HDL-subpopulation patterns in response to reductions in dietary total and saturated fat intakes in healthy subjects[1–3]

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ABSTRACT

Background: Little information is available about HDL subpopulations during dietary changes.

Objective: The objective was to investigate the effect of reductions in total and saturated fat intakes on HDL subpopulations.

Design: Multiracial, young and elderly men and women ($n = 103$) participating in the double-blind, randomized DELTA (Dietary Effects on Lipoproteins and Thrombogenic Activities) Study consumed 3 different diets, each for 8 wk: an average American diet (AAD: 34.3% total fat, 15.0% saturated fat), the American Heart Association Step I diet (28.6% total fat, 9.0% saturated fat), and a diet low in saturated fat (25.3% total fat, 6.1% saturated fat).

Results: HDL_2-cholesterol concentrations, by differential precipitation, decreased ($P < 0.001$) in a stepwise fashion after the reduction of total and saturated fat: 0.58 ± 0.21, 0.53 ± 0.19, and 0.48 ± 0.18 mmol/L with the AAD, Step I, and low-fat diets, respectively. HDL_3 cholesterol decreased ($P < 0.01$) less: 0.76 ± 0.13, 0.73 ± 0.12, and 0.72 ± 0.11 mmol/L with the AAD, Step I, and low-fat diets, respectively. As measured by nondenaturing gradient gel electrophoresis, the larger-size HDL_{2b} subpopulation decreased with the reduction in dietary fat, and a corresponding relative increase was seen for the smaller-sized $HDL_{3a, 3b}$, and $_{3c}$ subpopulations ($P < 0.01$). HDL_2-cholesterol concentrations correlated negatively with serum triacylglycerol concentrations on all 3 diets: $r = -0.46$, -0.37, and -0.45 with the AAD, Step I, and low-fat diets, respectively ($P < 0.0001$). A similar negative correlation was seen for HDL_{2b}, whereas $HDL_{3a, 3b}$, and $_{3c}$ correlated positively with triacylglycerol concentrations. Diet-induced changes in serum triacylglycerol were negatively correlated with changes in HDL_2 and HDL_{2b} cholesterol.

Conclusions: A reduction in dietary total and saturated fat decreased both large (HDL_2 and HDL_{2b}) and small, dense HDL subpopulations, although decreases in HDL_2 and HDL_{2b} were most pronounced. *Am J Clin Nutr* 1999;70:992–1000.

KEY WORDS Nutrition, diet, lipoproteins, saturated fat, triacylglycerols, race, women, HDL subpopulations, cardiovascular disease

INTRODUCTION

HDL cholesterol is an important negative risk factor for coronary heart disease (1–6). It is well recognized that HDL-cholesterol concentrations are affected by dietary modifications, generally increasing with diets high in saturated fat and cholesterol and decreasing when dietary fat is replaced with carbohydrates (7–10). The HDL subpopulation is metabolically dynamic and closely related to the metabolism of triacylglycerol-rich lipoproteins (11). Within the spectrum of HDL density, there is considerable particle heterogeneity, which is linked to differences in functional properties. A common technique to classify HDL subpopulations is based on differential precipitation, HDL_2 representing a less dense and HDL_3 a more dense subpopulation (12–15). Other characterizations are based on HDL particle size analyzed by gradient gel electrophoresis, in which 5 subpopulations can be identified (HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, and HDL_{3c}), and on HDL apolipoprotein (apo) specificity, with HDL particles containing only apo A-I (Lp A-I) compared with particles containing both apo A-I and apo A-II (Lp A-I/Lp A-II) isolated by immunologic methods (12, 16–19). The classifications of heterogeneity are independent, although somewhat overlapping, eg, HDL_2 contains a higher proportion of Lp A-I than Lp A-I/A-II and corresponds broadly to HDL_{2b} and HDL_{2a}. Previous studies indicated that metabolic and cardioprotective properties

differ primarily between HDL subpopulations. Thus, within the HDL spectrum, the less dense HDL_2 subpopulation has been linked to the cardioprotective effect of HDL, and this subpopulation also has been found to have a negative relation to serum triacylglycerol concentrations (12, 20–25). However, reductions in HDL_3-cholesterol concentrations also have been associated with cardiovascular risk (26). It has been well established that HDL heterogeneity is affected by drastic variations in dietary fat intake (8, 12), but considerably less information is available about HDL subpopulations during smaller changes in fat intake, more typical of the average food consumption pattern.

In the Dietary Effects on Lipoproteins and Thrombogenic Activities (DELTA) Study, a carefully controlled multicenter feeding study, the effect of a stepwise reduction of total and saturated fats on plasma lipid and lipoprotein patterns in healthy subjects was investigated (27). The diets used were designed to represent variations within a range recommended to the public, and the overall variation in fat intake was, therefore, more modest than in many previous studies. As detailed elsewhere, the study was designed to simultaneously obtain results in men and women, African Americans and non-African Americans, pre- and postmenopausal women, and younger and older men. Overall results on lipids and lipoproteins were published previously (27). Notably, also in this regimen, HDL-cholesterol concentrations decreased and triacylglycerol concentrations increased after a reduction in dietary total and saturated fats. In view of the previously established association between HDL_2 and triacylglycerol (12, 20–25), we hypothesized that a modest, stepwise reduction in total and saturated fat in the diet would influence primarily the larger HDL subpopulations. In the present study, we report on diet-induced changes in HDL-subpopulation distribution and HDL particle size.

SUBJECTS AND METHODS

Subjects

The DELTA Study design and the recruitment strategy were described in detail elsewhere (27). For protocol 1, healthy, normolipidemic subjects between 22 and 67 y of age were recruited. To be eligible for participation, baseline fasting plasma lipid concentrations had to be between the 25th and 90th percentiles for total cholesterol, below the 90th percentile for plasma triacylglycerol, and above the 10th percentile for HDL cholesterol—all adjusted for age, race, and sex (28). Each clinical research center (Columbia University, Pennington Biomedical Research Center, Pennsylvania State University, and the University of Minnesota) recruited 25–30 subjects and a total of 118 subjects were recruited. Of these 118 subjects, 103 (57 women and 46 men) completed all 3 feeding periods. The ethnic composition of the subjects was as follows: 77 whites and 26 African Americans. Of the women, 39 were premenopausal; of the men, 30 were <40 y of age.

Study design

The study was designed as a randomized, double-blind, 3-way crossover study, in which each subject consumed 3 different diets, each for 8 wk. Each dietary period was separated by 4–6 wk. The subjects consumed 2 meals each weekday in a cafeteria; meal consumption was supervised and it was ascertained that all food provided was consumed at these meals (27). A third packaged meal

and snacks were provided to all participants. Weekend meals, except for one self-selected dinner meal, were also packaged and provided to all participants.

