

## Rapid Analysis of Fatty Acids in Plasma Lipids

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A rapid and convenient procedure for the quantitative determination of the fatty acid composition of plasma lipids is described. Human plasma was applied directly to the preadsorbent zones of thin-layer silica gel plates with added antioxidant, internal standards and carriers. The thin-layer chromatography (TLC) plates were partially developed with methanol followed by chloroform/methanol (1:1, v/v), and then they were fully developed in hexane/diethyl ether/acetic acid (80:20:1, v/v/v) to separate the major classes of lipids. Silica gel from regions containing the separated lipids was scraped into screw-capped tubes and treated with boron trifluoride-methanol prior to gas chromatography. The method of direct application to TLC plates gave yields and compositions of fatty acids very similar to the method of applying extracted plasma lipids. This relatively simple method is suitable for analyzing the fatty acids in plasma lipids from a 50 microliter finger-tip blood samples from an individual, and it may be useful in wide-scale screening of different individuals to estimate the relative amounts of ingested polyunsaturated fatty acids.

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Highly unsaturated 20-carbon fatty acids (HUFA) influence the rate of biosynthesis of prostaglandins (1) and leukotrienes which, in turn, influence the frequency and severity of some diseases and disorders (2). The tissue abundances of the 20-carbon fatty acids of the n-3 and n-6 type reflect the relative amounts of these two types of fatty acid in the diet because neither of these can be formed *de novo* in animals or humans. Recent progress in understanding the quantitative relationships between the tissue composition of the various HUFA and the dietary intakes of 18:2n-6 and 18:3n-3 (3) make it seem likely that estimates of the amounts of n-3 and n-6 acids ingested might be made from analyses of the HUFA in plasma phospholipids. In addition, such estimates may be independently checked by using data on 18:2n-6 and 18:3n-3 in plasma triglycerides (3). A careful study of the role of the n-3 and n-6 fatty acids in diet-disease relationships can alert people to the prudent limits of dietary changes related to these fatty acids. For example, a longitudinal study

involving the range of dietary patterns seen in the U.S. and Japan could provide much useful information (4). One concept to be tested is whether a rapid chemical analysis of the n-3/n-6 pattern in plasma lipids would be more useful than the labor-intensive and highly variable nutrient assessment tools (e.g., ref. 5) currently available.

While considering a possible large-scale survey to correlate the relative amounts of n-3 and n-6 nutrients that are ingested with the frequency or severity of disease, we examined the feasibility of devising a rapid assay of the 20- and 22-carbon highly unsaturated fatty acids (HUFA) to reflect an individual's intake of n-3 and n-6 nutrients and the potential for eicosanoid biosynthesis. We wanted a method for analyzing fatty acid composition in plasma lipids that was simple, quantitative, and did not cause structural changes or side reactions of the fatty acids. Traditional methods for the extraction and separation of lipids (e.g., refs. 6-8) and the conversion to methyl esters (e.g., refs. 9-11) require several steps, and many different methods have been used to extract and analyze lipids in plasma. These methods use a number of organic solvents that are removed by evaporation in the absence of oxygen (employing an inert gas, usually nitrogen). After separating lipid classes by silica gel thin-layer chromatography (TLC), the resolved lipid classes are often extracted from the silica gel with an organic solvent that is then evaporated prior to preparing methyl esters from the lipid fractions.

This paper demonstrates the feasibility of direct application of small samples of plasma to the preadsorbent layer of a silica gel TLC plate and of transmethylation of lipid fractions without extraction of the lipid from silica gel. The utility and ease of this rapid method for fatty acid composition studies makes it suited for analyzing many samples with minimal effort.

### MATERIALS AND METHODS

Boron trifluoride-methanol (12% BF<sub>3</sub> in methanol) was obtained from Supelco, Inc. (Bellefonte, PA). Freshly opened reagent was redistributed to glass ampoules and sealed to prevent progressive deterioration during storage at 4°C. All solvents were of reagent grade. Fatty acids and their methyl esters, triglycerides, and cholesteryl esters were obtained from NuChek Prep (Elysian, MN) and phospholipids from Sigma Chemical Co. (St. Louis, MO). Vacutainers were obtained from Becton Dickinson Vacutainer Systems (Rutherford, NJ) and microhematocrit tubes and Sure-Seal tuber sealant were from American Scientific Products (McGaw Park, IL). Silica gel G preactivated thin-layer (250 μm thick) chromatography plates with preadsorbent zone were from Analtech (catalog #, Newark, DE). The DB-225 capillary gas-liquid chromatography (GLC) column was from J&W Scientific (Folsom, CA).

