The Effect of Endurance Exercise Training on Plasma Lipoprotein AI and Lipoprotein AI:AII Concentrations in Sedentary Adults

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The effect of 6 months of endurance exercise training on plasma concentrations of lipoprotein (Lp)AI and LpAI:AII was determined in 39 sedentary subjects (17 men, 22 women, average age, 57 years) with abnormal cholesterol concentrations (total cholesterol [TC] > 200 mg/dL, or high-density lipoprotein-cholesterol [HDL-C] < 35 mg/dL). Following exercise training, plasma LpAI concentrations increased (+5.9 ± 1.2 mg/dL; $P < .001$), but there was no change in total apolipoprotein (apo) A-I or LpAI:AII concentrations. The change in plasma LpAI concentration was positively correlated to changes in total HDL-C ($r = .495$, $P = .001$), the sum of HDL4-C_{nmr} + HDL5-C_{nmr} ($r = .417$, $P = .008$), and average HDL particle size ($r = .415$, $P = .009$), but not to changes in body composition or V)$o_2$max. In the 8 subjects with the greatest change in LpAI concentration following training, the size distribution of LpAI and LpAI:AII particles in plasma also was measured before and after training. In these subjects, the size distribution of LpAI:AII particles did not change with training, but there was a significant increase (0.1 nm; $P = .048$) in the peak size of the "medium" (7.8 to 9.8 nm) LpAI particles after training. In 7 subjects who served as age- and weight-matched sedentary controls, plasma concentrations of total apo A-I, the LpAI and LpAI:AII subfractions, and plasma lipoprotein-lipids did not differ significantly between baseline and final testing. These data indicate that endurance exercise training increases the average size and plasma concentrations of LpAI, but not LpAI:AII, particles, which may represent possible enhancements of reverse cholesterol transport and may provide insight into the role that exercise plays in reducing cardiovascular disease risk.

THE INVERSE RELATIONSHIP between plasma concentrations of high-density lipoproteins (HDL) and the development of coronary heart disease (CHD) in humans is well established. However, HDL are a heterogeneous group of particles that can be separated into subfractions based on their size,[1,2] density,[3] electrophoretic mobility,[4] and apolipoprotein (apo) content,[5] and these subfractions vary in terms of their metabolic, functional and clinical significance. Based on apo content, 2 major classes of HDL are generally recognized: HDL containing apo A-I without apoA-II are termed lipoprotein A-I (LpAI), while those that contain both apoA-I and apoA-II are designated lipoprotein A-I:A-II (LpAI:AII).[6] A number of factors indicate that LpAI particles are more cardioprotective than LpAI:AII, primarily because they are thought to play a greater role in reverse cholesterol transport (RCT).[7] Several studies have shown that plasma LpAI concentrations have a stronger inverse relationship to CHD risk than LpAI:AII concentrations,[8-10] although other studies have shown no difference.[11,12] Adolescents with a family history of CHD,[13] individuals with type III dyslipoproteinemia (a metabolic disorder leading to increased risk for atherosclerosis),[14,15] and individuals with type 2 diabetes[16] all have reduced concentrations of LpAI and/or elevations in LpAI:AII. In addition, octogenarians have significantly elevated concentrations of LpAI and reduced concentrations of LpAI:AII compared with younger controls,[17] suggesting a correlation between LpAI concentrations and longevity.

The metabolism and clinical significance of plasma LpAI particles may also depend on their size distribution. LpAI particles have been separated into small (7.2 to 8.2 nm), medium (8.2 to 9.8 nm), and large (9.8 to 12.2 nm) subfractions,[18] which contain at least 2, 3, or 4 apo A-I molecules, respectively, per particle.[19] Recent kinetic studies in nonhuman primates[20,21] indicate that "medium" and "large" LpAI represent an endpoint in the maturation of LpAI particles, thus increases in the plasma concentrations of these subfractions may serve as markers for an increase in RCT, and suggests that increases in the average size of LpAI particles may indicate a reduction in cardiovascular disease (CVD) risk.

A number of studies have examined the effect of exercise training on plasma concentrations of total apo A-I and apo A-II, the primary apo on HDL particles, but the results have been equivocal. Apo A-I concentrations have been shown to increase,[22-28] decrease,[29,30] or stay the same[25,31,32] following exercise training, while apo A-II concentrations generally have not changed.[27,32,33] However, it is not known whether exercise training has a differential effect on plasma concentrations of apo AI within the LpAI and LpAI:AII subfractions. In a cross-sectional study, Frey et al[34] found that a group of young sedentary and endurance trained men had similar apo AI concentrations within LpAI particles, but the apo AI concentration within LpAI:AII particles was greater in the endurance trained subjects. However, to our knowledge, there are no published reports that have distinguished the effect of exercise training on plasma concentrations of apo AI within LpAI and LpAI:AII particles. Thus, the primary purpose of this study was to determine whether there are changes in the plasma concentrations or size of LpAI and LpAI:AII particles following endurance

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exercise training.[35,36] We hypothesized that the apo AI concentration within LpAI, but not LpAI:AII, would increase following the exercise training intervention, and that there would be an increase in the average size of LpAI, but not LpAI:AII, particles.

