Exercise-mediated changes in high-density lipoprotein: Impact on form and function

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The goal of this systematic review was to assess the current understanding of the effects of exercise intervention on high-density lipoprotein (HDL) cholesterol (HDL-C) and changes in HDL function as well as modification of these effects by genomic factors. The reviewed studies demonstrate that exercise has modest effects on HDL-C with limited data suggesting an effect on HDL function. Genetic polymorphisms in proteins associated with HDL metabolism play a role in modifying the HDL-C response to exercise and possibly its function. Exercise as an intervention for patients at risk for cardiovascular events can lead to small improvements in HDL-C and potential changes in HDL function. There is an important modifier effect of genetics in determining these changes. [Am Heart J 2013;166:392-400.]

Recent data on the global prevalence of inactivity as a risk factor for cardiovascular disease are sobering and suggest that physical inactivity represents an insidious and mostly unrecognized risk factor that modulates susceptibility to cardiovascular disease. 1 On the other hand, the benefits of exercise on human cardiovascular health are clear and consistent, with exercise representing a foundation for risk modification in primary and secondary prevention of cardiovascular disease and diabetes. 2 Various agencies and associations have released statements and guidelines detailing exercise prescription for prevention of cardiovascular disease and maintenance of health. At least 30 minutes of moderate to vigorous exercise on most days of the week is recommended by most guidelines including the US Department of Health & Human Services Physical Activity Guidelines Advisory Committee Report. 2-5 The longer the total duration of physical activity/aerobic exercise training performed over the week, the greater the observed benefits. 4 The additive benefits of exercise on cardiovascular health are particularly appealing in light of the pleiotropic benefits of exercise and the diversity of mechanisms by which it facilitates cardiovascular health. An important mechanism through which exercise is believed to exert benefits on coronary heart disease is through its effects on lipoprotein metabolism. Specifically, previous studies have demonstrated an effect of physical activity on plasma lipoproteins. 6 Exercise is widely recognized and prescribed as a modality to improve high-density lipoprotein (HDL) cholesterol (HDL-C) and triglyceride (TG)-containing lipoproteins, which are commonly abnormal in patients with diabetes and/or insulin resistance; however, it is plausible that exercise-induced improvements in HDL function may be more important than changes in HDL-C. The purpose of this review is to provide information on what is known about HDL function and exercise and its determinants as well as to provide insights into the critical gaps in our information on the effects of exercise on HDL.

High-density lipoprotein and cardiovascular benefits

Epidemiologic studies have consistently shown that HDL-C is inversely associated with cardiovascular risk independent of other atherogenic lipoproteins (low-density lipoprotein [LDL] cholesterol [LDL-C] and non-HDL-C), with each SD increase in HDL-C in the Emerging Risk Factors Cohort (15 mg/dL) equating to a 22% decrease in coronary heart disease risk. 7 Clinical events are, however, quite common in patients with normal HDL-C levels, with as much as 44% of events in men and 43% of events in women occurring in individuals with HDL-C above these levels. Despite the consistency of epidemiology associating HDL-C as a protective factor, a number of lines of evidence have suggested that this relationship may not be as straightforward. These include evidence from post hoc analysis of randomized trials demonstrating a higher risk with HDL-C after adjustment for risk factors and apolipoprotein A-I (Apo A-I) levels, 8 evidence from Mendelian randomization studies demonstrating that polymorphisms in single or multiple genes that increase HDL-C may not necessarily
translate into reduction in myocardial infarction (MI) risk, and evidence from pharmacologic trials directed at increasing HDL-C levels with either niacin or with cholesterol ester transfer protein (CETP) inhibition that have not fared well.\textsuperscript{9,10} The consensus in the field is that alterations in HDL function are crucial in the modulation of cardiovascular risk. In the absence of pharmacologic options that may lower risk through HDL-specific pathways, there is a renewed interest in exercise and other lifestyle modifications for modulating HDL, particularly in those individuals with low HDL, a highly prevalent lipoprotein abnormality.