The 3 diets were as follows: *1*) an average American diet (AAD; 34.3% of energy as total fat: 15.0% saturated, 12.8% monounsaturated, and 6.5% polyunsaturated), *2*) the American Heart Association (AHA) Step I diet (28.6% of energy as total fat: 9.0% saturated, 12.9% monounsaturated, and 6.7% polyunsaturated), and *3*) a low-fat diet (25.3% of energy as total fat: 6.1% saturated, 12.4% monounsaturated, and 6.7% polyunsaturated fat). Details of the study design, food and menu preparation, and validation and monitoring of the diets are reported elsewhere (29). The diets were designed to provide ≈300 mg dietary cholesterol/d and the cholesterol content ranged from 267 to 285 mg/d. The diets were isoenergetic and designed to maintain body weight during the study. To ensure that the participants maintained their body weight, they were weighed twice weekly during the study and adjustments in energy intake were made if needed to maintain a stable body weight.

Analytic procedures

Blood samples were obtained weekly during the last 4 wk of each 8-wk dietary period after an overnight fast (27). Plasma and serum were isolated by centrifugation at $30\,000 \times g$ for 20 min at 4°C immediately after collection. Multiple aliquots of each were stored in cryovials at −80°C until the end of the study when all samples were analyzed. Concentrations of total cholesterol, triacylglycerol, and HDL cholesterol were assayed in the weekly samples at each research center by using standard enzymatic procedures. HDL-cholesterol concentrations were determined after precipitation of apo B–containing lipoproteins (30), and LDL-cholesterol concentrations were calculated by using the Friedewald formula (31). All laboratories participated in the Centers for Disease Control and Prevention Lipid Standardization Program; additional standardization procedures were also used.

HDL-subpopulation analyses were performed at Columbia University with plasma samples obtained during weeks 7 and 8 of all 3 diets by using a modification of the procedure described by Gidez et al (32). We established previously that a steady state was obtained at 4 wk and that in each dietary period, plasma lipid concentrations remained unchanged between 4 and 8 wk (27). Briefly, heparin and magnesium chloride were added to 0.5 mL plasma at a final concentration of 1.26 g/L and 91 mmol/L, respectively. After incubation for 10 min at room temperature, samples were centrifuged at $5000 \times g$ at 4°C in a table-top centrifuge for 1 h. The supernate was recovered and an aliquot obtained for analysis of total HDL-cholesterol concentrations. To 333 μL of the supernate, a 1/10 volume of dextran sulfate (molecular weight of 15 000; Genzyme, Cambridge, MA) was added. The mixture was incubated at room temperature for 20 min and thereafter centrifuged for 30 min at $5000 \times g$ at 4°C. The supernate, representing HDL_2, was removed and the cholesterol content was determined. HDL_3 concentrations were calculated as the difference between total HDL and HDL_2-cholesterol concentrations. For quality-control purposes, 3 individual sets of plasma samples, representing a wide variation in the HDL-subpopulation spectrum, were used. Each set of samples was divided into single-use aliquots and stored at −70°C before being used. In each assay, a set of controls was run with a 10-sample interval. The between-run CVs for HDL_2 and HDL_3 were 8% and 7%, respectively, at concentrations of 0.72 and 0.83 mmol/L, respectively.

HDL particle size was determined in 102 subjects at Pennington Biomedical Research Center by nondenaturing gradient gel electrophoresis on samples from weeks 5, 6, 7, and 8 by using a modification of the procedure published by Blanche et al (16). Briefly, concave acrylamide gradient gels (4–30%) were cast 4 or 8 at a time by using a GSC-8 gel-casting apparatus (Pharmacia, Uppsala, Sweden). The cast gels were allowed to polymerize overnight and were thereafter used immediately or stored for ≤1 wk wrapped in moist towels in plastic bags. Plasma samples (8 μL) were electrophoresed in a Pharmacia GE-2/4 electrophoresis apparatus in 90 mmol/L tris, 80 mmol/L boric acid, 3 mmol/L EDTA buffer, pH 8.3. The gels were pre-run for 15 min at 125 V before sample application. The samples were electrophoresed at 70 V for 15 min, followed by 125 V for 24 h. After electrophoresis, the gels were stained with Oil Red O (Sigma Chemical Co, St Louis) and scanned in a model GS-670 imaging densitometer (Bio-Rad Laboratories, Hercules, CA). The gels then were counterstained with Coomassie R-250 (Sigma Chemical Co) and rescanned. The lipid distribution across 5 subpopulations was determined (16). For quality-control purposes, 2 plasma samples representing extreme HDL distributions, stored frozen in single-use aliquots in 20% sucrose, were included in each run. The CVs for HDL_2 (HDL_{2b} + HDL_{2a}) and HDL_3 (HDL_{3a} + HDL_{3b} + HDL_{3c}), as estimated by gradient gel electrophoresis, averaged 9.8% and 11.1%, respectively.

Statistical analysis

Linear statistical models were fitted to the lipoprotein data by using the procedure MIXED in the SAS software package (SAS Institute Inc, Cary, NC). Subjects (ID) and diets were class variables. The syntax used for describing the repeated measures and random effects was REPEATED/SUBJECT = ID and RANDOM INT DIET/SUBJECT = ID. The model for each outcome variable included the 3 diets, 4 sex-age groups (men aged <40 y, men aged ≥40 y of age, premenopausal and postmenopausal women), 2 ethnic groups (African Americans and others), 3 apo E genotype groups (*E2/x, E3/3,* and *E4/x*), 4 field centers, interactions of all the above nondietary factors with the diets, and the 3 feeding periods. The effects of diet were estimated by weighting each relevant diet term's regression coefficient by the fraction of the study population for whom that diet term applied. For example, because 25 of 103 subjects had the *E4/3* or *E4/4 (E4/x)* apo E genotype, interactions of this factor with the diets were weighted by 0.2427 (25/103). Such weighting, based on observed frequencies, yields effects of diet for the total population that are independent of the model used.

RESULTS

As seen in **Table 1**, both HDL_2- and HDL_3-cholesterol concentrations decreased in a stepwise fashion as dietary saturated fat and total fat decreased. The lowest concentrations of both HDL subpopulations were observed during the low-fat diet. HDL_2-cholesterol concentrations differed significantly between all 3 diets (**Table 2**). Changes in HDL_3-cholesterol concentrations were smaller than those of HDL_2: concentrations with the AAD were significantly higher than those with the other 2 diets, whereas there was no significant difference in concentrations between the Step I and low-fat diets. The largest differences for both subpopulations were seen between the low-fat diet and AAD. In absolute terms, the decrease in HDL_2-cholesterol concentrations between these 2 diets was larger than that in HDL_3-cholesterol concentrations: 0.101 compared with 0.044 mmol/L, respectively. These

TABLE 1
HDL-cholesterol subpopulation distribution during the dietary intervention[1]

