Effect of dietary cholesterol and degree of fat unsaturation on plasma lipid levels, lipoprotein composition, and fecal steroid excretion in normal young adult men[1-3]

Suk Y Oh, PhD and Philip A Monaco, BSc

ABSTRACT Effect of four test diets differing in the ratio of polyunsaturated to saturated fats (P/S, 1.8 vs 0.28) and two cholesterol levels (1,000 vs 300 mg/day) for each level of the P/S ratio was determined on plasma lipid levels, lipoprotein compositions and concentrations, and fecal steroid excretion in a controlled diet study with 11 normal young men using a crossover design. Plasma cholesterol levels were significantly decreased by the diets high in P/S ratio regardless of the dietary cholesterol levels (14% decrease by high cholesterol and 20% in low cholesterol) while the diets low in P/S ratio increased cholesterol by an average of 24 and 22% in presence of high and low cholesterol, respectively. Lipids and apoproteins of lower-density lipoproteins were changed in accordance with those of plasma cholesterol but changes in high-density lipoprotein (HDL) appear to depend on both cholesterol content and P/S ratio. Dietary cholesterol level profoundly influenced the excretion of neutral sterols and diets high in P/S ratio significantly ($p < 0.05$) increased fecal bile acid extraction. The present study demonstrated that dietary polyunsaturated fats, when a moderate amount was consumed, were effective and beneficial hypocholesterolemic nutrients without reducing HDL-cholesterol. *Am J Clin Nutr* 1985;42:399–413.

KEY WORDS Dietary cholesterol, fat unsaturation, plasma lipids, lipoproteins, fecal steroids

Introduction

Attempts to relate dietary fat unsaturation and cholesterol to plasma lipid levels and lipoprotein composition have produced conflicting conclusions (1–5). It is clear, however, that the regular ingestion of large amount of saturated fat and cholesterol contributes to an elevation of plasma cholesterol and that polyunsaturated fat (PUF) effectively decreases it in humans (6–8). One of the key questions still to be answered from the results of such studies is whether a diet enriched in polyunsaturated fats has detrimental effect (1) on the pattern of cholesterol distributions among the plasma lipoprotein fractions. This question is particularly important since polyunsaturated fat is a widely used natural hypocholesterolemic nutrient (9). Unfortunately, much of the evidence for the inverse relationship between dietary PUF and high-density lipoprotein (HDL) cholesterol was obtained under unusual experimental conditions (1–2, 10–11). In the present study, a controlled-feeding study was conducted to elucidate the effect of dietary cholesterol and degree of fat unsaturation on plasma lipid levels and lipoprotein compositions, particularly those of HDL, in free-living normolipidemic young adult men.

Materials and methods

Subjects

Twelve young adult males were carefully selected from a pool of 25 university students on the basis of a

[1] From the Department of Foods and Nutrition, and Nutrition Research Institute, Oregon State University, Corvallis, OR.

[2] Supported by NIH Grant HL-26934.

[3] Address reprint requests to: Dr SY Oh, Division of Foods and Nutrition, College of Health, University of Utah, Salt Lake City, UT 84112.

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physical examination and medical history. Subjects with a family history of hyperlipidemia were not selected. None of the subjects were taking medications known to affect lipid metabolism. None of them were smokers. They were not permitted to drink alcohol during the study period. They were instructed not to change their routine physical activities. Prior to admission to the study each subject was individually counseled by a dietitian to keep dietary records of each meal and collect complete stool. One of three subjects on Diet D withdrew his participation from the study for personal reasons. Personal data on the subjects at admission and ranges of body weights through the study are given in **Table 1.**

During a 1-wk baseline period dietary records of ad libitum diets, complete stool collection and exercise logs were obtained for calculation of energy requirement for weight maintenance and for later analysis. Subsequently, subjects were randomly assigned to one of four dietary groups. The study was conducted on an outpatient basis. Subjects came to the metabolic kitchen and ate 3 meals/day. Snacks were taken out for consumption between meals or in the evening. Informed consent was obtained from each subject. The protocol of this study has been approved by the Human Subject Review Committee of Oregon State University. All diets recorded by the subjects during the baseline period were coded for batch processing by the CDC Cyber 170/172 system at the Oregon State University Computer Center. The 1981 update of the Ohio State Nutrient Data Base (12) was modified and used to determine the subject's nutrient intake. **Table 2** shows individual intake of major nutrients which are known to affect cholesterol metabolism.

Diets

Diets were prepared with ordinary food ingredients. Meats were ordered from a local butcher shop and frozen until used. Canned foods, margarines, and cereals were purchased in case lots. Milk, vegetables, and fruits were obtained from a local supermarket. Prerendered beef tallow was purchased from Tek-Lad (Madison, Wisconsin) and egg yolk was acquired from the poultry science department on campus.

There were four experimental diets differing in cholesterol content (300 or 1000 mg) and the ratio of polyunsaturated to saturated fat (P/S, 0.28 or 1.8). They were the diets high in P/S ratio and high cholesterol (P/H), low in P/S ratio and high cholesterol (S/H), high in P/S ratio and low cholesterol (P/L), and low in P/S ratio and low cholesterol (S/L). The composition and energy sources of the diets with fat modifications are given in **Table 3.** The diets were formulated by adding beef tallow or saffola margarine to alter P/S ratio and by using egg yolk to increase cholesterol level in the diet. The dietary exchanges were made in such a way that the total calorie intake remained constant. The addition of an ingredient to the diet was made at the expense of an equivalent amount of calories of another food. Approximately ⅔ of dietary fats came from the test fats. **Table 4** presents typical menus of P/L Diet and their nutrient content/standard servings. The basic food ingredients of menus for other diets were the same as those of P/L Diet. Nutrient values were calculated using the USDA Agricultural Handbook No 456 and Handbooks series 8-1 to 8-6. All nutrient intakes exceeded the 1980 RDA

TABLE 1
Characteristics of the subjects at admission and ranges of body weights during the study

Group*	Number of subjects	Age	Body weight (kg) Time (wk) T = 0	T = 6	T = 12	Mean energy intake for wt maintenance	Plasma lipid levels at baseline Chol†	TG†
						kcal/day	*mg/100 ml*	
I	3	23	68.3	69.2	69.2	3400	225	82
		24	73.8	72.8	71.9	3800	187	51
		22	68.3	67.4	67.4	3600	169	39
	Mean	23	70.1	69.7	69.7	3600	194	57
II	3	23	65.6	66.1	65.6	3400	168	46
		21	76.9	75.6	77.8	3850	145	55
		22	78.7	78.3	78.3	3600	189	51
	Mean	22	73.8	73.3	73.8	3616	167	50
III	3	23	67.0	67.4	67.4	3200	190	33
		22	78.3	76.4	76.9	3700	178	101
		23	71.0	70.1	71.0	3400	186	43
	Mean	23	72.9	71.5	71.9	3433	185	59
IV	2	21	68.8	70.1	67.4	3400	156	39
		21	58.8	58.8	58.8	2850	138	37
	Mean	21	63.8	64.3	62.9	3225	147	38

* Each diet-group was successively placed on two isocaloric diets for 6-wk/diet.
† Chol = Cholesterol, TG = Triglyceride.

