Response to reviewers

Resubmission of 21175 Version 1

Reviewer 3.

1. Per the reviewers' recommendation, the title has been changed to "Concordant lipoprotein and weight responses to dietary fat change in identical twins with divergent levels of exercise" (Underlined section is a replacement).

2. Abstracts now starts on a new page (This seems to have been a problem with my understanding of the PDF conversion).

3. Line 6 of the Abstracts now clearly states "Twenty-eight pairs of male monozygotic twins…"

4. Nine keywords have been added following the abstract.

5. Abstract is written in complete sentences. Abstract is 218 words, Introduction is 423 words, and Discussion is 816 words.

6. The last line of the Introduction has been deleted.

7. The concluding line of the Methods now states "Statistical analyses were performed using StatView version 5.0.1 (SAS Institute; Cary, North Carolina)."

8. The following explanation is provided in the Figure legend "The significance level is the probability that the adjusted product-moment correlation coefficient is zero." The footnote to table 1 had been changed to state: Statistical significance by paired t-test or product-moment (Pearson) correlation coefficient designated by * $P<0.05$; † $P<0.01$; § $P<0.005$; ¶ $P<0.001$. The footnote to table 2 has been changed to read "None of the dietary changes were significantly

different between the Running and Sedentary Twin by analysis of variance". The footnote to table 3 has been changed to "Significance levels from analysis of variance and the product-moment correlation are coded: * $p<0.05$; † $p<0.01$; § $p<0.005$; ¶ $p<0.001$".

9. Dietary records were not collected at baseline, only at the end of the high-fat and the low-fat diets. Table 2 shows the energy intake on each of the diets, and the footnote states that there were no significant differences between the running and sedentary twin.

10. We have added the baseline values for the areas of the LDL-distribution from gradient gel electrophoresis. The change data in table 3 are the differences between being on the high-fat and the low-fat diets from a cross-over experimental design. Table 1 presents the baseline data before the subjects went on any of the diets. Because of their high costs, analytic ultracentrifuge measurements were not made at the baseline visit (only at the end of each treatment) and therefore do not appear in table 1.

11. The following sentence has been added to both figure legends to clarify the purpose of the lines "The diagonal is not a line fitted to the observations but rather is drawn as reference to the locus of points where the changes are identical in the twin pairs."

Reviewer 1. We apologize for the careless typographical errors. We have reviewed the manuscript to ensure it is purged of any of the errors cited. Per the reviewers' recommendation, the title has been changed to "Concordant lipoprotein and weight responses to dietary fat change in identical twins with <u>divergent levels of exercise"</u> (Underlined section is a replacement).

1. The cut and paste errors have been corrected and the manuscript carefully reviewed for any other errors.
2. Corrected. Again we apologize for the errors.

3. Table 4 has been corrected to read table 3 and Figure 1 is correctly referenced.

4. We have removed the results for mg/dl and have presented all findings as mmol/L

5. The correct results are presented for cholesterol as mmol/L.

Reviewer 2.

1. Table 2 shows that there was no significant difference in the adherence to the two diets.

2. We have added the sentence that all subjects were carefully counseled to follow each of the diets (the order of the diets were assigned at random).

3. Yes.

4. The difference in the apo A-I response did not achieve statistical significance (P=0.07) and became less significant (P=0.91) with the adjustment for baseline differences in the running and sedentary twins' baseline apo A-I.. This was not discussed in the text because the unadjusted apo A-I differences were not significantly different between runners and nonrunners.

5. The variation in response is shown in Figures 1 and 2. The following has been added to the first paragraph of the discussion " Figures 1 and 2 show there was considerable variation in the weight, apo A-I, Lp(a), and LDL response in switching from a high-fat low-carbohydrate diet to a low-fat high-carbohydrate diet across individuals, and that much of this variation may be accounted for by genes."

Concordant lipoprotein and weight responses to dietary fat change in identical twins with divergent exercise levels.

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Running title: Lipoprotein changes due to dietary fat in twins

This work was supported in part by a grant from Dairy Management Incorporated and NIH R01 Grant HL-58621, and NIH Program Project Grant HL-18574 from the National Heart, Lung, and Blood Institute, and was conducted at Lawrence Berkeley National Laboratory through the U.S. Department of Energy under contract No. DEAC03-76SF00098.

Background/Objective: The purpose of this study is to test the extent that individual lipoprotein responses to diet can be attributed to genes in the presence of divergent exercise levels.
Design: Twenty-eight pairs of male monozygotic twins (one mostly sedentary, the other running an average of 50 km/week more than the sedentary twin) 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%.
Results: Despite the twins' differences in physical activity, the dietary manipulation produced significantly correlated changes ($P<0.05$) in the twin's total cholesterol ($r=0.56$), low-density lipoprotein (LDL)-cholesterol ($r=0.70$), large, buoyant LDL (S_f7-12, $r=0.52$), apo A-I ($r=0.49$), Lp(a) ($r=0.49$), electrophoresis measurements of LDL-I (LDLs between 26 and 28.5 nm diameter, $r=0.48$), LDL-IIB (25.2-24.6 nm, $r=0.54$), LDL-IV (22-24.1 nm, $r=0.50$), and body weights ($r=0.41$). Replacing fats with carbohydrates significantly decreased the size and ultracentrifuge flotation rate of the major LDL, the LDL mass concentrations of S_f7-12, LDL-I, high-density lipoprotein (HDL)-cholesterol and apo A-I, and significantly increased LDL-IIIA (24.7-25.5 nm diameter) and Lp(a).
Conclusions: Even in the presence of extreme exercise difference, genes significantly affect changes in LDL, apo A-I, Lp(a) and body weight when dietary fats are replaced with carbohydrates.