	AAD	Step I	Low fat
HDL cholesterol (mmol/L)	1.35 ± 0.29	1.25 ± 0.28	1.19 ± 0.26
HDL subpopulations (mmol/L)			
HDL_2	0.58 ± 0.21	0.53 ± 0.19	0.48 ± 0.18
HDL_3	0.76 ± 0.13	0.73 ± 0.12	0.72 ± 0.11
HDL size (%)			
HDL_{2b}	35.0 ± 7.9	33.6 ± 7.9	33.2 ± 8.3
HDL_{2a}	19.9 ± 2.2	19.8 ± 2.3	19.8 ± 2.3
HDL_{3a}	19.5 ± 2.6	19.9 ± 2.7	20.1 ± 2.9
HDL_{3b}	12.8 ± 2.9	13.3 ± 3.1	13.3 ± 3.2
HDL_{3c}	12.8 ± 4.6	13.5 ± 4.8	13.6 ± 4.9

[1] $\overline{x}$ ± SD. HDL-cholesterol concentrations are from reference 27. AAD, average American diet; Step I, American Heart Association diet. To convert values to mg/dL, multiply by 38.7.

decreases corresponded to 17.2% and 5.7%, respectively, of the HDL_2- and HDL_3-cholesterol concentrations with the AAD. Analysis of HDL size variation by gradient gel electrophoresis showed a similar pattern. The relative amounts of the largest HDL subpopulation, HDL_{2b}, decreased in a similar stepwise fashion with the decrease in dietary total and saturated fat (Tables 1 and 2). The changes in HDL_{2a} were less pronounced, but there was a small decrease between the AAD and Step I diets. Because these values are expressed in relative terms, there was a corresponding relative increase in HDL_{3a}, HDL_{3b}, and HDL_{3c} with a decrease in dietary fat intake. A common trend was seen for the 3 different HDL_3 particle size subpopulations, with similar relative increases in HDL_{3a}, HDL_{3b}, and HDL_{3c}, most pronounced for the smallest-sized subpopulation, HDL_{3c}.

The DELTA Study recruited subjects from different age, sex, and race groups to allow estimation of possible differences in diet-induced plasma lipid concentrations. As seen in **Table 3**, men had lower baseline HDL_2-subpopulation concentrations (determined by precipitation) with the AAD than did women. There were no significant differences in HDL_3 between men and women; there were no other significant differences in either HDL_2 or HDL_3 between age groups. Analysis of the HDL-subpopulation size pattern showed significant differences across sex, with more HDL_{2b} particles and fewer HDL_{3a}, HDL_{3b}, and HDL_{3c}

TABLE 2
Differences in HDL-cholesterol subpopulation concentrations during the dietary intervention[1]

	AAD − Step I	AAD − low fat	Step I − low fat
HDL subpopulations (mmol/L)			
HDL_2	−0.056 ± 0.10[2]	−0.101 ± 0.09[2]	−0.045 ± 0.10[2]
HDL_3	−0.028 ± 0.08[2]	−0.044 ± 0.08[3]	−0.016 ± 0.07
HDL particle size (%)			
HDL_{2b}	−1.41 ± 2.56[2]	−1.77 ± 2.61[2]	−0.36 ± 2.66
HDL_{2a}	−0.16 ± 0.82	−0.16 ± 0.83	0.00 ± 0.84
HDL_{3a}	0.42 ± 1.00[2]	0.56 ± 1.09[2]	0.14 ± 1.11
HDL_{3b}	0.51 ± 1.03[2]	0.57 ± 1.04[2]	0.07 ± 0.97
HDL_{3c}	0.65 ± 1.56[3]	0.79 ± 1.47[2]	0.14 ± 1.52

[1] $\overline{x}$ ± SD. AAD, average American diet.
[2] $P < 0.001$.
[3] $P < 0.01$.

TABLE 3
HDL-cholesterol subpopulation distribution in subgroups during the dietary intervention[1]

	AAD		Step I		Low fat	
	HDL_2	HDL_3	HDL_2	HDL_3	HDL_2	HDL_3
			mmol/L			
Black (n = 26)	0.59 ± 0.18	0.77 ± 0.15	0.54 ± 0.20	0.75 ± 0.13	0.49 ± 0.16	0.72 ± 0.11
White (n = 77)	0.58 ± 0.22	0.76 ± 0.13	0.53 ± 0.19	0.73 ± 0.12	0.48 ± 0.19	0.71 ± 0.12
Women (n = 57)	0.68 ± 0.20[2]	0.78 ± 0.12	0.61 ± 0.18[2]	0.76 ± 0.13	0.58 ± 0.17[2]	0.74 ± 0.11
Premenopausal (n = 39)	0.70 ± 0.20	0.76 ± 0.11	0.64 ± 0.19	0.75 ± 0.13	0.59 ± 0.18	0.71 ± 0.10
Postmenopausal (n = 18)	0.64 ± 0.19	0.82 ± 0.13	0.56 ± 0.13	0.79 ± 0.13	0.55 ± 0.16	0.78 ± 0.12
Men (n = 46)	0.46 ± 0.15	0.74 ± 0.14	0.43 ± 0.16	0.70 ± 0.11	0.37 ± 0.12	0.69 ± 0.12
<40 y (n = 30)	0.48 ± 0.16	0.76 ± 0.15	0.43 ± 0.14	0.71 ± 0.11	0.38 ± 0.12	0.70 ± 0.13
≥40 y (n = 16)	0.42 ± 0.15	0.70 ± 0.09	0.43 ± 0.20	0.68 ± 0.09	0.34 ± 0.11	0.67 ± 0.09

[1] $\bar{x}$ ± SD. AAD, average American diet.
[2] Significantly different from men, $P < 0.01$.

particles in women than in men (**Table 4**). For the different sex and age groups, HDL_{2b} was more numerous in premenopausal than in postmenopausal women, whereas other differences were not significant. In this study, we did not find any differences in HDL-subpopulation concentrations between African Americans and non-African Americans, by either differential precipitation or gradient gel electrophoresis. Additionally, overall HDL-cholesterol concentrations did not differ significantly between African Americans and non-African Americans (27).

