Influence of polyunsaturated fats on composition of plasma lipoproteins and apolipoproteins

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Abstract The mechanisms of the hypocholesterolemic effect of polyunsaturated fats (PUSF) are not well understood. One possibility is that these fats uniquely reduce the cholesterol content of lipoproteins. The present study was carried out to determine specifically whether the ratio of cholesterol-to-protein (or apoB) in LDL (or other lipoproteins) is reduced by PUSF; also, lipoprotein composition was examined for other possible changes. Eight men and two women with different levels of plasma cholesterol were studied on the metabolic ward for 8 weeks. They were given a diet high in saturated fats (SF) for 4 weeks and another rich in PUSF for 4 weeks. On PUSF diets, mean plasma cholesterol decreased by 25% (SF = 296 ± 27 (SEM) vs. PUSF = 223 ± 21 mg/dl) as did total plasma apoB (155 ± 8 vs. 116 ± 13 mg/dl). LDL-Cholesterol decreased by 26%, and LDL-apoB fell by 29%. The mean ratio of cholesterol-to-apoB did not change significantly (SF = 1.52 ± 0.04 vs. PUSF = 1.48 ± 0.07). Likewise, HDL-cholesterol decreased by 15% (SF = 51 ± 5 vs. PUSF = 43 ± 4 mg/dl), and total plasma apoA-I was reduced by 19% (95 ± 15 vs. 77 ± 6 mg/dl); also, no change in the cholesterol-to-apoA-I in HDL was noted. Finally, there were no changes in cholesterol/apoB or triglyceride/apoB ratios in VLDL despite a 23% decrease in plasma triglycerides on PUSF. Thus, the hypocholesterolemic effect of PUSF was uniform for all lipoproteins and usually was accompanied by a corresponding decrease in concentrations of apoprotein constituents.—**Vega, G. L., E. Groszek, R. Wolf, and S. M. Grundy.** Influence of polyunsaturated fats on composition of plasma lipoproteins and apolipoproteins. *J. Lipid Res.* 1982. **23:** 811–822.

Supplementary key words apoB • apoA-I • saturated fats • electroimmunoassay

A hypocholesterolemic action of polyunsaturated fats is well established, but the mechanisms involved have remained elusive. Several mechanisms have been proposed including changes in metabolism of cholesterol (1–8) and/or lipoproteins (9, 10). An intriguing hypothesis, proposed by Spritz and Mishkel (9), is that plasma cholesterol is decreased because lipid esters containing polyunsaturated fatty acids require more space in lipoproteins and thereby sterically exclude cholesterol. This hypothesis is consistent with their report of a decrease in cholesterol-to-protein ratios in low density lipoproteins (LDL) when polyunsaturated fats were substituted for saturated fats. Similar changes in LDL composition have been reported by other workers (11–14).

If polyunsaturated fats lower LDL-cholesterol by changing LDL chemical composition, this could have important clinical implications. For instance, particle number in LDL might not be reduced, and if not, lowering of LDL-cholesterol might not retard atherogenesis. Thus, we have reexamined the influence of polyunsaturated fats on lipoprotein composition. Special attention has been given to LDL, but other lipoproteins have been examined as well.

METHODS

Patients

Ten patients (eight men and two women) were studied on the Special Diagnostic and Treatment Unit of the Veterans Administration Medical Center, San Diego, CA. Their mean age was 52 years (21–65 years). Mean ideal body weight was 108 ± 14% (range 78–132%). One patient (No. 9) had familial hypercholesterolemia, and two (Nos. 2 and 10) had mild hypertriglyceridemia (Type IV hyperlipoproteinemia). The remaining seven had mild hypercholesterolemia.

Experimental design

Patients were hospitalized for 2 months. They were fed first a diet rich in saturated fats for 4 weeks, then one containing polyunsaturated fats for 4 weeks. In the first 2 weeks of each period blood was drawn for determination of cholesterol and triglycerides in total plasma and lipoprotein fractions. In the last 2 weeks, blood was drawn 3 times weekly for isolation and detailed analysis of lipoprotein concentration and composition.

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; DTNB, dithionitrobenzoic acid; TMU, tetramethylurea; EIA, electroimmunoassay.

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Diets

During hospitalization, the patients were given a liquid formula diet containing 40% of calories as fat, 45% as carbohydrate, and 15% as protein. The diet was given 5 times per day; calories were divided approximately equally between feedings. Lard supplied the saturated fats, and safflower oil, the polyunsaturates. Cholesterol intakes were less than 100 mg/day in both periods. Vitamin and mineral supplements were given daily, and caloric intake was adjusted to maintain total body weight at a constant level throughout the study.

Plasma total and lipoprotein lipids

Concentrations of total plasma cholesterol and triglycerides were determined on a Technicon Autoanalyzer II. Routine estimations of lipoprotein lipids were made according to standard Lipid Research Clinic methodology (15).

Isolation of plasma lipoproteins

Plasma collected in the last 2 weeks of each dietary period was used for isolation and analysis of lipoproteins according to the following procedures. Lipoproteins were isolated by preparative ultracentrifugation at densities of less than 1.019 g/ml, 1.019–1.063 g/ml, and 1.063–1.21 g/ml (16). These fractions were designated very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL), respectively. Isolation was carried out at 15°C in the presence of 0.1% sodium azide, 2 mM dithionitrobenzoic acid (DTNB), and 0.005% chloramphenicol. The isolated lipoproteins were dialyzed exhaustively against 0.85% saline containing the above reagents (except DTNB) at pH 7.4. The VLDL fractions from the six fractions of each dietary period were pooled for chemical analysis. The LDL and HDL fractions were not pooled; instead, chemical analysis was carried out on each isolated sample. Recovery of LDL and HDL after ultracentrifugation averaged 80% and 87%, respectively.

Analysis of lipids and lipoprotein subfractions

For VLDL and LDL, total cholesterol and triglycerides were determined by autoanalyzer (15), phospholipids by the method of Bartlett (17), and ratios of free to ester cholesterol were determined according to Roeschalau, Burnt, and Gruber (18). The same analyses were carried out on HDL except that the free/ester ratio of cholesterol was not measured.

