Effects of Dietary Polyunsaturated and Saturated Fat on the Properties of High Density Lipoproteins and the Metabolism of Apolipoprotein A-I

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ABSTRACT In this study we have investigated, in four normal males the effects of dietary saturated and polyunsaturated fat on the chemical composition and thermotropic properties of human high density lipoproteins (HDL) and have measured the influence of the diets on the metabolism of that fraction of HDL apolipoprotein A-I (apoA-I) that undergoes exchange in vitro and accounts for approximately two-thirds of the lipoprotein's apoA-I complement. When compared with the saturated fat diet, the polyunsaturated diet reduced plasma cholesterol ($24\%, P < 0.01$) by affecting the cholesterol content in the very low density lipoprotein ($\downarrow 25\%, P < 0.02$), low density lipoprotein ($\downarrow 20\%, P < 0.01$), and high density lipoprotein fractions ($\downarrow 33\%, P < 0.01$). Plasma triglyceride was also lowered (by $13\%, P < 0.01$). Furthermore, polyunsaturated fat ingestion caused a significant fall in the palmitate and stearate content of HDL triglyceride (41 and 37%, respectively), cholesteryl esters (29 and 35%), and phospholipids (17 and 9%) with a concomitant increase in the linoleate content of these moieties (157, 28, and 29%, respectively). The polyunsaturated diet also produced reciprocal changes in the percentage protein ($\downarrow 9\%, P < 0.02$) and phospholipid ($\downarrow 11.5\%, P < 0.01$) in HDL. These compositional changes were associated with an increase in the microscopic fluidity of the polyunsaturated HDL, although both diets had little effect on the fluidity parameters of HDL at body temperature. Rate zonal ultracentrifugation indicated that the HDL_2/HDL_3 ratio fell by 28% ($P < 0.05$) on the polyunsaturated fat diet. In addition to the above,

this diet reduced plasma apoA-I by 21% ($P < 0.01$). No change was seen in the fractional catabolic rate or the distribution of the apoprotein between intra-vascular and extravascular compartments on the two diets. However, when compared with the saturated diet, the synthetic rate of apoA-I was reduced by 26% during polyunsaturated fat feeding. The results show that polyunsaturated fat alters the chemical composition, thermotropic properties, and subfraction distribution of HDL without changing the fractional rate of catabolism of their major protein, apoA-I.

These findings deserve careful consideration in determining the applicability and efficacy of polyunsaturated fat diet therapy in the prevention of atherosclerosis in man.

INTRODUCTION

Over the last 60 yr, the per capita consumption of polyunsaturated fats has increased by 300% in the United States (2). The impetus for this change was provided by a number of human (3–6) and animal (7–10) studies that showed that the ingestion of saturated fats leads to an elevation of plasma cholesterol, whereas polyunsaturated fats produce the opposite effect. Widespread acceptance of this effect of diet modification has led to recommendations from the American Heart Association (11), the American Health Foundation (12), the Food and Nutrition Board of the National Research Council, and the American Medical Association (13) advocating changes in diet which include reduction of the total consumption of fat and cholesterol and substitution of polyunsaturated for saturated fat wherever possible.

Although the lipid lowering effects of dietary polyunsaturated fats appear to justify the above recommendations, their metabolic effects are not completely

understood. In addition to their hypocholesterolemic action, polyunsaturated fats also cause reduction in plasma triglyceride levels (14), increased excretion of fecal bile acids (15), alteration in the positional substitution of acyl groups in plasma triglycerides (16), and an increase in the proportion of polyunsaturated fatty acids in lipoprotein lipids (17–19) with resulting changes in the physical properties of the lipoproteins (19). The implications of these changes in lipoprotein structure relative to the metabolism of the complexes are not yet clear although Engelberg (14) found that lowering of serum triglycerides was correlated with an increase in lipoprotein catabolism, and studies in this laboratory (20, 21) have suggested a relationship between low density lipoprotein apolipoprotein (apoLDL)[1] catabolism and the fatty acid composition of LDL. To extend these observations, we have compared the effects of saturated vs. polyunsaturated dietary fat on the metabolism of human apolipoprotein A-I.

METHODS

Subjects. Four healthy adult males were studied in the General Clinical Research Center of the Methodist Hospital, Houston. None had clinical or biochemical evidence of cardiac, hepatic, renal, or endocrine disease. No medications were given for 4 wk before and throughout the study. Informed consent was obtained from each subject.

Diets. The effects of two isocaloric diet regimens on high density lipoprotein (HDL) apolipoprotein A-I (apoA-I) metab-

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Abbreviations used in this paper: apoA-I, apolipoprotein A-I, the major apoprotein of high density lipoproteins; HDL, high density lipoproteins, $1.063 < d < 1.21$ kg/liter; LDL, low density lipoproteins, $1.006 < d < 1.063$ kg/liter; TEMPO, 2,2,6,6-tetramethyl-piperidine-1-oxyl; VLDL, very low density lipoproteins of $d < 1.006$ kg/liter.

olism were investigated using the protocol outlined in Fig. 1. The caloric distribution of both diets was 20% protein, 40% carbohydrate, and 40% fat. The polyunsaturated/saturated fat ratio in study I (diet S) was 0.25 and in study II (diet P), 4.0. The cholesterol intake on each diet was 400 mg/day. The fatty acid composition of a typical saturated and polyunsaturated meal has been presented elsewhere (19). All meals were prepared in the Metabolic Kitchen of the Methodist Hospital General Clinical Research Center.