Keywords: Twins, Low-fat diet, high-carbohydrate diet, lipoproteins, Lp(a), physical activity, LDL-subclasses, apolipoproteins, cholesterol

The risk for coronary heart disease increases in association with higher plasma low-density lipoprotein (LDL)-cholesterol, triglycerides, and lipoprotein (a) (Lp(a)) levels and decreases in association with higher high-density lipoproteins (HDL)-cholesterol and apolipoprotein A-I levels and with the size and buoyancy of the LDL-particles {1,2}. Low-fat, high-carbohydrate diets decrease plasma concentrations LDL-cholesterol, HDL-cholesterol, apolipoprotein A-I, and increase Lp(a), and triglycerides {3}. The low-fat high-carbohydrate diets also produce a shift in the distribution of LDL's from larger, more buoyant particles to smaller denser particles {4}.

Individuals vary greatly in their lipoprotein responses to low-fat diets, some of this variation has been attributed to genes. Individuals having the apo E e4 allele experience greater reductions of LDL-cholesterol {5} and large, buoyant LDL (S_f7-12) {6} on low-fat, low-cholesterol diets than those lacking the allele. Polymorphisms in the apo B gene, signal peptide insertion allele, the LDL receptor gene, the MN blood group, and in the apo A-I promoter region are also reported to affect the LDL response to diet{5}. Low-fat diets induce a greater reduction in LDL-cholesterol and HDL_{2b} (the largest HDL particles) in individuals with a genetically influenced profile characterized by a predominance of small LDL particles than in those lacking this trait {7-9}.

Studies of monozygotic (MZ) twins provide evidence for genetic regulation in the absence of prior knowledge of the specific genes involved. Such studies provide a global test for genetic hypotheses while circumventing issues of multiple hypotheses testing that plague exploratory tests of multiple genetic loci {10}. For example, overfeeding and caloric expenditure in MZ twins causes weight gains and losses that correlate significantly within twin pairs {11,12}. However, to date only a small

proportion of the variation in body weight has been attributed to specific genes {13}.

The current study examines the effects of switching from high-fat low-carbohydrate to low-fat high-carbohydrate diets in MZ twins to assess the contribution of genes to the diet-induced changes in lipoproteins and body weight. Although it is often difficult to separate the effects of the twins' shared genotypes from their shared environment {14}, the current design minimizes the effect of the shared environment by: 1) deliberately choosing twins with divergent lifestyles (one physically active, one sedentary); 2) measuring the response to an experimental manipulation of diet (as opposed to observational twin studies that may be strongly affected by the shared environment).

Subjects and Methods

Twenty-nine pairs of identical male twins discordant for exercise participated in a crossover study of high-fat low-carbohydrate and low-fat high-carbohydrate diets. The twins were identified among current participants of the National Runners' Health Study and from announcements distributed at foot races through the Runner's World race participation program {15}. Criteria for eligibility were as follows: discordant for exercise (i.e., either one twin was sedentary and the other was running at least 32 km/wk or if both twins ran there was at least a 40 km/wk difference in running distance), no medication use likely to interfere with lipid metabolism, free of chronic disease, non-smoker, and willingness to abstain from alcohol and follow the prescribed diets over the twelve-week intervention. Each twin completed a questionnaire and signed a consent form approved by the Committee for the Protection of Human Subjects at Lawrence Berkeley National Laboratory, University of California, Berkeley.

The research used an outpatient setting with careful monitoring of dietary compliance. All participants were carefully counseled

by registered dieticians to follow the prescribed diets both before and during the experimental intervention. The twin-pairs received, in random order, a six-week low-fat solid-food diet (20% of total energy as fat, 65% as carbohydrates) and a six-week high-fat diet (40% fat, 45% carbohydrates) in a crossover design. The two experimental diets were designed to achieve a comparison of high- and low-fat intake by substitution of fat for carbohydrate without significant change in other major nutrients. Nutrient compositions of the diets were calculated using the Minnesota Nutrition Data System (NDS) software developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN, version 4.01. Registered dietitians supplied the participants with personalized menus demonstrating the number and size of servings for the experimental diets. Seven-day diets were prescribed to the participants representing 95% of total caloric intake as estimated from their baseline four-day food records; the remaining 5% were provided as food combinations that match the dietary composition of the prescribed diets which could be consumed ad-lib so that the total caloric intake could vary in response to the caloric intake required for satiety. The prescribed diets had to be eaten in their entirety within each 7-day period. The 5% additional calories could be consumed as one-half cup of low-fat milk with five vanilla wafers on the low-fat diet and as one teaspoon of peanut butter with eight wheat crackers on the high-fat diet. All subjects abstained from alcohol during the study. The staff contacted the subjects weekly during the study to verify adherence to the diet and to review the protocol. Compliance was assessed by four-day diet records and grocery receipts. One twin-pair did not complete the dietary intervention.

