Coordinate Changes in Levels of Human Serum Low and High Density Lipoprotein Subclasses in Healthy Men

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Measurement of serum lipoproteins by analytic ultracentrifugation revealed significant correlations involving subfractions of low density (LDL) and high density (HDL) lipoproteins in 81 men studied cross-sectionally at baseline and longitudinally during a 1-year exercise trial. One-year changes in lipoprotein levels in 38 exercising men and in 30 sedentary controls showed correlations that paralleled those observed at baseline. Positive correlations observed between plasma levels of larger, more buoyant LDL of flotation rate (Sf) 7 to 10 (LDL-I) and HDL2 may be due to processes that also coregulate changes in levels of these lipoprotein subclasses. Similarly, positive correlations among smaller, more dense LDL of Sf 2 to 6 (LDL-III), IDL, and HDL2 suggest that levels of these lipoprotein species are also coordinate regulated. An inverse correlation of change in LDL-I with change in LDL-III raises the possibility of product relationships between LDL in these categories. Thus, changes in lipoproteins which are related to coronary disease risk are not independent of one another, and the development of coronary disease may be influenced by processes linking the metabolism of individual IDL, LDL, and HDL components.

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Levels of serum low density lipoprotein (LDL) cholesterol are positively related to risk of coronary artery disease, and levels of high density lipoprotein (HDL) cholesterol are negatively related.1, 2 LDL and HDL cholesterol levels are generally thought to be regulated independently and to contribute independently to cardiovascular disease risk because their serum concentrations correlate weakly in cross-sectional studies.3

Serum mass concentrations of LDL subfractions in normal human subjects examined cross-sectionally, however, have been found to exhibit significant correlations with serum mass concentrations of HDL subclasses and very low density lipoproteins (VLDL).4 As defined by flotation rate (Sf) in the analytic ultracentrifuge procedure,4 these LDL subfractions are: Sf 12 to 20 (intermediate density lipoproteins or IDL); Sf 7 to 12 (larger LDL); and Sf 0 to 7 (smaller LDL). The latter two subgroups are of particular interest because of their correlations with concentrations of total HDL mass and the HDL2 subclass: positive for larger LDL and negative for smaller LDL. Furthermore, levels of VLDL mass (Sf 20 to 400) are correlated negatively with larger LDL and positively with smaller LDL.4

Density gradient ultracentrifugation and nondenaturing polyacrylamide gradient gel electrophoresis of LDL in normal subjects have revealed that both larger and smaller LDL subfractions include multiple distinct subobjects.5, 6 LDL subfractions isolated at mean density 1.031 g/ml were found to have a mean peak Sf rate of 8.4 in 12 normal subjects, and the protein mass of the LDL in this fraction correlated inversely with VLDL and positively with HDL total mass concentrations.5 In contrast, LDL subfractions isolated at mean density 1.041 g/ml had a mean peak Sf rate of 5.3, and the protein mass of the LDL in this fraction correlated positively with VLDL mass and inversely with HDL mass.5 These findings independently corroborated the correlations previously reported for larger and smaller LDL subfractions of Sf 7 to 12 and Sf 0 to 7 as measured by analytic ultracentrifugation.4 The results suggest that the metabolic relationships of LDL to VLDL and HDL components may vary for different LDL subtypes, and they raise the possibility of interdependence between HDL and LDL in their relationship to coronary heart disease (CHD) risk.

To better understand the nature and forms of the interrelationships among lipoprotein subclasses, correlational analyses were applied to repeated measurements of lipoprotein subfractions collected over a 1-year period in two groups of men who participated in a study on exercise. Specifically, the present report: 1) uses contour plots to provide a detailed description of the interrelationships among levels of VLDL, IDL, LDL, and HDL subclasses in terms of their individual flotation intervals, and 2) shows that changes in serum mass concentrations of lipoprotein subfraction concentrations over the course of 1 year exhibit correlations similar to those observed cross-sectionally.
Methods

Subjects

Subjects were 81 apparently healthy, sedentary men, ages 30 to 55 years (mean 45.7) recruited from among employees of Stanford University to participate in a controlled clinical trial of the effects of exercise conditioning on lipoprotein and other physiologic measurements. The exercise program consisted initially of three sessions per week of calisthenics, stretching, walking, or slow jogging, and a 25-minute work phase of running at 70% to 80% of capacity. After 2 to 3 weeks, the participants were requested to add a fourth and fifth day of exercise.