ApoA-I purification and labeling. Human plasma HDL was prepared by ultracentrifugal flotation between d 1.063 and 1.210 kg/liter (22), delipidated with diethyl ether:ethanol (3:1, vol/vol), and the apoproteins fractionated on Sephadex G-150 (23; Pharmacia Fine Chemicals, Piscataway, N. J.) using 0.1 M Tris/HCl, pH 8.6, containing 5.4 M urea and 0.01% disodium EDTA as elution buffer. The fractions containing apoA-I were pooled and dialyzed against 0.01 M Tris, pH 7.0/0.15 M NaCl/0.01% disodium EDTA. The protein gave a single band on sodium dodecyl sulfate disk gel electrophoresis (24) and had an amino acid composition comparable to that reported by others (25). Specific antibodies were raised in rabbits against the purified apoA-I (26). The apoA-I was radiolabeled with ^{125}I (Amersham/Searle, Arlington Heights, Ill.) using a modification (27) of the McFarlane technique (28).

Isolation and labeling of HDL. HDL was isolated from each subject in the postabsorptive state as described above, dialyzed against 0.05 M barbital buffer, pH 8.6, and labeled with ^{125}I-apoA by in vitro incubation for 30 min at 37°C. The incubation mixture contained approximately 4.0 mg of HDL protein and 100 μg of ^{125}I-apoA-I (100 μCi) in a total volume of 5.0 ml of 0.05 M sodium barbital buffer, pH 8.6. This procedure permits a mole for mole exchange of exogenous ^{125}I-apoA-I with endogenous apoA-I in the HDL particle and results in an uptake of radiolabeled apoA-I consistently >90% (27). After incubation, unbound ^{125}I-apoA-I was removed from the labeled HDL by ultracentrifugation (258,000 g for 18 h at 4°C) at d 1.225 kg/liter in an anglehead 65 Beckman rotor (Beckman Instruments, Palo Alto, Calif.). The top third from each ultracentrifuge tube, containing the apoA-I-labeled HDL, was removed carefully by aspiration and dialyzed against 0.01 M Tris/HCl, pH 7.0/0.15 M NaCl/0.01% disodium EDTA before sterilization and injection. In a study published
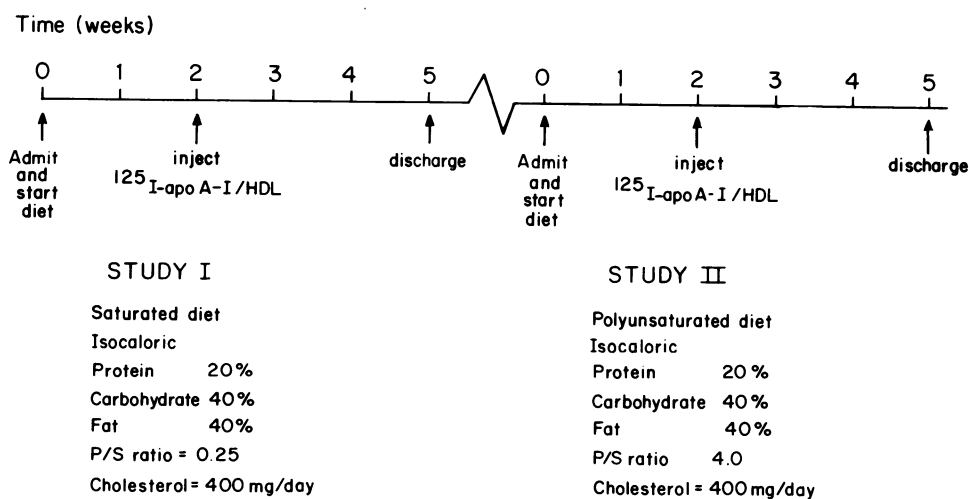
Time (weeks)

FIGURE 1   Plan of metabolic investigation of the effects of saturated vs. polyunsaturated fat diets on HDL metabolism.

elsewhere (27) we have found that the metabolism of ^{125}I-apoA-I intercalated into HDL by in vitro transfer is not directly comparable to that of the apoprotein labeled in the whole HDL particle, the former being catabolized 20% faster than the latter. The apoA-I turnover parameters presented in this report describe the metabolism of that component of HDL apoA-I that exchanges with free apoA-I in vitro (two-thirds of the HDL apoA-I complement). The faster rate of catabolism of this exchangeable apoA-I limits the utility of the HDL labeling technique but should not affect its application to the direct measurement of the influence of dietary fat saturation on the metabolism of the apoprotein.

Chemical analyses. Plasma cholesterol and triglyceride assays, and beta-quantification were performed as described in the Lipid Research Clinics Manual of Laboratory Operations (29).

Lipoprotein compositions were obtained as follows: Free and esterified cholesterol were measured by enzyme assay (Boehringer cholesterol kit, 15732, Boehringer Mannheim Biochemicals, Indianapolis, Ind.) using Boehringer cholesterol standards; triglyceride by a fluorometric procedure (29); phospholipid by phosphorus analysis (30); protein by the procedure of Lowry et al. (31) using a human albumin standard (Sigma Chemical Co., St. Louis, Mo.); and fatty acid analysis by gas liquid chromatography (19).

