Physical Fitness and Reverse Cholesterol Transport

Beata Olchawa, Bronwyn A. Kingwell, Anh Hoang, Laurence Schneider, Osamu Miyazaki, Paul Nestel, Dmitri Sviridov

Background—Physical exercise is associated with a decreased risk of cardiovascular disease, which may be partly caused by the effect of exercise on the lipoprotein profile. The most consistent effect of exercise on lipoprotein metabolism is an increase in high-density lipoprotein (HDL).

Methods and Results—Parameters of reverse cholesterol transport (RCT) in 25 endurance-trained male athletes were compared with 33 age-matched males enjoying an active lifestyle. VO2max was higher in athletes than in controls (53.4±1.2 versus 38.8±1.0 mL/min per kg; P<0.01). The following differences in parameters of RCT were found: (1) plasma HDL cholesterol and apoA-I levels were higher in athletes compared with controls (1.7±0.1 versus 1.4±0.1 mmol/L; P<0.001; and 145±2 versus 128±3 mg/dL; P<0.001, respectively). Both correlated with VO2max up to the value of 51 mL/min per kg; (2) preβ-HDL was higher in athletes than in controls (54±4 versus 37±3 μg/mL; P<0.001) and correlated positively with VO2max; (3) lecithin cholesterol: acyltransferase activity was higher in athletes (29.8±1.2 versus 24.2±1.4 mmol/µL per hour; P<0.005); and (4) the capacity of plasma to promote cholesterol efflux from macrophages was higher in athletes (18.8±0.8% versus 16.2±0.3%; P<0.03).

Conclusions—The likely reason for higher HDL concentration in physically fit people is increased formation of HDL from apoA-I and cellular lipids. (Arterioscler Thromb Vasc Biol. 2004;24:1087-1091.)

Key Words: atherosclerosis ■ lipoproteins ■ exercise ■ cholesterol

Physical exercise and fitness are considered key factors affecting overall risk of cardiovascular disease. It is an effective intervention for prevention and treatment of coronary artery disease (CAD) as well as for rehabilitation of coronary patients.1,2 Exercise favorably affects a number of cardiovascular risk factors, such as obesity, insulin resistance, and blood pressure; it also has a beneficial effect on the lipoprotein profile.3–5 The latter may be a significant contributor to the protective effect of exercise partly by lowering the concentrations of triglyceride-rich lipoprotein (TRL) and low-density lipoprotein (LDL), especially atherogenic small LDL.3,5 However, the most distinct and sustained effect of exercise on lipoproteins is the increase in circulating high-density lipoprotein (HDL), both as HDL cholesterol and the number of HDL particles.3,5,6 These changes likely reflect activity in the reverse cholesterol transport (RCT) pathway. RCT is a multistep dynamic process comprising removal of excess cholesterol from peripheral tissues, including arterial wall macrophages and delivery to the liver for excretion.7 Although epidemiological studies consistently show that the HDL concentration is inversely correlated with cardiovascular risk, HDL exerts a number of antiatherogenic properties8 and higher levels of HDL are not necessarily a reliable indicator of enhanced RCT.7 To investigate the effect of physical fitness on HDL and RCT, we studied several key steps in RCT in endurance-trained athletes and compared them with a reference group of physically active individuals. We found that higher levels of physical fitness were associated with enhanced HDL formation indicating a likely increase in RCT efficiency.

Methods

Study Subjects
Twenty five male athletes were recruited from triathlon (19), biathlon (3), running (2), and swimming (1) teams. The athletes were 23 to 42 years old (mean age: 33.6±1.1 years). The reference group (controls) consisted of 33 normally active males. Control subjects were not engaged in structured sporting activity and performed ≈3 30-minute bouts of moderate exercise per week. The age of control subjects was 24 to 44 years old (mean age: 30.8±1.0 years; P>0.05 versus athletes). Athletes were tested during the “off season” period, ie, at ≈2 months after or before competition. The amount of exercise during this period is relatively low, aiming to sustain rather than increase physical fitness. Neither athletes nor controls had exercised for at least 24 hours before the tests. None of the subjects was using any lipid-lowering medication, performance-enhancing drugs or food additives, drank alcohol excessively, or smoked. Subjects fasted for 12 hours before blood collection.