Twins reported to a local clinic of their choice to have their blood drawn at baseline and at the end of each six-week diet. All were required to have abstained for 12-14 hours from all food and vigorous activity. Plasma was prepared from venous blood collected in tubes containing Na2EDTA, 1.4 mg/mL. Samples were

drawn only on Mondays, Tuesdays, or Wednesdays and shipped overnight on wet ice to insure that they were delivered to our laboratory by Thursday morning. Before starting the study, all participants received an electronic scale for measuring their own body weight. Height and weight were also measured during the clinic visits.

Lipid and lipoprotein measureements Fasting plasma lipids were measured at baseline and after each six-week diet. Plasma concentrations of total cholesterol and triglycerides were measured by enzymatic procedures (ABA 200 instrument, Abbott Laboratories) {16}. HDL-cholesterol was measured by the dextran sulfate-magnesium precipitation of apo B containing lipoproteins followed by enzymatic determination of cholesterol {17,18}. Plasma LDL-cholesterol concentrations were calculated from the formula of Friedewald et al {19}. The laboratory remained certified by the Centers for Disease Control and Prevention lipid standardization program throughout the study. Apolipoproteins AI and B in plasma were measured by immunoturbidimetric assay {20,21}, using an ITA reagent kit reagent kit(Bacton Assay Systems, Inc., San Marcos, CA). Measurements are performed using the Express 550 analyzer according to kit instructions. Calibrators and controls are assigned quantitation levels based on the International Federation of Clinical Chemistry proposed Standard Reference Material SP1, and by participation in the IFCC/CDC directed Standardization Program. Intra- and inter-run coefficients of variation were within ±5%.

Fasting LDL particle diameters and LDL particle subclass intervals based on particle size were calculated from calibration curves using standards of known size {22}. Analyses are based on the area within the LDL-IVB (22.0-23.2 nm), LDL-IVA (23.3-24.1 nm), LDL-IIIB (24.2-24.6 nm), LDL-IIIA (24.7-25.5 nm), LDL-II (25.6-26.4 nm), and LDL-I (26.0-28.5 nm) particle size intervals {22,23}. Analytic ultracentrifugation was used to measure concentrations of total lipoprotein mass within multiple regions

for dense LDL (S_f0-7), buoyant LDL (S_f7-12), intermediate-density lipoproteins (IDL, S_f12-20) and very low-density lipoprotein (VLDL, S_f20-400) {24}.

Statistical analyses Fifteen pairs started with the high-fat diet and thirteen pairs started with the low-fat diet. Because the two diet sequences were not equally represented, the paired t-test was not used because temporal effects would not be eliminated by the analyses. We therefore computed separately the mean lipoprotein change in switching from a high to a low fat diet and the mean lipoprotein change in switching from the low to the high fat diets and their corresponding standard errors. We then calculated one half of the differences of the mean changes and their corresponding standard error (one half of the square root of the sum of the squared standard errors) to estimate separately the effect of the diet manipulation on the running twins' and the sedentary twins' lipoproteins while eliminating any temporal effects. The difference between the running and the sedentary twins' dietary response was calculated by subtracting the lipoprotein change within each twin pair and then analyzing the calculated differences as described above. Since none of the variables responded differently in the running and sedentary twins, we also analyzed the average of the twins' responses to assess the effect of the diet on lipoproteins with greater statistical power. Twin-pair correlations of the lipoprotein responses to the diets were calculated after adjusting for the diet sequence by regression analyses. Plots of the twins' responses are presented with adjustment to represent switching from the high to the low fat diet. Statistical analyses were performed using StatView version 5.0.1 software (SAS Institute; Cary, North Carolina).

Results

Baseline Table 1 presents the baseline characteristics of the twins. The running twins ran an average of 50 km per week more than the sedentary twins. Correspondingly, the running twins weighed significantly less than the sedentary twin, had significantly higher apo A-I and HDL-cholesterol and significantly lower triglycerides and apo B in plasma. The significantly higher mean Lp(a) concentration in the twins who ran was confirmed by the nonparametric sign test (24 runners had higher Lp(a) than their inactive twin brothers, P=0.0002). LDL peak particle diameter was also significantly larger in the running twin.

Consistent with their monozygosity, twin's heights were strongly correlated (r=0.92), as were their BMI's and weights. Despite substantial differences in physical activities, the twins exhibited strong, significant correlations for LDL-peak particle diameter, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, apolipoproteins A-I and B. They were also highly correlated for LDL-I, LDL-IIIA, LDL-IVA and LDL-IVB. The high correlation for Lp(a) was confirmed by nonparametric Spearman's correlation (rho=0.96).