Spin label paramagnetic resonance studies. Electron paramagnetic resonance studies were performed on HDL as described by Morrisett et al. (19), using the spin label probe 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO).

ApoA-I electroimmunoassay. A modification of the method of Laurell (32) was used. The procedure is described elsewhere (33).

Rate zonal ultracentrifugation. At the end of both diet periods, 15 ml of postabsorptive plasma was collected from each subject, and the HDL subfractions separated by rate zonal ultracentrifugation (34). The relative amount of HDL$_2$ and HDL$_3$ in each plasma sample was quantified from the area subtended by the appropriate segments of the absorption profile. The area of each subfraction peak was expressed as a percentage of the total HDL profile area.

Administration of ^{125}I-apoA-I/HDL and collection of samples. Each subject received 900 mg of KI daily in divided doses for 3 days before and throughout the period of the turnover study to minimize thyroidal uptake of [125]I. A weighed volume of autologous ^{125}I-apoA-I/HDL (25 μCi radioactivity; approximately 1 mg HDL apoprotein) was injected intravenously into each recipient at time zero, and 10-ml fasting venous blood samples were collected into disodium EDTA (1 mg/ml) after 10 min and subsequently at 24-h intervals for 17 days. The plasma volume of each subject was calculated from the isotope dilution that occurred during the first 10 min, after which time mixing was assumed to be complete. The results were in good agreement with a value of 4% of body weight. Sequential 24-h urine collections, whose completeness was evaluated by measuring creatinine excretion, were made throughout the 17-day period. 2-ml aliquots of the daily plasma and urine specimens were counted in a Packard Autogamma scintillation spectrometer (Packard Instrument Co. Inc., Downers Grove, Ill.) at the end of the turnover study to eliminate the need to correct for radioactive decay. Counting time was adjusted to reduce counting error to < ±2%. Every 3rd day, lipoprotein cholesterol, triglyceride, and apoA-I assays were performed on each plasma sample; and on days 1, 6, 13, the distribution of the ^{125}I-apoA-I among the plasma lipoproteins was determined (35) by 6% agarose gel chromatography (Bio-Gel A5m, 200–400 mesh, Bio-Rad Laboratories, Richmond, Calif.). At the end of each diet period, and after completion of the turnover studies, 100 ml of fasting plasma was obtained from each subject to isolate HDL (22). After recentrifugation, the HDL was used in chemical and physical studies.

Each individual was studied twice, first on diet S, then on diet P, the time interval between the two study periods being 5 wk.

Data handling. The plasma radioactivity clearance data were processed as described before (36) using the multicompartmental procedure of Matthews (37). Under steady-state conditions, this permits calculation of the fractional catabolic rate of the radioiodinated apoprotein (i.e., the fraction of the intravascular pool catabolized per day). This kinetic parameter can be estimated by an independent method (38) which expresses the fractional catabolic rate as the ratio of the total radioactivity excreted in the urine during each 24-h period to the mean plasma radioactivity in this period (i.e., the urine/plasma radioactivity ratio).

RESULTS

The validity of this study of the influence of dietary fat saturation levels on the structure of HDL and on

TABLE I
Effect of Dietary Fat Saturation Levels on Plasma Lipids and Lipoproteins

Subject	Age	Plasma cholesterol (n = 9)		Plasma triglyceride (n = 9)		VLDL cholesterol (n = 6)*		LDL cholesterol (n = 6)*		HDL cholesterol (n = 6)*	
		S‡	P	S	P	S	P	S	P	S	P
	(yr)	mg/dl		mg/dl		mg/dl		mg/dl		mg/dl	
1	22	171±13	122±9	125±14	109±15	20±5	15±4	109±15	80±6	43±15	27±3
2	19	205±11	152±11	72±13	63±19	11±3	9±3	148±20	111±9	46±7	31±6
3	21	211±11	170±6	102±8	88±11	18±4	12±2	147±16	124±4	45±14	32±2
4	22	239±19	189±8	82±14	70±7	15±4	10±4	174±21	147±4	51±14	34±3
Mean±1 SD		207±28	158+29	95±23	83±21	16±4	12±3	145±27	116±28	46±3	31±3
P§		<0.001		<0.01		<0.02		<0.01		<0.01	

* Obtained by beta-quantification (29).
‡ S, saturated diet; P, polyunsaturated diet.
§ Statistics obtained by paired *t* test.

