Fatty acid composition of human plasma lipoprotein fractions

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SUMMARY Serial samples of plasma were obtained from two fasting normal adult men who had consumed a diet with a slightly reduced fat content for 1 week previously. Three lipoprotein fractions were collected from each sample by ultracentrifugation at densities of 1.019, 1.063, and 1.21. The fatty acid distribution of cholesterol esters, triglycerides, and phospholipids of each lipoprotein fraction was determined.

The fatty acid compositions of corresponding lipid classes were very similar in the three lipoprotein fractions in each subject, although small but distinct differences did exist. Linoleic acid predominated in the cholesterol esters, oleic acid in the triglycerides, and palmitic acid was the major fatty acid in the phospholipids. The results suggest that in the postabsorptive state each lipid class originates from the same source in all three lipoproteins.

It is now well established that lipids in plasma exist almost entirely in the form of lipoproteins. Normal plasma contains several species of lipoproteins, differing in the nature of their protein moiety (1–3), and also in their relative content of the several classes of lipid (4–7). Relatively little detailed information is, however, available about the fatty acid composition of each individual lipoprotein. Lindgren and Nichols reported the fatty acid composition of each lipid class of each of three lipoprotein fractions, in eight samples of serum from seven adults (8). They found that the fatty acid compositions of the triglycerides and of the phospholipids were broadly similar among the three lipoproteins, but that the cholesterol ester composition of the very low density (density less than 1.006) lipoprotein differed from that of the others. Other reported investigations have dealt with the fatty acid composition of the several lipid classes of whole plasma or serum (8–11), of the phospholipids of serum and of serum lipoproteins (12) or of serum cholesterol esters (13). These recent studies have all employed gas–liquid chromatography (GLC) for fatty acid analyses. A study of the fatty acid composition of various lipid fractions of different lipoproteins, using the older alkali-isomerization method, has also been reported (14).

During the course of experiments on cholesterol ester metabolism in man, we have had occasion to collect serial samples of plasma for lipoprotein fractionation from two normal fasting men. Analysis of the fatty acid composition of each lipid class from each lipoprotein provided a comprehensive comparison of the fatty acid compositions of the different lipoproteins for each subject.

METHODS

The two normal subjects were a 39 year old Negro (subject WB) and an 18 year old white (subject EH) male. Both subjects ate their usual diets, except for a slightly reduced fat content, for 1 week preceding the study and during the period of blood collections. This was achieved by having the subject reduce his intake of certain foods, particularly whole milk and milk products, meats with high fat content, salad and cooking oil, rich desserts, and eggs. It was estimated that the fat content of the diet consumed during this period constituted approximately 30% of the total calories.

Blood samples of 50–100 ml were collected in syringes that had been moistened with a solution of heparin, and were immediately placed on ice. Samples were collected on each of three successive mornings after an overnight fast; additional fasting samples (particularly with EH) were collected during the 1st day. Blood samples were centrifuged at 2000 × *g* for 45 min in a refrigerated centrifuge, and the plasma was removed. Measured volumes of plasma (usually 10 ml) were extracted immediately (see text following). Larger measured volumes of plasma were then used for the serial separation of

lipoprotein classes as described by Havel, Eder, and Bragdon (4). Three lipoprotein fractions were collected from each sample by ultracentrifugation at densities (before centrifugation) of 1.019, 1.063, and 1.21. These centrifugations were carried out for 18, 22, and 28 hr, respectively, at 40,000 rpm and 15°, in the 40 rotor of a Spinco Model L ultracentrifuge.

