Efficacy of a Therapeutic Lifestyle Change/Step 2 diet in moderately hypercholesterolemic middle-aged and elderly female and male subjects

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Abstract **Lifestyle modification to decrease cardiovascular disease (CVD) risk has recently been reaffirmed by both the National Cholesterol Education Program and American Heart Association (AHA). Using a randomized crossover design, the Therapeutic Lifestyle Change (TLC)/Step 2 diet relative to a typical Western diet was assessed in 36 moderately hypercholesterolemic subjects in a clinical setting under isoweight conditions. Mean lipoprotein and apolipoprotein levels (fasting and non-fasting), fatty acid profiles, parameters of HDL metabolism, and glucose homeostasis were determined. Relative to the Western diet, the TLC/Step 2 diet resulted in 11% and 7% lower LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C), respectively, with no significant change in TG levels or total cholesterol-HDL-C ratio. Similar responses were observed in the non-fasting state. Linoleic (18:2n-6c) and α-linolenic (18:3n-3) acids increased at the expense of oleic acid (18:1n-9c) in the cholesteryl ester, TG, and phospholipid subfractions. The dietary changes had no significant effect on fractional esterification rate of HDL, phospholipid transfer protein (PLTP), or cholesterol ester transfer protein activities, or glucose and insulin levels. Female and male subjects responded similarly. The TLC/Step 2 diet resulted in a decrease in some CVD risk factors and no apparent adverse effects in others.**—Lichtenstein, A. H., L. M. Ausman, S. M. Jalbert, M. Vilella-Bach, M. Jauhiainen, S. McGladdery, A. T. Erkkilä, C. Ehnholm, J. Frohlich, and E. J. Schaefer. **Efficacy of a Therapeutic Lifestyle Change/Step 2 diet in moderately hypercholesterolemic middle-aged and elderly female and male subjects.** ***J. Lipid Res.*** **2002.** 43: **264–273.**

Supplementary key words therapeutic lifestyle change diet • Step 2 diet • saturated fat • cholesterol • low-density lipoprotein cholesterol • high-density lipoprotein cholesterol • fatty acids • diet • hypercholesterolemia

Dietary modification to reduce cardiovascular disease (CVD) risk remains the cornerstone of both the National Cholesterol Education Program (NCEP) and American Heart Association (AHA) recommendations for the treatment of hyperlipidemic individuals (1, 2). The recently revised dietary component of both organizations' guidelines center on reducing saturated fat and cholesterol intakes. These recommendations have evolved over time and are based on data from epidemiological observations, animal studies, and clinical trials (3–6). Current dietary recommendations for hypercholesterolemic individuals are to consume a diet meeting the following criteria: <7% of energy from saturated fat, <200 mg cholesterol per day, and 25% to 35% of energy from fat (1, 2).

Although specific criteria for intervention and outcome goals are predicated on the basis of LDL cholesterol (LDL-C) levels, dietary modification consistent with current recommendations frequently alters additional parameters that impact both positively and negatively on disease risk. These might include postprandial lipoprotein patterns (7, 8), HDL cholesterol (HDL-C) levels (9, 10), TG levels (11, 12), circulating fatty acid profiles (13, 14) and glucose homeostasis (15), and can directly impact on the overall therapeutic value of the intervention(s). For example, the physiological value of decreasing LDL-C levels, in the absence of a decrease in the total cholesterol-HDL-C ratio, has been questioned (16). Similarly, potential in-

Abbreviations: AHA, American Heart Association; CVD, cardiovascular disease; FER_{HDL}, cholesterol sterification rate; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Lp[a], lipoprotein [a]; NCEP, National Cholesterol Education Program; PC, phosphatidylcholine; PLTP, phospholipid transfer protein, TLC, therapeutic lifestyle change, VLDL-C, very low density lipoprotein cholesterol.

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creases in both fasting and non-fasting TG levels and a deterioration of glucose homoeostasis resulting from lower fat diets have been the subject of concern (17).

Assessing the efficacy of dietary modifications intended to reduce disease risk in humans and relate these findings to public health recommendations is a complicated undertaking. A multitude of factors such as age, gender, genetic make-up, initial serum cholesterol levels, and habitual diet can impact either individually or together on study outcomes and strongly influence the final conclusion. Hence, the efficacy of dietary modification to reduce CVD risk has been reported to be variable (18–20). The current study was designed to assess the utility of shifting older individuals with moderately elevated LDL-C levels from a diet similar to that consumed in Westernized countries to a diet consistent with current recommendations using commonly available foods. We investigated the effect of implementing such changes on a range of parameters associated with disease risk, lipoprotein profiles in the fasted and non-fasted state, measures of HDL metabolism, glucose and insulin levels, and fatty acid profiles. During the course of this work, the terminology changed from a Step 2 diet to the Therapeutic Lifestyle Change (TLC) diet. Therefore, both designations are used.

MATERIALS AND METHODS

Subjects

Thirty-six subjects (18 women and 18 men) with a mean age of 64 years (age range, 55 to 74 years) were selected to have LDL-C levels greater than 130 mg/dl (3.36 mmol/l) while consuming their usual diets. All subjects provided a medical history, underwent a physical examination, and had clinical chemistry analysis performed prior to enrollment. The subjects had no evidence of any chronic illness, including hepatic, renal, thyroid, or cardiac dysfunction. They did not smoke and were not taking medications known to affect plasma lipid levels (lipid-lowering drugs, β-blockers, diuretics, or hormones) or vitamin and/or mineral supplements. By design, subjects were older than 50 years, and all women were postmenopausal and not taking hormone replacement therapy. The study was approved by the Human Investigation Review Board of Tufts University and New England Medical Center, and all subjects gave informed consent prior to the start of the study.