TABLE II
Effect of Dietary Fat Saturation Level on HDL Composition

Subject	Free cholesterol		Cholesteryl esters		Triglyceride		Phospholipid		ApoA-I total protein		Phospholipid protein ratio		Protein	
	S*	P	S	P	S	P	S	P	S	P	S	P	S	P
	%		%		%		%		%				%	
1	2.7	2.4	22.7	21.8	4.5	4.8	26.6	30.6	66	67	0.61	0.76	43.5	40.4
2	2.9	2.9	23.8	22.8	4.1	4.3	26.1	29.8	65	66	0.61	0.74	43.0	40.2
3	3.3	3.1	24.5	24.6	4.4	4.6	29.2	31.4	65	65	0.76	0.87	38.6	36.3
4	2.9	3.4	23.5	27.1	3.8	4.8	25.6	28.2	52	65	0.58	0.77	44.1	36.5
Mean±1 SD	3.0±0.3	3.0±0.4	23.6±0.7	24.1±2.3	4.2±0.3	4.6±0.2	26.9±1.6	30.0±1.4	62±7	66±1	0.64±0.08	0.79±0.06	42.3±2.5	38.3±2.3
P	NS‡		NS		NS		<0.01		NS		<0.02		<0.02	

Each assay was performed in duplicate.
* S, saturated diet; P, polyunsaturated diet.
‡ Statistics obtained by paired t test.

the metabolism of HDL-associated ^{125}I-apoA-I depends on three factors. Firstly, we required to demonstrate that conversion from one to the other dietary regimen produced clearly discernible changes in plasma lipid saturation. Such changes would indicate that an adequate stimulus had been provided to alter HDL metabolism, if this indeed occurred. Secondly, to ensure the applicability of Matthews' mathematical procedure (37) which was used to analyze the data derived from the study, it was important to show that each subject maintained a steady state with respect to HDL metabolism throughout both study periods. Thirdly, in studies of protein metabolism using trace-labeling procedures, it is essential that the radioactive marker remains associated with the protein under investigation throughout its biological lifetime. This was particularly relevant to the present study because the radioactive marker, ^{125}I-apoA-I, had been incorporated initially into HDL by an in vitro transfer procedure and therefore might be susceptible to dissociation from its parent lipoprotein in vivo.

The efficacy of the polyunsaturated vs. the saturated fat diet in lowering plasma lipid concentrations is shown in Table I. The polyunsaturated fat produced significant reductions in total plasma cholesterol and triglyceride and also reduced the plasma levels of cholesterol associated with very low density lipoprotein (VLDL), LDL, and HDL. These findings, on the whole, corroborate and amplify the observations of other workers (3–8, 14, 15) and raise the possibility that the observed changes in the plasma lipid concentrations may be accompanied by changes in the composition of the plasma lipoproteins. Tables II and III indicate that this is so, at least for HDL. Ingestion

TABLE III
Fatty Acid Analysis of HDL Lipids from Subjects Fed Saturated and Polyunsaturated Fat Diets

Fatty acid	Mean percentage (±1 SD) in cholesteryl esters			Mean percentage (±1 SD) in triglycerides			Mean percentage (±1 SD) in phospholipids		
	S*	P	Change	S	P	Change	S	P	Change
	%			%			%		
14:0	4.5±2.5	2.1±1.2	NS‡	5.4±3.9	3.0±1.0	NS	2.4±2.3	0.3±0.3	NS
16:0	11.5±0.4	8.1±0.5	29 ($P < 0.01$)	25.8±1.6	15.3±1.7	41 ($P < 0.01$)	27.2±1.1	22.5±1.0	17 ($P < 0.05$)
16:1	2.7±0.6	1.0±0.4	NS	5.0±0.9	3.0±0.7	NS	0.9±1.0	—	—
18:0	1.4±0.4	0.9±0.2	35 ($P < 0.05$)	3.8±0.6	2.4±0.2	37 ($P < 0.01$)	14.5±1.3	13.2±1.1	9 ($P < 0.01$)
18:1	16.1±1.4	9.2±0.9	43 ($P < 0.01$)	40.4±4.8	24.9±2.6	38 ($P < 0.01$)	12.7±0.6	11.7±1.1	NS
18:2	53.3±3.2	68.1±2.1	28 ($P < 0.01$)	17.7±1.3	45.5±5.3	157 ($P < 0.01$)	23.0±1.6	29.6±1.2	29 ($P < 0.02$)
>18:2 ↓ <20:4	1.7±2.0	1.6±0.8	NS	1.2±1.1	3.7±1.5	NS	4.6±1.6	4.4±1.0	NS
>20:4	8.8±1.2	9.2±1.1	NS	1.5±0.5	2.2±0.1	NS	14.5±1.3	18.2±1.9	NS

* S, saturated diet; P, polyunsaturated diet.
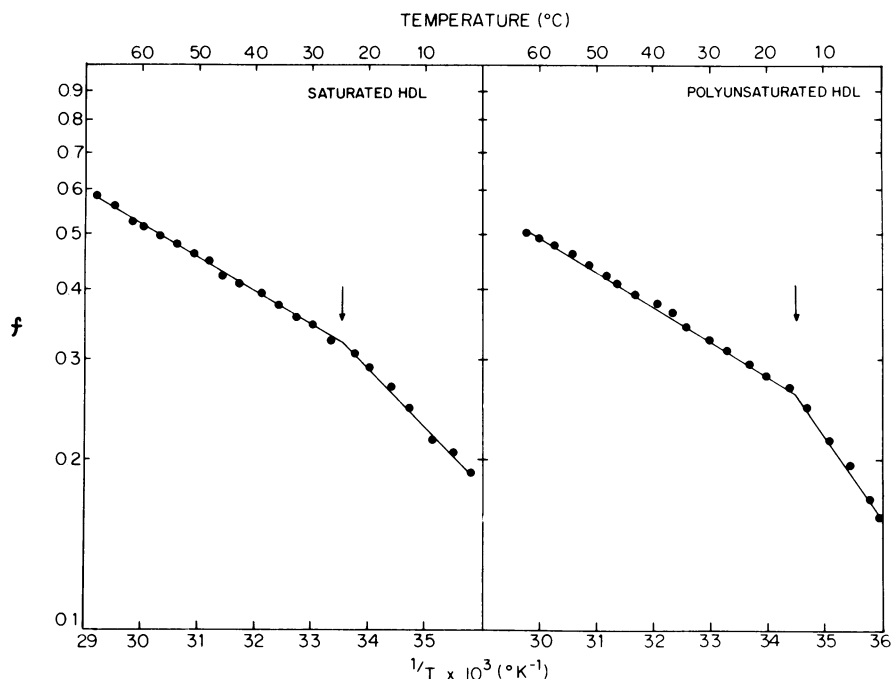‡ Statistics obtained by paired t test.

