LDL particle size and LDL and HDL cholesterol changes with dietary fat and cholesterol in healthy subjects

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Abstract   We have conducted a dietary trial in 54 men and 51 women with a wide range of fasting cholesterol values to examine the use of low density lipoprotein (LDL) particle size to predict the lipoprotein response to dietary fat and cholesterol. After a 2-week low fat period, subjects were given two liquid supplements in addition to their low fat diet for 3 weeks each, one containing 31–40 g of fat and 650–845 mg of cholesterol, the other fat free. LDL particle type was determined by 3–15% gradient gel electrophoresis. On multiple regression, LDL type was independently related to plasma triglyceride ($P < 0.001$), waist circumference ($P < 0.01$), and high density lipoprotein (HDL) ($P < 0.001$) accounting for 56% of the variance in LDL type in the whole group. Change in LDL cholesterol with dietary fat and cholesterol was unrelated to LDL particle size in either men or women. However, change in HDL cholesterol in men was strongly related to LDL particle type ($r = -0.52$, $P = 0.001$) and change in HDL_2 cholesterol in women was related to LDL particle type ($r = -0.40$, $P < 0.01$). In conclusion, we are unable to confirm the finding that LDL particle type can predict changes in LDL cholesterol following changes in dietary fat intake. However, LDL particle type can independently predict changes in HDL cholesterol in men and accounts for 27% of the variance.—Clifton, P. M., M. Noakes, and P. J. Nestel. **LDL particle size and LDL and HDL cholesterol changes with dietary fat and cholesterol in healthy subjects.** *J. Lipid. Res.* 1998. **39: 1799–1804.**

Supplementary key words   LDL size • fat distribution • gender • HDL • HDL_2 cholesterol

Whereas many studies have examined the lipid and lipoprotein responses to dietary fat and cholesterol in humans and have noted marked interindividual variability (1–6), there has been less definition of the attributes of individual subjects that may influence their response to dietary change. Factors that may influence the response include body mass index (BMI) (7, 8), the previous dietary intake of cholesterol (7), the basal level of plasma lipids (9, 10), age (8, 10), polymorphism of genes for apolipoprotein (apo)E (11–14), apoB (15, 16), apoAI (17), apoA-IV (18, 19), and low density lipoprotein (LDL) particle type (20).

Discrete subspecies of LDL based on particle size were

first described by Krauss and Burke over 10 years ago (21). Small LDL particles were found predominantly in men and were more common in obese subjects and in those subjects with elevated triglycerides and low high density lipoprotein (HDL) cholesterol (22). Subjects with a predominance of small LDL particles are at greater risk of coronary artery disease (23), but this has not been convincingly shown to be independent of plasma triglyceride, HDL cholesterol, or total cholesterol/HDL ratio (24–26). However, recent prospective data from the Quebec Cardiovascular study (27) demonstrated a 3.6-fold increase in risk comparing the bottom tertile of LDL size to the top tertile with no significant weakening of risk after adjustment for other lipoproteins. Dreon et al. (20) showed that men with small LDL on a high fat diet were more responsive to fat reduction with a fall in cholesterol twice as great as men with large LDL on the same diet. However, in our previous studies we have shown no relationship between change in LDL cholesterol with dietary fat and cholesterol changes and factors commonly related to LDL particle size such as plasma triglyceride, HDL cholesterol or body mass, so the finding from Krauss and Burke (21) appears surprising. In this study we sought to assess whether LDL particle type can be a useful predictor of the LDL and HDL cholesterol response to a change in dietary cholesterol and fat in both men and women.

METHODS

Healthy volunteers aged from 20 to 75 years were recruited by public advertisement. All were free of cardiac, renal, and hepatic disease. Subjects taking lipid-lowering medication were excluded; 20 women were on hormone replacement therapy, and only one woman was taking oral contraceptives. Fifty-four women and 55 men completed the study. Baseline characteristics are shown in **Table 1**. The total plasma cholesterol ranged from 3.4 to 8.8 mmol/l (mean ± SD 5.6 ± 1.1), triglyceride from 0.4 to 3.7

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein; PCR, polymerase chain reaction; BMI, body mass index; WHR, waist:hip ratio.