Mechanisms of exercise-mediated alterations in HDL

High-density lipoprotein cholesterol makes up 20% to 30% of total serum cholesterol. The major proteins associated with HDL include Apo A-I and apolipoprotein A-II, although there are approximately 48 other proteins that constitute the HDL proteome, together constituting approximately 10% of the lipoprotein particle.\textsuperscript{11} High-density lipoprotein promotes cholesterol homeostasis by reverse cholesterol transport (RCT) or the transfer of cholesterol, centripetally from peripheral tissues back to the liver.\textsuperscript{12,13} Briefly, new HDL particles are formed when the ATP-binding cassette transporter A1 (ABCA1) transfers lipids from the periphery to lipid-poor Apo A-I, and HDL grows in size via ATP-binding cassette transporter GI (ABCG1) on a number of cells including macrophages, which deliver cholesterol to lipid-rich HDL particles.\textsuperscript{14} Free cholesterol released from macrophages (via diffusion, interaction with ABCA1 and ABCG1 or by scavenger-receptor B1 [SR-B1]) is esterified by lecithin cholesterol acyltransferase (LCAT) in the HDL particle to cholesterol esters, with the HDL then being transported to the liver and intestine (transintestinal cholesterol excretion).\textsuperscript{12} A multitude of mechanisms have been postulated to modulate HDL function with exercise. Fundamentally, these pathways are nearly identical to those that are responsible for determining HDL composition and include alterations in cholesterol transporter, CETP, hepatic lipase, and lipoprotein lipase (LPL).

In humans, the best understood mechanism pertains to increased RCT, raised plasma pre-β-HDL levels, and increased LCAT activity. The first demonstration of exercise effects on HDL-C via CETP was seen as early as in 1993.\textsuperscript{13} In this study, 9 to 12 months of exercise training decreased CETP concentration by 13.2% in women and 14.2% in men and increased HDL-C by \(2.6 \pm 6.2\) mg/dL.\textsuperscript{15} Indirect mechanisms by which exercise may modify HDL function may include increase in nitric oxide (NO) bioavailability, which may decrease oxidative modification of HDL and thereby enhance its function.\textsuperscript{16} Exercise has been reported to increase LPL activity, although this is controversial.\textsuperscript{17} There is a direct correlation between plasma LPL activity and HDL, as LPL activity contributes to the maturation of the HDL particle via loading of cholesterol and proteins. Lipoprotein lipase activity is allosterically regulated by insulin, and in light of improvements in insulin resistance with exercise, improvement in LPL activity may contribute to improvement in HDL-C content and function. Antioxidants such as paraoxonase (PON) on the HDL particle may also confer protection. Paraoxonase has 3 isoforms (PON1, PON2, and PON3), all of which have been shown to inhibit LDL and cellular lipid oxidation in vitro.\textsuperscript{18} Of the PON isoforms, PON1 has been the most studied. The antioxidative function of HDL via enzymes such as PON has been postulated to play an important role in the development of atherosclerosis and may additionally represent mechanisms by which HDL exerts protective effects on the vasculature. Several other HDL-associated proteins may also mediate beneficial effects of exercise, including Apo A-I, but high-quality evidence to support this currently is lacking. An important role for HDL in modulating immune and endothelial function has recently been demonstrated.\textsuperscript{12,19} High-density lipoprotein attenuates toll-like receptor 4 signaling in innate immune cells such as macrophages and may help mediate the immune bolstering effects of exercise.\textsuperscript{20} High-density lipoprotein is well known to increase functional activity of endothelial nitric oxide synthase (eNOS) through transcriptional and posttranscriptional mechanisms.\textsuperscript{21} High-density lipoprotein binds to SR-B1 in caveolae, initiating a downstream signaling cascade leading to phosphorylation and activation of eNOS and leading to an increase in NO production. Nitric oxide is a prototypical antiatherogenic entity, with studies showing an atheroprotective role with a plethora of evidence linking exercise with favorable improvements in endothelial function. Low-intensity exercise may also exert beneficial effects on plasma lipids via peroxisome proliferator-activated receptor γ and peroxisome proliferator-activated receptor α pathways through a mechanism involving ABCA1 and ABCG1 up-regulation postexercise.\textsuperscript{22}

Studies on exercise and HDL

Studies of exercise on lipid parameters have been confounded by a number of variables that independently exert effects on lipid parameters. These include genetics and changes in diet as well as a multitude of environmental factors. Collectively, these make ascribing effects to exercise alone challenging. It may also be very difficult to tease out the hemodynamic effects of exercise from its effects on weight loss and body fat composition, especially when trials are conducted over longer durations. Indeed, a number of studies have found that a reduction in body fat is an important determinant of HDL-C response.\textsuperscript{23-25} Although most controlled clinical studies attempt to reduce the variability with non-exercise-related factors, in reality, it may be near impossible to rule out their influence in the HDL response.
Exercise and HDL-C levels

