

Effects of Dietary Cholesterol and Fatty Acids on Plasma Lipoproteins

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ABSTRACT The effects of dietary cholesterol and fatty acids on low density and high density lipoproteins (LDL and HDL) were studied in 20 young men. After 2-3 wk of evaluations on ad lib. diets, basal diets, which consisted of 15% protein, 45% carbohydrates, 40% fat, and 300 mg/day of cholesterol, were given for 4-5 wk (Basal₁). The ratio of dietary polyunsaturated to saturated fatty acids (P/S) for different groups of subjects were 0.25, 0.4, 0.8, or 2.5. 750 and 1,500 mg/d of cholesterol were added to the basal diets as 3 and 6 eggs, respectively. Total cholesterol and LDL cholesterol were lower in all subjects on the basal diets than on the ad lib. diets. Addition of 750 mg cholesterol to the diet with P/S = 0.25-0.4 raised LDL cholesterol by 16±14 mg/dl to 115% of basal diet values ($n = 11$, $P < 0.01$); 1,500 mg increased LDL cholesterol by 25±19 mg/dl to 125% ($n = 9$, $P < 0.01$). On the diet with P/S = 0.8, 750 mg produced insignificant increases in LDL cholesterol, but 1,500 mg produced increases of 17±22 mg/dl to 115% of basal ($n = 6$, $P < 0.02$). On the P/S = 2.5 diet, neither 750 nor 1,500 mg produced significant changes. Thus, both the cholesterol contents and P/S ratios of diets were important in determining LDL levels. The lipid and apoprotein compositions, flotation rates, molecular weights, and binding by cellular receptors of LDL were virtually unchanged by the addition of cholesterol to the diets high in saturated fat. These diets, therefore, caused an increase in the number of LDL particles of virtually unchanged physical and biological properties. On the

diet with low P/S ratio, HDL₂ rose, whereas this effect was absent on diets with high P/S ratios.

The response of LDL to dietary manipulations is consonant with epidemiologic data relating diets high in cholesterol and saturated fat to atherogenesis. The response of HDL₂, however, is opposite to that of its putative role as a negative risk factor. Further work is needed to clarify this interesting paradox.

INTRODUCTION

The relationships between dietary cholesterol, the relative proportions of dietary polyunsaturated to saturated fatty acids (P/S)¹ and plasma cholesterol levels have been extensively examined in cross-sectional population surveys (1-4), in outpatients who have been instructed in various experimental diets (5-12), and in metabolic ward studies (17-21). Cross-sectional surveys demonstrate poor correlations between reported dietary intakes and blood lipids but diets with high P/S consistently lower cholesterol levels in outpatient and inpatient studies (13-16). On the other hand, increases in dietary cholesterol produce variable rises in plasma cholesterol (17-22).

In contrast with the number of studies on total plasma cholesterol levels, only a few studies of human plasma lipoproteins have been reported (8, 9, 23). Our aim was to explore systematically the effects of dietary

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¹ Abbreviations used in this paper: Apo, apoprotein; DME, Dulbecco's modified Eagle's medium; HDL, high density lipoprotein; LDL, low density lipoprotein; P/S, ratio of dietary polyunsaturated to saturated fatty acids; VLDL, very low density lipoprotein.

cholesterol given as whole eggs at various dietary P/S (0.25–2.5) on the levels of the major plasma lipoproteins and apolipoproteins and to characterize the major plasma lipoproteins, especially low density and high density lipoproteins (LDL and HDL), in terms of their compositions, physical properties, and interactions with cells. A preliminary report of this study has been made (24).

METHODS

Study subjects. 20 healthy young men, 19 Caucasians and 1 Oriental, medical students or laboratory technicians aged 22–31 yr at the beginning of the study, volunteered to participate, having given informed consent. None had any evidence of physical or psychiatric disease. All denied allergies or intolerance to food. All had normal blood counts, urinalysis, and tests of liver, renal, and thyroid function. None was taking any medication.

Diets and feeding protocol. Diets were composed of solid foods, prepared in a diet kitchen, and consumed on site. Meat was obtained from a local meat processor, weighed into individual portions, wrapped in aluminum foil, and frozen. Individual servings of meat were baked as needed. Cereals were weighed and individually portioned into sealed plastic sandwich bags. Canned fruits, vegetables, juice, and margarine were purchased in case lots, milk was purchased in 1/2-pint containers from a local dairy, and fresh produce was purchased from a local supermarket. Basal diets (Basal₁ and Basal₂) consisted of 15% protein, 45% carbohydrate, and 40% fat (as percentages of calories), and 300 mg/d of cholesterol. P/S ratios were varied between 0.25, 0.4, 0.8, and 2.5. The following margarines were used: Clover Valley, P/S < 0.3, Parkay (stick), P/S = 0.45; Imperial (stick), P/S = 1.0; and Promise (tub), P/S = 2.5. A total P/S value for any given diet was calculated by averaging the P/S of all the fats over a 7-d period. Cholesterol (750 or 1,500 mg/d) was added to the different basal diets as 3 or 6 eggs/d, respectively, and macronutrients were adjusted to maintain P/S and calories. Food composition tables were used to calculate the nutrient compositions of the diets and calculations agreed well with chemical analyses of selected aliquots of the diets (R. E. Olson et al., unpublished observations). Body weights were measured 4 times/wk and found to vary by < 1 kg. 3–7-d cycle menus were used with caloric levels calibrated in increments of 100 K calories from 2,600 to 3,800. The subjects were allowed to drink a maximum of 12 12-oz portions of beer or alcohol equivalents weekly and instructed to record their alcohol intakes. No relationship was found between ethanol intake at these levels and responsiveness to dietary cholesterol. Each subject was given a daily multivitamin and mineral supplement to insure adequate nutrition. Water and minimal caloric seasonings and beverages were given ad lib. The regular dietary protocol (Fig. 1) consisted of 2–3 wk of ad lib. eating (ad lib.₁), 3–4 wk of basal diets (Basal₁), 4–5 wk of 3 eggs and 4–6 wk of 6 eggs, followed by return to the basal diet for 3 wk (Basal₂) and the ad lib. diet for 2–3 wk (ad lib.₂). 11 individuals (subjects 1–6, 10–14, Table I) ate diets with a P/S of 0.25 or 0.4 plus 3 eggs; nine (subjects 1–9) ate diets with a P/S of 0.25 plus 6 eggs. Six of these men (subjects 1–6) ate the diet with P/S = 0.25 plus first 3 and then 6 eggs. Six men (subjects 10–15) ate the diet with P/S = 0.8 plus 3 and 6 eggs, five of these men (subjects 10–14) also ate the diet with P/S = 0.4 plus 3 eggs, and are included with the P/S = 0.25–0.4 and

