

Lawrence Berkeley National Laboratory

Molecular Biophys & Integ Bi

Title

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

Permalink

<https://escholarship.org/uc/item/1mq9p793>

Journal

Lawrence Berkeley National Laboratory

Authors

Williams, Paul T.
Blanche, Patricia J.
Rawlings, Robin
et al.

Publication Date

2004-06-01

Peer reviewed

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 divergent levels of exercise” (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.

Paul T. Williams, Ph.D. 1

Patricia J. Blanche, M.S. 2

Robin Rawlings, R.N. 2

Ronald M. Krauss, M.D. 1,2

¹Lawrence Berkeley National Laboratory

Donner Laboratory

1 Cyclotron Road

Berkeley, CA 94720

Telephone: (510) 486-5633

Fax: (510) 486-5990

E-mail: ptwilliams@lbl.gov

(PT Williams is responsible for all correspondence)

² Children's Hospital Oakland Research Institute

5700 Martin Luther King Jr. Way

Oakland, CA 94609

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

1
2
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_f 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_{2b} (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 dietitians 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 Na₂EDTA, 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).

32 33 34 **Results** 35

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 $\pm$ SE: -117.69 ± 92.12 kcal/d), total fat ($0.53 \pm 0.82\%$), saturated fat ($0.12 \pm 0.22\%$), monounsaturated fat ($0.19 \pm 0.21\%$), polyunsaturated fat ($0.19 \pm 0.49\%$), carbohydrates ($-1.10 \pm 1.22\%$), protein ($0.58 \pm 0.51\%$) or dietary cholesterol (5.26 ± 15.21 mg/day).

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-IIIA. The
19 decrease in LDL-I and increase in LDL-IIIA 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-IIIA). 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).

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-IIIB 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-IIIB ($r=0.57$), 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-

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_f7-12 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.

36

- 1 [1] Castelli WP. Lipids, risk factors and ischaemic heart
2 disease. *Atherosclerosis*. 1996;124 Suppl:S1-9.
3
- 4 [2] Lamarche B, Lemieux I, Despres JP. The small, dense LDL
5 phenotype and the risk of coronary heart disease: epidemiology,
6 patho-physiology and therapeutic aspects. *Diabetes Metab*.
7 1999;25:199-211.
8
- 9 [3] Hegsted DM, Kritchevsky D. Diet and serum lipid
10 concentrations: where are we? *Am J Clin Nutr* 1997;65:1893-6
11
- 12 [4] Dreon DM, Fernstrom HA, Miller B, Krauss RM. Low-density
13 lipoprotein subclass patterns and lipoprotein response to a
14 reduced-fat diet in men. *FASEB J*. 1994;8:121-6.
15
- 16 [5] Dreon DM, Krauss RM. Diet-gene interactions in human
17 lipoprotein metabolism. *J Am Coll Nutr*. 1997;16:313-24.
18
- 19 [6] Dreon DM, Fernstrom HA, Miller B, Krauss RM. Apolipoprotein
20 E isoform phenotype and LDL subclass response to a reduced-fat
21 diet. *Arterioscler Thromb Vasc Biol*. 1995;15:105-11.
22
- 23 [7] Dreon DM, Fernstrom HA, Williams PT, Krauss RM. Reduced LDL
24 particle size in children consuming a very-low-fat diet is
25 related to parental LDL-subclass patterns. *Am J Clin Nutr*.
26 2000;71:1611-6.
27
- 28 [8] Dreon DM, Fernstrom HA, Williams PT, Krauss RM. A very low-
29 fat diet is not associated with improved lipoprotein profiles in
30 men with a predominance of large, low-density lipoproteins. *Am J*
31 *Clin Nutr*. 1999;69:411-8.
32
- 33 [9] Dreon DM, Fernstrom HA, Williams PT, Krauss RM. LDL subclass
34 patterns and lipoprotein response to a low-fat, high-carbohydrate
35 diet in women. *Arterioscler Thromb Vasc Biol*. 1997;17: 707-14.
36

1 [10] Ryman N, Jorde PE. Statistical power when testing for
2 genetic differentiation. Mol Ecol 200;10:2361-73

3
4 [11] Bouchard C; Tremblay A; Despres JP; Nadeau A; Lupien PJ;
5 Theriault G; Dussault J; Moorjani S; Pinault S; Fournier G. The
6 response to long-term overfeeding in identical twins. New Engl J
7 Med 1990; 322:1477-82.

