Independent effects of dietary saturated fat and cholesterol on plasma lipids, lipoproteins, and apolipoprotein E

E. A. Fisher, C. B. Blum, V. I. Zannis, and J. L. Breslow

Metabolism Division, Children's Hospital Medical Center; Department of Pediatrics, Harvard Medical School; Nutrition Department, Massachusetts Institute of Technology; and Department of Medicine, Columbia University College of Physicians and Surgeons

Abstract Nine normolipidemic males (18–37 years) were fed formula diets containing (as % of calories) egg white protein (15%), glucose polymer:sucrose, 3:1 (54%), and fats (31%) as one of the following: corn oil (corn), corn oil plus 1 gram/day cholesterol (corn+), coconut oil (coco), coconut oil plus 1 gram/day cholesterol (coco+). Two dietary periods of 18 days each were separated by 1 month during which plasma lipid levels returned to prestudy values. A given dietary period consisted of 9 days of either corn or coco feeding allowed by 9 days of corn+ or coco+, respectively. Fasting plasma samples were taken the last 3 days of each 9-day interval. Lipids were determined by standard procedures and the apoE levels in lipoprotein fractions isolated by discontinuous density gradient ultracentrifugation were determined by radioimmunoassay. The biochemical variables measured were: total plasma, VLDL, IDL + LDL, and HDL, cholesterol, triglyceride, and apoE levels, as well as the apoE of plasma d > 1.17 g/ml. The effects of apoE phenotype, the type of dietary oil (corn versus coco), the presence or absence of dietary cholesterol, and the day of sampling within triplicates on the above variables were assessed statistically. The type of oil had the only significant effect on any variable. At $P < 0.01$, the coconut oil diets were associated with significant elevations (as compared to corn oil) of the following nine variables: total, VLDL, IDL + LDL, and HDL cholesterol; total, VLDL, and IDL + LDL apoE; total and VLDL triglycerides.—**Fisher, E. A., C. B. Blum, V. I. Zannis, and J. L. Breslow.** Independent effects of dietary saturated fat and cholesterol on plasma lipids, lipoproteins, and apolipoprotein E. *J. Lipid Res.* 1983. **24:** 1039–1048.

Supplementary key words Saturated fat • cholesterol • apoE • dietary response

The effects of dietary saturated fat and cholesterol on human plasma lipid levels have been investigated for many years by a combination of cross-cultural population studies, diet recall studies (1–4), as well as by inpatient and outpatient feeding studies (5–21). The earlier investigations concentrated mainly on the effects of dietary cholesterol and fats with different ratios of polyunsaturated to saturated fatty acids (P/S ratio) on plasma cholesterol and triglyceride levels (2–13). In later studies, the effects of these nutrients on cholesterol absorption and excretion were also explored (24–27). More recent studies have measured the effect of dietary lipids on plasma lipoproteins and apolipoproteins (14–16, 18, 20–23). these studies have shown that both saturated fats and cholesterol elevate (2, 6, 7, 9, 11, 21–23) and polyunsaturated fats lower (2, 6, 14, 25, 26) plasma cholesterol levels. However, the relative effects of dietary cholesterol and fat saturation have been debated: some have suggested that the polyunsaturated fats play the major role (11), while others have suggested that cholesterol plays the major role (7, 8, 19). Some of these studies also indicate that dietary polyunsaturated fats decrease (14, 15, 25), saturated fats increase (11), and cholesterol has no effect on plasma triglycerides (8, 19). Another general conclusion of these studies is the presence of a large inter-individual variation in response to diet (6, 20, 25, 27). In several of the earlier studies, the subjects under study had various forms of hyperlipoproteinemia, diabetes, or atherosclerosis (6, 7, 11, 26). Since these conditions may be associated with abnormal lipoprotein metabolism, the results obtained may not be applicable to normal individuals. Thus, in spite of the extensive literature on the subject, important questions still exist regarding the relative effects of dietary saturated fats and cholesterol, separately and together, on the plasma lipid, lipoprotein, and apoprotein levels in normal individuals. Furthermore, available data indicate that diets with different P/S ratios may alter not only the concentrations of plasma and LDL cholesterol that are considered atherogenic (28, 29) but also the concentration of the HDL cholesterol (15, 20) that is considered an antiathero-