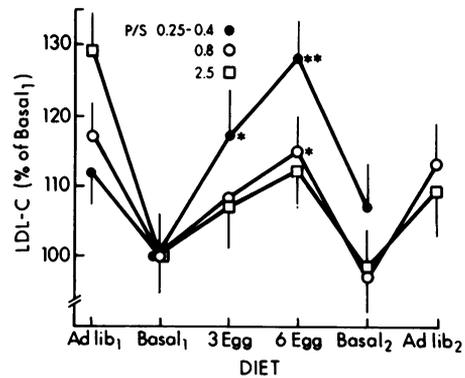


FIGURE 1 Dietary cholesterol-induced changes in fasting plasma LDL cholesterol concentrations (LDL-C). Results are means \pm 1 SEM, expressed as increments or decrements compared to the Basal₁ diet. (See Table II for numbers per group.) ** $P < 0.01$, * $P < 0.02$, by paired two-tailed t test.

3-egg group. Finally, six individuals (subjects 7, 16–20) had the diet with P/S = 2.5 plus 3 and 6 eggs, one of whom (subject 7) also ate the diet with P/S = 0.25 plus 6 eggs. Thus, six men ate two different P/S diets while the rest ate only one P/S diet.

Laboratory methods. Venous bloods (EDTA, 1 mg/ml) were drawn after subjects had fasted for 12–16 h. Lipoproteins were isolated from plasma by the combined ultracentrifugal and precipitation techniques of the Lipid Research Clinics (25). Coefficients of variation for cholesterol and triglyceride determinations were 3–4%. Apoproteins (Apo)A-I, A-II, B, C-II, C-III, and E were quantified by specific radioimmunoassays (26–30). The coefficients of variation of these assays ranged from 7 to 11%.

The compositions and physical properties of lipoproteins in selected plasmas were examined according to three independent methods: (a) ultracentrifugation at $d = 1.225$, followed by column chromatography on 4% agarose columns (31); (b) analytic ultracentrifugation (32); and (c) zonal ultracentrifugation (33, 34). Molecular weights and compositions of LDL were determined on chromatographic fractions as previously described (31, 32).

Sedimentation velocities were determined at densities of 1.20 and 1.063 g/ml (KBr 0.1 mM EDTA, 1.5 mM Na₂S₂O₃) in a Beckman Model E and analytical ultracentrifuge at 25°C, with double sector cells (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA). Flotation rates (S_f) were calculated from 5–6 Schlieren photographs taken at intervals during the run. S_f were corrected to partial specific volumes of 0.87 ml/g for HDL and 0.97 ml/g for LDL, to obtain S_f in a standard density solvent (35). Areas for HDL₂ and HDL₃ were estimated on the Nikon projector screen on centimeter graph paper (Nikon Inc., Garden City, NY).

Zonal ultracentrifugal separation and characterization of very low density lipoprotein (VLDL) plus LDL fractions were carried out in a continuous linear density gradient with a range of 1.00 to 1.30 at 15°C for 140 min at 42,000 rpm, in a Beckman Ti 14 zonal rotor (33). All solutions contained 1 mM EDTA and 0.1% sodium azide. For HDL separations, a three-step NaBR gradient spanning the density range 1.00–1.40 g/ml was generated and ultracentrifugation was done at 41,000 rpm at 12°C for 24 h (34). Rotor effluents were monitored continuously at 280 nm and triglycerides, free cholesterol, esterified cholesterol, (enzymatic triglyceride

TABLE I
Participation of Study Subjects in Various Dietary Protocols

Subject	P/S basal diet	Basal plus 3 eggs	Basal plus 6 eggs
1	0.25	+	+
2	0.25	+	+
3	0.25	+	+
4	0.25	+	+
5	0.25	+	+
6	0.25	+	+
7	0.25	-	+
	2.5	+	+
8	0.25	-	+
9	0.25	-	+
10	0.4	+	-
	0.8	+	+
11	0.4	+	-
	0.8	+	+
12	0.4	+	-
	0.8	+	+
13	0.4	+	-
	0.8	+	+
14	0.4	+	-
	0.8	+	+
15	0.8	+	+
16	2.5	+	+
17	2.5	+	+
18	2.5	+	+
19	2.5	+	+
20	2.5	+	+

+, Participated; -, did not participate.

and cholesterol kits from Boehringer Mannheim Biochemicals, Indianapolis, IN), lipid phosphorus (36), and protein (37) were determined in appropriately pooled fractions.

Cell culture. Normal human fibroblasts were cultured in Dulbecco's modified Eagle's medium (DME) containing 10% fetal calf serum for 7 d (38, 39). 48 h before the beginning of an experiment, the medium was changed to DME containing 10% lipoprotein deficient serum. To assess the cell interactions of LDL isolated from the experimental subjects, 5 µg/ml of normal ¹²⁵I-LDL (40) were added to the media along with increasing doses of experimental unlabeled LDL preparations isolated by zonal ultracentrifugation. Incubation was for 2 h at 37°C and then ¹²⁵I-LDL degradation and cell associated counts were determined. Triplicate incubations per data point for each subject were performed. Coef-

ficients of variation for the two assays averaged 16 and 13%, respectively.