There is substantial evidence from controlled clinical studies that aerobic exercise training increases HDL-C. The first study to demonstrate an increase in HDL-C with exercise was reported in 1979. Kelley and Kelly compiled 49 randomized controlled trials between 1955 and 2003 representing up to 67 outcomes from 2,990 men (1,741 exercise and 1,249 control). Aerobic exercise was shown to increase HDL-C in this meta-analysis by 2%. A meta-analysis by Kodama et al of 25 randomized controlled trials of exercise alone, without diet or drug therapy, found that aerobic exercise of 5.3 metabolic equivalents (64.8% of max aerobic capacity) significantly increased HDL-C by 2.53 mg/dL (pre-exercise mean HDL in the studies ranged from 49–67 mg/dL). Among exercise variables, exercise duration was found to be the most important determinant of increase in HDL-C on multivariate analysis. Minimal weekly exercise volume for increasing HDL-C level was estimated to be 900 kcal of energy expenditure or 120 minutes of exercise per week. Univariate regression analysis indicated that every 10-minute prolongation of exercise per session was associated with an approximately 1.4 mg/dL increase in HDL-C level. In contrast, there was no significant association between exercise frequency and intensity. Multiple meta-regression analyses demonstrated that subjects with higher total cholesterol (>220 mg/dL) or who were less obese (body mass index [BMI] <28) responded better to exercise training. Because exercise intensity, frequency, and duration as well as expression of energy expenditure often differ in studies, an aggregate increase in HDL-C for every MET of exercise cannot be determined; however, a level of exercise that elicits between 1,500 and 2,200 kcal/wk energy expenditure was associated with 3.5 to 6 mg/dL increases in HDL-C and TG reductions of 7 to 20 mg/dL. Additional benefits may be provided by an increase in training volume above these levels.

Genetic variables that modulate HDL-C response to exercise

High-density lipoprotein cholesterol levels are influenced to a considerable extent by genetics, with underlying genetic polymorphisms explaining up to 50% of the variation in HDL-C. Single nucleotide polymorphisms (SNPs) in Apo A-I, ABCA1, LPL, hepatic lipase (LIPC gene), CETP, and additional targets such as endothelial lipase (LIPG gene) can modulate HDL-C, functional aspects of HDL, and response to diet and exercise (Table 1). Each sequence variant that has been discovered to date explains only a small fraction (~2%) of the variation in HDL-C levels but collectively may have a major influence in determining HDL-C and response to environmental variables such as exercise.

A SNP in the Apo A1 gene promoter (rs670) has been reported to be associated with HDL-C as well as HDL-C response to dietary changes in polyunsaturated fat intake (Table 1). A cohort of healthy normolipidemic adults who volunteered for 6 months of supervised aerobic exercise (n = 75) were genotyped for this SNP. The change in total HDL-C after exercise was 0.8 ± 7.2 mg/dL (+1.7%) and was not statistically significant. The large HDL subfraction increased in the G homozygotes (baseline 45.8-57.4 mg/dL) and decreased in the A (pre-exercise HDL 52.3 ± 16.7 mg/dL) carriers (1.8 ± 6.6 mg/dL vs −6.1 ± 2.3 mg/dL, P < .0005). In contrast, the amount of the small HDL subfraction (HDL 1 + 2) decreased in G homozygotes and increased in A carriers (~1.3 ± 6.6 mg/dL vs 4.7 ± 1.2 mg/dL, P < .005). These results show that genetic variation at the APOA1 gene promoter is associated with HDL subfraction redistribution resulting from exercise training.

Several studies have demonstrated that hypofunctional alleles within CETP can result in an increase in HDL-C, with some of these associated with decreased cardiovascular risk and others showing no effect of risk or even increased risk. Decreases in CETP activity clearly influence HDL-C levels as a direct consequence of reduced exchange of cholesterol esters with apolipoprotein B–containing lipoproteins. This may, in turn, have implications for cholesterol efflux of the HDL particle and perhaps more importantly for cholesterol transport back the liver via LDL receptor mechanisms. In the prospective Women's Genome Health Study (WGHS), a subcohort of the Women's Health Study (n = 22,939 apparently healthy US women of European ancestry), 9 loci on 9 chromosomes had ≥1 SNP associated with HDL-C at genome-wide statistical significance (P < 5 × 10–8). Only SNPs near or in the CETP gene at 16q13 were associated with both HDL-C and risk of incident MI. Single nucleotide polymorphism rs708272, a common polymorphism in the CETP gene, was associated with a per-allele increase in HDL-C levels of 3.1 mg/dL and a concordant 24% lower risk of future MI (age-adjusted hazard ratio 0.76, 95% CI 0.62–0.94), consistent with the previous meta-analysis by Thompson et al that also had demonstrated that the rs708272 polymorphism was not only associated with reduced CETP activity/mass but also with higher HDL-C and reduced incidence of coronary artery disease (CAD). In the same cohort, when physical activity was evaluated as a predictor of HDL-C levels, the rs1800588 but not the rs708272 polymorphism demonstrated an effect modification with the change in HDL-C demonstrating a more robust increase in active versus inactive women.