Abbreviations: HLP, hyperlipoproteinemia; VLDL, very low density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; TG, triglyceride.

genic factor (29, 30). Other nutritional experiments with nonhuman species have shown that diets high in saturated fats and/or cholesterol cause accumulation of certain plasma lipoproteins (HDLc, βVLDL, IDL) that are rich in cholesteryl ester and apolipoprotein E (apoE) (31–40). Patients with type III hyperlipoproteinemia (type III HLP) receiving normal diets demonstrate lipoprotein abnormalities similar to those seen in cholesterol-fed animals. These include markedly increased plasma concentrations of apoE and the presence of β-migrating VLDL (40–45). Recent studies have shown that type III HLP results, at least in part, from a genetically determined alteration in the apoE polypeptide (46–49) rendering it poorly bound by specific cellular receptors (50–53). These observations taken together indicate that alterations in lipoprotein metabolism can be caused by genetic and dietary factors. Such alterations are manifested as changes in the concentration and composition of plasma lipoproteins.

The current study was undertaken in order to assess the effects of fat saturation and cholesterol on the levels of plasma lipids, lipoproteins, and apolipoprotein E in a relatively uniform population of young, normolipidemic, healthy males, by using formula diets with defined lipid and other nutrient composition.

METHODS

Plasma lipid, lipoprotein, and apoE analyses

Subjects resting in the sitting position for 5 min underwent venipuncture, and blood was obtained free-flowing without tourniquet constriction. Specimens were collected in tubes containing EDTA (1 mg/ml blood) and placed on ice. Plasma was obtained by centrifugation at 2500 rpm for 20 min in a refrigerated centrifuge at 4°C. The plasma was used for simultaneous determination of total cholesterol and triglycerides by the Technicon AutoAnalyzer II method as specified by the Laboratory Manual of the Lipid Research Clinics (54), and standardized by the Lipid Standardization Program of the Center for Disease Control in Atlanta. Another aliquot of plasma was used for lipoprotein fractionation by ultracentrifugation. During this procedure, 5 ml of plasma was overlayered with 1 ml of normal saline and centrifuged in a Beckman L5-65 ultracentrifuge for 18 hr at 120,000 *g* in a Beckman 40.3 rotor. After ultracentrifugation, the supernatant, VLDL, was harvested, dialyzed overnight against 4 l of water at 4°C, lyophilized, and used directly for two-dimensional gel electrophoretic analysis to determine apoE phenotypes, as previously described (47, 48). The volume of the infranatant solution was adjusted to 5 ml by addition of normal saline. An aliquot of the infranatant solution was treated with heparin and $MnCl_2$ to precipitate LDL; HDL remained in solution. Cholesterol and triglyceride concentrations were measured in the $d > 1.006$ g/ml infranatant both before and after treatment with heparin and $MnCl_2$. VLDL lipids were the difference between values for tota plasma and infranatant; LDL lipids were the difference between values for the infranatant before and after heparin-manganese precipitation, and HDL lipids were those of the infranatant after heparin-manganese precipitation.

Another 4-ml aliquot of plasma was subjected to discontinuous gradient ultracentrifugation as described by Redgrave, Roberts, and West (55). In this method, the plasma is adjusted to a density of 1.21 gm/ml with potassium bromide and is overlayered in a cellulose nitrate tube with 3 ml of each of the following solutions: potassium bromide of density 1.063 g/ml, potassium bromide of density 1.019 g/ml, and normal saline of density 1.006 g/ml. The tube is then spun at 40,000 rpm for 41 hr in a Beckman SW-41 rotor. The top ten 1-ml fractions were collected with Pasteur pipettes, with approximately 3 ml of remaining material forming the eleventh fraction. The 11 fractions were pooled as indicated. These pools designated A–E represented the density ranges shown that correspond roughly to VLDL, IDL, LDL, HDL, and lipoprotein-free plasma.

Pool	Fractions	Density (d) (g/ml)	Lipoprotein Class
A	1, 2	$d < 1.005$	VLDL
B	3, 4	$1.005 < d < 1.019$	IDL
C	5, 6, 7	$1.019 < d < 1.068$	LDL
D	8, 9, 10	$1.068 < d < 1.17$	HDL
E	11		density ≥ 1.17 g/ml

40 μl of each pool and of each whole plasma sample were lyophilized in siliconized tubes and apoE concentration was determined by radioimmunoassay (45). In this procedure, the samples of plasma were diluted 150-fold in a solution of 50 mM Na decyl sulfate, 100 mM NaCl, 50 mM Na phosphate, and 0.06% NaN_3 prior to assay. The samples of density gradient pools were diluted 50-fold in the same solution prior to assay.

