女、心血管疾病、血清脂肪酸。