Published in final edited form as:

Metabolism. 1995 January ; 44(1): 106–114.

The Associations of High-Density Lipoprotein Subclasses With Insulin and Glucose Levels, Physical Activity, Resting Heart Rate, and Regional Adiposity in Men With Coronary Artery Disease: The Stanford Coronary Risk Intervention Project Baseline Survey

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Abstract

We used nondenaturing polyacrylamide gradient gel electrophoresis to examine the associations of high-density lipoprotein (HDL) subclasses with adiposity, physical activity, resting heart rate (an indicator of sympathetic drive), and plasma insulin and glucose levels in 97 men with angiographically documented coronary artery disease. These men neither smoked nor used medications known to affect lipoproteins. The absorbency of protein stain was used as an index of mass concentrations at intervals of 0.01 nm within five HDL subclasses: HDL_{3c} (7.2 to 7.8 nm), HDL_{3b} (7.8 to 8.2 nm), HDL_{3a} (8.2 to 8.8 nm), HDL_{2a} (8.8 to 9.7 nm), and HDL_{2b} (9.7 to 12 nm). HDL peak diameter was determined from the predominant peak of the HDL particle distribution when plotted against particle diameter. Four men who were non-insulin-dependent diabetics as defined by a fasting glucose exceeding 140 mg/dL had significantly higher plasma HDL_{3b} levels and significantly smaller HDL peak diameters than nondiabetic men, and were therefore excluded from further analyses. In the remaining 93 nondiabetic men, plasma HDL_{3b} levels correlated positively with indices of truncal obesity (waist to hip ratio and subscapular skinfold), whereas plasma HDL_{2b} levels correlated negatively with indices of total adiposity (body mass index [BMI]) and truncal obesity (subscapular and abdominal skinfold). Fasting plasma insulin levels correlated negatively with HDL_{3a}, HDL_{2a}, and HDL_{2b}. Obesity significantly affected the relationships of resting heart rate with insulin and HDL subclasses. In heavier men (BMI > 25.8 kg/m²) but not in the less-obese men (BMI < 25.8 kg/m²), resting heart rate was negatively correlated with HDL_{3a}, HDL_{2a}, and HDL_{2b} levels and HDL peak diameter and positively correlated with fasting plasma insulin concentrations. Although the reported physical activity in heavier men also correlated with HDL_{3a}, HDL_{2a}, and resting heart rate, the associations of resting heart rate with HDL_{3a}, HDL_{2a}, and HDL_{2b} could not be attributed to activity level. These analyses suggest that the influences of plasma insulin, regional adiposity, physical activity, and resting heart rate on HDL involve specific HDL subclasses. In the presence of increased adiposity, sympathetic drive and physical inactivity may reduce levels of larger HDL and the peak diameter of the major HDL subspecies.

INTRODUCTION

High density lipoproteins (HDLs) can be characterized by particle size using nondenaturing polyacrylamide gradient gels [1]. Five HDL subclasses have so far been identified by this method: HDL_{3c} (7.2 to 7.8 nm in diameter), HDL_{3b} (7.8 to 8.2 nm), HDL_{3a} (8.2 to 8.8 nm), HDL_{2a} (8.8 to 9.7 nm), and HDL_{2b} (9.7 to 12 nm) [1]. Prior studies show that different HDL subclasses exhibit specific relationships to exercise [2], weight loss [2], age [3], menstrual status [4], adiposity [3], alcohol intake [3], post-menopausal estrogen replacement [3], other lipoproteins [5,6], and shared genetic and environmental influences within families [7]. Case-

control and angiographic studies suggest that coronary heart disease risk may be increased when HDL_{2b} is decreased relative to HDL_{3c} and HDL_{3b} [8–10].