FIGURE 2 Arrhenius plot of electron paramagnetic resonance spectra obtained from saturated and polyunsaturated HDL labeled with TEMPO.

of polyunsaturated fat, in comparison with saturated fat, caused a significant increase in the percent phospholipid in HDL with reciprocal changes in the apolipoprotein content (Table II). The percentages of free and esterified cholesterol and of triglyceride in HDL were unaffected by changes in dietary fat saturation. As has been shown by others (17–19), the polyunsaturated diet increased significantly the proportion of polyunsaturated fatty acids in the cholesteryl esters, triglycerides, and phospholipids of HDL, and de-creased the saturated fatty acids in these lipids. The major changes in fatty acid composition involved palmitate (16:0), stearate (18:0), oleate (18:1), and linoleate (18:2); the percentages of the first three fatty acids were significantly reduced in each lipid class as a result of the polyunsaturated fat diet (except in phospholipid oleate which was not significantly changed by diet treatment), whereas linoleate was uniformly increased in the HDL lipid fractions. These changes reflect the fatty acid composition of each diet. Overall, HDL triglycerides were most affected by polyunsaturated fat ingestion whereas phospholipids showed the least change. We predicted that these changes in saturation of the fatty acyl chains in HDL

TABLE IV
Effects of Dietary Fat Saturation Level on the Thermotropic Properties of HDL

	Thermotropic phase transition temperature	
Subject	S*	P
	°C	
1	24.8	15.0
2	27.0	17.0
3	24.0	19.5
4	30.0	20.0
Mean ± 1 SD	26.5 ± 2.6	17.9 ± 1.5

$P < 0.01$‡

* S, saturated diet; P, polyunsaturated diet.
‡ Paired t test.

TABLE V
Steady State Parameters on Saturated and Polyunsaturated Fat Diets

	Mean body weight ± 1 SD (n = 35)		Mean plasma apoA-I ± 1SD (n = 9)		Mean urine/plasma Radioactivity ratios ± 1 SD (n = 14)	
Subject	S*	P	S	P	S	P
	kg		mg/dl			
1	63.1 ± 0.3	63.0 ± 0.3	122 ± 1	92 ± 4	0.32 ± 0.05	0.2 ± 0.08
2	63.7 ± 0.2	64.5 ± 0.4	124 ± 4	101 ± 7	0.26 ± 0.04	0.30 ± 0.06
3	66.5 ± 0.3	65.4 ± 0.2	113 ± 6	95 ± 14	0.37 ± 0.04	0.32 ± 0.04
4	60.2 ± 0.2	69.8 ± 0.2	136 ± 3	104 ± 13	0.25 ± 0.03	0.26 ± 0.03

* S, saturated diet; P, polyunsaturated diet.

produced by polyunsaturated fat ingestion would result in significant changes in the microscopic fluidity of the lipoproteins. This prediction was confirmed by electron spin resonance studies of HDL isolated from the subjects' plasma at the end of each diet period. The amphiphilic spin label used (TEMPO) demonstrates temperature-dependent partitioning between the bulk aqueous phase and fluid lipid phase of lipoproteins that can be quantified from the amplitude changes evident in the hydrophobic and polar components of the TEMPO spectrum high field resonance line. Plots of the fluidity parameter, f, calculated from such spectra, vs. temperature indicate that lipoproteins, presumably because of their complexity, do not exhibit sharp phase transition temperatures. However, Arrhenius plots of ln f vs. $1/T$ gave a characteristic discontinuity for HDL as shown in Fig. 2. The transition temperatures for HDL obtained after both dietary regimens are presented in Table IV. Phase transition occurred at approximately 26.5° and 18°C on the saturated and polyunsaturated diets, respectively.