MATERIALS AND METHODS

Subjects

Forty–six subjects were recruited for the study. Thirty-nine (17 men, 22 women) were assigned to the exercise group, while 7 (3 men, 4 women) age- and weight-matched subjects served as sedentary controls. Eligibility requirements included: age 50 to 70 years; sedentary (< 40 minutes/week of aerobic activity for the 6 months prior to the study); total cholesterol (TC) concentrations greater than 200 mg/dL or HDL-C < 35 mg/dL; triglyceride (TG) concentrations < 400 mg/dL; nonsmoking; free of CVD; nondiabetic; body mass index (BMI) less than 35; not on lipid or glucose-lowering medications; normotensive, or hypertension controlled (blood pressure [BP] less than 160/99) by medications not affecting lipid or glucose metabolism; no history of ulcers or other bleeding disorders; and no other medical conditions that would preclude subjects from participating in a vigorous exercise training program. In addition, all women were postmenopausal (absence of menses for more than 2 years) and were required to maintain their current hormone replacement regimen (on or not on) for the duration of the study.

Seven subjects meeting all requirements for the study served as sedentary controls after being put on a waiting list for the exercise training program. These subjects underwent dietary stabilization (see below), then remained sedentary for 6 months while waiting for the next exercise cohort.

The experimental protocol was approved by the University of Maryland - College Park Institutional Review Board, and all subjects provided their written informed consent prior to starting the study.

Dietary Stabilization

All subjects meeting the preliminary requirements began a 6-week dietary instruction class and were stabilized on an American Heart Association (AHA) step 1 diet for at least 3 weeks prior to baseline testing. Compliance with this diet was monitored by the completion of 7-day food records in weeks 3 and 6 of the dietary class. Food records were analyzed for cholesterol intake, total caloric intake, and the percentage of calories from total fat, saturated fat, protein, and carbohydrate using Nutritionist IV software (N-Squared Computing, San Bruno, CA).

$\dot{V}O_2$max Testing

After subjects were stabilized on the AHA step I diet, those assigned to the exercise training group had their $\dot{V}O_2$max determined during graded treadmill walking or jogging using a modified Balke protocol,[37] with the grade adjusted 2% to 3% every 2 minutes during the test so that the total exercise time before the subject reached subjective exhaustion would be 8 to 12 minutes. $\dot{V}O_2$max was considered to have been achieved when at least 2 of the following criteria were met: (1) no further increase in oxygen uptake with an increase in work rate (< 150 mL/min); (2) age-predicted maximum heart rate; or (3) a respiratory exchange ratio of greater than 1.15.

Body Composition

Total body fat, lean body mass, and regional body fat were assessed by dual energy x-ray absorptiometry (DEXA) (model DPX-L; Lunar Corp, Madison, WI). This method has been described in detail elsewhere.[38] Intra-abdominal (IA) fat (visceral and subcutaneous [SC] adipose tissue areas) was assessed by computed tomography (CT) scan midway between L4 and L5 using a GE HiLight CT scanner (Philips Medical Systems, Philadelphia, PA) and a protocol outlined elsewhere.[39]

Plasma Lipoprotein-Lipid Measurements

Venous blood samples were drawn in the morning after a 12-hour fast for analysis of major plasma lipid concentrations and lipoprotein particle sizes. All baseline blood samples were drawn at the end of the 6-week dietary stabilization program, and at least 4 days following any exercise test. Plasma was isolated from these samples by centrifugation at 3,000 × *g* for 15 minutes at 4°C in the presence of 0.01% EDTA, then frozen at -70°C until analyzed. Plasma lipoprotein-lipid concentrations were measured by nuclear magnetic resonance spectroscopy (NMR), performed in the laboratory of Dr James Otvos, Lipomed, Inc (Raleigh, NC). The NMR analysis included determinations of both low-density lipoprotein (LDL) and HDL particle diameters, in addition to measurements of major lipoprotein-lipid concentrations. NMR differentiates 5 subclasses of HDL particles, termed HDL 1, 2, 3, 4, and 5, which have particle diameters of approximately 7.2 to 7.8 nm, 7.8 to 8.2 nm, 8.2 to 8.8 nm, 8.8 to 9.8 nm, and 9.8 to 12.2 nm, respectively. These roughly correlate in size and density to the 5 HDL subfractions historically designated as HDL3c, 3b, 3a, 2a, and 2b, respectively.

