

**Response to reviewers**  
**Resubmission of 21175 Version 1**

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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

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

4  
5 9. Dietary records were not collected at baseline, only at the end of the high-fat and the low-fat  
6 diets. Table 2 shows the energy intake on each of the diets, and the footnote states that there  
7 were no significant differences between the running and sedentary twin.

8  
9  
10 10. We have added the baseline values for the areas of the LDL-distribution from gradient gel  
11 electrophoresis. The change data in table 3 are the differences between being on the high-fat and  
12 the low-fat diets from a cross-over experimental design. Table 1 presents the baseline data  
13 before the subjects went on any of the diets. Because of their high costs, analytic ultracentrifuge  
14 measurements were not made at the baseline visit (only at the end of each treatment ) and  
15 therefore do not appear in table 1.

16  
17  
18 11. The following sentence has been added to both figure legends to clarify the purpose of the  
19 lines “The diagonal is not a line fitted to the observations but rather is drawn as reference to the  
20 locus of points where the changes are identical in the twin pairs.”

21  
22 Reviewer 1. We apologize for the careless typographical errors. We have reviewed the  
23 manuscript to ensure it is purged of any of the errors cited. Per the reviewers’ recommendation,  
24 the title has been changed to “Concordant lipoprotein and weight responses to dietary fat change  
25 in identical twins with divergent levels of exercise” (Underlined section is a replacement).

26  
27 1. The cut and paste errors have been corrected and the manuscript carefully reviewed for  
28 any other errors.

29 2. Corrected. Again we apologize for the errors.

30  
31 3. Table 4 has been corrected to read table 3 and Figure 1 is correctly referenced.

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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.”

1  
2 **Concordant lipoprotein and weight responses to dietary**  
3 **fat change in identical twins with divergent exercise**  
4 **levels.**

5  
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23

24 Running title: Lipoprotein changes due to dietary fat in twins

25

26 This work was supported in part by a grant from Dairy Management

27 Incorporated and NIH R01 Grant HL-58621, and NIH Program Project

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29 Institute, and was conducted at Lawrence Berkeley National

30 Laboratory through the U.S. Department of Energy under contract

31 No. DEAC03-76SF00098.

32

1  
2 **Background/Objective:** The purpose of this study is to test the  
3 extent that individual lipoprotein responses to diet can be  
4 attributed to genes in the presence of divergent exercise levels.

5 **Design:** Twenty-eight pairs of male monozygotic twins (one mostly  
6 sedentary, the other running an average of 50 km/week more than  
7 the sedentary twin) went from a 6-week 40% fat diet to a 6-week  
8 20% fat diet in a crossover design. The diets reduced fat  
9 primarily by reducing saturated and polyunsaturated fat (both  
10 from 14% to 4%), while increasing carbohydrate intake from 45% to  
11 65%.

12 **Results:** Despite the twins' differences in physical activity,  
13 the dietary manipulation produced significantly correlated  
14 changes ( $P < 0.05$ ) in the twin's total cholesterol ( $r = 0.56$ ), low-  
15 density lipoprotein (LDL)-cholesterol ( $r = 0.70$ ), large, buoyant  
16 LDL ( $S_{\text{f}}7-12$ ,  $r = 0.52$ ), apo A-I ( $r = 0.49$ ), Lp(a) ( $r = 0.49$ ),  
17 electrophoresis measurements of LDL-I (LDLs between 26 and 28.5  
18 nm diameter,  $r = 0.48$ ), LDL-IIB (25.2-24.6 nm,  $r = 0.54$ ), LDL-IV (22-  
19 24.1 nm,  $r = 0.50$ ), and body weights ( $r = 0.41$ ). Replacing fats with  
20 carbohydrates significantly decreased the size and  
21 ultracentrifuge flotation rate of the major LDL, the LDL mass  
22 concentrations of  $S_{\text{f}}7-12$ , LDL-I, high-density lipoprotein (HDL)-  
23 cholesterol and apo A-I, and significantly increased LDL-IIIA  
24 (24.7-25.5 nm diameter) and Lp(a).

25 **Conclusions:** Even in the presence of extreme exercise  
26 difference, genes significantly affect changes in LDL, apo A-I,  
27 Lp(a) and body weight when dietary fats are replaced with  
28 carbohydrates.