The overall response to the dietary changes in HDL_2- and HDL_3-cholesterol concentrations was similar among the subgroups (Table 3); in each subgroup, dietary effects were significant, as

TABLE 4
HDL size subpopulation distribution in subgroups during the decrease in dietary saturated fat[1]

Subgroup	HDL_{2b}	HDL_{2a}	HDL_{3a}	HDL_{3b}	HDL_{3c}
			%		
Blacks (n = 26)					
AAD	36.9 ± 7.2	20.4 ± 2.1	18.7 ± 2.6	11.7 ± 2.5	12.2 ± 4.5
Step I	35.5 ± 6.4	20.3 ± 2.1	19.1 ± 2.4	12.1 ± 2.3	12.9 ± 4.4
Low fat	35.4 ± 6.7	20.4 ± 2.3	19.0 ± 2.9	12.1 ± 2.2	13.0 ± 4.3
Whites (n = 76)					
AAD	34.3 ± 8.1	19.8 ± 2.2	19.8 ± 2.6	13.1 ± 3.0	13.1 ± 4.6
Step I	32.9 ± 8.2	19.6 ± 2.4	20.2 ± 2.7	13.7 ± 3.2	13.7 ± 4.9
Low fat	32.4 ± 8.7	19.5 ± 2.3	20.4 ± 2.8	13.8 ± 3.3	13.9 ± 5.0
Women (n = 57)					
AAD	38.4 ± 7.7[2]	20.3 ± 2.1	18.8 ± 2.7[2]	11.5 ± 2.6[2]	11.0 ± 4.0[2]
Step I	37.1 ± 7.5[2]	20.2 ± 2.3	19.3 ± 2.6[2]	11.9 ± 2.7[2]	11.5 ± 4.2[2]
Low fat	36.7 ± 8.1[2]	20.2 ± 2.3	19.4 ± 2.9[2]	12.0 ± 2.8[2]	11.7 ± 4.5[2]
Premenopausal women (n = 39)					
AAD	40.1 ± 7.5[3]	20.3 ± 2.0	18.5 ± 2.6	11.0 ± 2.5	10.1 ± 3.4
Step I	38.8 ± 7.3	20.2 ± 2.1	18.9 ± 2.7	11.4 ± 2.5	10.7 ± 3.5
Low fat	38.5 ± 8.2	20.3 ± 2.3	19.0 ± 3.0	11.4 ± 2.7	10.7 ± 3.8
Postmenopausal women (n = 18)					
AAD	34.8 ± 7.1	20.2 ± 2.5	19.6 ± 2.6	12.4 ± 2.6	12.9 ± 4.7
Step I	33.3 ± 6.7	20.2 ± 2.7	20.1 ± 2.2	13.0 ± 2.7	13.4 ± 5.1
Low fat	32.8 ± 6.6	20.0 ± 2.3	20.2 ± 2.3	13.1 ± 2.6	13.9 ± 5.1
Men (n = 45)					
AAD	30.6 ± 5.9	19.5 ± 2.3	20.3 ± 2.4	14.4 ± 2.5	15.1 ± 4.2
Step I	29.1 ± 5.9	19.3 ± 2.3	20.7 ± 2.5	15.0 ± 2.7	16.0 ± 4.3
Low fat	28.7 ± 6.0	19.2 ± 2.3	20.9 ± 2.7	15.1 ± 2.8	16.0 ± 4.3
Men aged <40 y (n = 29)					
AAD	31.3 ± 6.3	20.2 ± 2.1	20.4 ± 2.5	13.8 ± 2.5	14.2 ± 4.5
Step I	29.5 ± 6.3	19.9 ± 2.1	20.8 ± 2.8	14.5 ± 2.7	15.2 ± 4.6
Low fat	29.2 ± 6.6	19.8 ± 2.1	21.1 ± 3.0	14.7 ± 2.8	15.2 ± 4.3
Men aged ≥40 y (n = 16)					
AAD	29.3 ± 4.9	18.3 ± 2.1	20.2 ± 2.2	15.4 ± 2.4	16.9 ± 2.8
Step I	28.4 ± 5.1	18.1 ± 2.3	20.5 ± 2.1	15.8 ± 2.5	17.2 ± 3.4
Low fat	27.9 ± 5.1	18.1 ± 2.4	20.5 ± 1.9	15.9 ± 2.5	17.6 ± 3.8

[1] $\bar{x}$ ± SD. AAD, average American diet.
[2] Significantly different from men, $P < 0.01$.
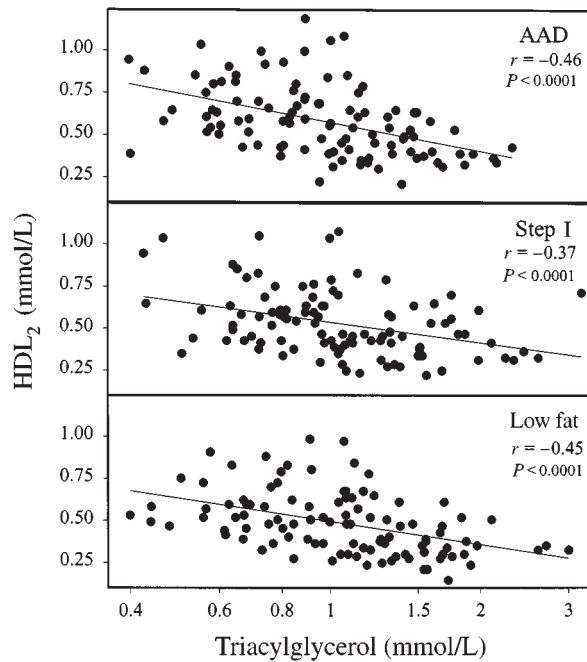[3] Significantly different from postmenopausal, $P < 0.01$.

FIGURE 1. Correlation between HDL_2-cholesterol and triacylglycerol concentrations for all subjects ($n = 103$) during the average American diet (AAD), the American Heart Association Step I diet, and a diet with a low total and saturated fat content (low-fat diet). To convert cholesterol and triacylglycerol to mg/dL, multiply by 38.7 and 88.5, respectively.
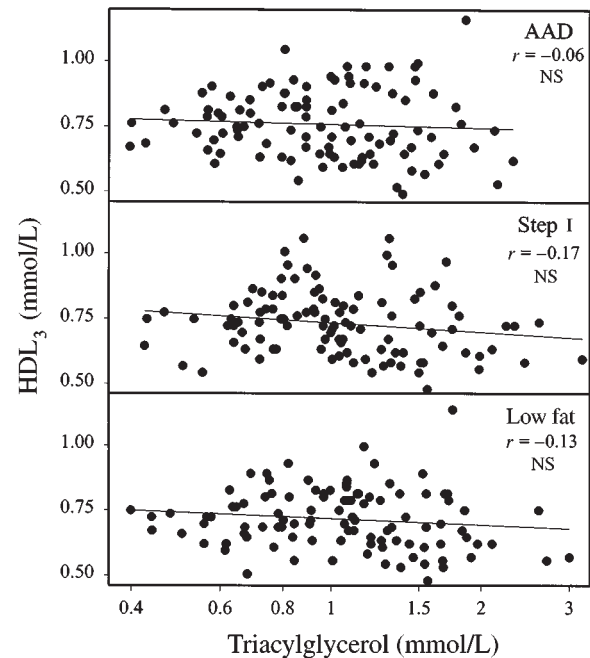

FIGURE 2. Correlation between HDL_3-cholesterol and triacylglycerol concentrations for all subjects ($n = 103$) during the average American diet (AAD), the American Heart Association Step I diet, and a diet with a low total and saturated fat content (low-fat diet). To convert cholesterol and triacylglycerol to mg/dL, multiply by 38.7 and 88.5, respectively.

for the whole group. In all subgroups, the decrease in HDL_2-cholesterol concentrations was significantly greater than the decrease in HDL_3-cholesterol concentrations ($P < 0.001$). When expressed in relative terms, decreases in HDL_2-cholesterol concentrations between the low-fat diet and the AAD ranged from 14.6% to 21.3%, being slightly higher in men, independent of age. Corresponding decreases in HDL_3-cholesterol concentrations between the subgroups was small, ranging from 3.7% to 6.4%. The observed changes between diets were similar in all subgroups by gradient gel electrophoresis, with a relative decrease in HDL_{2b} and a relative increase in HDL_{3a}, HDL_{3b}, and HDL_{3c} (Table 4). Thus, the overall response of HDL subpopulations to decreases in dietary total and saturated fat, on the basis of both differential precipitation and size classification, did not differ significantly among different sex, age, or race groups.

