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Effects of oil ingestion on lipoprotein fatty acids in man*

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SUMMARY

This report describes preliminary clinical investigations on the influence of safflower and olive oil ingestion on (*a*) the lipid composition of the major serum lipoprotein classes, (*b*) the fatty acid composition of the lipoprotein lipids, and (*c*) the fatty acid composition of the ultracentrifugal protein residue fraction. Significant glyceride increases occurred in the S_f 20-10^5 and the high-density lipoproteins (HDL). Fatty acid composition changes occurred in the glyceride moieties of the S_f 20-10^5, S_f 0-20, and HDL fractions. Marked alterations in the composition of the fatty acids associated with the ultracentrifugal protein residue fraction occurred following oil ingestion. The origin of the HDL lipid following oil ingestion is discussed in relation to the metabolism of the S_f 20-10^5 lipoproteins.

Various facets of the effects of acute fat ingestion on human serum lipids and lipoproteins have been investigated and reported (1–12). While these studies have helped to increase our understanding of certain specific aspects of the influence of fat ingestion on serum lipids, detailed investigations directed towards establishing an integrated picture of these phenomena have been lacking. To describe adequately the physical and chemical changes in serum lipids following fat ingestion, it is necessary, we feel, to evaluate the alterations occurring in (*a*) the concentrations of the major serum lipoprotein classes, (*b*) the concentrations of the lipid constituents (cholesterol esters, glycerides, phospholipids, and cholesterol) of these major lipoprotein classes, (*c*) the fatty acid compositions of these various esterified lipid constituents, and (*d*) the fatty acid composition of the albumin-bound free fatty acids (FFA). This report presents the results of preliminary studies in which most of the above variables were determined in humans following ingestion of vegetable oils (safflower and olive oil).

METHODS AND MATERIALS

Two fasting males (I. W. age 33 and B. W. age 41) were given 85 g of oil (safflower to I. W. and olive oil to B. W.) dispersed by a Waring blendor in 300 ml of skim milk containing a small amount of fruit flavoring. The oil preparation was well tolerated by both subjects. Blood was drawn (200 ml) immediately prior to oil ingestion and five hours afterwards. Directly after drawing of the blood, the serum was separated and subjected to preparative ultracentrifugal procedures (13) for the isolation of the following three major lipoprotein classes: the two low-density classes, S_f 20-10^5 and S_f 0-20, and the total class of high-density lipoproteins (HDL) d <1.20 and >1.063. All salt solutions used in the preparative ultracentrifugal procedures contained 0.05 g/liter of the disodium salt of ethylenediaminetetraacetic acid. Very low-density lipoprotein species, S_f 1,300-10^5, were separated from the serum of subject I. W. after oil ingestion, using low-speed ultracentrifugal techniques (14) with a swinging bucket rotor (15). In addition, the serum of subject I. W., before and after oil ingestion, was subjected to preparative and analytic ultracentrifugation and analyzed according to procedures previously described (16). A 3-ml aliquot of each of the sera was also directly adjusted to d = 1.21 by addition of 3 ml of a D_2O-$NaNO_3$ solution of d = 1.393, and subjected to preparative ultracentrifugation for separation of the lipoprotein-free

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TABLE 1. EFFECT OF SAFFLOWER OIL INGESTION ON SERUM LIPOPROTEIN CONCENTRATION

Sample	Lipoprotein Concentration (mg/100 ml)				
	S_f 20-400	S_f 8-20	S_f 0-8	$S_{f(1.20)}$ 4-8	$S_{f(1.20)}$ 0-4
Control	32	63	349	65	255
5 hr after oil	113	94	342	84	261

protein residue containing the bulk of the albumin-bound FFA. Errors in recovery in each of the above ultracentrifugal operations are estimated to be of the order of 5–10%.

Lipids were extracted from lipoprotein solutions by a modified method of Sperry et al. (17) and each total lipid extract was fractionated by silicic acid chromatography (18). The chromatographed lipids were quantified by infrared spectrophotometric techniques previously reported (19). The various lipid fractions were then transmethylated by the method of Stoffel et al. (20). FFA were extracted from protein solutions by the method of Dole et al. (21) and methylated by the procedure of Metcalfe et al. (22). The gas-liquid chromatographic system and chromatogram analysis techniques used for the fatty acid determinations have been described in detail elsewhere (23). The major fatty acids are tabulated according to the nomenclature proposed by Dole et al. (8). Minor and unidentified constituents are designated by: class A, methyl esters eluted before 16:0 (methyl palmitate); class B, methyl esters eluted between 16:0 and 18:0 (methyl stearate); class C, methyl esters eluted between 18:2 (methyl linoleate) and 20:4 (methyl arachidonate); and class D, methyl esters eluted after 20:4. These class designations refer to elution time interval positions of the minor and unidentified components relative to the more abundant identified esters using the diethylene glycol polysuccinate liquid phase. The error estimated for the over-all procedure of component fatty acid analysis is approximately 5% for the major components, which represent 10 to 90% of the reported fatty acid composition.