TABLE 2
Dietary intakes of key nutrients on the habitual diets*†

Number of subjects	Group	Energy	% Calories					P/S Ratio	Cholesterol
			Protein	Carbohydrates	Fat				
					Total	Saturated	Polyunsaturated		
		kcal							*mg/day*
3	I	3068	14	46	40	152	6.1	0.46	698
		4293	13	49	38	14.8	7.1	0.48	556
		3670	15	49	36	13.1	5.8	0.44	489
Mean ± SD		3677 ± 612	14 ± 1	48 ± 2	38 ± 2			0.46 ± .02	581 ± 106
3	II	3219	14	49	37	13.3	6.3	0.47	612
		3927	16	40	44	17.0	7.0	0.41	573
		3626	12	48	40	14.7	6.0	0.44	551
Mean ± SD		3590 ± 355	14 ± 2	46 ± 5	40 ± 4			0.44 ± .03	578 ± 30
3	III	3451	15	46	39	13.5	6.1	0.45	434
		4015	17	41	42	15.4	6.6	0.43	602
		2460	14	48	38	13.7	6.9	0.50	508
Mean ± SD		3308 ± 787	15 ± 2	45 ± 4	40 ± 2			0.46 ± .04	514 ± 84
2	IV	3304	16	47	37	13.4	6.3	0.47	529
		2728	13	46	41	15.7	6.6	0.42	477
Mean ± SD		3016 ± 407	15 ± 2	47 ± 1	39 ± 3			0.45 ± .04	503 ± 36

* Nutrient values were calculated from the Nutrient Data Base of the Ohio State University (12).
† Calculated from 1-wk dietary records.

values for males in the age group represented in this study.

Experimental design

A crossover design was used for the study over a 12-wk feeding period. Two isocaloric diets were successively tested on each group consisting of three subjects. Diet A was fed first for 6 wk followed by Diet B for another 6 wk in crisscross pattern as shown in **Figure 1.** The advantages of the crossover design are that each individual serves as a block in a completely randomized block design and that two isocholesterol diets differing in P/S ratio were tested on each subject.

Laboratory analyses

Blood samples were obtained from an antecubital vein in vacutainers containing 0.1% ethylenediaminetetraacetic acid (EDTA) after a 14-h overnight fast on T

TABLE 3
A typical basic diet menu (P/L Diet: high P/S ratio and low cholesterol)

Meal	Food	Amount	kcal	Meal	Food	Amount	kcal
		g				*g*	
Breakfast	Orange juice	249	122	Supper	Roast beef	114	214
	Sugar	4	15		M. Potatoes	½ c.	118
	Cornflakes	28	106		Gravy, Saffola	½ c.	177
	Whole wheat bread	56	134		Green beans	135	32
	Whole milk	244	157		Roll	50	156
	Bananas	150	128		Salad	1 c.	18
					Celery Seed dressing	17	97
Lunch	Turkey breast	85	134		Rye krisp	2 triple	50
	Saffola margarine	21	150		Peach half w/syrup		85
	Lettuce	25	3		Popcorn	18	69
	Hamburger bun	40	119		Saffola margarine	23	168
	Celery sticks	56	10		Butter	5	33
	Pnut butter-beef fat	20	133		Margarine	5	36
	Whole milk	244	157				
	Carrot cupcake	113	336		Total		3009
	Walnuts	8	52				

TABLE 4
Effect of dietary fat modifications on plasma lipid levels

Group	Diet period	Total cholesterol	Free cholesterol	Triglycerides	Phospholipids
			*mg/dl ± SD**		
I	Baseline	194.0 ± 30.9	66.3 ± 12.1	57.3 ± 22.2	172.3 ± 25.1
	P/H	167.0 ± 34.0†	50.3 ± 10.7†	40.7 ± 11.9†	144.7 ± 25.7†
	S/H	214.0 ± 35.1‡	60.3 ± 12.7	50.7 ± 7.5‡	212.3 ± 13.7†‖
II	Baseline	167.3 ± 22.0	54.0 ± 3.0	50.7 ± 4.5	156.3 ± 20.5
	S/H	199.3 ± 28.3†	64.3 ± 7.5†	70.3 ± 20.3†	197.7 ± 25.7†
	P/H	161.0 ± 22.1‡	54.7 ± 5.5‡	47.7 ± 10.8†‡	149.3 ± 23.0‡
III	Baseline	184.7 ± 6.1	65.7 ± 6.2	59.3 ± 36.5	172.0 ± 10.4
	P/L	147.7 ± 5.9§	45.3 ± 2.3§	38.7 ± 11.2†	144.3 ± 8.5§
	S/L	178.7 ± 8.5‡	55.3 ± 2.9†‡	67.0 ± 31.4‡	209.3 ± 10.4†‖
IV	Baseline	147.0 ± 23.9	41.5 ± 13.4	38.0 ± 1.4	127.5 ± 43.1
	S/L	180.5 ± 20.5†	53.0 ± 12.6	53.0 ± 2.8§	159.5 ± 48.8
	P/L	143.5 ± 26.1‡	42.5 ± 9.2‡	28.5 ± 10.6‖	147.5 ± 29.0

P/H: High P/S ratio, high cholesterol; S/H: Low P/S ratio, high cholesterol; P/L: High P/S ratio, low cholesterol; S/L: Low P/S ratio, low cholesterol.

* Mean ± SD of 3 subjects for groups I, II, III and 2 for IV.

† Significantly changed from the baseline at $p < 0.05$, paired t test.

‡ Significantly changed from the previous diet at $p < 0.05$, paired t test.

§ Significantly changed from the baseline at $p < 0.01$, paired t test.

‖ Significantly changed from the previous diet at $p < 0.01$, paired t test.

= 0, T = 6, T = 7, T = 9 and T = 12 wk and the plasma was separated by centrifugation at 1,860 × *g* for 30 min at 4°C in a refrigerated centrifuge. Fifty milliliters of blood/occasion was collected so that lipoproteins could be isolated. Blood samples were collected while subjects were on the ad libitum diet (T = 0) and at the end of the first diet (T = 6). During the second 6-wk diet period, blood samples were taken after 1 (T = 7), 3 (T = 9), and 6 (T = 12) wk on the second diet in order to determine the carry-over effect of the first diet on the plasma lipid levels. Plasma cholesterol (free and total) was determined by the cholesterol oxidase method as previously described (13). Triglycerides were quantitated by an Autoanalyzer II (Technicon, Tarrytown, NY) (14).