Subjects and diet protocol

The diet protocol was approved by both the Children's Hospital Medical Center and MIT Clinical Investigation Committees. Informed consent was obtained from each subject. Nonobese, healthy, white males were recruited. All had normal physical examinations and normal screening laboratory tests that included complete blood count, hepatic and renal function tests, blood glucose levels, and lipid analyses. These screening tests were repeated at the end of each dietary period. The subjects were compensated at the end of the study.

During the formula diet periods, drug and alcohol use as well as smoking were not permitted. The subjects had no significant illnesses or accidents while on the study and there were no significant changes in their weight or screening tests (except for lipid analyses) during the dietary period. The ages, heights, weights, and apoE phenotypes of the study subjects are indicated in **Table 1.**

The diets were provided in the following manner; approximately 75% of daily caloric intake was provided by a formula. Similar formulas have been used extensively by Dr. K. C. Hayes of the Harvard School of Public Health (56) in nonhuman primate studies. The basis ingredients of the diet were polycose (a glucose polymer, Ross Laboratories) plus sucrose (Domino brand table sugar) in a 3:1 ratio, egg white protein (Henningsen Co.), and one of the following fat sources: *a*) corn oil (Mazola brand, P/S ratio 4:1), *b*) corn oil plus 1 g per day of cholesterol (U.S.P., Sigma Chemical Co.), *c*) coconut oil (92% saturated fatty acids, Shade Foods, Inc.), *d*) coconut oil plus 1 g per day of cholesterol. Tap water was added to these basic ingredients to provide 48 oz of formula daily which was consumed in four 12-oz servings. Daily vitamins were provided as one One-A-Day capsule (Miles Laboratories). Vitamin E was supplemented (60 IU every other day) during the corn oil periods by using capsules (U.S.V. Laboratories). Minerals at RDA levels were added to the formula as a mixture of the appropriate salts. The 25% nonformula calories consisted of special muffins (low protein flour, sucrose, and either corn or coconut oil), 16 oz per day of orange juice and two apples per day. In addition, daily noncaloric intakes of one-third head of lettuce, two stalks of celery, decaffeinated black coffee (with artificial sweetener if desired), and sugarless chewing gum were allowed. The diets were prepared and dispensed under the supervision of experienced research dieticians in a clinical research center setting and the subjects were followed as outpatients. Caloric requirements were estimated at the beginning of the study from a diet history taken by a research dietician, and caloric intake was modified during the study to keep each subject's weight constant. The overall diet (formula and nonformula) contained the following percentage distribution of calories: 15% protein, 54% carbohydrate, and 31% fat. Approximately 1 month prior to the study, while the subjects were taking their ad-lib diets, fasting plasma was obtained on 3 consecutive days for determination of lipid, lipoprotein, and apoE levels.

Subsequently, there were two 18-day dietary periods, each separated by approximately 1 month during which plasma lipid levels returned to ad-lib values. A period consisted of 9 days of either corn oil or coconut oil as the fat source, followed immediately by 9 days of corn oil plus cholesterol or coconut oil plus cholesterol, respectively. Fasting plasma samples were taken the last 3 days of each 9-day interval.

Data analysis

The lipid data were used directly. The apoE data for the pooled ultracentrifuged fractions were adjusted to 100% recovery, assuming identical percentage losses for all fractions. This method of adjustment was supported by examining the triplicate data. Within a triplicate, although percentage recovery varied, the fractional distributions were similar. Overall recovery of apoE was 81%. Statistical analyses were done at the MIT computer center using Biomedical Computer Program P-Series (BMDP) nested analysis of variance programs (57).