Adiposity, insulin resistance, increased sympathetic drive, and dyslipoproteinemia often occur concurrently and are therefore thought to have a common etiology [11,12]. Their association is reported to be greatest in the presence of obesity [13]. The manifestations of increased sympathetic activity include elevated resting heart rate and blood pressure. Prior studies have shown that high resting heart rate is associated with dyslipoproteinemia {decreased concentrations of HDL₂ and increased concentrations of small low-density lipoproteins, intermediate-density lipoproteins, and very-low-density lipoproteins (VLDLs)} [14], elevated plasma insulin levels [15], increased development of atherosclerosis [16], and increased coronary heart disease risk [17–21].

This report examines the relationships of HDL subclasses to regional adiposity, resting heart rate, reported physical activity, and plasma insulin and glucose levels in nondiabetic men with coronary artery disease. The analyses to follow suggest that the influence of each of these factors on HDL involves specific subclasses. We also find that obesity may (1) exacerbate the dyslipoproteinemia associated with physical inactivity; and (2) be a necessary condition for sympathetic drive to affect plasma insulin and HDL concentrations.

SUBJECTS AND METHODS

Subject Selection

The data presented in this report were collected during the baseline visit for the Stanford Coronary Risk Intervention Project, a 4-year randomized clinical trial of multifactor risk reduction in 259 men and 41 women with angiographically documented coronary artery disease. The subjects were recruited among patients who received coronary arteriography at four local hospitals. All were free of congestive heart failure, severe pulmonary disease, intermittent claudication, and noncardiac life-threatening illness, and were less than 75 years old. All had at least one segment from a major coronary artery with lumen narrowing between 5% and 69% that was unaffected by revascularization procedures. Following arteriography, 145 of the 300 patients were medically treated, 139 received percutaneous transluminal coronary angioplasty, and 16 had coronary artery bypass graft surgery.

Too few women were free of cigarette usage or lipid-altering drugs to provide meaningful analyses. For this reason, women are excluded from the analyses to follow. Of the 259 men who entered the trial, 143 were excluded for using drugs that could potentially affect lipoprotein levels (lipid-lowering drugs, β -blockers, diuretics, antiadrenergic drugs, antiarrhythmic drugs, hypoglycemics, tranquilizers, antidepressants, and barbiturates), 14 were excluded for smoking, and five for missing gradient gel electrophoresis of HDL. Four men who were non-insulin-dependent diabetics as defined by a fasting glucose exceeding 140 mg/dL had significantly higher plasma HDL_{3b} levels and significantly smaller HDL peak diameters than nondiabetic men (Fig 1), and were therefore excluded from further analyses.

Clinical and Laboratory Measurements

Clinical measurements and fasting plasma samples were obtained at least 3 weeks after hospital discharge. Skinfolds were measured in triplicate at the subscapular, triceps, and suprailliac crest. Weight and height were measured with subjects wearing lightweight clothing or a hospital gown without shoes. Weight was measured to the nearest 0.1 kg using a balance scale. Height was measured to the nearest 0.5 cm with the patient standing erect, flat-footed, and with the head in the Frankfort horizontal plane. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Resting heart rate was the average of the last two values from three 15-second heart rate measurements taken with the subject seated

and having rested for 5 minutes. BMI and resting heart rate were measured during two clinic visits (usually within 2 weeks of one another), which were averaged for each subject to represent the subject. BMI measurements were correlated ($r = 0.99$) and the resting heart rate measurements were correlated ($r = 0.72$) between the first and second visits; their respective standard deviations for the differences between the two visits were 0.30 kg/m^2 and 7.37 beats per minute.

Reported smoking status was verified by measuring the carbon monoxide content of expired air and the thiocyanate concentration in plasma [23]. Dietary intake was determined by 4-day food records (Thursday through Sunday) using the Nutrition Coordinating Center protocols for data collection and coding and Version 13 data base for data analysis [24]. Participants were instructed and their records reviewed in person by trained diet coders.

Physical activity was reported using the Stanford Seven-Day Physical Activity Recall questionnaire [25]. Specifically, subjects were asked if they exercised regularly over the previous year and to recall the number of minutes over the previous week spent engaged in moderate, hard, and very hard activities. Activities were expressed in terms of total kilocalories per kilogram per day ($1 \text{ kcal} = 4.2 \text{ kJ}$).