Statistical computations. Values for the last 3 wk of each dietary period were averaged for each individual and used to compute overall means and standard deviations for the whole group of subjects for the various dietary periods. The means for the various dietary treatments (e.g., Basal₁, 3-egg, Basal₂) were then compared with each other. Differences (e.g., ΔLDL) between egg and basal periods were computed. Paired and unpaired *t* tests, as appropriate, were used to assess statistical significance. The computations were performed using the SAS statistical package available at the Washington University Computer Center (41).

RESULTS

Effect of dietary cholesterol on plasma lipoprotein lipid levels. The plasma lipids of all subjects decreased from their ad lib. values on the basal diet. Falls were greatest for those who changed from ad lib. to the diet with a P/S of 2.5 (Table II, Fig. 1). Ad lib. dietary cholesterol intakes and P/S were estimated to be 500-700 mg/d and 0.2-0.6, respectively. Dietary cholesterol when added to the basal diet to yield total

TABLE II
Effect of Dietary P/S and Cholesterol on Plasma Lipoprotein Lipid Levels

Diet	TC Total cholesterol	VLDL Triglyceride	LDL Cholesterol	HDL Cholesterol
mg/dl				
P/S 0.25-0.40				
Ad lib. ₁ (11)	188±29	88±43	118±24	49±6
Basal ₁ (11)	170±24	73±20	109±20	47±7
3 eggs (11)	188±24*	68±20	125±26†	50±6
Basal ₂ (11)	172±26	64±23	114±23	47±6
Ad lib. ₁ (9)	180±23	57±24	119±21	49±8
Basal ₁ (9)	164±20	66±19	102±19	47±5
6 eggs (9)	192±24†	48±15*	127±26*	52±8
Basal ₂ (9)	164±23	59±27	105±22	46±7
P/S 0.8				
Ad lib. ₁ (5)	197±40	62±28	135±40	45±12
Basal ₁ (6)	175±31	52±20	115±32	44±11
3 eggs (6)	180±28	64±15	124±29	50±10
6 eggs (6)	202±34*	61±15	132±33†	48±13
Basal ₂ (6)	174±28	53±9	113±24	51±14
P/S 2.5				
Ad lib. ₁ (6)	163±16	53±16	110±15	46±7
Basal ₁ (6)	141±23	59±21	85±22	45±8
3 eggs (6)	143±18	59±34	91±22	46±9
6 eggs (6)	151±23	45±11	95±23	46±8
Basal ₂ (6)	138±25	56±17	83±19	46±9

Mean±1 SD. Number of subjects are in parentheses. Comparison of means are between Basal₁ and egg periods.

* *P* < 0.01, by paired *t* test (two tailed).

† *P* < 0.02, by paired *t* test (two tailed).

TABLE III
Effect of Dietary P/S and Cholesterol on Plasma Apolipoprotein Levels

P/S	Eggs	ApoA-I	ApoA-II	ApoB	ApoC-II	ApoC-III	ApoE
mg/dl							
0.25-0.4	Basal ₁	103±16 (10)	36±9 (9)	83±21 (8)	4.9±1.6 (9)	13.1±3.1 (9)	4.2±0.7 (3)
0.25-0.4	3	109±16 (10)	38±11 (9)	102±30* (8)	5.5±2.4 (9)	13.8±3.3 (9)	4.6±1.0 (3)
0.25-0.4	Basal ₂	108±17 (10)	38±11 (9)	96±29 (8)	5.3±2.0 (9)	13.0±3.9 (9)	4.0±1.1 (3)
0.25-0.4	Basal ₁	106±13 (9)	32±8 (9)	75±15 (6)	4.0±1.6 (9)	11.9±3.1 (9)	4.2±0.7 (3)
0.25-0.4	6	111±10 (9)	29±6 (9)	92±18‡ (6)	4.4±2.1 (9)	12.1±1.9 (9)	4.0±0.3 (3)
0.25-0.4	Basal ₂	110±18 (9)	39±17 (9)	87±12 (6)	4.0±2.2 (9)	12.1±3.9 (9)	4.0±1.1 (3)
0.8	Basal ₁	111±10 (6)	42±6 (6)	108±21 (6)	5.2±1.2 (6)	12.7±2.8 (6)	3.3±1.1 (6)
0.8	3	123±6‡ (6)	39±5 (6)	129±31‡ (6)	4.4±1.0 (6)	14.6±3.0 (6)	3.2±1.2 (6)
0.8	6	128±18‡ (6)	38±5 (6)	146±40* (6)	4.4±0.9 (6)	14.6±3.0 (6)	3.5±1.3 (6)
0.8	Basal ₂	126±19 (6)	43±8 (6)	121±38 (6)	5.7±4.2 (6)	12.7±2.5 (6)	3.5±1.1 (6)
2.5	Basal ₁	90±18 (6)	36±4 (6)	89±19 (3)	3.6±1.1 (6)	10.1±2.2 (6)	3.8±0.7 (3)
2.5	3	91±9 (6)	33±5 (6)	100±20‡ (3)	4.2±0.9 (6)	11.3±2.0 (6)	4.4±0.5 (3)
2.5	6	93±21 (6)	32±7 (6)	97±21 (3)	4.1±0.7 (6)	10.0±3.4 (6)	4.9±1.1 (3)
2.5	Basal ₂	106±24 (6)	40±14 (6)	89±27 (3)	4.9±1.9 (6)	11.5±3.0 (6)	4.7±0.8 (3)

Plasma apoproteins were quantified by radioimmunoassay. Number of subjects is indicated in parentheses.

* $P < 0.01$ by paired two-tailed t test (vs. Basal₁, egg-free, diet).