Study design

This study was composed of two 32-day phases with a minimum interval of 2 weeks between diet phases during which period the subjects consumed either their habitual diets ad libitum or an alternate experimental diet. All subjects were provided with a diet designed to approximate that consumed by those individuals not complying with current dietary recommendations (termed Western diet) and a diet consistent with the NCEP (Adult Treatment Panel) (1) and AHA (2) recommendations (termed TLC/Step 2 diet) in randomized order. Previous work in this laboratory has indicated that under the specified study conditions, plasma lipid levels at the end of each 5-week period were independent of diet order or intervening phases (21). Additional diets were included in the randomization scheme but were not included in this analysis and addressed a different experimental question (22). The subjects were encouraged to maintain their habitual level of physical activity throughout the study period.

All food and drink were prepared and provided by the metabolic research unit of the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University for consumption on site, or were packaged for take-out. Packaging in containers appropriate for conventional or microwave ovens obviated the need to transfer food that could have resulted in losses. Subjects were required to report to our metabolic research unit at least four times per week, have their blood pressure and body weight measured at each visit, and eat at least one meal on-site. They were required to consume all that was provided to them and not supplement it with any food or drink with the exception of water and non-caloric beverages. During the final week of each diet phase, three fasting blood samples were obtained for lipid and apolipoprotein determinations. Once during the final week of each diet phase subjects consumed their three meals at standardized intervals, and one 4-h postprandial blood sample was obtained after the supper meal. Breakfast was served just after 0 h (8 AM), lunch at noon, and supper at 5 PM.

Study diets

The Western diet was designed to provide 15% of calories as protein, 47% carbohydrate, and 38% fat (16% saturated, 16% monounsaturated, and 6% polyunsaturated), and 180 mg cholesterol per 1,000 kcal. The TLC/Step 2 diet was designed to provide 15% of calories as protein, 58% carbohydrate, and 30% fat (7% saturated, 10% monounsaturated, and 10% polyunsaturated), and 75 mg cholesterol per 1,000 kcal. The diets were given number designations and the investigators, laboratory personnel, and study subjects were blinded as to the identity of the diets. Initial caloric levels were estimated using the Harris-Benedict formula and were adjusted, when necessary, to maintain the body weight. The mean daily caloric intake for the females was 2,105 ± 314 kcal (range, 1,900 to 2,500 kcal) and for the males was 2,803 ± 506 kcal (range, 2,000 to 4,250 kcal). At the beginning and end of the Western diet phase, body weights were 79.4 kg and 79.1 kg, respectively, and for the TLC/Step 2 diet phase were 79.4 kg and 79.1 kg, respectively.

Biochemical measurements

Fasting (14 h) blood samples were collected in tubes containing EDTA (0.15 percent final concentration). Non-fasting blood samples were collected in a similar manner 4 h after the evening meal in a subset of the study cohort (26 subjects; 13 female, 13 male). Plasma was separated by centrifugation at 1,100 × *g* at 4°C. VLDL was isolated from serum by ultracentrifugation at 109,000 × *g*, 4°C according to Lipid Research Clinics methodology (23). A modification of the dextran sulfate-magnesium chloride method was used to determine the concentration of HDL-2 and HDL-3. This allowed for the sequential precipitation of apolipoprotein B (apoB) containing lipoproteins (VLDL, IDL, and LDL), and then in a separate step, HDL-2 (24). Plasma, the 1.006 g/ml infranatant, HDL, and HDL-3 were assayed for total cholesterol and/or TG with a Spectrum CCX bichromatic analyzer (Abbott Diagnostics) using enzymatic reagents (25). Lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control, Atlanta, GA. Plasma glucose concentrations were determined using an enzymatic assay (Roche Laboratories, New Jersey, NY) and plasma insulin levels were measured using a human-insulin specific competitive binding radioimmunoassay (Linco Research Inc, St. Louis, MO).

Apolipoprotein A-I (apoA-I) and apoB were measured by immunoturbidometric assays using an Abbott Spectrum analyzer with reagents and calibrators from INCSTAR (Stillwater, MN) (26, 27). Within-run and between-run coefficients of variation of these assays were both less than 2% for apoB and 2.5% for apoA-I. Levels of apolipoprotein A-II (apoA-II) and apoA-I in particles

without apoA-II were measured by an elecroimmunodiffusion technique using commercially available agarose gels with polyclonal anti-apoA-II incorporated into the gels (Laboratories Sepia, France) (28). Levels of apoA-I in particles with apoA-I and apoA-II were calculated by difference. The coefficients of variation between runs for both measurements were 4% and 10%, respectively. Within each run, the coefficient of variation was approximately 4% for apoA-I and 7% for apoA-II. Lipoprotein [a] (Lp[a]) was quantified using an enzyme-linked immunoassay taking advantage of a monoclonal antibody as the first antibody that does not cross-react with plasminogen, and a polyclonal antibody as the second antibody directed against the apo[a] portion of the Lp[a] particle (Wampole Lab., Cranbury, NJ) (29).

Enzymes assays

The activity of CETP was measured in plasma after removal of endogenous VLDL and LDL by phosphotungstate and magnesium chloride precipitation as described previously (30).