A solution of internal standards (ISTD) with anti-

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Abbreviations: BHT, di-(*t*-butyl)-hydroxytoluene; CE, cholesteryl ester; FID, flameionization detector; GLC, gas-liquid chromatography; HUFA, highly unsaturated fatty acids; ISTD, internal standard mixture; PC, phosphatidylcholine; TG, triacylglycerol; TLC, thin-layer chromatography.

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oxidant and lipid carriers was prepared to contain the following in mg/mL: *tert*-butylhydroxytoluene (BHT), 2.00; dilauroyl phosphatidylcholine (PC) (12:0 PC), 3.700; diheptadecanoyl phosphatidylcholine (17:0 PC), 0.310; lauric acid (12:0), 3.34; heptadecenoic acid (17:1), 0.060; docosatrienoic acid (22:3), 0.022; tri-tridecanoylglycerol (13:0 TG), 2.810; triheptadecanoylglycerol (17:0 TG), 0.251; cholesteryl tridecanoate (13:0 CE), 3.84; cholesteryl heptadecanoate (17:0 CE), 0.270.

**Blood samples.** Blood was collected using heparinized microhematocrit tubes. The tubes were plugged by tube sealant and centrifuged at 3000 rpm for 15 min at 4°C. Volumes of plasma and red blood cells were estimated from the length of the tubes (35 mm = 25 mL). Plasma was removed with a Hamilton 50- $\mu$ L syringe (Reno, NV) and stored at -40°C prior to analysis. Alternately, blood was collected using Vacutainer Tubes which were then centrifuged at 3000 rpm (1500 g) for 30 min at 4°C. Plasma was removed and stored at -40°C. In each case, packed erythrocytes are also available for analysis of fatty acid composition (e.g., ref. 3).

**Extraction of lipids from plasma.** One-hundred  $\mu$ L of plasma was transferred to a Teflon-lined, screw-capped test tube, and 50  $\mu$ L of ISTD was added. Then 0.7 mL of water, 2.0 mL of methanol and 1.0 mL of chloroform (containing 100,  $\mu$ g BHT) were added in sequence with vigorous mixing after each addition; the lipids were then extracted and concentrated under a stream of nitrogen as noted above. The residue was dissolved in 100  $\mu$ L of chloroform methanol (2:1, v/v) for application to TLC plates.

**Thin-layer chromatography.** Fifty  $\mu$ L of ISTD was spotted on each 2-cm wide lane of the plate, and then either 75  $\mu$ L of plasma or extracts from 75,  $\mu$ L plasma were applied in alternate lanes onto the plate and allowed to air dry. The TLC plate was developed first in methanol to approximately 1.5 cm above the preadsorbent border, then air dried under the hood until the protein spot was no longer translucent. After evaporating the methanol, the plate was again developed to 1.5 cm with chloroform/methanol (1:1, v/v) to complete the extraction of lipids from the plasma protein, then air dried again under the hood for five to ten min to completely evaporate the solvent. The plate was then fully developed to 15 cm above the adsorbent band using hexane/diethyl ether/acetic acid (80:20:1, v/v/v). Paper tank liners were not used at any time during the TLC procedure. The resulting lipid bands were visualized by spraying the plate with rhodamine 6G (0.02% in 95% ethanol) and viewing the plate in UV light.  $R_f$  values were noted and individual bands were then scraped into screw-cap tubes.

**Transmethylation.** To each tube containing silica, 1.0 mL of methanol containing 50  $\mu$ g BHT was added. To the phospholipid, triglyceride and cholesteryl ester fractions, 1.41  $\mu$ g of 22:3 internal standard was added to follow HUF A recovery. All sample tubes were then treated with 1.0 mL of the BF<sub>3</sub> reagent, tightly capped and placed in a boiling water bath for 30 min. After heating, the tubes were withdrawn from the bath and cooled. Then, either methyl docosanoate (22:0 Me) or methyl tricosanoate (23:0 Me) was added in the quantity of 3.98  $\mu$ g as an internal reference for calculations

of the GLC results. The fatty acid methyl esters were extracted by adding 1.0 mL of hexane (with 50,  $\mu$ g BHT) and 3.0 mL of water, and the tubes were centrifuged to facilitate hexane separation. After centrifugation, the hexane extracts were removed and concentrated under a stream of nitrogen. The extracted derivatives from the cholesteryl ester (CE) band contained free cholesterol that was removed by passing the 1.0 mL layer through a 2 cm (1.0 mL) column of Florisil (or alumina) in a Pasteur pipette and rinsing with petroleum ether diethyl ether (9:1, v/v), collecting the second 2 mL for analysis.