It is well known from many previous studies that total HDL-cholesterol concentrations correlate negatively with serum triacylglycerol concentrations (33). In the present study, there was a significant negative correlation between HDL_2-cholesterol and serum triacylglycerol concentrations on all 3 diets (**Figure 1**). Because serum triacylglycerol concentrations were non-normally distributed, results were log transformed before analysis. In contrast, HDL_3-cholesterol concentrations did not correlate with serum triacylglycerol concentrations on any of the diets (**Figure 2**). The results indicate that during each dietary regimen, higher serum triacylglycerol concentrations were associated with lower concentrations of HDL_2 but not HDL_3 cholesterol. However, after adjustment for triacylglycerol concentrations, there was still a significant difference in HDL_2-cholesterol concentrations between

the 3 diets ($P < 0.0001$), showing a specific effect of diet on HDL_2 cholesterol. Overall, only $\approx 10\%$ of the difference in HDL_2-cholesterol concentrations between the 3 diets could be explained by changes in triacylglycerol concentrations. In contrast, there was no relation of either HDL-subpopulation concentration with LDL-cholesterol or total cholesterol concentrations (data not shown). To investigate whether the diet-induced decreases in HDL-subpopulation concentrations were associated with diet-induced increases in serum triacylglycerol concentrations, individual differences in these indexes were compared. As seen in **Figure 3**, there was a significant relation between changes in HDL_2-cholesterol concentrations and changes in serum triacylglycerol concentrations among all 3 diets. For HDL_3-cholesterol concentrations, there was a significant correlation with changes in serum triacylglycerol only when the AAD and low-fat diet were compared, representing the overall largest differences (**Figure 4**). For HDL size subpopulations, we found significant correlations between triacylglycerol concentrations and HDL_{2b}, HDL_{3a}, HDL_{3b}, and HDL_{3c} with all 3 diets (**Table 5**). In addition, changes in HDL_{2b} correlated negatively with changes in triacylglycerol concentrations when the Step I or the low-fat diet was compared with the AAD, whereas there was a positive correlation between changes in HDL_{3a} and HDL_{3b} and changes in triacylglycerol. HDL_{3c}, the smallest HDL subpopulation, correlated weakly with triacylglycerol concentrations on all diets, and changes in HDL_{3c} did not relate to changes in triacylglycerol. There were virtually no changes in HDL_{2a} between the diets and there was no relation between HDL_{2a} and triacylglycerol concentrations on any of the diets. As observed for the HDL_2 and HDL_3 subpopulations, between-diet changes in HDL size subpop-
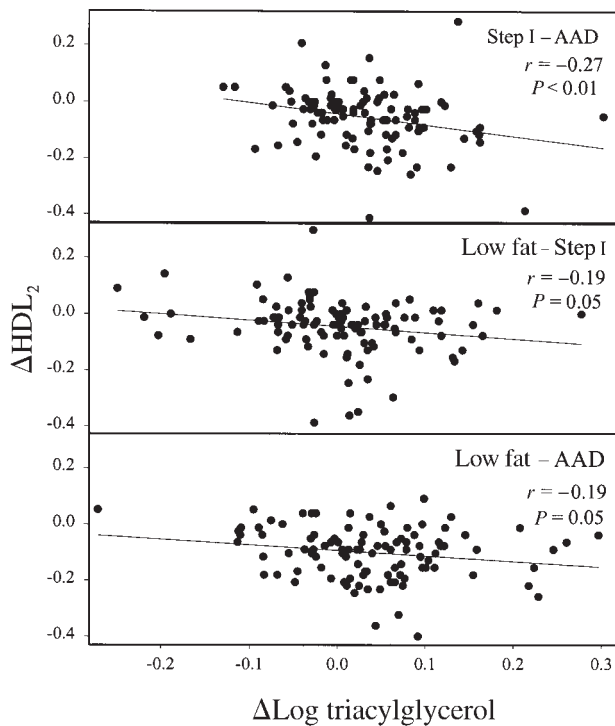
FIGURE 3. Correlation between changes (Δ) in HDL$_2$-cholesterol and log triacylglycerol concentrations for all subjects ($n = 103$) between the American Heart Association Step I diet and the average American diet (AAD), the diet with a low total and saturated fat content (low-fat diet) and the Step I diet, and the low-fat diet and the AAD.
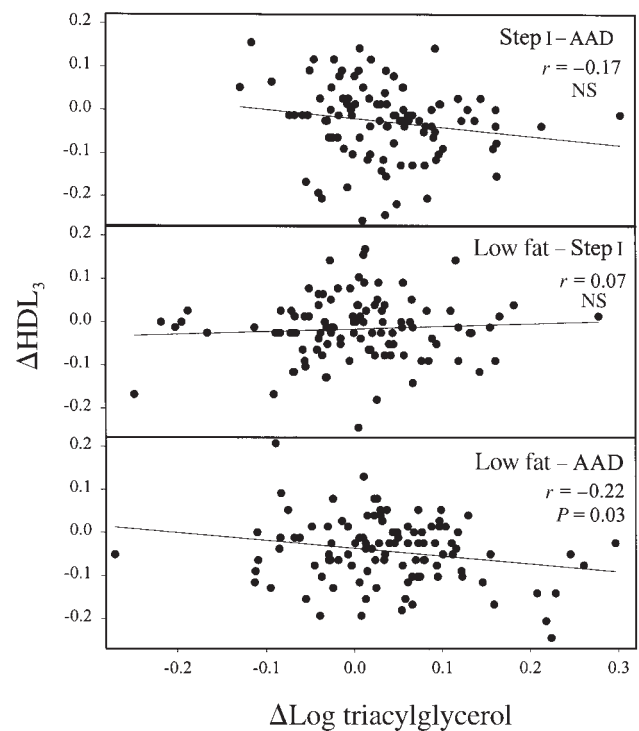

FIGURE 4. Correlation between changes (Δ) in HDL$_3$-cholesterol and log triacylglycerol concentrations for all subjects ($n = 103$) between the American Heart Association Step I diet and the average American diet (AAD), the diet with a low total and saturated fat content (low-fat diet) and the Step I diet, and the low-fat diet and the AAD.

ulations did not correlate significantly with changes in total or LDL cholesterol.

