Differential response to low-fat diet between low and normal HDL-cholesterol subjects

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Abstract Heart attacks frequently occur in normolipidemic subjects with low concentration of high density lipoproteins (≤35 mg/dL). A low-fat diet is generally recommended to patients with coronary heart disease. A low-fat diet decreases both low density (LDL) and high density lipoproteins (HDL). We have shown that on an Average American Diet, subjects with different HDL-cholesterol (HDL-C) levels have different HDL subpopulation profiles. In low HDL-C subjects (≤35 mg/dL), the apoA-I-only subpopulation α_1 is significantly decreased compared to individuals with normal HDL-C levels (>35 mg/dL). We hypothesized that as subjects with low HDL-C already have low HDL concentrations, the major decrease of HDL-C will occur in subjects with normal HDL-C when a low-fat diet is consumed. Normolipidemic male subjects consumed three diets differing in total fat and saturated fat composition (AAD: 37%, Step-1: 28%, Step-2: 24% total fat) for 6 weeks in a three-period double-blind randomized crossover design. Plasma lipids and apolipoproteins were determined and changes in distribution of HDL subpopulations were evaluated. As a result of a low-fat diet, low HDL-C individuals slightly decreased their HDL-C, but substantially decreased their LDL-C resulting in a significant improvement in the LDL-C/HDL-C ratio. However, subjects with normal HDL-C levels decreased both their LDL-C and HDL-C resulting in an unchanged LDL-C/HDL-C ratio. We also observed significant differences in response to low-fat diets in HDL-C and α_1 concentrations between low and normal HDL-C subjects. In the normal HDL-C group, consumption of a low-fat diet also resulted in redistribution of apoA-I-containing HDL subpopulations, indicated by a decrease in the large apoA-I-only α_1 subpopulation. These data demonstrate that male subjects with low HDL-C respond to a low-fat diet differently than individuals with normal HDL-C.—Asztalos, B., M. Lefevre, L. Wong, T. A. Foster, R. Tulley, M. Windhauser, W. Zhang, and P. S. Roheim. **Differential response to low-fat diet between low and normal HDL-cholesterol subjects.** ***J. Lipid Res.*** **2000.** 41: **321–328.**

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Epidemiological studies have shown that the majority of heart attacks occur in normolipidemic individuals with low high density lipoprotein (HDL) cholesterol (≤35 mg/dL) (1–4). Among normolipidemic subjects, HDL-C levels vary greatly (5). About 15%–20% of the male population has low HDL-C (≤35 mg/dL).

High density lipoprotein concentrations are influenced by a number of mutations of lipoprotein transport genes (6). These mutations cannot explain the great variation in plasma HDL levels in the general population; however, family and twin studies suggest that genetic factors influence plasma HDL concentrations. Recently, mutations of the intracellular cholesterol transport ABC1 gene (7–9) in Tangier disease (10, 11) patients show that a single mutation could substantially affect plasma HDL concentration.

The major protein constituent of HDL is apoA-I. ApoA-I concentration can be influenced by genetic and environmental factors, diet, and exercise (6). A high-fat diet increases apoA-I concentration by increasing production rate and decreasing fractional catabolic rate (FCR) without altering apoA-I mRNA levels (12, 13); the major regulations of apoA-I levels occur post-transcriptionally. The main determinant of the HDL-C level is FCR rather than the rate of synthesis or transport rates of apoA-I. Size of HDL particle is important because the size correlates inversely with FCR. Any genes that potentially influence the size of HDL particles, such as lipoprotein lipase (LPL), hepatic lipase (HL), lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), and phospholipid transfer protein (PLTP), will affect FCR of HDL.

A low-fat diet is generally recommended for patients with coronary heart disease (CHD) (14, 15). Numerous studies have shown that a low-fat diet decreases plasma

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; apo, apolipoprotein; FCR, fractional catabolic rate; LPL, lipoprotein lipase; HL, hepatic lipase; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; PLTP, phospholipid transfer protein; CHD, coronary heart disease; 2DE, 2-dimensional gel electrophoresis; AAD, Average American Diet.

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total, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterols, apoA-I and apoB concentrations, and also increases triglyceride concentration (16–19). Because low-fat diet decreases HDL-C concentration, recommendations for its use have been questioned (20, 21).