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TABLE 1. Baseline characteristics of subjects

	Women	Men
Age (years)	51.1 ± 9.5 (23–76)	50.0 ± 7.5 (30–66)
BMI (kg/m^2)	26.5 ± 4.8 (18.8–40.2)	26.6 ± 3.1 (20.8–34.4)
WHR	0.81 ± 0.06^a (0.70–0.96)	0.93 ± 0.06 (0.82–1.08)
Total cholesterol (mmol/L)	5.49 ± 1.08 (3.58–8.81)	5.66 ± 1.10 (3.40–8.04)
Triglyceride (mmol/L)	1.27 ± 0.54 (0.41–2.62)	1.61 ± 0.91 (0.53–3.72)
LDL cholesterol (mmol/L)	3.76 ± 0.98 (2.16–6.28)	4.07 ± 0.94 (2.16–6.26)
HDL cholesterol (mmol/L)	1.14 ± 0.31^a (0.52–2.05)	0.82 ± 0.26 (0.37–1.56)
HDL$_2$ cholesterol (mmol/L)	0.34 ± 0.17^a (0.08–0.89)	0.17 ± 0.11 (0.05–0.5)
HDL$_3$ cholesterol (mmol/L)	0.80 ± 0.19^a (0.44–1.16)	0.65 ± 0.18 (0.32–1.06)

Values given as mean $\pm$ SD; range in parentheses.
[a] $P < 0.001$ for difference between men and women.

mmol/l (1.4 ± 0.7) and HDL cholesterol from 0.4 to 2.1 mmol/l (1.01 ± 0.32). Their ages ranged from 23 to 75 years (50.6 ± 8.6 years) and BMI from 18.7 to 40.2 kg/m^2 (26.6 ± 4.0). The study was approved by the Human Ethics Committee of the CSIRO, Division of Human Nutrition and written consent was obtained from each subject.

Study design

Subjects were placed on a self-selected low fat diet (25% of energy as fat, cholesterol <250 mg/day, 1700 kcal day from diet records) based on lean meats and cereals which they followed for the duration of the study. After a 2-week baseline period, a milk-based liquid supplement was added to their low fat diet for the next 6 weeks. Two supplements were supplied, one containing 31 g of fat (56% saturated fat, 17% polyunsaturated) and 650 mg of cholesterol per 250 ml, the other containing no fat or cholesterol but isocaloric with the first. Men received an extra 70 ml (100 kcalories), so that for both men and women there was a difference of 15% of energy from fat between the two supplements. The background diet plus supplements contained 20% of energy from fat, 1900 kcal/day in the low fat phase, and 35% of energy from fat, 1900 kcal/day. The supplements were given in random order for 3 weeks each. The study was double blind. The supplements were very palatable and the volunteers could not reliably distinguish between them. A trained dietitian provided information on sources of dietary fat to enable the subjects to achieve the 25% fat baseline diet. Weighed food records were kept for 3 days in each experimental period. Nutrient intake was calculated from a computer database of foods based on the composition of Australian foods (28) and, in the case of the supplements, direct food analysis (Diet/1 Nutrient Calculation Software, Xyris Software). The diaries showed excellent compliance; 95% of supplements were consumed while 90% of the group achieved a fat difference between phases of between 7 and 25% (target 15%). Other lifestyle factors were also controlled throughout the study; subjects were instructed not to change their exercise patterns or their drinking habits for the duration of the study and leisure physical activity and alcohol consumption were monitored. As the subjects were middle-aged, walking and gardening were the major forms of exercise. Habitual heavy drinkers (more than 10% of energy as alcohol) were excluded. Only 50% of subjects drank any form of alcohol. There was only one light smoker (a woman) in the study. Compliance was monitored by questionnaire at each visit. There was no difference between men and women in the amount of physical activity nor in the amount of alcohol consumed and there were no changes across each experimental period. There was no difference between younger or older subjects in exercise patterns or alcohol consumption. Waist to hip ratio (WHR) was measured by assessing the minimum waist circumference at or just above

the umbilicus and the maximum hip circumference (usually at the level of the greater trochanter). Subjects were weighed at each visit and received further instruction from the dietitian. Body weights did not change significantly (71.2 kg in the low fat phase and 72.0 kg in the high fat/cholesterol phase).