‡ $P < 0.02$.

intakes of 1,800 mg/d had variable effects on plasma lipid and lipoprotein concentrations. Of particular importance in modulating the effect of dietary cholesterol was the P/S of the diet. The addition of 750 or 1,500 mg cholesterol to basal diets with P/S of 0.25-0.40 (Table II, Fig. 1) increased LDL cholesterol by 16±14 mg/dl to 115% of Basal₁ values, ($n = 11$, $P < 0.01$), and by 25±19 mg/dl to 125% of Basal₁ values ($n = 9$, $P < 0.01$), respectively. When these subjects were returned to the basal diet (Basal₂) their lipid values declined to values similar to those noted on Basal₁. In the six individuals fed the P/S = 0.25 basal diet to which were added first 750 and then 1,500 mg of cholesterol, mean increases were 19±16 and 34±20 mg/dl, respectively (120±18 and 135±23% of Basal₁). The differences between 750 and 1,500 mg of added cholesterol were significantly different from each other ($P < 0.001$, $n = 6$, paired t test). The increases in total cholesterol were due to increases in LDL cholesterol. No significant alterations in mean HDL cholesterol were noted (see HDL₂ and HDL₃ levels below). VLDL triglyceride decreased on the addition of 1,500 mg cholesterol (Table I).

On diets with a P/S of 0.8, the addition of 750 mg cholesterol caused an insignificant rise in LDL cholesterol (9±13 mg/dl to 108% of Basal₁, $P < 0.07$), but 1,500 mg/d produced an increase of 17±22 mg/dl to 115% of Basal₁ ($P < 0.02$). There were no significant changes in either HDL or VLDL cholesterol.

On the basal diet with high levels of linoleic acid (P/S = 2.5) levels of LDL cholesterol were much lower

(85-95 vs. 107-132 mg/dl on diets with P/S between 0.25 and 0.80), and there was no significant increase in LDL cholesterol at any level of cholesterol intake. Here too, mean levels of VLDL or HDL cholesterol were unchanged.

It is worth emphasizing that on each basal diet the addition of dietary cholesterol produced rises in LDL cholesterol that varied greatly from individual to individual. For example, on the diet with a P/S of 0.25, the addition of 750 mg cholesterol produced increases from 2 to 44 mg/dl, whereas 1,500 mg produced increases from 8 to 62 mg/dl; on the P/S = 2.5 diet, 750 mg produced changes from -16 to +29 mg/dl, and 1,500 mg from -9 to +18 mg/dl. Responses of the group were continuous rather than bimodal. Similar variability of individual responses to dietary cholesterol has been reported by others (22).

As noted, LDL cholesterol and total cholesterol levels fell when subjects changed from their ad lib.₁ diets to Basal₁ (Fig. 1). The addition of cholesterol to the basal diets tended to increase LDL cholesterol and total cholesterol up toward ad lib. levels, less so in diets with high P/S. The peak levels attained however were uniformly lower than ad lib. concentrations with one exception, that found when 6 eggs were added to the P/S = 0.25-0.4 diet (Fig. 1).

Effect of diet on apoprotein levels. ApoB increased significantly when cholesterol was added to the P/S = 0.25-0.4 and to the P/S = 0.8 basal diets (Table III). The increases in ApoB were of a magnitude similar to those of LDL cholesterol (Δ LDL cholesterol

TABLE IV
Effects of Dietary P/S and Cholesterol on Plasma ApoC and ApoE Density Distributions and on ApoA-I/ApoA-II Ratios

Diet		$d > 1.006$ / Plasma ApoC-II / ApoC-II	$d > 1.006$ / Plasma ApoC-III / ApoC-III	$d > 1.006$ / Plasma ApoE / ApoE	ApoA-I/ApoA-II
P/S	Eggs				
0.25	—	0.53±0.13 (9)	0.60±0.15 (9)	0.86±0.08 (3)	3.62±0.59 (6)
0.25	6	0.72±0.13* (9)	0.73±0.09† (9)	1.00±0.16 (3)	4.06±0.68 (6)
2.5	—	0.44±0.07 (5)	0.62±0.09 (6)	0.80±0.08 (3)	3.30±0.29 (5)
2.5	6	0.65±0.16 (5)	0.71±0.09† (6)	0.84±0.08 (3)	3.36±0.56 (5)

Levels of apoproteins were measured in total plasma and in $d > 1.006$ fractions by radioimmunoassays.

Results are means of mass ratios±1 SD; $n = 6-9$, subjects.

* $P < 0.01$ by paired two-tailed t tests.

† $P < 0.05$ by paired two-tailed t tests.

= 24 ± 26 , Δ ApoB = 20 ± 13 mg/dl), and LDL cholesterol/ $d > 1.006$ ApoB ratios (indices of LDL compositions), ranging from 1.20 to 1.41 for the different groups, were not significantly altered. Changes in ApoB were positively correlated with the increases in LDL cholesterol (Pearson r for Δ LDL cholesterol vs. Δ ApoB was 0.5, $n = 27$, $P < 0.01$). ApoA-I levels rose when cholesterol was added to the P/S = 0.8 basal diet, but for ApoA-I on other diets, and for the other apoproteins on all diets no consistent differences were found to be due to addition of cholesterol.

Although total levels of ApoC-II and ApoC-III did not change, when cholesterol was added to the basal diet having a low P/S, the density distributions of the some of apoproteins changed (Table IV). In those subjects who ate the P/S = 0.25 diet, a greater proportion of the total ApoC was found in the $d > 1.006$ lipoproteins. Since very little ApoC-II or ApoC-III are detected either in LDL or in the $d > 1.21$ fractions of plasma (29), these results suggested that ApoC had

shifted from VLDL to HDL. Compatible results were obtained for ApoE, but these were not statistically significant. Mean ApoA-I/ApoA-II ratios increased in those subjects eating the P/S = 0.25 and P/S = 0.80 diets (Tables III and IV), which suggests that HDL₂ may have increased relative to HDL₃ (34, 42).