High density lipoproteins are not homogeneous; they contain several subpopulations (22). Using nondenaturing two-dimensional gel electrophoresis (2DE), we separated 12 apoA-I-containing HDL subpopulations (22). Over 80% of HDL subpopulations have α electrophoretic mobility in the first dimension on agarose electrophoresis, and can be further separated on a nondenaturing polyacrylamide gradient gel into three distinct subpopulations according to size in the second dimension. The largest size (α_1) is an apoA-I-only particle corresponding approximately to the HDL_2 fraction (23). Smaller α_2 and α_3 particles correspond to HDL_3. In a previous study, we compared the distribution of HDL subpopulations in normolipidemic male subjects with low ($\leq$35 mg/dL) and normal (>35 mg/dL) HDL-C levels (23). We have shown that on an Average American Diet (AAD), healthy normolipidemic low HDL-C subjects have different concentrations of HDL subpopulations compared to normal HDL-C individuals. We observed that the HDL-α_1 subpopulation is lower and HDL-α_3 is higher in low HDL-C subjects compared to normal HDL-C individuals (23).

A decrease of the apoA-I-only LpA-I particle is associated with increased risk of CHD (24). As α_1 is an apoA-I-only particle, its decrease could be associated with increased risk of CHD. We also hypothesized that on a low-fat diet, the decrease of HDL-C concentration will occur mainly in subjects with normal HDL-C (>35 mg/dL). Therefore, in this study, we compared the response of plasma lipids/lipoproteins and HDL subpopulations to low-fat diets in healthy normolipidemic male subjects with low and normal HDL-C concentrations.

METHODS

Subjects

One hundred twenty male subjects (ages 22–65 years) were recruited to participate in a study examining the relationship between diet and risk factors for CHD. This study was based on a subset of 76 subjects who completed all diet periods. Participants were selected to have total plasma cholesterol and LDL cholesterol (LDL-C) between the 10th and 90th percentile, HDL-C above 25 mg/dL or below the 95th percentile, and triglycerides below the 95th percentile. Data were adjusted to that of NHANES II (25). Lipid cut-off points were selected to eliminate subjects with lipid disorders. Exclusion criteria included the presence of cardiovascular, renal, hepatic, endocrine, gastrointestinal or other systemic disease, body mass index (BMI) greater than 32 kg/m^2, and hypertension. During screening visits, physical examinations, blood chemistry, lipid profiles, as well as urinalyses, were performed. A history of drug or alcohol abuse was determined from medical questionnaires and psychological evaluations. Comparison of subjects at screening showed no difference (mean ± SE) in either age (37.9 ± 1.8 vs. 36.7 ± 1.5) or BMI (26.4 ± 0.8 vs. 25.0 ± 0.4) between low and normal HDL-C subjects.

All subjects indicated their willingness to participate in this study by signing the institutional approved consent form from the Pennington Biomedical Research Center (PBRC).

Diet and study design

The strength of this study is the use of a well-defined, rigorously controlled diet on a male-only population. The influence of low-fat diets on plasma lipids and apoA-I-containing HDL subpopulations was evaluated. The study randomized the sequence of three diets according to a double-blind, three-period crossover design. This design allowed each subject to be his own control.

Subjects were fed three diets differing in total fat and saturated fat content: *a*) an Average American Diet (AAD) containing 36.8% calories as total fat (14.1% saturated, 14.5% monounsaturated, 8.0% polyunsaturated, and 104 mg/1000 kcal cholesterol), 13.6% protein, and 49.6% carbohydrate; *b*) a diet similar to the National Cholesterol Education Program (NCEP) Step-1 diet containing 28.1% calories as fat (8.7% saturated, 11.5% monounsaturated, 7.9% polyunsaturated, and 76 mg/1000 kcal cholesterol), 13.9% protein, and 58.0% carbohydrate; *c*) a diet similar to the NCEP Step-2 diet containing 23.7% calories as fat (6.2% saturated, 9.7% monounsaturated, 7.8% polyunsaturated, and 63 mg/1000 kcal cholesterol), 14.4% protein, and 61.8% carbohydrate. Each day an extra complete meal was prepared for a "phantom subject." Prepared meals for each menu cycle were combined, composited, and chemically analyzed by the food chemistry laboratory of the Pennington Biomedical Research Center (PBRC). A 6-week-long diet period was chosen to enable stabilization of the lipoprotein endpoints (15). Data represent the sixth-week measurements of each diet period.