**Gas-liquid chromatography (GLC).** GLC was done on a Hewlett Packard Model 5890A Chromatograph (Hewlett Packard, Norwalk, CT) fitted with a Model 7673 Automatic Split injection system and a flame ionization detector (FID) using a bonded, flexible, fused silica capillary column (30 m  $\times$  0.25 mm I.D.; DB-225 coating thickness 0.25  $\mu$ m). The oven temperature was programmed from 140°C to 180°C at a rate of 20°C/min and then from 180°C to 240°C at 3°C/min and held at the final temperature for 2 min. When analyzing the cholesteryl ester fractions, the final temperature (240°C) was held for 34 min to elute cholesterol and its derivatives that may have eluted from the small florisil (or alumina) columns. The hydrogen carrier flow rate was 1.3 mL/min, and the nitrogen make-up gas was 28 mL/min. The injection port and detector temperatures were 250°C and 260°C, respectively. The autosampler was programmed to rinse the 10  $\mu$ L syringe, once with sample, pumped four times to remove air bubbles, and then inject 5  $\mu$ L of sample with a split ratio of about 9:1. The syringe was then rinsed five times with solvent. The peak areas were integrated and reported using a Hewlett Packard Model 3393A computing integrator and stored on the hard disk of a Hewlett Packard Vectra AT. The integrator was programmed with the theoretical relative response factors (12) of fatty acids to the flame detector to convert raw data to corrected peak areas. The stored results were converted to spreadsheet format (Lotus 1-2-3) by using Hewlett Packard "FileServer" software (HP3393/0123).

## RESULTS AND DISCUSSION

In preliminary attempts to avoid interference by the silica gel from the scraped TLC bands, we first extracted the lipids from the silica gel prior to transmethylation (Table 1). However, when comparing duplicate sets, we noted that the set that was scraped and the lipids extracted with chloroform/methanol (1:2, v/v) prior to transmethylation gave compositions and yields similar to the other set that was transmethyated directly without extraction from silica gel (Table 1). Thus extraction from the silica gel was not necessary for the analysis, and subsequent analyses omitted that time-consuming step. We also found that adding benzene recommended by others (10) was not beneficial with these samples (results not shown).

To further simplify the entire analysis of the methyl esters from plasma lipids, we tested the feasibility of omitting the extraction of lipids from the plasma prior to TLC. First, 50  $\mu$ L of ISTD was applied to the origin

TABLE 1

Esterification of Samples from TLC<sup>a</sup>

Fatty acid	Extracted μmol/mL	On silica gel μmol/mL
16:0	2.10 (0.14)	2.12 (0.07)
16:1n-7	0.33 (0.08)	0.25 (0.01)
18:0	0.69 (0.02)	0.74 (0.02)
18:1n-9	2.27 (0.06)	2.31 (0.00)
18:2n-6	2.86 (0.16)	3.06 (0.16)
18:3n-6	0.05 (0.01)	0.05 (0.00)
18:3n-3	0.05 (0.01)	0.04 (0.00)
20:3n-6	0.12 (0.01)	0.14 (0.01)
20:4n-6	0.56 (0.03)	0.65 (0.05)
20:5n-3	0.08 (0.01)	0.08 (0.01)
22:6n-3	0.19 (0.03)	0.23 (0.04)

<sup>a</sup>Aliquots of extracts of plasma of total lipids were applied to the silica gel and then scraped off for analysis. Values for standard deviation are given in parenthesis.