Determination of Total apo A-I, LpAI, and LpAI:AII Concentrations

For the purposes of this study, LpAI and LpAI:AII concentrations are defined as the concentration of apo A-I within LpAI and LpAI:AII particles, respectively. Total plasma apo A-I and LpAI concentrations were measured by an electroimmunoassay procedure[40] on Hydragel apo A-I/B and Hydragel LpAI electroimmunodiffusion gels (Sebia, Norcross, GA), respectively. Plasma concentrations of LpAI:AII were then calculated as the difference between the total apo A-I and LpAI concentrations, and thus represent the concentration of apo A-I within LpAI:AII particles.

Isolation of LpAI and LpAI:AII Particles

To determine the size distribution of LpAI and LpAI:AII particles before and after exercise training, LpAI and LpAI:AII particles were isolated by sequential immunoaffinity chromatography from fasting plasma samples from the 8 subjects who exhibited the largest increase in plasma LpAI concentrations following exercise training. Only 8 subjects were chosen for this preliminary analysis because the time and cost involved in isolating these particles prohibited us from performing these assays on all 39 subjects. The immunoaffinity columns used to isolate these particles were prepared by coupling monoclonal antibodies for either apo A-I or apo A-II to Affi-gel 10 (Bio-Rad, Hercules, CA) according to the manufacturer's directions. The immunoaffinity columns were equilibrated with phosphate-buffered saline (PBS) (0.01 mol/L Na phosphate, 0.15 mol/L NaCl, pH 7.4) before use. Plasma samples (4 mL) were applied to the anti-apo A-I column and allowed to mix for more than 2 hours. The flow through from the anti-apo A-I column was then collected and discarded, and the column was washed with PBS until the absorbance at 280 nm returned to baseline concentrations (which indicated that all unbound particles had been cleared from the column). The bound particles (apo A-I containing particles, ie, HDL) were then eluted with 1 bed volume (35 mL) of 3 mol/L NaSCN, pH 7.4, and immediately desalted over an 80-mL Sephadex G-25 (Pharmacia, Peapack, NJ) medium coarse column, dialyzed, and then concentrated in Centriflo CF25 membrane cones (Amicon, Bedford, MA). The concentrated elute from the anti-apo A-I column was then applied to the anti-apo A-II column and allowed to mix for more than 2 hours. The flow through from the anti-apo A-II column was collected,

and the column was washed with PBS until the absorbance at 280 nm returned to baseline concentrations. The flow through and the first 35 mL of wash were combined and concentrated. This represented the isolated LpAI fraction. The anti-apo A-II column was then eluted with NaSCN, desalted, dialyzed, and concentrated as described above for the anti-apo A-I column. The elute from the anti-apo A-II column contained the isolated LpAI:AII fraction. A differential electroimmunoassay[40] on Hydragel LpAI plates (Sebia) was used to test for the presence of apo A-II in the isolated LpAI sample and for the presence of LpAI particles in the isolated LpAI:AII samples. Only purified LpAI and LpAI:AII samples were used to determine particle size distributions by the methods discussed below.

Determination of LpAI and LpAI:AII Particle Size Distribution

To determine particle size distributions, LpAI and LpAI:AII particles isolated from the immunoaffinity columns were applied to precast 4% to 30% nondenaturing polyacrylamide gradient gel electrophoresis (GGE), as described elsewhere.[41] Following electrophoresis, a fixative (10% sulfosalicylic acid) was applied to the gels and allowed to mix at room temperature for more than 2 hours. The gels were then stained overnight with 0.02% Coomassie in 3.5% perchloric acid and then destained in 7.5% acetic acid until the background of each gel was clear.

The 4% to 30% gradient gels were then analyzed using a Nucleovision gel analyzer with GelExpert (version 3.5) software (NucleoTech Corp, San Mateo, CA). Particle sizes of the LpAI and LpAI:AII subfractions were determined from their migration distance relative to the migration distance of 5 standard proteins of known molecular weight and size (Pharmacia HMW standard). Using polyacrylamide GGE, past studies[18,19] have resolved HDL into 3 particle sizes, denoted "small" (7.2 to 8.2), "medium" (8.2 to 9.8 nm), and "large" (9.8 to 12.2 nm), respectively. However, only 1 or 2 distinct particle peaks were found in the LpAI and LpAI:AII samples analyzed here, which we designated as "medium" or "large" particles, having particle sizes in the range of approximately 7.8 to 9.8 nm or 9.8 to 12.2 nm, respectively. The gel analysis detected no particles in the 7.2 to 7.8 nm range, and the NMR analysis also indicated there were virtually no HDL particles less than 7.8 nm (corresponding to $HDL1_{nmr}$) in the plasma samples at baseline (1 subject did have a trace amount of HDL particles smaller than 7.8 nm at final testing), so the concentration of "small" particles was estimated to equal zero. The area under each particle peak detected was calculated to estimate the percentage of each LpAI or LpAI:AII size subfraction relative to the total. The position of each particle peak was also measured to compare the peak particle sizes before and after the training intervention.