Fasting plasma triglyceride and lipoprotein cholesterol levels were also measured during two clinic visits and averaged for each subject. Plasma concentrations of total cholesterol and triglycerides were measured in the Stanford Lipoprotein Research Laboratory by enzymatic procedures (ABA 200 instrument, Abbott Laboratories, Abbott Park, IL) [26,27]. The HDL cholesterol level was measured by dextran sulfate-magnesium precipitation [28] followed by enzymatic determination of cholesterol [26]. The laboratory remained certified by the Centers for Disease Control lipid standardization program throughout. Correlations between the first and second visit were $r = 0.75$ for triglycerides and $r = 0.87$ for HDL cholesterol, and their respective standard deviations for the differences between the two visits were 5.75 and 42.12 mg/dL . Plasma concentrations of HDL₂ mass and HDL₃ mass were measured by analytic ultracentrifugation [29]. The plasma glucose concentration was measured using the glucose oxidase method [30] and insulin concentration by radioimmunoassay [31] after an overnight fast and 1 hour after receiving a 100-g oral glucose load.

Electrophoresis of HDL in the ultracentrifuged $d \leq 1.20\text{-g/mL}$ fraction was performed on Pharmacia Electrophoresis Apparatus (GE 4-II) using slab gradient gels (PAA 4/30, Pharmacia, Piscataway, NJ) [1]. The protein-stained gradient gels were scanned with a mode/RFT densitometer (Transidyne, Ann Arbor, MI) at a wavelength of 603 nm . A mixture of four globular proteins (High Molecular Weight Calibration Kit, Pharmacia) was run on the central lane to calibrate for particle size. The HDL migration distances (R_f) were measured relative to the migration distance of the peak of bovine serum albumin, one of the protein standards [5]. The HDL distributions were converted from the migration distance scale to the particle diameter scale using a method previously described [5]. The HDL peak diameter was identified as the diameter of the maximum absorbency (highest point) of the HDL distribution when plotted along the diameter scale [5] (Fig 1).

Statistical Analysis

Student's *t* tests were used to evaluate differences in mean HDL levels between groups, and Pearson correlation coefficients were used to evaluate relationships between HDL levels and plasma insulin and glucose levels, adiposity, heart rate, and physical activity. The correlations and differences were computed at each 0.01 nm between 7.2 and 12 nm (ie, individually for all 481 diameter values). This approach enables us to study the relationship of HDL subclasses with other variables without necessarily assuming the shapes or particle size intervals of the

HDL subclasses. Partial correlation coefficients were used for adjustment. All significance levels are two-tailed.

Conversion from absorbency to plasma concentration is not necessary for analyzing protein-stained HDL by particle diameter, because the significance levels used in this report (ie, for t tests, Pearson's correlation coefficients, and partial correlation coefficients) are invariant to translations of scale or location. This means that the significance levels for absorbency will be identical to those based on unknown plasma concentrations when the conversion involves the addition and/or multiplication of numerical constants. In fact, different constants may be used at each diameter, so that variation in chromogenicity across the HDL particle size spectrum will not affect the results.

RESULTS

The 93 men had a mean ($\pm$ SD) age of 58.0 ± 9.3 years and a total cholesterol of 222.5 ± 39.4 mg/dL. Figure 1 displays the distribution of HDL by particle size. The curve was computed by averaging the heights of the individual HDL distributions at each diameter value. The approximate subclass intervals described by Blanche et al [1] are provided. Table 1 displays some of their other characteristics. Waist girth, hip girth, and abdominal skinfold measurements were introduced after the start of the study and therefore are missing in early recruits. Table 1 also includes the correlations of anthropometric and lipoprotein measurements with HDL cholesterol, HDL₂ and HDL₃ mass, triglycerides, BMI, and insulin and glucose levels when fasting and 1 hour after receiving a 100-g oral glucose load. The expected associations for HDL, triglyceride, and insulin levels were observed, namely (1) HDL cholesterol and HDL₂ mass correlated negatively with triglycerides, BMI, and fasting insulin; (2) HDL₂ mass also correlated negatively with abdominal and subscapular skinfolds and 1-hour insulin; and (3) plasma triglyceride levels correlated positively with fasting insulin and glucose, 1-hour insulin, and BMI. In addition, fasting and 1-hour insulin were positively correlated with BMI, and resting heart rate was positively correlated with fasting concentrations of triglycerides, insulin, and glucose. Table 1 also shows that the HDL peak diameter was negatively correlated with BMI and fasting concentrations of triglycerides, insulin, and glucose.

