Acute Changes in Serum Lipids and Lipoprotein Subclasses in Triathletes as Assessed by Proton Nuclear Magnetic Resonance Spectroscopy

Harry H. Yu, Geoffrey S. Ginsburg, Mary L. O’Toole, James D. Otvos, Pamela S. Douglas, Nader Rifai

Abstract—Exercise is associated with changes in lipids that may protect against coronary heart disease (CHD). In this study of 28 triathletes, we analyzed acute changes in serum lipid and lipoprotein concentrations after completion of the 1995 World Championship Hawaii Ironman Triathlon. With standard laboratory assays, we demonstrate significant decreases in total cholesterol, VLDL cholesterol, ApoB100, and Lp(a). Total HDL cholesterol increased significantly immediately after the race. With a novel proton NMR spectroscopy assay, we demonstrate that smaller diameter LDL particles, corresponding to small, dense LDL, declined by 62%. Moreover, larger HDL subclasses, whose levels are inversely associated with CHD, increased significantly by 11%. Smaller HDL subclasses, which have been directly associated with CHD in some studies, acutely decreased by 16%. Therefore, exercise not only acutely induces changes in lipoprotein concentrations among the standard species in a manner that favorably affects CHD risk, but also induces favorable changes in specific lipoprotein subclass size distribution that also may alter CHD risk independently of the total lipoprotein serum concentration. (Arterioscler Thromb Vasc Biol. 1999;19:1945-1949.)

Key Words: cholesterol ■ exercise ■ NMR ■ subclass

There is general acceptance that exercise results in changes that likely reduce the risk of developing cardiovascular disease and may slow the progression of established coronary artery disease. Chronic cardiovascular training results in changes in lipoproteins and apolipoproteins that reflect adaptation to the increased metabolic demands imposed by frequent, vigorous exercise. Moreover, the alterations in lipoproteins vary according to level of physical conditioning and intensity of exercise. Many of these changes consist of favorable alterations in apolipoproteins, LDL, HDL, and triglycerides. However, these lipoproteins are composed of heterogeneous species that have different atherogenic potential. For example, levels of small, dense LDL species and large HDL species demonstrate stronger direct and inverse associations, respectively, to atherosclerotic vascular disease. Moreover, lipoprotein size distribution is now commonly used in the assessment of cardiovascular risk and in the management of patients with hyperlipidemia and coronary heart disease (CHD).

Because lipoproteins change in size and composition with chronic endurance training and acute exercise, proton NMR spectroscopy provides a fast and reproducible method of measuring concentrations of lipoproteins of various sizes. In the present study, we used NMR to determine compositional changes in lipoproteins in triathletes whose chronic training and acute exercise reflect exercise and energy expenditure at the highest levels.

Methods

Subjects
The study population consisted of 28 highly trained volunteers, 22 men and 6 women, who completed the 1995 World Championship Hawaii Ironman Triathlon. Representing 8 different countries, all subjects were white with the exception of 2 Hispanic male athletes. Details of their training regimen have been reported previously. These athletes train 24 hours per week with a regimen consisting of 5 miles (8 km) of swimming, 205 miles (330 km) of cycling, and 47 miles (75 km) of running. Age, sex, and body mass index (BMI) data were obtained for participating athletes.

Biochemical and NMR Analyses
Specimens consisting of 25 mL of blood in sample tubes containing 0.1% EDTA or heparin were obtained 48 hours before the triathlon. Subjects did not participate in strenuous exercise at least 24 hours before the prerace samples were obtained. The triathlon consisted of a 2.4-mile (3.9 km) swim, a 112-mile (180.2 km) bicycle race, and a 26.2-mile (42.12 km) marathon. Specimens from the same subjects were obtained within 15 minutes of the completion of the race and immediately centrifuged and plasma stored at -70°C.

Our laboratory is certified by the National Heart, Lung and Blood Institute and Centers for Disease Control and Prevention Lipid
Standardization Program. Measurements of hematocrit and hemoglobin were performed by using standard methods. Total cholesterol and triglyceride levels were quantified enzymatically by using a Hitachi 911 autoanalyzer (Roche Diagnostics) according to the manufacturer's recommendations. Triglyceride measurement was corrected for endogenous glyerol. LDL cholesterol (LDL-C) and VLDL cholesterol (VLDL-C) were measured by β-quantification according to Lipid Research Clinics procedures. HDL cholesterol (HDL-C) levels were quantified after precipitation of ApoB100-containing particles by dextran sulfate and MgCl₂. ApoA1, ApoA2, ApoE, and ApoB100, and Lp(a) were determined by noncompetitive, immunonephelometric assays on the BN II analyzer (Dade Behring).