RESULTS

Although nonturbid before the experiment, the sera of both subjects were markedly lipemic following oil ingestion. The results of the analytic ultracentrifugation of the serum lipoproteins of subject I. W. before and five hours after ingestion of oil are tabulated in Table 1. These results show significant elevations in the concentrations of the S_f 20-400, S_f 8-20, and the $S_{f(1.20)}$ 4-8 lipoprotein classes after oil ingestion. Elevations in the levels of lipoprotein species of $S_f > 400$ were also noted but these could not be quantified by the techniques used.

Lipid analyses of the major lipoprotein classes before and five hours after oil ingestion are presented in Tables 2 and 3. In each subject the following observations are made: after oil ingestion (*a*) the S_f 20-10^5 lipid concentrations increased significantly, (*b*) the percentage lipid composition (in terms of cholesterol esters, glycerides, phospholipids, and cholesterol) of the S_f 20-10^5 remained relatively constant, (*c*) except for the glyceride concentration increase following safflower oil ingestion and the cholesterol concentration decrease following olive oil, the S_f 10-20 lipid concentrations showed no appreciable change, and (*d*) consistent increases occurred in HDL glyceride concentrations.

The fatty acid data for the esterified lipids of the S_f 1300-10^5 class following safflower oil ingestion are shown in Table 4. Control S_f1300-10^5 fatty acid compositions were not obtained due to the extremely low concentration of this lipoprotein class in the subject's serum prior to oil ingestion. Following safflower

TABLE 2. EFFECT OF SAFFLOWER OIL INGESTION ON LIPOPROTEIN LIPID CONCENTRATIONS

Lipoprotein Class	Sample	Lipid Concentration (mg/100 ml)			
		Cholesterol Esters	Glycerides	Cholesterol	Phospholipids
S_f 20-10^5	control	13.8	61.4	8.1	19.3
	5 hours after oil	28.8	134.6	14.6	38.4
S_f 0-20	control	170.4	19.8	36.3	86.9
	5 hours after oil	170.4	25.0	35.6	89.1
HDL	control	60.6	8.3	7.0	65.8
	5 hours after oil	63.1	15.6	7.9	79.8

TABLE 3. EFFECT OF OLIVE OIL INGESTION ON LIPOPROTEIN LIPID CONCENTRATIONS

Lipoprotein Class	Sample	Lipid Concentration (mg/100 ml)			
		Cholesterol Esters	Glycerides	Cholesterol	Phospholipids
S_f 20-10^5	control	7.0	32.9	7.7	11.9
	5 hours after oil	20.7	87.4	15.8	28.7
S_f 0-20	control	160.6	17.4	50.4	88.6
	5 hours after oil	159.3	17.9	40.1	87.9
HDL	control	76.1	8.6	13.3	101.6
	5 hours after oil	69.4	13.5	13.0	107.1

oil, however, this lipoprotein class increased markedly and Table 4 compares primarily the fatty acid composition of its glyceride moiety with that of the ingested oil. It is apparent that the fatty acid composition of the S_f 1300-10^5 glycerides following safflower oil ingestion is very similar to that of the oil fed. The fatty acid composition of the cholesterol esters is similar to that found in cholesterol esters of the other lipoprotein classes (see Table 5) following safflower oil ingestion. However, no arachidonic acid was detected in this ester of the S_f 1300-10^5 class, while in the remaining lipoprotein classes it made up from 5 to 7% of the fatty acids of the cholesterol esters.

The influence of safflower oil ingestion on the fatty acid compositions of the esterified lipids of the three major lipoprotein classes is shown in Tables 5, 6, and 7. By themselves these values indicate only shifts in fatty acid *composition*. To evaluate changes in the serum *concentration* of specific fatty acids it is necessary to combine these percentage data with the lipid concentration data of Table 2. In this section we will consider only changes in lipoprotein lipid composition. Composition changes appear in the following lipoprotein lipids five hours after safflower oil ingestion: (*a*) the cholesterol esters of the S_f 20-10^5 class, and (*b*) the glycerol esters of all of the broad lipoprotein classes investigated. In addition, consistent small changes are observed in the composition of the lipoprotein phospholipids after safflower oil ingestion. The major change in the above lipids is an increase in the percentage of linoleic acid.