The study was approved by the Alfred Hospital Ethics Committee and conducted in accordance with the Declaration of Helsinki of the World Medical Association.

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1087
Peak Pulmonary Oxygen Uptake
Peak pulmonary oxygen uptake (VO_2max: a measure of aerobic fitness) was determined during continuous incremental upright cycling to volitional exhaustion on an electronically braked ergometer. Expired air was analyzed for volume, O_2 and CO_2 using calibrated analyzers.

Blood Collection
After providing written informed consent the participants attended the Alfred Hospital at 9:00 AM. Blood (20 mL) was collected by a venepuncture into tubes containing streptokinase (Sigma; final concentration 150 U/mL) as the only anticoagulant. Blood was immediately cooled and plasma was isolated by centrifugation for 15 minutes at 4°C. Plasma was then aliquoted, snap-frozen in liquid nitrogen, and kept at −80°C. Aliquots were used once and not refrozen. It was demonstrated in preliminary experiments that under these conditions, the concentration of preβ-HDL remains the same as in fresh samples and does not change for at least 1 year. These strict cycling to volitional exhaustion on an electronically braked ergometer.

Lipids, Lipoproteins, Cholesterol Ester Transfer Protein, and Lecithin:Cholesterol Acyltransferase
Plasma total cholesterol (TC), triglyceride (TG), LDL cholesterol, apolipoprotein B (apoB), HDL cholesterol, and apolipoprotein A-I (apoA-I) were measured using spectrophotometric techniques with a Cobas-FARA centrifugal analyzer (Roche Diagnostic Systems). Preβ-HDL concentration was measured by enzyme-linked immunosorbent assay (ELISA) (Daichi Pure Chemicals, Tokyo, Japan). Standard samples were included in each round of determinations to account for the effect of interexperimental variations. The coefficient of variation for preβ-HDL assay was 8%. Changes in concentration of preβ-HDL determined by ELISA correlated with changes determined by 2-dimensional nondenaturating electrophoresis.

Cholesterol ester transfer protein (CETP) and lecithin:cholesterol acyltransferase (LCAT) concentrations were measured by ELISA (Daichii). CETP activity was measured by fluorometric assay (Roar Biomedical, New York, NY). LCAT activity was measured by the method of Bilici et al.

Cholesterol Efflux
Plasma cholesterol efflux was measured in 19 athletes and 24 controls (randomly selected). RAW-264.7 mouse macrophage cells were labeled by incubation with serum-containing medium containing [3H]cholesterol (Amersham; specific radioactivity 1.8 TBq/mmol; final radioactivity 75 kBq/mL) for 48 hours at 37°C. Cells were then washed 3 times with phosphate-buffered saline and incubated for 18 hours in serum-free medium containing 0.1% bovine serum albumin (essentially fatty-acid-free; Sigma). After the second incubation, cells were again washed and incubated for 2 hours with serum-free medium containing 2% plasma from subjects. Media and cells were then collected and radioactivity determined on a β-counter. Cholesterol efflux was defined as the amount of radioactivity in the medium divided by the amounts of radioactivity in the medium and cells ×100%. To account for interexperimental variations, a standard sample of plasma was included in each experiment.

Statistics
All results are expressed as mean±SEM. Group characteristics were compared by 1-way ANOVA. Correlations were calculated as Pearson product–moment correlation. In Figure 1, deviation from a linear relationship was defined by a breakpoint. The breakpoint was determined be arranging VO_2max values in ascending order, and including sequential values in the regression until inclusion of additional values caused a reduction in the Pearson correlation coefficient. The breakpoint was thus defined as the highest VO_2max value included in the linear regression.

Results
Physical Parameters and Non-HDL Lipids
The 2 groups investigated in this study were endurance-trained athletes and a normally active reference group (controls). Blood samples were taken at least 24 hours after exercise to exclude the effect of acute exercise. Choosing a normally active comparison group avoided a number of possible confounding factors, such as hypertriglyceridemia, obesity, and metabolic syndrome associated with sedentary lifestyle. The groups were well matched by age, body mass index (BMI) and waist-to-hip ratio (Table). There was no significant correlation between age, BMI, or waist-to-hip ratio and any lipid parameter. There was also no statistically significant difference between the 2 groups in plasma content of TC, TG, LDL cholesterol, and apoB (Table). Aerobic fitness measured as VO_2max was significantly higher in athletes (P<0.001) (Table).