The parameters used to define the maintenance of steady state during both phases of this metabolic study
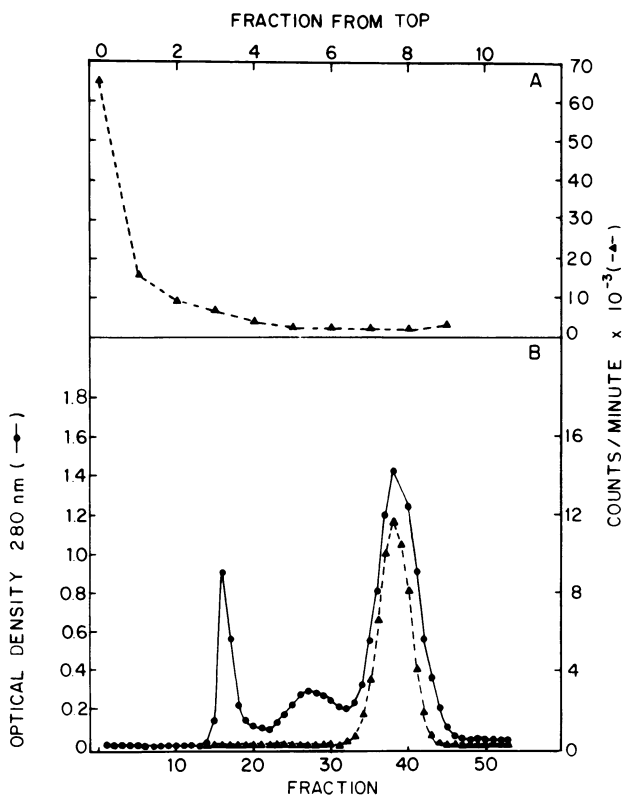
TABLE VI
Distribution of ^{125}I-ApoA-I Radioactivity during Polyunsaturated Fat Feeding

Subject	Days after injection	Percent plasma ^{125}I of $d < 1.225$ kg/liter		Percent plasma ^{125}I of $d < 1.225$ kg/liter in HDL on gel filtration‡	
	1	95.7		99.5	
1	6	93.5	93.6±2.1	98.4	96.5±4.3
	13	91.6		91.6	
	1	93.9		99.4	
2	6	91.3	90.6±3.6	98.4	97.4±2.6
	13	86.7		94.5	
	1	95.8		99.3	
3	6	94.6	94.8±1.0	98.1	93.9±8.3
	13	93.9		84.4	
	1	94.3		99.4	
4	6	95.5	93.7±2.2	98.4	96.0±5.1
	13	91.2		90.1	

Determinations were made on plasma collected on days 1, 6, and 13 after injection.

* Plasma lipoproteins were prepared by ultracentrifugation at $d = 1.225$ kg/liter and the percentage of total plasma radioactivity associated with the fraction determined.

‡ Plasma lipoproteins, prepared as described above, were chromatographed on a column containing 6% agarose and the percentage radioactivity associated with the HDL fraction determined.

are presented in Table V and include body weight, plasma apoA-I concentrations, and urine/plasma radioactivity ratios. Throughout both study periods, variation in daily body weight did not exceed 1% of the mean value for any subject. The variability of daily urine/plasma radioactivity ratios and of plasma apoA-I levels measured on five occasions throughout each study was 15.3 and 6.25%, respectively, from the mean value. We believe that these findings, when taken together, justify the conclusion that a steady state was maintained throughout both studies and consequently, the application of Matthews' (37) compartmental analysis procedure was warranted.

Ultracentrifugal flotation performed on plasma from each volunteer on day 1, 6, and 13 after injection demonstrated that the ^{125}I-apoA-I retained its association with a plasma component of < 1.225 kg/liter throughout both study periods. Overall, on the polyunsaturated diet, a mean of 93.2±8% of the total radioactivity appeared at the top of the centrifuge tube (Fig. 3A, Table VI). Gel filtration of this material through 6% agarose demonstrated that 96.0±1.5% of the radioactivity that floated had the elution characteristics of HDL (Fig. 3B, Table VI). The flotation and chromatographic characteristics of the radioactive material were unaffected by diet regimen. From these data,
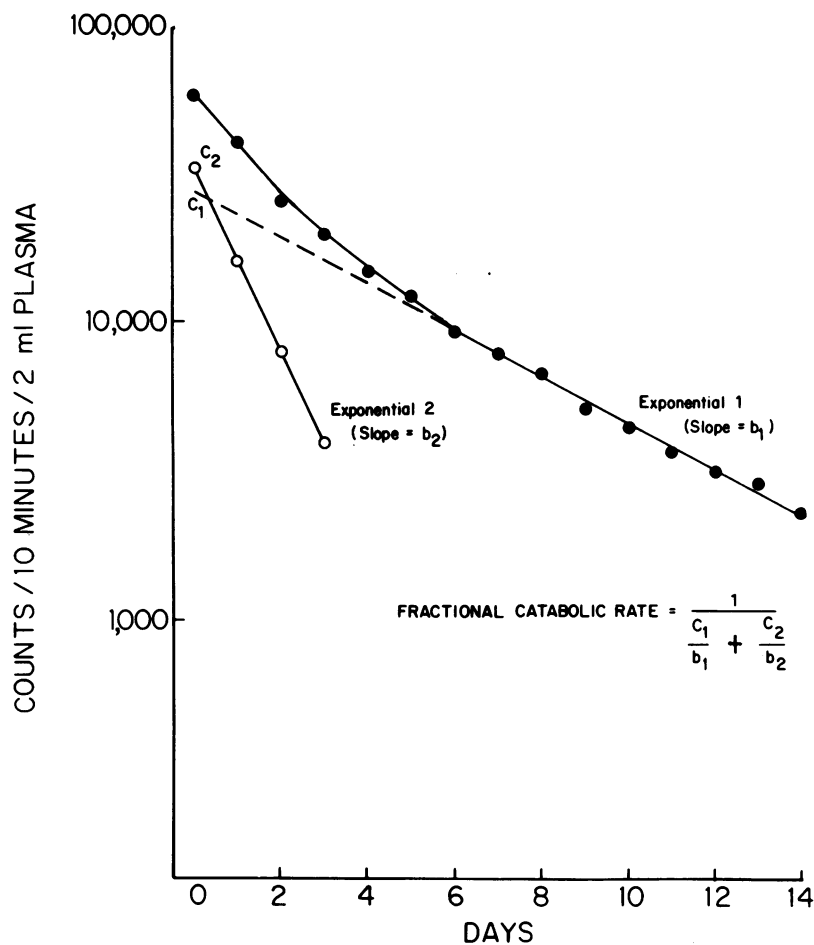