Phospholipid transfer protein (PLTP) activity in plasma was quantified by assessing the transfer of radioactively labeled phosphatidylcholine (PC) in PC-liposomes to HDL-3 according to the method of Damen et al. (31) with minor modifications (32, 33).

Fractional esterification rate of HDL was determined by an isotopic assay method (34, 35). ApoB containing lipoproteins were precipitated from the serum with phosphotungstic acid and magnesium chloride. A trace amount of tritiated cholesterol was applied to a paper disk and incubated with the sample to allow for spontaneous transfer, and after incubation the radioactivity in the free and esterified cholesterol fractions was quantified. The cholesterol sterification rate (FER_{HDL}) was calculated as the percentage of label found in cholesteryl ester relative to the total radioactivity in the sample.

Fatty acid profiles of lipid subfractions

The procedure for fatty acid analysis has been described previously (36). Briefly, serum samples were extracted in chloroform-methanol (2:1, v/v) and the cholesteryl ester, TG, and phospholipid fractions were separated by solid-phase extraction with aminopropyl columns. The fatty acids from each fraction were transmethylated with 14% boron trifluoride in methanol and analyzed using a gas chromatograph (HP 5890 Series II; Hewlett-Packard Company, Waldbronn, Germany) equipped with an HP-FFAP capillary column (25 m, 0.20 mm i.d., 33 μm film thickness; Hewlett-Packard, Palo Alto, CA) for analysis of fatty acids other than *trans* and with a fused-silica capillary column (100 m, 0.25 mm i.d., 0.33 μm film thickness; Supelco, Bellefonte, PA) for analysis of *trans* fatty acids. The molar percentage proportions of fatty acids in serum lipid fractions were calculated.

Statistical analysis

The data were entered into a spreadsheet and exported for analysis by using SAS for Windows, version 8.1 (SAS Institute, Cary, NC). Prior to the analysis, descriptive statistics and graphs (PROC UNIVARIATE and PROC MEANS) were used to summarize the overall effects of diets and distributions of the outcome measures. When violations of the basic testing assumptions were noted, square root or logarithmic transformations of the data were used. Paired *t*-tests were carried out for each outcome measure.

RESULTS

The nutrient compositions of the diets as determined by chemical analysis of the food were similar to the target values (**Table 1**). The fatty acid profile of the TLC/Step 2 diet met the criteria for saturated fat and monounsaturated fat, whereas it exceeded the recommendation for polyunsaturated fat by 2%. This resulted from the decision to use a single source of added fat, soybean oil, to adjust the total fat content of the diet while keeping the saturated fat content within TLC/Step 2 guidelines. The cholesterol levels of the diets were somewhat less than anticipated, likely due to discrepancies in the food tables as has previously been reported (37). Diverging from the TLC/Step 2 guidelines, dietary cholesterol was calculated for individual subjects on the basis of total caloric intake rather than total amount of cholesterol per day to achieve more dietary uniformity among study subjects given the metabolic nature of the study design. This resulted in mean cholesterol intakes of 345 ± 51 and 460 ± 83 mg/day for female and male subjects, respectively, while subjects consumed the Western diet; and 139 ± 21 and 185 ± 33 mg/day for female and male subjects, respectively, while subjects consumed the TLC/Step 2 diet. These cholesterol intakes were consistent with current population intakes during the Western diet period and with NCEP recommendations during the TLC/Step 2 diet period, with the exception of the three male subjects whose daily energy intake exceeded 3,030 calories. As would be predicted on the basis of displacing fat with carbohydrate containing foods, the fiber content of the TLC/Step 2 diet was higher than that of the Western diet.

TABLE 1. Nutrient composition of diets as determined by chemical analysis of food[a]

Nutrient	Western	Step 2	Difference
Total energy, mean, calories			
Protein	17	16	−1
Carbohydrate	45	56	+11
Fat	39	28	−11
SFA	15	7	−8
MUFA	15	8	−7
PUFA	8	12	+4
Dietary cholesterol level mean, mg/1,000 calories	164	66	−98
Dietary fiber mean, g/1,000 calories	10	16	+6

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids.
[a] Percentages may not equal 100 because of rounding.

By design, the study subjects were middle-aged and elderly women and men, with moderately elevated LDL-C levels (**Table 2**). Both female and male subjects had somewhat elevated body mass indexes, characteristic of older individuals presenting with moderate hypercholesterolemia. Prior to the beginning of the controlled feeding period, the mean serum cholesterol concentration was 245 ± 33 mg/dl (6.34 ± 0.85 mmol/l), and the LDL-C concentration was 167 ± 28 mg/dl (4.32 ± 0.72 mmol/l), indicating that the subjects participating in the study were from a subset of the population who are prime targets for dietary modification. Otherwise, the characteristics of the study subjects were unremarkable. The female subjects had somewhat higher HDL-C levels and more favorable total cholesterol-HDL-C ratios than the male subjects.