Each plasma lipoprotein and whole plasma sample was extracted with 25 volumes of chloroform–methanol, 2:1 (v/v). The extraction mixture was split into two phases by addition of five volumes of 0.05% H_2SO_4, and the entire chloroform phase was collected and evaporated to dryness under a stream of nitrogen. The total lipid so obtained was chromatographed on 5-g columns of silicic acid (Unisil, 100–200 mesh, Clarkson Chemical Co., Williamsport, Pa.), using a modification of the method of Horning, Williams, and Horning (15). Serial elutions were carried out with 45 ml of 10% benzene in hexane; 90 ml of 21% benzene in hexane; 50 ml of benzene, then 100 ml of chloroform; and 100 ml of methanol. The four fractions contained, respectively: hydrocarbons, cholesterol esters, triglycerides plus free cholesterol plus free fatty acids (FFA), and phospholipids. The separations were checked repeatedly to verify that quantitative recovery of cholesterol esters, uncontaminated by triglycerides, was being achieved. This was done by repeatedly chromatographing known mixtures of H^3-labeled cholesterol esters and C^{14}-labeled triglycerides, and determining the efficiency of chromatographic separation by simultaneous assay of the column fractions for both isotopes. Labeled cholesteryl linoleate and tripalmitin were always included in the standard mixtures. Recovery of more than 98% of the H^3-cholesterol esters was always achieved in the second column fraction, and this fraction was contaminated less than 1% with triglyceride-C^{14}. A detailed discussion of the efficiency of the separation of a small amount of cholesterol ester in the presence of a large excess of triglyceride has been presented elsewhere (16). The column fractions were evaporated to dryness under nitrogen, and stored under nitrogen at −20° in a small measured volume or benzene or chloroform (for phospholipids).

Aliquots of the appropriate column fractions were analyzed for cholesterol, lipid phosphorus, and triglyceride, and were then referred back to the corresponding concentration in the original plasma. Cholesterol was determined by the method of Sperry and Webb (17); lipid phosphorus by the method of Fiske and Subbarow (18); and triglyceride by the method of Jover (19). The phospholipid concentration was calculated by multiplying the lipid phosphorus value by 25.

Free fatty acids were extracted from portions of each triglyceride fraction by extracting a hexane solution of it with 0.1 N NaOH in 50% ethanol (20). The extracted triglyceride solutions were then used for GLC. The FFA were recovered, when desired, by acidification of the ethanolic NaOH and extraction of the FFA with hexane.

The triglyceride (plus free cholesterol) samples from each of the lipoproteins from two plasma samples of EH were analyzed for the presence of either diglyceride or monoglyceride by thin-layer chromatography (TLC). After removal of free cholesterol as its digitonide complex, 75–100 μg of triglyceride samples were chromatographed as single spots on thin layers of Silica Gel G in both benzene–ethyl acetate, 5:1, and hexane–ethyl ether–acetic acid, 60:40:2. The plates were sprayed with Rhodamine 6G and viewed under ultraviolet light.

The distribution of phospholipids in each lipoprotein from one plasma sample of EH was determined in a fashion similar to that of Doizaki and Zieve (21). After TLC of portions of the phospholipid fraction from each lipoprotein in $CHCl_3$–CH_3OH–H_2O, 140:50:7, the separated phospholipids were made visible with Rhodamine 6G, and the corresponding areas of Silica Gel G scraped from the plates and analyzed directly for phosphorus. Blank areas provided appropriate controls.

Fatty acid methyl esters were prepared from each sample by heating small portions of lipid (less than 0.5 mg) under nitrogen at 60° overnight, in 2 ml of absolute methanol containing 2% H_2SO_4 and 0.2% benzene, using a screw cap vial with a lined cap. The fatty acid methyl esters were extracted as described elsewhere (16). Methanolysis of the phospholipid samples was carried out with 5% H_2SO_4 in methanol, and extra precautions were taken during the extraction of the methyl esters from these samples, as described in detail elsewhere (22).

The fatty acid methyl esters were analyzed by GLC, using a model 25 Barber-Colman chromatograph equipped with a radium-containing argon ionization detector. The samples from WB were chromatographed on a coiled column, 8 ft × 0.2 in., containing 12% ethylene glycol succinate polyester on 60–80 mesh Chromosorb W. The cholesterol ester fractions from the whole plasma samples of EH were also analyzed with this column. All the other samples from EH were chromatographed on a coiled column, 6 ft × 0.25 in., containing 12% stabilized diethylene glycol succinate polyester on 70–80 mesh Anakrom AB. Both columns were purchased as prepacked columns from Analabs, Inc. The column was at 170° and the detector cell at 220°, operated at 900 v. The argon pressure was 30 psi, and methyl stearate emerged from both columns after 9.5 min. The columns and instrument were calibrated with N.I.H. methyl ester standards A to F, kindly provided by the Metab-

olism Study Section of the National Institutes of Health. The identity of the fatty acid methyl esters in the biological samples was determined by comparison of the retention times with those of a variety of reference standard methyl esters provided by the Metabolism Study Section. All analyses were continued for longer than the retention time of methyl arachidonate. At least one analysis from each lipid class of each lipoprotein was carried out for three times the retention time of methyl arachidonate in case fatty acids with longer retention times were present; significant amounts of such fatty acids were never observed. Mass distributions were calculated from the GLC records by triangulation.