DISCUSSION

A major finding of the present study was that the dietary response to a reduction in total and saturated fats in the diet varied between different HDL subpopulations. We addressed these effects by measuring 2 different HDL-subpopulation properties: differential precipitation and size. Independent of the method used, the most pronounced response was seen for the less dense, larger HDL subpopulations (HDL$_2$ and HDL$_{2b}$), which decreased with a decrease in total and saturated fat intakes. The concentrations of this larger HDL subpopulation correlated significantly with serum triacylglycerol concentrations during all diets. In addition, the between-diet changes observed for the larger HDL subpopulations correlated significantly with between-diet changes in serum triacylglycerol concentrations. The results showed simultaneous changes in triacylglycerol, HDL$_{2b}$, and HDL$_2$ during dietary modification within the normal food intake range and suggest a close metabolic relation between these subpopulations.

The design of the study offered several important advantages. First, the number of subjects enrolled was larger than in most other metabolic ward studies and offered possibilities to address diversities in race and sex. In addition, the use of modest and realistic dietary changes makes the results relevant to clinical practice. Another strength was the use of 2 different and independent methods to assess HDL-subpopulation changes. Each

method addresses unique properties of HDL and the results, therefore, do not necessarily overlap (12). Although concentrations of HDL subpopulations are measured in the stepwise precipitation procedure, lipid staining is used to estimate the relative concentrations of the size subpopulations. Therefore, it was reassuring that the results with stepwise precipitation were corroborated by those with lipid staining. It is important to emphasize that HDL size subpopulation data are presented in relative terms, with an increase in relative concentrations of HDL$_{3a}$, HDL$_{3b}$, and HDL$_{3c}$ with the Step I and low-fat diets. When the changes in HDL subpopulations derived by precipitation were expressed in relative terms, there was an increase in the HDL$_3$ subpopulation from 56.6% to 59.7%. Thus, overall, both procedures gave similar results, showing reductions in the larger, less dense HDL subpopulations in response to the dietary changes.

It has been well established that the higher the HDL-cholesterol concentration, the lower the risk of atherogenesis, and, in epidemiologic studies, low HDL-cholesterol concentrations are a risk factor for coronary heart disease (1–6). HDL-cholesterol concentrations vary widely in the population and are affected by the action of many genetic and metabolic factors (11, 34–37). It is well established that the composition of the diet affects HDL-cholesterol concentrations, with an increase during higher intakes of saturated fat and a decrease when saturated fat is replaced with unsaturated fat or carbohydrates (7). In addition to diet, other environmental factors, such as exercise, body weight, and drug use influence HDL cholesterol (7, 11, 35). Because of this complex regulation of HDL-cholesterol concentrations, the

TABLE 5
Correlation between HDL size subpopulations and serum triacylglycerol concentrations[1]

	HDL$_{2b}$	HDL$_{2a}$	HDL$_{3a}$	HDL$_{3b}$	HDL$_{3c}$
AAD					
r	−0.41	−0.05	0.43	0.43	0.21
P	<0.001	NS	<0.001	<0.001	0.034
Step I					
r	−0.41	−0.03	0.36	0.45	0.21
P	<0.001	NS	<0.001	<0.001	0.035
Low fat					
r	−0.49	−0.03	0.48	0.50	0.24
P	<0.001	NS	<0.001	<0.001	0.013
Step I vs AAD					
r	−0.43	−0.12	0.34	0.39	0.29
P	<0.001	NS	<0.001	<0.001	0.003
Low fat vs AAD					
r	−0.39	0.05	0.37	0.35	0.14
P	<0.001	NS	<0.001	<0.001	NS

[1] AAD, average American diet.

underlying mechanisms for the cardioprotective role of HDL remains to be elucidated. Emerging data during recent years have focused attention on the role of HDL subpopulations. The HDL fraction is heterogeneous, containing particles with various contents of apolipoproteins, cholesterol, triacylglycerol, and phospholipids. Because of the complexity of HDL metabolism and composition, many subpopulation classification systems have been used. Of the HDL subpopulations measured by precipitation (HDL$_2$ and HDL$_3$), HDL$_2$ has been most consistently linked to the antiatherogenic effect of HDL (38–42). This subpopulation has been affected more by large changes in dietary fat intake, whereas the response in HDL$_3$-cholesterol concentrations has been less pronounced (43–47). However, studies in which there were more modest changes in dietary fat intake have not always shown an effect on HDL$_2$ (48). In some previous studies, as the replacement of dietary fat with carbohydrate increased, HDL-cholesterol concentrations decreased accordingly, particularly concentrations of the larger HDL subpopulations (7, 49, 50). The carbohydrate-induced decrease in HDL was shown to be associated with changes in apo A-I metabolism (51, 52). However, several of these studies used diets with large variations in fat and carbohydrate contents (8, 12); therefore, it was important to extend these findings to the general population by using more modest and commonly occurring dietary differences.

HDL has a complex metabolic relation to chylomicron, VLDL, and LDL metabolism (35). High triacylglycerol and low HDL-cholesterol concentrations usually occur together clinically and there is an exchange of cholesterol and triacylglycerol between HDL and triacylglycerol-rich lipoproteins in the circulation. In normolipidemic subjects, VLDL concentrations have been reported to be rate-limiting for the net transfer of cholesterol esters from HDL to VLDL, whereas cholesteryl ester transfer protein concentrations are suggested to be more important in hypertriglyceridemia (53, 54). In the present study, serum triacylglycerol concentrations increased with the stepwise replacement of fat with carbohydrate in normolipidemic subjects. This could have occurred in part because of carbohydrate-induced increases in hepatic VLDL output (55). The increased availability of VLDL-associated triacylglycerols could potentially stimulate cholesteryl ester transfer from HDL to VLDL, as suggested by the present decrease in

HDL-cholesterol concentrations in parallel with the increase in triacylglycerol concentrations during a reduction in dietary fat intake. This is of particular interest because the subjects were normolipidemic, with modest changes in mean triacylglycerol concentrations between the 3 diets (AAD: 0.96 mmol/L; Step I diet: 1.04 mmol/L; low-fat diet: 1.05 mmol/L; the only significant difference was between the AAD and the other 2 diets (27). HDL$_2$ cholesterol has shown a stronger correlation with serum triacylglycerol than has HDL$_3$ cholesterol (20–25) and it is conceivable that an increased exchange between VLDL triacylglycerol and primarily HDL$_2$ cholesteryl ester with the Step I and low-fat diets could have contributed to the decrease in HDL$_2$- and HDL$_{2b}$-cholesterol concentrations. We found significant correlations not only between triacylglycerol concentrations and HDL$_2$- and HDL$_{2b}$-cholesterol concentrations during all 3 diets, but also between changes in triacylglycerol concentrations and in HDL$_2$- and HDL$_{2b}$-cholesterol concentrations, indicating a dynamic metabolic relation between triacylglycerol-rich lipoproteins and the larger, less dense HDL subpopulation in these normolipidemic subjects. However, the changes in triacylglycerol concentrations only explained a small part (10%) of the interdietary differences in HDL$_2$-cholesterol concentrations; after adjustment for differences in triacylglycerol concentrations, significant differences in HDL$_2$-cholesterol concentrations remained between the 3 diets. These results suggest that the HDL-lowering effect observed after a reduction in dietary saturated fat was due to several mechanisms.