Switching from the high to the low fat diet Table 2 shows the reported nutrient intake from 7-day food records for the running and sedentary twins on the two diets. The dietary goals were achieved on both diets. The changes in mean nutrient intake from switching from the high-fat low-carbohydrate diet to the low-fat high carbohydrate diet were not significantly different between the running and sedentary twin for total energy intake (mean •Exercise-•Sedentary±SE: -117.69 ± 92.12 kcal/d), total fat (0.53 ± 0.82%), saturated fat (0.12±0.22%), monounsaturated fat (0.19±0.21%), polyunsaturated fat (0.19±0.49%), carbohydrates (-1.10±1.22%), protein (0.58±0.51%) or dietary cholesterol (5.26±15.21 mg/day).

Table 3 shows that decreasing dietary fat significantly decreased HDL-cholesterol in both the running and the sedentary twins. Apolipoprotein A-I also decreased significantly in the running twins, and marginally in the sedentary twins. The decreases in both HDL-cholesterol and apo A-I were significant when the running and sedentary twins' data were average, as was the increase in mean plasma Lp(a) concentrations.

Table 3 also presents the changes in VLDL and LDL in response to decreasing fat and increasing carbohydrates. Mean LDL-peak particle diameter and the LDL-peak flotation rate decreased in both the sedentary and exercising twins. Mass concentrations of buoyant LDL also decreased significantly in both. Correspondingly, changes in LDL-peak diameter, LDL-peak flotation rate, and buoyant LDL were strongly significant when running and sedentary twins were averaged. The additional statistical power for detecting change when running and sedentary twins were averaged revealed significant increases in LDL-IIIA. The decrease in LDL-I and increase in LDL-IIIA were significant in the sedentary twins but not the running twins (p=0.10 for LDL-I and P=0.07 for LDL-IIIA). VLDL-mass concentrations increased in the running twin but not in their sedentary brothers (P=0.55), or the pooled twin-pairs (P=0.11). The lipoprotein responses to the diets were not significantly different between the running and sedentary twins (Tables 3).

Concordance within twin-pairs Increased dietary fat did not significantly change body weight (Table 3). However, there was considerable variability to the body weight response to the diets, and the responses were significantly correlated within twin pairs (r=0.41, Figure 1). Despite the substantial differences in physical activity, changes in apo A-I were strongly correlated within twin pairs, as were changes in Lp(a) (Figure 1).

The strongest correlation between the running and sedentary twins' lipoproteins was the correlation in the LDL-cholesterol response when switching from a high to a low fat diet (Figure 2). Table 3 suggests that the within-pair correlation for changes in LDL-cholesterol reflects within-pair concordant changes in the most buoyant LDL (S_f7-12) and LDL-I. Twins were also significantly correlated for changes in LDL-IIB and LDL-IV (Table 3).

The correlation between the twins' lipoprotein changes could not be attributed to concordance in their adherence to the dietary protocol. The correlations for changes in %protein, %carbohydrate and dietary cholesterol were all nonsignificant (0.06•r•0.08) when switching from the high-fat low-carbohydrate diet to the low-fat high-carbohydrate diet. One of the twin pairs reported concordantly low changes in total and saturated fat intake and one of the other twin pairs reported concordantly low changes in polyunsaturated fat intake. Excluding these two twin pairs eliminated the significant twin correlation between changes in total % fat intake (r=0.36 reduced to r=-0.15),% saturated fat intake (r=0.58 reduced to r=0.14), %monounsaturated fat intake (r=0.36 reduced to r=0.18), and %polyunsaturated fat intake (r=0.36 reduced to r=-0.13) when switching between diets. Eliminating these two twin pairs had almost no detectable effect on the twin correlations for changes in apo A-I (r=0.47), total cholesterol (r=0.56), LDL-cholesterol (r=0.70), Lp(a) (r=0.47), LDL-I (r=0.40), LDL-IIB (r=0.57), LDL-IVA (r=0.50), LDL-IVB (r=0.49), and large buoyant LDL-mass (r=0.58) in going from the high-fat low-carbohydrate diet to the low-fat high-carbohydrate diet.

Discussion

The lipoprotein changes produced in these twenty-eight twins confirms previous reports by ourselves and others that switching from a high-fat low-carbohydrate diet to a low-fat high-

carbohydrate diet decreases HDL-cholesterol, and apo A-I and increases Lp(a) {25-27}. The diet also decreased the size and buoyancy of the LDL-particle distribution, due to reductions in LDL-particles of S_f7-12 and 26-28.5 nm diameter (LDL-I). In addition, gradient gel electrophoresis revealed significant increases in LDL-IIIA. Figures 1 and 2 show there was considerable variation in the weight, apo A-I, Lp(a), and LDL response in switching from a high-fat low-carbohydrate diet to a low-fat high-carbohydrate diet across individuals, and that much of this variation may be accounted for by genes.

Whereas our previous studies held total caloric intake constant or manipulated calorie intake to hold body weight constant {4,6,7,8} we prescribed 95% of caloric intake and allowed each subject to supplement their diets with food combinations in accordance with individual preferences to achieve satiety while maintaining the nutrient composition of the diets, thereby more realistically reflecting the implementation of these diets in free-living unsupervised populations. This approach was taken because weight and lipoprotein changes that occur for real-life exposure to these diets may differ from those observed when caloric intake or body weights are forced to remain constant. For example, reductions in dietary fat have been reported by others to increase triglyceride and total-cholesterol/HDL-cholesterol ratio under weight-maintenance conditions but not under ad lib conditions leading to weight loss {28}.