Participants were provided with all food during the study. Investigators were blinded as the samples were identified by clinic numbers. The subjects were also blinded as they were not told of their dietary assignments or dietary sequence. The different diets were outwardly identical with most of the changes in fat visibly hidden. The only difference the subjects could have perceived would have been changes in the volumes and proportion of food items. On weekdays, subjects consumed breakfast and dinner at the PBRC dining facility. Meal trays were inspected after each meal to ensure that all food items were consumed. Weekday packaged lunches were distributed at breakfast; evening snacks were distributed at dinner. A daily compliance questionnaire was administered to determine whether the subjects had eaten all their supplied food items, and whether they had consumed food items other than those provided. Weekend meals were packaged and distributed on Friday. Meals were prepared at four energy levels (2200, 2600, 3000, and 3400 kcal/day). One hundred kcal unit foods, similar in composition to the assigned diet, were used for energy adjustments. Subjects started on the energy level most closely matching their estimated energy requirement. Body weight was measured twice weekly. If a subject's weight differed from the initial level by more than 1 kg, the subject was transferred to another energy level or the number of unit foods was changed until the weight returned to within 1 kg of the initial value.

Processing blood

At the end of the 6-week dietary period, overnight fasting blood samples were collected in tubes containing 1.2 g/L EDTA and immediately placed on ice. Plasma was isolated by centrifugation at 30,000 *g* min and stored in liquid nitrogen. For two-dimensional electrophoresis (2DE), plasma samples were collected as described previously (22, 23).

Chemical methods

Serum total cholesterol, HDL-C, and triglycerides were measured using enzymatic assays on a Beckman Synchron CX5 auto-

mated chemistry analyzer. HDL-C was determined after precipitation of the non-HDL fractions by dextran sulfate [50,000 molecular weight; DMA] (26). Interassay coefficient of variation was less than 2%. ApoA-I and apoB were measured on a Beckman Array analyzer. Interassay coefficients of variation were less than 7%.

Low (≤35 mg/dL) and normal (>35 mg/dL) HDL-C categories were based on values obtained at the end of the AAD period.

HDL subpopulations

The 2DE for HDL subpopulations were carried out as previously described (22, 23). Plasma was frozen and stored in liquid nitrogen until samples were processed by 2DE. Four microliters of plasma was applied and run on agarose in the first dimension followed by electrophoresis into a 3% to 35% nondenaturing concave gradient polyacrylamide gel at 280 V for 24 h at 10°C. In this 2DE system, the agarose electrophoresis differs from the one generally used in that the agarose contains no albumin. Electrophoretic transfer, fixing, blocking, and immunolocalization were performed as described previously (22). Bound radioactivity was quantified by PhosphorImager analysis (27). Monospecific polyclonal anti-apoA-I antibodies used for these studies were produced in goats in our laboratory as previously described (22). For secondary antibody, F(ab′)2 fragments of anti-goat gamma globulins were used (Zymed). Subpopulations were characterized by: *1*) charge (preβ, α, and preα) based on their relative mobility with respect to albumin, and *2*) size determined from molecular weight standards run simultaneously in the same gel. With this system, we were able to separate and quantify apoA-I-containing subpopulations. Criteria for designation of HDL subfractions of α_1, α_2, and α_3 (22) are based on integration of α-migrating HDL. Three distinct peaks are observed, providing a basis for the designation of α_1, α_2, and α_3. Quantification was calculated based on total pixel volumes of the areas. Ninety percent of HDL can quantitatively be recovered after 2DE with less than 8% of interassay coefficient of variation for the α migrating subpopulation (22, 23).

It should be considered that values presented in the absolute concentration of HDL subpopulations are the result of determination of percent distribution of HDL subpopulations (total pixel volumes of areas) multiplied by apoA-I concentrations. Therefore, a decrease of apoA-I concentration will influence absolute values. In our case, we observed a decrease in α_1 and an increase of α_3 percent distribution (data not shown) in normal HDL subjects.

Statistical analysis

Descriptive statistics (scatterplots, means, and standard errors) were examined and data were transformed to Gaussian distributions when appropriate. Analysis of variance (ANOVA), corresponding to the crossover design (28, 29), verified the designed absence of carry-over effects as well as the absence of interaction between diet and time. The subsequent reduced model ANOVA tested the hypothesis of no difference in response to the three dietary regimens and to dietary regimens within low and normal HDL-C groups. After ANOVA, planned (a priori) comparisons tested the hypothesis of no difference between AAD and each of the low-fat diets. Subjects were classified into HDL-C groups after collection of samples but before analysis. All analyses were developed using SAS® (30).