Analysis of apolipoprotein B (apoB)

ApoB mass by Lowry procedure. Total protein in VLDL, LDL, and HDL was determined by the method of Lowry et al. (19) as modified by Sata, Havel, and Jones (20). For VLDL and LDL, apoB was estimated by tetramethylurea (TMU) precipitation described by Kane et al. (21) and its amount was calculated as the difference between total protein and that determined on the supernatant after TMU precipitation. This method will be referred to as the TMU-Lowry procedure. The coefficient of variation for protein measurements was less than 2%.

For the procedure of Lowry et al. (19), bovine serum albumin (BSA) (98% pure by electrophoresis, Calbiochem, San Diego, CA) was used as the standard. The water content of BSA was determined by drying a sample of crystals at 125°C to constant weight. The mass of the hydrated BSA, which was actually used for the standard solution, could thereby be corrected for its water content. According to Kane et al. (21), human apoB and anhydrous BSA have the same chromogenicity (factor = 1.0).

ApoB mass by electroimmunoassay. To obtain an independent measurement of the effect of polyunsaturated fats on apoB concentration in plasma and LDL, the Laurell system (22) of electroimmunoassay (EIA) was modified. Normal human LDL (d 1.03–1.05 g/ml) was used to prepare antibodies in goats.[2] The antisera were shown to react specifically with LDL and not with any other serum protein.

For apoB standard, the apoB content of isolated VLDL + LDL (d < 1.070 g/ml) was determined by the TMU-Lowry procedure described above. Percent recovery of apoB during isolation of VLDL + LDL was determined by dividing cholesterol recovery in this fraction by that obtained by the precipitation method using heparin-manganese (15).

Samples for electrophoresis were diluted (1:20 v/v) with a buffer consisting of 20 mM barbital, 70 mM Tris, pH 8.6, and 0.34 mM calcium lactate, 8 M urea, and 0.05% nondiet P-40 (BDH Gallard Schlessinger, Carle Place, NY). This sample treatment generally resulted in rockets with a configuration of a relatively well-defined triangle. Samples were stored at −20°C for less than 2 weeks within this period (23). ApoB standard was stored at −70°C.

EIA was conducted as follows: 20 μl of anti-LDL B antisera was mixed with 20 ml of melted agarose at a temperature of 54°C. One percent agarose (Seakem agarose; EEO 0.16–0.19; Marine Colloids Div. FMC Corporation, Rockland, ME) and 1.5% polyethylene glycol 6000 (Baker AR, Phillipsburg, NJ) were mixed in 20 mM barbital, 70 mM Tris, 34 mM calcium lactate, and 0.1% sodium azide, pH 8.6. The resulting agarose-containing gel was cast into glass plates 200 × 100 × 1

[2] Antibody to LDL was prepared in collaboration with Dr. Paul S. Roheim, Louisiana State University Medical Center, New Orleans, LA.

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mm. Wells 4 mm in diameter were perforated into the gel. Both standard and unknown plasma samples were treated as described above. Diluted samples (10 μl) were added to the wells. Each unknown sample was placed between two standards. A total of 15 standards was thus alternated with an equal number of unknown samples.

Electrophoresis of the samples was carried out by applying a field strength of 2.7 volts/cm for 17 hr at 16°C. Plates were stained with 0.05% Coomassie Brilliant Blue R-250 (Eastman Kodak, Rochester, NY) dissolved in 4.5:4.5:1 (v/v/v) ethanol–water–acetic acid. Destaining was carried out with the same solvent system.

The areas contained within the rockets were determined by triangulation. An isosceles triangle was constructed using the lines delineating the perimeter of the rocket, and the area of the triangle was determined. Inter- and intra-assay variation ranged between 3% to 6%.

A formula for the routine estimation of LDL apoB was developed relating plasma apoB to lipoprotein lipids. Albers[3] has shown that the apoB/TG ratio in VLDL of normotriglyceridemic subjects is approximately 0.085; in 145 normal subjects he found that the VLDL-TG/apoB ratio was 11.7 ± 1.2 (SEM). Therefore, apoB in VLDL may be calculated from estimated VLDL-TG. The latter value was determined by the method of Meyers, Phillips, and Havel (24). Thus, we reasoned that LDL-apoB can be calculated from the following equation:

$$\text{apoB}_{\text{LDL}} = \text{apoB}_{\text{plasma}} - 0.085 \text{ VLDL-TG}$$

This equation would seem satisfactory for patients with relatively low plasma TG who would have a low VLDL-apoB compared to LDL-apoB. It would not necessarily hold for patients with hypertriglyceridemia.

Analysis of apolipoprotein A-I (apoA-I)

Total plasma apoA-I was measured by EIA essentially as described for apoB. However, the analysis of apoA-I differed in two respects. First, a field strength of 72 volts per cm for a period of 3 hr and a sample dilution of 1:40 was used. Linearity for apoA-I assay was established between 0.15 and 0.32 mg per 10 μl. Quantitation of rockets also was carried out by triangulation. Inter- and intra-assay variation ranged between 2–8%. Samples held for apoA-I assays and standards were stored at −70°C.

ApoA-I antiserum was prepared in rabbits. The antigen (apoA-I) was isolated by gel chromatography on Sephadex G-200; its purity was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Specificity of the antigen was tested by immunodiffusion (25) against LDL, apoA-I, and apoA-II. The antiserum reacted only with apoA-I.

ApoA-I standard for EIA was the same plasma utilized in the apoB assay. This plasma was calibrated for apoA-I content using a serial dilution of isolated apoA-I of known concentration. The protein content of isolated apoA-I was determined by the method of Lowry et al. (19). Estimations of apoA-II by EIA were not made.