FIGURE 3 Plasma distribution of ^{125}I-apoA-I on day 6 after injection of ^{125}I-apoA-I/HDL. (A) ^{125}I-apoA-I flotation at $d < 1.225$ kg/liter. (B) Bio-Gel A5m (200–400 mesh) eluate profile at 280 nm of $d < 1.225$ kg/liter plasma lipoproteins.

$$\text{FRACTIONAL CATABOLIC RATE} = \cfrac{1}{\cfrac{c_1}{b_1} + \cfrac{c_2}{b_2}}$$

FIGURE 4   Typical plasma decay profile of ^{125}I-apoA-I/HDL.

TABLE VII
Effects of Saturated and Polyunsaturated Fat Diets on Kinetic Parameters of ApoA-I Metabolism

Subject	Plasma apoA-I concentration		Plasma volume		ApoA-I plasma pool‡		ApoA-I intravascular	
	S*	P	S	P	S	P	S	P
	mg/dl		*ml*		*mg*		*%*	
1	122	92	2,520	2,520	3,080	2,320	64.2	83.4
2	124	101	2,550	2,580	3,160	2,610	66.7	75.6
3	113	95	2,660	2,620	3,010	2,490	61.3	67.0
4	136	104	2,770	2,790	3,760	2,900	73.2	70.5
Mean±1 SD	124±9	98±5	2,625±114	2,628±116	3,253±344	2,580±244	66±5	74±7
P	<0.01‡‡		NS		<0.01		NS	

* S, saturated diet; P, polyunsaturated diet.
‡ The product of apoA-I concentration and plasma volume.
§ Obtained by Matthews' kinetic analysis of plasma decay curve (36).
‖ Ratio of 24 h urinary radioactivity to mean plasma radioactivity in the same time period.
¶ The product of calculated fractional catabolic rate and apoA-I pool size.
** Absolute rate of catabolism of apoA-I (in milligrams per day) divided by body weight (in kilograms).
‡‡ Paired *t* test.

we concluded that the injected ^{125}I-apoA-I/HDL tracer maintained its integrity throughout the study periods, and this conclusion was substantiated by the finding that the injected radioactivity was distributed in the body in a manner consistent with its continued association with a high molecular weight species.

A typical ^{125}I-apoA-I/HDL plasma decay curve is shown in Fig. 4 and, like the decay profile for LDL (36), can be adequately described by two exponential components whose slopes and y-axis intercepts were used to calculate the fractional catabolic rate of the ^{125}I-apoA-I/HDL (37). The urine/plasma radioactivity ratio, measured at daily intervals throughout the study, provided an independent assessment of the same kinetic parameter (38). The constancy of this ratio is a useful monitor of steady-state and tracer homogeneity during the period of study (see Table V). The kinetic data calculated are presented in Table VII. It is evident that polyunsaturated fat therapy produced a significant reduction ($P < 0.01$) in the plasma apoA-I level. This effect may have been caused by increased catabolism or decreased synthesis of the apoprotein, or by a combination of both. As no increase in the fractional rate of catabolism of the exchangeable apoA-I could be demonstrated either by calculation from the plasma data or by direct measurements of the urine/plasma ratio, we infer that the synthetic rate of the apoprotein (which is equivalent to the absolute catabolic rate under steady-state conditions) is lowered as a result of the polyunsaturated fat diet.

The change produced in plasma apoA-I levels by ingestion of the diet high in polyunsaturated fat had an effect on the distribution of the HDL subfractions (HDL_2 and HDL_3) in the plasma. Specifically, there was a 28% fall in the HDL_2:HDL_3 plasma ratio during polyunsaturated fat feeding (Table VIII).

DISCUSSION

That ingestion of polyunsaturated fat by normal subjects causes a reduction in plasma cholesterol, triglyceride, and phospholipid is well documented (3–6, 14, 15, 39, 40). The cholesterol-lowering effect has been shown to result largely from a decrease in plasma LDL. Some investigators have claimed that a fall in HDL cholesterol also contributes to this effect (40), although others have failed to confirm this observation (39, 41). Our findings in healthy young males (Table I) indicate that 60% of the polyunsaturated fat-induced cholesterol lowering resulted from a reduction in the LDL fraction, augmented by significant decreases in VLDL and HDL cholesterol (producing 8 and 32% of the reduction, respectively).