Figure 2 displays the correlations between HDL absorbency and plasma insulin and glucose concentrations. Significant correlations ($P \leq 0.05$) are designated by the solid portions of the bars at the bottom of each graph. Plasma insulin concentrations were negatively correlated with plasma HDL_{3a}, HDL_{2a}, and HDL_{2b} levels in fasting plasma and with fasting HDL_{2a} and HDL_{2b} levels 1 hour after receiving the glucose load. Postload plasma glucose levels but not fasting glucose were negatively correlated with fasting HDL_{2a}.

Adiposity, Resting Heart Rate, and Exercise

Figure 3 shows that plasma HDL_{2b} levels were inversely correlated with BMI and subscapular and abdominal skinfolds. The ratio of waist to hip girths also correlated within the HDL_{2b}, albeit significantly over a narrow diameter range. Both waist to hip girth and subscapular skinfolds correlated with portions of HDL_{3b}.

The correlations of Fig 4 show that resting heart rate was negatively correlated with HDL_{2a} and HDL_{2b} and that these relationships primarily involve the more obese men. Specifically, when the data were divided at the 50% percentile of BMI for this sample, resting heart rate in the heavier men ($25.8 \text{ kg/m}^2 < \text{BMI} < 36.3 \text{ kg/m}^2$) correlated significantly with HDL_{3a}, HDL_{2a}, and HDL_{2b}, whereas resting heart rate in the leaner men ($20.6 \text{ kg/m}^2 < \text{BMI} < 25.8 \text{ kg/m}^2$) showed no relationship to HDL subclasses. The relationships are in fact significantly different between the heavier and leaner men for HDL between 8.29 and 8.95 nm (analyses not displayed). Although reported physical activity also correlated with HDL_{3a} and

HDL_{2a} (*middle panel*, Fig 4) and weakly with resting heart rate ($r = -0.25$, $P = .09$) in heavier men, the bottom panel of Fig 4 suggests that physical activity does not account for the associations between resting heart rate and the HDL_{2a} and HDL_{2b} subclasses. Similar results were obtained when subscapular skinfold measurements were used to dichotomize the sample into the upper (17.6 to 40 mm) and lower (9.5 to 17.5 mm) halves of distribution, and HDL levels were correlated with resting heart rate (significant for HDL between 8.82 and 11.84 nm for men with thicker skinfolds and nonsignificant for those with thinner skinfolds) and reported physical activity (significant for HDL between 8.41 and 9.85 nm for men with thicker skinfolds and nonsignificant for those with thinner skinfolds, results not displayed).

Figure 5 displays the relationships of resting heart rate with HDL peak diameter and fasting insulin levels. The data are again divided by the 50% percentile of BMI. In the leaner men, resting heart rate was unrelated to both peak diameter and fasting insulin, whereas in the heavier men resting heart rate was inversely related to HDL peak diameter (regression slope ($\beta \pm$ SE): -0.011 ± 0.003 nm per beat per minute, $P < .0001$) and concordantly related to fasting insulin ($\beta \pm$ SE: 0.425 ± 0.132 pmol/L per beat per minute, $P < 0.002$). The difference in regression slopes between heavier and leaner men was significant for both HDL peak diameter (difference in slopes, -0.010 ± 0.004 , $P = 0.02$) and fasting insulin concentrations (difference, 0.42 ± 0.17 , $P = 0.02$). In contrast, adiposity does not appear to affect the relationship between the HDL peak diameter and fasting plasma insulin (difference in slopes, -0.004 ± 0.006 , $P = 0.56$) or triglyceride concentrations (difference in slopes, -0.0003 ± 0.0007 , $P = 0.68$, analyses not displayed).