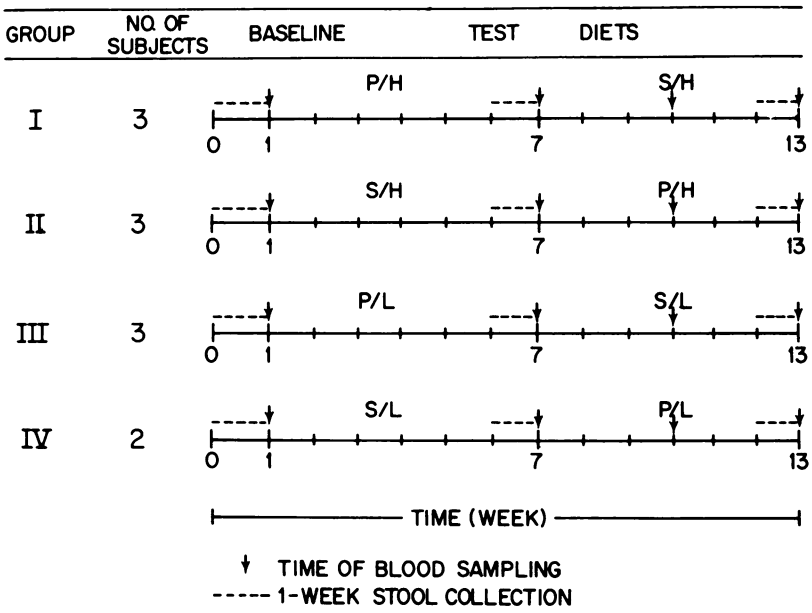

FIG 1. Experimental design with dietary protocol in a crossover pattern. Each group of subjects was sequentially placed on two different diets differing only in P/S ratio.

Phospholipids were measured by the Bartlett method (15). Plasma lipoprotein fractions were isolated by sequential preparative ultracentrifugation as described by Havel et al (16). Ultracentrifugation was performed at 4°C in a Beckman 50 Ti Rotor using a Beckman model L5-75 ultracentrifuge (Beckman, Fullerton, CA). In operation of the ultracentrifuge the $\omega^2 t$ integrator was employed to exactly reproduce the total centrifugal effect ($\omega^2 dt$) for isolation of lipoproteins from blood samples obtained over different times. Four major lipoprotein fractions (VLDL, d < 1.006 g/ml; LDL, d = 1.006–1.063 g/ml; HDL_2, d = 1.063–1.125 g/ml; HDL_3, d = 1.125–1.21 g/ml) were sequentially isolated by tube slicing technique and subsequently dialyzed for 24 h with three changes of dialysate which consisted of 0.15 M NaCl containing 0.01% EDTA, pH 7.0.

Cholesterol, triglycerides, and phospholipids of isolated lipoproteins were assayed by the same procedures used for plasma lipids. Total protein was measured by the Lowry method (17) with bovine serum albumin as the standard. For determination of apo B concentration in LDL, apo B was precipitated by tetramethylurea (TMU) as described previously (2, 18) and protein in the supernatant was determined by Lowry method. The difference in protein values between the intact lipoprotein and that in the supernatant after TMU precipitation was used as the apo B concentration.

The cholesterol values of the four lipoprotein classes in the present study were obtained by assaying cholesterol contents in the four lipoprotein fractions and presented without correcting them by the percent of cholesterol recovery as a fraction of the total plasma cholesterol concentration. Therefore, our data of HDL cholesterol do not represent the plasma HDL cholesterol values which were obtained by the polyanion precipitation method employed by others (1–4, 10–11).

Apoprotein A-I, A-II of HDLs and apoproteins B, C_{III} and E of VLDL were measured by rocket electroimmunoassay (19) in the laboratory of Dr Peter Alaupovic at Oklahoma Medical Research Foundation. Briefly, the electrophoresis was performed using 2% rabbit antiserum to each apoprotein under assay in 1.8% agarose (type A37, Accurate Chemical Co) in 50 mM diethylbarbiturate buffer (pH 8.5) as supporting medium for the antisera. The electrophoresis was carried out at 10 V/cm for 4–6 h depending on the assay. Antibodies were produced in rabbits for apo A-I and goats for B, C_{III} and E apoproteins.

Analysis of fecal steroids

Feces were collected for 1 whole wk between two fecal dye markers, FDC Blue #1 (50 mg of the dye plus 200 mg methylcellulose) in a gelatin capsule which were orally taken on day 1 and day 7 during the periods as indicated in Figure 1. The pooled feces were weighed and homogenized in a paint-blender with a measured volume of water. Aliquots of each homogenate were transferred into weighed plastic containers and dried in a freezer dryer. The dried fecal aliquots were ground with a mortar and pestle and stored at −20°C until they were used for steroid analysis.

Neutral and acidic fecal steroids were extracted from 0.5 g of dried fecal powder in the presence of two internal standards, 5α-cholestane for neutral steroids and hyodeoxycholic acid for acidic steroids, respectively. The extraction procedures of Grundy et al (20) were used.

Chromatographic analysis. The petroleum ether and the ethyl acetate extracts were dried under nitrogen for quantitation in gas chromatography. All gas chromagraphic analyses were performed in a Hewlett Packard model 401 with flame ionization detector. For the neutral sterol analysis, 1 or 2 μl aliquot from 100 μl hexane solution was directly injected on the column without derivatization. 3% OV-17 on 100/120 mesh Gas Chrom Q was packed into a 6-ft glass column with 2 mm ID. The column temperature was 260°C and the helium (carrier gas) flow rate was 33 ml/min.

For the bile acid analysis a shorter column (3′ × 2 mm ID) was packed with 3% SP-2250 on 100/120 mesh Supelcoport (Bellefonte, PA). The column temperature was 270°C and helium flow was 40 ml/min. The dried bile acid extract was methylated with 3 ml of fresh Methanolic-HCl (3%), and redissolved in 100 μl of ethyl acetate to inject onto the column. The gas chromatography was equipped with a reporting integrator (H/P model 3390A, Hewlett Packard, Avondale, PA) which printed out percent of areas of each bile acid peak by reference to the internal standard, hyodeoxycholic acid.

Statistical analysis

The effect of treatment order of the two diets on each subject group was determined by the statistical treatise as described in Appendix. All results were expressed as mean SD. Analysis of variance for crossover design (21) was used to determine whether there was significant effect of treatments on the parameters determined. Statistical analysis was performed on the data between periods within each group. (A paired two-tailed *t* test was used to compare data obtained at the beginning and at the end of each diet.) For example, data obtained from Group I at the end of Diet A were compared with those of the baseline (T = 0) and the data obtained at the end of the experiment were compared with those obtained at the beginning of the second diet (B).

Results

Characteristics of individual subjects who successfully completed the requirements of the study are presented in Table 1. Although the mean body weight of Group IV was noticeably lower than that of other groups due to the random assignment of the subjects to each dietary group, the body weights of the subjects were maintained constant throughout the experiment.