29

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

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

14  
15 Individuals vary greatly in their lipoprotein responses to low-  
16 fat diets, some of this variation has been attributed to genes.  
17 Individuals having the apo E e4 allele experience greater  
18 reductions of LDL-cholesterol {5} and large, buoyant LDL (S<sub>f</sub>7-12)  
19 {6} on low-fat, low-cholesterol diets than those lacking the  
20 allele. Polymorphisms in the apo B gene, signal peptide insertion  
21 allele, the LDL receptor gene, the MN blood group, and in the apo  
22 A-I promoter region are also reported to affect the LDL response  
23 to diet{5}. Low-fat diets induce a greater reduction in LDL-  
24 cholesterol and HDL<sub>2b</sub> (the largest HDL particles) in individuals  
25 with a genetically influenced profile characterized by a  
26 predominance of small LDL particles than in those lacking this  
27 trait {7-9}.

28  
29 Studies of monozygotic (MZ) twins provide evidence for genetic  
30 regulation in the absence of prior knowledge of the specific  
31 genes involved. Such studies provide a global test for genetic  
32 hypotheses while circumventing issues of multiple hypotheses  
33 testing that plague exploratory tests of multiple genetic loci  
34 {10}. For example, overfeeding and caloric expenditure in MZ  
35 twins causes weight gains and losses that correlate significantly  
36 within twin pairs {11,12}. However, to date only a small

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

3  
4 The current study examines the effects of switching from high-fat  
5 low-carbohydrate to low-fat high-carbohydrate diets in MZ twins  
6 to assess the contribution of genes to the diet-induced changes  
7 in lipoproteins and body weight. Although it is often difficult  
8 to separate the effects of the twins' shared genotypes from their  
9 shared environment {14}, the current design minimizes the effect  
10 of the shared environment by: 1) deliberately choosing twins  
11 with divergent lifestyles (one physically active, one sedentary);  
12 2) measuring the response to an experimental manipulation of diet  
13 (as opposed to observational twin studies that may be strongly  
14 affected by the shared environment).

15

## 16 **Subjects and Methods**

17

18 Twenty-nine pairs of identical male twins discordant for exercise  
19 participated in a crossover study of high-fat low-carbohydrate  
20 and low-fat high-carbohydrate diets. The twins were identified  
21 among current participants of the National Runners' Health Study  
22 and from announcements distributed at foot races through the  
23 Runner's World race participation program {15}. Criteria for  
24 eligibility were as follows: discordant for exercise (i.e.,  
25 either one twin was sedentary and the other was running at least  
26 32 km/wk or if both twins ran there was at least a 40 km/wk  
27 difference in running distance), no medication use likely to  
28 interfere with lipid metabolism, free of chronic disease, non-  
29 smoker, and willingness to abstain from alcohol and follow the  
30 prescribed diets over the twelve-week intervention. Each twin  
31 completed a questionnaire and signed a consent form approved by  
32 the Committee for the Protection of Human Subjects at Lawrence  
33 Berkeley National Laboratory, University of California, Berkeley.

34

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

1 by registered dieticians to follow the prescribed diets both  
2 before and during the experimental intervention. The twin-pairs  
3 received, in random order, a six-week low-fat solid-food diet  
4 (20% of total energy as fat, 65% as carbohydrates) and a six-week  
5 high-fat diet (40% fat, 45% carbohydrates) in a crossover design.  
6 The two experimental diets were designed to achieve a comparison  
7 of high- and low-fat intake by substitution of fat for  
8 carbohydrate without significant change in other major nutrients.  
9 Nutrient compositions of the diets were calculated using the  
10 Minnesota Nutrition Data System (NDS) software developed by the  
11 Nutrition Coordinating Center (NCC), University of Minnesota,  
12 Minneapolis, MN, version 4.01. Registered dietitians supplied the  
13 participants with personalized menus demonstrating the number and  
14 size of servings for the experimental diets. Seven-day diets  
15 were prescribed to the participants representing 95% of total  
16 caloric intake as estimated from their baseline four-day food  
17 records; the remaining 5% were provided as food combinations that  
18 match the dietary composition of the prescribed diets which could  
19 be consumed ad-lib so that the total caloric intake could vary in  
20 response to the caloric intake required for satiety. The  
21 prescribed diets had to be eaten in their entirety within each 7-  
22 day period. The 5% additional calories could be consumed as one-  
23 half cup of low-fat milk with five vanilla wafers on the low-fat  
24 diet and as one teaspoon of peanut butter with eight wheat  
25 crackers on the high-fat diet. All subjects abstained from  
26 alcohol during the study. The staff contacted the subjects weekly  
27 during the study to verify adherence to the diet and to review  
28 the protocol. Compliance was assessed by four-day diet records  
29 and grocery receipts. One twin-pair did not complete the dietary  
30 intervention.