Partial Correlation Analyses

Figure 6 displays the results of partial correlation analyses between HDL absorbency and fasting insulin, BMI, subscapular skinfold, resting heart rate, and reported physical activity. The wide bars designate the range of diameter values that are significant when adjusted for fasting insulin, BMI, subscapular skinfold, resting heart rate, reported physical activity, alcohol intake (kcal/d), carbohydrate intake (% of total calories), or plasma triglyceride concentrations. The following results were observed: (1) insulin: Negative correlations with HDL_{3a}, HDL_{2a}, and HDL_{2b} remain significant when adjusted for all variables except plasma triglyceride levels. Adjustment for plasma triglycerides eliminates the significant association with HDL_{3a} and smaller-diameter HDL_{2a}; (2) BMI: Adjustment for fasting plasma insulin or triglycerides eliminates the negative correlation with HDL_{2b} levels. The positive correlation between BMI and HDL_{3b} achieves significance when adjusted for plasma insulin or triglycerides, resting heart rate, physical activity, or alcohol intake, presumably because these additional sources of variation are eliminated; (3) subscapular skinfold: Adjustment for fasting plasma insulin or triglycerides eliminates the negative correlation with HDL_{2b} levels. The positive correlations with HDL_{3b} persist for all adjustments except BMI; (4) resting heart rate in heavier men: The correlations with HDL_{3a} and smaller HDL_{2a} are eliminated when adjusted for fasting plasma insulin or triglyceride levels or physical activity; however, the correlations with HDL_{2b} levels are unaffected; and (5) physical activity in heavier men: The correlations with HDL_{3a} and HDL_{2a} are generally unaffected by the adjustments. The association is significant over a more restricted diameter range when adjusted for plasma triglyceride levels.

DISCUSSION

Insulinemia and Adiposity

In nondiabetic men, fasting plasma insulin concentrations were inversely correlated with plasma HDL_{3a}, HDL_{2a}, and HDL_{2b} levels (Fig 2). Presumably this reflects the effects of insulin resistance on HDL_{3a}, HDL_{2a}, and HDL_{2b} subclasses, since plasma insulin concentrations and insulin resistance are essentially linearly related [32]. The relationship between insulin and the

HDL particle distribution is apparently unaffected by adiposity, suggesting that insulin exerts a more direct effect on lipoprotein metabolism. Studies of diabetic subjects show that plasma levels of triglycerides are reduced and of HDL cholesterol are increased by oral hypoglycemic agents or insulin therapy [33]. In addition to increasing very-low-density lipoprotein (VLDL) production, hyperinsulinemia also appears to reduce VLDL clearance, possibly due to reduced synthesis of the lipoprotein lipase enzyme that hydrolyzes triglycerides [34,35]. Reductions in HDL_{3a}, HDL_{2a}, HDL_{2b}, and HDL peak diameter may be due in part to the increased availability of triglyceride-enriched lipoproteins for cholesteryl ester-triglyceride exchange.

Figure 3 shows that three measures of upper-body adiposity (waist to hip girth, subscapular skinfold, and abdominal skinfold) and overall adiposity (BMI), were negatively correlated with HDL_{2b}. The association between HDL₂ and waist to hip girth has been observed cross-sectionally [36–38] and longitudinally [39]. The partial correlations of Fig 6 suggest that metabolic factors associated with insulin resistance and elevated triglycerides could mediate in part the reduction in HDL_{2b} associated with adiposity. Salomma et al [40] have hypothesized the primacy of insulin resistance in mediating associations between adiposity and lipoproteins in men. Taskinen et al [41] reported that insulin therapy increases HDL₂ and reduces HDL₃ concomitant to a twofold increase in adipose tissue lipoprotein lipase activity. Eckel and Yost have reported that increases in HDL₂ cholesterol after weight loss are strongly correlated with a change in the responsiveness of adipose tissue lipoprotein lipase to insulin [42].