RESULTS

The concentrations of each lipid class in whole plasma and in each plasma lipoprotein fraction varied only slightly brom sample to sample, for each subject. Both subjects had low normal concentrations of serum lipids and lipoproteins. The concentrations of esterified cholesterol, triglyceride, and phospholipid in each lipoprotein fraction are listed in Tables 2 to 4. The distribution of lipids within each lipoprotein generally conformed to the distributions reported by Bragdon, Havel, and Boyle (5) for pooled sera. The percentage esterification of the total cholesterol differed significantly in each of the three lipoproteins, as previously noted by Bragdon et al. (5). For subject WB, the per cent of cholesterol present as ester in whole plasma and in each of the three lipoproteins (in order of increasing lipoprotein density) was 72.5 ± 0.5, 61.5 ± 1.4, 71.2 ± 0.4, and 79.1 ± 0.5, respectively. The corresponding values for subject EH were 69.5 ± 0.2, 58.3 ± 1.4, 70.7 ± 0.2, and 79.9 ± 0.2.

The fatty acid compositions of the several lipid classes of whole plasma are presented in Table 1. Each lipid class displayed a distinct fatty acid pattern, with linoleic acid (18:2) predominating in the cholesterol esters, oleic acid (18:1) in the triglycerides and FFA, and palmitic acid (16:0), to a lesser extent, in the plasma phospholipids. These distributions for cholesterol ester,

TABLE 1 FATTY ACID COMPOSITIONS OF THE LIPIDS OF WHOLE PLASMA

Fatty Acid*	Relative Retention Time†	% of Total Fatty Acids (Mean ± Standard Error‡)			
		Cholesterol Esters	Triglycerides	Phospholipids	FFA
Subject WB		(n = 12)‡	(n = 2)	(n = 2)	(n = 2)
14:0	0.29	0.3	0.9	0.2	1.2
unid.	0.39	0.1	0.2	0.2	0.4
unid.	0.45	0.2	0.1	0.6	—
16:0	0.54	11.8 ± 0.25	26.5 ± 0.35	34.9 ± 1.3	23.7 ± 0.70
16:1	0.65	1.8 ± 0.04	2.8 ± 0.33	0.5	2.1 ± 0.20
(17:0)	0.75	0.2	0.4	0.4	0.6
unid.	0.86	0.2	0.2	0.4	0.6
18:0	1.00	1.2 ± 0.05	3.0 ± 0.52	14.5 ± 0.12	16.9 ± 3.8
18:1	1.17	24.6 ± 0.33	43.6 ± 0.57	11.5 ± 0.59	45.5 ± 4.4
18:2	1.51	50.9 ± 0.43	14.7 ± 1.07	18.2 ± 0.76	7.0 ± 0.14
20:0	1.86	0.9	0.4	0.7	—
20:1	2.08	0.3	1.1	—	0.5
(20:3)	3.25	—	—	2.5 ± 0.31	—
20:4	3.75	7.7 ± 0.18	6.0 ± 0.35	15.5 ± 0.63	1.5 ± 0.50
Subject EH		(n = 8)	(n = 5)	(n = 4)	(n = 5)
14:0	0.28	0.6	0.9	0.2	1.6
unid.	0.38	0.2	0.3	0.2	0.3
unid.	0.43	0.1	0.1	0.1	0.2
16:0	0.53	11.2 ± 0.20	24.7 ± 1.3	33.1 ± 0.39	24.7 ± 0.34
16:1	0.64	2.3 ± 0.05	3.4 ± 0.27	0.4	4.4 ± 0.06
unid.	0.85	0.5	0.3	0.2	0.5
18:0	1.00	0.9	2.8 ± 0.26	14.0 ± 0.07	9.4 ± 0.49
18:1	1.18	21.4 ± 0.46	46.6 ± 1.3	12.2 ± 0.11	44.4 ± 1.2
18:2	1.54	56.0 ± 0.65	17.4 ± 0.82	22.5 ± 0.73	11.0 ± 0.48
20:0	1.88	1.0	0.6	0.7	0.4
20:1	2.17	—	0.9	—	1.2
unid.	2.77	—	—	0.3	0.7
(20:3)	3.51	—	—	3.6 ± 0.19	—
20:4	4.00	5.8 ± 0.42	1.9 ± 0.20	12.5 ± 0.16	1.1

* Number of carbon atoms: number of double bonds. The symbol "unid." means unidentified. Presumptive identifications are enclosed in parentheses.