TABLE 1. Characteristics of subjects

Subject	Age	Diet Order[a]	Height	ApoE Phenotype	Weight (kg)[b]			
					Corn Oil Diets		Coconut Oil Diets	
					Beginning	End	Beginning	End
			cm					
JB	37	1	193	E4/3	87.8 ± 0.4[c]	87.9 ± 0.1	88.3 ± 0.4	87.3 ± 0.2
EF	30	1	180	E3/3	73.8 ± 0.6	73.5 ± 0.2	74.3 ± 0.5	73.9 ± 0.5
PF	24	2	178.8	E3/3	80.4 ± 0.7	79.5 ± 0.5	80.8 ± 0.3	80.2 ± 0.4
DJ	21	1	171.5	E3/3	69.5 ± 0.3	69.8 ± 0.3	70.1 ± 0.2	69.6 ± 0.6
BM	27	1	175.3	E3/2	67.9 ± 0.4	67.4 ± 0.2	68.1 ± 0.0	68.2 ± 0.1
DM	29	1	180	E3/3	83.5 ± 0.4	83.7 ± 0.1	83.3 ± 0.3	83.7 ± 0.4
DR	18	1	196.2	E3/2	84.5 ± 0.2	83.8 ± 0.3	85.0 ± 0.3	84.5 ± 0.1
JS	21	1	182.9	E3/3	73.1 ± 0.1	74.3 ± 0.6	74.3 ± 0.3	74.7 ± 0.6
TS	22	2	176.5	E3/2	73.3 ± 0.2	72.3 ± 0.5	72.5 ± 0.6	72.6 ± 0.1

[a] 1, Corn oil diets first; 2, coconut oil diets first.
[b] Based on first and last 3 days of each dietary period.
[c] Means ± SD.

TABLE 2. Ad-lib diets: lipid, lipoprotein, and apoE levels[a]

	JB	EF	PF	DJ	BM	DM	DR	JS	TS
Total chol.	168 ± 6	157 ± 2	168 ± 3	162 ± 3	136 ± 7	130 ± 8	118 ± 3	147 ± 3	164 ± 4
VLDL chol.	15 ± 6	3 ± 4	13 ± 11	21 ± 3	14 ± 8	7 ± 6	22 ± 2	10 ± 0	17 ± 6
LDL + IDL chol.	118 ± 1	111 ± 6	112 ± 6	93 ± 2	73 ± 3	87 ± 3	57 ± 2	112 ± 1.4	111 ± 10
HDL chol.	35 ± 2	45 ± 1	43 ± 3	50 ± 4	47 ± 2	38 ± 2	41 ± 3	25 ± 2	36 ± 2
Total TG	48 ± 5	52 ± 4	38 ± 10	45 ± 15	42 ± 4	32 ± 8	73 ± 21	82 ± 1	57 ± 7
VLDL TG	31 ± 5	20 ± 9	29 ± 10	23 ± 15	22 ± 6	12 ± 6	50 ± 33	46 ± 5	29 ± 8
LDL + IDL TG	16 ± 2	27 ± 2	8 ± 9	22 ± 1	20 ± 3	14 ± 13	21 ± 4	36 ± 4	29 ± 1
HDL TG	2 ± 1	4 ± 2	1 ± 2	0 ± 0	0.5 ± 0.7	5 ± 9	1 ± 1	0 ± 0	0 ± 0
Total apoE	25.6 ± 1.7	26.7 ± 3.6	24.6 ± 0.4	30.0 ± 5.5	27.8 ± 3.1	19.3 ± 2.0	25.6 ± 4.0	25.3 ± 6.6	29.3 ± 2.3
VLDL apoE	0.9 ± 0.3	1.0 ± 0.3	1.6 ± 0.7	3.7 ± 2.0	2.5 ± 0.5	2.1 ± 0.8	2.2 ± 0.7	1.8 ± 0.04	1.8 ± 1.5
IDL apoE	0.5 ± 0.02	0.8 ± 0.5	0.3 ± 0.1	0.7 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	1.1 ± 1.3
LDL apoE	2.2 ± 1.8	1.9 ± 1.6	2.6 ± 1.0	4.5 ± 0.1	3.9 ± 1.4	4.3 ± 1.7	1.8 ± 1.1	3.6 ± 0.1	3.5 ± 1.8
HDL apoE	9.2 ± 0.3	9.3 ± 2.1	14.3 ± 6.4	12.1 ± 1.6	13.6 ± 2.3	8.6 ± 1.0	11.8 ± 2.2	8.9 ± 0.3	8.9 ± 1.5
d > 1.17 g/ml ApoE	12.6 ± 0.6	13.8 ± 5.5	5.8 ± 1.8	8.9 ± 0.1	7.3 ± 0.7	4.3 ± 0.9	9.0 ± 1.9	10.7 ± 0.6	14.0 ± 4.4

[a] Means ± SD.