Hepatic lipase hydrolyzes phospholipids and TG in HDL and influences RCT. Four specific polymorphisms in the promoter region of the hepatic lipase gene (LIPC) have been implicated with reduced hepatic lipase activity and increased HDL-C, of which 3 are quite prevalent
(Table I). However, previous studies have demonstrated an increased risk for CAD, particularly in triple heterozygotes and triple homozygotes, both of which were associated with an increase in HDL-C but an increased risk when adjusted for age, sex, and HDL-C. However, more recent and larger studies that have examined this association have not found an association with increased risk. In the WGHS, the per-minor-allele increase in HDL-C for rs1800588 at LIPC was greater in triple heterozygotes and triple homozygotes, both of which demonstrated an increased risk for CAD, particularly in triple heterozygotes and triple homozygotes, whereas no major effect of an active lifestyle was seen comparing heterozygous carriers with homozygous G-allele carriers. In a small prospective study that evaluated the LIPC rs1800588 polymorphism (−514C>T) on lipoprotein changes in response to 24 weeks of exercise in sedentary overweight individuals (baseline HDL-C of 44 ± 1 mg/dL), the −514T minor allele significantly affects training-induced changes in VLDL-TG (−22% vs +7%, P < .05, CC vs CT gene alleles, respectively) and increases in HDL-C (increase of 7% vs 22% vs +7%, P < .05, CC vs CT gene alleles, respectively). There were also genotype-specific changes in LPL activity, with LPL increasing only in CC subjects (P < .006) and hepatic lipase (HL) decreasing only in CT subjects (P < .007). Reductions in TG and VLDL-TG and increases in HDL-C were significantly correlated with changes in LPL, but not HL, activity only in CC subjects. This suggests that the LIPC−514C>T variant significantly affects training-induced changes in VLDL-TG and HDL through an association with increased LPL activity. Because exercise regulates LPL activity, it is possible that the effect of exercise on LIPC expression is influenced by polymorphisms or by promoter variants in high linkage disequilibrium. For instance, although the LPL

### Table I. Polymorphisms in genes associated with HDL-C levels and risk for CAD; effect modification by exercise

<table>
<thead>
<tr>
<th>Candidate gene(s)</th>
<th>Locus</th>
<th>Effect modification with exercise</th>
<th>Association with MI/CVD</th>
<th>SNPs identifiers*</th>
<th>Description, allele (MAF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo A-I</td>
<td>11q23.3</td>
<td>Unclear</td>
<td>Unclear</td>
<td>rs670</td>
<td>Promoter, -75G/A (T = 0.185/404)</td>
</tr>
<tr>
<td>LIPC (hepatic lipase)</td>
<td>15q22.1</td>
<td>Yes</td>
<td>No</td>
<td>rs1800558 (-514)</td>
<td>+2070895 (-250)</td>
</tr>
<tr>
<td>LPL</td>
<td>8p21.3</td>
<td>Yes</td>
<td>No</td>
<td>rs328</td>
<td>Exon (G = 0.0962/210)</td>
</tr>
<tr>
<td>CETP</td>
<td>16q13</td>
<td>Yes</td>
<td>Unclear</td>
<td>rs1800776</td>
<td>rs1532624</td>
</tr>
<tr>
<td>LIPG (endothelial lipase)</td>
<td>18q21.1</td>
<td>No</td>
<td>No</td>
<td>rs1800588</td>
<td>rs708272 (TaqIB)</td>
</tr>
<tr>
<td>PLTP</td>
<td>20q13.12</td>
<td>Not known</td>
<td>No</td>
<td>rs6589566</td>
<td>Intronic, A</td>
</tr>
<tr>
<td>APOC1, APOC2, APOC4, APOE</td>
<td>19q13.32</td>
<td>Not known</td>
<td>Unclear</td>
<td>rs4420638</td>
<td>rs157580</td>
</tr>
<tr>
<td>APOA1, APOA4, APOA5, APOC3</td>
<td>11q23.3</td>
<td>Not known</td>
<td>Unclear</td>
<td>rs6389566</td>
<td>Intronic, A→G (G = 0.1131/247)</td>
</tr>
<tr>
<td>ABCA1</td>
<td>9q31.1</td>
<td>Not Known</td>
<td>Unclear</td>
<td>rs3905000</td>
<td>rs1883025</td>
</tr>
<tr>
<td>GALT2 (UDP-N-Acetyl-α-D-Galactosamine:polypeptide N-acetylglactosaminyl transferase 2)</td>
<td>1q32</td>
<td>Not known</td>
<td>Unclear</td>
<td>rs4825614</td>
<td>Intronic, A→G (G = 0.3668/801)</td>
</tr>
<tr>
<td>PPP1R3B</td>
<td>8p23</td>
<td>Not known</td>
<td>Unclear</td>
<td>rs3748140</td>
<td>Exonic, G→A (T = 0.0325/71)</td>
</tr>
</tbody>
</table>