† Relative to methyl stearate (18:0), taken as 1.00.

‡ Standard errors have not been calculated for fatty acids comprising less than 1.5% of the total. The symbol n means number of separate samples (of plasma or plasma lipoprotein); the symbol (—) means not detected.

TABLE 2 CHOLESTEROL ESTER FATTY ACID COMPOSITIONS OF PLASMA LIPOPROTEINS

	Subject WB			Subject EH		
	Plasma Lipoprotein of Density			Plasma Lipoprotein of Density		
	<1.019	1.019–1.063	1.063–1.21	<1.019	1.019–1.063	1.063–1.21
Concentration* (mg/100 ml).....	15.5 ± 1.6	75.4 ± 2.6	30.1 ± 1.2	12.4 ± 1.1	44.3 ± 1.8	20.0 ± 1.0
No. of samples.....	4	4	4	6	7	7
			% of total fatty acids†			
16:0	13.6 ± 0.99	12.0 ± 0.37	11.6 ± 0.22	9.4 ± 0.18	10.5 ± 0.17	11.2 ± 0.26
16:1	1.8 ± 0.09	1.6 ± 0.07	1.7 ± 0.05	2.2 ± 0.08	2.2 ± 0.05	2.4 ± 0.08
18:0	1.5 ± 0.20	1.1 ± 0.06	1.3 ± 0.05	1.2	0.9	0.9
18:1	26.9 ± 0.99	23.9 ± 0.24	23.6 ± 0.58	24.2 ± 0.24	20.8 ± 0.19	21.2 ± 0.37
18:2	48.2 ± 1.7	52.1 ± 0.35	52.5 ± 0.62	55.4 ± 0.47	57.7 ± 0.59	57.1 ± 0.70
20:0	0.7	0.5	0.6	0.8	0.8	0.5
20:4	5.2 ± 0.36	7.6 ± 0.23	7.0 ± 0.40	5.7 ± 0.31	6.1 ± 0.34	5.5 ± 0.31

* Concentration of esterified cholesterol.

† Mean ± standard error of the mean, where SE = $\sqrt{\Sigma d^2/n(n-1)}$. Minor components (less than 0.5% of the total fatty acids) have been omitted from Tables 2–4.

triglyceride, and phospholipid fatty acids are similar to those observed by other investigators (8, 9, 11). The composition of the FFA was fairly similar to that of the triglycerides, but with considerably more stearic acid (18:0) and significantly less of the polyunsaturated linoleic and arachidonic (20:4) acids. The two subjects showed fairly similar distributions for each lipid class; small but distinct differences did, however, exist between them.

The fatty acid compositions of the cholesterol esters, triglycerides, and phospholipids of each of the three lipoprotein fractions, for each subject, are shown in Tables 2, 3, and 4. Minor components (less than 0.5% of the total fatty acids) are not listed in these tables. It should be noted that the standard errors listed in the tables reflect both the biological variation between samples and the technical errors involved in the measurements. In order to reduce the technical contribution to the standard error, many of the samples were analyzed more than once; where this was done only the mean values for a sample were used as representing the composition of that sample. Each table lists the number of samples which were analyzed to determine each distribution. The very small size of the standard errors demonstrates the reproducibility of serial samples of plasma from the same individual under the particular conditions used in this study.

Inspection of Tables 2 to 4 reveals that for each subject the fatty acid patterns of each lipid class were similar in all three lipoproteins. Despite these similarities, small but statistically significant differences did exist in the fatty acid composition of each lipid class, among the three lipoproteins. Both subjects had more oleic acid in the cholesterol esters of the very low density (<1.019) lipoproteins. In both subjects the triglycerides of the very low density lipoproteins contained less arachidonic acid than did the triglycerides of the other lipoproteins. In addition, several small but significant differences were