of each TLC lane to provide antioxidant, carriers and internal standards. Then, lipid extracts from 75 μL of plasma were applied to the preadsorbent zone of the TLC plate, and in an adjacent zone a corresponding amount (75 μL) of plasma was applied directly (without

extracting first). The brief development to 1.5 cm with methanol was used to dehydrate the applied samples, and denature the lipoprotein complexes to facilitate the chromatography. A second brief development with methanol was occasionally used prior to a 1.5-cm development with chloroform/methanol (1:1, v/v) to further condition the plate. After these solvents had been evaporated from the plate, the plate was fully developed, and the lipids were located, scraped into tubes, and transmethylated and analyzed as described in the Materials and Methods section. Other labs have examined the method of direct application (13), but the small plasma samples were sufficient only for fluorescence measurements of the separated lipids. The present method employs larger amounts of plasma with methanol to speed the dehydration of the larger aqueous samples needed for fatty acid analyses.

The gas chromatographic conditions using capillary columns described in the Materials and Methods section provided excellent resolution of all methyl esters of interest within 20 min (Fig. 1). The low initial oven temperature was programmed to permit elution of the solvent and reagent impurities and the medium chain length carrier methyl esters (12:0 and 13:0) prior

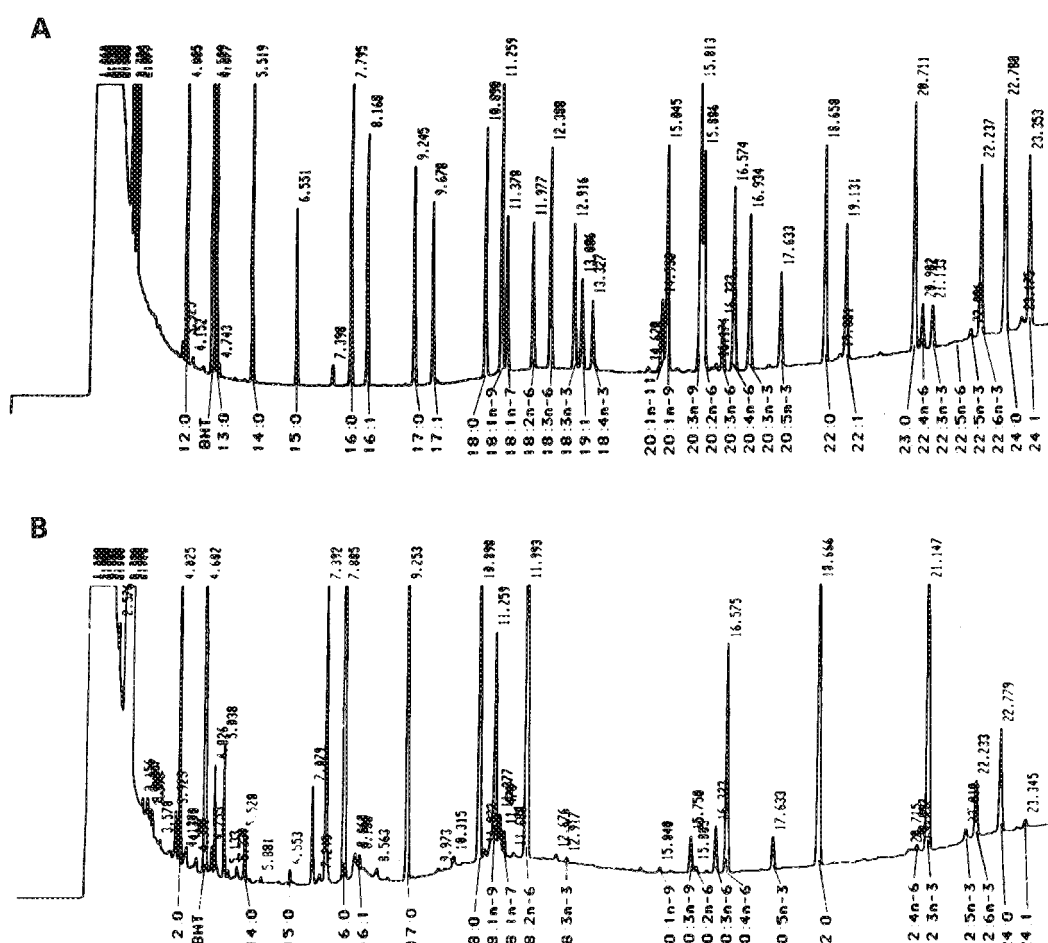


FIG. 1. Gas chromatographic separation of methyl esters and standards. Separation of components of (A) the standard reference calibration mixture; and (B) a representative sample of esters from the plasma phospholipid region which contains the accompanying standards and carrier materials.