8
9 [12] Poehlman ET; Tremblay A; Marcotte M; Perusse L; Theriault G;
10 Bouchard C. Heredity and changes in body composition and adipose
11 tissue metabolism after short-term exercise-training. European
12 Journal of Applied Physiology and Occupational Physiology,
13 56:398-402, 1987

14
15 [13] Rankinen T, Perusse L, Weisnagel SJ, Snyder EE, Chagnon YC,
16 Bouchard C. The human obesity gene map: the 2001 update. Obes Res.
17 2002;10:196-243.

18
19 [14] Kamin LJ, Goldberger AS. Twin studies in behavioral
20 research: a skeptical view. Theor Popul Biol 2002;61:83-95

21
22 [15] Williams PT. Relationship of distance run per week to
23 coronary heart disease risk factors in 9,920 male runners.
24 Archives of Internal Medicine 1997; 157:191-8.

25
26 [16] Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic
27 determination of total serum cholesterol. Clin Chem. 1974;20:470-
28 475.

29
30 [17] Hainline A Jr, Karon J, Lippel K, eds. Manual of Laboratory
31 Operations: Lipid and Lipoprotein Analysis. 2ed. Washington DC:
32 US Government Printing Office, National Heart Lung and Blood
33 Institute, Lipid Research Clinics Program; 1982, DHEW publication
34 no NIH 75-628 (revised)

[18] Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg²⁺ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem. 1982;28:1379-1388.

[19] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. Clin Chem. 1972;18:499-502.

[20] Rifai N, King ME. Immuno- turbidimetric assays of apolipoproteins A, AI, AII and B in Serum. Clin. Chem.1986; 32:957-961

[21] Smith SJ, Cooper GR, Henderson LO, Hannon WH. An international collaborative study on standardization of apolipoproteins A-I and B. Part I. Evaluation of a lyophilized candidate reference and calibration material.Clin Chem. 1987;33:2240-9.

[22] Nichols AV, Krauss RM, Musliner TA. 1986. Nondenaturing polyacrylamide gradient gel electrophoresis. Methods in Enzymology 128:417-431

[23] Krauss RM, Burke DJ. 1982. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res 23: 97-104

[24] Lindgren FT, Jensen LC, Hatch FT. The isolation and quantitative analysis of serum lipoproteins. In Nelson GJ, eds. Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism. New York, NY: John Wiley and Sons; 1972:181-274

[25] Schaefer EJ, Levy RI, Ernst ND, Van Sant FD, Brewer HB. The effects of low cholesterol, high polyunsaturated fat, and low fat diets on plasma lipid and lipoprotein cholesterol levels in

1 normal and hypercholesterolemic subjects. Am J Clin Nutr 34:
2 1758-1763, 1981

3
4 [26] Brinton EA, Eisenberg S, Breslow JL. A low-fat diet
5 decreases high density lipoprotein (HDL) cholesterol by
6 decreasing HDL apolipoprotein transport rates. J Clin Invest 85:
7 144-51, 1990

8
9 [27] Hayek T, Ito Y, Azrolan N, Verdery RB, Aalto-Setälä K,
10 Walsh A, Breslow JL. Dietary fat increases high density
11 lipoprotein (HDL) levels both by increasing the transport rates
12 and decreasing fractional catabolic rates of HDL cholesterol
13 ester and apolipoprotein (apo) A-I. J Clin Invest 1993; 91, 1665-
14 71.