RESULTS

The influence of low-fat diets was studied in normolipidemic male subjects. **Table 1** compares mean lipid and apoprotein levels among three diets (AAD, Step-1, and Step-2) for all subjects combined. Compared to subjects on AAD, Step-1 and Step-2 diets significantly decreased the concentrations of plasma total cholesterol, LDL-C, HDL-C, apoA-I, and apoB and increased triglycerides. On the Step-2 diet, a 10% decrease of HDL-C was observed. To illustrate that on a low-fat diet there is a substantial increase of subjects with low HDL-C (≤35 mg/dL), we obtained a histogram of HDL-C concentration on AAD and on the Step-2 diet (**Fig. 1**). This figure shows that when subjects consumed the Step-2 diet, there was an increase in subjects with low HDL-C (<35 mg/dL). On AAD, 33% of subjects had low HDL-C levels (≤35 mg/dL); however, on the Step-2 diet, the majority of subjects (55%) had low HDL-C levels, which is associated with increased risk for CHD (1–4). This decrease of HDL-C occurred mostly in subjects with normal HDL-C concentration (data not shown).

TABLE 1. Plasma lipids and apolipoproteins by diet

Variable	n	AAD	Step-1	Step-2
			mg/dl	
TC	75	185.4 ± 3.5	176.3 ± 3.1[c]	168.0 ± 3.3[c]
HDL-C	76	40.7 ± 1.0	38.1 ± 1.0[c]	36.8 ± 0.9[c]
LDL-C	74	125.6 ± 3.0	115.8 ± 2.8[c]	109.2 ± 2.6[c]
TG[d]	75	93.6 ± 7.4	106.8 ± 7.4[c]	111.1 ± 8.1[c]
ApoA-I	77	121.7 ± 1.6	116.8 ± 1.5[c]	114.4 ± 1.5[c]
ApoB	77	96.3 ± 2.3	92.7 ± 2.2[b]	89.1 ± 2.3[c]
LDL-C/HDL-C[e]	73	3.23 ± 0.10	3.20 ± 0.10	3.10 ± 0.10[a]
HDL-C/ ApoA-I[d,e]	76	0.333 ± 0.005	0.325 ± 0.006[a]	0.320 ± 0.006[b]

Values given as means ± SE. To convert values for cholesterol to mmol/L, divide by 38.67. To convert values for triglycerides to mmol/L, divide by 88.54.

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.0001$: planned (a priori) comparisons test (Step-1–AAD) and (Step-2–AAD).

[d] Log (1n) transformed for analysis.

[e] Unitless quantity.

Considering our earlier observation that low HDL-C subjects have different distributions of HDL subpopulations compared to individuals with higher HDL-C levels (23), we evaluated the response to low-fat diets separately in subjects with low (≤35 mg/dL) and normal (>35 mg/dL) HDL-C.

Table 2 shows lipid and apoprotein levels on the three diets in low (≤35 mg/dL) and normal (>35 mg/dL) HDL-C individuals. The response to low-fat diets in plasma total cholesterol and LDL cholesterol values was similar both in low and normal HDL-C groups. In the normal HDL-C group, consumption of Step-1 and Step-2 diets resulted in a significant decrease, both in HDL-C and apoA-I concentrations. However, in the low HDL-C group, HDL-C and apoA-I did not decrease on the Step-1 diet, and decreased only slightly on the Step-2 diet. On low-fat diets, LDL-C decreased and triglyceride concentration increased in both groups.

The low-fat diet also differentially influenced the atherosclerotic index in normal and low HDL-C individuals (Table 2). In low HDL-C subjects, consumption of the low-fat diet resulted in an improvement of the atherosclerotic

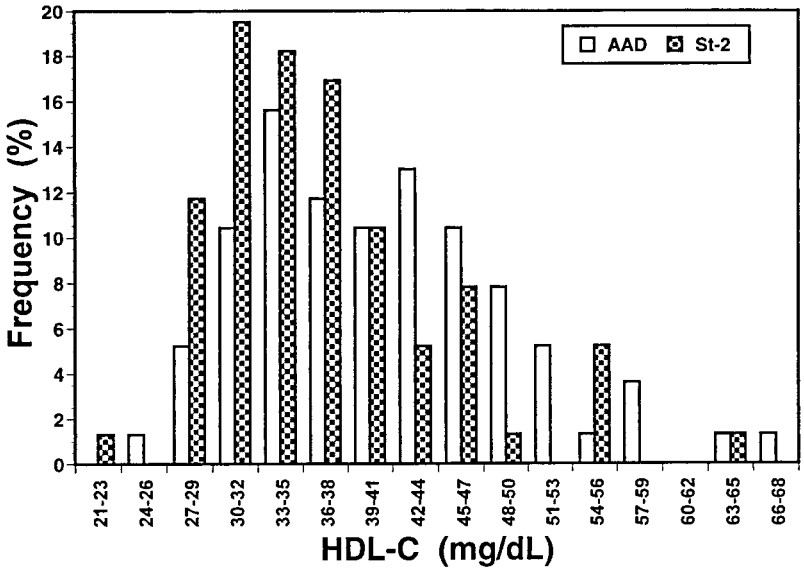

Fig. 1. Histogram of HDL-C.

index; the LDL-C/HDL-C ratio of low HDL-C subjects decreased significantly. Normal HDL-C individuals did not change their LDL-C/HDL-C ratio, as both LDL-C and HDL-C decreased. A decrease in the HDL-C/apoA-I ratio was also observed in normal HDL-C subjects both on Step-1 and Step-2 diets, suggesting a change in the chemical composition of HDL subpopulations.