Other studies report that the association between adiposity and HDL₂ is only partially explained by fasting insulin [37,43], and it is unlikely that the adiposity-HDL_{2b} relationship is solely the metabolic consequence of insulin resistance. Adipocytes may affect HDL concentrations directly, ie, plasma HDL cholesterol levels are inversely correlated with HDL₂ and HDL₃ binding to adipocytes, with the affinity for binding to adipocytes being greater for HDL₂ than for HDL₃ [44,45].

Sympathetic Drive

A Western life-style, characterized by high dietary intake of fat, low carbohydrate intake, and sedentary existence, appears to increase sympathetic drive [46–49]. Figures 4 and 5 suggest that resting heart rate (an indicator of sympathetic drive) was negatively correlated with HDL peak diameter and HDL_{2a} and HDL_{2b} levels, and positively correlated with fasting insulin levels in the presence of obesity.

There is disagreement as to whether sympathetic drive is increased by elevated plasma insulin concentrations stemming from peripheral insulin resistance [50] or whether the plasma insulin concentration is increased by elevated sympathetic drive [51]. Elevated plasma insulin may increase heart rate and stimulate the sympathetic nervous system [52]. O'Hara et al [53] showed that application of the euglycemic-hyperinsulinemic clamp to increase insulin without altering plasma glucose increases sympathetic drive equally in both nonobese and obese individuals [53]. This is in contrast to Fig 5, which suggests that the relationship of resting heart rate with plasma insulin levels is different in leaner and more obese individuals. The discrepancy between our results and theirs may argue against hyperinsulinemia as an antecedent to higher heart rate in our sample.

Increased sympathetic drive may increase plasma insulin levels, which in turn may affect lipoprotein metabolism. High sympathetic drive increases plasma catecholamine levels. Catecholamines stimulate the release of fatty acids by adipose tissue, particularly in intra-abdominal fat cells, which are more responsive to the lipolytic actions of catecholamines than the gluteal fat cell [54] and more resistant to the ability of insulin to inhibit catecholamine-induced lipolysis [55]. This may promote insulin resistance because the free fatty acids compete with glucose for oxidation by muscle, so that glucose uptake is reduced and higher serum

glucose levels in plasma result [56]. Higher plasma insulin levels may follow through peripheral insulin resistance and islet cell dysfunction [57]. Excess free fatty acids also inhibit hepatic insulin uptake [54,58]. By releasing fatty acids directly into portal circulation, sympathetic stimulation of intra-abdominal fat cells may also lead to enhanced VLDL production [54,59]. This may account for the increases in VLDL production during stimulation of the α -adrenergic receptors [60]. Increased VLDL concentrations may reduce HDL_{2a}, HDL_{2b}, and HDL peak diameter through enhanced cholesteryl ester-triglyceride exchange [61]. Increased fatty acid concentrations in plasma could also directly affect cholesteryl ester-triglyceride exchange through increased binding of cholesteryl ester transfer protein to lipoproteins [62]. Thus, both insulin and plasma lipoproteins may be affected by sympathetic drive via the release of fatty acids by adipose tissue. As the direct intermediary, the amount of adipose tissue (particularly visceral or abdominal fat) could affect the insulin and lipoprotein responses to the primary effects of heightened sympathetic drive. The larger adipocytes of heavier men may also play a role, given that the release of free fatty acids from adipose tissue increases linearly with the size of the fat cell [63].

Physical Activity

Our previous intervention studies show that running increases both HDL₃ and HDL₂ concentrations. It is our belief that several metabolic effects may be involved. Specifically, we report elsewhere that initially sedentary men who ran an average of 13.4 km/wk for 1 year appear to increase HDL_{2b} through metabolic processes associated with weight loss and to increase HDL_{2a} and HDL_{3a} through processes that are largely independent of weight loss [64]. There is a considerable difference in the level of strenuous physical activity between the men studied here and the men who participated in our previous running study. Nevertheless, the distinct effects of physical activity and BMI on HDL subclasses are suggested in the present report. Whereas adiposity appears to be associated with HDL_{2b} (Fig 3), reported physical activity appears to be principally associated with HDL_{3a} and HDL_{2a} levels in heavier men (Fig 4). The correlations of Fig 4 are consistent with the report by Ruys et al [65] suggesting that HDL₃ cholesterol production is increased in trained as compared with untrained arm muscles [66]. Although Keins and Lithell [66] reported increased arteriovenous HDL₂ cholesterol production across exercising leg muscles, the increase may involve the HDL_{2a} component [64].