31  
32 Twins reported to a local clinic of their choice to have their  
33 blood drawn at baseline and at the end of each six-week diet. All  
34 were required to have abstained for 12-14 hours from all food and  
35 vigorous activity. Plasma was prepared from venous blood  
36 collected in tubes containing Na<sub>2</sub>EDTA, 1.4 mg/mL. Samples were



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

7  
8 *Lipid and lipoprotein measurements* Fasting plasma lipids were  
9 measured at baseline and after each six-week diet. Plasma  
10 concentrations of total cholesterol and triglycerides were  
11 measured by enzymatic procedures (ABA 200 instrument, Abbott  
12 Laboratories) {16}. HDL-cholesterol was measured by the dextran  
13 sulfate-magnesium precipitation of apo B containing lipoproteins  
14 followed by enzymatic determination of cholesterol {17,18}.  
15 Plasma LDL-cholesterol concentrations were calculated from the  
16 formula of Friedewald et al {19}. The laboratory remained  
17 certified by the Centers for Disease Control and Prevention lipid  
18 standardization program throughout the study. Apolipoproteins AI  
19 and B in plasma were measured by immunoturbidimetric assay  
20 {20,21}, using an ITA reagent kit reagent kit (Bacton Assay  
21 Systems, Inc., San Marcos, CA). Measurements are performed using  
22 the Express 550 analyzer according to kit instructions.  
23 Calibrators and controls are assigned quantitation levels based  
24 on the International Federation of Clinical Chemistry proposed  
25 Standard Reference Material SP1, and by participation in the  
26 IFCC/CDC directed Standardization Program. Intra- and inter-run  
27 coefficients of variation were within  $\pm 5\%$ .

28  
29 Fasting LDL particle diameters and LDL particle subclass  
30 intervals based on particle size were calculated from calibration  
31 curves using standards of known size {22}. Analyses are based  
32 on the area within the LDL-IVB (22.0-23.2 nm), LDL-IVA (23.3-24.1  
33 nm), LDL-IIIB (24.2-24.6 nm), LDL-IIIA (24.7-25.5 nm), LDL-II  
34 (25.6-26.4 nm), and LDL-I (26.0-28.5 nm) particle size intervals  
35 {22,23}. Analytic ultracentrifugation was used to measure  
36 concentrations of total lipoprotein mass within multiple regions

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

4  
5 *Statistical analyses* Fifteen pairs started with the high-fat diet  
6 and thirteen pairs started with the low-fat diet. Because the  
7 two diet sequences were not equally represented, the paired t-  
8 test was not used because temporal effects would not be  
9 eliminated by the analyses. We therefore computed separately the  
10 mean lipoprotein change in switching from a high to a low fat  
11 diet and the mean lipoprotein change in switching from the low to  
12 the high fat diets and their corresponding standard errors. We  
13 then calculated one half of the differences of the mean changes  
14 and their corresponding standard error (one half of the square  
15 root of the sum of the squared standard errors) to estimate  
16 separately the effect of the diet manipulation on the running  
17 twins' and the sedentary twins' lipoproteins while eliminating  
18 any temporal effects. The difference between the running and the  
19 sedentary twins' dietary response was calculated by subtracting  
20 the lipoprotein change within each twin pair and then analyzing  
21 the calculated differences as described above. Since none of the  
22 variables responded differently in the running and sedentary  
23 twins, we also analyzed the average of the twins' responses to  
24 assess the effect of the diet on lipoproteins with greater  
25 statistical power. Twin-pair correlations of the lipoprotein  
26 responses to the diets were calculated after adjusting for the  
27 diet sequence by regression analyses. Plots of the twins'  
28 responses are presented with adjustment to represent switching  
29 from the high to the low fat diet. Statistical analyses were  
30 performed using StatView version 5.0.1 software (SAS Institute;  
31 Cary, North Carolina).