RESULTS

Effect of dietary fats and cholesterol on plasma lipids, lipoproteins, and apoE

The age, weight, height, apoE phenotype, and the diet sequence employed for the nine subjects are shown in Table 1. Fasting plasma samples were taken from each subject on 3 consecutive days several weeks prior to beginning formula feeding. The mean levels of lipids, lipoproteins, and apoE were determined and the results are shown in **Table 2.** The biochemical variables measured were total plasma, VLDL, IDL + LDL, and HDL cholesterol, triglycerides (TG), and apoE, as well as apoE of d > 1.17 gm/ml. All 13 variables measured were within the normal range in every subject.

Subjects then ingested formula diets as described in the Methods section and were sampled during the last 3 days of each dietary period. The mean levels of lipids, lipoproteins, and apoE for all subjects on each day of sampling are shown in **Table 3.** Using a nested analysis of variance design, only the type of oil (corn versus coco) had any statistically significant ($\alpha < 0.01$) effect on lipid or lipoprotein variables (**Table 4**). At $P < 0.01$, the coconut oil compared to the corn oil formula diets were associated with elevations of total, VLDL, IDL + LDL, and HDL cholesterol; total, and VLDL triglycerides; and total, VLDL, and IDL + LDL apoE. In this particular dietary protocol, the addition of 1 g/day of USP cholesterol to the diet had no measurable effect on plasma lipid, lipoprotein, or apoE levels. In addition, there were no significant interactions between saturated fat and cholesterol feeding. In these subjects, it appeared that the effects of the type of oil (corn or coco) were, if not complete, at least in a plateau phase, since sampling plasma at the end of days 7, 8, and 9 did not show a significant secular trend in the variables measured.

The data were also analyzed by comparing the formula diets to the ad-lib diets (**Table 5**). When compared to ad-lib diets, the corn ± cholesterol diets significantly decreased the total plasma IDL + LDL and HDL cholesterol concentrations without significantly affecting VLDL cholesterol, triglycerides, or apoE concentrations. When compared to ad-lib diets, the coconut ± cholesterol diets significantly increased total plasma and VLDL triglycerides, and VLDL, IDL + LDL and d > 1.17 g/ml apoE values.

Assessment of apoE phenotypes and ad-lib lipoprotein levels as predictors of dietary response

Of the nine subjects studied, five had the apoE phenotype E3/3 and three had the apoE phenotype E3/2. The data were repartitioned into subgroups according

TABLE 3. Formula diets: lipid, lipoprotein, and apoE levels. Results for entire group of subjects (n = 9)[a]