The criteria for selection into the study were: serum total cholesterol <300 mg/dl, serum triglyceride <500 mg/dl, moderate or no alcohol consumption, and no use of drugs known to affect lipid metabolism. Baseline plasma triglyceride levels were greater than 200 mg/dl in six subjects (maximum 365 mg/dl) and plasma cholesterol levels were greater than 265 mg/dl in three subjects (maximum 291 mg/dl). Of the 81 original subjects, 48 were assigned at random to a running program (designated the exercise group in the text) and 33 did not engage in exercise conditioning (control group). Lipids and lipoproteins were measured at baseline and at 6 months and 1 year after baseline. For the purposes of the present analyses, 7 of the original 81 men were excluded from longitudinal analysis because of incomplete data. In addition, six men were excluded because they reported in diet records that they voluntarily modified their intake of total calories or of major dietary components to lose weight. Therefore, cross-sectional results are presented at baseline for all 81 men; longitudinal results are presented for the 38 exercisers and 30 controls who were sampled at baseline, 6 months, and 1 year and who did not go on special diets during the study.

Laboratory Procedures

Blood was collected from subjects who had fasted overnight (12 to 14 hours) into evacuated tubes containing merthiolate (1 mg/20 ml blood). After the blood had clotted at room temperature for 1 hour, serum samples were obtained from two low-speed centrifugations (30 minutes at 1500 rpm in IEC). Serum concentrations of lipoproteins were measured within 2 weeks of collection by analytic ultracentrifugation as the sum of mass within arbitrary flotation intervals for LDL (Sf 0 to 12, 11 intervals), IDL (Sf 12 to 20, four intervals), and VLDL (Sf 20 to 400, 14 intervals). The peak Sf rate of LDL was measured as the Sf rate at which LDL mass was greatest for each subject. In addition, total HDL mass was measured by analytic ultracentrifugation for 15 flotation intervals (F720), and levels of two major HDL subclasses, HDL2 (F720 0 to 3.5) and HDL2 (F720 3.5 to 9.0). Levels of cholesterol and triglyceride were measured by the method of the Lipid Research Clinics in plasma (EDTA, 1 mg/ml) obtained at the same time as the serum sample.

Statistical Calculations

The relationships between lipoprotein mass concentrations were assessed cross-sectionally by using the base-line measurements and longitudinally by using the difference scores that were computed by subtracting the baseline measurements from the 1-year measurements. The strengths of the relationships among concentrations of the major lipoprotein subclasses were assessed by Spearman's rho (r) correlation coefficients. The results are presented in tabular form. Spearman correlations were also calculated between the serum mass concentrations of individual flotation intervals and the results displayed graphically as contour plots. Spearman's correlation coefficients provide a nonparametric test for significant linear association among the ranks of the variables, have high efficiency when the data are in fact normal, and are robust to outliers. Superscripts are used to designate significance at p ≤ 0.05 (*), p ≤ 0.01 (†), and p ≤ 0.001 ($) in Table 2 and the text. There is less power to detect significant correlations between changes in lipoprotein concentrations than between lipoprotein concentrations cross-sectionally due to the smaller sample sizes when exercisers (n = 38) and controls (n = 30) are analyzed separately.