TABLE 3 TRIGLYCERIDE (TG) FATTY ACID COMPOSITIONS OF PLASMA LIPOPROTEINS

	Subject WB			Subject EH		
	Plasma Lipoprotein of Density			Plasma Lipoprotein of Density		
	<1.019	1.019–1.063	1.063–1.21	<1.019	1.019–1.063	1.063–1.21
TG concentration (mg/100 ml).....	47.3*	10.6	5.1	41.1 ± 1.5	5.6 ± 0.2	2.8 ± 0.2
No. of samples.....	2	2	2	5	5	5
			% of total fatty acids			
14:0	0.9	0.7	0.8	1.0	0.7	0.8
16:0	28.8 ± 0.39	25.0 ± 0.61	24.2 ± 0.24	25.0 ± 1.4	21.1 ± 0.94	22.5 ± 1.2
16:1	3.0 ± 0.37	2.5 ± 0.34	2.6 ± 0.40	3.5 ± 0.33	3.0 ± 0.11	3.2 ± 0.26
18:0	2.8 ± 0.33	3.4 ± 0.57	4.1 ± 0.98	3.0 ± 0.31	3.2 ± 0.08	2.8 ± 0.21
18:1	44.6 ± 2.1	44.4 ± 1.1	40.4 ± 0.83	46.2 ± 1.5	48.8 ± 0.86	47.2 ± 0.98
18:2	14.1 ± 1.3	14.3 ± 0.54	13.6 ± 1.1	17.2 ± 0.82	17.1 ± 0.45	18.2 ± 0.75
20:0	—	1.1	0.9	0.7	0.9	0.7
20:1	1.0	—	—	1.2	1.0	0.7
20:4	3.5 ± 0.22	7.1 ± 0.69	12.0 ± 0.93	1.5 ± 0.11	3.6 ± 0.23	3.2 ± 0.38

* Standard errors are not given for the triglyceride concentrations of WB, because only one set of such measurements was made.

TABLE 4 PHOSPHOLIPID (PL) FATTY ACID COMPOSITION OF PLASMA LIPOPROTEINS

	Subject WB			Subject EH		
	Plasma Lipoprotein of Density			Plasma Lipoprotein of Density		
	<1.019	1.019–1.063	1.063–1.21	<1.019	1.019–1.063	1.063–1.21
PL concentration (mg/100 ml)	23.6 ± 6.1	63 ± 5.8	52	24.2 ± 3.7	56 ± 3.2	68 ± 2.9
No. of samples	2	2	1	5	5	5
	% of total fatty acids					
16:0	35.5 ± 1.3	37.2 ± 1.2	32.7	32.0 ± 0.29	34.8 ± 0.27	30.9 ± 0.12
16:1	0.5	0.5	0.4	0.5	0.5	0.4
18:0	14.6 ± 0.10	14.3 ± 0.15	14.4	14.8 ± 0.36	14.3 ± 0.16	14.5 ± 0.12
18:1	11.9 ± 0.74	11.2 ± 0.37	12.0	12.5 ± 0.15	11.9 ± 0.12	12.5 ± 0.15
18:2	17.7 ± 0.45	16.4 ± 0.51	19.1	22.8 ± 0.53	21.4 ± 0.68	22.1 ± 0.41
20:0	0.9	1.6	0.7	0.9	1.1	0.6
(20:3)	2.5 ± 0.20	2.5 ± 0.43	2.4	3.8 ± 0.28	3.9 ± 0.30	3.9 ± 0.18
20:4	15.2 ± 1.2	15.2 ± 0.38	17.2	12.0 ± 0.27	11.2 ± 0.32	14.2 ± 0.29

observed, in only one of the two subjects, in both the cholesterol ester and triglyceride distributions. Thus, for example, in subject WB the cholesterol esters of the very low density lipoprotein contained less arachidonic acid than did the cholesterol esters of the other lipoproteins, and the triglycerides of the high density lipoprotein contained much more arachidonic acid than did the triglycerides of the other lipoproteins.

Several small differences were also observed between the phospholipid patterns of the different lipoproteins of both subjects. These phospholipid differences included the presence of more palmitic and less oleic acid in the density 1.019 to 1.063 lipoprotein than in either of the other two lipoproteins, and more arachidonic acid in the high density lipoprotein than in either of the other lipoproteins.

In order to determine whether the triglyceride differences among the different lipoproteins might be due to different concentrations of di- or monoglycerides in the three lipoproteins, the triglyceride samples from two sets of lipoproteins of EH were analyzed by TLC. No di- or monoglyceride spots were observed in any of the samples. It was estimated that these compounds would have been detected had they been present at a level of 5% or more of the triglyceride concentration. It is hence unlikely that the triglyceride differences derived from different concentrations of partial glyceride in the three lipoproteins.