Analysis of the composition of plasma HDL at the end of both diet periods (Table II) indicated that the percentage of cholesterol in the lipoprotein was unchanged by diet, and we concluded that the fall in HDL cholesterol resulted from an overall decrease in the plasma HDL concentration. Polyunsaturated fat ingestion, however, did cause a significant increase in HDL phospholipid, reciprocated by a fall in protein (and apoA-I). A similar change in the phospholipid/protein ratio in LDL was observed by Spritz and Mishkel (41) as a result of polyunsaturated diet therapy. They suggested that because unsaturated fatty acids occupy a greater area than saturated acids, they increase the space occupied by the lipids into which they are incorporated. By extrapolation, the

TABLE VII (*Continued*)

(a)Calculated§		(b)[∥] From urine/plasma ratios		Absolute catabolic rate of apoA-I[¶]		Absolute catabolic rate of apoA-I[**]	
S	P	S	P	S	P	S	P
				mg/day		*mg/kg/day*	
0.35	0.29	0.32	0.29	1,060	680	16.8	10.8
0.27	0.26	0.26	0.31	860	690	13.4	10.6
0.36	0.32	0.37	0.32	1,070	790	16.0	12.0
0.25	0.26	0.25	0.26	940	750	13.6	10.8
0.31±0.05	0.28±0.03	0.30±0.06	0.30±0.03	983±101	728±52	15.0±1.7	11.1±0.6
NS		NS		<0.02		<0.02	

The header "Fractional catabolic rate (pools/day)" spans the (a)Calculated and (b) From urine/plasma ratios columns.

TABLE VIII
Effect of Saturated and Polyunsaturated Fat Diets on Plasma HDL Subfraction Distribution*

| | Percentage of initial (saturated) HDL | | | | | | | |
| | Total HDL | | HDL$_2$ | | HDL$_3$ | | HDL$_2$/HDL$_3$ ratio | |
Subject	S‡	P	S	P	S	P	S	P
1	100	99.9	7.8	3.3	92.1	96.6	0.085	0.034
2	100	98.3	7.6	6.4	92.4	91.9	0.082	0.069
3	100	91.1	18.3	14.0	81.7	77.2	0.225	0.181
4	100	97.0	7.8	5.3	92.1	91.7	0.084	0.058
P	<0.2§		<0.05		NS		<0.05	

* HDL subfractionation performed by rate zonal ultracentrifugation (34).
‡ S, saturated diet; P, polyunsaturated diet.
§ Paired t test.

29% increase in HDL phospholipid linoleate that occurred in our study as a result of polyunsaturated fat feeding may limit the space available for apoprotein binding on the surface of the HDL and consequently produce a significant increase in the phospholipid/protein ratio.

The fatty acid composition of plasma HDL (Table III) was directly correlated with the saturation level of the ingested fat. With respect to the saturated fat diet, ingestion of polyunsaturated fat caused significant enrichment of linoleate in all HDL lipids (cholesteryl esters, triglyceride, and phospholipid) accompanied by a fall in palmitate and stearate in these fractions. One result of these compositional changes was an alteration of the thermotropic properties of HDL whose microscopic fluidity was increased by polyunsaturated fat feeding. The HDL phase transition occurred on the polyunsaturated diet over the range 15–20°C, in accord with the findings of Morrisett et al. (19). However, on the saturated diet, the transition temperatures we observed were significantly lower than in this earlier study. This difference may be explained by the fact that on the saturated diet, the HDL phospholipids from the study of Morrisett et al. contained 38% less linoleic acid than we observed (Table III). The reason for these differences is the variability in individual response to modification of the saturation level of dietary fat. Because the phase transitions on both diets occurred well below body temperature, and at 37°C the fluidity parameters were not significantly different (Fig. 2), the observed alteration of apoA-I metabolism could not be explained on the basis of this physical parameter.

Previous studies suggest that changes in the fatty acid composition of lipoproteins may influence directly their rate of catabolism. For example, Thompson et al.

(20), by intralipid infusion, increased the oleate:linoleate ratio in LDL cholesteryl esters and demonstrated a decrease in LDL apoprotein catabolism. Also, Soutar and co-workers (42) showed that the rate of acyl group transfer by lecithin:cholesterol acyl transferase, an enzyme intimately associated with HDL metabolism (43), was dependent on the fatty acid component of the substrate. Furthermore, lipoprotein lipase, another major enzyme involved in lipoprotein metabolism, hydrolyzes unsaturated triglycerides faster than saturated triglycerides (44). We expected that the diet-induced alterations in HDL lipid saturation might produce changes in catabolism of the lipoprotein and of its major apoprotein, apoA-I, which would account for the fall in plasma HDL we observed. It is evident from Table VII that catabolism of apoA-I as measured by the method reported here was not affected by the change in diet. On the contrary ingestion of the polyunsaturated diet was associated with a decreased rate of synthesis of this apoprotein. In other words, polyunsaturated fat feeding appears not to exert its effect upon apoA-I catabolism, secondary to alterations of the physicochemical properties of HDL but rather by modifying the rate of apoA-I synthesis or secretion or both.

Our observations are germane to the therapeutic efficacy of polyunsaturated fats in combating the development of atherosclerotic heart disease. The undoubted hypocholesterolemic effect of this treatment (with its implied benefits) must be weighed against the potentially detrimental reduction in HDL because this lipoprotein is widely believed (45, 46) to have antiatherogenic properties. However, we must make it clear that the diets in this study were extreme examples of those normally encountered in current clinical practice. The data presented here provide a base line for further studies designed to quantify the effects of polyunsaturated fat diets.

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