Compared to normal HDL-C subjects, low HDL-C subjects had a decrease in the HDL-α_1 subpopulation, an apoA-I-only particle (23). As the low-fat diet decreases HDL-C concentrations, we evaluated whether the low-fat diet would alter the distribution of HDL subpopulations. The influence of the low-fat diet on the distribution of HDL subpopulations in a representative subject with normal HDL-C is illustrated in **Fig. 2**. As this subject changed from AAD to the Step-2 diet, there was a specific decrease in the largest α_1 HDL particle.

A histogram of the α_1 subpopulation on AAD and the Step-2 diet is presented in **Fig. 3** for the combined samples. On a low-fat diet, concentration of the α_1 subpopulation shifted towards lower levels. The distribution of α_1 concentrations peaked between 14–15 mg/dL on the Step-2 diet compared to the peak observed between 22–25 mg/dL on AAD. The decrease of α_1 occurred only in subjects with normal HDL-C (data not shown).

Table 3 shows the differential response to low-fat diets in HDL subpopulations between individuals with low and normal HDL-C. We observed a decrease in the concentra-

TABLE 2. Plasma lipids and apolipoproteins by diet and HDL-C category

Variable	AAD Level of HDL-C	n	AAD	Step-1	Step-2
	mg/dl			*mg/dl*	
TC	≤35	25	181.7 ± 5.9	173.9 ± 4.9	163.3 ± 5.9[c]
	>35	50	187.2 ± 4.5	177.4 ± 4.0[c]	170.3 ± 3.9[c]
HDL-C	≤35	25	31.8 ± 0.6	31.2 ± 0.9	30.5 ± 0.7[a]
	>35	51	45.1 ± 1.0	41.5 ± 1.1[c]	39.9 ± 1.0[c]
LDL-C	≤35	25	124.0 ± 4.9	113.9 ± 3.9[b]	105.2 ± 4.4[c]
	>35	48	126.5 ± 3.9	117.1 ± 3.8[c]	111.4 ± 3.3[c]
TG[d]	≤35	24	128.9 ± 17.5	140.9 ± 17.7	143.4 ± 18.4
	>35	50	77.7 ± 5.9	91.5 ± 6.0[c]	96.7 ± 7.4[c]
ApoA-I	≤35	25	108.8 ± 1.7	106.6 ± 1.8	104.0 ± 1.9[b]
	>35	51	127.7 ± 1.7	121.4 ± 1.7[c]	119.1 ± 1.6[c]
ApoB	≤35	25	99.2 ± 3.7	96.1 ± 3.5	90.9 ± 3.6[c]
	>35	51	95.0 ± 3.0	91.4 ± 2.8[a]	88.5 ± 3.0[c]
LDL-C/HDL-C[e]	≤35	25	3.92 ± 0.15	3.71 ± 0.15	3.49 ± 0.16[b]
	>35	48	2.87 ± 0.10	2.93 ± 0.12	2.89 ± 0.11
HDL-C/ApoA-I[d,e]	≤35	25	0.293 ± 0.005	0.293 ± 0.007	0.291 ± 0.007
	>35	51	0.353 ± 0.005	0.340 ± 0.006[b]	0.335 ± 0.007[c]

Values given as means ± SE.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; planned (a priori) comparisons test (Step-1–AAD) and (Step-2–AAD).
[d] Log (1n) transformed for analysis.
[e] Unitless quantity.