Exercise Training Protocol

Subjects assigned to the exercise training program exercised 3 times/week for 6 months on stairstepping machines, rowing ergometers, treadmills, stationary bicycles, and ski machines in the presence of study personnel. Initial exercise sessions consisted of 20 minutes of exercise corresponding to 50% of each subject's heart rate reserve (Karvonen method), assessed using heart rate monitors (Polar, Brooklyn, NY). Each week the subjects increased the duration of their workouts by 5 minutes until they reached 40 minutes/session in week 5. The intensity of the workouts then increased by 5%/week until they reached an intensity of 70% heart rate reserve in week 9. Starting in week 10, subjects were instructed to add a weekend 45- to 60-minute unsupervised low-intensity walk to their exercise programs. Subjects then maintained this intensity and duration of exercise for the remainder of the program (24 weeks total).

Subjects assigned to the control group completed dietary stabilization and maintained an AHA step 1 diet, but did not participate in the training program and were instructed to maintain the same activity level (less than 40 minutes of aerobic exercise activity/week) they had at baseline.

Dietary Monitoring

Subjects completed 7-day food records every 8 weeks during the training program to ensure compliance with an AHA step I diet. Furthermore, subjects were weighed once a week to ensure they did not lose significant weight during the program. Any subject who lost more weight than expected from their increased energy expenditure associated with exercise training was counseled to increase their energy intake.

Final Testing

Following the completion of the exercise-training intervention, all subjects underwent a final $\dot{V}O_2$max test, and body composition was again measured via DEXA and CT scans. In addition, fasting blood samples were drawn 24 to 36 hours following an exercise training session to reassess all plasma lipoprotein-lipid concentrations and lipoprotein particle sizes. Subjects in the nonexercising control group had blood samples drawn 6 months after baseline samples were drawn.

Statistics

K-S normality tests indicated that all variables were normally distributed, so parametric statistics were used to analyze all data. Paired sample *t* tests were utilized to assess training-related effects on all variables in this study, including changes in major lipoprotein-lipid concentrations, average lipoprotein particle sizes, body composition measures, and $\dot{V}O_2$max. Correlation analysis and multiple linear regression were used to assess the independent determinants of changes in lipoprotein size, concentration, and lipid concentrations. All data are presented as mean ± SE. $P < .05$ was accepted as statistically significant.

As stated above, to qualify for this study, each subject had to have an elevated TC (> 200 mg/dL) and/or a low HDL-C concentration (< 40 mg/dL). Only 3 of the 39 subjects in the exercise training group qualified on the basis of a low HDL-C level only, so the data for these 2 groups were combined.

RESULTS

Physical characteristics at baseline and final testing for the subjects in the exercise training group are presented in Table 1. Changes in body mass indices and lipoprotein sizes and concentrations following exercise training did not differ between women on and not on hormone replacement therapy (HRT) or between males and females (data not shown), so all data in the study are pooled across gender. On average, the subjects in the exercise group lost approximately 1 kg of body weight ($P = .004$), which included a significant reduction in both percent body fat and IA fat mass, and $\dot{V}O_2$max increased by 17% ($P < .001$) at final testing. For the 7 control subjects, the average age was 56, and average weight at baseline and final testing was 76.2 kg and 76.6 kg, respectively (P = not significant [NS]).

Changes in Lipoprotein Size and Concentrations

Table 2 shows the average size and concentrations of the major lipoprotein fractions at baseline and final testing in the exercise and control groups. For the control group, there was no significant change in any of the lipoprotein-lipid concentrations measured over the 6-month time period. For the exercise group,

Table 1. Physical Characteristics of Subjects in Control and Exercise Training Groups at Baseline and Final Testing

	Baseline	Final Test	*P* Value
Age			
Exercise	57.3 ± 1.3	—	—
Control	56.3 ± 0.5	—	—
Weight (kg)			
Exercise	79.4 ± 2.5	78.3 ± 2.4	.004*
Control	76.2 ± 4.4	76.6 ± 4.3	.482
Body fat (%)			
Exercise	35.8 ± 1.3	34.8 ± 1.3	<.001*
Lean body weight (kg)			
Exercise	47.5 ± 1.9	48.0 ± 1.8	.059
Intra-abdominal fat (cm^2)			
Exercise	136.1 ± 7.0	119.8 ± 6.0	<.001*
Subcutaneous fat (cm^2)			
Exercise	305.0 ± 18.3	301.6 ± 17.7	.503
$\dot{V}o_2$max (mL/kg/min)			
Exercise	25.3 ± 0.7	29.4 ± 0.8	<.001*

NOTE. All values are listed as mean ± SE. In exercise group, n = 39 (17 men, 22 women). In control group, n = 7 (4 men, 3 women).