Lipoprotein distributions, physical properties, and composition. Neither the cholesterol/protein or cholesterol/phospholipid ratios nor the molecular weights of LDL were significantly altered by dietary cholesterol (Table V). On analytic ultracentrifugation, S_f rates of LDL increased by ~ 0.4 U ($P < 0.025$) (Table V) and the heterogeneity of LDL increased slightly as evidenced by slightly broader Schlieren peaks (not shown). On zonal ultracentrifugation (performed on six individuals eating the P/S = 0.25 and on six eating the P/S = 2.5 diet), LDL flotation properties remained virtually constant when either 750 or 1,500 mg of cholesterol was added (not shown). These data suggest that adding cholesterol to the basal diets high in saturated

TABLE V
Effects of Dietary P/S and Cholesterol on LDL Composition

P/S	Eggs	C/Pr	C/PL	Molecular weights	$S_f = 1.063$
		mass ratios		daltons $\times 10^6$	
0.25	—	1.55±0.10	1.47±0.08	2.93±0.26	8.7±0.2
0.25	3	1.44±0.03	1.40±0.07	2.94±0.15	—
0.25	6	1.55±0.03	1.48±0.07	2.91±0.18	8.1±0.3
2.5	—	1.54±0.07	1.45±0.06	2.94±0.07	8.0±1.1
2.5	3	1.46±0.03	1.36±0.04	3.02±0.05	—
2.5	6	1.37±0.12	1.44±0.01	3.00±0.10	7.7±1.5

C/Pr and C/PL are cholesterol/protein and cholesterol/phospholipid mass ratios, respectively. Molecular weights and ratios were determined by chemical analysis of LDL fractions isolated from plasma by column chromatography (32). S_f rates were determined by analytic ultracentrifugation. Results are means±1 SD; $n = 3$ subjects per data point.

fats resulted primarily in increased numbers of LDL particles, although some slight increase in heterogeneity may have occurred.

HDL also was examined by analytic (Table VI) and zonal ultracentrifugation (not shown), the latter was performed on two individuals eating the P/S = 0.25 diet before and after addition of 1,500 mg/d cholesterol. Both methods indicated that the mass of HDL₂ increased relative to HDL₃. The flotation rate of HDL₃ rose as cholesterol was added to the diet with a low P/S, but not when added to the diet with a high P/S.

Lipoprotein-cell interactions. LDL fractions isolated by zonal ultracentrifugation from the plasmas of subjects eating basal and egg-containing diets competed equally well with ¹²⁵I-LDL for cellular association and degradation (Fig. 2). Furthermore, the amount of intracellular cholesterol ester accumulation produced by LDL collected before and after egg feeding also was not different (data not shown).

DISCUSSION

The main aim of these studies was the characterization of the change in the distribution of the major plasma lipoprotein classes in young men when the cholesterol content of various basal diets were altered. Normolipidemic young men were chosen to obviate any fluctuations due to the menstrual cycle (43). Because of the limited nature of the study population and because only fasting plasma samples were examined, it is not clear whether the results can be extrapolated to post-cibal plasmas, or to plasmas of women, older individuals, or persons with hyperlipidemia. However, it is likely that the results are generally representative of

TABLE VI

Effect of Dietary P/S and Cholesterol on HDL S_f Rates and on the Relative Masses of HDL₂ and HDL₃

Diet		HDL ₃	HDL ₂	HDL ₃
P/S	Eggs			
		S _f = 1.2	weight %	
0.25	—	3.2±0.3	7±2	93±12
0.25	3	3.3±0.1	25±3	75±3*
0.25	6	3.6±0.4*	26±3	74±3*
2.5	—	3.0±0.1	12±2	88±12
2.5	3	2.8±0.3	12±2	88±12
2.5	6	3.4±0.1*	14±3	86±13

S_f rates were obtained by analytic ultracentrifugation, weight % represent corrected HDL₂ and HDL₃ areas under the Schlieren curves relative to each other.

* P < 0.02 by paired two-tailed t test, n = 3 subjects at each P/S ratio.

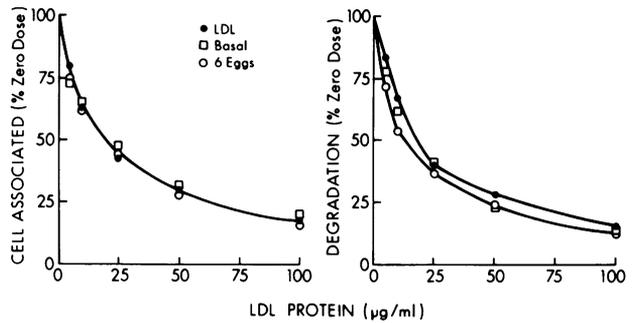


FIGURE 2 Effects of dietary cholesterol on interaction of LDL with the LDL receptor of cultured normal human fibroblasts. Cells were cultured in 30-mm cluster dishes for 5 d in DME-10% fetal calf serum and for 48 h in DME-10% LPDS. Incubations were for 2 h at 37°C in DME-Hepes, pH 7.4, 10% LPDS, 5 µg/ml ¹²⁵I-LDL (specific radioactivity 147 cpm/ng) and increasing doses of the indicated unlabeled lipoproteins (expressed as lipoprotein-protein) isolated by zonal ultracentrifugation. 100% ¹²⁵I-LDL cell associated and degraded were measured in the absence of unlabeled LDL, and were 248 and 306 ng/mg cell protein, respectively. (In behavior these LDL were indistinguishable from other LDL preparations used in other experiments; see reference 39.) LDL, the normal LDL preparation carried in each assay as the overlap standard. Results are means for six individuals fed the diet with the P/S of 0.25. Each point for each individual was measured in triplicate.

the postabsorptive lipoprotein compositions and levels of young men.

Both the cholesterol content and the P/S of the diets were important in determining changes in the levels of lipoproteins in fasting plasma. On diets with low P/S (0.25–0.4), the addition of 750 mg cholesterol/d increased the plasma LDL cholesterol and ApoB values. On the diet with the highest P/S (2.5) even additional 1,500 mg cholesterol produced no significant changes. Some (6), but not all workers (15, 44), find moderating effects of the degree of saturation of fatty acids on the effects of dietary cholesterol.

The major diet cholesterol-induced changes in total cholesterol were due to LDL cholesterol. Analytic and zonal ultracentrifugation (Table V) and column chromatography each revealed that increased numbers of LDL particles of virtually unchanged size, shape, density, and composition were responsible for the rises in LDL cholesterol. Moreover, the interactions of LDL with fibroblast LDL receptor were unaltered by diet (Fig. 2). The increased numbers of particles could have resulted from enhanced hepatic secretion of cholesterol-rich lipoproteins (45, 46) or by accumulation of metabolic products of intestinal lipoproteins in plasma (47).