TABLE 2. Characteristics of the subjects at the time of screening for the study

Variable	Females n = 18	Males n = 18	All n = 36
Age (years)[a]	67 ± 4	60 ± 7	63 ± 6
Body mass index (kg/m^2)	26.6 ± 2.4	28.1 ± 3.4	27.4 ± 3.0
		mg/dl	
Total-cholesterol[b]	253 ± 32	237 ± 33	245 ± 33
VLDL-C[b]	31 ± 13	28 ± 11	29 ± 12
LDL-C[b]	167 ± 30	167 ± 26	167 ± 28
HDL-C[b]	53 ± 11	42 ± 9	48 ± 11
TG[c]	158 ± 71	138 ± 55	148 ± 64
TC/HDL-C[d]	4.95 ± 1.27	5.75 ± 1.09	5.35 ± 1.21

TC, total cholesterol.
[a] Values are expressed as mean ± SD.
[b] To convert mg/dl to mmol/l multiply cholesterol by 0.0259.
[c] To convert mg/dl to mmol/l multiply TG by 0.0113.
[d] Values expressed as ratios, not mg/dl.

Consuming a TLC/Step 2 diet, relative to a Western diet, resulted in an 11% decrease in LDL-C (**Table 3**, **Fig. 1**). The difference was similar in both female (−12%) and male (−11%) subjects. The pattern was similar if the data were expressed as non-HDL-C (total cholesterol − HDL-C). Concomitant with the lower LDL-C levels after the period when the subjects consumed the TLC/Step 2 diet, HDL-C levels were 7% lower and again, the effect in females (−8%) and males (−7%), was similar. The decreased HDL-C levels attributable to the TLC/Step 2 diet resulted primarily from lower HDL-2 than HDL-3 cholesterol levels. No significant difference in the total cholesterol-HDL-C ratio caused by diet for either the group as a whole or on the basis of gender was observed.

Mean serum TG levels tended to be higher after subjects consumed the TLC/Step 2 diet, relative to the Western diet. The response was highly variable among subjects (Fig. 1) and the differences did not reach statistical significance. Interestingly, the TG elevation was more prominent in male than female subjects. There was little effect of diet on VLDL cholesterol (VLDL-C) levels.

ApoB and apoA-I levels were lower after subjects consumed the TLC/Step 2 diet relative to the Western diet, following a pattern similar to that of LDL-C and HDL-C, respectively, although the difference in apoB levels did not reach statistical significance in the male subjects. The lower levels of apoA-I appeared to be accounted for by differences in apoA-I in Lp[A-I/A-II] particles rather than apoA-I in Lp[A-I] only particles. Somewhat surprisingly, higher levels of apoA-II were observed after the subjects consumed the TLC/Step 2 diet relative to the Western diet, a difference that reached statistical significance for the group as a whole.

The subjects participating in the study had a relatively wide range of Lp[a] levels. There was no significant effect of diet on mean levels. Analyzing the data from those individuals with Lp[a] levels above 35 mg/dl did not change this conclusion (data not shown).

Non-fasting serum lipid and lipoprotein levels were determined 4 h after the evening meal. Differences in serum lipid and lipoprotein levels between the two diets in the non-fasting state were of a somewhat smaller magnitude, particularly for LDL-C levels in male subjects, than in the fasting state (**Table 4**). In all cases, the direction of the changes observed in the fasting state were maintained in the non-fasting state. No significant effect of diet on the ratio of total cholesterol-HDL-C was observed in the non-fasting state. Although the TLC/Step 2 diet resulted in a somewhat higher mean

TABLE 3. Effect of a Western and TLC/Step 2 diet on fasting serum lipid, lipoprotein, and apolipoprotein levels

Variable	Western Diet	Step 2 Diet	*P*	% Change
Total cholesterol (mg/dl)[a,b]	250 ± 33	225 ± 32	<0.001	−9
Female	250 ± 30	225 ± 29	<0.001	−10
Male	249 ± 37	226 ± 35	0.005	−9
VLDL-C (mg/dl)[b]	28 ± 10	28 ± 13	0.736	+7
Female	26 ± 11	25 ± 11	0.825	+5
Male	29 ± 8	32 ± 14	0.515	+9
LDL-C (mg/dl)[b]	175 ± 31	154 ± 28	<0.001	−11
Female	173 ± 28	153 ± 32	<0.001	−12
Male	177 ± 34	155 ± 25	0.004	−11
Non-HDL-C (mg/dl)[b]	202 ± 32	182 ± 33	<0.001	−10
Female	199 ± 30	178 ± 31	0.001	−10
Male	206 ± 34	186 ± 34	<0.001	−9
HDL-C (mg/dl)[b]	47 ± 11	43 ± 9	<0.001	−7
Female	51 ± 11	47 ± 9	0.007	−8
Male	43 ± 9	40 ± 7	0.002	−7
HDL-2-C[b]	15 ± 8	12 ± 6	<0.001	−15
Female	18 ± 9	15 ± 7	0.007	−16
Male	12 ± 6	9 ± 5	0.004	−14
HDL3-C[b]	32 ± 5	31 ± 4	0.059	−2
Female	33 ± 5	32 ± 4	0.198	−3
Male	31 ± 5	30 ± 4	0.175	−2
TC/HDL-C	5.53 ± 1.12	5.43 ± 1.24	0.393	—
Female	5.04 ± 0.99	4.99 ± 1.17	0.700	—
Male	6.01 ± 1.26	5.86 ± 1.19	0.453	—
TG (mmol/l, mg/dl)[c,d]	133 ± 45	143 ± 64	0.265	+7
Female	129 ± 43	131 ± 51	0.796	+1
Male	136 ± 48	155 ± 74	0.138	+13
ApoB (mg/dl)	141 ± 23	132 ± 26	<0.000	−6
Female	140 ± 23	131 ± 24	<0.001	−7
Male	141 ± 25	133 ± 28	0.089	−5
ApoA-I (mg/dl)	157 ± 23	147 ± 23	<0.001	−6
Female	164 ± 23	153 ± 23	<0.001	−7
Male	150 ± 21	141 ± 22	0.004	−6
ApoA-I in Lp[A-I] (mg/dl)	44 ± 13	43 ± 11	0.565	0
Female	47 ± 14	47 ± 13	0.933	0
Male	40 ± 12	39 ± 8	0.401	−1
ApoA-I in Lp[A-I/A-II] (mg/dL)	114 ± 19	106 ± 20	0.005	−6
Female	117 ± 20	107 ± 19	0.016	−8
Male	110 ± 18	105 ± 21	0.152	−5
ApoA-II (mg/dl)	30 ± 7	33 ± 7	0.012	+13
Female	29 ± 6	33 ± 7	0.073	+16
Male	30 ± 8	32 ± 7	0.079	+11
Lp[a] (mg/dl)[d]	20 ± 19	19 ± 18	0.941	−2
Female	23 ± 22	22 ± 20	0.378	−2
Male	17 ± 15	17 ± 15	0.276	+1