The implications of reducing the HDL cholesterol and specific HDL-subpopulations with low-fat diets are controversial (33). Previous studies suggest that a decrease in overall HDL- or HDL$_2$- and HDL$_{2b}$-cholesterol concentrations in conjunction with a diet-induced increase in serum triacylglycerol concentrations did not indicate an increased risk of coronary heart disease (7, 34, 49, 50, 56, 57). In addition, several intervention studies showed that reductions in saturated fat and cholesterol intakes are associated with a decreased incidence in atherosclerotic cardiovascular disease and improved mortality (58–60). Thus, reductions in HDL cholesterol, primarily in HDL$_2$ and HDL$_{2b}$ cholesterol, may not be harmful if coupled with reductions in LDL cholesterol. In parallel with the changes in HDL subpopulations, there was a reduction in LDL cholesterol and apo B concentrations in the present study, clearly compatible with a decreased risk of coronary heart disease (27). The decrease in LDL cholesterol and apo B concentrations, suggesting a decrease in the number of circulating LDL particles, may offset any potential negative effects of a decrease in overall HDL, HDL$_2$, or HDL$_{2b}$ concentrations.

In conclusion, a gradual decrease in total and saturated fat intakes resulted in a significant decrease in HDL$_2$- and HDL$_{2b}$-cholesterol concentrations in all age, sex, and race groups, whereas changes in HDL$_3$ cholesterol and HDL$_{3a}$-, HDL$_{3b}$-, and HDL$_{3c}$-cholesterol concentrations were less prominent. Although the quantitative effect of changes in serum triacylglycerol concentrations on differences in HDL subpopulations was limited, there was a close association between changes in concentrations of HDL$_2$ and HDL$_{2b}$ and changes in serum triacylglycerol concentrations. The results indicate that dietary changes suggested to be prudent for a large segment of the population will primarily affect the concentrations of the most prominent antiatherogenic HDL subpopulation. However, the simultaneous decrease in the atherogenic LDL subpopulation will most likely offset any potential negative effect on cardiovascular risk.

REFERENCES

1. Barr DP, Russ EM, Eder HA. Protein lipid relationship in human plasma. II. In atherosclerosis and related conditions. Am J Med 1951;11:480–93.
2. Miller GJ, Miller NE. Plasma high-density lipoprotein concentration and development of ischemic heart disease. Lancet 1975;1:16–9.
3. Gordon DJ, Rifkind BM. High density lipoprotein—the clinical implications of recent studies. N Engl J Med 1989;321:365–74.
4. Rhoads GG, Gulbrandsen CL, Kagan A. Serum lipoproteins and coronary heart disease in a population study of Hawaii Japanese men. N Engl J Med 1976;294:293–8.
5. Miller NE, Thelle DS, Forde OH, Mjos OD. The Tromso Heart Study: high-density lipoprotein and coronary heart disease: a prospective case-control study. Lancet 1977;1:965–8.
6. Gordon DJ, Probstfield JL, Garrison RJ. High-density lipoprotein cholesterol and cardiovascular disease. Circulation 1989;79:8–15.
7. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arterioscler Thromb 1992;12:911–9.
8. Williams PT, Dreon DM, Krauss RM. Effects of dietary fat on high-density-lipoprotein subclasses are influenced by both apolipoprotein E isoforms and low-density-lipoprotein subclass patterns. Am J Clin Nutr 1995;61:1234–40.
9. Grundy SM, Denke MA. Dietary influences on serum lipids and lipoproteins. J Lipid Res 1990;31:1149–72.
10. Schaefer EJ, Levy RI, Ernst ND Van Sant FD, Brewer HB Jr. The effects of low cholesterol, high polyunsaturated fat, and low fat diets on plasma lipid and lipoprotein cholesterol levels in normal and hypercholesterolemic subjects. Am J Clin Nutr 1981;34:1758–63.
11. Tall AR. Metabolic and genetic control of HDL cholesterol levels. J Intern Med 1992;231:661–8.
12. Silverman DI, Ginsburg GS, Pasternak RC. High-density lipoprotein subfractions. Am J Med 1993;94:636–45.
13. Schaefer EJ, Foster DM, Jenkins LL, Lindgren FT, Berman M, Levy RI. The composition and metabolism of high density lipoprotein subfractions. Lipids 1979;14:511–22.
14. DeLalla OF, Elliott HA, Gofman JW. Ultracentrifugal studies of high density serum lipoproteins in clinically healthy adults. Am J Physiol 1954;179:333–7.
15. Anderson DW, Nichols AV, Pan SS, Lindgren FT. High density lipoprotein distribution: resolution and determination of three major components in a normal population. Atherosclerosis 1977;29:161–79.
16. Blanche PJ, Gong EL, Forte TM, Nichols AV. Characterization of human high-density lipoproteins by gradient gel electrophoresis. Biochim Biophys Acta 1981;665:408–19.
17. Verdery RB, Benham DF, Baldwin HL, Goldberg AP, Nichols AV. Measurement of normative HDL subfraction levels by Gaussian summation analysis of gradient gels. J Lipid Res 1989;30:1085–95.
18. Li Z, McNamara JR, Ordovas JM, Schaefer EJ. Analysis of high density lipoproteins by a modified gradient gel electrophoresis method. J Lipid Res 1994;35:1698–711.
19. Cheung MC, Albers JJ. Characterization of lipoprotein particles isolated by immunoaffinity chromatography: particles containing A-I and A-II and particles containing A-I but not A-II. J Biol Chem 1984;259:12201–9.
20. Miller NE. Associations of high-density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atherosclerosis. Am Heart J 1987;113:589–97.
21. Robinson D, Ferns GA, Bevan EA, Stocks J, Williams PT, Galton DJ. High density lipoprotein subfractions and coronary risk factors in normal men. Arteriosclerosis 1987;7:341–6.
22. Ballantyne F, Clark RS, Simpson HS, Ballantyne D. High density and low density lipoprotein subfractions in survivors of myocardial infarction and in control subjects. Metabolism 1982;31:433–7.
23. Hamsten A, Walldius G, Dahlen G, Johansson B, de Faire U. Serum lipoproteins and apolipoproteins in young male survivors of myocardial infarction. Atherosclerosis 1986;59:223–35.
24. Patsch JR, Prasad S, Gotto AM, Patsch W. High density lipoprotein 2: relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. J Clin Invest 1987;80:341–7.
25. Williams PT, Krauss RM, Vranizan KM, Stefanick ML, Wood PDS, Lindgren FT. Associations of lipoproteins and apolipoproteins with gradient gel electrophoresis estimates of high density lipoprotein subfractions in men and women. Arterioscler Thromb 1992;12:332–40.
26. Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins and the risk of myocardial infarction. N Engl J Med 1991;325:373–81.
27. Ginsberg HN, Kris-Etherton P, Dennis B, et al. Effects of reducing dietary saturated fatty acids on plasma lipids and lipoproteins in healthy subjects: The DELTA Study, protocol 1. Arterioscler Thromb Vasc Biol 1998;18:441–9.
28. Johnson CL, Rifkind BM, Sempos CT, et al. Declining serum total cholesterol levels among US adults: the National Health and Nutrition Examination Surveys. JAMA 1993;269:3002–8.
29. Dennis BH, Stewart P, Wang CH, et al. Diet design for multicenter controlled feeding trials: The DELTA program. J Am Diet Assoc 1998;98:766–76.
30. Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem 1977;23:882–4.
31. Friedewald WF, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1977;18:499–502.
32. Gidez LI, Miller GJ, Burstein M, Slagle S, Eder HA. Separation and quantitation of human plasma high density lipoproteins by a simple precipitation procedure. J Lipid Res 1982;23:1206–23.
33. NIH Consensus Conference. Triglyceride, high-density lipoprotein and coronary heart disease. JAMA 1993;269:505–10.
34. Tall AR. Plasma lipid transfer proteins. J Lipid Res 1986;27:361–7.
35. Eisenberg S. High density lipoprotein metabolism. J Lipid Res 1984;25:1017–58.