CVD, Cardiovascular disease; MAF, minor allele frequency. * Single nucleotide polymorphisms with P values < 10−8 adapted from Ridker et al, Thompson et al, Kathiresan et al, Teslovich et al and Anand et al.
gene is on chromosome 8 and the LIPC gene is on chromosome 15, the -514C>T variant may contribute to increases in LPL activity with exercise through “trans” effects on functional polymorphisms located on other chromosomes. Similar interactions between the -514C>T variant and LPL activity were seen in the Health, Risk Factors, Exercise Training, and Genetics Family Study (n = 662). White and black homozygotes (-514TT) had lower baseline HDL cholesterol and lower hepatic lipase activity that persisted postexercise training. Both black and white individuals homozygous for -514CC had higher LPL at baseline and after exercise training (increase in LPL was seen postexercise).44

Lipoprotein lipase is a major regulator of TG-rich lipoproteins and HDL and has been known to modulate responses of HDL-C to exercise. The impact of exercise on LPL is, however, variable, with some studies demonstrating an increase, whereas others show no change.45,46 The extent to which regular physical activity modifies the effect of recently discovered SNPs on HDL-C levels or their effect on risk of MI has not been well studied, particularly among women. In the WGPS, SNPs in LPL (8q21) and CETP (16q13) were associated with elevations in HDL-C. The per-allele increase in HDL-C and Apo A-I is higher in the inactive women with rs10096633 compared with those without the polymorphism. This increase in HDL-C in those with minor-allele carrier status at the LPL rs10096633 SNP genotype translated into reduced risk of MI in active (hazard ratio 0.51, 95% CI 0.30-0.86) but not among inactive women (hazard ratio 1.13, 95% CI 0.79-1.61, P for interaction <.007). In contrast to this stratification of risk by physical activity status, carrier status at the CETP SNP rs1800775 was associated with a reduced risk of MI regardless of activity level (hazard ratio 0.72, 95% CI 0.57-0.92, P for interaction <.71).47

Apolipoprotein E (APOE) physically interacts with lipases such as hepatic lipase and LPL, and the 3 common haplotypes of the APOE gene (ε2, ε3, and ε4) yield protein isoforms (ε2, ε3, and ε4, respectively) that are functionally different. Prior studies, including a meta-analysis and data from INTERHEART, have reported an association between ε2 carriage and increased risk for MI when compared with ε3 homozygotes, whereas the ε2 isoform is associated with lowered risk with evidence of a gene dose effect.48,49 In a study involving normolipidemic men and women, postheparin plasma lipase activities were measured in a group of patients stratified according to APOE genotypes. The ε2/ε3, ε3/ε3, and ε4/ε3 are the most common genotypes comprising 12%, 62%, and 21% of the population, respectively. In men, but not women, hepatic lipase was higher in the ε2/ε3 group compared with ε4/ε3 (P = .01) and ε3/ε3 (P = .05). Neither sex nor APOE genotype affected baseline LPL activity. Training decreased HbA1c by 5.2%, whereas a differential response to exercise was noted dependent on APOE genotype once corrected from baseline insulin levels. Exercise decreased LPL activity in ε3/ε3 compared with the combined increases of 6.6% in ε2/ε3 and 12% in ε4/ε3 (P = .018 vs ε3/ε3).50