The relative constancy of LDL physical properties and percentage compositions differs from the results reported for some nonhuman primates, where marked

alterations in LDL molecular weight, size, and composition (32) and LDL interactions with cells (48) were noted when these animals were fed high-cholesterol and high-fat diets. These differences may relate to the fact that the levels of LDL in monkeys rose much more (fivefold over basal) than in man even though diet cholesterol intakes in man and monkeys were similar (0.5–1.0 mg/kcal per d), and levels of LDL were directly related to LDL molecular weight in monkeys.

Mean HDL cholesterol levels did not change when eggs were added to the basal diets (Table II). The lack of rise may be due to the relative insensitivity of the heparin-MnCl₂ procedure to small changes in HDL concentrations. Some ApoE containing HDL (HDL_c) or Lp(a) may have been precipitated along with LDL (49, 50), which would result in an underestimation of HDL cholesterol. In fact, on column chromatographic analysis (32), HDL cholesterol did rise by ~6 mg/dl when 1,500 mg cholesterol was added to diets with low P/S (data not shown). Nevertheless, as eggs were added to the low P/S basal diets, HDL₂ increased relative to HDL₃ both by analytic (Table VI) and zonal ultracentrifugation. HDL₂ did not change significantly in those subjects who were eating the diet with high P/S.

The feeding of high-fat, high-cholesterol diets to several species of animals stimulates the appearance of β -VLDL and HDL_c in their plasmas (51–54). These lipoproteins may be identified in and isolated from plasma by combined ultracentrifugal and electrophoretic procedures (54), but adequate specific methods for their quantitation have not been developed to date. β -VLDL contains more ApoE than does α_2 VLDL (55) and HDL_c contains more than LDL or HDL (54). Although we did not identify these lipoprotein moieties in plasma directly, we did quantify ApoE in whole plasma. Total plasma ApoE did not change (Table II), which confirms the finding of others (9, 56), and suggests that large amounts of these lipoproteins did not accumulate in plasma as a result of the dietary changes. Of course, the potential atherogenic importance of β -VLDL and/or HDL_c may not be reflected adequately by their levels in plasma, but rather by their interactions with vascular cells or interstitium.

The connection between the diet-induced changes in the plasma lipoproteins we measured and atherosclerosis is not clear, because it is difficult to reconcile the responses of the lipoproteins with epidemiologic data. High-cholesterol, high-fat diets are associated with high plasma levels of total and LDL cholesterol and with high risk of coronary artery disease, whereas HDL cholesterol, particularly HDL₂ is thought to be a negative risk factor (57–59). The data presented above demonstrate that potentially atherogenic diets raise not only LDL but also HDL₂ levels. Elsewhere,

we and others have shown that high carbohydrate (60, 61) and vegetarian diets (62) and diets with high P/S (11, 18–21, 23), which are thought to be antiatherogenic, lower both LDL and HDL levels. Clearly, there is a discrepancy between the behavior of HDL as a risk factor in population studies and its responses to manipulations by dietary means, whereas the responses of LDL are consistent. Further work is necessary to clarify this interesting paradox.

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REFERENCES

1. Shekelle, R. B., A. MacMillan Shryock, O. Paul, M. Lopper, J. Stamler, S. Liu, and W. J. Raynor, Jr. 1981. Diet, serum cholesterol, and death from coronary heart disease. *N. Engl. J. Med.* **304**: 65–70.
2. Nichols, A. B., C. Ravenscroft, D. E. Lamphiear, and L. D. Ostrander. 1976. Daily nutritional intake and serum lipid levels: the Tecumseh Study. *Am. J. Clin. Nutr.* **29**: 1384–1392.
3. Kannell, W. B., and T. Gordon. 1970. The Farmington Diet Study: diet and the regulation of serum cholesterol. Department of Health, Education, and Welfare Report, Section 24. Washington, D. C.
4. Keys, A., J. T. Anderson, O. Mickelsen, S. F. Adelson, and F. Fidanza. 1956. Diet and serum cholesterol in man: lack of effect of dietary control. *J. Nutr.* **59**: 39–56.
5. Stein, E. A., D. Mendelsohn, M. Fleming, G. D. Barnard, K. J. Carter, P. S. du Toit, J. D. L. Hansen, and I. Besohn. 1975. Lowering of plasma cholesterol levels in free-living adolescent males: use of natural and synthetic polyunsaturated foods to provide balanced fat diets. *Am. J. Clin. Nutr.* **28**: 1204–1216.
6. The National Diet Heart Study Final Report. 1968. American Heart Association Monograph No. 18. American Heart Association, Inc., Dallas, TX. 27–421.
7. Nestel, P. J., N. Havenstein, H. M. Whyte, T. J. Scott, and L. J. Cook. 1973. Lowering of plasma cholesterol and enhanced sterol excretion with the consumption of polyunsaturated ruminant fats. *N. Engl. J. Med.* **288**: 379–381.
8. Mistry, P., N. E. Miller, M. Laker, W. R. Hazzard, and B. Lewis. 1981. Individual variation in the effects of dietary cholesterol on plasma lipoproteins and cellular cholesterol homeostasis in man. *J. Clin. Invest.* **67**: 493–502.
9. Applebaum-Bowden, D., W. R. Hazzard, J. Cain, M. C. Cheung, R. S. Kushwaha, and J. J. Albers. 1979. Short-term egg yolk feeding in humans. *Atherosclerosis*. **33**: 385–396.
10. Flynn, M. A., G. B. Nolph, T. C. Flynn, R. Kahrs, and G. Krause. 1979. Effects of dietary egg on human serum cholesterol and triglycerides. *Am. J. Clin. Nutr.* **32**: 1051–1057.