Analytic isoelectric focusing of VLDL apoproteins

VLDL was delipidated with ethanol–ether 3:1 (v/v) at 4°C. The apoproteins were solubilized in 0.05 M Tris-Cl, 0.06 M sodium decyl sulfate in 8 M urea, pH 8.2, and 2% β-mercaptoethanol. Analytical isoelectric focusing was carried out in 8% polyacrylamide cylindrical gels (IEF-PAGE) using ampholine (LKB, Uppsala, Sweden) of pH range 4.0–6.0. Anolyte and catholyte were 0.01 M H_3PO_4 and 0.02 M NaOH, respectively. Electrophoresis was conducted at a constant power of 3 watts for 2 hr and 7 watts for 4 hrs at 4°C. The gels were stained for 16 hr in 0.075% Coomassie Brilliant Blue R-250 (Eastman Kodak, Rochester, NY) dissolved in 25% ethanol and 9% acetic acid. Gels were destained in 25% ethanol and 9% acetic acid. Densitometric scans of the gels were performed in a Transidyne Densitometer (Model 2500–2520, Transidyne General Corporation, Ann Arbor, MI) at a wave length of 560 nm. Linearity of the system was established after focusing of various amounts of VLDL apoproteins (150–250 μg of protein) and scanning of these gels. Percent distributions of apoE isoforms, and apoC-II and C-III were obtained.

Analytic SDS-PAGE of HDL apoproteins

Delipidation of HDL and solubilization of apoproteins was carried out in the same way as for VLDL except that β-mercaptoethanol was not used. SDS-PAGE in 12% acrylamide was conducted in a 0.1 M phosphate, pH 7.0, and 0.1% SDS; 2.5 mA per gel was used for 15 hr (26). Gels were stained for 1 hr in 0.5% Coomassie Brilliant Blue R-250 (Eastman Kodak, Rochester, NY) dissolved in 25% methanol and 9% acetic acid. The gels were destained in 9% acetic acid for 24 hr. Densitometric scanning was carried out as described above, and the percent distribution of apoproteins A-I, A-II, D, and C's was obtained.

Lipid and protein concentrations of lipoproteins

The concentrations of lipids and proteins in each lipoprotein fraction were determined from concentrations of lipoprotein-cholesterol, estimated by standard LRC procedures (15), and the chemical composition of each lipoprotein. The ratios of each component to cholesterol (determined in composition measurements) was multiplied times the lipoprotein-cholesterol (determined by

[3] Albers, J. J. Personal communication.

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LRC procedures) to obtain the concentration of each component. This procedure thus corrected for any losses that may have occurred in isolation of lipoproteins.

Statistical analysis

All statistical comparisons between results for feeding of saturated fats and polyunsaturated fats were done by paired *t*-test.

RESULTS

Plasma lipids and apoB

The effects of the two diets on plasma levels of total lipids and apoB are shown in **Table 1.** All patients except one had a significant reduction of plasma cholesterol on polyunsaturated fats; the mean reduction was 25% (range 13–32%). Most also showed a decrease in plasma TG

TABLE 1. Plasma lipid and apoB concentrations

Patient	Diet	Plasma Cholesterol	Plasma Triglycerides	Plasma ApoB
		mg/dl ± SD (n = 6)[a]		
1	SF	248 ± 29	170 ± 40	
	PUSF	216 ± 12	129 ± 32	
	Δ(%)	−32 (13)	−41 (25)	
	$P <$	0.0005	0.01	
2	SF	300 ± 6	381 ± 23	176 ± 8
	PUSF	205 ± 18	224 ± 48	137 ± 8
	Δ(%)	−95 (32)	−157 (41)	39 (22)
	$P <$	0.0005	0.0005	0.0005
3	SF	282 ± 10	165 ± 16	152 ± 5
	PUSF	205 ± 16	114 ± 7	114 ± 1
	Δ(%)	−77 (28)	−51 (31)	38 (25)
	$P <$	0.0005	0.0005	0.0005
4	SF	239 ± 9	163 ± 25	
	PUSF	238 ± 21	163 ± 24	
	Δ(%)	−1 (1)	0 (0)	
	$P <$	N.S.	N.S.	
5	SF	270 ± 2	102 ± 12	116 ± 6
	PUSF	177 ± 7	97 ± 8	80 ± 1
	Δ(%)	−93 (35)	−5 (5)	36 (31)
	$P <$	0.005	N.S.	0.0005
6	SF	295 ± 9	233 ± 25	133 ± 6
	PUSF	199 ± 13	203 ± 17	107 ± 4
	Δ(%)	−96 (33)	−30 (13)	26 (20)
	$P <$	0.005	0.05	0.0025
7	SF	505 ± 28	114 ± 12	258 ± 36
	PUSF	382 ± 19	87 ± 7	187 ± 9
	Δ(%)	−123 (24)	−27 (24)	71 (28)
	$P <$	0.0005	0.005	0.005
8	SF	241 ± 8	95 ± 18	95 ± 3
	PUSF	168 ± 7	64 ± 7	77 ± 3
	Δ(%)	−73 (30)	31 (33)	16 (17)
	$P <$	0.005	0.025	0.0005
9	SF	278 ± 5	199 ± 27	149 ± 5
	PUSF	201 ± 15	150 ± 23	108 ± 10
	Δ(%)	−77 (28)	49 (25)	41 (28)
	$P <$	0.0005	0.005	0.0005
10	SF	304 ± 19	327 ± 24	
	PUSF	240 ± 32	276 ± 22	
	Δ(%)	−64 (21)	51 (16)	
	$P <$	0.0005	0.05	
Mean ± SD	SF	296 ± 77	195 ± 95	154 ± 53
	PUSF	223 ± 60	151 ± 67	116 ± 38
	Δ(%)	73 (25)	44 (23)	38 (25)
	$P <$	0.0005	0.005	0.0025

[a] The values shown in this table represent the mean ± SD obtained by analysis of six separate samples from each dietary period.
N.S., not significant.

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(mean decrease, 23%; range 5–41%). Total plasma apoB was estimated by EIA in seven patients; all patients had a significant reduction (mean decrease, 25%).

Low density lipoprotein (LDL)

Concentrations of lipid and protein components in LDL are shown in **Table 2.** Total cholesterol in LDL fell during feeding of polyunsaturates by an average of 26% (range 4–42%). Similar changes were noted for free and esterified cholesterol, phospholipids, and triglyceride. Since all lipid components declined similarly, the average lipid composition of LDL was not altered. Likewise, total protein and $apoB_{TMU}$ in LDL fell by 25% and 29%, respectively. Thus, polyunsaturates lowered all LDL components.