36. Barter PJ, Hopkins GJ, Ha YC. The role of lipid transfer proteins in plasma lipoprotein metabolism. Am Heart J 1987;113:538–42.

37. Zhong S, Sharp DS, Grove JS, et al. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl transfer protein gene despite increased HDL levels. J Clin Invest 1996;97:2917–23.

38. Miller NE, Hammett F, Saltissi S, et al. Relation to angiographically defined coronary artery disease to plasma lipoprotein subfractions and apolipoproteins. Br Med J 1981;282:1741–4.

39. Campos H, Roederer GO, Lussier-Cacan S, Davignon J, Krauss RM. Predominance of large LDL and reduced HDL₂ cholesterol in normolipidemic men with coronary artery disease. Arterioscler Thromb Vasc Biol 1995;15:1043–8.

40. Wilson HM, Patel JC, Skinner ER. The distribution of high density lipoproteins in coronary survivors. Biochem Soc Trans 1991;18:1175–6.

41. Johansson J, Carlson LA, Landou C, Hamsten A. High density lipoprotein and coronary atherosclerosis: a strong relation with the largest HDL particles that is confined to normotriglyceridemic subjects. Arteriosclerosis 1991;11:174–82.

42. Johansson J, Olsson AG, Bergstrand L, et al. Lowering of HDL₂ᵦ by probucol partly explains the failure of the drug to affect femoral atherosclerosis in subjects with hypercholesterolemia. A probucol quantitative regression Swedish trial (PQRST) report. Arterioscler Thromb Vasc Biol 1995;15:1049–56.

43. Kasim SE, Martino S, Kim PN, et al. Dietary and anthropometric determinants of plasma lipoproteins during a long-term low-fat diet in healthy women. Am J Clin Nutr 1993;57:146–53.

44. Ehnholm C, Huttunen JK, Pietinen P, et al. Effect of a diet low in unsaturated fatty acids on plasma lipids, lipoproteins and HDL subfractions. Arteriosclerosis 1984;4:265–9.

45. Shepherd J, Packer CJ, Patsch JR, Gotto AM, Taunton OD. Effects of dietary saturated and polyunsaturated fat on the properties of high density lipoproteins and the metabolism of apoprotein AI. J Clin Invest 1978;61:1582–92.

46. Clevidence BA, Judd JT, Schatzkin A, et al. Plasma lipid and lipoprotein concentrations of men consuming a low-fat, high-fiber diet. Am J Clin Nutr 1992;55:689–94.

47. Schaefer EJ, Lichtenstein AH, Lamon-Fava S, et al. Efficacy of a National Cholesterol Education Program Step 2 diet in normolipidemic and hypercholesterolemic middle-aged and elderly men and women. Arterioscler Thromb Vasc Biol 1995;15:1079–85.

48. Mensink RP, Katan MP. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol on healthy women and men. N Engl J Med 1989;321:436–41.

49. Knuiman JT, West CE, Katan MB, Hautvast GAJ. Total cholesterol and high density lipoprotein cholesterol levels in populations differing in fat and carbohydrate intake. Arteriosclerosis 1987;7:612–9.

50. West CE, Sullivan DR, Katan MB, Halferkamps IN, van der Torre HW. Boys from populations with high-carbohydrate intake have higher fasting triglyceride levels than boys from populations with high-fat intake. Am J Epidemiol 1990;131:271–82.

51. Blum CB, Levy RI, Eisenberg S, Hall M, Goebel RH, Berman M. High density lipoprotein metabolism in man. J Clin Invest 1977;60:795–807.

52. Brinton EA, Eisenberg S, Breslow JL. A low-fat diet decreases high density lipoprotein (HDL) cholesterol by decreasing HDL apolipoprotein transport rates. J Clin Invest 1990;85:144–51.

53. Mann CJ, Yen FT, Grant AM, Bihain BE. Mechanism of plasma cholesteryl ester transfer in hypertriglyceridemia. J Clin Invest 1991;88:2059–66.

54. McPherson R, Mann CJ, Tall AR, et al. Plasma concentrations of cholesteryl ester transfer protein (CETP) in hyperlipoproteinemia: relationship to CETP activity and other lipoprotein variables. Arteriosclerosis 1991;11:797–804.

55. Melish J, Le N-A, Ginsberg H, Steinberg D, Brown WV. Dissociation of apoprotein B and triglyceride production in very-low-density lipoproteins. Am J Physiol 1980;239:E354–62.

56. Katan MB, Zock PL, Mensink RP. Dietary oils, serum lipoproteins, and coronary heart disease. Am J Clin Nutr 1995;61(suppl):1368S–73S.

57. Grundy SM. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. N Engl J Med 1986;314:745–8.

58. Hjermann I, Holme I, Velve Byre K, Leren P. Effect of diet and smoking intervention on the incidence of coronary heart disease: report from the Oslo Study Group of a randomized trial in healthy men. Lancet 1981;2:1303–10.

59. Ornish D, Brown SE, Scherwitz LW, et al. Can lifestyle changes reverse coronary heart disease? The Lifestyle Heart Trial. Lancet 1990;336:129–33.

60. Watts GF, Lewis B, Brunt JNH, et al. Effects on coronary artery disease of lipid-lowering diet, or diet plus cholestyramine, in the St. Thomas' Atherosclerosis Regression Study (STARS). Lancet 1992;339:563–9.