Analysis of the distribution of phospholipids in one set of lipoproteins of subject EH revealed the presence of distributions similar to those observed by Nelson and Freeman (12) and by Phillips (23). The percentage of total phospholipid present as lecithin and as sphingomyelin, respectively, in each lipoprotein, was: density <1.019, 74.8 and 16.4%; density 1.019 to 1.063, 67.8 and 25.2%; density 1.063 to 1.21, 78.5 and 13.2%. Small amounts of cephalin and of lysolecithin were also found in each lipoprotein. The presence of relatively more sphingomyelin in the 1.019 to 1.063 (beta) lipoprotein than in the other lipoproteins has been observed repeatedly (12, 23, 24). Nelson (25) has also reported the results of gas chromatographic analyses of the fatty acid patterns of purified serum phospholipids. Sphingomyelin contained relatively more palmitic and less oleic acid than did lecithin. It is thus probable that the phospholipid fatty acid pattern differences observed here were largely due to the different phospholipid distributions in the three lipoproteins.

DISCUSSION

The data presented here demonstrate that the fatty acid composition of each lipid class was similar in all three plasma lipoproteins in these two normal fasting men. Nevertheless small but statistically significant differences did exist between the three lipoproteins, for each lipid class. These differences were so small that most of them were established with confidence only because of the fact that analyses were conducted on multiple samples from the same subject, thus providing results with very small standard errors. Some of these small differences were observed in both subjects, but some were observed in only one or the other of the two subjects.

The fatty acid compositions of the lipids of whole plasma were similar to those observed by others (8, 9, 11). Cholesterol esters, triglycerides, and phospholipids each displayed a distinctive fatty acid pattern. The fairly close resemblance between the composition of the FFA and triglyceride fractions of whole plasma was not surprising in view of the available evidence, in both man (26) and the rabbit (27), that the chief source of circulating triglyceride fatty acids in the postabsorptive state is the circulating FFA. Fatty acid analyses were not carried out on the FFA associated with the three lipoprotein fractions. Such analyses would not have been

meaningful, since it has been shown that media of high ionic strength, such as those employed here for lipoprotein fractionation, displace FFA from serum albumin, resulting in a much greater percentage of FFA becoming associated with the lipoproteins (28). This artifactual displacement of FFA is undoubtedly one of the reasons for the high concentrations of FFA which have been reported to be associated with the high density lipoproteins (29).

One of the most interesting aspects of lipoprotein structure is the fact that many of the lipids undergo in vitro exchange between different lipoproteins. This in vitro exchange occurs rapidly with free cholesterol (30–32), and results in complete equilibration of free cholesterol between lipoproteins. A slower exchange has also been demonstrated with phosphatides (33, 34) and with triglycerides (27); the maximum extent of equilibration which could result from in vitro exchange has not been established for these two lipid classes. In contrast, virtually no exchange of cholesterol esters occurs in vitro (31, 32).

Despite this absence of cholesterol ester exchange, no greater differences were observed between lipoproteins in the fatty acid composition of the cholesterol esters than in the triglycerides or phospholipids. As discussed above, the phospholipid differences probably derived from the different distributions of the various phosphatides within the phospholipid fractions in the three lipoproteins. The cholesterol ester and triglyceride differences, however, undoubtedly reflected true, albeit small, differences in the fatty acid compositions of these single lipid classes.

Even more striking than the differences between the lipoproteins were the strong similarities in their fatty acid compositions for each lipid class. These similarities suggest that in the postabsorptive state each lipid class originates from the same source in all three lipoproteins.