Table 3 shows cholesterol/apoB ratios in LDL with

TABLE 2. Lipid and protein concentrations of LDL

Patient	Diet	Total Cholesterol	Esterified Cholesterol	Unesterified Cholesterol	Triglyceride	Phospholipid	Total Protein	ApoB (TMU)
		mg/dl ± SD (n = 6)[a]						
1	SF	167 ± 18	121 ± 14	46 ± 6	23 ± 3	108 ± 21	125 ± 25	121 ± 26
	PUSF	142 ± 11	106 ± 8	36 ± 6	19 ± 2	79 ± 7	86 ± 9	85 ± 12
	Δ(%)	25 (15)	14 (12)	10 (22)	4 (17)	29 (23)	39 (31)	36 (30)
	$P <$	0.0005	0.005	0.0005	0.0025	0.0005	0.0005	0.0005
2	SF	184 ± 6	140 ± 8	43 ± 6	46 ± 5	117 ± 11	160 ± 10	151 ± 10
	PUSF	127 ± 15	95 ± 13	30 ± 3	27 ± 7	75 ± 10	105 ± 12	99 ± 12
	Δ(%)	57 (31)	45 (32)	13 (30)	19 (41)	42 (36)	55 (34)	52 (34)
	$P <$	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005
3	SF	215 ± 7	159 ± 5	57 ± 4	28 ± 3	139 ± 12	154 ± 17	150 ± 7
	PUSF	158 ± 2	111 ± 8	47 ± 3	22 ± 1	98 ± 9	104 ± 5	100 ± 2
	Δ(%)	57 (27)	48 (30)	10 (18)	6 (21)	41 (29)	50 (32)	50 (33)
	$P <$	0.0005	0.0005	0.0005	0.025	0.0005	0.0005	0.0005
4	SF	173 ± 18	126 ± 14	47 ± 4	31 ± 3	113 ± 13	124 ± 18	121 ± 17
	PUSF	180 ± 19	139 ± 17	49 ± 3	31 ± 5	119 ± 13	124 ± 12	118 ± 13
	Δ(%)	−7 (4)	−5 (4)	2 (4)	0 (0)	−6 (5)	0 (0)	3 (2)
	$P <$	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
5	SF	187 ± 2	131 ± 2	56 ± 2	32 ± 3	115 ± 4	115 ± 2	104 ± 1
	PUSF	108 ± 6	76 ± 3	32 ± 3	28 ± 6	67 ± 4	77 ± 7	70 ± 2
	Δ(%)	79 (42)	55 (42)	24 (43)	6 (19)	48 (42)	38 (33)	34 (33)
	$P <$	0.0005	0.0005	0.0005	0.0025	0.0005	0.0005	0.0005
6	SF	205 ± 8	148 ± 4	57 ± 10	35 ± 6	123 ± 11	133 ± 6	127 ± 3
	PUSF	127 ± 3	91 ± 3	33 ± 4	26 ± 3	75 ± 6	100 ± 9	92 ± 3
	Δ(%)	78 (38)	57 (39)	24 (42)	9 (26)	48 (39)	33 (25)	35 (28)
	$P <$	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005
7	SF	430 ± 10	310 ± 24	119 ± 4	34 ± 1	276 ± 38	280 ± 40	274 ± 96
	PUSF	325 ± 7	239 ± 19	85 ± 5	26 ± 1	176 ± 6	190 ± 11	184 ± 25
	Δ(%)	105 (24)	71 (23)	34 (29)	8 (24)	100 (6)	90 (32)	90 (33)
	$P <$	0.0005	0.0005	0.0005	N.S.	0.0005	0.0005	0.0005
8	SF	158 ± 3	113 ± 2	44 ± 4	17 ± 6	95 ± 5	103 ± 6	95 ± 6
	PUSF	108 ± 8	76 ± 8	30 ± 5	11 ± 3	67 ± 5	77 ± 7	74 ± 6
	Δ(%)	50 (32)	37 (33)	14 (32)	6 (35)	28 (29)	26 (25)	21 (22)
	$P <$	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005
9	SF	201 ± 8	144 ± 5	55 ± 3	27 ± 3	132 ± 11	148 ± 1	145 ± 9
	PUSF	141 ± 10	98 ± 13	41 ± 6	21 ± 5	91 ± 8	106 ± 9	101 ± 11
	Δ(%)	60 (30)	46 (32)	14 (25)	6 (22)	41 (31)	42 (28)	44 (30)
	$P <$	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005
10	SF	200 ± 13	150 ± 10	50 ± 3	34 ± 3	74 ± 4	155 ± 23	141 ± 24
	PUSF	147 ± 31	113 ± 22	34 ± 10	21 ± 4	97 ± 22	110 ± 20	106 ± 19
	Δ(%)	53 (27)	37 (25)	16 (32)	13 (38)	−23 (31)	42 (28)	35 (25)
	$P <$	0.0005	0.0005	0.0005	0.0005	0.005	0.0005	0.005
Mean ± SD	SF	212 ± 79	154 ± 57	58 ± 22	31 ± 8	129 ± 58	150 ± 50	143 ± 50
	PUSF	156 ± 63	114 ± 48	42 ± 16	23 ± 6	94 ± 33	108 ± 32	103 ± 32
	Δ(%)	56 (26)	40 (26)	16 (28)	8 (26)	35 (27)	42 (28)	40 (28)
	$P <$	0.001	0.001	0.001	0.02	0.01	0.001	0.001

[a] See footnote *a*, Table 1.
N.S., not significant.