The fatty acid composition data following olive oil ingestion for the cholesterol esters and glycerides (phospholipids were lost) of the three lipoprotein classes are presented in Tables 8 and 9. Changes were observed in the S_f 20-10^5 cholesterol esters and the glycerol esters of the S_f 20-10^5, S_f 0-20, and HDL. The major change was an increase in the percentage of oleic acid. The cholesterol ester changes were much smaller following olive oil than after safflower oil, but were larger

TABLE 4. FATTY ACID COMPOSITION OF S_f 1300-10^5 LIPIDS AFTER SAFFLOWER INGESTION AND OF THE SAFFLOWER OIL FED

Fatty Acids*	Fatty Acid Composition (per cent of total methyl esters)			
	Cholesterol Esters	Phospholipids	Glycerides	Fed Safflower Oil
Class A	4.0	2.6	0.4	0.1
16:0	11.2	23.6	8.5	5.5
16:1	3.3	3.6	0.3	0.1
Class B	1.3	1.3	N.D.†	0.1
18:0	3.2	17.9	2.9	2.3
18:1	20.8	12.7	13.2	11.4
18:2	53.2	27.7	72.7	79.9
Class C	3.2	3.6	1.7	0.7
20:4	N.D.	7.0	0.3	N.D.
Class D	N.D.	N.D.	N.D.	N.D.

* Defined in Methods section
† None detected.

TABLE 5. EFFECT OF SAFFLOWER OIL INGESTION ON FATTY ACIDS IN LIPOPROTEIN CHOLESTEROL ESTERS

Fatty Acids*	Fatty Acid Composition (per cent of total methyl esters)					
	S_f 20-10^5 Fraction		S_f 0-20 Fraction		HDL Fraction	
	Control	5 Hours After Oil	Control	5 Hours After Oil	Control	5 Hours After Oil
Class A	2.1	1.3	1.0	0.8	0.8	1.1
16:0	11.4	9.5	10.4	10.3	9.5	9.1
16:1	2.6	2.2	2.3	2.3	2.2	2.3
Class B	1.1	0.8	0.9	0.7	0.5	0.8
18:0	3.2	2.0	1.5	1.5	1.1	1.2
18:1	22.5	20.4	19.2	19.4	18.3	18.3
18:2	47.6	55.0	54.4	55.3	57.0	56.4
Class C	3.7	2.9	3.1	2.8	3.2	2.7
20:4	5.3	5.9	6.4	6.5	7.4	7.2
Class D	0.5	N.D.†	0.8	0.6	N.D.	1.0

* Defined in Methods section.
† None detected.

than the estimated error in the fatty acid analysis. The possible error introduced by biological variation was not determined, but judging from the constancy of the fatty acid compositions of the S_f 0-20 and HDL cholesterol esters in both experiments, it is reasonable to assume that it is quite small during the experimental period. This assumption is also made for the fatty acid composition data obtained for the other lipid components. The possibility of glyceride contamination of the cholesterol esters during silicic acid chromatography has been carefully avoided in this investigation.

The composition data for the ultracentrifugal protein residue containing the FFA are found in Table 10. Significant alterations in fatty acid composition were detected after oil ingestion. In the safflower oil experiment the percentage of linoleic acid increased, while in the olive oil experiment the percentage of oleic acid rose. Possible limitations on the interpretation of these FFA composition data are discussed below.

TABLE 6. EFFECT OF SAFFLOWER OIL INGESTION ON FATTY ACIDS IN LIPOPROTEIN GLYCERIDES

	Fatty Acid Composition (per cent of total methyl esters)					
	S_f 20-10^5 Fraction		S_f 0-20 Fraction		HDL Fraction	
Fatty Acids*	Control	5 Hours After Oil	Control	5 Hours After Oil	Control	5 Hours After Oil
Class A	2.4	1.4	1.8	1.9	1.9	1.7
16:0	22.7	16.1	22.0	18.6	22.3	18.2
16:1	3.5	2.2	2.7	2.7	3.1	2.6
Class B	1.0	0.9	0.9	1.1	1.1	1.1
18:0	4.2	3.5	5.3	4.6	6.2	4.8
18:1	39.8	24.4	40.4	30.3	37.7	26.9
18:2	21.0	47.1	20.6	37.2	21.2	40.1
Class C	4.3	3.2	3.4	2.2	2.9	2.3
20:4	1.0	1.2	1.8	1.4	1.6	1.3
Class D	0.2	N.D.	1.2	N.D.	2.0	1.1

* Defined in Methods section.
† None detected.