TC, total cholesterol.
[a] Values are expressed as mean ± SD, n = 18 females, n = 18 males.
[b] To convert mg/dl to mmol/l multiply cholesterol by 0.0259.
[c] To convert mg/dl to mmol/l multiply TG by 0.0113.
[d] Numbers log transformed prior to analysis.

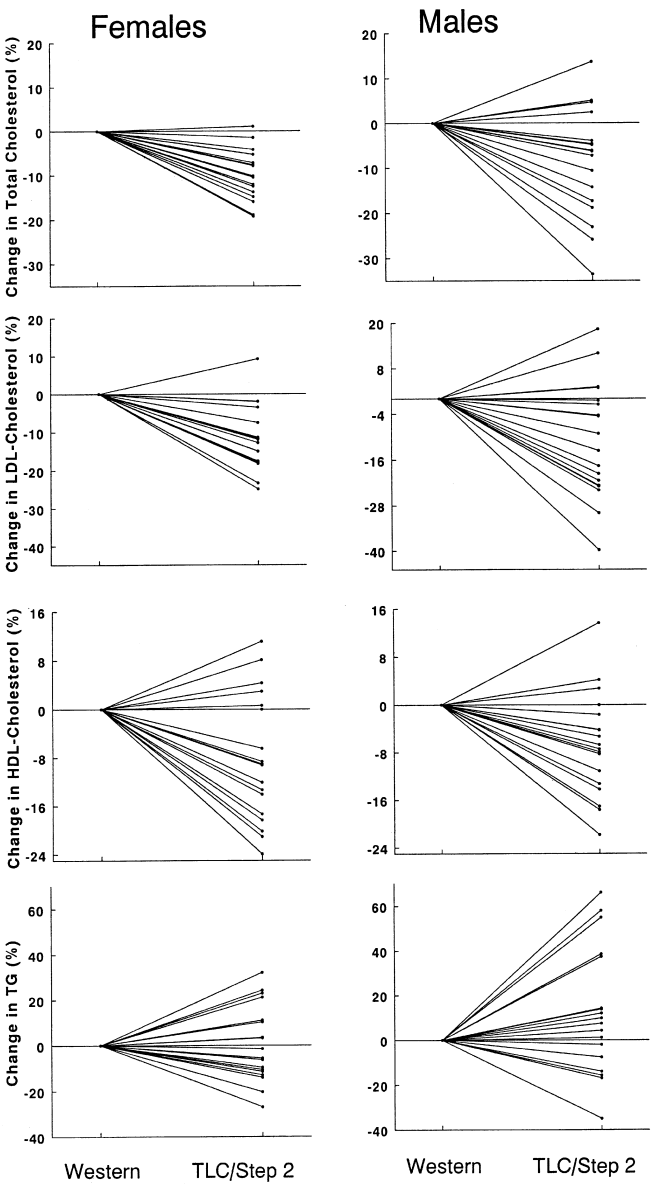

Fig. 1. Percent change in serum lipid levels after subjects consumed a Western and a TLC/Step 2 diet.

non-fasting TG level, the difference did not reach statistical significance.

Shifts in the fatty acid profile of the cholesteryl ester, TG, and phospholipid fractions in serum were consistent with the differences in the fatty acid profile of the diet (**Table 5**). Although the actual fatty acid profiles of each of the lipid subfractions differed, the general patterns were similar. The major changes include an increase in linoleic (18:2n-6c) and α-linolenic (18:3n-3) acids at the expense of oleic acid (18:1n-9c). The magnitude of the changes were most pronounced in the serum cholesteryl ester and TG subfractions.

In an effort to explain the differences observed in lipoprotein cholesterol levels, especially the potentially detrimental fall in HDL-C levels resulting from shifting individuals from a Western to a TLC/Step 2 diet, selected measures of cholesterol metabolism, the FER_{HDL}, and activities of CETP and PLTP were assessed (**Table 6**).