15
16 [28] Schaefer EJ, Lichtenstein AH, Lamon-Fava S, McNamara JR,
17 Schaefer MM, Rasmussen H, Ordovas JM. Body weight and low-density
18 lipoprotein cholesterol changes after consumption of a low-fat ad
19 libitum diet. JAMA 1995; 274:1450-1455.

20
21 [29] Williams PT, Superko HR, Haskell WL, Al derman EL, Blanche
22 PJ, Holl LG, Krauss RM. Smallest LDL particles are most strongly
23 related to coronary disease progression in men.
24 Arteriosclerosis, Thrombosis, (submitted)

25
26 [30] Williams PT, Vranizan KM, Krauss RM. Correlations of plasma
27 lipoproteins with LDL subfractions by particle size in men and
28 women. J Lipid Res. 1992;33:765-774.

29
30 [31] Boerwinkle E, Leffert CC, Lin J, Lackner C, Chiesa G, Hobbs
31 HH. Apolipoprotein(a) gene accounts for greater than 90% of the
32 variation in plasma lipoprotein(a) concentrations. J Clin Invest.
33 1992;90:52-60.

34
35 [32] Mensink RP, Zock PL, Kester AD, Katan MB. of dietary fatty
36 acids and carbohydrates on the ratio of serum total to HDL

1 cholesterol and on serum lipids and apolipoproteins: a meta-
2 analysis of 60 controlled trials. *Am J Clin Nutr.* 2003
3 May;77(5):1146-55.

4
5 [33] Williams PT, Krauss RM, Vranizan KM, Wood PD. Changes in
6 lipoprotein subfractions during diet-induced and exercise-induced
7 weight loss in moderately overweight men. *Circulation* 1990; 81:
8 1293-1304.

9
10 [34] Williams PT, Krauss RM, Wood PD, Lindgren FT, Giotas C,
11 Vranizan K: Lipoprotein subfractions of runners and sedentary
12 men. *Metabolism* 1986; 35: 45-52.

13
14 [35] Williams PT, Krauss RM, Stefanick ML, Vranizan KM, Wood PDS.
15 Effects of low-fat diet, calorie restriction and running on
16 lipoprotein subfraction concentrations in moderately overweight
17 men. *Metabolism* 1994; 43:655-663.

18
19 [36] Despres JP; Tremblay A; Nadeau A; Bouchard C. Physical
20 training and changes in regional adipose tissue distribution.
21 *Acta Medica Scandinavica. Supplementum* 1988, 723:205-12.

22
23 [37] Hubinger L, Mackinnon LT, Lepre F. Lipoprotein(a) [Lp(a)]
24 levels in middle-aged male runners and sedentary controls. *Med*
25 *Sci Sports Exerc* 1995;27:490-6

26
27 [38] Cardoso GC, Posadas C, Orvananos OO, Peniche C, Zamora J,
28 Aguilar R, Holguin JA, Raynaud AS, Morrisett JD, Guevara J Jr.
29 Long distance runners and body-builders exhibit elevated plasma
30 levels of lipoprotein(a). *Chem Phys Lipids.* 1994, 67-68:207-21

31
32 [39] Mackinnon LT, Hubinger LM. Effects of exercise on
33 lipoprotein(a). *Sports Med.* 1999;28:11-24

34
35 [40] Dreon DM, Fernstrom HA, Campos H, Blanche P, Williams PT,
36 Krauss RM. Change in dietary saturated fat intake is correlated

- 1 with change in mass of large low-density-lipoprotein particles in
- 2 men. *Am J Clin Nutr.* 1998;67:828-36.

