Behavioral versus genetic determination of lipoproteins and adiposity in identical twins discordant for exercise

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Short title: Identical twins discordant for exercise

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Background: Lipoprotein and weight differences between vigorously active and sedentary MZ twins are used to: 1) estimate the effects of training while controlling for genotype; 2) estimate genetic concordance in the presence of divergent lifestyles.

Methods and Results: 35 pairs of monozygotic twins (25 male, 10 female) recruited nationally who were discordant for vigorous exercise (running distances differ • 40 kilometers in male and •32 km in female twins). The active twins ran an average (±SE) of 63.0 ± 20.4 km/wk, while the mostly-sedentary twins averaged 7.0±13.5 km/wk. The active twins had significantly lower BMI (difference±SE: -2.12±0.57 kg/m2, P=0.0007) and significantly higher HDL-cholesterol (0.13±0.04 mmol/L, P=0.004), HDL_2 (2.71 ± 1.04 units, P=0.01) and apoA-I (0.10±0.03 g/L, P=0.004). Despite the difference in lifestyle, when adjusted for sex, the correlations between the discordant MZ twin pairs were significant (P<0.01) for HDL-cholesterol (r=0.69), apo A-I (r=0.58), and HDL_2 (r=0.67). There was no significant MZ twin correlation for BMI (r=0.17). None of the active twins having an overweight twin were themselves overweight.

Conclusions: Behavior (vigorous exercise) may reduce genetic influences on BMI. In contrast, genetic (or shared environment) substantially influence HDL even in the presence of extreme behavioral differences. There may be greater individual control over moderate degrees of obesity, whereas low HDL may be largely predetermined and less effectively treated by vigorous exercise.

Physical activity is recommended for the hygienic treatment of both obesity and low-plasma concentrations of high-density lipoproteins (HDL) {1-3}. Obesity is an increasingly prevalent condition in Westernized societies that raises the risks for hypertension, type-II diabetes, and coronary heart disease {4}. Low HDL-cholesterol is also a risk factor for coronary heart disease and has received greater emphasis in the most recent National Cholesterol Education Program Adult Treatment Panel guidelines (ATP III) than in previous guidelines, reflecting its wider recognition as an important, treatable risk factor {1}. The relative contribution of environment and genes to determining adiposity and HDL are pivotal in setting realistic hygienic treatment goals for individuals and populations. Specifically, a large environmental contribution would suggest the potential for successful behavioral or environmental interventions whereas a large genetic component may suggest these risk factors are largely predetermined.

Studies in twins suggest a substantial heritable component to obesity (or the susceptibility to obesity in permissive environments). Twin studies suggest that sixty to ninety percent of the variation in adiposity is genetic {5}. Genes are estimated to account for approximately 70% of the variation in maximum lifetime BMI and adult weight gain {6}. Plasma HDL concentrations also appear to be influenced significantly by genes {7}, and estimates from path analysis models suggest that the genetic heritability of HDL-cholesterol is greater than its cultural heritability {8-12}.

Although vigorous exercise effectively increases HDL {1,13} and lowers body fat{1,3,14}, its effectiveness relative to the genetic influences on these traits is not known. Nor can its effectiveness be accurately inferred from prior twin or family studies that are relevant only to the largely sedentary populations from which they were drawn. This report examines the potential for physical activity to raise HDL-cholesterol and reduce weight in identical twins discordant for vigorous exercise. By comparing them to the active twin, the sedentary twin provides an estimate of the effects of genotype and shared environment on the

lipoprotein and body weight in the absence of exercise. Specifically, lipoprotein and weight differences between vigorously active and sedentary twin are used to: 1) estimate the effects of training while controlling for genetic background; 2) estimate the degree of genetic similarity in the presence of divergent lifestyles. In addition, we estimate the possible bias of self-selection due to genetic background and shared environment by contrasting the co-twin differences with those obtained from cross-sectional studies.

Methods and materials

We identified male and female participants of the National Runners' Health Study {15,16} who reported having a living, monozygotic twin, 18-74 years old, who resided within the United States, and who had no prior history of heart disease, diabetes, or cancer (except skin cancer). We required weekly running distances to differ by at least 40 kilometers (25 miles) in male and at least 32 km (20 miles) in female twins. Three weeks prior to a blood draw, the twins adjusted their alcohol consumptions to the level of the twin whose intake was lowest. We excluded twins discordant for tobacco use, medications, oral contraceptives, or postmenopausal estrogen replacement.