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## Results

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

12  
13 Consistent with their monozygosity, twin's heights were strongly  
14 correlated ( $r=0.92$ ), as were their BMI's and weights. Despite  
15 substantial differences in physical activities, the twins  
16 exhibited strong, significant correlations for LDL-peak particle  
17 diameter, total cholesterol, triglycerides, HDL-cholesterol, LDL-  
18 cholesterol, apolipoproteins A-I and B. They were also highly  
19 correlated for LDL-I, LDL-IIIA, LDL-IVA and LDL-IVB. The high  
20 correlation for Lp(a) was confirmed by nonparametric Spearman's  
21 correlation ( $\rho=0.96$ ).

22  
23 *Switching from the high to the low fat diet* Table 2 shows the  
24 reported nutrient intake from 7-day food records for the running  
25 and sedentary twins on the two diets. The dietary goals were  
26 achieved on both diets. The changes in mean nutrient intake from  
27 switching from the high-fat low-carbohydrate diet to the low-fat  
28 high carbohydrate diet were not significantly different between  
29 the running and sedentary twin for total energy intake (mean  
30 •Exercise-•Sedentary $\pm$ SE:  $-117.69 \pm 92.12$  kcal/d), total fat ( $0.53$   
31  $\pm 0.82\%$ ), saturated fat ( $0.12\pm 0.22\%$ ), monounsaturated fat  
32 ( $0.19\pm 0.21\%$ ), polyunsaturated fat ( $0.19\pm 0.49\%$ ), carbohydrates ( $-$   
33  $1.10\pm 1.22\%$ ), protein ( $0.58\pm 0.51\%$ ) or dietary cholesterol  
34 ( $5.26\pm 15.21$  mg/day).

35

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

8  
9 Table 3 also presents the changes in VLDL and LDL in response to  
10 decreasing fat and increasing carbohydrates. Mean LDL-peak  
11 particle diameter and the LDL-peak flotation rate decreased in  
12 both the sedentary and exercising twins. Mass concentrations of  
13 buoyant LDL also decreased significantly in both.  
14 Correspondingly, changes in LDL-peak diameter, LDL-peak flotation  
15 rate, and buoyant LDL were strongly significant when running and  
16 sedentary twins were averaged. The additional statistical power  
17 for detecting change when running and sedentary twins were  
18 averaged revealed significant increases in LDL-III A. The  
19 decrease in LDL-I and increase in LDL-III A were significant in  
20 the sedentary twins but not the running twins ( $p=0.10$  for LDL-I  
21 and  $P=0.07$  for LDL-III A). VLDL-mass concentrations increased in  
22 the running twin but not in their sedentary brothers ( $P=0.55$ ), or  
23 the pooled twin-pairs ( $P=0.11$ ). The lipoprotein responses to the  
24 diets were not significantly different between the running and  
25 sedentary twins (Tables 3).

26  
27 *Concordance within twin-pairs* Increased dietary fat did not  
28 significantly change body weight (Table 3). However, there was  
29 considerable variability to the body weight response to the  
30 diets, and the responses were significantly correlated within  
31 twin pairs ( $r=0.41$ , Figure 1). Despite the substantial  
32 differences in physical activity, changes in apo A-I were  
33 strongly correlated within twin pairs, as were changes in Lp(a)  
34 (Figure 1).

35

1 The strongest correlation between the running and sedentary  
2 twins' lipoproteins was the correlation in the LDL-cholesterol  
3 response when switching from a high to a low fat diet (Figure 2).  
4 Table 3 suggests that the within-pair correlation for changes in  
5 LDL-cholesterol reflects within-pair concordant changes in the  
6 most buoyant LDL ( $S_{f7-12}$ ) and LDL-I. Twins were also  
7 significantly correlated for changes in LDL-IIIB and LDL-IV (Table  
8 3).