Laboratory measurements

Blood samples were taken on two occasions at the end of the baseline period and on three occasions at the end of each experimental period after a 12-h fast. Plasma was separated by low speed centrifugation and immediately frozen at $-70°C$. All measurements were performed in one run at the end of the trial. Plasma and lipoprotein lipids were measured on a Cobas Bio centrifugal analyzer (Roche Diagnostica, Basel, Switzerland) using Roche enzymatic kits and control sera. HDL$_2$ and HDL$_3$ subfractions were prepared by selective precipitation (29) using Dextralip (Sochibo, France) and magnesium. LDL was calculated with the Friedewald equation (30). LDL particle type was assessed by non-denaturing gradient gel electrophoresis of the total lipoprotein fractions (isolated by ultracentrifugation at $d < 1.21$ g/ml fraction) as described by McNamara et al. (22) with the gel buffer, running and staining conditions exactly as described by Krauss et al. (21), using well characterized 3–15% commercial gels (Gradipore Pty Ltd, Sydney, Australia). In a pooled plasma sample LDL$_1$ was defined as the major LDL band in the density range 1.019–1.033 g/ml, LDL$_2$ the larger and LDL$_3$ the smaller in the range 1.033–1.038, LDL$_4$ and LDL$_5$, 1.038–1.050 g/ml, and LDL$_6$ and LDL$_7$, 1.050–1.063. There were no subjects with LDL types 6 and 7 although 15–16% of men would be expected to fall into this category. The macroglobulin standard used (diameter 17 nm) migrated 18–19 mm after running at 200 V for 12 h while a standard LDL of 26 nm diameter migrated 10 mm. The coefficient of variation of the standard LDL migration was 5.2% (within and between gels, three LDL replicates on thirty gels). The apparent diameter of the LDL types outlined above was calculated from the mean of two separate gels and the LDL diameters of the study samples converted to LDL types. Thus in this study LDL diameter of >26.0 nm defined type 1, 25.6–26.0 nm type 2, 25.0–25.6 nm type 3, 24.6–25 nm type 4, and 24–24.6 nm type 5.

ApoE genotype was determined by polymerase chain reaction (PCR) with appropriate primers as described by Hixson and Vernier (31). Insulin was measured by commercial radio-immuno-assay (Pharmacia, Uppsala, Sweden).

Statistics

Covariate analysis of variance, correlations, regression analysis, and 2-tailed paired t-test were performed using SPSS on a personal computer (SPSS Inc., Chicago, IL). All of the data except

plasma triglyceride were normally distributed, so this variable was log transformed before statistical testing.

RESULTS

Men had smaller LDL particles than women in both the low fat and the high fat phases (LDL type 3.08 ± 1.1 vs. 2.07 ± 1.24, $P < 0.001$ during the high fat phase). The distribution of LDL particle types during the high fat phase is shown separately for men and women in **Fig. 1** and **Fig. 2**. There was no difference in LDL particle type between the low fat and high fat phases. Although HDL cholesterol levels seem low, in our hands the dextralip method for HDL_2 and HDL_3 produces a value 20% lower than the PEG method for total HDL cholesterol. **Table 2** outlines the baseline anthropometric and lipoprotein variables in subjects divided into LDL particle type (combining types 1 and 2 and 4 and 5 to produce groups of comparable size in men).

As expected, men and women with the smallest LDL size had higher plasma triglyceride levels ($P < 0.001$) and men had greater abdominal and total obesity than subjects with large LDL particles ($P < 0.01$). Total and LDL cholesterol were similar in both groups. In men, HDL_3 cholesterol ($P < 0.01$) differed between the groups while, in women, HDL cholesterol and HDL_2 cholesterol differed ($P < 0.001$ and $P < 0.01$, respectively).