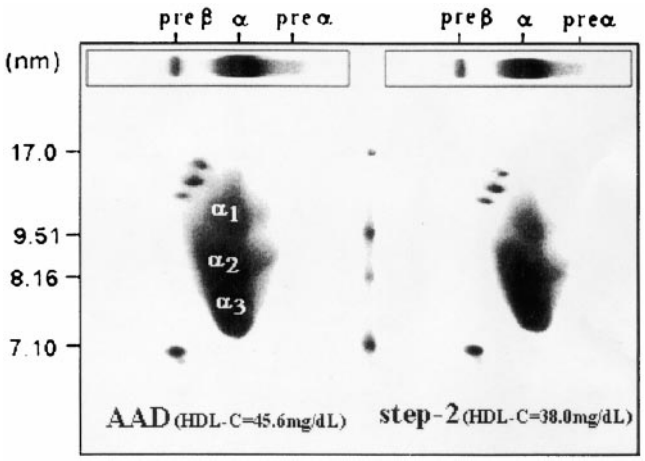

Fig. 2. A low-fat diet alters the distribution of HDL subpopulations. 2DE pattern of a representative subject with normal HDL-C 45.6 mg/dL on AAD and on the Step-2 diet 38.0 mg/dL. Plasma was electrophoresed in the first dimension in agarose followed by application of the agarose strip to the top of a nondenaturing 3–35% polyacrylamide gel and subsequently electrophoresed. In the middle, ^{125}I-labeled Pharmacia high-molecular-weight standard was applied. The horizontal insert on top represents apoA-I distribution on a duplicate agarose strip.

TABLE 3. Absolute concentration of alpha subpopulations by diet and HDL-C category

Subpopulation	AAD Level of HDL-C	n	AAD	Step-1	Step-2
	mg/dl			*mg/dl*	
α_1 ApoA-I[d]	≤35	22	15.1 ± 0.9	15.9 ± 0.8	14.6 ± 0.7
	>35	46	24.5 ± 0.9	22.3 ± 0.9[c]	21.2 ± 0.8[c]
α_2 ApoA-I	≤35	22	33.9 ± 1.1	32.8 ± 1.3	31.7 ± 1.0[a]
	>35	46	41.8 ± 0.8	39.9 ± 0.7[b]	38.6 ± 0.8[c]
α_3 ApoA-I[d]	≤35	22	35.2 ± 1.5	33.1 ± 1.4[a]	33.9 ± 1.5
	>35	46	33.3 ± 1.1	32.1 ± 1.1	34.2 ± 1.1

Values given as means ± SE.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; planned (a priori) comparisons test (Step-1–AAD) and (Step-2–AAD).
[d] (1n) transformed for analysis.

tion of the α_1 and α_2 subpopulations, both on Step-1 and Step-2 diets in normal HDL-C subjects. In contrast, the concentration of α_1 HDL subpopulations did not change in low HDL-C subjects. No statistically significant change was observed in preβ in either group while preα_1 decreased.

Differences in response to the low-fat diet between low and normal HDL-C groups can be better demonstrated by comparing their response to low-fat diets (**Table 4**). A significant difference ($P < 0.001$) was observed in response to the low-fat diet in the decrease of HDL-C and α_1 between low and normal HDL-C groups. On low-fat diets, the changes with respect to total cholesterol, LDL-C, triglycerides, and apoB were similar in the normal and low HDL-C groups. We also compared these differences in response to the atherosclerotic index (LDL-C/HDL-C) and found a statistically significant improvement (decrease) of the LDL-C/HDL-C ratio in low HDL-C subjects [a decrease of −0.43 compared to an increase of 0.02 in normal HDL-C individuals ($P < 0.005$)].

DISCUSSION

Data from the Framingham study (2) as well as data from Assmann et al. (31) showed that the incidence of CHD increased in subjects with low HDL-C despite normal total cholesterol (<200 mg/dL) or LDL-cholesterol (<135 mg/dL). Clinical trials and epidemiological studies suggest that an increase of 1 mg/dL HDL-C results in a 2–3% decrease in coronary risk while an increase of 1 mg/dL LDL-C results in a 1% increase in the risk of CHD. Thus, every mg/dL change in HDL-C concentration provides a 3-fold greater contribution to the prediction of CHD than does a 1 mg/dL change of LDL-C (1, 4, 14, 18).

A number of studies describe the influence of a low-fat diet on lipid response (13–16) showing that both LDL and HDL cholesterols decrease. Concomitant decreases