The present data (Table 2) displayed that the subjects have consumed typical American diet which is characterized by high fat, low P/S ratio and relatively high cholesterol during the baseline period. The subjects' mean daily energy consumption of 3,432 kcal was ~500 kcal greater than the RDA (22) of 2,900 kcal for men, ages 19–22 yr. This discrepancy in energy intake may be due, in

TABLE 5
Effect of dietary fat modifications on composition of lipoproteins

Group	Diet period	Total cholesterol	Free cholesterol	Triglycerides	Phospholipids	Protein
				VLDL (mg/dl ± SD)		
I	Baseline	6.3 ± 2.8	3.3 ± 1.5	31.3 ± 18.5	6.9 ± 1.3	11.3 ± 4.7
	P/H	7.0 ± 3.9	3.0 ± 1.0	25.3 ± 5.0	6.3 ± 0.7	8.7 ± 3.5
	S/H	9.3 ± 1.9*	5.0 ± 2.0†	35.3 ± 6.1†	10.5 ± 1.6*	10.0 ± 4.7
II	Baseline	4.5 ± 1.6	3.4 ± 1.6	36.0 ± 7.3	4.7 ± 0.9	10.7 ± 5.7
	S/H	7.7 ± 4.1	3.3 ± 1.5	45.3 ± 16.1	6.3 ± 1.5	13.3 ± 4.5
	P/H	3.4 ± 2.3†	2.1 ± 0.6	33.0 ± 8.9†	4.3 ± 0.6†	9.3 ± 2.5
III	Baseline	3.6 ± 1.7	2.3 ± 2.0	34.7 ± 16.7	6.3 ± 1.1	10.3 ± 2.6
	P/L	4.0 ± 0.6	2.5 ± 1.2	24.0 ± 7.0	5.9 ± 0.6	8.3 ± 2.2
	S/L	11.1 ± 4.5‡§	4.3 ± 2.5	47.3 ± 27.5†	11.0 ± 1.2‡§	10.7 ± 2.0
IV	Baseline	3.4 ± 0.5	2.2 ± 0.5	24.0 ± 2.5	5.8 ± 1.4	8.5 ± 2.1
	S/L	5.9 ± 0.4*	3.5 ± 1.5	35.5 ± 6.7*	7.5 ± 0.7	10.0 ± 2.8
	P/L	4.9 ± 0.6†	2.9 ± 0.8	18.0 ± 6.3†	4.8 ± 1.8†	7.0 ± 3.4
				LDL (mg/dl ± SD)		
I	Baseline	96.0 ± 26.5	26.7 ± 9.6	9.7 ± 3.1	52.0 ± 15.6	61.0 ± 3.0
	P/H	77.7 ± 24.4*	19.3 ± 7.0*	8.0 ± 3.6	41.3 ± 10.6	50.7 ± 9.2*
	S/H	125.0 ± 25.1*§	32.3 ± 14.5†	9.3 ± 6.1	69.0 ± 24.5*§	70.0 ± 29.1*§
II	Baseline	87.3 ± 11.4	23.7 ± 3.1	8.3 ± 0.6	49.2 ± 5.0	57.0 ± 8.0
	S/H	108.0 ± 21.0*	26.3 ± 7.5	12.3 ± 4.9*	54.7 ± 8.8	63.7 ± 10.6
	P/H	86.7 ± 18.6	22.7 ± 5.0	5.0 ± 1.0*§	46.7 ± 6.7†	56.3 ± 17.9
III	Baseline	96.3 ± 4.0	26.3 ± 3.1	9.0 ± 2.0	47.0 ± 3.6	56.7 ± 4.7
	P/L	74.0 ± 4.6‡	17.3 ± 2.3‡	7.7 ± 1.5	36.0 ± 2.6‡	51.3 ± 3.8*
	S/L	88.0 ± 7.8*§	22.7 ± 3.1*§	9.5 ± 3.0	47.0 ± 4.6§	50.3 ± 6.8*
IV	Baseline	72.5 ± 10.6	22.0 ± 4.2	8.0 ± 2.8	40.5 ± 12.0	47.5 ± 3.0
	S/L	94.5 ± 7.8*	24.5 ± 3.5	9.5 ± 0.7	54.0 ± 7.8‡	53.0 ± 4.2*
	P/L	63.5 ± 10.6§	18.0 ± 1.4*§	5.5 ± 0.7*§	34.5 ± 5.7§	41.0 ± 2.8*†
				HDL_2 (mg/dl ± SD)		
I	Baseline	23.0 ± 7.8	4.7 ± 2.1	2.7 ± 0.6	36.0 ± 9.6	38.7 ± 10.1
	P/H	26.0 ± 6.6	5.7 ± 2.1	2.0 ± 1.0	38.0 ± 4.6	35.7 ± 10.4
	S/H	29.7 ± 10.1*	7.7 ± 3.2*	2.3 ± 0.6	39.3 ± 6.8	38.3 ± 13.3
II	Baseline	18.7 ± 3.5	5.3 ± 2.5	2.3 ± 1.2	24.7 ± 1.2	36.7 ± 1.5
	S/H	24.7 ± 1.5‡	5.4 ± 1.8	2.7 ± 0.6	30.7 ± 6.7*	37.3 ± 2.6
	P/H	27.3 ± 0.6†‡	6.0 ± 2.0	2.5 ± 0.6	28.7 ± 4.5	39.3 ± 2.1
III	Baseline	26.7 ± 10.7	7.3 ± 2.9	2.3 ± 0.6	29.7 ± 7.0	40.7 ± 12.9
	P/L	24.0 ± 3.6	6.1 ± 2.1	2.7 ± 0.6	27.0 ± 3.5	41.0 ± 7.5
	S/L	26.3 ± 5.1	6.3 ± 1.5	2.0 ± 1.0	30.7 ± 3.2	45.6 ± 13.9
IV	Baseline	14.5 ± 3.5	3.5 ± 0.7	2.5 ± 0.7	21.5 ± 5.0	19.5 ± 2.1
	S/L	18.5 ± 15.0	4.5 ± 1.5	2.8 ± 1.2	23.6 ± 6.2	27.5 ± 4.9*
	P/L	15.0 ± 4.2	5.0 ± 1.4	2.0 ± 0.4	22.5 ± 2.9	28.5 ± 12.0*
				HDL_3 (mg/dl ± SD)		
I	Baseline	25.7 ± 3.2	3.0 ± 1.0	5.1 ± 1.0	31.3 ± 3.8	91.3 ± 21.4
	P/H	28.0 ± 3.0	3.3 ± 0.6	5.7 ± 0.6	46.3 ± 5.0‡	94.0 ± 10.5
	S/H	31.0 ± 5.3*	5.7 ± 1.2‡	4.7 ± 1.2	45.7 ± 9.0‡	91.9 ± 19.5
II	Baseline	25.3 ± 2.3	3.0 ± 0.5	4.3 ± 0.6	36.0 ± 3.5	86.0 ± 7.0
	S/H	28.7 ± 4.5	3.4 ± 1.2	6.3 ± 1.6*	41.7 ± 7.1	88.3 ± 14.5
	P/H	30.3 ± 2.1‡	4.7 ± 0.6*	4.5 ± 0.5‡	39.7 ± 4.2	98.7 ± 14.2*