As part of a telephone interview, each twin provided us the name of a local clinic or hospital where it would be convenient to have their blood drawn. Blood was drawn after a 12 hour fast and 24 hours after the most recent vigorous exercise on Mondays, Tuesdays, or Wednesdays to ensure their delivery to our laboratory by Thursday. The local clinic also measured height and weight, and returned these data with the processed blood along with the signed consent form. The samples were shipped on wet ice by overnight carrier on the same day of collection, and arrived at our laboratory within 18 hours.

All participants received a VHS video tape providing 16 minutes of instruction for completing the four-day diet record, a 0 to 16 oz. diet scale for weighing foods, a food record for recording food intake on Thursday, Friday, Saturday, and Sunday, and a pre-paid, pre-addressed

envelope for returning the record. The food records were sent to the Central Dietary Data Entry Center located at Children's Hospital Medical Center, Cincinnati, Ohio for coding. Nutrient analyses were based on the extensive food data base from the NHLBI nutrient assessment system used by the Lipid Research Clinics {17}. Weekly running distance and participation in any other exercise was determined by questionnaires and follow-up telephone interviews.

Laboratory measurements The plasma were analyzed for concentrations of cholesterol {18}, triglyceride {19} and HDL-cholesterol, measured directly after precipitation of apoprotein B containing lipoproteins in plasma {20}. LDL cholesterol was calculated by subtraction of estimated very low density lipoprotein and measured HDL cholesterol from the measured total cholesterol and triglyceride in plasma. Lipid assays were enzymatic end-point measurements utilizing enzyme reagent kits (Ciba-Corning Diagnostics Corp., Oberlin, Ohio) and a CIBA Corning Express 550 automated analyzer. The measurements were standardized through the CDC-NHLBI Lipid Standardization Program. Enzyme-linked immunosorbent assays were used for the measurements of apo A-I and B {21}.

Electrophoresis was used to determine the levels of HDL within five subclass intervals. The plasma d• 1.20 g/ml fraction was obtained after single spin ultracentrifugation (114,000 g, 24 hours, 10°, Beckman 50.3 rotor). Electrophoresis of HDL in the ultracentrifuged d • 1.20 g/ml fraction was done on a Pharmacia Electrophoresis Apparatus (GE 4-II) using slab gradient gels as described by Blanche et al.{22} Following electrophoresis, plasma lipoproteins derived from ultracentrifugally isolated fractions were stained for protein content. The stained gradient gels were scanned with a model RFT densitometer (Transidyne Corp., Ann Arbor, MI) at a wave length of 603 nm. A mixture of four globular proteins (HMW Calibration Kit) was run on the central lane to calibrate them for particle size. The HDL-migration distances (R_f) were measured relative to the migration distance of the peak of bovine serum albumin. Differences between twins were computed for each of the five HDL subfractions in the total d>1.006 plasma fraction [HDL_{3c} (7.2-7.8 nm

diameter), HDL_{3b} (7.8-8.2 nm), HDL_{3a} (8.2-8.8 nm), HDL_{2a} (8.8-9.7), and HDL_{2b} (9.7-12.9 nm) {22}.

Statistical analyses Mean differences were evaluated by paired t-test (verified nonparametrically by the sign-rank test) and correlations were assessed by Pearson correlation coefficients (verified nonparametrically by the Spearman's correlation). Linear regression was used to adjust for sex and other covariates.

Results

Thirty-five pairs of MZ twins (10 female, 25 male) discordant for exercise participated in the study. They averaged (±SD) 40.5± 6.8 years in age. There was a 51.97±16.83 km mean difference in the average weekly running distance between twins. The active twins ran an average of 63.0 ± 20.4 km/wk, while the mostly-sedentary twins averaged 7.0±13.5 km/wk. There were no significant correlations for the active and more-sedentary twins' intakes of total calories (r=0.38), or the percent of total calories from carbohydrates (r=0.29), fat (r=0.10), saturated fat (r=0.16), monounsaturated fat (r=0.10), polyunsaturated fat (r=0.05). The significant twin correlation for grams of alcohol intake (r=0.45) reflect our instruction to adjust intake to the lower of the twins intakes within each pair.

