Rapid Extraction of Triglycerides from Human Adipose Tissue with Petroleum Ether

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A rapid method has been developed for extracting neutral lipid from small samples of human adipose tissue, with a petroleum ether ("Skellysolve B") as the extracting solvent. It is coupled with a simple method of transesterification, to convert the purified triglycerides to fatty acid methyl esters. Virtually no phospholipid is extracted by the petroleum ether. Analysis of the extracted lipid by gas–liquid chromatography yielded fatty acid distributions comparable to those obtained by chloroform–methanol extraction.

Additional Keyphrases *fatty acids • gas–liquid chromatography • phospholipids • chloroform–methanol extraction • methyl esterification*

For routine determinations of the fatty acid composition of adipose tissue an extraction procedure more rapid than that of Folch et al. (*1*) is proposed, to decrease the time needed for analysis and to minimize errors caused by handling. With heptane, neutral lipid is quantitatively extracted and phospholipid partly extracted from plasma lipoprotein lyophilized on a starch matrix (*2*), and mouse adipose tissue has been extracted with petroleum ether to analyze fatty acid composition (*3*), but in the latter study no detailed comparison was made with the chloroform–methanol extraction procedure. This suggests that lipids may be quantitatively extracted from saline-suspended adipose tissue with Skellysolve B. This procedure is shown here to give extracts having fatty acid patterns comparable to those obtained with chloroform–methanol as the extracting solvent.

Material and Methods

Samples of human depot fat, removed at autopsy from nine individuals and stored in sodium chloride solution (9 g/liter) at −60°C, were used in our comparison of extraction procedures. Eight samples were taken from the abdomen, one from the retroperitoneal region.

All glassware used for the experiment was washed in detergent and rinsed with chloroform–methanol (2:1, by vol).

About 10 g of each sample, with saline, was ground in a glass mortar to a pasty mass. Four 0.2–1.1 g aliquots of each sample were removed with a transfer pipet to 7-ml test tubes. Two portions of each adipose tissue sample were extracted with chloroform–methanol by the method of Folch et al. (*1*). After washing with water, the lower phase was evaporated under nitrogen at 45°–50°C, and the sample (CM)[2] was resuspended in 2 ml of chloroform. Two portions of each adipose tissue sample were extracted with 2 ml of redistilled SSB (Skelly Oil Co., Kansas City, Mo. 64141). Each was mixed for 5 s and allowed to set for 15 min. Half of the CM and SSB extracts were transferred to 5-ml ampuls, which had previously been rinsed with chloroform–methanol (2:1, by vol). The solvent was evaporated under nitrogen, and 2 ml of sulfuric acid in methanol (2:98, by vol) was added. Each ampul was purged with nitrogen, sealed, and heated at 65°C overnight. The ampuls were allowed to cool and stored in the dark.

When an ampul was opened, 2 ml of SSB was added and mixed in with a vortex-type mixer for 5 s. After the phases had separated, about 0.2 ml of the upper layer was transferred to a "microflex" tube (Kontes Glass Co., Vineland, N. J. 08360), and 0.5–1.5 μg was injected into the gas–liquid chromatograph.

Gas–liquid chromatography. Gas–liquid chromatography of methyl esters was performed with a Model 402 chromatograph (Hewlett-Packard, Dayton, Ohio 45439) with two pre-tested 185-cm U-shaped columns packed with 80/100 mesh "Chromsorb W," with 14% ethylene glycol succinate as the liquid phase (Applied Science Laboratories, Inc., State College, Pa. 16801). The helium carrier-gas flow rate was 60 ml/min, and

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[2] Nonstandard abbreviations used: CM refers to the sample extracted into chloroform–methanol. SSB refers to the solvent Skelly Solve B. The spelling "Skelly Solve B" is used by our supplier. The alternative spelling (Concise Chemical and Technical Dictionary) is "Skellysolve B."

temperatures of flash heater, column, and detector were 250°, 180°, and 225°C, respectively. The composition of each sample was calculated by using virtual retention time multiplied by peak height (*4*). The precision of the column was checked with the NIH Fatty Acid Standard, KD (Applied Science Laboratories).

Results, as percentage composition, for 21 analyses of KD (four to six done per day) are given in Table 1. Labeled values for the KD standard and our computed values do not correspond because the method uses virtual retention time. However, since all samples were calculated in the same way, and the same chromatographic columns were used throughout this study, this source of variation is common to all our experimental data.

Thin-layer chromatography, triglyceride, and phosphorus assays. For this procedure, samples of 245 to 437 mg of adipose tissue were homogenized with a Teflon-glass homogenizer (Arthur H. Thomas, Philadelphia, Pa. 19105) after 5 ml of sodium chloride solution (9 g/liter) and either 6 ml of chloroform–methanol (2:1, by vol) or 2 ml of SSB were added to the sample. After 20 passes with the homogenizer pestle, each sample was transferred to a 125-ml Erlenmeyer flask with either 60 ml of chloroform–methanol or 75 ml of SSB, and either extracted for 2 h or stored overnight. The chloroform–methanol fractions and the SSB portions of the SSB–saline preparations were filtered through Büchner glass funnels with fritted discs and diluted to 100 ml with the corresponding solvent. An aliquot (25 ml) of each sample was transferred to 40-ml centrifuge tubes, and the samples in chloroform–methanol were washed according to the method of Folch et al. (*1*).