	Sample	Corn	Corn+	Coco	Coco+
Total chol.	1	114 ± 14	118 ± 11	166 ± 27	180 ± 29
	2	112 ± 12	117 ± 13	169 ± 31	178 ± 31
	3	115 ± 13	115 ± 14	168 ± 27	174 ± 30
VLDL chol.	1	13 ± 7	15 ± 7	20 ± 9	26 ± 10
	2	10 ± 5	17 ± 5	23 ± 17	34 ± 12
	3	14 ± 5	11 ± 5	20 ± 6	22 ± 13
LDL + IDL chol.	1	68 ± 16	69 ± 17	105 ± 25	116 ± 28
	2	69 ± 14	66 ± 16	104 ± 22	108 ± 36
	3	68 ± 15	71 ± 17	106 ± 29	112 ± 32
HDL chol.	1	34 ± 6	34 ± 5	41 ± 7	38 ± 7
	2	34 ± 6	34 ± 7	42 ± 9	37 ± 9
	3	34 ± 6	33 ± 8	43 ± 8	40 ± 7
Total TG	1	59 ± 17	49 ± 15	79 ± 43	99 ± 22
	2	54 ± 13	57 ± 14	82 ± 46	103 ± 31
	3	56 ± 18	51 ± 11	86 ± 43	102 ± 42
VLDL TG	1	39 ± 17	32 ± 14	43 ± 21	65 ± 20
	2	33 ± 16	39 ± 15	50 ± 27	73 ± 29
	3	34 ± 17	32 ± 11	53 ± 22	71 ± 40
LDL + IDL TG	1	18 ± 7	18 ± 3	36 ± 26	35 ± 18
	2	20 ± 5	18 ± 4	32 ± 22	30 ± 12
	3	22 ± 3	19 ± 4	33 ± 23	31 ± 16
HDL TG	1	1.7 ± 4	0.2 ± 1	0 ± 0	0 ± 0
	2	0.3 ± 1	0.2 ± 1	0 ± 0	0 ± 0
	3	0.3 ± 1	0.2 ± 1	0 ± 0	0 ± 0
Total apoE	1	24.1 ± 2.7	24.2 ± 3.8	32.0 ± 6.2	32.2 ± 8.5
	2	23.8 ± 2.9	24.6 ± 3.8	32.7 ± 9.2	29.9 ± 8.8
	3	24.1 ± 5.0	24.6 ± 4.5	31.7 ± 7.9	30.7 ± 6.6
VLDL apoE	1	2.1 ± 1.2	2.9 ± 1.9	5.3 ± 3.8	6.8 ± 3.6
	2	1.7 ± 0.9	3.4 ± 2.3	5.7 ± 5.3	7.2 ± 3.0
	3	1.8 ± 1.4	2.1 ± 1.7	6.3 ± 5.5	6.5 ± 2.7
LDL + IDL apoE	1	3.3 ± 1.5	3.4 ± 2.7	9.8 ± 4.0	7.3 ± 3.3
	2	4.3 ± 1.8	3.4 ± 1.5	8.3 ± 4.2	8.0 ± 3.4
	3	3.6 ± 1.8	4.8 ± 3.2	8.3 ± 3.9	7.3 ± 2.5
HDL apoE	1	10.3 ± 3.2	10.0 ± 2.3	10.4 ± 3.2	10.9 ± 2.9
	2	9.9 ± 2.4	10.4 ± 3.7	10.6 ± 3.2	8.9 ± 2.0
	3	10.9 ± 3.9	10.6 ± 3.3	10.7 ± 3.2	10.2 ± 2.1
d > 1.17 g/ml ApoE	1	8.5 ± 3.8	7.8 ± 2.1	6.5 ± 4.4	7.4 ± 3.1
	2	7.8 ± 3.3	7.6 ± 2.5	8.0 ± 4.8	5.8 ± 3.8
	3	7.8 ± 2.7	7.1 ± 2.6	6.5 ± 2.6	6.7 ± 3.2

[a] Means ± SD.

to apoE phenotype and re-analyzed with apoE phenotype as a factor. The single subject with the E4/3 phenotype was excluded from this analysis. In this small sample, including only two apoE phenotypes, there was no significant effect of apoE phenotype on any of the variables measured.

In further analysis of the data presented in this report, we sought to determine whether a subject's lipoprotein level during the ad-lib diet period had any value in predicting the magnitude of the change in the value of a variable associated with a change in dietary oils (defined as dietary response). The means of the total, LDL, and HDL cholesterol and total triglyceride levels from the ad-lib period for each subject were examined to see if they correlated with dietary responses of total, LDL, and HDL cholesterol, and total triglycerides. In this analysis, only the ad-lib HDL cholesterol levels had a significant correlation, $P < 0.05$ (**Table 6**). The prestudy HDL cholesterol values best predicted the change in total cholesterol, LDL cholesterol, and total triglycerides. However, prestudy HDL cholesterol levels did not predict diet-induced changes in HDL cholesterol itself. Furthermore, ad-lib total cholesterol, LDL cholesterol, and total triglyceride were not significant pre-

TABLE 4. Summary of statistical results[a]

Variable	Effect of			Interactions among Variables
	Dietary Oil	Cholesterol	Day	
Total chol.	<0.0001			
VLDL chol.	<0.01			
IDL + LDL chol.	<0.0001		<0.03	
HDL chol.	<0.002			
Total TG	<0.0001			(oil, chol.) <0.02
VLDL TG	<0.002			
IDL + LDL TG	<0.05			
HDL TG				
Total apoE	<0.002			
VLDL apoE	<0.003	<0.04		
IDL + LDL apoE	<0.002			
HDL apoE				
d > 1.17 g/ml ApoE	<0.04			(oil, chol., day) < 0.05

[a] Numbers in the columns are *P* values. Because of the group size and the many comparisons, only *P* values < 0.01 are considered to be significant. *P* values not shown are >0.05.

dictors of plasma lipid and lipoprotein changes in response to a saturated fat-containing diet.