Table 1 displays the mean BMI and lipoprotein concentrations in the active and mostly-sedentary twins, and their co-twin differences (±SE). The active twins had significantly lower BMI (P=0.0007) and significantly higher HDL-cholesterol (P=0.004) and apoA-I (P=0.004). There were no significant differences in the twins' plasma apo B (P=0.12), LDL-cholesterol (P=0.59), or triglyceride concentrations (P=0.43), or daily alcohol intake (difference±SE: 15.5 ± 16.6 ml, P=0.36). The BMI, HDL-cholesterol, and apo A-I co-twin differences were also significant for the 25 male twin pairs considered separately. The female co-twin differences were consistent with those of the males for HDL-cholesterol, apo A-I and BMI (presumably nonsignificant because there were only ten pairs). Table 2 displays the co-twin differences for the five HDL

subclasses as measured by gradient gel electrophoresis. The active twins had significantly higher HDL_2 (P=0.01), specifically significantly higher HDL_{2a} (P=0.004) and marginally higher HDL_{2b} (P=0.08). Among males, the levels of HDL in the active twins were significantly higher for HDL_{3a} (P=0.007), HDL_{2a} (P=0.0004) and HDL_{2b} (P=0.02) vis-a-vis the mostly-sedentary twins.

Figures 1 and 2 compare the active twin (vertical axes) with their mostly-sedentary twins (horizontal axes). The 35 plotted points represent the corresponding values of the twin pairs. The diagonal line represents the locus of equivalent values. Points fall above the diagonal when the active twins have higher values than the mostly-sedentary twins, and below the diagonal when the converse is true. The points lie above the diagonal (active greater than mostly sedentary) significantly more often than expected by chance for HDL-cholesterol (25 above vs 10 below the diagonal, P=0.009 by sign test), apo A-I (23 vs 12, P=0.04), and HDL_2 (27 vs 8, P=0.002). They also lie significantly more often above the diagonal for HDL_{2b} (25 vs 10, P=0.02) and HDL_{2a} (26 vs, 9, P=0.006), but not HDL_{3a} (23 vs 12, P=0.09), HDL_{3b} (13 vs 22, P=0.18) or HDL_{3c} (19 vs. 16, P=0.74, analyses not displayed). Figure 2 shows that the majority of points for BMI lie below the diagonal (27 vs 8, P=0.002), representing the lower BMI of the active twin.

When adjusted for sex, the correlations between the discordant MZ twin pairs were significant (P<0.01) for height (r=0.82), HDL-cholesterol (r=0.69), apo A-I (r=0.58), LDL-cholesterol (r=0.72), apoB (r=0.70), triglycerides (r=0.42), HDL_{3b}(r=0.52), HDL_{2a}(r=0.64), HDL_{2b}(r=0.62), and HDL_2(r=0.67), but not HDL_{3c}(r=0.23), or HDL_{3a}(r=0.20). Figure 1 shows that despite the big differences in weekly vigorous activity, HDL-cholesterol, apo A-I and HDL_2 are strongly related within twin pairs. Adjustment for alcohol intake did not alter the significance of the mean differences nor the significance of the MZ twin correlations. In addition, the observed concordance does not appear to be a methodological artifact from twins being occasionally analyzed within the same batch of samples. Specifically concordance was also demonstrated across different methodologies for measuring HDL, i.e., there is a strongly significant

correlation between the active twins' HDL_2 from gradient gel electrophoresis and the mostly-sedentary twins' HDL-cholesterol that is estimated by precipitation, and between the active twins' HDL-cholesterol and the mostly-sedentary twins' HDL_2 (r=0.67 for both).

We found no significant MZ twin correlation for BMI (Figure 2). Thirteen of the mostly-sedentary twins were moderately overweight (BMI>25 kg/m^2), as compared to only two of the active twins. None of the active twins having an overweight twin were themselves overweight. The twins provided data on their greatest lifetime weight, which was used to estimate the effect of exercise since under sedentary condition, individuals are expected to gain weight as they age. The reduction in BMI from the time of greatest lifetime weight was greater in those active twins with an overweight twin than in those with a lean twin (difference = -1.6 kg/m^2, p=0.06). This difference is particularly strong among males (-2.6 kg/m^2, p=0.02). This suggests that those active twins having an overweight twin had historically lost more weight (presumably due to running) than those not having an overweight twin.

