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Direct methylation from mouse plasma and from liver and brain homogenates

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Abstract

The analysis of fatty acid composition of plasma and tissue is important as a method for studying lipid nutrition. We investigated the possibility of direct methylation of fatty acids by BF_3-methanol from plasma and from liver and brain homogenates without lipid extraction. There were no ghost peaks in the chromatogram produced by the direct methylation method. The 18:0 percentages were significantly higher in the direct methylation method than in the lipid extraction method. There were not remarkable differences in fatty acid composition in the direct methylation and methylation after lyophilization methods. Furthermore, the recovery ratio of the internal standard in the direct methylation method was higher than that in the lipid extraction method. The difference of fatty acid composition with lipid extraction may be caused by the change of lipid class extraction. Therefore, the direct methylation method without lipid extraction is the most suitable for determining fatty acid composition in plasma and tissue.

Keywords: Fatty acid; Boron trifluoride; Plasma; Liver; Brain

The n-3 polyunsaturated fatty acids (n-3PUFAs),[1] such as 20:5n-3 and 22:6n-3, play important roles in human health and development [1,2]. Therefore, there are many studies of the effects of dietary fat which contain n-3 PUFAs, and these studies are important for the prevention of lifestyle-related diseases [3,4]. Moreover, the analysis of fatty acid composition in plasma and tissue is significant for lipid nutrition research [5].

Fatty acid composition is usually analyzed by gas–liquid chromatography (GLC). Numerous methods for derivatizing these compounds for GLC have been developed, e.g., boron trifluoride (BF_3)-methanol [6,7], HCl-methanol [8,9], and KOH-methanol [10] methods. However, the presence of water inhibits the generation of fatty acid methyl esters from lipids [10,11]. Therefore, the analysis of fatty acid composition from fresh body samples is generally derived after lipid extraction or lyophilization [12]. However, these operations require much time and effort before fatty acid derivatization.

The BF_3-methanol derivatization method has been adopted as an officially published method, and its operation is simple [13–15]. The purpose of this study was to improve methods for making BF_3-methanol rapidly. To this end, we clarified that fatty acids composed of plasma and of liver and brain homogenates can be analyzed without lipid extraction.

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[1] *Abbreviations used:* n-3PUFAs, n-3 polyunsaturated fatty acids; FFA, free fatty acid; PL, phospholipid; TG, triacylglycerol; ME, methyl ester; DMA, dimethylacetal.

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Materials and methods

Reagents

Free fatty acid (FFA), phosholipid (PL), triacylglycerol (TG), and the sterol ester standards *cis*-9,11,13,15,17,19-docosahexaenoic acid (22:6n-3), phosphatidylcholine, dioleoy (18:1n-9), tripalmitoylglycerol (16:0), and cholesteryl myristate (14:0) (Sigma 99% pure) were used. The fatty acid ester standards heneicosanoic acid methyl ester (21:0ME) and tricosanoic acid methyl ester (23:0ME) (Sigma 99% pure) were used. The 14% BF_3-methanol solvent was purchased from Sigma–Aldrich.

Animals

Male mice of Crj:CD-1(ICR) strain were obtained from Charles River Japan (Atsugi, Kanagawa, Japan). These animals were fed a 5% lard diet for 3 months prior to the study.

Preparation of plasma samples and liver and brain homogenates

The mice were fasted for 24 h before being anesthetized with diethyl ether. Blood was collected from the inferior vena cava with a heparinized syringe and put into ice-cold tubes. The plasma was separated by centrifugation at 900*g* for 20 min at 4 °C. After the blood was collected, the liver and brain were removed and homogenized with 1/15 mol/L phosphate-buffered saline (pH 7.4) and with 0.32 M sucrose, respectively, using a Teflon-glass homogenizer. Plasma samples and liver and brain homogenates were stored at −30 °C until required for lipid analyses. The lipid characteristics of plasma and of liver and brain homogenates are presented in Table 1.

Lipid extraction and fatty acid derivatization

The total lipids were extracted from the plasma samples and from the liver and brain homogenates by the method of Folch et al. [16]. One milligram of each lipid standard sample was taken in a scrum cap glass tube containing 1 mg of 23:0 ME as an internal standard. One milliliter of 0.5 mol/ml NaOH-methanol was added to each sample and the sample was boiled at 100 °C. For fresh samples, 150 µl plasma and 100 µl liver and brain homogenates were taken in a scrum cap glass tube containing 0.1 mg internal standard. One milliliter of 0.5 mol/ml NaOH-methanol was then added, and the sample was boiled at 100 °C for 15 min. The sample was cooled to room temperature, 2 ml of BF_3-methanol was added, and the sample was boiled at 100 °C for 20 s. The sample was again cooled to room temperature, 1 ml of isooctane was added, and the mixture was shaken. In the measurement of recovery ratio of the internal standard, 1 ml isooctane containing 0.1 mg 21:0ME was added. Five milliliters of saturated NaCl solution was then added, and the mixture was agitated thoroughly. When the isooctane layer separated from the aqueous lower phase, the isooctane layer was transferred to a glass vial. After isooctane was evaporated, adequate hexane was added, and the sample was injected into the GLC system.

Table 1
Lipid content of plasma sample and liver and brain homogenates

	Total cholesterol	Triacylglycerol	Phospholipids
Plasma (mg/dl)	130.6	67.5	201.8
Liver homogenate (mg/g)	6.6	70.1	34.8
Brain homogenate (mg/g)	37.9	—	22.4

Fatty acid analyses

The fatty acids were measured using a gas chromatograph (GC-18A, Shimadzu, Kyoto, Japan) equipped with a fused silica capillary column (Supelcowax 10; 30 m × 0.25 mm i.d.; Supelco, Bellefonte, USA) and operated with a Class 10 workstation (Shimadzu). The carrier gas was helium (flow 1 ml/min) with a split injection of 40:1. The temperature profiles were as follows: initial temperature, 175 °C; heating rate, 1 °C/min; final temperature, 220 °C (15 min isolation); injector temperature, 250 °C; and detector temperature, 270 °C. The fatty acids were identified by comparing the retention times with those of standard purified fatty acids.

Statistical analyses

All results are expressed as means ± SD. The statistical significance of differences in fatty acid percentages obtained by these methods was determined by the Statistica statistical program package (StatSoft, OK, USA). The methods were compared using the Spjotvoll/Stoline test or *t* test at $p < 0.05$.

Results

The time course of methyl ester production from sterol ester is shown in Fig. 1. The sterol ester was converted to methyl ester in 15 min. TG, PL, and FFA were satisfactorily transferred to the methyl ester with NaOH-methanol or BF_3-methanol at 100 °C within 20 s.

The predominant fatty acid compositions and content of the plasma from mice are presented in Table 2. The 18:0 percentages were significantly higher in the methods