The present finding of only very small differences between the cholesterol esters of the three lipoproteins contrasts with the results of Lindgren and Nichols (8) and Lindgren, Nichols, and Wills (35), who found a markedly different cholesterol ester composition in the very low density lipoprotein fraction, compared with the other lipoproteins. The serum samples which these investigators analyzed, however, were apparently not obtained in the postabsorptive state. Kayden, Karmen, and Dumont (36) have shown that the composition of the cholesterol esters in the very low density serum lipoprotein fraction changes during fat absorption, in accordance with the composition of the dietary fat. Similar changes were not observed in the cholesterol esters of the higher density lipoproteins. It is thus possible that the differences observed by Lindgren et al. primarily reflected the dietary status of their subjects. Evidence in support of this conclusion derives from the markedly different cholesterol ester composition seen in the very low density lipoprotein fraction of two samples obtained 1 month apart from the same subject (8). It should also be noted that the very low density lipoprotein fraction obtained by Lindgren et al. consisted of material which floated at density 1.006, rather than 1.019 as used here. In addition, the silicic acid column fractionation employed by these workers may not have quantitatively separated cholesterol esters from triglycerides, since they remarked (8) on the difficulties involved in the separation of cholesterol esters from triglycerides, particularly in the presence of relatively large amounts of triglyceride. The column separations employed here were rigorously standardized and repeatedly checked for quantitative validity.

Finally, it should be noted that the results presented here were obtained with only two male subjects, in the postabsorptive state and on a diet with a slightly reduced fat content. Both subjects had low normal serum lipoprotein levels. Although consistent results were obtained with both subjects, it is certainly possible that these results may only reflect the particular subjects and conditions employed, and may not be representative of a larger population. Further data on a more representative and larger population, under a variety of conditions, would be very desirable.

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Note Added in Proof:

Since acceptance of the above manuscript for publication, analyses of lipoprotein fatty acid patterns have also been carried out on a single sample of plasma obtained from a 49 year old white man with atherosclerotic heart disease, one year after myocardial infarction, and with mild diabetes mellitus requiring no specific therapy. This subject (subject JG) ate his usual diet for the period preceding blood collection. Blood was collected after an overnight fast, and the plasma was processed as described above. In contrast to subjects WB and EH, subject JG had high normal levels of plasma lipids, with approximate whole plasma concentrations of cholesterol of 235, of phospholipids of 200, and of total lipid of 700 mg/100 ml plasma.

Table 5 shows the fatty acid compositions of the cholesterol esters, triglycerides, and phospholipids from whole plasma, and from each of the three plasma lipoprotein fractions. The per cent distribution of the FFA from

TABLE 5 Fatty Acid Compositions of Plasma Lipoprotein Lipids in Subject JG

Fatty Acid	Whole Plasma	Plasma Lipoprotein of Density		
		<1.019	1.019–1.063	1.063–1.21
		% of total fatty acids		
Cholesterol esters				
14:0	1.2	0.8	1.4	1.0
16:0	14.2	12.8	14.7	15.7
16:1	4.4	4.0	4.5	4.4
18:0	1.3	1.4	1.4	2.0
18:1	24.5	25.9	23.7	24.8
18:2	45.4	45.5	45.0	43.1
20:0	1.4	2.0	2.0	1.9
20:4	7.7	7.6	7.6	7.6
Triglycerides				
14:0	2.5	2.6	1.8	2.3
16:0	33.2	31.1	27.2	28.7
16:1	4.2	4.4	3.8	3.2
18:0	4.3	4.6	5.4	5.4
18:1	41.7	42.5	39.9	40.0
18:2	9.9	11.0	11.2	12.0
20:0	0.4	0.5	1.2	1.3
20:1	1.1	1.1	2.0	2.5
20:4	1.5	1.2	6.1	3.9
Phospholipids				
14:0	0.5	0.5	0.4	0.3
16:0	34.5	33.6	35.8	33.1
16:1	1.0	0.9	0.9	1.1
18:0	18.4	18.6	18.2	17.7
18:1	11.8	12.7	11.7	11.3
18:2	14.9	15.7	14.7	15.0
20:0	0.6	1.0	0.6	1.2
(20:3)	5.0	3.8	5.2	5.0
20:4	12.7	12.0	11.2	14.0

whole plasma was: 14:0, 2.5; 16:0, 36.1; 16:1, 2.7; 18:0, 13.4; 18:1, 36.5; 18:2, 8.7; and 20:4, trace. The fatty acid patterns seen with subject JG were similar to those observed with subjects WB and EH. Strong similarities were seen in the fatty acid compositions of the three lipoproteins, for each lipid class. Small differences were observed between the three lipoprotein fractions, for each lipid class; these differences qualitatively resembled those previously seen with subjects WB and EH. The similarities between the three lipoproteins were much more striking, however, than the observed differences.

The similarity in the findings obtained with subject JG, compared to subjects WB and EH, suggests that these results may indeed be representative of a larger population, at least of American males.

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