Another important practical question to consider is to what extent are changes in HDL-C and/or Apo A-I regulated by genetics, particularly when exercise and diet are both varied significantly and at the same time, as is so often the case in practical terms. In a study of genetic sib-pairs, 28 pairs of male monozygotic twins (1 twin mostly sedentary, the other running an average of 50 km/wk) went from a 6-week 40% fat diet to a 6-week 20% fat diet in a crossover design. The diets reduced fat primarily by reducing saturated and polyunsaturated fat (both from 14% to 4%) while increasing carbohydrate intake from 45% to 65%. Despite the twins’ differences in physical activity, the dietary manipulation produced significantly correlated changes (P < .05) in the twins’ total cholesterol (r = 0.56), LDL cholesterol (r = 0.70), and Apo A-I (r = 0.49). These results suggest that underlying genetics strongly influences response to dietary manipulation despite significant differences in exercise levels.51

Exercise and changes in HDL form and function

Studies from as early as the 1980s showed that exercise training can alter HDL subfractions, providing a clue that exercise is capable of modulating HDL metabolism and, hence, possibly its function.52 Table II presents articles from 2007 to 2012 that analyzed HDL function in response to exercise intervention in humans. There were 6 published studies on aerobic exercise and HDL function between the years 2007 and 2012. Some of the studies were confounded by a concurrent weight loss program during the period of study; in addition, some patients were obese or diabetic and on medication. Of the 6 studies, HDL-C levels increased in 1,53,54 decreased in 1,55 and did not change in 4.56-59 Lecithin cholesterol acyl-transferase, cholesterol efflux, and antioxidant activity were found to increase in multiple studies.53,54,56,57 Five of the studies used an exercise stimulus of appropriate duration (>8 weeks) and frequency to see physiological improvements in fitness.55,59 One study was only 6 weeks long.54 The exercise intensity was sufficient to increase aerobic fitness in 4 of the intervention studies.54,56,58,59 Two described the physical activity intervention only as “brisk” walking or cycling or an increase in steps per day with no mention of intensity.55,57 In 2 of the studies, concomitant weight loss rendered the interpretation of results difficult.55,57 In the study by Casella-Filho et al.,56 patients with metabolic syndrome underwent moderate intensity exercise training for 3 months on bicycle ergometers. Blood was sampled before and after training for biochemical analysis, PON1 activity, and HDL subfraction composition and antioxidative capacity. No changes were observed in HDL-C, LDL-C, and Apo A-I following 3 months of exercise, but PON1 activity was significantly increased after training along with
enhanced protective effects of exogenously added HDL2a or HDL3b from the metabolic syndrome group in preventing CuSO4-mediated LDL oxidation. Training increased the ability of HDL particles to accept free cholesterol and cholesterol ester from a standardized lipidic nanoemulsion in the metabolic syndrome group without changes in phospholipid transfer. Ribeiro et al studied the effect of a 4-month aerobic training program in type II diabetic patients and compared their metabolic responses including assessment of HDL subfractions, oxidative capacity, and cholesterol efflux with a group of sedentary type II diabetic patients and healthy controls. Exercising improved maximal oxygen consumption and reduced waist circumference but did not modify body weight, BMI, plasma LDL-C, HDL-C, TG, glucose, insulin, or homeostasis model of assessment - insulin resistance. The HDL3 composition did not change, and its efflux capacity (measured by efflux of radioactively labeled cholesterol from J774 cells) was unaltered by aerobic training. In diabetic patients but not in healthy controls, aerobic training improved 15% the HDL3 protective effect against LDL maximal oxidation rate in the fasting state. The difference in results between the studies of Ribeiro et al and Casella-Filho et al may relate experimental differences in measuring cholesterol efflux. In another study from the same group, the impact of 18 weeks of aerobic exercise training in type 2 diabetes mellitus (DM) and in healthy control subjects was evaluated. The lag time for LDL oxidation (LAG) and the maximal rate of conjugated diene formation was evaluated by incubating plasma HDL2 and HDL3 from patients before and after exercise with LDL from pooled healthy donors’ plasma. Eighteen weeks of aerobic