Caveats and Summary

Caution is warranted when extrapolating our results to men without overt coronary artery disease. Men with coronary disease tend to have decreased levels of total HDL cholesterol, HDL₂ cholesterol, and apolipoprotein A-I [67,68] and a smaller proportion of HDL protein within the HDL_{3a}, HDL_{2a}, and HDL_{2b} intervals [8]. However, the correlations of Table 1 are all consistent with those reported for coronary heart disease-free men, suggesting that the factors affecting HDL levels in men with and without coronary artery disease may be similar. Our results suggest that the influences of insulin status, adiposity, resting heart rate, and physical activity on HDL involve specific HDL subclasses. Studies that report the absence of significant relationships of HDL₃ to total adiposity [69], upper-body obesity [37,38], and non-insulin-dependent diabetes mellitus [70] could be missing important alterations in HDL_{3b}. However, these results are derived on a specialized sample of men, those with coronary heart disease, and their generalizability to the larger population remains to be determined.

Acknowledgments

We wish to thank Laura Holl, Charlotte Brown, and Bahareh Sahami for laboratory analysis of gradient gel electrophoresis, and the staff of the Stanford Coronary Risk Intervention Project for their efforts in completing the clinical trial.

Supported in part by Grants No. HL-02183, HL-24462, and HL-28292 from the National Heart, Lung, and Blood Institute, and conducted at Stanford University and Lawrence Berkeley Laboratory (Department of Energy Grant No. DE-ACO3-76SFO0098 to the University of California).

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Fig 1.

Mean absorbency of protein-stained HDL by particle size in four untreated non-insulin-dependent diabetic men (fasting plasma glucose > 140 mg/dL) and 93 nondiabetic men (top)), and the mean differences between diabetic and nondiabetic men (bottom). The curves were computed by averaging the heights of the individual HDL distributions at each diameter value. The solid portions of the bar at the bottom of the graph designate those diameter values that achieve statistical significance for the differences ($P \leq 0.05$) for two sample t test. The predominant HDL peak diameters are indicated, and the subclass intervals defined by Blanche et al [1] are provided for reference. The untreated diabetics had significantly smaller HDL peak diameter (mean $\pm$ SD: 7.86 ± 0.06 vs. 8.21 ± 0.20 nm) than nondiabetic men and significantly higher HDL_{3b},

Fig 2.

Pearson correlation coefficients between plasma insulin and glucose levels and the absorbency of protein-stained HDL by particle size. The solid portions of the bars at the bottom of the graphs designate the range of diameter values that correlate significantly at $P \leq 0.05$. The subclass intervals defined by Blanche et al [1] are provided for reference.

Fig 3.

Pearson correlation coefficients between adiposity and absorbency of protein-stained HDL by particle size. The solid portions of the bars at the bottom of the graphs designate the range of diameter values that correlate significantly at $P \leq 0.05$. The subclass intervals defined by Blanche et al [1] are provided for reference.

Fig 4.

Pearson correlation coefficients of resting heart rate (top) and reported physical activity (middle) with absorbency of protein stained HDL by particle size. Partial correlations between resting heart rate and protein-stained HDL adjusted for physical activity are shown in the bottom panel. Results are presented for all 93 men, Low-BMI men ($\text{BMI} \leq 25.8 \text{ kg/m}^2$, $n = 47$), and high-BMI men ($\text{BMI} \geq 25.8 \text{ kg/m}^2$, $n = 46$). The solid portions of the bars at the bottom of the graphs designate the range of diameter values that correlate significantly at $P \leq 0.05$. The subclass intervals defined by Blanche et al [1] are provided for reference.