TABLE 4. Effect of a Western and TLC/Step 2 diet on non-fasting serum lipid, lipoprotein and apolipoprotein levels

Variable	Western Diet	Step 2 Diet	*P*	% Change
Total cholesterol (mg/dl)[a]	235 ± 30	217 ± 31	<0.001	−7
Female	236 ± 33	219 ± 32	<0.001	−7
Male	233 ± 27	216 ± 31	0.065	−7
VLDL-C (mg/dl)[b]	34 ± 14	33 ± 14	0.677	+7
Female	30 ± 12	32 ± 10	0.390	+22
Male	39 ± 15	35 ± 16	0.351	−8
LDL-C (mg/dl)[b]	156 ± 25	143 ± 26	0.006	−8
Female	158 ± 30	142 ± 31	0.002	−10
Male	153 ± 20	143 ± 22	0.219	−5
HDL-C (mg/dl)[b]	45 ± 11	42 ± 9	0.012	−5
Female	48 ± 12	45 ± 10	0.066	−6
Male	41 ± 8	38 ± 7	0.102	−4
HDL2-C[b]	14 ± 9	11 ± 6	0.041	−15
Female	17 ± 10	13 ± 7	0.148	−17
Male	11 ± 6	9 ± 5	0.043	−14
HDL3-C[b]	31 ± 5	30 ± 5	0.810	0
Female	31 ± 4	31 ± 7	0.924	0
Male	30 ± 5	29 ± 4	0.588	0
TG (mmol/l, mg/dl)[c,d]	198 ± 84	217 ± 111	0.200	+11
Female	185 ± 73	215 ± 91	0.066	+15
Male	211 ± 96	219 ± 131	0.813	+7
TC/HDL-C[b]	5.51 ± 1.32	5.42 ± 1.22	0.465	—
Female	5.09 ± 1.15	5.10 ± 1.28	0.973	—
Male	5.93 ± 1.38	5.74 ± 1.10	0.383	—
ApoB (mg/dl)	137 ± 19	127 ± 25	0.065	−8
Female	148 ± 25	141 ± 26	0.144	−5
Male	128 ± 8	116 ± 20	0.222	−5
Apo A-I (mg/dl)	134 ± 19	124 ± 15	0.055	−7
Female	130 ± 19	124 ± 11	0.223	−4
Male	137 ± 20	125 ± 19	0.160	−6

TC, total cholesterol.
[a] Values are expressed as mean ± SD, n = 13 females, n = 13 males.
[b] To convert mg/dl to mmol/l multiply cholesterol by 0.0259.
[c] To convert mg/dl to mmol/l multiply TG by 0.0113.
[d] Numbers log transformed prior to analysis.

There were no significant effects of diet on these parameters. Similarly, shifting from a Western to a TLC/Step 2 diet had no significant effect on plasma insulin or glucose levels, factors potentially associated with an atherogenic profile (Table 6).

DISCUSSION

Shifting moderately hypercholesterolemic individuals from a diet formulated to approximate that currently consumed in Westernized countries to a diet meeting the most recent TLC/Step 2 criteria by decreasing saturated fat by 8% of energy and cholesterol by 98 mg/1,000 calories resulted in an 11% decrease in LDL-C levels, a 7% decrease in HDL-C levels, and no significant effect in the total cholesterol-HDL-C ratio or TG, glucose, and insulin levels. A similar trend was observed if the data were expressed as non-HDL-C. For individuals with LDL-C levels in the borderline high risk range this response may be sufficient to forestall the use of lipid lowering medications (1). On a population-wide basis it has been estimated that a de-

TABLE 5. Fatty acid profiles (mol% of total) of serum cholesteryl ester, TG, and phospholipid fractions at the end of each experimental diet phase[a]

	Cholesteryl Ester		TG		Phospholipid	
	Western	Step 2	Western	Step 2	Western	Step 2
14:0	0.96 ± 0.21	0.80 ± 0.22[d]	2.35 ± 0.69	2.27 ± 0.71	0.57 ± 0.13	0.54 ± 0.12
16:0	12.38 ± 0.83	11.98 ± 1.06[b]	26.2 ± 2.62	24.92 ± 3.94[b]	30.59 ± 0.90	30.53 ± 1.05
16:1	2.46 ± 0.73	2.10 ± 0.67[c]	3.58 ± 1.01	3.30 ± 0.91[b]	0.65 ± 0.18	0.60 ± 0.17
18:0	0.98 ± 0.17	0.84 ± 0.24[c]	3.32 ± 0.55	3.03 ± 0.84[b]	13.70 ± 0.85	13.88 ± 1.06
18:1n-7c	1.07 ± 0.016	1.02 ± 0.20	1.99 ± 0.30	1.90 ± 0.36	1.14 ± 0.18	1.19 ± 0.23[b]
18:1n-9c	15.47 ± 1.95	12.03 ± 2.61[d]	31.93 ± 2.73	27.26 ± 3.90[d]	7.28 ± 1.11	6.23 ± 1.22[d]
18:1t	1.01 ± 0.55	1.19 ± 1.21	3.79 ± 1.22	3.37 ± 1.74	1.80 ± 0.53	1.72 ± 0.72
18:2n-6c	53.16 ± 4.10	57.54 ± 4.38[d]	20.04 ± 3.77	26.37 ± 5.60[d]	21.00 ± 2.39	22.99 ± 2.54[d]
18:2t	1.79 ± 0.38	1.85 ± 0.66	1.72 ± 0.41	1.59 ± 0.56	0.66 ± 0.22	0.68 ± 0.31
18:3n-3	0.61 ± 0.11	0.75 ± 0.29[c]	1.41 ± 0.38	2.21 ± 0.87[d]	0.22 ± 0.05	0.29 ± 0.08[d]
18:3n-6	0.86 ± 0.32	0.89 ± 0.36	0.51 ± 0.19	0.62 ± 0.27[c]	0.14 ± 0.04	0.15 ± 0.06
20:4n-6	7.36 ± 1.51	7.06 ± 1.77	1.61 ± 0.57	1.49 ± 0.49	9.77 ± 1.64	9.12 ± 1.56[b]
20:5n-3	0.80 ± 0.20	0.84 ± 0.46	0.31 ± 0.13	0.37 ± 0.21[c]	0.88 ± 0.25	0.90 ± 0.55
22:6n-3	0.47 ± 0.13	0.48 ± 0.19	trace	trace	3.73 ± 0.70	3.47 ± 0.90[b]