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TABLE 2

Comparison Between Direct Application and Prior Extraction<sup>a</sup>

	Phospholipid		Non-esterified acids	
	Direct	Extracted	Direct	Extracted
14:0	0.4 (1.1)	0.6 (0.1)	2.9 (0.6)	5.0 (0.8)
16:0	33.7 (1.1)	31.7 (0.7)	43.9 (3.6)	43.0 (4.0)
16:1	1.3 (0.2)	1.0 (0.0)	3.4 (0.2)	2.8 (0.3)
18:0	13.7 (1.4)	14.2 (1.1)	9.2 (0.3)	10.3 (0.8)
18:1	11.2 (1.0)	11.1 (0.6)	25.7 (0.8)	24.2 (2.1)
18:2n-6	21.1 (2.0)	22.1 (1.9)	11.4 (0.4)	10.2 (0.8)
18:3n-6	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.4 (0.0)
18:3n-3	0.6 (0.1)	0.5 (0.1)	0.6 (0.1)	0.9 (0.1)
20:2/20:3	0.9 (0.1)	0.8 (0.3)	0.4 (0.0)	0.6 (0.1)
20:3n-6	2.7 (0.3)	3.0 (0.3)	0.3 (0.1)	0.4 (0.1)
20:4n-6	9.1 (1.0)	9.5 (0.8)	1.1 (0.1)	1.2 (0.1)
20:5n-3	1.1 (0.3)	1.2 (0.2)	0.4 (0.0)	0.7 (0.1)
22:4n-6	1.0 (0.4)	0.5 (0.2)	0.0 (0.0)	0.0 (0.0)
22:5n-3	1.1 (0.2)	0.8 (0.2)	0.5 (0.1)	0.8 (0.0)
22:6n-3	3.3 (0.4)	3.5 (0.3)	0.7 (0.1)	1.0 (0.0)
$\mu\text{moles/mL}$	4.3	4.6	0.7	0.8
	Triglycerides		Cholesteryl esters	
	Direct	Extracted	Direct	Extracted
14:0	2.9 (0.5)	3.0 (0.6)	1.1 (0.1)	2.2 (0.3)
16:0	34.2 (4.0)	35.4 (5.0)	14.7 (0.7)	17.2 (1.3)
16:1	4.3 (0.5)	4.5 (0.7)	2.4 (0.4)	2.9 (0.4)
18:0	4.8 (1.0)	5.1 (1.4)	1.9 (0.5)	1.2 (0.1)
18:1	33.9 (5.7)	33.1 (6.6)	17.3 (0.2)	15.8 (0.6)
18:2n-6	16.4 (3.1)	15.9 (3.6)	52.6 (0.9)	46.4 (2.5)
18:3n-6	0.0 (0.0)	0.8 (0.2)	1.3 (0.1)	1.3 (0.2)
18:3n-3	1.0 (0.4)	0.7 (0.1)	0.5 (0.1)	0.6 (0.0)
20:2/20:3	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
20:3n-6	1.2 (0.7)	0.3 (0.1)	1.2 (0.2)	4.8 (0.2)
20:4n-6	1.4 (0.2)	1.3 (0.2)	6.7 (0.1)	5.7 (0.2)
20:5n-3	0.6 (0.0)	0.9 (0.2)	1.0 (0.1)	1.1 (0.0)
22:4n-6	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
22:5n-3	0.5 (0.1)	0.5 (0.1)	0.0 (0.0)	0.0 (0.0)
22:6n-3	0.8 (0.1)	0.7 (0.1)	0.6 (0.0)	0.3 (0.1)
$\mu\text{moles/mL}$	2.8	2.7	3.0	3.8

<sup>a</sup>Fatty acid composition is expressed as the mean weight percent based on eight different samples with the standard error of the mean given in parenthesis.

to the esters of the major naturally occurring fatty acids. The capillary column resolved 18:1n-9 from 18:1n-7, 20:2n-6, and 22:6n-3 from the 24-carbon derivatives, 24:0 and 24:1. Resolution of all major fatty acids was achieved in 25 min, and the standard mixture of methyl esters shown in Figure 1A was frequently used to confirm analytical performance and to recalibrate retention time assignments made by the integrating calculator. When resolution of 20:2n-6 from 20:3n-9 was not required, excellent resolution of all the other esters could be obtained in less than 20 min. The use of a robotic autosampler permitted overnight analysis of more than 30 samples, and data stored on hard disc was converted to a standard spreadsheet format for publication.