ApoE distribution in lipoprotein fractions following density gradient ultracentrifugation

The present studies provide insight into the effects of diets on the distribution of apolipoprotein E among lipoproteins. As shown in Tables 2 and 3, apoE of normal subjects is found predominantly in HDL and in d > 1.17 gm/ml fractions, and to a lesser extent in VLDL, IDL, and LDL. The relative distribution of apoE was not affected significantly by the corn oil diet as compared to the ad-lib diets. However, the coconut oil diet shifted the apoE towards lipoproteins of lower density (VLDL, IDL, and LDL).

DISCUSSION

The present studies were undertaken in order to assess the effects of dietary cholesterol and saturated fats on plasma levels of lipids, lipoproteins, and apoE in normal human males using well-defined nutritional protocols. In these experiments, diets containing 31% of calories in the form of coconut oil produced statistically significant elevations in total, VLDL, IDL + LDL, and HDL cholesterol; total, VLDL, and IDL + LDL apoE; and total and VLDL triglycerides when compared to the corn oil-containing diets. Dietary cholesterol had no significant effect on any variable assayed. It is possible that longer treatment with cholesterol might have resulted in an effect. However, in our experimental de-

TABLE 5. Group ad-lib versus dietary period values[a]

	Ad-lib	Corn	Coco
Total chol.	150 ± 18[b]	115 ± 12 (*P* <0.001)	172 ± 28 (*P* < 0.05)
VLDL chol.	14 ± 6	13 ± 5	23 ± 7 (*P* < 0.02)
IDL + LDL chol.	97 ± 21	68 ± 15 (*P* < 0.001)	109 ± 28
HDL chol.	40 ± 8	34 ± 5 (*P* < 0.01)	40 ± 7
Total TG	52 ± 16	54 ± 11	92 ± 30 (*P* < 0.001)
VLDL TG	29 ± 12	34 ± 11	59 ± 16 (*P* < 0.001)
IDL + LDL TG	21 ± 12	19 ± 3	33 ± 19
HDL TG	2 ± 2	0.4 ± 0.7	0 ± 0
Total apoE	26.0 ± 3.1	24.4 ± 3.4	31.2 ± 7.9
VLDL apoE	2.0 ± 0.8	2.7 ± 1.3	6.2 ± 3.5 (*P* < 0.01)
IDL + LDL apoE	3.6 ± 1.4	3.9 ± 1.5	7.6 ± 2.7 (*P* < 0.01)
HDL apoE	10.7 ± 2.2	10.5 ± 2.4	10.1 ± 1.9
d > 1.17 g/ml ApoE	9.6 ± 3.5	7.9 ± 2.4	6.6 ± 2.5 (*P* < 0.01)

[a] The means from the ad-lib periods were compared to the corresponding means from the corn and coco dietary periods. The data from the dietary periods were not separated in terms of cholesterol content since previous statistical analyses (Table 4) did not demonstrate a significant cholesterol effect.

[b] Means ± SD. All *P* values not shown are >0.05.

TABLE 6. Significant (α = 0.05) correlation between ad-lib lipid levels and dietary response

Response to Diet	Ad-lib Variable	Correlation Coefficient	*P* Value
ΔTotal cholesterol	HDL cholesterol	−0.69	<0.04
ΔLDL cholesterol	HDL cholesterol	−0.71	<0.04
ΔTotal triglycerides	HDL cholesterol	−0.81	<0.01

sign, although the complete effect of dietary cholesterol may not have been expressed by day 9, some significant effect should have been evident. The fact that sampling after days 7, 8, and 9 (Table 3) of cholesterol ingestion showed no trend suggests that, under the conditions of our study, a cholesterol effect would not be manifest in any reasonable period of time. In addition, others (2, 8, 11, 19, 20, 58–60) have noted that most dietary effects on serum lipids have stabilized within 10 days to 2 weeks.