11. Grundy, S. M., and E. H. Ahrens, Jr. 1970. The effects of unsaturated dietary fats on absorption, excretion, synthesis, and distribution of cholesterol in man. *J. Clin. Invest.* **49**: 1135-1152.
12. Mahley, R. W., T. L. Innerarity, T. P. Bersot, A. Lipson, and S. Margolis. 1978. Alterations in human high-density lipoproteins, with or without increased plasma-cholesterol, induced by diets high in cholesterol. *Lancet*. **II**: 807-809.
13. Mattson, F. H., B. A. Erickson, and A. M. Kligman. 1972. Effect of dietary cholesterol on serum cholesterol in man. *Am. J. Clin. Nutr.* **25**: 589-594.
14. Connor, W. E., R. E. Hodges, and R. E. Bleiler. 1961. Effect of dietary cholesterol upon serum lipids in man. *J. Lab. Clin. Med.* **57**: 311-343.
15. Keys, A., J. T. Anderson, and F. Grande. 1965. Serum cholesterol response to changes in the diet. II. The effects of cholesterol in the diet. *Clin. Endocrinol. Metab.* **14**: 759-765.
16. Lin, D. S., and W. E. Connor. 1980. The long term effects of dietary cholesterol upon the plasma lipids, lipoproteins, cholesterol absorption, and the sterol balance in man: the demonstration of feedback inhibition of cholesterol biosynthesis and increase bile acid excretion. *J. Lipid Res.* **21**: 1042-1051.
17. Connor, W. E., M. T. Cerqueira, R. W. Connor, R. B. Wallace, R. Malinow, and H. R. Casdorph. 1978. The plasma lipids, lipoproteins, and diet of the Tarahumara Indians of Mexico. *Am. J. Clin. Nutr.* **31**: 1131-1142.
18. Ahrens, E. H., Jr., J. Hirsch, W. Insull, Jr., T. T. Tsaltas, R. Blomstrand, and M. L. Peterson. 1957. The influence of dietary fats on serum lipid levels in man. *Lancet*. **I**: 942-953.
19. Woods, P. D. S., Shioda, Ryuzo, and L. W. Kinsell. 1966. Dietary regulation of cholesterol metabolism. *Lancet*. **I**: 604-607.
20. Spritz, N., and M. A. Mishkel. 1969. Effects of dietary fats on plasma lipids and lipoproteins. An hypothesis for the lipid-lowering effect of unsaturated fatty acids. *J. Clin. Invest.* **48**: 78-86.
21. Connor, W. E., D. T. Witiak, D. B. Stone, and M. L. Armstrong. 1969. Cholesterol balance and fecal neutral steroid and bile acid excretion in normal men fed dietary fats of different fatty acid composition. *J. Clin. Invest.* **48**: 1363-1375.
22. Nestel, P. J., and A. Poyser. 1976. Changes in cholesterol synthesis and excretion when cholesterol intake is increased. *Clin. Endocrinol. Metab.* **25**: 1591-1599.
23. Shephard, J., C. J. Packard, S. M. Grundy, D. Yeshurun, A. M. Gotto, Jr., and O. D. Taunton. 1980. Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. *J. Lipid Res.* **21**: 91-99.
24. Olson, R. E., W. Patsch, M. Epstein, L. L. Rudel, and G. Schonfeld. 1980. Effect of dietary cholesterol on serum lipids and lipoproteins in man. *Am. J. Clin. Nutr.* **33**: 915.
25. Lipid Research Clinics Program. 1974. Manual of Laboratory Operations. Lipid and Lipoprotein Analysis. Department of Health, Education, and Welfare Publication No. (NIH) 75-628. Vol. 1.
26. Schonfeld, G., and B. Pflieger. 1974. The structure of human high density lipoprotein and the levels of apolipoprotein A-I in plasma as determined by radioimmunoassay. *J. Clin. Invest.* **54**: 236-246.
27. Schonfeld, G., J. Chen, W. F. McDonnell, and I. Jeng. 1977. Apolipoprotein A-II content of human plasma high density lipoproteins measured by radioimmunoassay. *J. Lipid Res.* **18**: 645-655.
28. Schonfeld, G., R. S. Lees, P. K. George, and B. Pflieger. 1974. Assay of total plasma apolipoprotein B concentration in human subjects. *J. Clin. Invest.* **53**: 1458-1467.
29. Schonfeld, G., P. K. George, J. Miller, P. Reilly, and J. Witztum. 1979. Apolipoprotein C-II and C-III levels in hyperlipoproteinemia. *Clin. Endocrinol. Metab.* **28**: 1001-1010.
30. Falko, J. M., G. Schonfeld, J. L. Witztum, J. B. Kolar, S. W. Weidman, and R. Steelman. 1980. Effects of diet on Apo-E levels and on the Apo-E subspecies in human plasma lipoproteins. *J. Clin. Endocrinol. Metab.* **50**: 521-528.
31. Rudel, L. L., J. A. Lee, M. D. Morris, and J. M. Felts. 1974. Characterization of plasma lipoproteins separated and purified by agarose-column chromatography. *Biochem. J.* **139**: 89-95.
32. Rudel, L. L., L. L. Pitts, and C. A. Nelson. 1977. Characterization of plasma low density lipoproteins of non-human primates fed dietary cholesterol. *J. Lipid Res.* **18**: 211-222.
33. Patsch, J. R., S. Sailer, G. Kostner, F. Sandhofer, A. Holasek, and H. Braunsteiner. 1974. Separation of the main lipoprotein density classes from human plasma by rate zonal ultracentrifugation. *J. Lipid Res.* **15**: 356-366.
34. Patsch, W., G. Schonfeld, A. M. Gotto, Jr., and J. R. Patsch. 1980. Characterization of human high density lipoproteins by zonal ultracentrifugation. *J. Biol. Chem.* **255**: 3178-3185.
35. Fisher, W. R., M. E. Gravade, and J. L. Mauldin. 1971. Hydrodynamic studies of human low density lipoproteins. Evaluation of the diffusion coefficient and the preferential hydration. *Biochemistry*. **10**: 1622-1629.
36. Bartlett, G. R. 1958. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**: 466-468.
37. Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
38. Goldstein, J. L., and M. S. Brown. 1974. Binding and degradation of low density lipoproteins by cultured human fibroblasts. *J. Biol. Chem.* **249**: 5153-5162.
39. Ostlund, R. E., Jr., B. Pflieger, and G. Schonfeld. 1979. Role of microtubules in low density lipoprotein processing by cultured cells. *J. Clin. Invest.* **63**: 75-84.
40. Bilheimer, D. W., S. Eisenbert, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary *in vitro* and *in vivo* observations. *Biochim. Biophys. Acta.* **260**: 212-221.
41. Nie, N. H., C. H. Hull, J. G. Jenkins, K. Steinbrenner, and D. H. Bent. 1970. Statistical Package for the Social Sciences. McGraw Hill Publications, New York. 2nd edition.
42. Cheung, M. C., and J. J. Albers. 1977. The measurement of apolipoprotein A-I and A-II levels in men and women by immunoassay. *J. Clin. Invest.* **60**: 43-50.
43. Gustafson, A., L. Lillienberg, and A. Svanborg. 1974. Human plasma high density lipoprotein composition during the menstrual cycle. *Scand. J. Clin. Lab. Invest.* **33**: 63-70.
44. Anderson, J. T., F. Grande, and A. Keys. 1976. Independence of the effects of cholesterol and degree of saturation of the fat in the diet on serum cholesterol in man. *Am. J. Clin. Nutr.* **29**: 1184-1189.
45. Swift, L. L., N. R. Manowitz, G. D. Dunn, and V. S. LeQuire. 1980. Isolation and characterization of hepatic