Lipoprotein subclass profiles were measured by proton NMR spectroscopy as described previously. In brief, this method exploits the fact that each lipoprotein particle in plasma broadcasts a lipid NMR signal with distinct frequencies and shape, the intensity of which is proportional to its lipid mass concentration. The NMR spectra of each plasma specimen (0.7 mL) is acquired on a dedicated 360-MHz spectrometer (Analogic Corp) under defined conditions (47°C). By deconvoluting the composite lipid methyl group signal envelope that appears in the spectrum at ~0.8 ppm and comparing this to reference spectrum of lipoprotein subclasses, the concentrations of 15 subclasses of VLDL, LDL, and HDL are derived simultaneously. The diameter ranges of VLDL and LDL subclasses were determined by calibration, using purified subfractions isolated by a combination of ultracentrifugation and agarose gel filtration chromatography, and characterized for size distribution by electron microscopy. The calibration standards used for determination of the HDL subclass diameters were also isolated by ultracentrifugation and agarose gel filtration, but characterized for size distribution by polycrylamide gradient gel electrophoresis.

The NMR-derived HDL subclasses, H5, H4, H3, H2, and H1 (mean diameter, 11.5, 9.4, 8.5, 8.0, and 7.4 nm, respectively) are closely related to the 5 HDL subclasses that are designated HDL2b, HDL2a, HDL3a, HDL3b, and HDL4a, respectively, in the gradient gel electrophoresis literature. HDL subclasses correspond to the sum of H4 and H5, and HLDL, corresponds to the sum of H1, H2, and H3. Small LDL refers to the NMR-derived L1 subclass (mean diameter, 19.0 nm) and large LDL refers to the sum of L2 and L3 (mean diameter, 20.5 and 22.0 nm, respectively). NMR also provides 6 VLDL subclasses, V1 through V6, with particle diameters of ~29.0 to 150.0 nm.

**Statistical Analysis**

The general linear modeling procedure for repeated measures was used to evaluate the statistical differences between prerace and postrace parameters, adjusting for age, sex, and BMI. Subject ages, triglycerides, chylomicrons, Lp(a), and HDL particle sizes were log-transformed before analyses. A probability value of ≤0.05 was deemed statistically significant. All calculations were performed with Microsoft Excel 97 (Microsoft Corp) and SAS, Version 6.12 (SAS Institute Inc). The influence of exercise-associated plasma volume changes on lipoprotein levels was corrected by using hemoglobin (Hb) and hematocrit (Hct) measurements, according to the Dill and Costill formula (% of Plasma Volume Change=100×[(preHb/postHb)×(1−postHct)/(1−preHct)]−100). ¹³

**Results**

The mean age was 35 years (range, 24 to 51 years). As expected in this group of lean, elite athletes, BMI was low with relatively low variability, ie, mean BMI was 22.4 ± 1.8 (range, 19.3 to 25.3). The only significant differences between male and female athletes were in BMI (mean, 22.8 and 20.7, respectively; P=0.001) and baseline HDL size (9.3 and 9.7 nm, respectively; P=0.020).

Prrace and postrace measurements adjusted for age and sex are shown in the Table. Total cholesterol decreased significantly by 7% (P=0.023), and triglycerides decreased by 23% (P=0.036). Mean HDL-C increased from 43 to 56 mg/dL, a 30% increase (P=0.0001). LDL-C did not change significantly. VLDL-C decreased 52% (P<0.001). Total cholesterol: HDL-C ratio and LDL-C: HDL-C ratio decreased significantly (P=0.0001).

ApoA1, ApoA2, and ApoE did not change significantly after the triathlon. However, ApoB100 demonstrated a 12% reduction (P=0.0001) after the race. Lp(a) also decreased by 18% (P=0.0006) when adjusted for age, sex, and BMI.

As measured by NMR, small LDL species (L1 subclass) decreased 38% in P=0.03) immediately after the race (Figure). However, larger LDL species did not change significantly. NMR analysis demonstrated a 16% reduction in HDL₂ subclasses (P=0.0001) and a 20% increase in HDL₃ subclasses (P=0.0007). Accordingly, average HDL size increased by 2.7% (P=0.0001).

**Discussion**

Endurance exercise results in changes in standard lipoprotein species and lipoprotein subclasses in patterns that may convey protection against atherosclerotic disease.
After this ultraendurance event, total cholesterol fell significantly, which is consistent with some14–16 but not all studies17 of prolonged exercise. Whether this change is sustained chronically in athletes is uncertain, however. In most observational studies, total cholesterol is not significantly lower than in inactive, matched controls, regardless of training intensity.