DISCUSSION

Ultracentrifugal lipoprotein concentration data show that following safflower oil ingestion there is an increase in $S_{f(1.20)}$ 4-8 lipoprotein species in addition to the expected elevation in S_f 20-400 lipoproteins. This concentration increase in $S_{f(1.20)}$ 4-8 species is associated with a significant elevation in the glyceride content of the total HDL lipid fraction. In humans, elevations of HDL phospholipid concentrations have been reported by Havel et al. (6), while, in dogs, Hillyard et al. (24) observed increases of all lipid constituents of d = 1.063–1.107 lipoproteins after a fat meal. Some elevation in HDL phospholipids after oil ingestion was also noted in the present investigation but it was much smaller than the glyceride elevation. The fact that cholesterol ester and phospholipid change so little compared with glycerides argues against an increase in level of the usual type of HDL molecules. Either new types of HDL molecules, high in glyceride content, must be appearing or glyceride is attaching to the usually occurring HDL species. Thus, the observed increase in HDL species may arise in part from the metabolism of very low density lipoprotein (S_f 1,300-10^5) which may yield some HDL-type molecules relatively high in glyceride content. Indirect evidence for this possibility may be drawn from the study of Scanu et al. (25) in which they observed the appearance of radioactivity in the HDL fraction following the administration of protein-labeled (I^{131}) chylomicrons to dogs. On acute ingestion of oil, there may occur a detectable "pile-up" of some HDL species that may be involved in the "clearance" of the very low-density lipoprotein species. Further evidence for some metabolic association between serum glycerides and HDL may be drawn from existing re-

TABLE 7. EFFECT OF SAFFLOWER OIL INGESTION ON FATTY ACIDS IN LIPOPROTEIN PHOSPHOLIPIDS

	Fatty Acid Composition (per cent of total methyl esters)					
	S_f 20-10^5 Fraction		S_f 0-20 Fraction		HDL Fraction	
Fatty Acids*	Control	5 Hours After Oil	Control	5 Hours After Oil	Control	5 Hours After Oil
Class A	1.9	1.3	1.4	1.3	1.7	1.5
16:0	22.6	21.6	23.1	23.3	23.0	21.7
16:1	1.1	0.9	0.9	0.8	1.0	0.9
Class B	1.1	0.8	1.0	0.8	0.9	1.0
18:0	15.6	15.7	14.8	14.9	14.8	14.9
18:1	12.9	12.1	12.0	11.2	12.0	12.0
18:2	25.9	28.2	25.9	28.4	24.6	27.1
Class C	5.3	5.6	5.7	5.3	5.8	5.4
20:4	8.9	9.8	9.2	8.8	10.1	10.4
Class D	4.8	7.6	6.0	5.3	6.2	5.2

* Defined in Methods section.

TABLE 8. EFFECT OF OLIVE OIL INGESTION ON FATTY ACIDS IN LIPOPROTEIN CHOLESTEROL ESTERS

	Fatty Acid Composition (per cent of total methyl esters)					
	S_f 20-10^5		S_f 0-20		HDL	
Fatty Acids*	Control	5 Hours After Oil	Control	5 Hours After Oil	Control	5 Hours After Oil
Class A	1.8	1.3	0.7	0.6	0.7	0.7
16:0	9.6	9.6	9.2	9.8	9.0	9.0
16:1	2.8	2.5	2.0	2.1	2.1	2.1
Class B	0.3	0.4	0.2	0.2	0.3	0.3
18:0	1.5	1.5	0.8	0.8	0.8	0.9
18:1	17.6	21.1	15.9	15.9	15.4	15.9
18:2	54.6	52.2	59.4	59.1	58.8	58.1
Class C	3.8	2.5	2.3	2.4	2.4	2.3
20:4	8.1	8.4	8.8	8.5	9.8	9.9
Class D	N.D.†	0.6	0.8	0.7	0.9	0.8

* Defined in Methods section.
† None detected.