III	Baseline	29.7 ± 3.2	4.0 ± 1.0	3.0 ± 1.0	44.7 ± 7.6	81.7 ± 10.2
	P/L	27.3 ± 2.5	3.3 ± 0.6	5.0 ± 2.1	40.7 ± 2.2	78.3 ± 5.1
	S/L	32.7 ± 3.2†	5.0 ± 2.0	4.7 ± 2.6	48.7 ± 1.3†	96.0 ± 18.4
IV	Baseline	26.5 ± 3.5	3.0 ± 1.4	3.5 ± 1.4	38.5 ± 9.1	86.0 ± 7.1
	S/L	32.0 ± 1.0‡	2.5 ± 0.3	5.0 ± 1.0	44.5 ± 8.7	101.0 ± 5.0‡
	P/L	28.0 ± 1.4‡	3.5 ± 0.7	3.5 ± 0.7	40.0 ± 2.8	93.1 ± 2.8*†

Diet periods are defined in footnote to Table 4. Numbers represent mean ± standard deviation (SD).
* Significantly changed from the baseline at $p < 0.05$, paired t test.
† Significantly changed from the previous diet at $p < 0.05$, paired t test.
‡ Significantly changed from the baseline at $p < 0.01$, paired t test.
§ Significantly changed from the previous diet at $p < 0.01$, paired t test.

part, to the individual physical activity level of college students and partially to a plentiful supply of dormitory foods.

The effect of the polyunsaturated vs saturated fats with low or high cholesterol in each diet on the plasma lipid levels of plasma is shown in Table 4. The polyunsaturated fat diets significantly decreased plasma total cholesterol levels by an average of 16% ($p < 0.05$) in the presence of high dietary cholesterol and by an average of 20% ($p < 0.01$) in the presence of low cholesterol depending on whether the subjects started to eat the diets from a baseline point or from a time point after they had consumed diets rich in saturated fatty acids (ie, S/H or S/L). In contrast, the diets rich in saturated fatty acids with high or low cholesterol (S/H) significantly ($p < 0.05$) increased plasma cholesterol by approximately 24% while the same diet with low cholesterol (S/L) enhanced plasma cholesterol level by 22% again regardless what diet the subjects had consumed prior to the test diets. It appears that the cholesterol-lowering effect of the polyunsaturated fatty acids was greater than the cholesterol-enhancing effect of dietary cholesterol on plasma cholesterol levels. The order effect of feeding two different diets in a consecutive manner was tested according to the statistical treatise which was described earlier in the statistical analysis section. The paired t test for the order effect on changes in plasma total cholesterol level was not significant ($p < 0.05$), meaning that the order of test diets did not significantly influence the magnitude of changes in plasma cholesterol concentrations. Plasma-free cholesterol levels were altered by the modifications of dietary fat as were total cholesterol levels. Therefore, the percentage of free cholesterol to total cholesterol was not significantly changed. Plasma levels of triglycerides and phospholipids were also significantly ($p < 0.05$) lowered by the diet high in polyunsaturated fats with either high or low cholesterol in the diets and increased by the diets high in saturated fatty acids.

The effects of dietary fat modifications on the compositions of plasma lipoproteins are presented in **Table 5.** It appears that LDL-cholesterol levels have followed the pattern of the changes in plasma cholesterol levels but with greater magnitude. When compared with the saturated-fat diets, the polyunsaturated-fat diets lowered LDL-cholesterol measured in the LDL-fraction isolated by ultracentrifugation as described earlier in the laboratory analyses by an average of 19 and 28% ($p < 0.05$) in the presence of high or low cholesterol in the diet, respectively. In comparison, LDL-cholesterol levels in subjects fed the saturated fat diets were markedly increased ($p < 0.05$) by an average of 42 and 25% depending on high and low levels of dietary cholesterol. The VLDL-cholesterol was also decreased by the diets rich in polyunsaturated fats ($p < 0.05$) and slightly increased by the saturated-fat diets. Changes in HDL composition show that the cholesterol concentrations of HDL subfractions, HDL_2 and HDL_3, appear to be influenced by the interaction between amounts of cholesterol and the type of fat in diet. In the presence of high dietary cholesterol, both saturated and polyunsaturated fats significantly increased HDL-cholesterol ($p < 0.05$); while with low dietary cholesterol, only diets high in saturated fats significantly ($p < 0.05$) elevated HDL-cholesterol levels and the polyunsaturated fat diets slightly decreased the cholesterol contents of both HDL_2 and HDL_3. There were concomitant changes in free cho-

lesterol, phospholipids, and total protein along with those in total cholesterol. There were no significant changes in triglyceride contents of lipoproteins except those of VLDL which were significantly ($p < 0.05$) decreased by diets high in polyunsaturated fats and increased by diets rich in saturated-fed fat.

Figure 2 shows changes in the plasma total cholesterol levels measured at different time points. There were almost mirror images of the graphical lines drawn from the cholesterol values on the diet P/H vs S/H or the diet P/L vs S/L, which differ only in the P/S ratio. When switched to the second diet after 6 wk on the first diet there was a carry-over effect of the first diet on the plasma cholesterol levels during the first week of the second diet period. Judging from the plasma cholesterol values measured at week 6, 7, 9, and 12 (during the second diet period), the effects of dietary fat modification on plasma cholesterol concentrations required feeding the diet for at least 6 wk to obtain the maximum dietary effect.

The effects of the four diets on major apoprotein concentrations of the lipoprotein fractions are shown in **Table 6.** Apo B levels of VLDL and LDL were significantly reduced by the polyunsaturated-fat diets (P/H and P/L) but increased by the saturated-fat diets containing either high or low cholesterol. The reduction of apo B levels of LDL by the polyunsaturated fats was 25% on the high-cholesterol diet and 12% on the low-cholesterol diet. In contrast, the saturated fats increased the apo B of LDL by 28 and 12% in the presence of the high- and low-cholesterol diets, respectively. The diet containing high polyunsaturated fat and high cholesterol raised apo A-I levels in HDL_2 but not at the significant level ($p < 0.05$), while the polyunsaturated-fat diet with low cholesterol slightly reduced the apoprotein. The saturated-fat diets tended to increase apo A-I of HDL_2 and HDL_3 regardless of the amount of cholesterol in the diets. There were significant increases ($p < 0.05$) of apoprotein E concentrations of HDL_2 on the S/H diet. The S/L diet also elevated apo E concentrations in HDL_2 fractions. Although not significant at $p < 0.05$, the diets high in polyunsaturated fats tended to lower apo E levels in HDL_2 fractions. Other sporadic changes in apoproteins lack consistency so that meaningful interpretation of such changes may not be practical.