Analyses using only sedentary twins. The mostly-sedentary twins included 25 sedentary twins (9 female, 16 male) who did not run at all or engage in other vigorous activity (mean±SD: 56.29± 18.16 km/wk less than their active twin). Results similar to the complete sample were obtained when the analyses were restricted to this subset of twin pairs. The active twin weighed significantly less than the sedentary twin and had significantly higher HDL-cholesterol (difference±SE: 0.14 ± 0.05 mmol/L, P=0.005), apo A-I (0.09±0.04 g/L, P=0.02), HDL_2 (3.16±1.17 units, P=0.01), HDL_{2a} (1.51±0.61 units, P=0.01), and HDL_{2b} (1.64±0.65 units, P=0.01). There were no significant differences for plasma LDL-cholesterol (-0.08±0.14 mmol/L, P=0.56), triglycerides (-0.07±0.05 mmol/L, P=0.16), apo B (-0.39±0.29 mmol/L, P=0.19), HDL_{3c} (0.04±0.15 units, P=0.80), HDL_{3b} (-0.32±0.26 units, P=0.22), or HDL_{3a} (0.69 ±0.65 units, P=0.30). Table 3 presents the means for the active and sedentary twins and their differences by sex. The active male twins had significantly lower BMI and significantly higher HDL-cholesterol than their sedentary twin, and marginally higher apo A-I. When adjusted for

sex, the correlations between the discordant MZ pairs were significant ($P<0.01$) for height ($r=0.78$), HDL-cholesterol ($r=0.66$), apo A-I ($r=0.52$), LDL-cholesterol ($r=0.85$), apoB ($r=0.83$), triglycerides ($r=0.78$), HDL_{3b}($r=0.60$), HDL_{2a}($r=0.54$), HDL_{2b}($r=0.65$), and HDL_2($r=0.67$), but not HDL_{3c}($r=0.38$), HDL_{3a}($r=0.05$) or BMI ($r=0.15$).

Discussion

These results suggest that behavior (vigorous exercise) can mitigate genetic influences on BMI. In contrast, genetic (or shared environment) substantially influence plasma HDL concentrations even in the presence of extreme behavioral differences. The prescription of vigorous exercise to reduce weight is likely to be much more effective than the prescription of vigorous exercise to raise HDL. The public health promotion of vigorous exercise may be far more successful when body weight is targeted rather than HDL-cholesterol. Our results suggest there may be individual control over moderate degrees of obesity, whereas low HDL levels may be largely predetermined and less effectively treated by vigorous exercise. These analyses do not preclude the possibility that within the population, there may be a minority of individuals in whom weight is primarily genetic or HDL is susceptible to intervention.

The strong correlations we observed for HDL-cholesterol, apo A-I (the major apolipoprotein of HDL), and HDL2 (the lighter, larger fraction of HDL) are consistent with the high level of heritability reported by others on presumably mostly sedentary populations. These include estimates of genetic heritability from twin and pedigree studies for HDL-cholesterol (35% {23}, 34% {24}, 59%{25}, 66% {26}, 74% of HDL variation {27}), HDL_2-cholesterol (37% {28}, 50% {24}), and apo A-I (50 to 58% {29}, 66% {30}). In contrast, absence of any significant association between the adiposity of the running and sedentary twin was unexpected. Prior studies of twins (presumably mostly sedentary) reveal heritability estimates that range from 0.6 and 0.9 {5}. Over 300 genes, markers, and chromosomal regions have been related to adiposity or weight {31}. Shared environmental influences do not appear to explain the strong correlation of adiposity in identical twins. Specifically, adoption studies show

little concordance between parents' and their adoptive children's adiposity {32}, and studies of identical twins raised apart suggest that the shared environments within families may have less influence on BMI than genes {33}.

Our findings are qualitatively consistent with prior cross-sectional studies that show male and female runners have higher plasma HDL-cholesterol concentrations and lower body weight than sedentary men and women {15,16}. The higher HDL-cholesterol reflects higher plasma concentrations of the larger particles (HDL_{2b}, HDL_{2a}, and HDL_{3a} subclasses) {34.35}. These lipoprotein and weight differences may explain in part the lower risks of cardiovascular disease and total mortality in physically active and cardiovascularly fit men and women compared to those who are inactive and unfit {36}. Differences in LDL-cholesterol and triglycerides did not achieve statistical significance between the active and sedentary twin. This is in contrast to several cross-sectional and intervention studies that suggest running decreases plasma triglyceride and LDL-cholesterol concentration in men {15} but not necessarily women {16}.

Particularly in men, the more physically active twin had higher plasma levels of HDL_{3a}, and HDL_2 (including both HDL_{2a} and HDL_{2b}). Despite the differences in activity, plasma levels of HDL_{3b}, HDL_{3a}, HDL_{2a} and HDL_{2b} correlated significantly between the active and more sedentary twin. These differences and associations reflect are reflective of two overlapping HDL particle distributions that differ in their apolipoprotein compositions: HDL containing both apo A-I and apo A-II with includes major components within HDL_{3b}, HDL_{3a} and HDL_{2a} subclasses, and HDL containing apo A-I and no apo A-II with major components within the HDL_{3c}, HDL_{3a} and HDL_{2b} subclasses. The two HDL distributions have minimal exchange of their apo A-I {37} and metabolic interconversions between HDL subclasses appear to occur predominantly within each distribution {38}.