The unique study design revealed significant within-pair correlations in the twins' lipoprotein responses to the dietary manipulations despite their divergent lifestyles. The strongest correlation was for changes in LDL-cholesterol. Although several genes have been linked to LDL-cholesterol change during dietary manipulation {5}, these are unlikely to account for the 49% of the variance in LDL-cholesterol change our study attributes to the twins' genes or shared environment. Analytic ultracentrifugation and gradient gel electrophoresis suggest that

the concordance in the twins LDL-cholesterol response involves buoyant LDL-particles of S_f7-12 and large LDL particles of the LDL-I subclass. The agreement among three independent LDL measurements involving three separate methodologies confirms the concordant LDL-cholesterol response to the diet.

Diet-induced changes in the LDL-IIB subclass were also significantly correlated within twin-pairs, as were changes in LDL-IV. The LBL-IVB subclass is a relatively minor portion of the LDL distribution that has recently been shown to have an independent association with coronary disease progression {29}. Table 3 shows a discontinuity in the concordance of the MZ-twin diet response between LDL-IIB and LDL-IV that is similar to the discontinuities we have previously reported when LDL-subclasses are correlated with atherosclerosis {29} and other lipoproteins {30}.

The high MZ correlation for Lp(a) measured cross-sectionally is consistent with the finding that over 90% of the variation in Lp(a) concentrations is accounted for by the apo(a) gene {31}. Our data (Table 3) also suggests a strong genetic influence on the Lp(a) response to diet.

We recognize that free-living populations could be less likely to follow controlled diets than subjects for whom food is supplied. However, we have now completed several studies of men and women with similar dietary protocols {4,6,7,9}. Our success in implementing these studies is reflected both in diet records and by the finding that mean lipid responses conform to those predicted from previous controlled feeding studies {32}.

We defined divergent lifestyles with respect to different levels of physical activity. As shown in Table 1, runners weighed significantly less than their sedentary twin, had lower plasma concentrations of triglycerides and apolipoprotein B, higher plasma concentrations of HDL-cholesterol, apo A-I, and larger

LDL-peak particle diameter. Although these lipoprotein and weight differences are well documented between vigorously active and inactive men {33-35}, Table 1 shows that these differences persist when controlling for genetic effects, an important consideration because the lipoprotein response to exercise is affected by genes {36}. Genes presumably also partially explain why sedentary men with high HDL-cholesterol run longer weekly distances when enrolled in a training program than those with low HDL-cholesterol. The running twins also had higher concentrations of Lp(a) than their sedentary brothers, which has not been consistently observed by others {37-39}, but may have been discernible in our study design because we matched for genotype (i.e., Table 1 shows a strong genetic concordance for Lp(a) values).

Our results suggest there are genes that strongly influence the LDL-cholesterol response to diet, even in the presence of large differences in physical activity. These genes appear to primarily affect the dietary response of the larger, more buoyant LDL particles. Previous studies have indicated that these particles are more strongly associated with changes in saturated fat intake than are other LDL species {40}. Even the most physically active men are susceptible to the effects of diet on HDL-cholesterol, apo A-I, and large buoyant LDL concentrations and the size and buoyancy of the predominant LDL particles. The prominent role genes play in regulating lipoproteins response to diet is evident whether following ab lib dietary choices (Table 1) or large dietary perturbations in carbohydrate and fat consumption, regardless of the level of physical activity (Table 3). Moreover, our analyses support earlier observations indicative of the genetic regulation of weight change following environmental perturbation {11,12}. Based on these results we believe that detailed analyses using genetic association or linkage studies are warranted to identify the causes of the associations of diet with lipoprotein and weight.

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Table 1. Baseline characteristics of MZ twins				
	Runner (mean±SD)	Sedentary (mean±SD)	Difference (mean±SE)	Correlation
Running distance (km)	52.56 ± 20.75	2.39 ± 4.68	50.17 ± 3.77¶	
Body mass index (kg/m^2)	23.49 ± 1.6	25.27 ± 3.11	-1.78 ± 2.51¶	0.69¶
Apolipoprotein A-I (g/L)	1.21 ± 0.21	1.11 ± 0.16	0.1 ± 0.03§	0.64¶
Apolipoprotein B (g/L)	0.83 ± 0.18	0.92 ± 0.22	-0.09 ± 0.03§	0.79¶
Triglycerides (mmol/L)	0.97 ± 0.51	1.46 ± 0.93	-0.49 ± 0.14§	0.57§
Total cholesterol (mmol/L)	4.66 ± 0.89	4.74 ± 0.93	-0.08 ± 0.11	0.78¶
HDL-cholesterol (mmol/L)	1.32 ± 0.39	1.09 ± 0.3	0.23 ± 0.05¶	0.76¶
LDL-cholesterol (mmol/L)	2.9 ± 0.13	2.98 ± 0.14	-0.08 ± 0.1	0.71¶
Lp(a) (mmol/L)	0.6 ± 0.7	0.48 ± 0.53	0.12 ± 0.04¶	0.99¶
LDL-peak particle diameter (nm)	26.61 ± 0.86	26.28 ± 0.93	0.33 ± 0.12†	0.75¶
LDL-I (area)	2233.07 ± 794.82	1923.86 ± 833.10	309.21 ± 853.83	0.45*
LDL-IIA (area)	1574.93 ± 669.83	1460.80 ± 511.01	114.13 ± 757.84	0.20
LDL-IIB (area)	2951.08 ± 6663.64	1680.17 ± 710.88	1270.91 ± 6719.14	0.03
LDL-IIIA (area)	1195.24 ± 748.79	1278.60 ± 815.35	-83.36 ± 593.68	0.71¶
LDL-IIIB (area)	349.71 ± 181.54	396.69 ± 451.77	-46.98 ± 469.40	0.10
LDL-IVA (area)	412.11 ± 165.34	413.48 ± 379.96	-1.37 ± 349.95	0.39*
LDL-IVB (area)	332.97 ± 242.31	337.79 ± 240.85	-4.82 ± 243.73	0.49†
Statistical significance by paired t-test or product-moment (Pearson) correlation coefficient designated by * P<0.05; † P<0.01; § P<0.005; ¶ P<0.001				