A typical chromatogram of methyl esters from plasmas and phospholipids (Fig. 1B) illustrates the dominance of 16:0, 18:0, 18:1 and 18:2, relative to the small amounts of HUFA that are measured. The peaks for internal standards and antioxidant provide quality control data that ensure valid interpretations. If the methyl ester sample has been evaporated (concentrated) too

vigorously prior to analysis, the carrier esters, 12:0 and 13:0, will be decreased, and if autoxidation has occurred, the relative ratios of 22:3n-3 to 17:0 will be decreased. In control assays without added standard, the very small peaks in the region of 17:0 and 17:1 were negligible (<1%) relative to the amount of internal standard routinely added. To diminish the risk of spontaneous loss of solvent (hexane; bp 69°C) with concomitant autoxidation, 50  $\mu\text{L}$  of decane (bp 174°C) was added, permitting samples to be kept at 10°C for weeks without loss of any of the polyunsaturated esters.

Separation of the lipid classes using the TLC conditions as described gave good resolution of PL ( $R_f$ , 0.0-0.15), cholesterol ( $R_f$ , 0.20-0.26), non-esterified acids ( $R_f$ , 0.30-0.44), TG ( $R_f$ , 0.60-0.76), and cholesteryl esters ( $R_f$ , 0.85-0.92). The lipid recoveries of 17:1 (or 17:0) and 22:3n-3 in each lipid fraction (using either direct application or the extraction method) indicated that the standard HUFA was recovered intact without any autoxidative loss during the analytical procedures. In addition, the fatty acid compositions of the four

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TABLE 3

Results With Different Amounts of Plasma Applied to TLC<sup>a</sup>

$\mu\text{L}$ Applied	120	100	80	40	20	10	Mean
Phospholipids							
16:0	29.62	29.20	29.06	27.98	29.53	33.53	29.82 (1.74) <sup>b</sup>
16:1	2.02	1.29	1.72	1.78	1.20	1.40	1.57 (0.29)
18:0	14.65	15.02	15.12	15.98	14.88	15.68	15.22 (0.46)
18:1n-9	9.91	9.22	9.70	9.99	10.75	11.08	10.11 (0.63)
18:1n-7	1.36	1.35	1.58	1.99	1.48	1.58	1.56 (0.21)
18:2	26.96	26.98	26.48	23.55	24.16	21.98	25.02 (1.91)
18:3n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0.00)
18:3n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0.00)
20:3n-6	1.40	1.50	1.60	2.03	1.61	1.38	1.59 (0.22)
20:4n-6	8.30	8.68	8.70	8.62	8.48	7.16	8.32 (0.54)
20:5n-3	1.36	1.29	1.43	1.12	1.25	0.96	1.24 (0.16)
22:5n-3	0.64	0.97	0.55	0.88	1.02	0.51	0.76 (0.20)
22:6n-3	2.50	3.27	2.83	3.90	4.25	2.63	3.23 (0.65)
Total $\mu\text{g}/\mu\text{L}$	0.88	0.96	0.96	1.00	0.99	0.92	0.95 (0.04)
Triglycerides							
16:0	21.16	20.84	21.14	21.67	21.82	23.09	21.62 (0.74)
16:1	2.52	2.18	2.66	2.76	2.56	4.13	2.80 (0.62)
18:0	5.57	5.80	5.90	6.88	6.79	8.05	6.50 (0.85)
18:1n-9	32.19	32.70	32.07	31.81	31.51	28.87	31.53 (1.24)
18:1n-7	2.10	2.12	2.16	2.01	2.00	2.02	2.07 (0.06)
18:2	24.79	24.92	24.27	23.19	22.70	20.23	23.35 (1.61)
18:3n-6	1.13	0.99	1.09	1.10	1.02	1.38	1.12 (0.13)
18:3n-3	1.30	1.27	1.33	1.21	1.20	1.35	1.28 (0.06)
20:3n-6	0.37	0.38	0.42	0.49	0.49	0.38	0.42 (0.05)
20:4n-6	1.42	1.54	1.50	1.40	1.35	1.35	1.43 (0.07)
20:5n-3	0.72	0.77	0.77	0.70	0.67	0.75	0.73 (0.04)
22:5n-3	0.49	0.44	0.45	0.48	0.69	0.36	0.49 (0.10)
22:6n-3	1.94	1.88	1.96	1.98	2.40	2.55	2.12 (0.26)
Total $\mu\text{g}/\mu\text{L}$	0.37	0.40	0.41	0.46	0.45	0.50	0.43 (0.04)