HDL
Plasma concentrations of HDL cholesterol and apoA-I were higher in athletes compared with controls. On average, HDL

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>Athletes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO_2max (mL/min per kg)</td>
<td>38.8±1.0</td>
<td>53.4±1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2±0.4</td>
<td>23.9±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>1.00±0.01</td>
<td>0.98±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.9±0.1</td>
<td>5.2±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.9±0.2</td>
<td>3.2±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>apoB (mg/dL)</td>
<td>73.4±3.0</td>
<td>75.9±3.5</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.1±0.1</td>
<td>0.9±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.4±0.1</td>
<td>1.7±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>apoA-I (mg/dL)</td>
<td>128±3</td>
<td>145±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Preβ-HDL</td>
<td>37±3</td>
<td>54±4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LCAT mass (μg/mL)</td>
<td>7.0±0.3</td>
<td>6.6±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>LCAT activity (mmol/μL per h)</td>
<td>24.2±1.4</td>
<td>29.8±1.2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>CETP mass (μg/mL)</td>
<td>1.7±0.1</td>
<td>2.0±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>CETP activity (mmol/mL per h)</td>
<td>73±2</td>
<td>67±3</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Cholesterol efflux (%)</td>
<td>16.2±0.3</td>
<td>18.8±0.8</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Cholesterol efflux per apoA-I unit</td>
<td>0.13±0.01</td>
<td>0.13±0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean±SEM is shown.

N = 25 athletes and 33 controls, except for cholesterol efflux studies when n=19 athletes and 24 controls.
cholesterol was 21% higher and apoA-I was 13% higher in athletes (Table). HDL cholesterol correlated positively and significantly \((r=0.6; P<0.001)\) with aerobic fitness at lower values of \(\text{VO}_2\max\), but linear dependence ceased to be significant when higher \(\text{VO}_2\max\) values were included (Figure 1A). The breakpoint of the linear relationship was determined to be 51 mL/min per kg. Above this point, there was no statistically significant correlation between HDL cholesterol and \(\text{VO}_2\max\). A similar relationship was observed between aerobic fitness and plasma concentrations of apoA-I \((r=0.6; P<0.001)\) (Figure 1B).

Mean concentration of pre\(\beta\)-HDL was 46% higher in athletes compared with control subjects (Table). Plasma concentration of pre\(\beta\)-HDL correlated with \(\text{VO}_2\max\) \((r=0.4; P<0.01)\) (Figure 2) and with plasma levels of HDL cholesterol \((r=0.4 P<0.01)\) and apoA-I \((r=0.4; P<0.002)\) (Figure 3A and 3B).

**LCAT**

The concentration of LCAT was similar for athletes and control subjects; however, the activity of LCAT was 23% higher in athletes \((P<0.005)\) (Table). There was no correlation between LCAT activity or mass and HDL cholesterol, apoA-I, and pre\(\beta\)-HDL concentrations in the plasma. There was also no correlation between LCAT mass and activity.

**CETP**

There was no difference between athletes and controls in either CETP plasma content or CETP activity (Table). There was also no correlation between CETP mass or activity and HDL cholesterol, apoA-I, pre\(\beta\)-HDL, TG, LCAT activity, or mass.

**Cholesterol Efflux**

Cholesterol efflux was tested as the capacity of plasma to promote cholesterol efflux from a macrophage cell line. Mean cholesterol efflux to plasma samples from athletes was 16% higher compared with that from controls \((P<0.03)\) (Table). However, when cholesterol efflux was related to the concentration of apoA-I (which reflects the number of HDL particles), the difference between the 2 groups was eliminated (Table). We conclude that enhanced cholesterol efflux to plasmas from athletes was caused by a greater number of HDL particles, rather than to an increased ability of HDL particles to promote cholesterol efflux. Accordingly, there were significant positive correlations between cholesterol efflux and HDL cholesterol and apoA-I concentrations in plasma \((r=0.4; P<0.02\) for HDL cholesterol and \(r=0.3; P<0.05\) for apoA-I) (Figure 4A and 4B).