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T**able 2. Mean nutrient intake (±SE) on high to a low-fat diets**				
	High Fat, low carbohydrate		Low Fat, high carbohydrate	
	Runners	Sedentary	Runners	Sedenta
Energy (kcal)	2676.8 ± 358.2	2713.5 ± 369.5	2631.1 ± 323.5	2550.1 ± 3
Total Fat (%)	39.2 ± 3.0	39.1 ± 3.7	20.8 ± 1.8	21.2 ± 1
Saturated Fat (%)	12.4 ± 1.2	12.4 ± 0.9	4.6 ± 0.7	4.7 ± 0.
Monounsaturated Fat (%)	12.0 ± 0.7	11.7 ± 0.9	9.4 ± 0.9	9.3 ± 0.
Polyunsaturated	12.2 ± 2.0	12.2 ± 0.6	4.8 ± 0.4	5.0 ± 0.
Carbohydrates (%)	46.4 ± 3.1	46.6 ± 3.3	63.8 ± 2.1	63.0 ± 3
Protein (%)	15.8 ± 0.9	15.6 ± 0.8	16.4 ± 1.0	16.8 ± 2
Cholesterol (mg)	324.9 ± 58.0	327.1 ± 42.7	311.7 ± 50.4	319.2 ± 4
None of the dietary changes were significantly different between the Running and Sedentary Twin				

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Table 3. Mean changes in MZ twins' weight, apolipoprotein, and lipoprotein concentra switching from a six-week high fat to a six-week low-fat diet

	Runner (mean ± SE)	Sedentary (mean ± SE)	Difference (mean ± SE)	Average (mean ± SE)	
ΔWeight (kg)	-0.05 ± 0.31	-0.11 ± 0.24	0.05 ± 0.29	-0.08 ± 0.24	0
Δ apolipoprotein A-I (g/L)	-0.08 ± 0.02¶	-0.04 ± 0.02*	-0.04 ± 0.02	-0.06 ± 0.02§	0
Δ apolipoprotein B (g/L)	0.02 ± 0.02	0.04 ± 0.03	-0.02 ± 0.03	0.03 ± 0.02	0
ΔTriglycerides (mmol/L)	0.19 ± 0.06†	-0.24 ± 0.27	0.43 ± 0.3	-0.02 ± 0.13	-
ΔTotal Cholesterol (mmol/L)	-0.16 ± 0.08	-0.15 ± 0.11	-0.01 ± 0.1	-0.16 ± 0.09	0
ΔHDL-cholesterol (mmol/L)	-0.14 ± 0.04§	-0.07 ± 0.02§	-0.07 ± 0.03	-0.1 ± 0.02¶	0
ΔLDL-cholesterol (mmol/L)	-0.12 ± 0.07	-0.07 ± 0.1	-0.05 ± 0.07	-0.1 ± 0.08	0
ΔLp(a) (μmol/L)	0.06 ± 0.03	0.1 ± 0.05	-0.04 ± 0.05	0.08 ± 0.04*	0
ΔLDL-peak diameter (nm)	-5.2 ± 1.0¶	-3.5 ± 1.0§	-1.7 ± 1.3	-4.3 ± 0.7¶	0
ΔLDL-I (area)	-164.4 ± 96.2	-261.9 ± 89.9†	97.6 ± 93.1	-213.1 ± 80.6*	0
ΔLDL-IIA (area)	-51.1 ± 77.5	-151.9 ± 114.1	100.9 ± 116.2	-101.5 ± 78.3	0
ΔLDL-IIB (area)	194.9 ± 111.5	248.6 ± 143.2	-53.7 ± 121.2	221.7 ± 113.1	0
ΔLDL-IIIA (area)	210.5 ± 107.8	276.4 ± 109.8*	-65.9 ± 132.9	243.5 ± 86.2†	0
ΔLDL-IIIB (area)	37 ± 30.6	-22.8 ± 72.3	59.8 ± 78.9	7.1 ± 39	-
ΔLDL-IVA (area)	-7.1 ± 33.2	-23.2 ± 48.2	16.1 ± 42.2	-15.2 ± 35.6	0
ΔLDL-IVB (area)	38.8 ± 45.2	38.4 ± 49.6	0.4 ± 50.6	38.6 ± 40.1	0
Peak flotation rate (Sf)	-0.5 ± 0.1¶	-0.3 ± 0.1§	-0.2 ± 0.2	-0.4 ± 0.1¶	0
VLDL-mass (mg/dL)	17 ± 8.5*	9.3 ± 14.4	7.4 ± 18.5	13 ± 7.6	-
IDL-mass (mg/dL)	2.9 ± 2.1	1.7 ± 2.3	1.1 ± 3.1	2.2 ± 1.6	0
Large, buoyant LDL-mass (mg/dL)	-17.3 ± 4.3¶	-13.2 ± 5.2*	-4.8 ± 4.8	-15.6 ± 4.2¶	0
Small, dense LDL-mass (mg/dL)	-0.9 ± 6.1	7.8 ± 7.4	-10 ± 8.9	2.7 ± 5.1	0