TABLE 9. EFFECT OF OLIVE OIL INGESTION ON FATTY ACIDS IN LIPOPROTEIN GLYCERIDES

Fatty Acids*	Fatty Acid Composition (per cent of total methyl esters)					
	S_f 20-10^5		S_f 0-20		HDL	
	Control	5 Hours After Oil	Control	5 Hours After Oil	Control	5 Hours After Oil
Class A	2.4	1.6	0.9	0.6	1.8	1.6
16:0	25.5	22.3	22.3	20.2	22.9	22.5
16:1	4.5	3.4	3.6	2.8	3.7	3.6
Class B	0.5	0.3	1.2	1.0	1.5	0.9
18:0	3.1	2.8	5.9	4.1	6.3	4.1
18:1	36.8	46.5	38.2	42.8	35.6	41.6
18:2	22.1	19.9	20.7	20.3	20.0	19.8
Class C	3.7	2.1	3.3	3.1	4.2	2.9
20:4	1.2	1.4	3.1	4.2	2.2	1.7
Class D	0.3	N.D.†	0.9	0.8	2.0	1.4

* Defined in Methods section.
† None detected.

ports (26, 27) of similarities in the protein moieties of chylomicrons and HDL. Additional chemical information on the $S_{f(1.20)}$ 4-8 species, both under fasting and post-prandial conditions, is now being obtained for evaluation of their possible origin.

Some direct attachment of additional glyceride to normal HDL may also play a part in the observed HDL changes and studies are in progress to evaluate *in vitro* interaction of glycerides with HDL species. Evidence (28) is available for the attachment of HDL to artificial glyceride emulsions of very large particle size, but no data are available on the binding of small numbers of glyceride molecules by intact HDL molecules.

The glyceride increase in HDL species following oil ingestion suggests a possible metabolic relationship between this lipoprotein class and serum glycerides. Thus, certain species of HDL might actively participate in serum glyceride transport and metabolism, and, as a result, might play a regulatory role on serum levels of S_f 20-10^5 lipoproteins. That this may be the case is suggested from the lipoprotein data on human populations by deLalla et al. (29). In these data, significant inverse relationships are found between serum concentrations of S_f 20-400 species and lipoproteins of $d < 1.107$ and >1.063 (HDL_2). In particular, the data of deLalla show that females, who at all ages have mean serum HDL_2 levels approximately double those in males, exhibit significantly lower mean S_f 20-400 levels than males at all ages. Further, the recent studies by Fredrickson et al. (30) on Tangier disease, where there is apparently an almost complete absence of serum HDL, also support the above hypothesis that HDL species may play a regulatory role in determining S_f 20-10^5 concentrations. In this unique disease, a significant elevation of $d < 1.019$ lipoproteins and glycerides has been reported. Other disease states manifesting elevated S_f 20-10^5 levels (such as idiopathic hyperlipemia xanthoma tuberosum, and glycogen storage disease) have also been reported to be associated with lower levels of HDL species (31, 32).

TABLE 10. FATTY ACID COMPOSITION* OF THE ULTRACENTRIFUGAL PROTEIN RESIDUE FRACTION FOLLOWING OIL INGESTION

Fatty Acids†	Safflower Oil Ingestion		Olive Oil Ingestion	
	Control	5 Hours After Oil	Control	5 Hours After Oil
Class A	2.1	1.1	0.9	0.8
16:0	18.7	14.3	21.7	19.4
16:1	2.1	1.4	2.3	2.1
Class B	1.8	1.0	1.1	0.6
18:0	14.4	7.8	9.8	5.8
18:1	29.5	21.4	35.8	46.2
18:2	21.1	47.1	21.2	19.9
Class C	5.3	2.4	3.2	2.3
20:4	2.4	1.9	3.1	1.9
Class D	2.6	1.7	1.0	1.1

* Values shown represent concentrations of components as a percentage of the total fatty acid methyl esters detected.
† Defined in Methods section.

Bragdon et al. (9) studied the effects of oil ingestion on the fatty acids of the total mixture of lipids in the chylomicron class and reported an almost identical composition for this mixture and the ingested oil. In similar studies, Farquhar et al. (33) separated the total lipid mixture extracted from the chylomicron class into two fractions: (A) the nonphospholipid fraction, and (B) the phospholipid fraction. Their fatty acid composition analyses indicated that the fraction (A) resembled the fed fat and that the fraction (B) reflected the fed fat, but to a lesser extent than (A). In the present study, the S_f 1,300-10^5 glyceride moiety is very similar in fatty acid composition to the ingested oil, while the fatty acid compositions of its cholesterol esters and phospholipids resemble those observed in the S_f 20-10^5 following oil ingestion.