The CM samples were dried under nitrogen and the residues were dissolved in SSB, then dialyzed according to the method of Van Beers (*5*). The dialysis takes place across a rubber membrane and allows separation of phospholipids from neutral lipids since the former polymerize in SSB whereas the latter do not, and readily pass through the membrane. The material inside the rubber membrane was transferred to 15-ml screw-cap tubes for digestion and phosphorus assay (*6*). Other fractions from the original chloroform–methanol or SSB extraction were removed for thin-layer chromatography. Each of the samples was chromatographed together with a phosphatidyl choline standard (Applied Science Labs.) on prewashed-activated (110°C, 1 h) "Camag" silica gel without binder (Chemie-Erzeugnisse und Adsorptionstechnik AG, Switzerland), spread on plates. The solvent system used was chloroform–methanol–acetic acid–water (25:5:4:1, by vol). The developed plates were sprayed with the molybdate reagent of Dittmer and Lester (*7*).

Triglycerides from the undialyzed samples were analyzed by the procedure of Block and Jarrett (*8*), omitting steps required for removal of phospholipid and glucose because phospholipid was negligible or absent and glucose was removed by the procedure of Folch et al. (*1*) or excluded by extraction with SSB.

Results and Discussion

The amounts of the six major fatty acids found in the samples of adipose tissue were calculated. No significant difference in the respective relative amounts of myristic, palmitic, palmitoleic, stearic, and oleic acids was found between the nine samples (two replicates per sample) extracted with chloroform–methanol and the nine samples (two replicates per sample) extracted with SSB (Table 2). A difference ($P < .05$) was found for linoleic acid, which represents 3.65% of the mean value for the percentage of linoleic acid. The distribution of fatty acids in adipose tissue by other investigators is summarized by Insull and Bartsch (*9*).

Table 1. Comparison of Labeled and Analyzed Fatty Acid Distributions of NIH Standard KD

Fatty acid (methyl ester)	Labeled composition, %	Analyzed composition, % mean ± SD
14:0[a]	11.79	14.04 ± 0.92
16:0	23.65	25.93 ± 0.42
16:1	6.89	6.54 ± 0.27
18:0	13.11	13.08 ± 0.80
18:1	44.56	40.41 ± 0.93

[a] The numbers to the left of the colon refer to fatty acid chain length and the numbers to the right of the colon refer to number of unsaturated double bonds; e.g., 14:0 signifies 14 carbon atoms with no double bonds.

Table 2. Percentage Distribution of Fatty Acids in Extracts of Human Adipose Tissue[a]

Fatty acid	Chloroform–methanol extraction	Skellysolve B extraction	Standard error of differences
14:0	3.07 ± 1.03	3.00 ± 0.91	0.10
16:0	23.8 ± 2.53	23.8 ± 2.56	0.15
16:1	6.14 ± 1.87	6.19 ± 1.68	0.14
18:0	5.61 ± 1.78	5.49 ± 1.71	0.12
18:1	53.4 ± 2.03	53.5 ± 2.24	0.20
18:2	7.90 ± 2.95	8.19 ± 2.86[b]	0.12

[a] Each value represents the mean ±SD for nine adipose tissues (two replicates per sample).

[b] Significantly greater ($P < .05$) than the fatty acids from the chloroform–methanol extraction,

$$t = \frac{7.90 - 8.19}{.35/9} = 2.5$$

We tested the hypothesis that adipose tissue phospholipids are not extracted along with triglycerides when SSB is the extracting solvent. Thin-layer chromatography was performed on samples containing 8.40 to 17.3 mg of triglyceride. When the chromatographic plate was sprayed with molybdic acid to demonstrate phospholipid phosphorus, a spot appeared with the same R_f as phosphatidyl choline for all samples extracted with chloroform–methanol. (In one instance another spot, migrating close to the triglyceride spot, was barely visible.) In all instances, no phospholipid was discernible in the SSB fractions. The lower limit for detection of phospholipid is 0.36 μg of lecithin).

Means of values obtained for triglycerides extracted by the chloroform–methanol and SSB procedures were compared by analysis of variance; there was a significantly lower extraction by the SSB procedure (Table 3). Mean phospholipid-phosphorus (Table 3) by SSB extraction was 1.12 μg/g, about 0.05 the mean phospholipid-phosphorus obtained by the chloroform–methanol extraction. A test of significance was unnecessary, because of the marked difference in means and the obvious lack of overlapping of the two distributions. Despite the small losses of triglyceride and lack of phospholipid-phosphorus in SSB as compared to chloroform–methanol extractions of adipose tissue, there was no striking difference in fatty acid distribution.

We conclude that the method presented here is specifically applicable to small samples of adipose tissue removed with a Christakis syringe[3] (*10*), and that this new extraction method does not remove significant amounts of phospholipids from adipose tissue.

[3] This method has been used to obtain small adipose tissue samples for fatty acid determination in relation to dietary management of hyperlipidemia associated with coronary disease proneness. In the routine method for adipose tissue fatty acids, the adipose tissue–saline mixture in the test tube from the Christakis syringe is mixed with redistilled SSB, the two phases allowed to separate, and the supernatant phase transferred to glass ampuls for transesterification with H_2SO_4–methanol.

Table 3. Adipose Tissue Triglyceride and Phospholipid-Phosphorus Analyses

Extraction procedure	Triglyceride mg/g[a]	Phospholipid-P μg/g[a]	No. experiments
Chloroform–methanol	782[b]	22.7 ± 5.29	7
Skellysolve B	723	1.12 ± 0.74	7

[a] Grams of tissue wet weight.
[b] Significantly greater than Skelly Solve B, $F = 15.70$, see Table 2.

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