9  
10 The correlation between the twins' lipoprotein changes could not  
11 be attributed to concordance in their adherence to the dietary  
12 protocol. The correlations for changes in %protein,  
13 %carbohydrate and dietary cholesterol were all nonsignificant  
14 ( $0.06 < r < 0.08$ ) when switching from the high-fat low-carbohydrate  
15 diet to the low-fat high-carbohydrate diet. One of the twin pairs  
16 reported concordantly low changes in total and saturated fat  
17 intake and one of the other twin pairs reported concordantly low  
18 changes in polyunsaturated fat intake. Excluding these two twin  
19 pairs eliminated the significant twin correlation between changes  
20 in total % fat intake ( $r=0.36$  reduced to  $r=-0.15$ ), % saturated fat  
21 intake ( $r=0.58$  reduced to  $r=0.14$ ), %monounsaturated fat intake  
22 ( $r=0.36$  reduced to  $r=0.18$ ), and %polyunsaturated fat intake  
23 ( $r=0.36$  reduced to  $r=-0.13$ ) when switching between diets.  
24 Eliminating these two twin pairs had almost no detectable effect  
25 on the twin correlations for changes in apo A-I ( $r=0.47$ ), total  
26 cholesterol ( $r=0.56$ ), LDL-cholesterol ( $r=0.70$ ), Lp(a) ( $r=0.47$ ),  
27 LDL-I ( $r=0.40$ ), LDL-IIIB ( $r=0.57$ ), LDL-IVA ( $r=0.50$ ), LDL-IVB  
28 ( $r=0.49$ ), and large buoyant LDL-mass ( $r=0.58$ ) in going from the  
29 high-fat low-carbohydrate diet to the low-fat high-carbohydrate  
30 diet.

### 31 32 Discussion

33  
34 The lipoprotein changes produced in these twenty-eight twins  
35 confirms previous reports by ourselves and others that switching  
36 from a high-fat low-carbohydrate diet to a low-fat high-

1 carbohydrate diet decreases HDL-cholesterol, and apo A-I and  
2 increases Lp(a) {25-27}. The diet also decreased the size and  
3 buoyancy of the LDL-particle distribution, due to reductions in  
4 LDL-particles of  $S_f$ 7-12 and 26-28.5 nm diameter (LDL-I). In  
5 addition, gradient gel electrophoresis revealed significant  
6 increases in LDL-IIIA. Figures 1 and 2 show there was  
7 considerable variation in the weight, apo A-I, Lp(a), and LDL  
8 response in switching from a high-fat low-carbohydrate diet to a  
9 low-fat high-carbohydrate diet across individuals, and that much  
10 of this variation may be accounted for by genes.

11  
12 Whereas our previous studies held total caloric intake constant  
13 or manipulated caloric intake to hold body weight constant  
14 {4,6,7,8} we prescribed 95% of caloric intake and allowed each  
15 subject to supplement their diets with food combinations in  
16 accordance with individual preferences to achieve satiety while  
17 maintaining the nutrient composition of the diets, thereby more  
18 realistically reflecting the implementation of these diets in  
19 free-living unsupervised populations. This approach was taken  
20 because weight and lipoprotein changes that occur for real-life  
21 exposure to these diets may differ from those observed when  
22 caloric intake or body weights are forced to remain constant. For  
23 example, reductions in dietary fat have been reported by others  
24 to increase triglyceride and total-cholesterol/HDL-cholesterol  
25 ratio under weight-maintenance conditions but not under ad lib  
26 conditions leading to weight loss {28}.

27  
28 The unique study design revealed significant within-pair  
29 correlations in the twins' lipoprotein responses to the dietary  
30 manipulations despite their divergent lifestyles. The strongest  
31 correlation was for changes in LDL-cholesterol. Although several  
32 genes have been linked to LDL-cholesterol change during dietary  
33 manipulation {5}, these are unlikely to account for the 49% of  
34 the variance in LDL-cholesterol change our study attributes to  
35 the twins' genes or shared environment. Analytic  
36 ultracentrifugation and gradient gel electrophoresis suggest that

1 the concordance in the twins LDL-cholesterol response involves  
2 buoyant LDL-particles of S<sub>f</sub>7-12 and large LDL particles of the  
3 LDL-I subclass. The agreement among three independent LDL  
4 measurements involving three separate methodologies confirms the  
5 concordant LDL-cholesterol response to the diet.

6  
7 Diet-induced changes in the LDL-IIB subclass were also  
8 significantly correlated within twin-pairs, as were changes in  
9 LDL-IV. The LBL-IVB subclass is a relatively minor portion of  
10 the LDL distribution that has recently been shown to have an  
11 independent association with coronary disease progression {29}.  
12 Table 3 shows a discontinuity in the concordance of the MZ-twin  
13 diet response between LDL-IIB and LDL-IV that is similar to the  
14 discontinuities we have previously reported when LDL-subclasses  
15 are correlated with atherosclerosis {29} and other lipoproteins  
16 {30}.

17  
18 The high MZ correlation for Lp(a) measured cross-sectionally is  
19 consistent with the finding that over 90% of the variation in  
20 Lp(a) concentrations is accounted for by the apo(a) gene {31}.  
21 Our data (Table 3) also suggests a strong genetic influence on  
22 the Lp(a) response to diet.