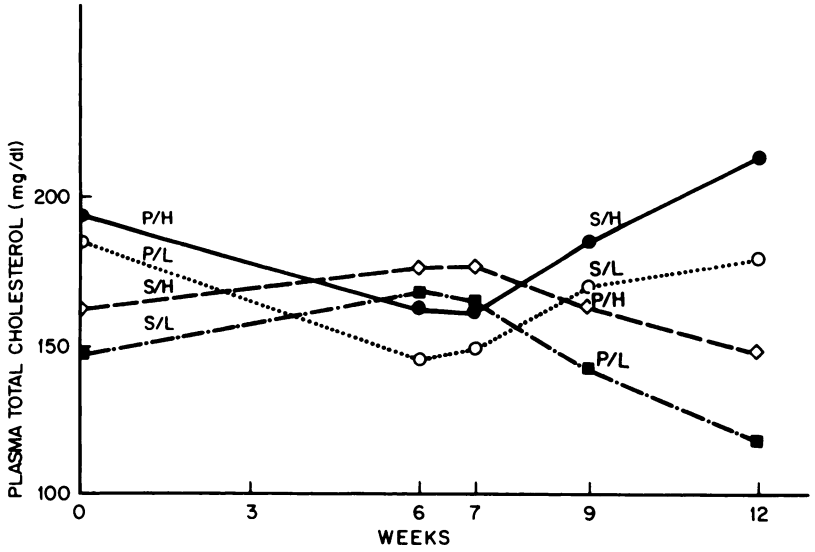

FIG 2. Effect of dietary cholesterol and P/S ratio on plasma cholesterol levels. P/H diet, 1,019 mg cholesterol/day and P/S ratio, 1.95; S/H diet, 1,028 mg cholesterol/day and P/S ratio, 0.24; P/L diet, 285 mg cholesterol/day and P/S ratio, 1.84; S/L diet, 279 mg cholesterol/day and P/S ratio 0.23.

Fecal excretions of neutral and acidic steroids are given in **Table 7.** The P/H diet which contained a high P/S ratio and high cholesterol significantly increased the excretions of both neutral steroids ($p < 0.01$) and bile acids ($p < 0.05$) when compared to those of the baseline. The group on S/H diet which contained low P/S ratio but high cholesterol also excreted significantly more neutral steroids ($p < 0.05$) but not bile acids. The P/L diet slightly increased neutral steroid excretion. The S/H diet did not change the fecal steroid excretion. There were no noticeable variations in the mean intake of plant sterols among the groups except the group on P/L diet whose intake of plant sterol was changed from 83 to 105 mg/day.

Discussion

The purpose of this study was to determine the effect of degree of fat unsaturation and amount of dietary cholesterol on the plasma lipid levels and the compositions of plasma lipoproteins, particularly the high-density lipoprotein subfractions. The cholesterol contents and P/S ratios of the present diets were above the average American intakes but within the upper or lower normal ranges of ordinary intakes of the American population. The ranges of cholesterol levels and the P/S ratios contained in the diets of this study were not extreme so that the present diets low in cholesterol and high in P/S ratio may be adopted and maintained in a free-living state.

Throughout the study periods, caloric adjustments were made as required to maintain body weight within 2 lb variation. The 11 subjects who completed the study have maintained their body weights within that range. Participants in this study were well-motivated and complied well with the diets.

What our present data revealed was that the plasma cholesterol levels of the subjects were increased by lowering the P/S ratio and decreased as the consequence of increasing the P/S ratio. Our findings are very compatible to most studies (1–3, 6–8, 10–11, 23–25). The amount of cholesterol in the diets played a less influential role in determining

TABLE 6
Effect of dietary cholesterol and P/S ratio on apoprotein concentration (mg/dl)

Group	Diet period	VLDL			LDL	HDL$_2$			HDL$_3$	
		B	C_m	E	B	A-I	A-II	C_m	A-I	A-II
I	B*	4.3 ± 2.6	2.7 ± 0.9	3.7 ± 0.7	61.3 ± 8.0	25.8 ± 6.0	6.6 ± 2.4	1.8 ± 0.3	58.1 ± 8.7	24.1 ± 7.4
	P/H	3.3 ± 0.6	1.8 ± 0.7	3.0 ± 0.5	48.0 ± 3.5†	28.6 ± 7.2	8.2 ± 1.5	1.7 ± 0.6	56.3 ± 7.2	23.0 ± 5.5
	S/H	5.2 ± 1.7‡	3.2 ± 0.9	2.9 ± 0.4	63.3 ± 8.4§	31.8 ± 5.2	8.6 ± 2.6	2.9 ± 0.5‡	60.0 ± 4.6	23.3 ± 0.7
II	B*	3.9 ± 0.7	2.3 ± 0.8	2.7 ± 0.3	53.7 ± 8.5	21.4 ± 5.1	7.2 ± 1.8	1.8 ± 0.4	50.9 ± 8.1	20.9 ± 2.2
	S/H	4.8 ± 1.1	3.4 ± 0.9	3.3 ± 0.6	66.3 ± 7.5‖	24.6 ± 4.7	7.5 ± 1.4	2.1 ± 0.3	55.5 ± 5.2	21.7 ± 2.2
	P/H	3.8 ± 0.2	3.8 ± 0.9‖	2.7 ± 0.4	48.0 ± 8.2§	24.5 ± 3.2	7.3 ± 2.5	2.8 ± 0.6	56.0 ± 4.9	21.2 ± 2.1
III	B*	2.5 ± 0.4	2.2 ± 0.9	3.0 ± 0.4	51.7 ± 4.7	22.2 ± 5.6	6.8 ± 1.6	3.2 ± 0.4	61.3 ± 13.1	27.6 ± 6.2
	P/L	2.0 ± 0.7	2.2 ± 1.0	2.9 ± 0.3	46.3 ± 3.8‖	20.2 ± 4.0	7.2 ± 1.6	2.0 ± 0.3	61.2 ± 16.3	25.2 ± 4.9
	S/L	3.5 ± 0.4‖	2.4 ± 0.7	2.6 ± 0.3	52.0 ± 6.7	25.7 ± 6.2	8.4 ± 2.2	2.8 ± 0.4	63.3 ± 10.3	26.9 ± 0.9
IV	B*	3.0 ± 1.4	1.3 ± 0.4	2.7 ± 0.3	47.5 ± 3.5	16.7 ± 2.6	5.8 ± 1.6	2.6 ± 0.2	42.7 ± 1.6	18.5 ± 1.0
	S/L	4.5 ± 0.4	2.7 ± 0.6	2.7 ± 0.3	53.0 ± 4.3‖	20.7 ± 5.0	7.6 ± 1.2‖	2.0 ± 0.1	47.2 ± 2.0‖	20.4 ± 1.0
	P/L	3.5 ± 1.3	1.6 ± 0.7	2.8 ± 0.2	46.0 ± 3.8§	18.3 ± 4.1	7.3 ± 1.8‖	2.6 ± 0.4	43.6 ± 4.7‖	22.9 ± 2.0

Diet periods are defined in footnote to Table 4. Numbers represent mean ± standard deviation (SD).
* Baseline
† Significantly changed from the baseline at $p < 0.01$, paired *t* test.
‡ Significantly changed from the previous diet at $p < 0.05$, paired *t* test.
§ Significantly changed from the previous diet at $p < 0.01$, paired *t* test.
‖ Significantly changed from the baseline at $p < 0.05$, paired *t* test.