It is of interest to compare the estimated effect of exercise from the discordant twin design with those achieved experimentally or observed

cross-sectionally. The present analyses suggest that HDL-cholesterol increased 0.100 mg/dL per km run (5.5 mg/dL HDL-cholesterol difference divided by a 57.35 km difference in weekly distance run). Elsewhere, we reported that experimentally one year training was reported to increase HDL-cholesterol by 1.4 mg/dL in men who averaged 13.9 km/wk, or 0.100 mg/dL per km run {39}. Both designs control for genetic effects (i.e., the genotype being constant for both MZ twins and experimentally-induced changes within an individual) and yield consistent estimates. The mean HDL-cholesterol difference between active and sedentary female MZ twins was 4.40 mg/dL. Based on the 45.4 km/wk difference in their running distance we estimate that HDL increased 0.097 mg/dL per km/wk run, which is consistent with the prior two estimates. However, our previous large cross-sectional studies of runners suggested a greater increase in HDL-cholesterol per km run than discordant MZ twin or experimental studies: 0.136 mg/dL per km run in men {15} and 0.133 mg/dL per km run in women {16}. These cross-sectional estimates are 36% higher in men and 37% higher in women than the twin or experimental estimates.

Most cross-sectional studies of physical activity do not compare runners of different weekly distances but rather physically active vis-a-vis sedentary individuals {35}). For example, the National Health and Nutrition Examination Survey III reported a mean HDL-cholesterol of 45.7 mg/dL for men {40}. If we were to estimate the effects of exercise on HDL-cholesterol by subtracting the runners' HDL-cholesterol from that of the NHANES sample, and divided this difference by the 57.35 km week difference in running distance (assuming the NHANES men were all sedentary), then we would estimate that HDL-cholesterol increases 0.189 mg/dL per km (a conservative estimate because if some of the NHANES men are vigorously active then denominator would be even smaller). This estimate is 89% higher than those obtained from twins or derived experimentally.

We believe that the inflated cross-sectional estimates are largely due to self selection, perhaps selection of genetic profiles affecting both the propensity to exercise and high HDL-choleterol. We have demonstrated in two separate training studies that sedentary men who have higher HDL-

cholesterol at baseline will run longer weekly distances at the end training program compared to men who start out with low HDL {41,42}. We speculated that high baseline HDL may identify individuals genetically endowed with a high proportion of slow-twitch red muscle fibers. These fibers are more adaptive to endurance exercise and are relatively enriched with lipoprotein lipase (an enzyme that promotes higher HDL){42}. The high HDL-cholesterol levels of the sedentary twin (50.6 mg/dl, Table 3) suggest that just the ability to run (as represented by their more active brother) genetically confers high HDL-cholesterol. The sedentary twins were also relatively lean and had generally low triglycerides.

The contrasting estimates, depending upon whether genetic effects are controlled for or not, may have important implications concerning the presumed magnitude of the benefits of physical activity. Prospective epidemiological studies have repeatedly shown that fit, physically-active men and women are at less risk for cardiovascular disease then unfit, inactive men and women. These associations will overestimate the benefits of changing physical activity if 48% of the cross-sectional association is due to self-selection. Although HDL-cholesterol is only one of several risk factors for cardiovascular disease, we believe that genetic factors may also predispose individuals to be more active and have lower cardiovascular disease risk {36}. Attempts to circumvent this bias by relating change in physical activity between two baseline visits to disease endpoints during subsequent follow-up may incur other biases due to measurement error {43}.

Theoretically, randomized, controlled clinical trials should provide the most definitive proof that increasing physical activity causes HDL-cholesterol to increase and body weight to decrease {13,44}. In practice, however, the levels of exercise achieved in training studies scarcely ever approach the exercise differential observed cross-sectionally. The HERITAGE family study produced small increases in HDL-cholesterol (1.1 mg/dL in men and 1.4 mg/dL in women {45}) and small decreases in weight (0.9 pounds in men and 0.4 pounds in women {46}) after 20 weeks of

training. In another study, one year training was reported to increase HDL-cholesterol by 1.4 mg/dL in men who averaged 13.9 km/wk {14}.