After an acute bout of prolonged, aerobic exercise, LDL-C is generally lower14,16,18,19 or unchanged.17,20 In the present study, the total postrace LDL-C did not decrease significantly. The effect of chronic training on LDL-C also is unresolved. Although many cross-sectional studies have demonstrated lower LDL-C levels in endurance athletes, other investigations, including longitudinal studies, have not been consistent in their conclusions. In general, LDL is unchanged or reduced with training. However, many of the studies that demonstrate a decrease in LDL-C also correlate these changes with the distance run each week.15,21

LDL concentrations are determined primarily from formation from VLDL remnants via a “salvage pathway,” and ApoB/ApoE receptor–mediated uptake. With regular strenuous aerobic training22–24 and acutely after exercise,25 lipoprotein lipase (LPL) activity increases, which enhances catabolism of VLDL, formation of VLDL remnants, and production of LDL. Therefore, LDL uptake and catabolism may also be enhanced with training.

LDL is comprised of subclasses that have distinct biochemical and associated cardiovascular risk characteristics depending on LDL particle size. A pattern of increased relative concentration of small, dense LDL particles, referred to as phenotype B by Austin et al, has been associated with an increased risk of myocardial infarction and ischemic heart disease.26–28 This risk may be independent of total LDL-C but not of triglycerides.29 One manner in which exercise may affect cardiovascular benefit is by altering metabolism of these small, atherogenic LDL species. With chronic, intense cardiovascular training, these species have been shown to decrease with little or no change in larger LDL species and intermediate density lipoproteins (IDL).30 In a similar manner, in our group of athletes, small LDL particles decreased significantly by 62% and larger LDL subclasses did not. Overall, LDL size was unchanged, probably reflecting the low concentration of these small LDL species relatively to the overall spectrum of LDL particles.

Alterations in LDL composition associated with training may be mediated by changes in hepatic triglyceride lipase (HTGL) activity. High HTGL activity has been correlated with increased small, dense LDL and phenotype B in patients with CHD.31 Although HTGL may not change with a single exercise session,23 training can result in chronic reduction in HTGL activity,22 which may lead to lower concentrations of small LDL particles.

Many studies have demonstrated an increase in HDL-C with an acute bout of exercise. However, thresholds of energy expenditure23 and duration24 may need to be achieved before HDL changes significantly. In this study of an extraordinarily high energy expenditure, total serum HDL-C increased significantly after the race.

Similar to LDL, HDL subclasses have different metabolic and vascular properties depending on particle size. The larger, 8.5- to 11.5-nm mean diameter HDL particles, corre-
of free fatty acids with increasing levels of exercise. Indeed, enhanced clearance of exogenous triglycerides resulting from enhanced LPL activity has been previously demonstrated in endurance athletes.\(^{18,25}\)

ApoA1, the major apolipoprotein found in HDL, has been correlated inversely with CHD risk.\(^{51}\) ApoA2, on the other hand, may be associated with atherogenesis.\(^{52}\) In most studies of chronic training, ApoA1 does not change significantly or does not change independently of other factors such as weight loss. In addition, previous studies do not demonstrate a consistent response to a single exercise session. In our subjects, neither ApoA1 nor ApoA2 changed significantly although total HDL-C concentrations and mean HDL particle size increased. This supports an overall increase in the mass (cholesterol content) and size of HDL, especially HDL₂, without an increase in the ApoA1 or ApoA2 content.

Elevated ApoB100 levels may confer an increased risk for CHD. In the present study, ApoB100 acutely decreased after the race, which is consistent with the observed decreases in CHD. In the present study, ApoB₁₀₀ acutely decreased after exercise, but some have demonstrated modest elevation in the days after exercise.\(^{19,45}\)

Baseline lipid analyses demonstrate favorable cardiovascular risk profiles in our study population of elite athletes. After a single session of strenuous exercise, we show that lipoprotein species that constitute well-established CHD risk factors (total cholesterol, HDL, and total cholesterol:HDL-C ratio), as well as potentially important determinants of risk [triglycerides, ApoB, Lp(a), and LDL-C:HDL-C ratio], change favorably. Moreover, with NMR spectroscopy, we demonstrate changes in small LDL particles and HDL size consistent with a pattern of cardiovascular disease risk reduction. Thus, exercise may reduce cardiovascular risk by altering quantitative as well as qualitative lipoprotein subclass distributions.

References


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