- Golgi lipoproteins from hypercholesterolemic rats. *J. Clin. Invest.* **66**: 415-425.
46. Dolphin, P. J. 1981. Serum and hepatic nascent lipoproteins in normal and hypercholesterolemic rats. *J. Lipid Res.* **22**: 971-989.
 47. Redgrave, T. G., and D. A. Snibson. 1977. Clearance of chylomicron triacylglycerol and cholesteryl ester from the plasma of streptozotocin-induced diabetic and hypercholesterolemic hypothyroid rats. *Clin. Endocrinol. Metab.* **26**: 493-503.
 48. St. Clair, R. W., J. J. Mitschelen, and M. Leight. 1980. Metabolism by cells in culture of low density lipoproteins of abnormal composition from non-human primates with diet induced hypercholesterolemia. *Biochim. Biophys. Acta.* **618**: 63-79.
 49. Albers, J. J., V. G. Cabana, G. R. Warnick, and W. R. Hazzard. 1975. Lp(a) lipoprotein: relationship to sinking pre-beta lipoprotein, hyperlipoproteinemia, and apolipoprotein B. *Clin. Endocrinol. Metab.* **24**: 1047-1054.
 50. Mahley, R. W., and T. L. Innerarity. 1977. Interaction of canine and swine lipoproteins with the low density lipoprotein receptor of fibroblasts as correlated with heparin/manganese precipitability. *J. Biol. Chem.* **252**: 3980-3986.
 51. Mahley, R. W., and K. S. Holcombe. 1977. Alterations of the plasma lipoproteins and apoproteins following cholesterol feeding in the rat. *J. Lipid Res.* **18**: 314-324.
 52. Mahley, R. W., K. H. Weisgraber, and T. Innerarity. 1976. Atherogenic hyperlipoproteinemia induced by cholesterol feeding in the Patas monkey. *Biochemistry.* **15**: 2979-2985.
 53. Mahley, R. W., T. L. Innerarity, K. H. Weisgraber, and D. L. Fray. 1977. Canine hyperlipoproteinemia and atherosclerosis accumulation of lipid by aortic medial cells in vivo and in vitro. *Am. J. Pathol.* **87**: 205-226.
 54. Mahley, R. W., K. H. Weisgraber, and T. Innerarity. 1974. Canine lipoproteins and atherosclerosis. II. Characterization of the plasma lipoproteins associated with atherogenic and nonatherogenic hyperlipidemia. *Circ. Res.* **35**: 713-733.
 55. Mahley, R. W., T. L. Innerarity, M. S. Brown, Y. K. Ho, and J. L. Goldstein. 1980. Cholesteryl ester synthesis in macrophages: stimulation by β -very low density lipoproteins from cholesterol-fed animals of several species. *J. Lipid Res.* **21**: 970-980.
 56. Tan, M. H., M. A. Dickson, J. J. Albers, R. J. Havel, M. C. Cheung, and L. J. Vigne. 1980. The effects of a high cholesterol saturated fat diet on serum high density lipoprotein cholesterol ApoA-I and ApoE levels in normolipidemic humans. *Am. J. Clin. Nutr.* **33**: 2559-2566.
 57. Pooling Project Research Group. 1978. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight, and ECG abnormalities to incidence of major coronary events: final report of the Pooling Project. *J. Chronic Dis.* **31**: 201-306.
 58. Miller, G. J. 1980. High density lipoproteins and atherosclerosis. *Annu. Rev. Med.* **31**: 97-108.
 59. Castelli, W. P., J. T. Doyle, T. Gordon, C. G. Hames, M. C. Hjortland, S. B. Hulley, A. Kagan, and W. J. Zukel. 1977. HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. *Circulation.* **55**: 767-772.
 60. Gonen, B., W. Patsch, I. Kuisk, and G. Schonfeld. 1981. The effect of short-term feeding of a high carbohydrate diet on HDL subclasses in normal subjects. *Clin. Endocrinol. Metab.* **30**: 1125-1129.
 61. Schonfeld, G., S. W. Weidman, J. L. Witztum, and R. M. Bowen. 1976. Alterations in levels and interrelations of plasma apolipoproteins induced by diet. *Clin. Endocrinol. Metab.* **25**: 261-275.
 62. Burslem, J., G. Schonfeld, M. A. Howald, S. W. Weidman, and J. P. Miller. 1978. Plasma apoprotein and lipoprotein lipid levels in vegetarians. *Clin. Endocrinol. Metab.* **27**: 711-719.