TABLE 7
Effect of dietary fat modifications on fecal excretion of neutral steroids, bile acids and plant sterols

Group	Diet period	Neutral steroids	Bile acids	Plant sterols
			mg/day	
I	Baseline	443 ± 83	284 ± 29	98 ± 21
	P/H	759 ± 183*	375 ± 31†	101 ± 18
	S/H	703 ± 178*	321 ± 30†‡	104 ± 18
II	Baseline	511 ± 73	292 ± 34	112 ± 26
	S/H	823 ± 159*	323 ± 35	109 ± 24
	P/H	928 ± 201*	358 ± 37†	108 ± 24
III	Baseline	498 ± 97	274 ± 28	83 ± 14
	P/L	555 ± 118	294 ± 23	105 ± 17†
	S/L	454 ± 130	290 ± 29	102 ± 16†
IV	Baseline	453 ± 107	315 ± 31	89 ± 28
	S/L	482 ± 87	304 ± 30	95 ± 26
	P/L	491 ± 73	321 ± 34	107 ± 25

Diet periods are defined in footnote to Table 4. Numbers represent mean ± standard deviation (SD).
* Significantly increased from the baseline at $p < 0.01$, paired t test.
† Significantly increased from the baseline at $p < 0.05$, paired t test.
‡ Significantly changed from the previous diet at $p < 0.05$, paired t test.

the plasma cholesterol level. This conclusion was drawn from Figure 2 which shows mirror images of the changes in plasma cholesterol concentrations of subjects fed diets which contained the same level of cholesterol but differed in P/S ratio. It has been suggested that a higher intake of polyunsaturated fatty acids may diminish the effect of high dietary cholesterol on the plasma cholesterol concentration (26). In studies to determine the effect of various levels of dietary P/S ratios and different amounts of cholesterol on plasma lipoproteins, Schonfeld et al (27) found that both the cholesterol contents and P/S ratios of diets were important in determining plasma and LDL cholesterol levels.

Several metabolic studies have presented unequivocal evidence that saturated fats, per se, raised concentrations of cholesterol in man as compared to other dietary constituents (11, 28–30). Keys et al (31) and Hegsted et al (32) have derived mathematical equations from metabolic study data to quantitatively express the direct effect of dietary fats on the plasma cholesterol level. The equations express that saturated fats raise the plasma cholesterol by approximately twice as much as polyunsaturated fats lower it. An expression of the effect of dietary cholesterol on plasma cholesterol was also included in the equations. Other investigators have reported that dietary cholesterol may have an independent effect on the plasma cholesterol levels (33–36). In our study, the equations were found to be reasonably reliable. The dietary variables have not independently altered the free cholesterol levels in plasma judging from the similar trends of changes in free cholesterol as those in total cholesterol. The observed changes in free cholesterol are in agreement with others (1–2, 37).

The plasma lipid (Table 4) showed that the two diets which were high in polyunsaturated fats significantly ($p < 0.05$) decreased plasma triglyceride (TG) levels. The other diets high in saturated fats with or without high cholesterol did not affect much of the plasma TG levels. Previous studies (1, 38–39) have shown more dramatic decreases in plasma TG concentration when they raised the P/S ratio in the diet. Some investigators employed P/S ratios up to 5.0 (1, 23).

The reduction of plasma cholesterol by the high-polyunsaturated fat diets appeared to result from significant ($p < 0.05$) drops (19% by P/H and 28% by P/L) in LDL cholesterol. This decrease in LDL cholesterol by polyunsaturated fat was not accompanied by a decrease in HDL-cholesterol as reported earlier (1–2). Since our LDL- and HDL-cholesterol values represent cholesterol of LDL and HDL fractions isolated from sequential ultra-

centrifugation described by Havel et al (16), they may not be directly comparable with those obtained by the combination method of ultracentrifugation and heparin-Mm^{2+} precipitation technique. The cholesterol level of the diet high in polyunsaturated fats apparently played an important role in determining the HDL-cholesterol level. In the presence of high cholesterol, the polyunsaturated-fat diet (P/H) noticeably but not significantly increased HDL_2 and HDL_3 cholesterol while the same diet with low cholesterol (P/L) tended to decrease HDL-cholesterol level. According to the survey data of Vessby et al (40), who investigated the relationship between diet and HDL, nearly all studies showed a decrease in HDL cholesterol during lipid lowering treatment with diets enriched in polyunsaturated fatty acids. Although the calorie source of these diets was ~40% from fat, most of the diets contained low cholesterol. In the present study, both diets P/H and P/L rich in polyunsaturated fats decreased the calculated ratio between LDL/HDL cholesterol (not shown here). Our data on LDL-cholesterol changes are comparable to the 16% decrease in LDL cholesterol level of five female subjects on a diet containing P/S ratio of 1.7 for 35 days (41) and in 11 subjects fed a diet with a P/S ratio of 2.0 and 250–300 mg/day cholesterol for 2 wk (11). A more extreme P/S ratio of 4.0 (400 mg/day cholesterol) caused a 23% decrease of LDL-cholesterol after 5 wk on the diet (1) or 26% (2) after 4 wk on the high-safflower oil and very low-cholesterol diet. The observed decreases in cholesterol levels in plasma and LDL by the diets high in polyunsaturated fats are in accordance with the previous findings of others (3, 42) who tested diets containing 1 or 1.5 P/S ratio and low cholesterol. Our data suggest that the degree of dietary P/S ratio might be very crucial in influencing the HDL cholesterol level. Brussard et al (4, 43), in a controlled 16-wk trial with moderate amount of fat containing high polyunsaturated fats, found that the HDL-cholesterol level was unchanged when compared to that of diet low in polyunsaturated fat. In the same studies, they showed that HDL-cholesterol was depressed and VLDL were elevated by the low-fat high-carbohydrate diet. The authors suggested that the decrease of HDL-cholesterol was not due to the high P/S ratio but a low total fat and low cholesterol content in the diets. Many other studies (4, 38, 43–44) did not observe reduction in HDL-cholesterol by polyunsaturated-fat diet. The studies in which the polyunsaturated fats were found to lower HDL-cholesterol have used either hyperlipidemic subjects (2, 10) or inpatients who were confined in a metabolic ward during the study (1–2, 10–11, 23–24, 40). Otherwise, the diets were often liquid formula (2) or low-fat, low-cholesterol (4, 10) or extremely polyunsaturated-fat diets (1–2, 23, 45) or short period of feeding (11, 23–24).

A high LDL/HDL cholesterol ratio has been associated with a high risk of coronary heart disease (CHD) (46). In previous studies, reduction of serum cholesterol levels during treatment with fat-modified diets has been associated with a decreased incidence of CHD (47–48). However, the relative significance of LDL cholesterol in relation to HDL is not known yet. The present study suggests that the response of HDL cholesterol to dietary polyunsaturated fats was variable depending on the amount of cholesterol in the diet.