23  
24 We recognize that free-living populations could be less likely to  
25 follow controlled diets than subjects for whom food is supplied.  
26 However, we have now completed several studies of men and women  
27 with similar dietary protocols {4,6,7,9}. Our success in  
28 implementing these studies is reflected both in diet records and  
29 by the finding that mean lipid responses conform to those  
30 predicted from previous controlled feeding studies {32}.

31  
32 We defined divergent lifestyles with respect to different levels  
33 of physical activity. As shown in Table 1, runners weighed  
34 significantly less than their sedentary twin, had lower plasma  
35 concentrations of triglycerides and apolipoprotein B, higher  
36 plasma concentrations of HDL-cholesterol, apo A-I, and larger

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

15  
16 Our results suggest there are genes that strongly influence the  
17 LDL-cholesterol response to diet, even in the presence of large  
18 differences in physical activity. These genes appear to  
19 primarily affect the dietary response of the larger, more buoyant  
20 LDL particles. Previous studies have indicated that these  
21 particles are more strongly associated with changes in saturated  
22 fat intake than are other LDL species {40}. Even the most  
23 physically active men are susceptible to the effects of diet on  
24 HDL-cholesterol, apo A-I, and large buoyant LDL concentrations  
25 and the size and buoyancy of the predominant LDL particles. The  
26 prominent role genes play in regulating lipoproteins response to  
27 diet is evident whether following ab lib dietary choices (Table  
28 1) or large dietary perturbations in carbohydrate and fat  
29 consumption, regardless of the level of physical activity (Table  
30 3). Moreover, our analyses support earlier observations  
31 indicative of the genetic regulation of weight change following  
32 environmental perturbation {11,12}. Based on these results we  
33 believe that detailed analyses using genetic association or  
34 linkage studies are warranted to identify the causes of the  
35 associations of diet with lipoprotein and weight.

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1

<b>Table 1. Baseline characteristics of MZ twins</b>				
	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 <sup>2</sup> )	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				

2



1

	High Fat, low carbohydrate		Low Fat, high carbohydrate	
	Runners	Sedentary	Runners	Sedentary
Energy (kcal)	2676.8 $\pm$ 358.2	2713.5 $\pm$ 369.5	2631.1 $\pm$ 323.5	2550.1 $\pm$ 323.5
Total Fat (%)	39.2 $\pm$ 3.0	39.1 $\pm$ 3.7	20.8 $\pm$ 1.8	21.2 $\pm$ 1.8
Saturated Fat (%)	12.4 $\pm$ 1.2	12.4 $\pm$ 0.9	4.6 $\pm$ 0.7	4.7 $\pm$ 0.7
Monounsaturated Fat (%)	12.0 $\pm$ 0.7	11.7 $\pm$ 0.9	9.4 $\pm$ 0.9	9.3 $\pm$ 0.9
Polyunsaturated	12.2 $\pm$ 2.0	12.2 $\pm$ 0.6	4.8 $\pm$ 0.4	5.0 $\pm$ 0.4
Carbohydrates (%)	46.4 $\pm$ 3.1	46.6 $\pm$ 3.3	63.8 $\pm$ 2.1	63.0 $\pm$ 2.1
Protein (%)	15.8 $\pm$ 0.9	15.6 $\pm$ 0.8	16.4 $\pm$ 1.0	16.8 $\pm$ 1.0
Cholesterol (mg)	324.9 $\pm$ 58.0	327.1 $\pm$ 42.7	311.7 $\pm$ 50.4	319.2 $\pm$ 50.4
None of the dietary changes were significantly different between the Running and Sedentary Twin				