In summary, the discordant twin study design provides the advantages of both cross-sectional association studies (large phenotypic effects) and training studies (controlling for genotype) without the self-selection bias of cross-sectional association studies or the small phenotypic response of training studies. The design yields estimates of the increase in HDL-cholesterol per km run per week that agrees very well with those derived experimentally, and show that the projected benefits are likely to accrue beyond the limited training distances that can be usually achieved experimentally. HDL-cholesterol, apo A-I, HDL_{3a} and HDL_2 (including HDL_{2a} and HDL_{2b}) all appear to increase with exercise. However, genetic influences appear to be a greater determinent of HDL levels than exercise, and the large differences in HDL-cholesterol between sedentary men and runners are likely to be due to a large part to genetic differences between runners and nonrunners. Merely sharing the same genes as a dedicated runners appears sufficient to bestow a desirable level of HDL-cholesterol in the absence of activity. This can be further improved upon by the addition of vigorous activity.

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Table 1. Differences in lipoproteins, adiposity and body mass index in identical twins discordant for exercise

	Active twins mean ± SD	Mostly sedentary twins mean ± SD	Difference ± SE
Males and Females (N=35)			
BMI (kg/m2)	22.01 ± 1.72	24.13 ± 3.20	-2.12 ± 0.57§
HDL-cholesterol (mmol/L)	1.51 ± 0.34	1.37 ± 0.33	0.13 ± 0.04§
HDL-cholesterol (mg/dL)	58.29 ± 13.31	53.09 ± 12.61	5.20 ± 1.70§
LDL-cholesterol (mmol/L)	3.05 ± 0.84	3.12 ± 1.10	-0.07 ± 0.13
LDL-cholesterol (mg/dL)	117.86 ± 32.36	120.60 ± 42.31	-2.74 ± 5.08
Triglycerides (mmol/L)	0.94 ± 0.46	1.01 ± 0.55	-0.07 ± 0.09
Triglycerides (mg/dL)	82.95 ± 40.86	89.51 ± 48.50	-6.56 ± 8.19
apo A-I (g/L)	1.54 ± 0.21	1.44 ± 0.20	0.10 ± 0.03§
apoB (g/L)	7.44 ± 2.22	7.96 ± 2.66	-0.52 ± 0.32
Males (N=25)			
BMI (kg/m2)	22.46 ± 1.60	24.30 ± 2.17	-1.84 ± 0.49§
HDL-cholesterol (mmol/L)	1.48 ± 0.39	1.34 ± 0.35	0.14 ± 0.05§
HDL-cholesterol (mg/dL)	57.08 ± 14.87	51.56 ± 13.48	5.52 ± 2.12§
LDL-cholesterol (mmol/L)	3.05 ± 0.89	3.25 ± 1.21	-0.20 ± 0.17
LDL-cholesterol (mg/dL)	117.80 ± 34.42	125.64 ± 46.71	-7.84 ± 6.56
Triglycerides (mmol/L)	0.96 ± 0.51	1.07 ± 0.60	-0.12 ± 0.12
Triglycerides (mg/dL)	84.52 ± 44.92	95.04 ± 52.68	-10.52 ± 10.91
apo A-I (g/L)	1.55 ± 0.24	1.44 ± 0.22	0.10 ± 0.04§
apoB (g/L)	7.50 ± 2.42	8.18 ± 3.01	-0.69 ± 0.42
Females (N=10)			
BMI (kg/m2)	20.89 ± 1.57	23.71 ± 5.09	-2.82 ± 1.61
HDL-cholesterol	1.59 ± 0.21	1.47 ± 0.25	0.11 ± 0.08

(mmol/L)			
HDL-cholesterol (mg/dL)	61.30 ± 8.11	56.90 ± 9.67	4.40 ± 2.91
LDL-cholesterol (mmol/L)	3.06 ± 0.73	2.80 ± 0.68	0.26 ± 0.14
LDL-cholesterol (mg/dL)	118.00 ± 28.23	108.00 ± 26.44	10.00 ± 5.35
Triglycerides (mmol/L)	0.89 ± 0.34	0.86 ± 0.39	0.04 ± 0.10
Triglycerides (mg/dL)	79.04 ± 30.04	75.70 ± 34.45	3.34 ± 8.90
apo A-I (g/L)	1.53 ± 0.14	1.44 ± 0.15	0.09 ± 0.05
apoB (g/L)	7.29 ± 1.72	7.39 ± 1.44	-0.10 ± 0.41