Cholesterol efflux did not correlate with pre\(\beta\)-HDL concentration or with LCAT or CETP mass or activity.

**Discussion**

The effect of physical exercise on plasma HDL levels has been demonstrated in a number of intervention studies. Long-term,\(^3\) short-term,\(^1,1^3\) and even single-session\(^1^4\) exercise all increase HDL cholesterol levels. A similar effect was found in several cross-sectional studies.\(^5\) The present study was aimed at: (1) investigating potential mechanisms mediating the effect of exercise on HDL levels; and (2) determining whether exercise induced increases in HDL cholesterol reflects increased functionality of RCT.

The major finding of this study was that physical fitness was associated with increments in plasma concentrations of HDL cholesterol, apoA-I, pre\(\beta\)-HDL, activity of LCAT in plasma, and in the capacity of plasma to promote cholesterol efflux from macrophages.
Higher levels of HDL cholesterol and apoA-I in physically fit people was an expected finding and the magnitude was consistent with other cross-sectional and intervention studies. Dependence of HDL cholesterol and apoA-I on the level of aerobic fitness was linear up to a value of \( V_o,max \) of 51 mL/min per kg; however, further increases in fitness were not accompanied by a further increase in HDL levels (Figure 1). Previous studies have also observed a low threshold in relation to the amount of exercise required to initiate an increase in HDL. Such a threshold was not observed in the current study, which is likely because of our normally active rather than sedentary control group. The presence of an upper threshold is a new finding, because the effect on HDL cholesterol was thought to be proportional to physical activity even at higher levels of fitness. This finding, however, is consistent with conclusions of Durstine et al, who found a significant difference in HDL cholesterol between “recreational” and “good” runners, but not between “good” and “elite” runners. The reasons for the existence of an upper threshold are not clear. We speculate that it might be related to the threshold in the increase of muscle mass associated with increasing fitness or factors linked to the catabolism of HDL.

Another novel finding of this study was that higher levels of fitness were accompanied by higher concentrations of plasma pre\( \beta \)-HDL. This finding is consistent with previously reported increases in pre\( \beta \)-HDL after exercise. We have previously demonstrated that pre\( \beta \)-HDL is generated during passage of blood from artery to vein across human leg muscles and that this can be substantially stimulated by exercise. Because athletes have a bigger muscle volume, we speculate that muscle may be an important source of higher pre\( \beta \)-HDL levels in athletes.

Pre\( \beta \)-HDL is considered to be the first product of lipida- tion of minimally lipidated apoA-I and a likely acceptor of cellular cholesterol. Plasma levels of pre\( \beta \)-HDL are higher in patients with hypercholesterolemia, CAD, and obesity. Because the magnitude of changes in pre\( \beta \)-HDL concentration usually exceed that in HDL cholesterol, and because pre\( \beta \)-HDL levels rapidly respond to treatment (eg, weight loss), it has been suggested that pre\( \beta \)-HDL may be a better marker of RCT than HDL cholesterol. We have previously suggested that pre\( \beta \)-HDL might be a marker of formation of HDL from minimally lipidated apoA-I (both synthesized de novo and recycled during HDL remodeling) and cellular cholesterol, a rate-limiting step in RCT. Consistent with our previous results, there was no correlation between pre\( \beta \)-HDL concentration and the capacity of plasma to promote cholesterol efflux, suggesting that pre\( \beta \)-HDL is more likely to be a product rather than an initiator of the efflux. Higher levels of pre\( \beta \)-HDL in dyslipidemia might therefore reflect higher activity of RCT in response to accumulation of cellular cholesterol caused by higher levels of plasma cholesterol or result from increased remodeling of triglyceride-enriched HDL by hepatic lipase in obese and dyslipidemic patients. It is, however, premature to draw conclusions regarding the relationship between pre\( \beta \)-HDL concentration and atherosclerosis. Asztalos et al reported either no difference in pre\( \beta \)-HDL between men with or without CAD or a higher pre\( \beta \)-HDL/\( \alpha \)-HDL ratio in subjects with more severe coronary artery stenosis. Miida et al have reported higher pre\( \beta \)-HDL values among Japanese men with CAD. The mechanisms responsible for such differences between patients with CAD and healthy subjects have not been studied and may be multiple. The concentration of pre\( \beta \)-HDL in plasma is a reflection of the rate of its formation (result of cholesterol efflux and lipolysis of triglyc- eride-rich lipoproteins) and remodeling (through the action of LCAT). Thus, the level of pre\( \beta \)-HDL could reflect the sum of both these steps and should be interpreted in a context of changes in other parameters of RCT.