Results

Table 1 presents means and standard deviations for lipoprotein concentrations in the 81 men who agreed to participate in the 1-year exercise trial. Also presented are the means and standard deviations for 1-year changes in lipoproteins for the 30 control subjects and the 38 exercise subjects who had complete data and who did not report going on special diets during the study. Mean changes in lipoprotein concentration were not significantly different for exercisers and controls at the end of the 1 year of intervention, ostensibly because many runners failed to achieve the level of exercise needed to promote increased HDL and decreased LDL concentrations. The correlational analyses that follow assess the covariation in lipoprotein changes among individuals.

Interrelationships among Lipoprotein Mass Concentrations

The interrelationships among lipoprotein mass concentrations of different flotation rates are summarized graphically as contour plots in Figures 1 and 2. Solid contour lines designate levels of equivalent positive correlation and broken contour lines designate levels of equivalent negative correlation. Shading is used to denote flotation rates with serum mass concentrations that correlate significantly (p ≤ 0.05). The correlations between lipoprotein mass concentrations for major groupings of flotation intervals are also presented in tabular form (Table 2).

Figure 1 (top) displays the contour plot for the intercorrelations of LDL and IDL mass by particle flotation rates and the contour plot for LDL and IDL mass versus VLDL mass. The contour plot for the interrelationships among LDL and IDL flotation rates shows a correlation of unity along the diagonal and decreasing correlations as flotation intervals become further apart. The contour lines are reflected symmetrically with respect to the diagonal. The plots reveal: 1) individual flotation intervals within the Sf 2 to 6 region correlate positively with all flotation intervals within IDL of Sf 12 to 20 and VLDL of Sf 20 to 350 and negatively with
LDL intervals within Sf 7 to 10, 2) the Sf 12 to 20 IDL flotation intervals correlate negatively with LDL of Sf 7 to 9 and positively with VLDL of Sf 20 to 350, and 3) intervals within LDL Sf 7 to 9 and VLDL of Sf 20 to 350 region are negatively correlated.

In other analyses (not displayed), LDL peak Sf rate correlated negatively with all flotation intervals within VLDL of Sf 20 to 350, IDL of Sf 12 to 20, and LDL of Sf 2 to 6 and positively with individual flotation intervals of LDL of Sf 7 to 10. Plasma triglyceride concentrations correlated positively with individual flotation intervals of Sf 20 to 400, Sf 10 to 20, and Sf 2 to 6 and negatively with Sf 7 to 9.

The interrelationships for 1-year changes in the low to very low density lipoprotein mass concentrations appear in Figure 1 for controls (center) and exercisers (bottom). An inverse correlation between LDL of Sf 3 to 5 and Sf 7 to 10 is present in all plots. The positive correlations of IDL versus LDL of Sf 2 to 6 and negative correlation of LDL versus LDL Sf 7 to 9 observed at baseline do not achieve significance for changes in the mass of individual flotation intervals. Table 2 also shows that changes in LDL peak Sf correlate negatively with changes in Sf 2 to 6 and positively with changes in Sf 7 to 10 and that plasma triglyceride change correlates positively with changes in Sf 20 to 400 and negatively with changes in Sf 7 to 10.

Figure 2 (top) graphically depicts regions of strong and weak cross-sectional correlations between LDL mass concentrations and LDL, IDL, and VLDL mass concentrations by flotation rate. Vertical and horizontal axes correspond to HDL flotation rates (Ff 20) and LDL, IDL, and VLDL flotation rates, respectively. For HDL, individual flotation intervals of Ff 20 3.5 to 8 all exhibit negative relationships with LDL flotation intervals of Sf 7 to 10, IDL intervals of Sf 14 to 20, and VLDL intervals of Sf 20 to 250 and positive relationships with LDL intervals of Sf 7 to 10. These correlations also involve HDL of Ff 20 2.5 to 3.5. HDL flotation intervals within Ff 20 0 to 1.5 correlate positively with LDL flotation intervals of Sf 3 to 6 and IDL of Sf 12 to 20. As for the cross-sectional correlations shown in Figure 1, none of these relationships differed when the sample size was restricted to the 68 men whose 1-year change data were analyzed (data not shown).