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apoB being estimated by both the TMU-Lowry procedure and EIA. By the former method, three patients (Nos. 1, 2, and 3) showed a significant increase in ratio on polyunsaturates; four others (Nos. 5, 6, 8, and 9) had a significant decrease, and the remainder showed no change. Those who had declines in ratios had relatively high ratios on saturated fats, and they had large reductions in LDL-cholesterol (range 30–53%). For the whole group, the mean ratio of cholesterol-to-apoB in LDL was not significantly lower on polyunsaturates. LDL-apoB was measured by EIA in only seven patients. Four patients had a significant decrease in cholesterol/apoB ratio in LDL, and three of those (Nos. 5, 6, 8) also showed a reduced ratio with the TMU-Lowry proce-

TABLE 3. Cholesterol/apoB ratios in LDL

Patient	Diet	LDL-C/B_{TMU}[a]	LDL-C/B_{EIA}[a]	% Plasma apoB
		Ratio ± SD (n = 6)[b]		
1	SF	1.38 ± 0.1		
	PUSF	1.78 ± 0.1		
	Δ(%)	+0.4 (26)		
	P <	0.0005		
2	SF	1.22 ± 0.1	1.22 ± 0.04	86 ± 1
	PUSF	1.28 ± 0.02	1.14 ± 0.01	90 ± 3
	Δ(%)	+0.06 (5)	−0.08 (7)	−4 (5)
	P <	0.05	0.0125	N.S.
3	SF	1.48 ± 0.2	1.45 ± 0.1	94 ± 1
	PUSF	1.59 ± 0.1	1.46 ± 0.1	95 ± 1
	Δ(%)	+0.11 (7)	+0.01	−1 (1)
	P <	0.025	N.S.	N.S.
4	SF	1.41 ± 0.1		
	PUSF	1.42 ± 0.1		
	Δ(%)	+0.01 (1)		
	P <	N.S.		
5	SF	1.79 ± 0.1	1.71 ± 0.1	97 ± 1
	PUSF	1.52 ± 0.1	1.42 ± 0.1	96 ± 1
	Δ(%)	−0.27 (15)	−0.19 (17)	1 (1)
	P <	0.0005	0.005	N.S.
6	SF	1.64 ± 0.1	1.74 ± 0.1	89 ± 2
	PUSF	−1.35 ± 0.1	1.33 ± 0.1	88 ± 2
	Δ(%)	0.19 (18)	0.41 (24)	1 (1)
	P <	0.0005	0.0025	N.S.
7	SF	1.84 ± 0.04	1.70 ± 0.2	96 ± 1
	PUSF	1.78 ± 0.2	1.77 ± 0.04	96 ± 0.4
	Δ(%)	0.06 (3)	−0.07 (4)	0 (0)
	P <	N.S.	N.S.	N.S.
8	SF	1.69 ± 0.1	1.80 ± 0.1	95 ± 1
	PUSF	1.45 ± 0.03	1.48 ± 0.1	96 ± 1
	Δ(%)	−0.24 (12)	−0.32 (18)	1 (1)
	P <	0.0025	0.0025	N.S.
9	SF	1.44 ± 0.1	1.47 ± 0.03	92 ± 2
	PUSF	1.35 ± 0.03	1.43 ± 0.1	93 ± 2
	Δ(%)	−0.09 (6)	0.04 (3)	−1 (1)
	P <	0.05	N.S.	N.S.
10	SF	1.30 ± 0.1		
	PUSF	1.30 ± 0.1		
	Δ(%)	0 (0)		
	P <	N.S.		
Mean ± SD	SF	1.52 ± 0.1	1.58 ± 0.2	93 ± 4
	PUSF	1.48 ± 0.2	1.43 ± 0.2	93 ± 3
	Δ(%)	0.04 (3)	0.15 (9)	0 (0)
	P <	N.S.	N.S.	N.S.

[a] LDL-cholesterol/LDL-apoB.
[b] See footnote *a*, Table 1.
N.S., not significant.

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dure. The percent of total apoB in LDL also is shown in Table 3. Between 86 and 97% of total apoB was determined to be in LDL; this percentage was the same for both periods.

Very low density lipoproteins (VLDL)

Lipid and protein concentrations of VLDL are presented in **Table 4.** The mean concentration of each component of VLDL was reduced during intake of polyunsaturated fat. In general, VLDL composition was not changed. In particular, ratios of cholesterol or triglyceride to apoB in VLDL were not statistically altered by polyunsaturates (**Table 5**). The cholesterol/apoB ratio was remarkably constant for the two periods. There were more fluctuations in triglyceride/apoB ratios, but no consistent trends could be discerned. About 7% of plasma total apoB was present in VLDL in both periods.

The pattern of apoproteins in VLDL is shown in **Table 6.** Polyunsaturates did not change proportions of apoB and TMU-soluble proteins. The latter were subjected to isoelectric focusing in seven patients, and no significant alterations were noted in apoC-II/apoC-III ratios, in the isoform distribution of apoE, or in $apoE_3$/ $apoE_2$ ratios.

High density lipoproteins (HDL)

Table 7 gives concentrations of lipids and proteins in HDL of eight patients. Polyunsaturates significantly reduced cholesterol, phospholipids, and proteins by 15%, 12%, and 21%, respectively. Total plasma apoA-I also was decreased significantly, but no changes were found in relative proportions of apoproteins A-I, A-II, D, and total C. For saturated fats the distribution of the apoproteins was: A-I, 50 ± 5%; A-II, 20 ± 3%; D, 3 ± 1%;

TABLE 4. Lipid and protein concentrations of VLDL

Patient	Diet	Total Cholesterol	Esterified Cholesterol	Unesterified Cholesterol	Triglyceride	Phospholipid	Total Protein	ApoB (TMU)
				mg/dl[a]				
1	SF	28	15	13	126	45	26	16
	PUSF	20	8	11	87	27	20	12
	Δ(%)	8 (29)	7 (47)	2 (15)	39 (31)	18 (40)	6 (23)	4 (25)
2	SF	101	58	41	295	122	72	37
	PUSF	45	23	21	172	65	46	18
	Δ(%)	56 (55)	35 (60)	20 (49)	123 (42)	57 (47)	26 (36)	19 (51)
3	SF	39	21	17	113	46	32	22
	PUSF	11	5	5	67	12	9	7
	Δ(%)	28 (72)	16 (76)	12 (71)	46 (41)	34 (74)	23 (72)	15 (68)
4	SF	35	18	16	107	53	28	16
	PUSF	29	16	12	107	52	28	14
	Δ(%)	6 (17)	2 (11)	4 (25)	0 (0)	1 (2)	0 (0)	2 (13)
5	SF	32	20	10	57	27	17	12
	PUSF	16	9	6	44	16	15	7
	Δ(%)	16 (50)	11 (55)	4 (40)	13 (23)	11 (41)	2 (12)	5 (42)
6	SF	69	40	27	171	66	47	34
	PUSF	47	26	19	148	49	36	21
	Δ(%)	22 (32)	14 (35)	8 (30)	23 (13)	17 (26)	11 (23)	13 (38)
7	SF	19	11	8	55	21	20	9
	PUSF	17	10	5	40	17	16	9
	Δ(%)	2 (11)	0 (0)	3 (38)	15 (27)	4 (19)	4 (20)	0 (0)
8	SF	19	10	7	54	15	13	6
	PUSF	8	5	3	20	8	6	3
	Δ(%)	11 (58)	5 (50)	4 (57)	34 (63)	7 (54)	7 (54)	3 (50)
9	SF	72	41	29	150	66	45	33
	PUSF	36	20	15	100	38	31	11
	Δ(%)	36 (50)	21 (51)	14 (48)	50 (33)	28 (42)	14 (31)	22 (6)
Mean ± SD	SF	46 ± 28	26 ± 16	19 ± 11	125 ± 76	51 ± 32	33 ± 19	21 ± 1
	PUSF	25 ± 14	14 ± 8	11 ± 7	87 ± 51	32 ± 20	23 ± 13	11 ± 6
	Δ(%)	21 (46)	12 (46)	8 (42)	38 (30)	19 (37)	10 (30)	10 (48)
	P <	0.01	0.005	0.01	0.005	0.005	0.025	0.025