The present data show that oil ingestion influences the fatty acid composition of the S_f 20-10^5, S_f 0-20, HDL, and the ultracentrifugal protein residue fraction. Bragdon et al. (11) studied the effects of corn oil ingestion on the fatty acid compositions of total lipid extracts from various lipoprotein classes and reported changes in composition based on the ratios of linoleic to oleic acids. These authors found shifts in this ratio primarily in the chylomicrons, lipoproteins of $d < 1.019$, and the fraction of $d > 1.21$. Since total lipid extracts were used for the evaluation of fatty acid composition changes, only gross changes in lipoprotein fatty acid composition could be detected.

An approximate calculation has been made for the fatty acid composition of the glyceride increment in the

S_f 20-10^5 and the HDL classes for both oils studied. Considering only the major fatty acids, we find for the safflower oil study the following fatty acid composition for this glyceride increment for the S_f 20-10^5: 16:0, 11%; 18:1, 12%; and 18:2, 69%; for the HDL: 16:0, 14%; 18:1, 15%; and 18:2, 61%. For the olive oil study we find for the S_f 20-10^5: 16:0, 20%; 18:1, 51%; and 18:2, 18%; for the HDL: 16:0, 21%; 18:1, 53%; and 18:2, 21%. Although the values of these increments approach the fatty acid distributions of the fed oils (safflower: 16:0, 5%; 18:1, 11%; 18:2, 80%; and olive: 16:0, 15%; 18:1, 64%; 18:2, 16%), they are somewhat different. The fatty acid composition of the total S_f 20-10^5 glyceride increment however is very similar to that of the S_f 1,300-10^5 species. Since the fatty acid compositions of the glyceride increments in the major lipoprotein classes are not identical with the ingested oil, some dilution is probably taking place. This dilution may be due in part to exchange of the newly ingested glyceride with endogenous glycerides. Bragdon et al. (11), found no detectable *in vitro* exchange between chylomicrons and lipoprotein fractions from their measurements of linoleic to oleic acid ratios in the total lipid extracts from the various lipoprotein fractions. In the present report an indirect estimate of the extent of the exchange that may be occurring can be made by inspection of the fatty acid composition changes in the S_f 0-20 following olive oil ingestion. In this particular fraction there was little if any increase in glyceride content and changes in fatty acid composition would thus be expected to be due primarily to exchange.

In conflict with the observations of Dole et al. (8), Bragdon et al. (9) reported significant shifts in the linoleic to oleic acid ratio of the $d > 1.21$ fraction following corn oil ingestion. In studies on the effects of long-term ingestion of corn oil, Hirsch et al. (34) also observed FFA composition changes reflecting the composition of the fed oil. In the present report major alterations are found in the composition of this fraction following safflower and olive oil ingestion. The changes are significant and are in the direction of the fatty acid composition of the fed oil. These values, although indicative of composition changes in the albumin-bound FFA, may not represent the exact *in vivo* composition because of two technical factors. The first factor may be a minimal but unavoidable hydrolysis of some serum lipids during the ultracentrifugal procedures (21), and the second may be some differential displacement of fatty acids from albumin to lipoproteins under the influence of the relatively high ionic strengths used during the ultracentrifugal procedures (35). To minimize the possible influence of the first of these, the serum was separated without delay and immediately adjusted to $d = 1.21$ and centrifuged for 24 hours at 40,000 rpm at a temperature of 17°. To minimize the second factor, a D_2O-$NaNO_3$ solution was used for the density adjustment instead of an aqueous solution of higher salt content such as H_2O-NaBr. The D_2O-$NaNO_3$ solution, however, may have nonetheless produced some differential displacement of FFA from the albumin in spite of its lower salt content. Quantitative contributions of these two factors were not evaluated in the present study. Bragdon et al. (11) found no significant change in the linoleic to oleic acid ratio of the $d > 1.21$ fraction upon *in vitro* incubation of chylomicrons with fasting serum.

It is interesting to note that following safflower oil ingestion the fatty acid composition of the ultracentrifugal protein residue fraction is very similar to that of the S_f 20-10^5 glycerides. This suggests that the composition changes in the residue fraction may be due either to the binding of FFA released during *in vivo* hydrolysis of ingested oil, or to the appearance of some of the ingested glyceride in this fraction following oil ingestion.

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