Contour plots are also displayed for the correlations between changes in serum HDL versus IDL and LDL mass concentrations in controls (Figure 2, center) and exercisers (Figure 2, bottom) after 1 year. The shaded regions of these plots indicating correlations significant at p ≤ 0.05 are constrained to regions of higher correlations in exercisers (r > 0.32 and r < -0.32) and controls (r > 0.38 and r < -0.38) compared to the baseline plot (r = 0.22 and r < -0.22) because of the reduced sample size for changes in lipoprotein mass concentrations between baseline and 1 year. The longitudinal relationships for changes in lipoprotein mass concentrations between baseline and 1 year show similarities to those seen cross-sectionally but show several areas of departure from the cross-sectional relationships: 1) the positive correlation of HDL3 and smaller LDL is not significant for the 1-year change data, 2) the concordant relation between HDL and LDL change in exercisers involves HDL3 of Ff 20 1.5 to 2.5, rather than Ff 20 0 to 1.5, and 3) the inverse relationship between HDL2 and IDL and VLDL involves the entire Sf 12 to 250 region cross-sectionally, but is restricted to the Sf 20 to 50 region for longitudinal change data in exercisers and controls.

One-year changes in HDL3 concentrations, estimated as the total lipoprotein mass of Ff 20 3.5 to 9, correlated significantly with one-year changes in levels of larger LDL of Sf 7 to 10 (exercisers, r = 0.60; controls, r = 0.47), levels of smaller LDL of Sf 2 to 6 (exercisers, r = -0.65; controls, r = -0.51), LDL peak Sf (exercisers, r = 0.67; controls, r = 0.49); and triglyceride concentrations (exercisers, r = -0.31; controls, r = -0.51).

Discussion

Our results provide confirmation of our earlier findings that significant interrelationships exist cross-sectionally among serum levels of individual LDL and HDL subfractions in normal subjects. More importantly, the major relationships seen cross-sectionally are found to apply to changes in lipoproteins within individuals over the course of 1 year. The contour plots for correlations between concentrations of individual flotation intervals display the lipoprotein interrelationships at the maximum resolution currently achievable for the analytic ultracentrifuge. The correlations showing the greatest strength and consistency involve two major subpopulations of LDL: larger, more buoyant LDL of Sf 7 to 10, and smaller, more dense LDL of Sf 2 to 6. These Sf groupings, which are based on
Correlations of Baseline Concentrations

Correlations of One-Year Changes

Control

Exercise
The results of the contour plot analysis indicate that the relationships previously described for larger and smaller LDL of Sf 1 to 12 and 0 to 7, respectively, are due primarily to variations within narrower Sf intervals. The major species within these Sf intervals have been designated LDL-I and LDL-III, respectively. It is possible that the inverse correlation between these two LDL components may have obscured weaker relationships involving LDL-II, which has an intermediate size and density distribution that overlaps with those of LDL-I and LDL-III. Metabolic pathways cannot be directly inferred from the negative correlations of LDL-I versus LDL-III, respectively. Alternatively, changes in production of LDL species are derived from catabolism of larger, more dense LDL species are derived from catabolism of larger, more buoyant LDL. Alternatively, changes in production of LDL-I may be reciprocally linked to changes in input of LDL-I and LDL-III, respectively. It is possible that the extent of exchange of LDL triglyceride with cholesteryl ester in IDL and larger LDL influences the production of smaller LDL by subsequent lipolysis, as suggested recently. The correlations involving changes in HDL revealed major differences in relationships for the HDL2 and HDL3 subclasses. A number of previous observations suggest metabolic processes that might contribute to the correlations involving HDL2 and HDL3 subclasses reported here. It has been reported that the fractional catabolic rate of HDL2 (especially HDL2c) is inversely related to levels of triglyceride or VLDL, possibly accounting for the reciprocal relations of HDL2 and VLDL changes. Furthermore, levels of HDL2, and more recently larger LDL, have been positively correlated with levels of adipose tissue lipoprotein lipase. In contrast, reduced activity of hepatic lipase has also been shown to be associated with concomitant increases in mass of HDL2 and more buoyant LDL. Thus, lipolytic activities, in conjunction with flux of triglyceride-rich lipoproteins, may contribute to the coordinate regulation of HDL2 and large LDL. There is as yet no basis for explaining the relationship of HDL2 change with change in IDL or the observation that IDL changes were associated with changes in HDL2 of slightly higher flotation rate in runners than in controls.