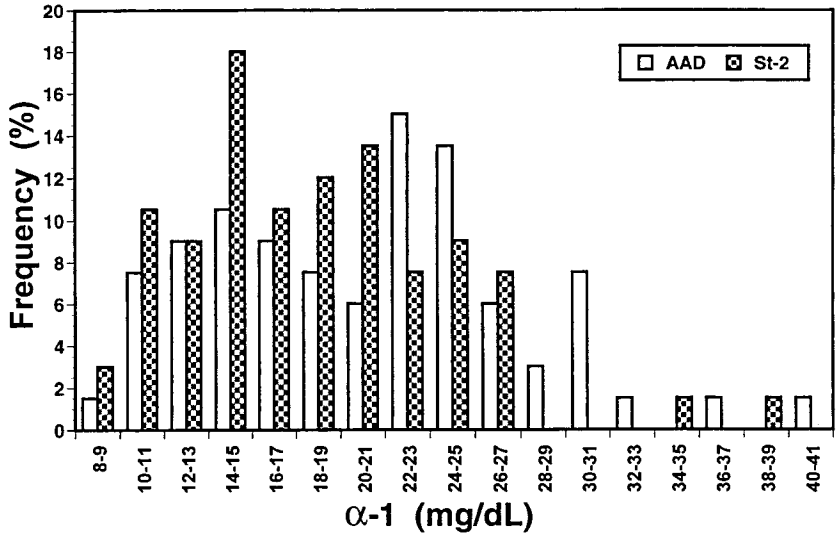

Fig. 3. Histogram of HDL-α_1 subpopulations.

TABLE 4. Difference in response to low-fat diet between normal and low HDL-C subjects

Variable	Step-1 ⩽35 (n = 22–25)	Step-1 >35 (n = 46–51)	Step-2 ⩽35 (n = 22–25)	Step-2 >35 (n = 46–51)
	mg/d1		*mg/d1*	
TC	−7.8 ± 3.3	−9.8 ± 2.8	−18.4 ± 4.6	−16.9 ± 2.5
HDL-C	−0.6 ± 0.9	−3.6 ± 0.6[a]	−1.2 ± 0.6	−5.2 ± 0.7[b]
LDL-C	−10.2 ± 3.0	−9.4 ± 2.7	−18.9 ± 3.7	−15.1 ± 2.1
TG	12.0 ± 12.1	13.8 ± 4.4	14.5 ± 8.9	19.0 ± 4.4
ApoA-I	−2.2 ± 1.8	−6.4 ± 1.6	−4.8 ± 1.6	−8.6 ± 1.6
ApoB	−3.1 ± 1.9	−3.7 ± 1.5	−8.3 ± 2.3	−6.5 ± 1.5
α_1	0.9 ± 0.7	−2.2 ± 0.6[a]	−0.5 ± 0.6	−3.3 ± 0.6[a]
α_2	−1.1 ± 1.3	−1.8 ± 0.7	−2.2 ± 0.9	−3.2 ± 0.7
α_3	−2.1 ± 0.9	−1.1 ± 0.9	−1.3 ± 0.9	0.9 ± 0.9
LDL-C/HDL-C	−0.20 ± 0.11[a]	0.06 ± 0.07	−0.43 ± 0.13[c]	0.02 ± 0.06

Lipid and lipoprotein differences in response were calculated for each subject when diets were changed from AAD to low fat; means ± SE are shown.

[a] $P < 0.01$; [b] $P < 0.001$; [c] $P < 0.005$; *t*-test for independent samples.

of LDL-C and HDL-C concentrations led to a questioning of the beneficial effects of a low-fat diet (17). Our results from the combined populations (Table 1) are consistent with these findings.

We observed that on AAD, normolipidemic low HDL-C subjects (<35 mg/dL) have different distributions of apoA-I-containing HDL subpopulations than individuals with normal HDL-C (>35 mg/dL) (23). On the basis of these data, we postulated that feeding a low-fat diet would elicit a differential response in subjects with different levels of HDL-C. We hypothesized that as subjects with low HDL-C already have low HDL concentrations, the major decrease of HDL-C would occur in subjects with normal HDL.

Because diet influences the concentration of HDL, it was important that classification be done in a steady-state dietary regimen. In this study, classification of subjects into low and normal HDL-C groups was based on HDL-C determinations obtained after consuming an AAD for 6 weeks.

We compared changes in lipid–lipoprotein profiles separately (Table 2) of individuals with low HDL-C and normal HDL-C when a low-fat diet was consumed. In the low HDL-C subjects, levels of HDL-C and apoA-I slightly decreased while α_1 remained unchanged and LDL-C decreased. However, in normal HDL-C individuals, LDL-C, HDL-C, apoA-I, and α_1 HDL subpopulations decreased. The decrease of HDL-C and apoA-I in the low HDL-C group was observed only on the Step-2 diet and was small compared to the substantial decrease in the normal HDL-C group (Table 2). When the difference in response (Table 4) between low and normal HDL-C groups was compared, a significant difference was observed in HDL-C and α_1 concentrations only in subjects with normal HDL-C.