*Denotes a statistically significant change from baseline values at $P < .05$.

there was no significant change in either TC or LDL-C concentrations, but HDL particle size and total HDL-C concentrations increased significantly (+1.3%, $P = .004$; and 8.8%, $P = .001$, respectively). The increase in total HDL-C was primarily due to increases in the largest HDL-C subfractions ($HDL4_{nmr}$ and $HDL5_{nmr}$). Average TG concentrations were reduced by approximately 14 mg/dL, although this change was not statistically significant ($P = .053$).

Changes in apo AI Concentrations

Table 3 shows the changes in plasma concentrations of total apo AI and the apo AI concentration within the LpAI and LpAI:AII subfractions, while Table 4 shows the size distribution of the LpAI and LpAI:AII particles at baseline and final testing. There was a significant increase in LpAI concentrations (≈ 12%) ($P < .001$) in the training group, despite no change in either total apo A-I or LpAI:AII concentrations. In the control group, there was no significant change in plasma concentrations of either total apo A-I or the LpAI and LpAI:AII subfractions.

Changes in LpAI and LpAI:AII Particle Sizes

Plasma samples from the 8 subjects with the largest exercise training-induced changes in LpAI concentrations were used to measure changes in LpAI and LpAI:AII particle sizes. These eight subjects (4 men, 4 women) had a mean increase in plasma LpAI concentration of 17.2 ± 2.0 mg/dL, compared with an increase of 5.9 ± 1.2 mg/dL for the whole group ($P < .001$), but did not differ from the remaining group in terms of any other lipid, physical, or demographic characteristics (data not shown). Table 4 shows the peak particle sizes within the LpAI and LpAI:AII subfractions, as well as the percentage of LpAI particles classified as "large" (9.8 to 12.2 nm) before and after the exercise training intervention. When the isolated LpAI samples were run on 4% to 30% polyacrylamide gradient gels to separate these particles into different sized bands (see Materials and Methods), 7 of the 8 subjects were found to have 2 bands of LpAI particles, 1 ranging from 7.8 to 9.8 nm (denoted "medium") and the second spanning 9.8 to 12.2 nm (denoted "large"), while 1 subject had only 1 detectable band, which corresponded to "medium"-sized LpAI particles. For each of the LpAI:AII samples, only 1 band was evident, corresponding to "medium"-sized particles. No band in either the LpAI or LpAI:AII fractions were detected in the region of the gels corresponding to "small" HDL (7.2 to 7.8 nm) particles. There was no significant change in the ratio of "medium" to "large" LpAI particles after training, and there also was no change in the position of either the "large" LpAI or "medium" LpAI:AII particle peaks, indicating no change in the peak particle sizes of these subfractions. However, there was a significant increase in the peak position of the bands corresponding to "medium" LpAI particles, indicating that the average size of the "medium" particles increased by 0.08 nm ($P = .048$) with training.

Table 2. Lipoprotein Sizes and Lipoprotein-Lipid Concentrations Before and After Exercise Training

	Exercise Training Group (n = 39)		Control Group (n = 7)	
Lipoprotein-lipid	Baseline	Final Test	Baseline	Final Test
TC (mg/dL)	214 ± 5.5	214 ± 5.7	225 ± 9.2	236 ± 14.6
LDL-C (mg/dL)	141 ± 4.1	141 ± 3.6	151 ± 6.9	161 ± 13.2
HDL-C (mg/dL)	40.9 ± 1.9	43.9 ± 1.9*	43.4 ± 3.7	41.6 ± 2.8
HDL avg size (nm)	8.7 ± 0.1	8.8 ± 0.1*	8.7 ± 0.1	8.6 ± 0.1
$HDL5\text{-}C_{nmr}$ (mg/dL)	2.9 ± 0.9	4.0 ± 0.9	1.6 ± 1.0	1.0 ± 0.6
$HDL4\text{-}C_{nmr}$ (mg/dL)	11.1 ± 1.0	14.9 ± 1.1*	14.8 ± 3.4	16.4 ± 2.9
$HDL3\text{-}C_{nmr}$ (mg/dL)	14.3 ± 1.8	11.4 ± 1.5	8.3 ± 4.3	4.4 ± 1.9
$HDL2\text{-}C_{nmr}$ (mg/dL)	12.6 ± 1.3	13.6 ± 1.1	18.8 ± 3.3	19.9 ± 2.0
$HDL1\text{-}C_{nmr}$ (mg/dL)	0.0 ± 0.0	0.0 ± 0.0	0 ± 0	0.0 ± 0.0
$HDL1 + 2 + 3\text{-}C_{nmr}$ (mg/dL)	26.9 ± 0.8	25.1 ± 0.7*	27.0 ± 1.8	24.3 ± 1.1
$HDL4 + 5\text{-}C_{nmr}$ (mg/dL)	14 ± 1.5	18.8 ± 1.6*	16.5 ± 3.5	17.4 ± 3.0
TG (mg/dL)	146 ± 9.9	132 ± 9.6	144 ± 30.0	135 ± 20.0

NOTE. All values are listed as mean ± SE.