The finding of a negative correlation of LDL peak Sf rate
**Figure 2.** Top. Contour plots of the correlations between serum concentrations of individual high density lipoprotein (HDL) flotation ($F_{1.20}$) intervals and low density (LDL), intermediate density (IDL), and very low density (VLDL) lipoprotein flotation ($S_f$) intervals measured cross-sectionally at baseline (n = 81). Bottom. Contour plots of the correlations between changes in serum mass concentrations of HDL and LDL, IDL, and VLDL flotation intervals between baseline and 1 year in non-exercising (Control, n = 30) and exercising (Exercise, n = 38) men. Solid contour lines designate levels of equivalent negative correlation. Shaded areas designate regions with correlations that are significant at $p < 0.05$. 
with plasma VLDL levels confirms previous reports.\textsuperscript{22, 23, 24} Since S1 rate is proportional to particle molecular weight,\textsuperscript{9} this observation could be interpreted as indicating that plasma triglyceride influences the mass and size of LDL particles.\textsuperscript{18} However, peak S1 rate can also be influenced by changes in concentrations of LDL subtypes with differing S1 values (Table 2). Therefore, the negative correlations of LDL peak S1 with triglyceride, VLDL, and IDL and the positive correlations with HDL\textsubscript{2} could be influenced by relationships involving multiple LDL subclasses, although relationships involving the molecular weight of the most abundant species are also possible.

It is possible that genetic factors contribute to the correlations reported here. Recent family studies have indicated a genetic basis for a trait characterized by a predominance of LDL-III in conjunction with relative increases in VLDL and IDL and reductions in HDL\textsubscript{2}.\textsuperscript{25} Preliminary analyses indicate that up to 25\% to 30\% of normal subjects may be carriers of this trait and that it is fully expressed in the heterozygous state after about age 40.\textsuperscript{26} Thus the prevalence of this trait could contribute to the cross-sectional correlations involving smaller, denser LDL subtypes in the populations reported here and previously.\textsuperscript{4} However, the longitudinal correlations of LDL-III vs. LDL-I and HDL\textsubscript{2} and of LDL-I vs. HDL\textsubscript{2} were found in men in the present study irrespective of whether LDL-III was the major LDL component at baseline (Krauss RM and Williams PT, unpublished data). Therefore, the metabolic processes responsible for these coordinate lipoprotein changes are not dependent on the LDL subtraction phenotype.

The present observations lend further support to the suggestion made previously\textsuperscript{4} that the positive contribution of LDL and the negative contribution of HDL to coronary disease risk may not be independent of one another. Based on the correlations shown here, increased coronary risk associated with reductions of HDL could be related to reciprocal increases of IDL or smaller, denser LDL or conceivably to parallel reductions of a "protective" component within the larger LDL subclasses. Increased coronary disease risk has been reported in normocholesterolemic patients with elevated plasma apoprotein B levels.\textsuperscript{26} Studies of LDL subtypes in these patients,\textsuperscript{27} as well as in other patients with the related disorder, familial combined hyperlipidemia,\textsuperscript{28} have revealed an increase in LDL protein content due to an abundance of particles within the LDL-III subclass. The present finding that changes in this LDL fraction are inversely related to changes in HDL\textsubscript{2} provides further support for the concept that levels of smaller, denser LDL subtypes may be predictive of coronary disease risk.

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References


Index Terms: analytic ultracentrifugation • exercise • flotation rate • high density lipoproteins • intermediate density lipoproteins • lipoproteins • low density lipoproteins • triglyceride • very low density lipoproteins
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