Significance levels are coded:* $p<0.05$; † $p<0.01$; § $p<0.005$; ¶ $p<0.001$

Table 2. Differences in protein-stained HDL-total subclass intervals in identical twins discordant for exercise

	Females	Males	Males and females together
HDL_{3c} (area)	37 ± 178	191 ± 216	127 ± 162
HDL_{3b} (area)	-94 ± 443	-414 ± 283	-323 ± 263
HDL_{3a} (area)	-661 ± 1218	1347 ± 459 §	773 ± 493
HDL_{2a} (area)	263 ± 1130	1945 ± 469 ¶	1464 ± 474 §
HDL_{2b} (area)	-772 ± 1176	2058 ± 795 *	1250 ± 687
HDL_2 (area)	-509 ± 2119	4003 ± 1116 †	2714 ± 1044 †

Significance levels are coded:* $p<0.05$; † $p<0.01$; § $p<0.005$; ¶ $p<0.001$

Table 3. Differences in lipoproteins, adiposity and body mass index in discordant identical twins in which the less active twin does not run.

	Active twins mean ± SD	Sedentary twins mean ± SD	Difference ± SE
Males (N=16)			
BMI (kg/m2)	22.66 ± 1.65	24.14 ± 2.13	-1.48 ± 0.61*
HDL-cholesterol (mmol/L)	1.45 ± 0.33	1.31 ± 0.26	0.14 ± 0.06*
HDL-cholesterol (mg/dL)	56.06 ± 12.73	50.56 ± 9.99	5.50 ± 2.15*
LDL-cholesterol (mmol/L)	2.94 ± 0.93	3.23 ± 1.36	-0.29 ± 0.18
LDL-cholesterol (mg/dL)	113.50 ± 35.79	124.63 ± 52.69	-11.13 ± 6.87
Triglycerides (mmol/L)	0.86 ± 0.39	0.96 ± 0.36	-0.11 ± 0.06
Triglycerides (mg/dL)	75.69 ± 34.27	85.06 ± 31.99	-9.38 ± 5.12
apo A-I (g/L)	1.51 ± 0.23	1.42 ± 0.21	0.10 ± 0.05
apoB (g/L)	7.13 ± 2.56	7.78 ± 2.90	-0.65 ± 0.38
Females (N=9)			
BMI (kg/m2)	20.82 ± 1.64	23.56 ± 5.38	-2.75 ± 1.79
HDL-cholesterol (mmol/L)	1.59 ± 0.22	1.45 ± 0.26	0.13 ± 0.08
HDL-cholesterol (mg/dL)	61.22 ± 8.60	56.11 ± 9.91	5.11 ± 3.16
LDL-cholesterol (mmol/L)	3.11 ± 0.76	2.82 ± 0.72	0.29 ± 0.15
LDL-cholesterol (mg/dL)	120.00 ± 29.18	108.89 ± 27.88	11.11 ± 5.85
Triglycerides (mmol/L)	0.88 ± 0.36	0.90 ± 0.39	-0.01 ± 0.10
Triglycerides (mg/dL)	78.04 ± 31.69	79.22 ± 34.58	-1.18 ± 8.58
apo A-I (g/L)	1.53 ± 0.15	1.44 ± 0.16	0.09 ± 0.06
apoB (g/L)	7.46 ± 1.74	7.37 ± 1.53	-0.08 ± 0.41