Studies suggest that increased risk of CHD is associated with a decrease of the large HDL_2 (32) and apoA-I-only particles (24). Low HDL-C subjects have a decrease in α_1 concentration (23), the largest HDL subpopulation (22). According to our data, α_1 is an apoA-I-only particle (23). At the present time, we do not know whether the increased incidence of CHD in low HDL-C subjects is associated with a decrease of α_1 subpopulations. It is possible that increased risk of CHD is associated not only with lower levels of HDL-C but also with a greater decrease in α_1 concentrations. Our recent data from patients undergoing angiography has suggested that subjects with advanced atherosclerosis have a disproportional decrease of α_1 compared to HDL or apoA-I (B. F. Asztalos, R. Milani, L. Wong, E. Schaefer, and P. S. Roheim, unpublished results).

The mechanism responsible for the differential response of normal and low HDL-C subjects to a low-fat diet may be explained by the regulation of the ABC1 gene. Recently, it has been reported that in Tangier's disease (10, 11), the ABC1 gene influencing intracellular cholesterol transport is mutated (7–9, 33). It is possible that consuming a low-fat diet will result in the down-regulation of the ABC1 gene in subjects with normal HDL-C. This hypothesis is testable as it has been shown that this gene can be modulated (34). More studies on the role of this gene will advance our understanding of the regulation of HDL levels.

Another mechanism for the explanation of our finding is differences in hepatic lipase and CETP activities between normal and low HDL-C subjects when a low-fat diet is consumed. Hepatic lipase activity is inversely proportional to HDL-C concentration and influences the HDL_2 fraction (35–37), which corresponds approximately to the α_1 subpopulation. Increased CETP activity has been shown in subjects with low HDL-C (38). Consumption of a low-fat diet results in an increase in TG concentration in subjects with normal HDL-C favoring enhanced cholesteryl ester transfer by CETP (39). Thus, increased CETP and hepatic lipase activity after consumption of a low-fat diet in normal HDL subjects could be responsible for the decreased concentration of HDL-α_1 subpopulation. Cholesteryl ester transfer also results in redistribution of HDL subpopulations resulting in a decrease of large cholesterol-rich HDL particles (α_1). The fractional catabolic rates of apoA-I correlate inversely with HDL size (39, 40). Therefore, as a consequence of the decrease in the large HDL (α_1) particle, there is an increase in the fractional catabolic rate of HDL resulting in a decrease in apoA-I concentration (40, 41). Our data show that in normolipi-

demic individuals with normal HDL-C levels, consumption of a low-fat diet produces an increase of small HDL particles, relatively poor in cholesterol, manifesting in the decrease of the HDL-C/apoA-I ratio.

Phospholipid transfer protein activity may also be responsible for the observed changes as it favors the formation of larger-sized cholesterol-rich particles (42). Decrease of phospholipid transfer protein activity may result in a decrease of formation of larger-sized cholesterol-rich HDL particles as well as a decrease in HDL-C concentration.

In this study, we compared the response to low-fat diets of subjects with low and normal HDL-C. Low-fat diets resulted in a significant decrease in HDL-C both on Step-1 and Step-2 diets in subjects with normal HDL-C (Table 2). Low HDL-C subjects did decrease their HDL-C on the Step-2 diet slightly. We believe that this decrease is clinically insignificant. The atherosclerotic index LDL-C/HDL-C (Table 2) did not change in individuals with normal HDL-C; while in low HDL-C subjects, it decreased significantly ($P < 0.01$) from 3.92 to 3.49 (11%). In addition, low HDL-C subjects did not decrease their α_1 concentration (Table 3). When we compared the response to low-fat diets (Table 4), we observed a significant difference between low and normal HDL subjects in HDL-C and in α_1 concentrations. These data suggest that a low-fat diet is not uniformly beneficial.

Our data suggest that plasma HDL concentration is an important consideration in recommending low-fat diets. However, we believe additional studies will be desirable to confirm this observation. These studies were conducted on healthy, normolipidemic male subjects and the low-fat diet in this study was achieved mainly through a decrease in saturated fat content. Valuable information would be gained if these studies would be replicated by substituting the decreased saturated fat in the diet with monounsaturated fat and comparing the lipid/lipoprotein responses of normal and low HDL-C subjects.

The applicability of these findings to women, both pre- and post-menopausal, should also be evaluated. It would also be important to evaluate whether, under hyperlipidemic conditions, HDL-C concentrations might also be included in the guidelines for recommending a low-fat diet.

We conclude that an isocaloric low-fat diet is beneficial to normolipidemic, low HDL-C subjects, but is equivocal for individuals with normal HDL-C.

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