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

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 ~21 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

Received October 14, 1998; revision accepted January 14, 1999.
From the Department of Medicine (H.H.Y.) and the Cardiovascular Division (G.S.G., P.S.D.), Beth Israel Deaconess Medical Center, and the Departments of Laboratory Medicine and Pathology (H.H.Y., N.R.), Children’s Hospital, Harvard Medical School, Boston, Mass; the Department of Orthopaedic Surgery (M.L.O.), University of Tennessee-Memphis, Tenn; and the Department of Biochemistry (J.D.O.), North Carolina State University, Raleigh, NC.
Correspondence to Nader Rifai, PhD, Department of Laboratory Medicine, Children’s Hospital, Boston, MA 02115. E-mail rifai@a1.tch.harvard.edu
© 1999 American Heart Association, Inc.
Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org

1945
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 300-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 polyacrylamide gradient gel electrophoresis. The NMR-derived HDL subclasses, H5, H3, H4, H2, 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 HDL2a, HDL2b, HDL3, HDL3c, and HDL3d, respectively, in the gradient gel electrophoresis literature. HDL subclasses correspond to the sum of H4 and H3, and HDL 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 prereace and postrace parameters, adjusting for age, sex, and BMI. Subject ages, triglycerides, chlomycinor, 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).

Prereace 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 HDL1 subclasses (P = 0.0001) and a 20% increase in HDL2 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.
Yu et al  Acute Lipoprotein Changes in Triathletes 1947

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 expenditure33 and duration34 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, corresponding to HDL2 and HDL2a, as defined by polyacrylamide gel electrophoresis, are cardioprotective, whereas smaller, 7.5- to 8.0-nm mean diameter HDL species, corresponding to HDL3a and HDL3b, may not confer the same benefit or may be associated with atherogenesis.23,35 Higher HDL2 and lower HDL3 have been measured in elite endurance runners when compared with inactive controls, with HDL2 correlating with time spent running each week (r=0.673, P<0.05).24 Previous studies of single exercise sessions have not shown consistent changes in HDL subclasses. However, an aerobic training threshold of intensity and duration may need to be achieved before HDL composition is altered. In our subjects, there was an 11% increase in postrace HDL2 and a 16% decrease in postrace HDL3 subclasses.

The mechanism for the increased HDL2 associated with prolonged, chronic training26 and a single exercise session25 may be enhanced LPL activity in skeletal tissue and, possibly, in adipose tissue. Augmented transfer of surface components from triglyceride-rich lipoproteins (ie, VLDL and chylomicrons) catalyzed by this enzyme increase the larger, more cholesterol-laden HDL2 species.

A second mechanism by which HDL2 species may be increased chronically is a decrease in HTGL activity that catalyzes the conversion of HDL2 to HDL3. A reduction in enzymatic activity may result in the accumulation of the larger HDL2 species and decrease the quantity of the smaller HDL3 species.32

A third explanation for acute and chronic changes in HDL composition may be related, in part, to reduced cholesteryl ester transfer protein (CETP) activity.37 CETP catalyzes the transfer of VLDL triglycerides for HDL cholesteryl esters. The resulting triglyceride-rich HDL is metabolized by HTGL resulting in the reduction of the core volume of HDL. Human and knockout mice studies have demonstrated that decreased CETP levels or mutations in CETP result in increased HDL and relative HDL2 composition.38

Immediately after the completion of the triathlon, the total cholesterol:HDL-C ratio and LDL-C:HDL-C ratio decreased significantly. Increased total cholesterol:HDL-C ratio has been strongly associated with cardiovascular risk.39,40 Moreover, an increased LDL-C:HDL-C ratio has been associated with coronary disease in diabetics41 whereas a decrease has been associated with attenuated progression of CHD on angiography.42 Thus, exercise acutely improved these ratios that reflect the balance between atherogenic and antiatherogenic factors.

Triglycerides have been shown to be an independent risk factor for CHD29 or predict risk for coronary disease when LDL-C:HDL-C ratio is elevated43 or when HDL-C is low.44 Total triglycerides decreased significantly (P=0.036), which is consistent with previous studies demonstrating significant decreases in triglycerides that correlate with race duration.45–47 In the present study, total VLDL-C was also significantly reduced without a change in size distribution after exercise (Table). Our observation is congruent with other studies that have shown significant decreases in VLDL-C immediately after prolonged activity with large energy requirements.16,19 The baseline levels of serum triglycerides48 and VLDL-C49,50 are lower in endurance athletes. These changes may reflect a muscle and liver glycogen depletion state with an upregulation of LPL and enhanced use
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.\textsuperscript{18,25}

ApoA1, the major apolipoprotein found in HDL, has been correlated inversely with CHD risk.\textsuperscript{51} ApoA2, on the other hand, may be associated with atherogenesis.\textsuperscript{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 HDL\textsubscript{2}, 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 VLDL-C. This suggests decreased secretion or production of these lipoprotein species or increased clearance from circulation, possibly by hepatic and adipose ApoB receptors.

Elevation in Lp(a) has been associated with increased risk of cardiovascular disease in many studies.\textsuperscript{53} However, there are few data regarding the acute and chronic effects of exercise on Lp(a) levels. Our data demonstrate a significant decrease in Lp(a) with acute exercise. Other reports have demonstrated no significant change with an acute bout of exercise, but some have demonstrated modest elevation in the days after exercise.\textsuperscript{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 sub-class distributions.

References


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 and Nader Rifai

doi: 10.1161/01.ATV.19.8.1945
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/19/8/1945

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/