Plasma insulin was related to LDL particle type (men $P < 0.01$ and women $P < 0.001$), but any relationship was lost once plasma triglyceride and HDL cholesterol were accounted for. With men and women, combined LDL particle type (1 to 5) was accounted for on linear regression by plasma triglyceride ($P < 0.001$), waist circumference ($P < 0.01$), and HDL cholesterol ($P < 0.001$), with 56% of the variance explained. Gender as an independent variable was eliminated from the regression equation.

The changes in fat and cholesterol intake are shown in **Table 3** while the changes in lipid and lipoprotein levels
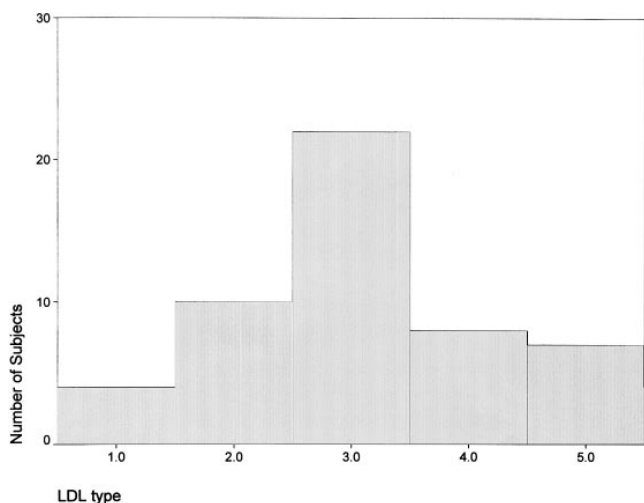
Fig. 1. Distribution of LDL particle types in men on a high fat diet as assessed by 3–15% non-denaturing gradient gel electrophoresis.
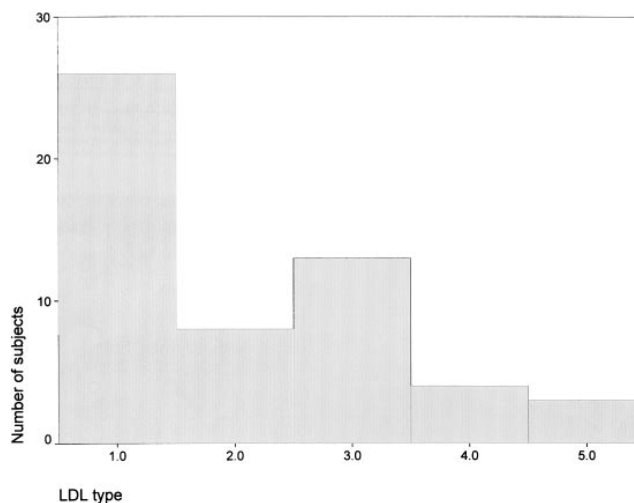
Fig. 2. Distribution of LDL particle types in women on a high fat diet as assessed by 3–15% non-denaturing gradient gel electrophoresis.

with the diet are shown in **Table 4**. An increase in energy from fat from 20% to 35% and a cholesterol increase of 560 mg/day led to a rise in LDL cholesterol of 0.29 mmol/L, a rise in HDL cholesterol of 0.15 mmol/L in women and 0.11 mmol/L in men, and a fall in triglyceride of from 0.14 in women to 0.24 mmol/L in men. The change in total cholesterol, LDL cholesterol, or triglyceride was unrelated to LDL type in the high fat phase in both men and women (**Table 5**). Adjustment for baseline lipid levels, age, or BMI did not alter this. BMI did not change overall in the study and change in BMI was unrelated to dietary response. However, the change in HDL cholesterol was greater in men with the largest LDL particles (-0.16 vs. -0.03 mmol/L, $P < 0.05$) and the change in HDL_2 cholesterol (-0.13 vs. -0.05 mmol/L, $P < 0.05$) was greater in women in the large LDL group. Change in HDL cholesterol in men was strongly related to LDL particle type ($r = -0.52$, $P = 0.001$) and change in HDL_2 cholesterol in women was related to LDL particle type ($r = -0.40$, $P < 0.01$). On multiple regression in men, the LDL peak type during the high fat phase could predict 27% of the variance in the change in HDL cholesterol with a change in dietary fat and cholesterol, independently of baseline HDL, BMI (or change in BMI) and WHR. In women, LDL particle type as a predictor was not independent of baseline HDL_2 cholesterol or WHR; these two variables could account for 31% of the variance in the change in HDL_2 cholesterol. These differences in HDL and HDL_2 cholesterol and the lack of differences in LDL cholesterol could not be accounted for by differences in dietary intake between subjects with large or small LDL particles. The change in fat between phases (14.9 ± 4.7 vs. $17.1 \pm 6.0\%$ energy for men, 16.5 ± 4.7 vs. $15.7 \pm 5.0\%$ for women) and cholesterol (286 ± 84 vs. 325 ± 101 mg/1000 kcal for men and 277 ± 73 vs. 290 ± 82 kcal for women) was the same in the upper and lower LDL particle size groups. ApoE phenotype was a minor predictor of the change in HDL cholesterol. Those subjects with an