[a] Values shown represent the mean of duplicate determinations on a single pool of six samples.

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TABLE 5. Ratios of cholesterol and triglyceride to apoB in VLDL

Patient	Diet	VLDL-C/B[a]	VLDL-TG/B[a]
1	SF	1.81	8.12
	PUSF	1.70	7.40
	Δ(%)	0.11 (6)	0.72 (9)
2	SF	2.71	7.93
	PUSF	2.44	9.31
	Δ(%)	0.27 (10)	+1.38 (17)
3	SF	1.75	5.06
	PUSF	1.70	10.10
	Δ(%)	0.05 (3)	+5.04 (100)
4	SF	2.20	6.73
	PUSF	2.07	7.60
	Δ(%)	0.13 (6)	+0.87 (13)
5	SF	2.14	4.60
	PUSF	2.20	6.00
	Δ(%)	+0.06 (3)	+1.40 (30)
6	SF	2.04	5.08
	PUSF	2.17	6.89
	Δ(%)	+0.13 (6)	+1.81 (36)
7	SF	2.14	6.29
	PUSF	2.00	4.60
	Δ(%)	0.14 (7)	1.69 (27)
8	SF	2.18	7.25
	PUSF	3.14	6.25
	Δ(%)	0.96 (44)	1.00 (14)
9	SF	2.18	4.53
	PUSF	3.14	8.71
	Δ(%)	+0.96 (44)	+4.18 (92)
Mean ± SD	SF	2.3 ± 0.5	6.2 ± 1
	PUSF	2.1 ± 0.3	7.4 ± 2
	Δ(%)	0.15 (7)	1.2 (2)
	P <	N.S.	N.S.

[a] These ratios were derived from the values shown in Table 4.

and total C's 28 ± 7%; for polyunsaturated fats percentages were 51 ± 5, 23 ± 5, 3 ± 1, and 24 ± 2, respectively.

Ratios of cholesterol to total protein (and to plasma A-I) in HDL are presented for seven patients in **Table 8;** no alterations were noted in either. Furthermore, A-I/A-II ratios in HDL did not change; the mean ratio for subjects on saturated fats was 2.6 ± 0.4, and for subjects on polyunsaturated fats, it was 2.4 ± 1.0.

DISCUSSION

The mechanisms of plasma cholesterol lowering by polyunsaturated fats are poorly understood. Changes in metabolism of cholesterol and/or bile acids have been reported (1–4), but not always confirmed (5–8). Alterations in apolipoprotein metabolism also have been reported (10, 12). In addition, a reduction in cholesterol-carrying capacity of lipoproteins has been postulated (9). To complicate the picture, the actions of polyunsaturates are not limited to LDL; reductions in VLDL and HDL also have been found (9–12, 14).

The present study was designed primarily to examine effects of polyunsaturated fats on concentrations of each constituent of the major lipoproteins. Of particular concern was whether reduction of LDL-cholesterol is accompanied by a simultaneous decrease in LDL-apoB. If LDL-cholesterol, but not LDL-apoB, is reduced, as suggested by Spritz and Mishkel (9), the usefulness of polyunsaturates for retardation of atherogenesis might be questioned. Recently, Sniderman et al. (27) reported that LDL-apoB is a better predictor of risk for coronary heart disease (CHD) than is LDL-cholesterol. For this reason, full chemical characterization of LDL was carried out in ten patients during exchange of polyunsaturated fats for saturated fats. Six samples from each period were subjected to complete analysis to enhance the reliability of the results. The data showed that the composition of LDL was basically unchanged with LDL lowering by polyunsaturates. Mean decreases in LDL-cholesterol and LDL-apoB averaged 26% and 29%, respectively. These results show unequivocally that polyunsaturated fats lower LDL-apoB, and theoretically therefore should retard atherogenesis.

Our findings generally are not in agreement with those of other investigators. For instance, Spritz and Mishkel (9) reported a reduction in mean cholesterol/protein ratio in LDL from 1.43 to 1.16 when polyunsaturated fats were substituted for saturated fats. These authors thus postulated that polyunsaturates lower LDL-cholesterol by expanding the space occupied by unsaturated cholesteryl esters and phospholipids; this change could reduce the quantity of cholesterol that can be incorporated into LDL. The results of other workers are consistent with this hypothesis (11–14).

How can the differences between our findings and those of others be explained? First, the composition of LDL was examined in much more detail in the present study than previously. Multiple samples were taken in each period to enhance reliability. Improved methods for estimating total protein and apoB in LDL also were used. In particular, protein was determined with a modification (20) of the method of Lowry et al. (19) that removes turbidity caused by lipids. Other improvements of the Lowry procedure, which were not previously known, also were employed (28, 29).