### Table II. Analysis of HDL function in response to exercise intervention in humans

<table>
<thead>
<tr>
<th>Study population (n)</th>
<th>Exercise mode, intensity, duration, frequency</th>
<th>HDL function measurement (method of measurement and results)</th>
<th>Salient findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese/overweight women (n = 100)</td>
<td>6 month low-intensity exercise (increase of 5000 steps per day as measured by a pedometer)</td>
<td>In vitro RCT with (3)H-cholesterol-labeled BHK cells incubated with 1% ApoB-depleted serum. PON1 activity, ORAC, and endothelial eNOS activation assays in endothelial cells incubated with HDL (n = 49). Time interval between exercise bouts and serum sampling not reported.</td>
<td>Weight loss noted in patients with a ↓ in HDL-C (−3 ± 9 mg/dL, P = .016). Cholesterol efflux capacity by ABCA1 ↓ by 10% (P = .006); efflux capacities by the ABCG1 and SR-B1 transporters not altered. ORAC ↓ 15%.</td>
</tr>
<tr>
<td>Sedentary metabolic syndrome subjects (n = 30)</td>
<td>3 times per week and 45 min per session on a bicycle ergometer. Training intensity began at anaerobic threshold and was progressively intensified.</td>
<td>Apo A-I and serum PON1 activity, HDL subfraction composition, and antioxidative capacity. Lipid transfer to HDL assayed in vitro using a labeled nanoemulsion as the lipid donor. Serum sampled 18 h postexercise.</td>
<td>Training ↓ TG but did not change LDL-C or HDL-C. Exercise ↑ HDL subfractions’ antioxidative capacity and PON1 activity. Metabolic syndrome group had compositional changes in the smallest HDL subfraction associated with increased free cholesterol and cholesterol ester transfers to HDL, reaching normal values.</td>
</tr>
<tr>
<td>Healthy Japanese men (n = 7; 59-67 y)</td>
<td>Cycle ergometer training at lactate threshold for 60 min/d, 5 times/wk for 6 wk.</td>
<td>LCAT activity (LCAT, dipalmitoyl lecithin substrate method), HDL2-C/HDL3-C (agarose electrophoresis method), Apo A-I/ApoB measures (turbidimetric immunomassay). Serum sampled 2 d postexercise.</td>
<td>6 wk of training ↑ HDL-C, HDL2-C, HDL3-C, and LCAT levels but did not affect apolipoproteins</td>
</tr>
<tr>
<td>Healthy obese female volunteers (n = 15), BMI &gt;29</td>
<td>9 wk of 5 aerobic sessions/wk (60 min)</td>
<td>Nonesterified fatty acids measured enzymatically; Apo A-I and Apo B by immunoturbidimetric method. In vitro RCT using macrophages with 14C cholesterol. Time interval between exercise bouts and serum sampling not reported.</td>
<td>No change in HDL-C. Slight but significant ↓ of ApoB and Apo A-I. After outliers were removed, cholesterol efflux increased by 1.8 % (ie, 14% of baseline value), and the calculated statistical significance of cholesterol efflux became significant.</td>
</tr>
<tr>
<td>Type 2 DM (n = 14) and healthy subjects (12)</td>
<td>18-wk monitored aerobic exercise training consisting of 40-min cycle ergometry sessions, 3 times a week.</td>
<td>Plasma and lipoprotein measurements. Apo A-I and ApoB were measured by immunoturbidimetry. Time interval between exercise bouts and serum sampling not reported.</td>
<td>No change in TG, HDL-C, ApoB, non-HDL-C. ↓ in LDL oxidation (lag time/min) in the presence of HDL2 vs control after 18 wk of exercise. The % of Apo A-I in HDL2 was higher in diabetics compared with control at 18 wk.</td>
</tr>
<tr>
<td>Type 2 DM patients (n = 21) and healthy subjects (n = 11)</td>
<td>4-month monitored aerobic exercise training plan performed on a cycle ergometer. Sessions lasted 40 min, 3 times a week.</td>
<td>LPL mass determined by ELISA (Daichi Pure Chemicals Company, Tokyo, Japan). HDL2 oxidation, PON activity, and cell cholesterol efflux also measured. Serum sampled 48 h postexercise.</td>
<td>No change in HDL-C among groups before or after training. After training, postprandial pre-β 1-HDL level was significantly ↓ 24% in DM group alone. In the experimental groups, the percentage of cell cholesterol efflux elicited by HDL3 used as lipid acceptor did not differ between the basal and final periods. PON activity did not change.</td>
</tr>
</tbody>
</table>