TABLE 2. Baseline lipid values and LDL particle type

	Men			Women		
	Type 1 and 2 (n = 14)	Type 3 (n = 22)	Type 4 and 5 (n = 15)	Type 1 and 2 (n = 34)	Type 3 (n = 13)	Type 4 and 5 (n = 7)
Total cholesterol, mmol/L	5.21 ± 1.07	5.93 ± 1.17	5.68 ± 1.05	5.36 ± 1.09	5.81 ± 1.24	5.53 ± 0.63
Triglyceride, mmol/L	0.99 ± 0.33^a	1.56 ± 0.83	2.27 ± 0.98	1.07 ± 0.45^a	1.46 ± 0.45	1.90 ± 0.52
LDL cholesterol, mmol/L	4.09 ± 1.12	4.17 ± 0.84	3.95 ± 0.83	3.68 ± 0.97	3.44 ± 1.00	4.20 ± 0.84
HDL cholesterol, mmol/L	0.98 ± 0.22	0.85 ± 0.21	0.78 ± 0.33	1.30 ± 0.27^a	0.92 ± 0.21	0.85 ± 0.18
HDL$_2$ cholesterol, mmol/L	0.18 ± 0.11	0.18 ± 0.11	0.17 ± 0.12	0.40 ± 0.18^b	0.23 ± 0.10	0.24 ± 0.10
HDL$_3$ cholesterol, mmol/L	0.75 ± 0.12^b	0.65 ± 0.18	0.53 ± 0.17	0.46 ± 0.19	0.30 ± 0.16	0.25 ± 0.10
BMI, kg/m^2	24.1 ± 2.2^b	27.6 ± 3.3	27.5 ± 2.2	25.9 ± 4.6	26.2 ± 6.2	30.0 ± 3.8
WHR, cm	0.90 ± 0.04	0.94 ± 0.05	0.95 ± 0.06	0.79 ± 0.07	0.81 ± 0.06	0.85 ± 0.06
Waist circumference, cm	87.7 ± 6.7^b	97.0 ± 7.7	98.9 ± 9.6	82 ± 12	87 ± 12	96 ± 12
Insulin, mU/ml	5.4 ± 1.8^b	8.6 ± 4.1	10.7 ± 5.6	6.1 ± 2.1^a	8.3 ± 3.4	15.8 ± 8.8
Age, yr	53.4 ± 6.5	48.0 ± 7.7	49.6 ± 7.4	49.8 ± 10.9	52.8 ± 6.4	54.1 ± 9.2

Values are given as mean $\pm$ SD.
[a] $P < 0.000$; [b] $P < 0.01$, ANOVA.

E2/E3 genotype had a larger change in HDL ($P = 0.04$) and HDL$_3$ cholesterol ($P = 0.015$) compared to those with an E3/E3 genotype. There was no relationship between menopausal status, use of hormone replacement therapy, exercise pattern or alcohol intake, and change in LDL or HDL cholesterol, nor did these factors alter the relationship between LDL particle type and dietary response.