[a] Values are expressed as mean ± SD, n = 18 females, n = 18 males.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

crease in total cholesterol levels of 10% would decrease CVD incidence by about 30% and the mortality by 13% (38–40). Even a less robust outcome would have a positive impact on reducing the economic and social burden associated with CVD.

HDL-C levels decreased about 7% when subjects consumed the TLC/Step 2 diet relative to the Western diet. The decline in HDL-C was attributed to a decrease in the HDL-2 and not the HDL-3 subfraction. Reduced levels of HDL-2 have been associated with increased risk of developing CVD (41). HDL-2, the larger cholesteryl ester rich fraction, rather than HDL-3, the smaller cholesteryl ester poor fraction, is more responsive to alterations in environmental factors, so our observations were not unexpected (42–45). However, with respect to interpretation of the data in light of CVD risk, it should be noted that our understanding of changes in HDL-C levels resulting from dietary modification is somewhat incomplete. A decline in HDL-C in response to a decline in saturated fat intake may be a physiologically appropriate response to a decrease in LDL-C levels, and it is only when there is a discordant response of LDL and HDL-C, resulting in an increased ratio, that a person's risk of developing CVD is increased. In a relative sense, the mean absolute change in LDL-C was 21 mg/dl and HDL-C was 4 mg/dl.

TABLE 6. Fraction esterification rate of HDL (FER_{HDL}), phospholipid transfer protein (PLTP) and cholesterol ester transfer protein (CETP) activities and insulin and glucose levels at the end of each experimental diet phase

	Western	Step 2	*P*
FER_{HDL} (%/h)	20.7 ± 5.2	21.0 ± 5.7	0.718
Females	20.1 ± 5.1	20.9 ± 7.1	0.504
Males	21.4 ± 5.3	21.1 ± 4.0	0.758
PLTP ($\mu mol^{-1} \cdot h^{-1} \cdot l^{-1}$)	5,334 ± 2,002	5,333 ± 1,919	0.994
Females	5,612 ± 2,147	5,324 ± 2,011	0.292
Males	5,056 ± 1,864	5,342 ± 1,880	0.221
CETP ($nmol^{-1} \cdot h^{-1} \cdot ml^{-1}$)	13.52 ± 3.76	13.12 ± 5.07	0.559
Females	13.54 ± 4.45	13.84 ± 5.36	0.768
Males	13.52 ± 3.05	12.40 ± 4.82	0.257
Insulin ($\mu U/ml$)	12.6 ± 6.9	11.2 ± 4.9	0.080
Females	13.3 ± 8.7	11.8 ± 5.9	0.292
Males	11.9 ± 4.7	10.7 ± 3.9	0.175
Glucose (mmol/l)	92 ± 8	92 ± 11	0.823
Females	92 ± 10	91 ± 11	0.662
Males	92 ± 7	92 ± 5	0.930

HDL metabolism is partially determined by the activities of PLTP and CETP, and FER_{HDL} (32, 46–49). In an attempt to understand the underlying mechanisms for the change in HDL-C levels, we measured the activities of these factors. No difference in the FER_{HDL}, PLTP, or CETP activity was observed. The relatively small change in HDL-C levels and the high degree of variability in response to the parameters assessed among study subjects may have limited our ability to identify a mechanism. Alternately, the parameters measured may not have been the putative ones responsible for the decrease in HDL-C levels observed.

There was no significant effect of diet on the total cholesterol-HDL-C ratio. This result is attributable to declines in both total cholesterol and HDL-C levels. Similar results were obtained when the data were expressed as the LDL-C-HDL-C ratio (data not shown). The question arises as to whether, in the absence of a decrease in the total cholesterol-HDL-C ratio, would the dietary modifications instituted result in a decrease in CVD risk? The most predictive measure of CVD risk is LDL-C level, and current guidelines have been established on the basis of this parameter (1). In the absence of evidence otherwise, at this point it seems appropriate to conclude that a decrease in LDL-C level not accompanied by an increase in the total cholesterol-HDL-C ratio can be interpreted to decrease in CVD risk.

There was no significant effect of the TLC/Step 2 diet, relative to the Western diet, on mean fasting TG levels.

There was a wide range of responses among the study subjects, even when they were selected to have normal plasma glucose levels. This lack of a significant increase in TG levels is in contrast to that commonly observed in response to a decrease in fat intake (50–59). The absence of a significant mean increase in TG levels is likely due, in part, to the relatively modest decrease in the fat content of the diet and adequate length of the study period. These two factors have been identified as being major determinants of TG response to dietary modification (60). The TCL/Step 2 diet as formulated for the current study would be classified as a reduced fat, not a very low fat, diet. It is in the later scenario where the most dramatic effects on TG levels have been reported (17). Additionally, the higher fiber content of the TLC/Step 2 diet, generally characteristic of reduced fat diets achieved via an increase in foods of plant origin, may also have had an attenuating effect on plasma TG levels (16, 17).