When compared with previous nutritional studies, our findings demonstrate similarities, but are different in some respects (5–23). We found that saturated fats increase plasma triglyceride levels as previously reported (11). Also in agreement with previous studies, we find that a diet high in polyunsaturated fats, even when supplemented with substantial amounts of cholesterol, has a plasma cholesterol-lowering effect and that saturated fat has a plasma cholesterol-elevating effect (6, 14, 19, 25, 26). Previous studies have shown that saturated fats enriched in myristic acid exert the greatest effect in elevating plasma cholesterol levels (2). The coconut oil used for our study is rich in myristic acid (17.6%) and our results are consistent with these earlier findings. Overall, the coconut oil we used consisted of 77% saturated fatty acids, chain length C12 or longer. The content of unsaturated fatty acids was only 8%, which is the lowest of the naturally occurring fat sources commonly used in dietary experiments to provide saturated fatty acids (61). It should be noted that corn oil consists of predominantly long-chain fatty acids and our experiments do not separate any effects of chain length distribution differences from differences in the content of saturated fatty acids. However, any of the common fat sources that would be rich in long-chain saturated fatty acids have a significant (28–67%) content of unsaturated fatty acids (61). In future studies, the use of mixtures of individual fatty acids would allow more control over this dietary variable.

In our study, dietary cholesterol had no significant effect on plasma cholesterol levels. Previous studies have indicated that dietary cholesterol either increased or did not alter plasma cholesterol levels (2, 7, 8, 19–21). In addition, it has been reported by others that dietary cholesterol and saturated fats act synergistically in elevating plasma cholesterol levels (9). However, our data did not reveal such synergistic effects. The reasons for the discrepancy between our results and those of others are not clear. The data of Corey and Hayes (62) from studies of nonhuman primates indicate that dietary cholesterol is absorbed to a normal extent when presented in a formula such as the one we used. They found 36–55% absorption of USP cholesterol included in formula diets similar to the ones we used for a variety of nonhuman primate species. This range is comparable to values found in humans in studies using egg yolk cholesterol (19, 63). Thus, the lack of an observed metabolic effect of dietary cholesterol in the present study is unlikely to be caused by poor absorption of the cholesterol from the dietary formula. One might speculate that another dietary component, lacking in our subjects' diets, perhaps phospholipids, may be needed for metabolic effects of dietary cholesterol to be evidenced. Our data do not address this question directly, so we can only speculate on the possibility.

We found statistically significant increases in plasma and lipoprotein apoE levels when we compared the coco with the corn diets; however, total apoE levels remained within the accepted normal limits for plasma apoE (45). In accord with other recent studies of similar design, we did not observe any change in plasma apoE levels when large quantities of cholesterol were added to either the corn or coconut oil based diets (16, 21, 22). Of considerable interest is the observation that although apoE in normal subjects on ad-lib and corn oil diets is found mainly in HDL and to a lesser extent in VLDL, IDL, and LDL, the coconut oil diet shifts the apoE distribution towards VLDL, IDL, and LDL. Redistribution of apoE caused by dietary fat has been attributed to the transfer of this apoprotein from HDL to the lipoproteins of lower density (64). The observed increase in apoE on the coco diets may result from synthesis of apoE-containing lipoprotein particles, decreased catabolism, or a combination of both processes. It should be noted that the apoE data for the lipoprotein fractions were obtained from samples separated by density gradient ultracentrifugation. Blum (45, 64, 65) has noted that ultracentrifugation can cause artifacts in quantitative results as compared to results obtained by agarose column chromatography. However, since the apoE dis-

tributions obtained from both methods are similar, the effects of ultracentrifugation are nonspecific and comparisons between identically processed samples are valid.

In agreement with others (6, 20, 25, 27), we have observed considerable interindividual variation in the response of the subjects to dietary cholesterol and saturated fats. This variation may reflect individual differences in the regulation of specific metabolic pathways for cholesterol, saturated fats, or plasma lipoproteins. These observations of interindividual differences in response to dietary perturbations prompted us to assess various ad-lib lipoprotein values and apoE phenotypes as predictors of diet-induced changes in plasma lipids and lipoproteins. We found that only ad-lib HDL cholesterol levels gave a significant correlation with diet induced changes in lipoprotein levels ($P < 0.05$). This intriguing finding must be considered preliminary because it is based on the study of only nine subjects and because it emerged from a search of numerous potential relationships, a procedure that may yield spurious results. Thus, additional study of a larger number of subjects is needed to confirm the finding of a relationship between ad-lib HDL-cholesterol levels and the degree of diet-induced changes in total plasma cholesterol, LDL cholesterol, and plasma triglycerides.

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