DISCUSSION

In this study we have demonstrated for the first time that LDL particle type is a major independent predictor in men of the change in HDL cholesterol with a change in dietary fat and cholesterol. LDL particle type accounted for 27% of the variance in the change in HDL cholesterol and was independent of baseline HDL cholesterol and waist to hip ratio. In women, although LDL particle type predicted the change in HDL$_2$ cholesterol, it was not independent of baseline HDL$_2$ cholesterol or waist to hip ratio. However, we have failed to confirm the observations of Dreon et al. (20) that LDL particle type is a predictor of the change in LDL cholesterol with a dietary fat reduction. This confirms our previous observations (8) that plasma triglyceride and HDL cholesterol, factors closely linked to LDL particle size, were unrelated to the change in LDL cholesterol with a dietary fat and cholesterol change. However, there are some differences between the two studies: the change in fat in this study was 15% compared with the 22% change in fat in the study by Dreon et al. (20) and cholesterol intake changed in our study while it was unchanged in the latter. The overall effect of these differences would be a smaller change in LDL cholesterol. The change in LDL cholesterol in our study was 0.28 mmol/L for men with large LDL and 0.33 mmol/L for men with small LDL compared with the larger changes in the Dreon study (0.38 mmol/L for type A and 0.76 for type B) with much greater variation in response in our study (CV of 160% vs. 64%). Although this limited LDL response might weaken any relationship with LDL particle size, it should not completely obscure it if it existed.

Although the duration of our study was shorter than the study by Dreon et al. (20) (3 weeks versus 6 weeks) and the subjects may not have achieved the maximum possible change in LDL cholesterol, their ranking in terms of response would be expected to be the same. The relationship between LDL particle size and plasma triglyceride and HDL cholesterol and between LDL particle size and fat distribution was in accord with previous data (23) and our study population had normal body weights and normal plasma triglyceride levels. LDL particle size also did not change with the dietary change despite a change in triglyceride of 0.20 mmol/L. The process of LDL size

TABLE 3. Dietary intake

Nutrient	Baseline	Low Fat Phase	High Fat Phase
Energy (kcal)	1712 ± 425	2026 ± 493	1921 ± 441
% Protein	19.7 ± 4.3	20.0 ± 3.3	18.0 ± 2.3
% Carbohydrate	51.1 ± 9.0	59.3 ± 7.0	46.3 ± 6.7^a
% Fat	26.2 ± 7.3	20.7 ± 5.4	35.7 ± 5.9^a
% Monounsaturated fat	9.0 ± 3.0	7.1 ± 2.4	12.1 ± 2.6^a
% Polyunsaturated fat	5.4 ± 2.2	4.3 ± 1.7	3.7 ± 1.6^a
% Saturated fat	9.0 ± 3.2	7.5 ± 2.5	14.9 ± 3.4^a
Cholesterol (mg)	183 ± 85	182 ± 90	748 ± 117^a

Values are given as mean $\pm$ SD.
[a] $P < 0.001$ difference between low and high fat diets.

TABLE 4. Lipid values in low and high fat phases

	Women		Men	
	Low Fat Phase	High Fat Phase	Low Fat Phase	High Fat Phase
	mmol/L		mmol/L	
Total cholesterol	5.51 ± 1.11	5.88 ± 1.17^a	5.71 ± 1.15	6.00 ± 1.10^a
Triglyceride	1.49 ± 0.67	1.35 ± 0.57^a	2.05 ± 1.22	1.81 ± 0.98^b
LDL cholesterol	3.75 ± 0.96	4.04 ± 1.04^a	3.95 ± 0.92	4.24 ± 0.99^a
HDL cholesterol	1.08 ± 0.26	1.23 ± 0.31^a	0.83 ± 0.23	0.94 ± 0.28^a
HDL$_2$ cholesterol	0.28 ± 0.14	0.37 ± 0.18^a	0.17 ± 0.12	0.21 ± 0.15^a
HDL$_3$ cholesterol	0.79 ± 0.20	0.86 ± 0.19^a	0.63 ± 0.15	0.71 ± 0.20^a

Values are given as mean $\pm$ SD.
[a] $P \leq 0.001$ for difference between low and high fat diets.
[b] $P < 0.05$.