*Denotes a statistically significant change from baseline values at $P < .05$.

Table 3. Apo AI Concentrations Before and After Exercise Training

Apo Fraction	Exercise Training Group (n = 39)		Control Group (n = 7)	
	Baseline	Final Test	Baseline	Final Test
Total apo AI (mg/dL)	167 ± 4.3	169 ± 3.7	152 ± 5.7	152 ± 7.4
Apo AI within LpAI (mg/dL)	51 ± 2.2	57 ± 2.3*	44 ± 7.4	44 ± 8.5
Apo AI within LpAI:AII (mg/dL)	116 ± 3.3	112 ± 3.4	109 ± 8.4	107 ± 10.4

NOTE. All values are listed as mean ± SE.

*Denotes a statistically significant change from baseline values at $P < .05$.

Correlations With Changes in LpAI and LpAI:AII Concentrations

For the exercise training group, correlations between changes in LpAI and LpAI:AII with HDL-C and HDL particle size changes are shown in Table 5, while correlations with changes in body composition indices are shown in Table 6. The change in LpAI concentration was positively correlated to the change in total HDL-C, the change in average HDL particle size, and the change in the sum of $HDL4_{nmr}$-C + $HDL5_{nmr}$-C, but was not correlated to baseline concentrations of either total apo A-I ($r = -.205$; $P = .212$) or LpAI ($r = -.186$; $P = .257$). There also was no correlation between the change in LpAI or HDL-C and changes in either $\dot{V}O_2$max or indices of body composition (body weight, BMI, percent fat mass, IA fat mass, and SC fat mass). In a multiple regression analysis that included the ΔHDL size, Δtotal HDL-C, and ΔHDL4 + 5-C_{nmr} as independent variables, the change in total HDL-C independently predicted the change in LpAI, explaining 25% of the variance in the change in LpAI concentrations with training ($y = 0.7336 * \Delta$HDL-C + 3.73; $r^2 = .2448$; $P = .001$). In addition, the change in LpAI:AII was positively correlated with the change in total HDL-C, the change in the sum of HDL1-C_{nmr} + HDL2-C_{nmr} + HDL3-C_{nmr}, and the change in lean body weight (LBW).

Table 4. LpAI and LpAI:AII Particle Sizes Before and After Exercise Training

	Mean ± SE (nm)	Difference (nm)
Peak particle size (nm)		
Large (> 9.8 nm) LpAI particles		
Baseline (n = 7)*	10.3 ± 0.10	−0.04 ± 0.14
Final testing (n = 7)*	10.3 ± 0.04	($P = .774$)
Medium (8.2 to 9.8 nm) LpAI particles		
Baseline (n = 8)	8.1 ± 0.04	0.08 ± 0.04
Final testing (n = 8)	8.2 ± 0.03	($P = .048$)†
LpAI:AII particles		
Baseline (n = 7)	8.3 ± 0.07	−0.05 ± 0.06
Final testing (n = 7)	8.2 ± 0.10	($P = .385$)
Large LpAI (%)		
Baseline (n = 7)*	20.2 ± 2.7	0.35 ± 2.09
Final testing (n = 7)*	20.5 ± 3.6	($P = .861$)

NOTE. All values are listed as mean ± SE.

*Eight subjects were tested, but 1 had no evidence of large LpAI particles.

†Denotes a statistically significant change from baseline levels at $P < .05$.

DISCUSSION

The results of this study support the primary hypothesis that LpAI, but not LpAI:AII, concentrations increase as a result of 6 months of endurance exercise training in older sedentary men and women. This hypothesis was based on several lines of evidence. First, past studies have shown that exercise training-induced increases in both HDL-C and HDL mass are primarily due to increases in the HDL_2 subfractions ($HDL4_{nmr}$ and $HDL5_{nmr}$).[42] Second, the proportion of LpAI is greater in the larger HDL subfractions,[43] and in particular, large LpAI particles (9.8 to 12.2 nm) represent the majority of HDL_{2b} ($HDL5_{nmr}$).[6] Thus, any increases in the large HDL subfractions should occur primarily as a result of increases in the concentration of LpAI, and not LpAI:AII, particles and the present data support this hypothesis.