2

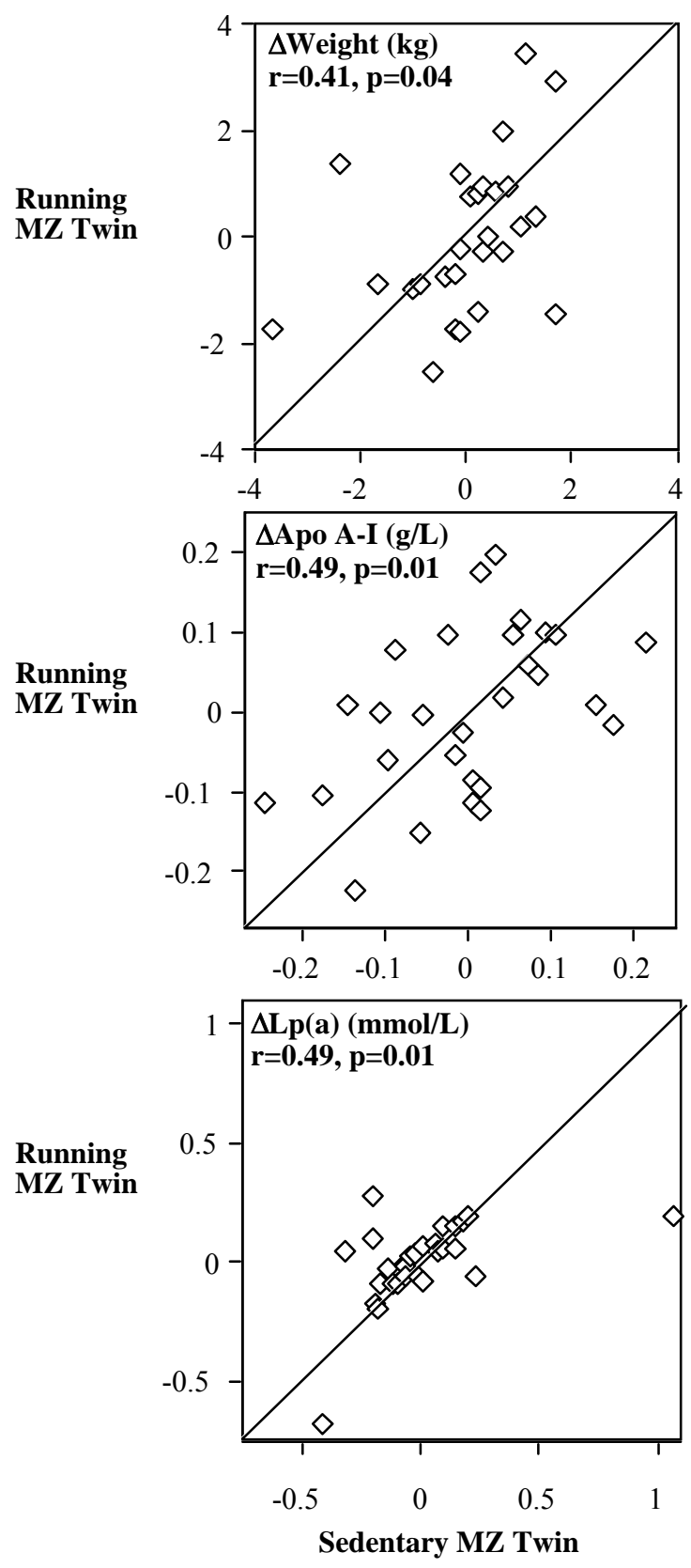
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<b>Table 3. Mean changes in MZ twins' weight, apolipoprotein, and lipoprotein concentrations switching from a six-week high fat to a six-week low-fat diet</b>					
	Runner (mean ± SE)	Sedentary (mean ± SE)	Difference (mean ± SE)	Average (mean ± SE)	
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<0.05; † p<0.01; § p<0.005; ¶ p<0.001					



1  
2  
3 Figure 1. Changes in weight and plasma apolipoprotein A-I and  
4 Lp(a) concentrations when switching from a six-week high-fat diet  
5 (40%) to a six-week low-fat diet (20% fat) in 28 MZ twins  
6 discordant for physical activity. The significance level is the  
7 probability that the product-moment correlation coefficient is  
8 zero. The diagonal is not a line fitted to the observations but  
9 rather is drawn as reference to the locus of points where the  
10 changes are identical in the twin pairs.





1  
2 Figure 2. Changes in plasma concentrations of LDL-cholesterol,  
3 LDL-I and buoyant LDL ( $S_{\text{f}}7-12$ ) when switching from a six-week  
4 high-fat diet (40%) to a six-week low-fat diet (20%) in 28 MZ  
5 twins discordant for physical activity (27 pairs for buoyant  
6 LDL). The diagonal is drawn as reference to the locus of points  
7 where the changes are identical in the twin pairs. The  
8 significance level is the probability that the product-moment  
9 correlation coefficient is zero. The diagonal is not a line  
10 fitted to the observations but rather is drawn as reference to  
11 the locus of points where the changes are identical in the twin  
12 pairs.

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