Fig 5.

Regression lines depicting the relationships of resting heart rate with the predominant HDL peak diameter (top) and fasting insulin levels (bottom). Separate lines are shown for the Low-BMI man ($\text{BMI} \leq 25.8 \text{ kg/m}^2$, $n = 47$) and high-BMI men ($\text{BMI} \geq 25.8 \text{ kg/m}^2$, $n = 46$). Significance levels refer to the difference in slopes between the low-and high-BMI men.

Fig 6.

Partial correlational analyses between HDL protein levels and fasting plasma insulin, BMI, subscapular skinfold, resting heart rate, and reported physical activity. Expanded portions of the lines designate the range of diameter values that correlate significantly at $P \leq 0.05$ when adjusted. *In heavier men only ($25.8 \text{ kg/m}^2 \leq \text{BMI} \leq 36.3 \text{ kg/m}^2$).

Sample characteristics and correlations with HDL cholesterol and mass concentrations, triglycerides, BMI, and plasma insulin and glucose concentrations

Table 1

	Mean±SD	Correlations with:								
		HDL cholesterol	HDL ₂ mass	HDL ₃ mass	Triglycerides	BMI	Insulin		Glucose	
							Fasting	1-h	Fasting	1-h
Triglycerides (mg/dL)	123.84 ± 54.84	-0.43 [‡]	-0.39 [‡]	-0.13	1.00	0.32 [‡]	0.51 [‡]	0.37 [‡]	0.31 [‡]	0.20
HDL cholesterol (mg/dL)	44.83 ± 10.85	1.00	0.77 [‡]	0.61 [‡]	-0.43 [‡]	-0.23 [*]	-0.35 [‡]	-0.16	0.11	-0.07
HDL peak diameter (nm)	8.21 ± 0.20	0.49 [‡]	0.51 [‡]	0.25 [*]	-0.49 [‡]	-0.24 [*]	-0.43 [‡]	-0.20	-0.20 [*]	-0.14
BMI (kg/m ²)	26.40 ± 3.31	-0.23 [*]	-0.29 [‡]	0.04	0.32 [‡]	1.00	0.39 [‡]	0.37 [‡]	0.07	0.19
Waist to hip ratio	0.94 ± 0.05	-0.09	-0.21	0.05	0.21	0.52 [‡]	0.15	0.11	0.09	0.20
Abdominal skinfold (mm)	25.84 ± 8.61	-0.15	-0.31 [*]	-0.16	0.22	0.71 [‡]	0.16	0.09	0.02	0.24
Subscapular skinfold (mm)	18.95 ± 5.98	-0.18	-0.25 [*]	-0.08	0.16	0.66 [‡]	0.31 [‡]	0.26 [*]	0.14	0.22 [*]
Fasting insulin (pmol/L)	14.40 ± 8.13	-0.35 [‡]	-0.29 [‡]	-0.02	0.51 [‡]	0.39 [‡]	1.00	0.38 [‡]	0.30 [‡]	0.12
1-h insulin (pmol/L)	119.01 ± 87.73	-0.16	-0.22 [*]	0.09	0.37 [‡]	0.37 [‡]	0.38 [‡]	1.00	0.14	0.42 [‡]
Fasting glucose (mg/dL)	96.71 ± 15.68	0.11	0.00	0.19	0.31 [‡]	0.07	0.30 [‡]	0.14	1.00	0.41 [‡]
1-h glucose (mg/dL)	147.71 ± 50.13	-0.07	-0.15	-0.09	0.20	0.19	0.12	0.42 [‡]	0.41 [‡]	1.00
Resting heart rate (bpm)	66.18 ± 9.08	-0.15	-0.17	-0.07	0.37 [‡]	0.00	0.30 [‡]	-0.01	0.22 [*]	-0.05

NOTE. Sample sizes are 93 men, except for subscapular skinfold and fasting waist to hip ratio (n = 59), and abdominal skinfold (n = 58).

* P ≤ 0.05

[‡] P ≤ 0.01

[‡] P ≤ 0.001.