In the fasting state there was little difference in the plasma lipid response of female and male subjects to the dietary modification. All women in the current study were postmenopausal and not using hormone replacement therapy. The somewhat greater LDL-C and HDL-C, and TG response in male relative to female subjects that we previously reported was not observed in the current study (61). Inclusion of pre-menopausal females in the prior study, which accounted for about 50% of the females, is the likely explanation. Similar responses of females and males in LDL-C and TG levels to diets designed to optimize lipoprotein profiles have been reported previously (62–66). The HDL-C response was similar in the females and males participating in the current study, consistent with some, but not all, previous work (12, 61–66).

Changes in the non-fasting lipid and lipoprotein levels were similar to those observed in the fasting state, albeit, of a somewhat smaller magnitude for the group as a whole. Although increases in TG levels after subjects consumed the TLC/Step 2 diet were somewhat greater than those in the fasting state, the differences were not significant. Concluded from these data is that the dietary modifications as implemented did not result in a postprandial atherogenic profile (7).

The response of non-fasting lipid and lipoprotein levels was slightly more pronounced in female relative to male subjects. LDL-C levels were lowered by twice as much. In contrast, changes in TG levels were about 2-fold higher in the female compared with male subjects, although in only one case were the differences statistically significant. To our knowledge this gender difference in the non-fasting lipoprotein profile in response to dietary modification has not previously been reported and awaits further validation.

The difference in the fatty acid profile of the two diets was reflected in the fatty acid profile of the major lipid subclasses. The most pronounced shifts occurred in the cholesteryl ester and TG subclasses. The lower proportion of cholesterol oleate, and higher proportion of cholesterol linoleate and cholesterol alpha-linolenate, suggests a potentially less atherogenic particle was formed after subjects consumed the TLC/Step 2 relative to the Western diet, an additional benefit of the dietary intervention (67, 68). Further modifications in the TCL/Step 2 diet, for example, inclusion of fish, would have increased plasma eicosapentaenoic acid and docosahexaenoic acid and may have contributed additional benefit (69, 70).

Shifting from a Western-type diet to a TLC/Step 2 diet resulted in no significant effect on fasting glucose or immunoreactive insulin levels. Data from previous work in this area has been somewhat inconsistent (15). In some studies, decreasing the fat content of the diet had a negative affect on glycemic control (63, 71–75), whereas in other studies no such effect was observed (59, 76–78). Reconciling the differences in the data is difficult. The magnitude of difference in the fat content of the diets, changes in body weight during the study period, and the presence of established (i.e., type 2 diabetes), or a predisposition to abnormalities in glucose metabolism (i.e., excess body weight) need to be factored in. The relatively modest decrease in the fat content of the diet, lack of change in body weight, and normal glucose levels at screening likely contributed to the lack of significant effect of dietary intervention on glucose and insulin levels, and suggest that modest decreases in dietary fat did not result in a detrimental effect of insulin and glucose levels.

Some large-scale studies in free-living populations have reported a less robust response (about 5% reduction) to a TLC/Step 2 diet (19, 20). The results of these studies are in contrast to the LDL reductions reported in the current study, studies of like design (79–87), and other studies of free-living individuals (62, 63, 88). The discrepancy in results among studies may be related to the uncertainty associated with self-reported dietary data used to assess compliance, the self selection of non-diet responsive individuals to volunteer for studies that have the potential for a pharmacological intervention, and the absolute differential in the saturated fat, cholesterol, and possibly fiber content of the baseline and experimental diets actually achieved.

A potential limitation in broadening the interpretation of the study results to a larger group of individuals is that each diet was consumed over a relatively short period of time. However, we have previously demonstrated that when the level and type of dietary fat was altered over a 24-week period, subjects attained a maximal lipoprotein response by 4 weeks and maintained that level of response over the subsequent 20-week period (21). A second potential limitation in broadening the interpretation of the study is that body weights were kept consistent by design throughout the study period. Although some data suggest that decreasing the fat content of the diet results in weight loss, the percent decrease in dietary fat in those studies was much greater than that employed in the current study. Furthermore, the efficacy of decreasing dietary fat to initiate and maintain body weight loss is highly controversial (89, 90).

In summary, changing from a Western to a TLC/Step 2 diet improved the lipoprotein profile, and resulted in an increase in the proportion of circulating lipids containing polyunsaturated cholesteryl esters without adversely af-

fecting the total cholesterol-HDL-C ratio or TG, glucose, and insulin levels. Changes in the non-fasting state were similar to those observed in the fasting state. These changes would shift some individuals that, on the basis of elevated LDL-C levels and other established risk factors, would be candidates for pharmacological intervention to individuals that could use dietary and lifestyle means to decrease CVD risk.

The authors would like to thank the staff of the Metabolic Research Unit, including Helen Rasmussen, M.S., R.D., for the expert care provided to the study subjects, and Ritva Keva (Helsinki, Finland) for her technical assistance. We would also like to gratefully acknowledge the cooperation of the study subjects, without whom this investigation would not have been possible. This work was supported by grant HL 54727 from the National Institutes of Health, Bethesda, MD, research grants from the Finnish Foundation for Cardiovascular Research, and the U.S. Department of Agriculture, under agreement No. 58-1950-9-001. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

Manuscript received 19 July 2001 and in revised form 10 October 2001.

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