TABLE 5. Effect of LDL particle size on dietary fat and cholesterol change

| | Difference: High Fat-Low Fat Diet | | | | | |
| | Men | | | Women | | |
	Type 1 and 2 (n = 14)	Type 3 (n = 22)	Type 4 and 5 (n = 15)	Type 1 and 2 (n = 34)	Type 3 (n = 13)	Type 4 and 5 (n = 7)
	mmol/L			*mmol/L*		
Total cholesterol	0.41 ± 0.38	0.21 ± 0.43	0.30 ± 0.48	0.34 ± 0.47	0.46 ± 0.60	0.30 ± 0.41
Triglyceride	−0.11 ± 0.33	−0.28 ± 0.76	−0.30 ± 0.94	−0.12 ± 0.21	−0.15 ± 0.50	−0.27 ± 0.41
LDL cholesterol	0.29 ± 0.35	0.23 ± 0.35	0.30 ± 0.48	0.23 ± 0.45	0.42 ± 0.49	0.29 ± 0.55
HDL cholesterol	0.16 ± 0.17[a]	0.11 ± 0.09	0.03 ± 0.09	0.17 ± 0.14	0.10 ± 0.10	0.13 ± 0.17
HDL_2 cholesterol	0.05 ± 0.07	0.03 ± 0.05	0.02 ± 0.03	0.13 ± 0.13[b]	0.04 ± 0.08	0.05 ± 0.09
HDL_3 cholesterol	0.12 ± 0.10[a]	0.07 ± 0.07	0.02 ± 0.09	0.05 ± 0.07	0.07 ± 0.10	0.09 ± 0.10

[a] $P < 0.05$, Type 1 and 2 vs. Type 4 and 5, by Duncan's posthoc test.
[b] $P < 0.05$, Type 1 and 2 vs. Type 3, by Duncan's posthoc test.

modulation may take longer than 3 weeks, but this is an issue separate from the relationship of LDL size to dietary response and does not confound the association.

In contrast to the changes in LDL cholesterol, changes in HDL cholesterol in men and HDL_2 cholesterol in women were related to LDL particle size. Thus men with large LDL particles (type 1 and 2) had changes in HDL cholesterol that were five times as great as those seen in men with small particles (type 4 and 5) [0.16 vs. 0.03 mmol/L]. Most of this difference was accounted for by HDL_3 cholesterol. Similar changes were seen in HDL_2 cholesterol in women with large LDL compared to women with small LDL (0.13 vs. 0.04 mmol/L), which was not accounted for by differences in menopausal status or use of hormones. We have previously shown that these factors do not influence the HDL response to diet (32). Similar changes were seen by Dreon et al. (20) although the difference was not as marked and it did not have statistical significance. However, when gradient gel electrophoresis of HDL is performed, there are significant differences between type A and type B men (33). The type A men had a marked decrease in HDL subfractions 3a, 2a, and 2b with increases in 3b, while the type B men had no significant change in their HDL profile. The change in the amount of type 2b HDL was significantly different between the two phenotypic groups. Thus we are in agreement with Williams, Dreon, and Krauss (33) that men with large LDL do not derive as much potential benefit from a low fat diet as men with small LDL because the reductions in HDL cholesterol are greater in the former, although we could not confirm that there were differences in the change in LDL cholesterol. We are also in agreement with these investigators that the E2 allele also appears to be associated with an adverse effect of a low fat diet on HDL cholesterol. However, in other circumstances the type A phenotype may be advantageous. Overweight type A men have a greater rise in HDL cholesterol with weight loss than type B men with the same degree of weight loss (34). Thus the men with pattern A had a 0.09 mmol/L increase in HDL_2 cholesterol and a 0.08 mmol/L increase in HDL_3 cholesterol compared with 0.03 and 0.06 mmol/L, respectively, in men with pattern B. On gradient gels this is reflected in increases in HDL_{2a} and HDL_{2b} subfractions. In this study

(33) the change in LDL cholesterol was slightly, but not significantly greater in men with large LDL.

In conclusion there appears to be little doubt that men with large LDL have an HDL that is more responsive to changes in dietary fat and cholesterol and changes in weight. Whether changes in LDL cholesterol are related to LDL size is not clear and further studies are required.

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