ApoB, Apolipoprotein B; ELISA, enzyme-linked immunosorbent assay.
exercise in type II diabetic patients improved the ability of HDL3 to reduce LAG and conjugated diene formation. In the presence of HDL2, the lower baseline LAG in type 2 DM equaled that of control patients after aerobic exercise training, whereas conjugated diene formation remained unchanged. Serum PON activity was unchanged with exercise in type II diabetic patients despite lowering of serum lipid hydroperoxides. In contrast to the positive effects on cholesterol efflux in these studies with exercise, Aicher et al55 evaluated the effects of low-intensity exercise in conjunction with weight loss in women (n = 100, 60 African American) over a period of 6 months. The results were dominated by the effects of weight loss, making the conclusions on exercise itself hard to interpret. Participants achieved an average weight loss of 2.2 ± 3.9 kg (P < .001), associated with reductions in both LDL-C (−6 ± 21 mg/dL, P = .004) and HDL-C (−3 ± 9 mg/dL, P = .016, baseline HDL-C of 55 ± 14 mg/dL). Cholesterol efflux capacity by the ABCA1 transporter decreased by 10% (P = .006), whereas efflux capacities by the ABCG1 and SR-B1 transporters were unaltered. Neither PON1 activity nor eNOS activation significantly changed.

Changes in HDL subclass may have important implications for future cardiovascular events,60 and several studies have examined the impact of exercise on HDL subclass. As an example, a short-term training study of 6 weeks increased maximal oxygen consumption as well as HDL-C, HDL2-C, HDL3-C, and LCAT levels. Low-density lipoprotein cholesterol, total cholesterol, apolipoproteins, TG levels, insulin, hemoglobin A1c, and ureic acid levels did not change. It is possible that more dramatic changes would be seen after a longer training intervention.54

An 18-week exercise training program did not change HDL-C level but did improve the antioxidant capacity of HDL2 and HDL3 only in diabetic patients. No change in antioxidant capacities was noted after acute exercise bouts, nor were there any differences in PON1 activity in the groups after the training period.58 Likewise, no change was detected in PON1 activity or eNOS activation after a 6-month training intervention, but oxygen radical absorbance capacity (ORAC) was decreased 15%.55

Resistance training and HDL

Seventeen articles from the years 2007 to 2012 discuss HDL level and resistance exercise, but only 1 addresses HDL function with resistance exercise. The study authors measured the level of ABCA1 expression after an exercise session, but direct measurement of function was not performed.61 The purposes of the study were to measure ABCA1 expression in human peripheral blood lymphocytes in response to a single session of circuit resistance, to compare the effects of different resistance intensities, and to correlate ABCA1 expression and plasma HDL-C in 20 young females. The exercise program consisted of 9 major muscle group exercises (8 repetitions per exercise, 3 non-stop circuits with 1-minute rest between circuits, 26 minutes total duration) using free weights. Intensity was set at 40%, 60%, or 80% of each individual’s 1-repetition maximum. No significant change was observed in HDL-C immediately after the exercise sessions, but ABCA1 expression increased. Low to moderate intensity resistance exercise had more of an effect on ABCA1 expression than did high intensity. The significance of these results is unclear and was not investigated further in this study. Two of the resistance studies that did not address functional aspects of HDL looked at a single bout of exercise only, and 7 compared aerobic to resistance training programs. Six of the studies showed a significant increase in HDL-C with the resistance exercise. Ten showed no change, and 1 showed a decrease. Although there appears to be a contradiction with regard to the ability of resistance exercise to increase HDL levels, a meta-analysis of studies from 1955 to 2007 suggests that resistance training has less of an effect on HDL than aerobic training.62 Of the 7 studies that compared resistance training to aerobic training, 2 in which HDL was unchanged showed a significant increase with aerobic training alone. However, studies also suggest that resistance plus aerobic training may be better than either alone.63,64

Summary

Exercise has modest effects on HDL-C and may modulate its function. Multiple gaps exist in the relationship...
between exercise and HDL function. Figure summarizes current pathways that may be influenced by exercise. The following areas could be important in defining the role of exercise in improving functional aspects of HDL: (1) development of assays for assessing dysfunctional HDL; (2) the mechanisms that produce dysfunctional HDL and how exercise may delay transition to a dysfunctional state; (3) unbiased approaches to probe changes in HDL such as proteomic or transcriptomic approaches including changes in micro-RNAs that regulate HDL in response to exercise; (4) the appropriate frequency, duration, and intensity of exercise in modulating HDL function; (5) the impact of aerobic versus resistance or both aerobic and resistance training combined on HDL function; and (6) the differential impact of genetics in HDL function.

Disclosures
Funding support: No extramural funding was used to support this work. The authors are solely responsible for the design and conduct of this study, study analyses, the drafting and editing of the manuscript, and its final contents.

References