The procedure of Lowry et al. (19), however, was not used in all other studies for estimating apoB. For example, Vessby et al. (14) using EIA reported that polyunsaturated fats did not reduce total plasma apoB in patients with hyperlipoproteinemias of Types IIa and IIb, but a significant reduction was found in Type IV

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TABLE 6. VLDL apoproteins

Patient	Diet	B	TMU-Soluble	E_3	E_2	E_1	E_3/E_2	C-II	$C\text{-}III_0$	$C\text{-}III_1$	$C\text{-}III_2$	C-II/C-III
		% of total protein		*% of VLDL-E*			*ratio*	*% of VLDL-C*				*ratio*
2	SF	52	48	48	29	24	1.66	16	13	41	30	0.19
	PUSF	40	60	42	30	28	1.40	14	20	36	31	0.25
3	SF	70	30	58	27	15	2.15	32	13	32	23	0.47
	PUSF	77	23									
5	SF	71	29	41	34	24	1.21	13	12	40	36	0.15
	PUSF	50	50	46	31	23	1.48	11	14	39	36	0.12
6	SF	71	29	51	31	17	1.65	26	18	31	27	0.34
	PUSF	60	40	54	31	15	1.74	28	21	28	23	0.39
7	SF	44	56	45	36	18	1.25	16	8	48	28	0.19
	PUSF	56	44	53	29	18	2.94	19	10	43	29	0.23
8	SF	44	56	50	26	24	1.92	12	5	42	41	0.14
	PUSF	50	50	46	42	12	1.10	17	12	37	33	0.20
9	SF	74	26	48	29	24	1.66	14	19	37	29	0.16
	PUSF	37	63	38	31	31	1.22	18	19	34	29	0.22
Mean ± SD	SF	60 ± 12	40 ± 12	49 ± 5	30 ± 4	21 ± 4	1.64 ± 0.3	18 ± 8	13 ± 5	39 ± 6	31 ± 6	0.23 ± 0.1
	PUSF	53 ± 12	47 ± 12	47 ± 6	32 ± 5	21 ± 7	1.65 ± 0.7	18 ± 6	16 ± 5	36 ± 5	30 ± 4	0.26 ± 0.1
	$P <$	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

patients. In contrast, we consistently observed a decrease in total plasma apoB, as determined by EIA as well as by the Lowry procedure, in patients with mild hypercholesterolemia. In another study, Durrington et al. (11) measured serum total apoB by radioimmunoassay, and they observed a significant reduction on polyunsaturates. Although they found a mean reduction of the cholesterol/apoB ratio in LDL, the decrease was not statistically significant.

Although polyunsaturates lowered *both* cholesterol and apoB in LDL in our study, responses were somewhat variable from patient to patient. Four patients had a greater fall in LDL-cholesterol than in LDL-apoB, and hence the ratio decreased significantly. These patients had among the highest ratios of cholesterol-to-apoB in LDL when on saturated fats. Still, three other patients had a rise in ratio, and the decrease in the other four may have been due partly to the phenomenon of "regression to the mean". Nonetheless, it is possible that polyunsaturated fats may decrease the cholesterol/apoB ratio in LDL of some patients; the results of the current study indicate, however, that this mechanism cannot explain the major portion of LDL-cholesterol lowering.

Changes in the LDL-cholesterol/apoB ratios, noted in several of our patients, indicate that these ratios are not fixed. In fact, ratios ranged from 1.22 to 1.89 while the patients were on the saturated fat diet. The ratio commonly is high in familial hypercholesterolemia (30), as observed in our single patient with this disease. The reason is unknown, but delayed clearance of LDL (30) may allow time for greater accumulation of cholesterol esters through prolonged action of lecithin:cholesterol acyltransferase (LCAT). This mechanism is supported by the observation that inhibition of bile acid absorption decreases the cholesterol/apoB ratio in LDL (31, 32); bile acid diversion apparently accelerates clearance of LDL (33–35), and therefore should minimize acquisition of cholesteryl esters by this lipoprotein. Thus, the decrease in ratio in our four patients with initially high values may be due to enhanced clearance of LDL-apoB, as reported for polyunsaturated fats by Shepherd et al. (12). In our view, a reduction in cholesterol/apoB ratio in LDL is more likely explained by this mechanism than by the original hypothesis of Spritz and Mishkel (9).

Turning to VLDL, polyunsaturated fats reduced every constituent significantly without changing their relative proportions. Specifically, no changes were found in the ratios of either cholesterol or triglyceride to apoB, and apoproteins E, C-II, and C-III apparently decreased equally. Also, the distribution of apoE isoforms and of polymorphic forms of apoC-III were unaltered, as were C-II/C-III ratios. Finally, the distribution of apoB between VLDL and LDL was not changed by polyunsaturates.

Lastly, polyunsaturated fats affected HDL similarly to LDL and VLDL. HDL-cholesterol fell significantly by an average of 15%, phospholipids by 12%, and total protein by 21%. The magnitudes of these reductions were not statistically different one from the other. Also, total plasma apoA-I decreased by 8% in the eight patients studied. No alterations were noted in distributions of apoproteins A-I, A-II, D, or total C in HDL.

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TABLE 7. Lipid and protein concentrations in HDL