Significance levels are coded:* $p<0.05$; † $p<0.01$; § $p<0.005$; ¶ $p<0.001$

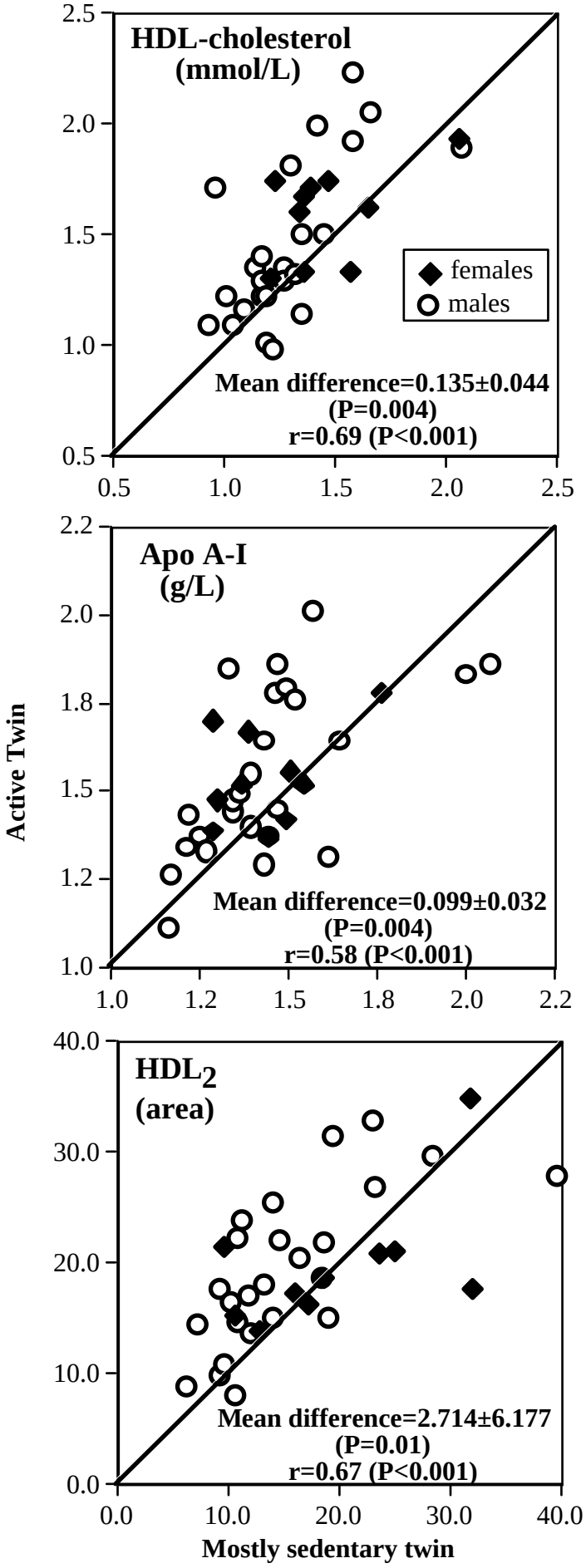
HDL-cholesterol
(mmol/L)
females
males
Mean difference=0.135±0.044
(P=0.004)
r=0.69 (P<0.001)
Apo A-I
(g/L)
Mean difference=0.099±0.032
(P=0.004)
r=0.58 (P<0.001)
HDL2
(area)
Mean difference=2.714±6.177
(P=0.01)
r=0.67 (P<0.001)
Active Twin
Mostly sedentary twin

Figure 1. Plot of plasma levels of HDL-cholesterol, HDL2 and apo A-I in the active(vertical axes) and mostly-sedentary twins (horizontal axes). The 35 plotted points represent the corresponding values of the twin pairs. The diagonal line represents the locus of equivalent values. Points fall above the diagonal when the runners have higher values than the sedentary twin, and below the diagonal when the converse is true.

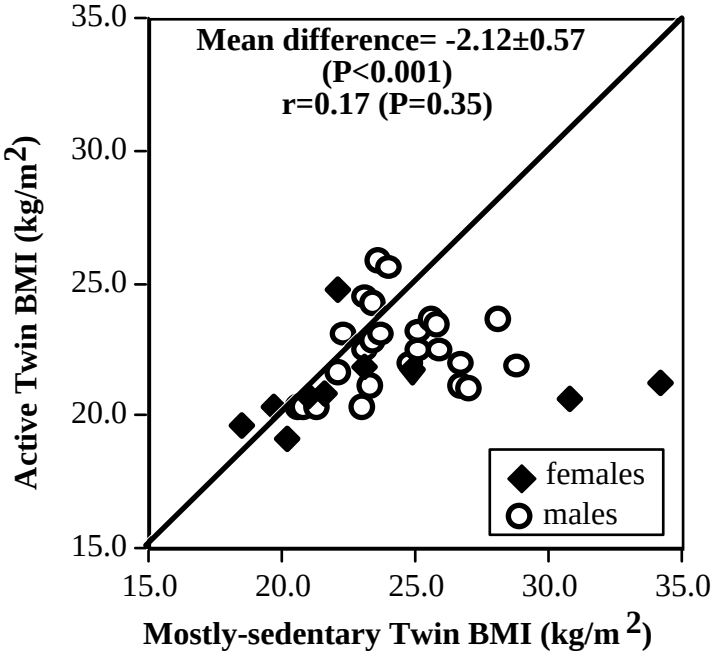

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Figure 2. Plot of body mass index (BMI) in the active (vertical axes) and mostly-sedentary twins (horizontal axes), showing no significant relationship.