1

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				

2

1

Table 2. Mean nutrient intake ($\pm$SE) on high to a low-fat diets				
	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 $\pm$ 3.1
Protein (%)	15.8 $\pm$ 0.9	15.6 $\pm$ 0.8	16.4 $\pm$ 1.0	16.8 $\pm$ 2.1
Cholesterol (mg)	324.9 $\pm$ 58.0	327.1 $\pm$ 42.7	311.7 $\pm$ 50.4	319.2 $\pm$ 42.7
None of the dietary changes were significantly different between the Running and Sedentary Twin				

2

1

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

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

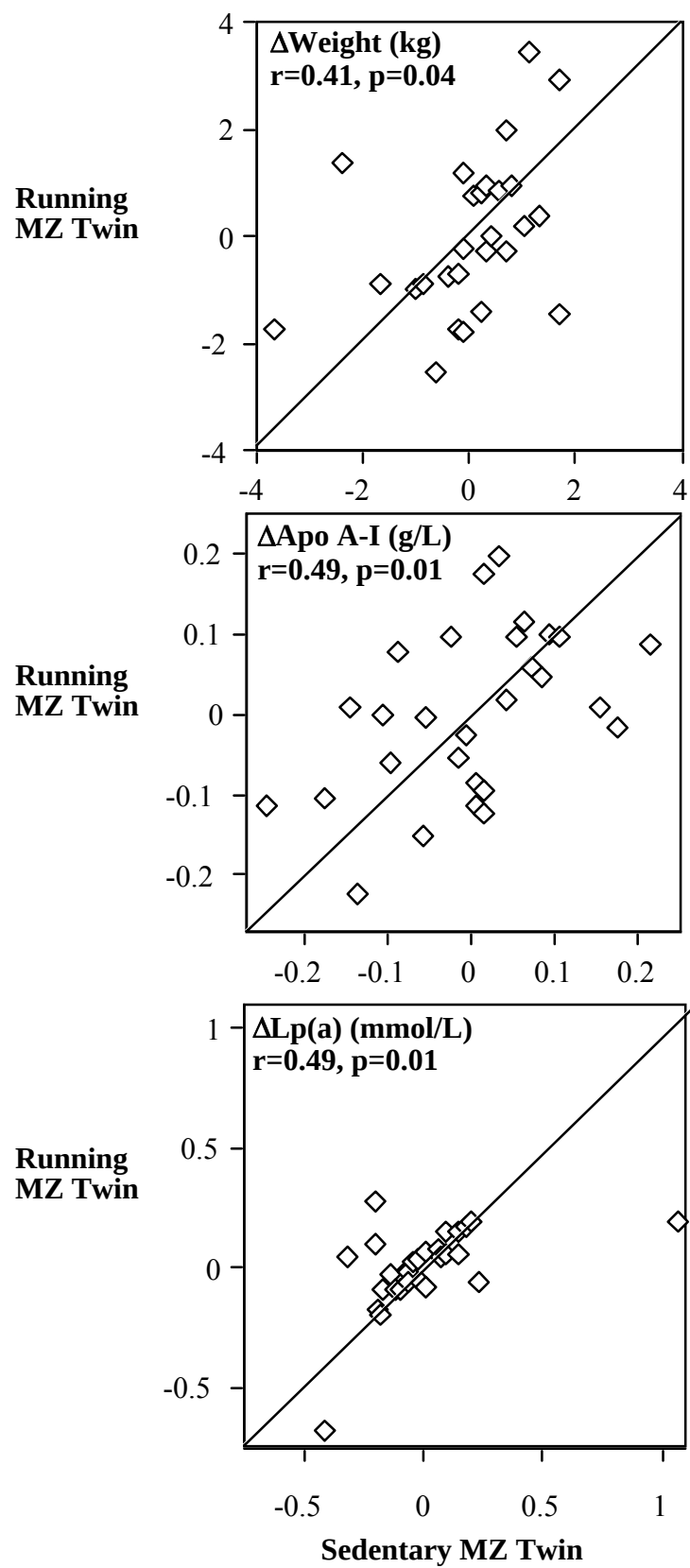


Figure 2. Changes in plasma concentrations of LDL-cholesterol, LDL-I and buoyant LDL (S_f 7-12) when switching from a six-week high-fat diet (40%) to a six-week low-fat diet (20%) in 28 MZ twins discordant for physical activity (27 pairs for buoyant LDL). The diagonal is drawn as reference to the locus 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.