Patient	Diet	Total Cholesterol	Triglyceride	Phospholipid	Protein	Plasma
			mg/dl ± SD (n = 6)			
1	SF	57 ± 6	21 ± 4	88 ± 11	180 ± 12	
	PUSF	52 ± 4	23 ± 4	82 ± 16	136 ± 12	
	Δ(%)	5 (9)	+2 (10)	6 (7)	44 (24)	
	$P <$	N.S.	N.S.	N.S.	0.005	
2	SF	40 ± 4	40 ± 7	97 ± 8	157 ± 17	74 ± 8
	PUSF	39 ± 5	25 ± 2	85 ± 12	148 ± 20	80 ± 11
	Δ(%)	1 (3)	15 (38)	12 (12)	9 (6)	6 (8)
	$P <$	N.S.	0.005	0.05	N.S.	N.S.
3	SF	35 ± 2	24 ± 3	50 ± 9	115 ± 16	90 ± 11
	PUSF	26 ± 1	25 ± 2	40 ± 3	80 ± 5	62 ± 12
	Δ(%)	9 (4)	+1 (4)	10 (20)	35 (30)	28 (31)
	$P <$	0.005	N.S.	0.05	0.005	0.005
5	SF	70 ± 4	23 ± 2	122 ± 9	218 ± 10	123 ± 29
	PUSF	57 ± 4	25 ± 4	100 ± 12	183 ± 31	105 ± 10
	Δ(%)	13 (19)	+2 (9)	22 (18)	35 (16)	18 (5)
	$P <$	0.005	N.S.	0.01	0.025	0.025
6	SF	40 ± 4	27 ± 4	70 ± 6	121 ± 15	97 ± 6
	PUSF	34 ± 6	29 ± 6	59 ± 12	107 ± 18	96 ± 9
	Δ(%)	6 (15)	+2 (7)	11 (16)	14 (12)	1 (1)
	$P <$	0.1	N.S.	0.1	0.1	N.S.
7	SF	59 ± 4	25 ± 3	88 ± 6	180 ± 16	101 ± 9
	PUSF	46 ± 3	22 ± 2	57 ± 3	125 ± 12	66 ± 7
	Δ(%)	13 (22)	3 (12)	31 (35)	55 (31)	35 (35)
	$P <$	0.005	0.05	0.005	0.005	0.005
8	SF	67 ± 6	24 ± 3	118 ± 11	223 ± 21	96 ± 9
	PUSF	54 ± 3	24 ± 4	100 ± 9	159 ± 9	65 ± 4
	Δ(%)	13 (19)	0 (0)	18 (15)	64 (29)	31 (32)
	$P <$	0.005	N.S.	0.025	0.005	0.005
9	SF	40 ± 4	27 ± 1	84 ± 11	150 ± 17	84 ± 9
	PUSF	38 ± 6	29 ± 4	75 ± 10	123 ± 9	67 ± 5
	Δ(%)	2 (5)	+2 (7)	9 (11)	23 (15)	17 (20)
	$P <$	N.S.	N.S.	0.1	0.025	0.005
Mean ± SD	SF	51.0 ± 14	26.4 ± 6	89.6 ± 24	168.0 ± 40	95 ± 15
	PUSF	43.3 ± 11	25.3 ± 3	78.8 ± 21	133.1 ± 32	77 ± 17
	Δ(%)	8 (15)	1 (4)	11 (12)	35 (21)	18 (19)
	$P <$	0.005	N.S.	0.005	0.005	0.0125

Our findings on HDL-cholesterol support those of Shepherd et al. (10, 12). In two separate studies they found that polyunsaturates decreased HDL-cholesterol by 20% and 33%. Vessby et al. (14) likewise found a 16% decrease in HDL-cholesterol in patients with Type IIa hyperlipoproteinemia, but no change in Type IIb. No reduction in HDL-cholesterol was found by Spritz and Mishkel (9). These differences between studies cannot be explained readily, but they may be related to differences in methodology, types of patients under study, or kinds of diets employed. Shepherd et al. (10) also noted that apoA-I levels fell by 21% in four patients, and rate zonal ultracentrifugation indicated that the HDL_2/HDL_3 ratio fell by 28% in patients receiving high polyunsaturates (10). On the basis of the work of Cheung and Albers (36), a decrease in the HDL_2/HDL_3 ratio would have been expected to cause a corresponding decrease in the ratio of apoA-I to apoA-II; we found no change in the latter ratio which might suggest a discrepancy with the results of Shepherd et al. (10).

In summary, polyunsaturated fats were found to reduce cholesterol in each of the three major lipoprotein fractions. Indeed, we found no predominant decrease in cholesterol in one lipoprotein class compared to another. Furthermore, apoproteins in each fraction generally were reduced in proportion to the fall in cholesterol. Although we cannot rule out the possibility that polyunsaturated fats may preferentially lower cholesterol more than apoproteins in *some* patients, most patients appear to have uniform decreases in all lipoprotein constituents. This suggests that the major action of polyunsaturates is to alter either the production or clearance of the plasma

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TABLE 8. Ratios of cholesterol to protein and cholesterol to A-I in HDL

Patient	Diet	HDL-Chol/ Protein	HDL-Chol/ Plasma A-I	A-I/A-II
		ratio ± SD		
2	SF	0.26 ± 0.01	0.54 ± 0.01	2.4
	PUSF	0.26 ± 0.01	0.45 ± 0.02	2.5
	Δ(%)	0 (0)	0.05 (9)	
	$P <$	N.S.	N.S.	
3	SF	0.31 ± 0.04	0.40 ± 0.04	2.7
	PUSF	0.33 ± 0.03	0.44 ± 0.06	2.7
	Δ(%)	+0.02 (6)	+0.04 (10)	
	$P <$	N.S.	N.S.	
5	SF	0.32 ± 0.01	0.66 ± 0.1	2.8
	PUSF	0.32 ± 0.1	0.53 ± 0.1	2.8
	Δ(%)	0 (0)	0.13 (20)	
	$P <$	N.S.	N.S.	
6	SF	0.33 ± 0.02	0.42 ± 0.04	2.1
	PUSF	0.32 ± 0.01	0.36 ± 0.1	2.3
	Δ(%)	0.01 (3)	0.06 (14)	
	$P <$	N.S.	N.S.	
7	SF	0.33 ± 0.03	0.59 ± 0.1	3.3
	PUSF	0.37 ± 0.03	0.70 ± 0.1	2.5
	Δ(%)	+0.04 (12)	+0.11 (19)	
	$P <$	0.05	0.025	
8	SF	0.30 ± 0.004	0.70 ± 0.01	2.2
	PUSF	0.34 ± 0.01	0.83 ± 0.04	9.2
	Δ(%)	+0.04 (13)	+0.13 (19)	
	$P <$	0.0005	0.0005	
9	SF	0.27 ± 0.01	0.48 ± 0.02	2.4
	PUSF	0.31 ± 0.01	0.59 ± 0.04	2.6
	Δ(%)	+0.04 (15)	0.11 (23)	
	$P <$	0.0005	0.0005	
Mean ± SD	SF	0.30 ± 0.03	0.54 ± 0.1	2.6 ± 0.4
	PUSF	0.32 ± 0.03	0.56 ± 0.2	2.4 ± 1
	Δ(%)	+0.02 (7)	+0.02 (4)	0.2 (8)
	$P <$	N.S.	N.S.	N.S.

lipoproteins and not to change the relative proportions of their lipid and apoprotein components.

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