The results of previous exercise training studies that have measured changes in total apo A-I concentrations have provided equivocal results, as apo A-I concentrations have been shown to increase,[22,23,26-28] decrease,[30] or stay the same[31-33,44] following training. However, to our knowledge, this is the first exercise training study to measure changes in the apo A-I concentration within the LpAI and LpAI:AII subfractions. Because LpAI particles are believed to be the primary "antiatherogenic" HDL subfraction,[8-10] the increases in LpAI concentrations seen here may provide insight into the role that exercise plays in helping prevent the development of CVD. Furthermore, because increases in apo A-I levels were seen in the LpAI, but not LpAI:AII subfraction, these findings add to the evidence[45] indicating that LpAI and LpAI:AII particles differ metabolically.

The mechanisms responsible for the preferential increases in

Table 5. Correlations Between Changes in LpAI and LpAI:AII Concentrations With Changes in HDL Particle Size and HDL-C in the Exercise Training Group

	ΔLpAI	ΔLpAI:AII
ΔHDL size	$r = .415$; $P = .009$*	$r = .238$; $P = .144$
ΔTotal HDL-C	$r = .495$; $P = .001$†	$r = .353$; $P = .027$*
ΔHDL5-C_{nmr}	$r = .184$; $P = .263$	$r = .300$; $P = .064$
ΔHDL4-C_{nmr}	$r = .254$; $P = .118$	$r = -.218$; $P = .182$
ΔHDL3-C_{nmr}	$r = .274$; $P = .092$	$r = .351$; $P = .028$*
ΔHDL2-C_{nmr}	$r = .133$; $P = .420$	$r = -.240$; $P = .142$
ΔHDL4 + 5-C_{nmr}	$r = .417$; $P = .008$*	$r = -.022$; $P = .893$
ΔHDL3 + 2 + 1-C_{nmr}	$r = .168$; $P = .306$	$r = .445$; $P = .005$*

*Denotes a statistically significant correlation at $P < .05$ (n = 39).

†Denotes a statistically significant correlation after a Bonferroni correction was applied for testing multiple (16) correlations ($P < .003$).

Table 6. Correlations Between Changes in LpAI, LpAI:AII, and HDL-C Concentrations With Changes in Body Composition Indices and $\dot{V}O_2$max in the Exercise Training Group

	ΔLpAI	ΔLpAI:AII	ΔHDL-C
ΔWT	$r = .052$ ($P = .772$)	$r = .277$ ($P = .088$)	$r = .091$ ($P = .582$)
ΔTotal fat %	$r = -.075$ ($P = .651$)	$r = -.167$ ($P = .310$)	$r = -.166$ ($P = .313$)
ΔIA fat	$r = -.177$ ($P = .281$)	$r = .166$ ($P = .133$)	$r = -.053$ ($P = .750$)
ΔSC fat	$r = -.114$ ($P = .488$)	$r = .142$ ($P = .389$)	$r = -.124$ ($P = .454$)
ΔLBW	$r = -.139$ ($P = .399$)	$r = .406$ ($P = .010$)*	$r = .059$ ($P = .720$)
Δ$\dot{V}o_2$max	$r = .223$ ($P = .172$)	$r = .054$ ($P = .744$)	$r = .123$ ($P = .454$)

*Denotes a statistically significant correlation at $P < .05$ (n = 39).

LpAI size and concentration in this study are not clear. The change in LpAI was positively correlated to the change in mean HDL particle size, total HDL-C change, and the change in the sum of the largest 2 HDL-C subfractions (HDL4-C_{nmr} + HDL5-C_{nmr}), so it is possible that similar mechanisms are responsible for each of these changes. However, despite significant increases in $\dot{V}o_2$max and significant reductions in body weight and percent body fat, the magnitude of these changes was quite small and was not correlated with the changes in either LpAI or HDL-C levels.

Increases in HDL size are promoted by the changes in the activity of several enzymes that play central roles in lipoprotein metabolism, including increases in lipoprotein lipase (LPL) and lecithin-cholesterol acyl transferase (LCAT), and reductions in hepatic lipase (HL) and cholesterol ester transfer protein (CETP).[46] However, we did not measure the activities of these enzymes here because, to our knowledge, their affect on apo concentrations has not been established. Several exercise-training studies have demonstrated concomitant increases in LPL activity and HDL-C concentrations, along with reductions in HL activity.[32,47-50] However, significant correlations between changes in HDL-C and exercise-induced changes in lipase activities have not been demonstrated.[47-50] This has led to speculation that changes in LCAT and CETP activities may be primarily responsible for the exercise-induced increases in the larger HDL-C subfractions, although few studies have tested this hypothesis. Two studies[51,52] have demonstrated increases in LCAT activity with exercise training, although changes in LCAT activity[31] and LCAT mass[53] were not found in others, and it was not stated whether correlations between the change in HDL-C and LCAT activity or mass were seen in any of these studies. In addition, CETP mass (CETP activity was not measured) was found to decrease with training,[50] but was not correlated with changes in HDL-C concentrations, although baseline CETP concentrations were correlated with changes in HDL-C.