<sup>a</sup>Fatty acid composition is expressed as weight percent.<sup>b</sup>Values are the mean with the standard deviation given in parentheses.

major lipid fractions were the same with both methods. The fatty acid compositions of the triglycerides and non-esterified fatty acids were primarily comprised of 16:0, 18:0, 18:1 and 18:2 (90%) with only 0.6 to 1% of the n-3 fatty acid, 18:3n-3, and very small amounts of the 20- and 22-carbon HUFA (Table 2). In contrast, the plasma phospholipids contained significant amounts of both n-6 HUFA (20:3n-6, 2.7%; 20:4n-6, 9.1%) and n-3 HUFA (20:5n-3, 1.1%; 22:5n-3, 1.1% 22:6n-3, 3.3%) and also higher levels of 18:2n-6 (21%). The absence of 18:2n-6 and 18:3n-3 from Table 2 illustrates how minor components may not be detected quantitatively with such small samples.

The fatty acid compositions for the major types of plasma lipids (Table 2) are similar to those reported in 1979 (14) for typical Americans, although the level of 18:2n-6 in the plasma TG of the young Chicagoans in this study (15.9%) is slightly higher than the 14.4% reported 10 years ago for semi-rural individuals (14). On the other hand, both values are higher than the 12.5% reported for Finnish individuals (15). There is good general agreement among the three studies that fatty acids in plasma phospholipids are about 45% saturated, about 35% C<sub>16</sub> and C<sub>18</sub> unsaturated, and

about 20% HUFA. Historical increases in dietary 18:2n-6 may underlie some differences found in the literature. For example, a recent report (16) showed that total plasma fatty acids contained 35% 18:2n-6 as compared to only 27% observed in 1979 (14). Unfortunately, the recent work failed to resolve the major lipid classes, making it difficult to discern whether the high level of 18:2n-6 was attributable to greater ingestion (reflected in triglyceride 18:2n-6) or to greater contents of cholesterol esters (with their typical high contents of 18:2n-6). The relatively low content of 16:1 and high contents of 18:2n-6 and 20:4n-6 in the 1987 report (16) suggests that individuals in that study ingested greater amounts of n-6 nutrients as compared to the Minnesota individuals in 1973 to 1975, who were described in the 1979 report (14).

Because wide range screening of plasma fatty acids from different individuals would be easiest with a single finger-tip sample (50  $\mu\text{L}$  of blood; 20  $\mu\text{L}$  of plasma), we tested the analytical procedure with small amounts of plasma. Table 3 compares the results with direct application to the TLC plate of different amounts of plasma from the same individual, and it indicates that 10  $\mu\text{L}$  samples may be unsatisfactory. Analyses were

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performed routinely on phospholipids and triglycerides because the very low concentration of non-esterified fatty acid in plasma requires large samples (>100  $\mu$ L) for reliable assay, and the cholesteryl esters require additional treatment to remove cholesterol that interferes in chromatography. Analytical results with aliquots of 5 to 150  $\mu$ L of plasma were very similar (results not shown), although the triglyceride fraction scraped from the TLC plate showed a systematic rise in the calculated amount of methyl stearate (18:0) with progressively smaller samples, indicating that a slight impurity co-chromatographing with methyl stearate on gas chromatography had been present in that TLC region. All other fatty acids appeared to be measured without such appreciable interference.

The present streamlined method permits collecting blood samples from 12 to 20 individuals in the morning, resolving the lipid fractions by TLC, converting them to methyl esters in the afternoon, and analyzing overnight the fatty acid composition of the phospholipids and triglycerides with the aid of the autosampler. We believe that the demonstrated success of this combination of methods provides clinical epidemiologists with a suitable tool for large-scale screening of plasma samples from selected populations.

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