It is important to recognize that anti-pre\( \beta \)-HDL antibody used in the assay reacts with epitopes of apoA-I masked during apoA-I lipidation but exposed on pre\( \beta \)-HDL and lipid-free apoA-I. However, when human plasma or delipidated apoA-I were separated on 2-dimensional nondenaturing electrophoresis, no fraction corresponding to lipid-free apoA-I was found in plasma. When plasma was pretreated with the anti pre\( \beta \)-HDL antibody the pre\( \beta \)-HDL fraction was the only fraction that had shifted, no other fraction being affected by the antibody. This evidence indicates that there are no detectable amounts of fully delipidated apoA-I in human plasma.

The capacity of plasma from athletes to promote cholesterol efflux was enhanced compared with controls, but the increased number of HDL particles fully accounted for this effect. This indicates that athletes are capable of producing more HDL particles, but the ability of these HDL particles to promote cholesterol efflux is unchanged.

Contributing to the higher HDL cholesterol in the athletes was their higher LCAT activity, an observation consistent with that of Lehmann et al and Gupta et al. It may be speculated that the increase in LCAT activity without an increase in LCAT mass is secondary to the higher concentra- tion of its major substrate and possible carrier, pre\( \beta \)-HDL, which is consistent with the fact that we found no correlation between plasma mass and activity of LCAT despite at least 2- to 3-fold variation in each of the parameters. It has been demonstrated by Chung et al that activity of LCAT depends on its distribution among different lipoproteins, with LCAT residing on lipoproteins other than HDL, being only minimally active. Possibly, lack of correlation between LCAT mass and activity reflects distribution of LCAT between lipoprotein subfractions.

Recent studies on the process of RCT suggest that it is likely to be comprised of the following steps: (1) efflux of cellular cholesterol on lipid-free or minimally lipidated apoA-I. The process is most likely modulated by ABCA1 and results in formation of pre\( \beta \)-HDL particles; (2) further efflux of cholesterol to pre\( \beta \)-HDL with the formation of larger discoid HDL particles; (3) esterification of cholesterol with LCAT resulting in formation of spherical HDL; (4) maturation of HDL: formation of large HDL particles either by acquiring and esterifying additional cholesterol from other lipoproteins or by fusion of smaller HDL particles; and (5) remodeling of mature HDL by the action of CETP, phospho- lipid transfer protein (PLTP), hepatic lipase, and scavenger receptor type B1 (SR-B1) with the formation of smaller HDL particles and minimally lipidated apoA-I. The latter may...
undergo further lipidation with cellular cholesterol. Higher levels of HDL cholesterol, which mainly represent mature HDL particles, may be caused by increased formation of these particles (“anabolic” arm of RCT, stages 1 to 4) or caused by retarded remodeling of mature HDL (“catabolic” part, stage 5). The former situation would likely indicate increased functionality of RCT, whereas the latter would be indicative of a dysfunctional RCT.

Summarizing our findings, we established that a high level of fitness is accompanied by enhanced capacity of plasma to promote cholesterol efflux (step 1 of RCT), increased concentration of preβ1-HDL (steps 1 and 2), increased activity of LCAT (step 3), and higher concentration of HDL cholesterol (step 4). All these steps represent the anabolic arm of RCT and are involved in formation of mature HDL. However, at least 1 key parameter of the catabolic arm of RCT, CETP, was unchanged. We hypothesize that higher levels of HDL cholesterol in athletes may be a consequence of increased formation of HDL from apoA-I and cellular cholesterol, enhanced flow of cholesterol along the RCT pathway, and hence increased functionality of RCT. Similar changes in RCT were observed when formation of HDL was induced by intravenous infusion of apoA-I/phospholipid discs in humans30 or oral administration of dimyristoylphosphocholine to apoE−/− mice.31 The latter was accompanied by marked reduction of atherosclerosis.

Acknowledgments

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References

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