In summary, while it is likely that these enzymes (LPL, HL, LCAT, and CETP) play a significant role in the exercise-induced changes in both HDL-C concentrations and HDL particle size, the relative role that each enzyme plays in causing these changes is not known. Furthermore, although changes in HDL-C and particle size were correlated with changes in LpAI concentrations in this study, the mechanisms by which these enzymes could affect changes in the apo A-I concentrations within HDL are uncertain.

It was hypothesized that there would be an increase in the average size of LpAI, but not LpAI:AII particles. The average peak size of the "medium" LpAI particles in the 8 subjects tested increased significantly by approximately 0.1 nm. Coincidentally, the NMR analysis found that the average increase in HDL particle size for all 39 subjects was also about 0.1 nm (see Table 2). Whether or not an increase of this magnitude in either the average size of total HDL or the "medium" LpAI subfraction is important physiologically is uncertain. However, because the magnitude of the increase in both of these was similar, it can be speculated that the change in average HDL particle size may have been caused primarily by an increase in the average size of the LpAI particles in the "medium" size range. The NMR analysis also indicated that there was a significant increase in HDL4-C_{nmr}. An increase in the cholesterol content of $HDL4_{nmr}$ particles suggests there was an increase in either $HDL4_{nmr}$ particle size and/or the concentration of $HDL4_{nmr}$ particles, as cholesterol concentrations in any given HDL subfraction are unlikely to increase significantly without a concomitant change in either the size or number of particles in that subfraction. $HDL4_{nmr}$ particles are defined as having diameters between 8.8 to 9.8 nm,[54] which corresponds to the large end of the spectrum of LpAI particles denoted here as "medium" (7.8-9.8nm). As such, this line of evidence also suggests that the increase in average HDL particle size may have been due to an increase in the average size of medium LpAI particles. In addition, the NMR analysis indicated that HDL5-C_{nmr} concentrations increased by 1.0 ± 0.5 mg/dl, although this increase only approached significance ($P = 0.055$). Regardless, past studies have shown that "large" LpAI particles represent the majority of HDL_{2b} particles ($HDL5_{nmr}$),[6] and no LpAI:AII particles in this size range were detected in this study. Consequently, it follows that any increase in the HDL5-C_{nmr} concentrations were likely due to changes in the size or concentration of "large" LpAI. Despite these lines of evidence, many more subjects need to be investigated before it can be established if the increases in HDL size, HDL4-C_{nmr} and HDL5-C_{nmr} are due to preferential changes in particular LpAI or LpAI:AII subfractions.

We sought to determine whether the changes in LpAI concentrations were due to preferential increases in the medium and/or large subfraction(s), which, as stated above, may indicate an increase in reverse cholesterol transport. However, because the assays used to isolate and quantify these subfractions are extremely time-consuming, it was not feasible to measure the LpAI size subfractions in all 39 subjects. Instead, plasma samples from the eight subjects exhibiting the greatest changes in total LpAI concentrations were chosen for this preliminary analysis. It was hypothesized that choosing sub-

jects with the largest changes in total LpAI concentrations may increase the likelihood of detecting changes in LpAI particle size, if changes occur. However, it is probable that the magnitude of change in LpAI particle size may have been greater if the subjects with the largest increases in HDL size had been chosen for this analysis instead. The increase in average HDL particle size for the eight subjects chosen was 0.24 nm, which was higher than the average increase of 0.11 nm, but only about half of the average for the eight subjects with the largest increases in HDL size (0.42 nm). Regardless, the changes in the particle sizes seen here may not be representative of the changes for all subjects due to this selection bias.

In contrast to other studies, only two LpAI particle peaks were detected in most subjects, one corresponding to "large" particles, and one roughly to "medium" particles as historically designated, but none to "small" particles. The same pattern of two major LpAI peaks, one corresponding to "large" and one to "medium" particles, were found when similar procedures were employed in other studies.[6,19,55,56] However, in these other studies, minor peaks were apparently detected in many subjects that corresponded to "small" LpAI particles. The reason for the absence of small particles in the subjects in this study is not known; however, the NMR analysis performed on the whole plasma samples detected "small" ($HDL1_{nmr}$) particles in only 1 of the 39 subjects tested, which is similar to what has been seen in other studies,[57] and supports the evidence for a lack of "small" LpAI and LpAI:AII particles found here.

In conclusion, the present results indicate that endurance exercise training causes a preferential increase in plasma concentrations of LpAI, while LpAI:AII concentrations were unchanged. There also was evidence that the average size of the LpAI particles was increased, likely due to an increase in the average size and/or concentration of particles within the $HDL4_{nmr}$ and/or $HDL5_{nmr}$ size range (8.8 – 12.2 nm). The increase in LpAI size and concentration are believed to represent possible enhancements of reverse cholesterol transport and may provide insight into the role that exercise plays in reducing the risk of developing cardiovascular disease.

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