Significance levels from analysis of variance and the product-moment correlation are coded: * p<0 p<0.01; § p<0.005; ¶ p<0.001

Figure 1. Changes in weight and plasma apolipoprotein A-I and Lp(a) concentrations when switching from a six-week high-fat diet (40%) to a six-week low-fat diet (20% fat) in 28 MZ twins discordant for physical activity. The significance level is the probability that the product-moment correlation coefficient is zero. The diagonal is not a line fitted to the observations but rather is drawn as reference to the locus of points where the changes are identical in the twin pairs.

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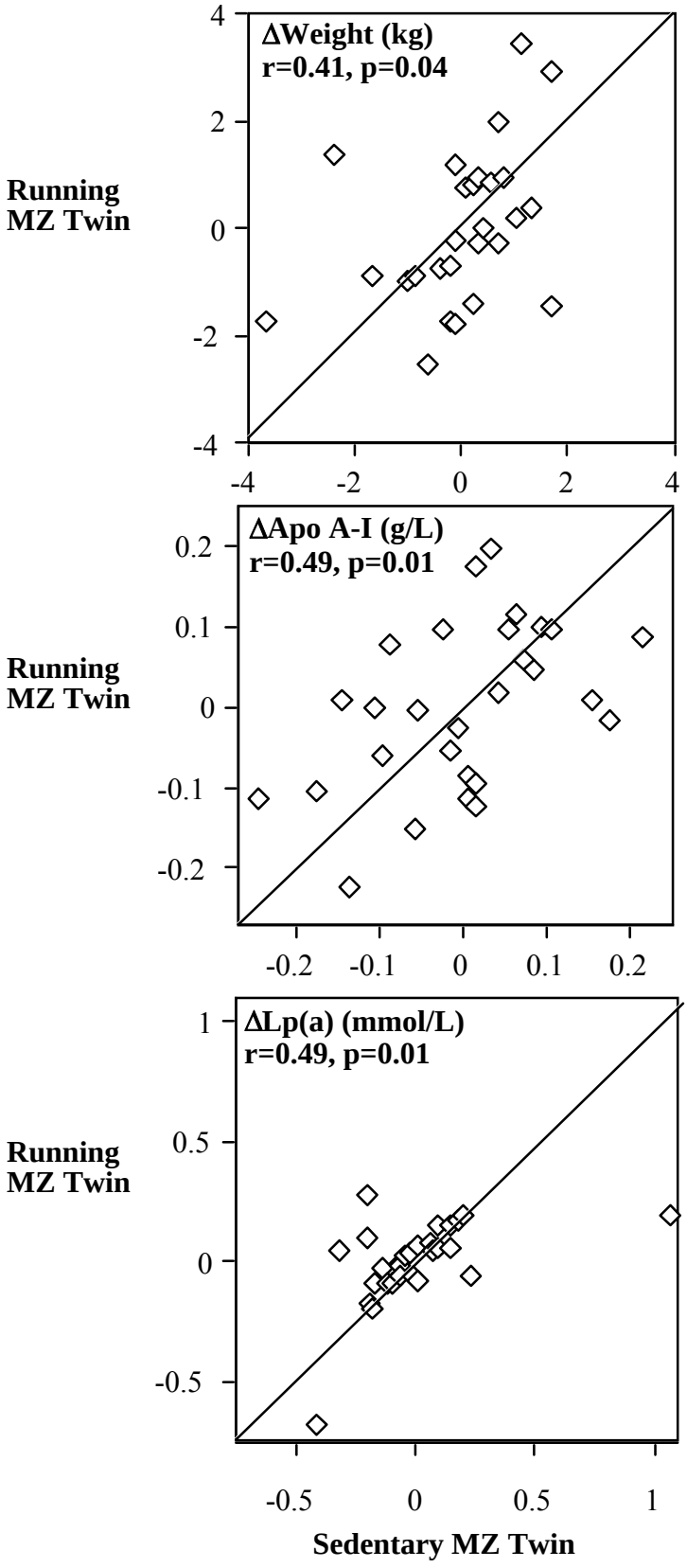
ΔWeight (kg)
r=0.41, p=0.04
ΔApo A-I (g/L)
r=0.49, p=0.01
ΔLp(a) (mmol/L)
r=0.49, p=0.01
Running MZ Twin
Running MZ Twin
Running MZ Twin
Sedentary MZ Twin