Although the controlled diet study with outpatients has the disadvantage of not being sure of whether the subjects comply 100% with the diet, it does have an advantage over a study conducted with inpatients who have been confined in a metabolic ward for a prolonged time. Deprival of regular exercise and effects of psychological stress might cause tremendous deviation from normal lipid metabolism of free-living populations. The 6-wk duration of feeding each test diet in the present study appears to be reasonable in detecting changes in cholesterol concentrations of plasma and lipoprotein fractions because the half-life ($t_{1/2}$) of the second body cholesterol pool which represents the turnover of whole body cholesterol was reported to be 38 days (49). Most previous studies of feeding test diets were conducted 2 to 8 wk (1–2, 4, 8, 10–11, 27, 37–38, 44, 50) except a few long-term studies (3, 43) in which the effect of test diets on plasma cholesterol was already apparent after 4 wk. However, in order to study a new state of cholesterol equilibrium in man, it might be necessary to feed diets for a much longer period of time (51).

The electroimmunoassay (EIA) of apoproteins revealed that the changes in apo B contents in VLDL and LDL were similar to those of VLDL and LDL cholesterol suggesting that parallel changes occurred to LDL-cholesterol and apo B when dietary fats exert its influence. Our findings are in good agreement with those reported by Vega et al (2) who found that polyunsaturated fats significantly reduced both LDL cholesterol and LDL apo B. Durrington et al (45) also observed a significant reduction of LDL apo B on polyunsaturated-fat diet. Recently Schonfeld et al (27) reported that most apoprotein concentrations were not changed by diets high or low in P/S ratio with high or low cholesterol. The only significant changes in apoprotein concentration were apo B levels between the basal diet and those with high cholesterol in their study. The authors reported that apo A-I levels of the groups on the diets containing P/S ratio of 0.8 and 3 to 6 eggs/day were significantly increased from those of the basal diet. In the present study, the changes in apo A-I and A-II of the combined HDL fractions (HDL_2 and HDL_3) were more pronounced. Our data on the levels of apo A-I in HDL fractions revealed that the saturated-fat diets S/H and S/L increased the concentrations of apo A-I while only the P/H diet significantly ($p < 0.05$) elevated the apo A-I levels of HDLs. However, the P/L diet containing high polyunsaturated fats but low cholesterol reduced the apo A-I level suggesting that dietary cholesterol might have an independent effect on this apoprotein. It appears that dietary cholesterol mitigated the HDL-lowering effect of polyunsaturated fats and caused a significant increase in apo A-I levels in HDLs. The fact that apo A-I concentrations in HDLs were increased by the diets high in cholesterol regardless of the type of fat suggests that polyunsaturated fats, per se, may neither decrease apo A-I nor cholesterol in HDL fractions. Other investigators found that diets low in fat and cholesterol decreased the HDL-cholesterol levels in man (44). The significant increase of apo A-I in HDL by the saturated fat concurs with previous observations (11) that HDL-cholesterol and apo A-I were increased by diets high both in saturated fat and cholesterol in six normolipidemic subjects. We found considerable changes in apo E in HDL_2 on the saturated-fat diet but not in VLDL. Others have not found changes of apo E in lipoprotein fractions by dietary fat modifications (50). Our data imply that the increase in HDL cholesterol might be related to the increment of apo E in the lipoproteins.

Our findings that feeding high cholesterol resulted in increased excretion of total fecal steroids are in agreement with the earlier findings that excessive consumption of cholesterol increases fecal steroid outputs (52–54). Our data revealed that the excretion of neutral sterols was far greater than that of bile acids. The excretion of neutral sterols were increased by an average of 71 and 61% by the high-cholesterol diets, P/H and S/H, respectively, while bile acid excretions were enhanced by an average 32 and 11%. The data suggest that when exogeneous cholesterol intake was increased, a compensating mechanism was in play to prevent progressive accumulation of cholesterol in body. Quintao et al (52) demonstrated that when cholesterol was absorbed in excessive amounts, reexcretion occurred mainly in the form of the neutral sterol; there was no enhanced conversion of cholesterol into bile acids. In contrast, animals responded to the feeding of cholesterol with a substantial increase in excretion of bile acid (53). Polyunsaturated fats apparently exerted some influence in the enhanced excretion of neutral sterols as well as acidic steroids. The effect of polyunsaturated fats on fecal steroid output was pronounced in the diet containing high cholesterol. Others have shown that polyunsaturated fats increase bile acid excretion in humans (54–55). A study in a normolipidemic young man showed increased sterol excretion with a diet high in polyunsaturated fats and cholesterol (56).

There were no significant changes in excretion of both neutral and acidic steroids on low-cholesterol diets containing either high or low P/S ratios. However, on the diet with high P/S ratio subjects tended to excrete more neutral and acidic steroids compared to the baseline values.

In conclusion, we have shown from this study that polyunsaturated fats had a greater effect in lowering the plasma cholesterol level than dietary cholesterol, while saturated fats

increased it. The present data also showed that the elevated plasma cholesterol levels by a diet high in cholesterol and saturated fats were reversed when the diets were modified to contain high amounts of polyunsaturated fats. Although the changes in plasma cholesterol levels were accompanied by the compatible changes in VLDL and LDL cholesterol, those of HDL did not always follow the changes in plasma cholesterol levels. The diets containing high saturated fat with or without high cholesterol increased the levels of both cholesterol and apo A-I of HDL fractions. In contrast, the diet high in P/S ratio and high in cholesterol increased the cholesterol and apo A-I levels of HDL while the same diet with low cholesterol decreased them. The diets high in cholesterol (P/H and S/H) significantly increased the excretion of fecal sterols and the diets high in polyunsaturated fats (P/H and P/L) enhanced bile acids excretion in stool. The present data suggest that moderate amounts of polyunsaturated fats may be consumed as effective cholesterol lowering nutrients by persons on a regular American diet containing high fat and moderate amount of cholesterol.

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Appendix A

Test of treatment order

The effect of dietary treatment order was examined as the following format. The treatment order of two diets for Group I may be illustrated as

P/H		S/H
y_1	y_2	y_3

Then the treatment difference, P/H − S/H, is

$$y_2 - y_1 - (y_3 - y_2) = -y_1 + 2y_2 - y_3. \quad (1)$$

Since there were three subjects for group I, the mean,

$$\bar{Z}_1 = -\bar{y}_1 + 2\bar{y}_2 - \bar{y}_3 \quad (2)$$

and the variance of $\bar{Z}_1$ is S_1^2. Likewise, the dietary treatment of Group II is illustrated as

S/H		P/H
y_4	y_5	y_6

Thus, the treatment difference, P/H − S/H, for group II is

$$y_6 - y_5 - (y_5 - y_4) = y_4 - 2y_5 + y_6 \quad (3)$$

the mean

$$\bar{Z}_2 = \bar{y}_4 - 2\bar{y}_5 + \bar{y}_6 \quad (4)$$

and the variance of $\bar{Z}_2$ is S_2^2. Therefore,

$$t = \frac{\bar{Z}_1 \bar{Z}_2}{\sqrt{\frac{2S^2p}{3}}},$$

where

$$S^2p = \frac{2 - S_1^2 + 2 \cdot 5_2^2}{4}$$

is pooled variance. The null hypothesis for existence of an order effect if denied when t test is not significant.