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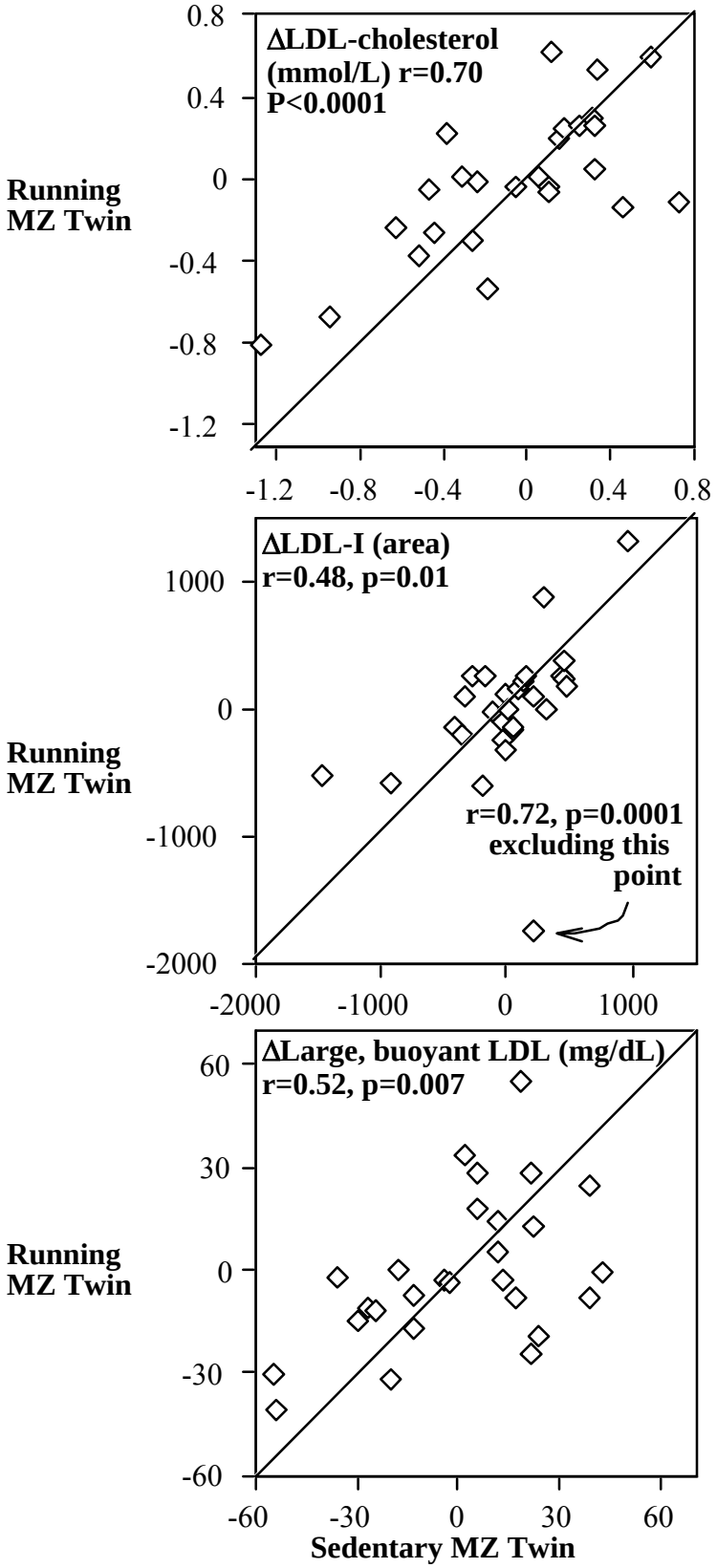
Figure 2. Changes in plasma concentrations of LDL-cholesterol, LDL-I and buoyant LDL (S_f7-12) when switching from a six-week high-fat diet (40%) to a six-week low-fat diet (20%) in 28 MZ twins discordant for physical activity (27 pairs for buoyant LDL). The diagonal is drawn as reference to the locus of points where the changes are identical in the twin pairs. The significance level is the probability that the product-moment correlation coefficient is zero. The diagonal is not a line fitted to the observations but rather is drawn as reference to the locus of points where the changes are identical in the twin pairs.


ΔLDL-cholesterol
(mmol/L) r=0.70
P<0.0001
Running
MZ Twin
0.8
0.4
0
-0.4
-0.8
-1.2
-1.2
-0.8
-0.4
0
0.4
0.8
ΔLDL-I (area)
r=0.48, p=0.01
r=0.72, p=0.0001
excluding this
point
Running
MZ Twin
1000
0
-1000
-2000
-2000
-1000
0
1000
ΔLarge, buoyant LDL (mg/dL)
r=0.52, p=0.007
Running
MZ Twin
60
30
0
-30
-60
-60
-30
0
30
60
Sedentary MZ Twin

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