STUDIES ON LIPID EXTRACTION BY THREE DIFFERENT METHODS IN THE SERUM AND WHOLE BLOOD

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Abstract

Many studies concerning health effects of extremely toxic compounds such as PCDDs, PCDFs and dioxin-like PCBs have so far been reported using their concentrations on lipid weight basis. We, however, have pointed out this type of concentration is not good for their toxic and/or risk evaluations due to the difficulties of reproducible and quantitative lipid extractions from the blood and breast milk, and maybe, other human samples. In this study, we examined the quantitative lipid extraction from the serum and whole blood with three methods, that is, accelerated solvent extraction (ASE) with acetone/n-hexane, and solvent systems of acetone/n-hexane and ethanol/n-hexane. Concentrations of total lipids extracted from the serum by ethanol/n-hexane were the highest, 452±111 mg/dl, then acetone/n-hexane, 359±54.4 mg/dl and the lowest ASE, 312±63.9 mg/dl. Concentrations of total lipids extracted from the whole blood were as follows: ethanol/n-hexane; 410±115 mg/dl, acetone/n-hexane; 317±47.5 mg/dl and ASE; 299±54.8 mg/dl. Therefore, in these three methods, the solvent system of ethanol/n-hexane was considered the best method. Furthermore, we are now under investigation concerning much quantitative lipid extraction from the serum.

Introduction

Many studies concerning adverse health effects of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (DL-PCBs) and PCBs so far have been reported in particular on growth retardation of fetus and infants [1, 2], birth weight [3~7], neuro-developmental dysfunction [1, 2, 8~10], thyroid deficiency[1, 2, 11, 12], immune deficiency[1, 2, 13~16], reproduction and/or fertility [1, 2], diabetes [17~19]and cancer [1, 2]. In these studies, however, in order to evaluate their toxic effects, their concentrations on lipid weight basis have been employed and we have pointed out that this type of concentration is not good for their toxic and/or risk evaluations, due to the difficulties of reproducible and quantitative lipid extractions from biological samples such as the blood and breast milk [20, 21] and also, of course, from other human tissues/organs. This problem is very important because in order to investigate health effects and risks of toxic compounds for human, we usually use this kind of concentration, as mentioned above. Therefore, we are now inspecting the quantities and qualities of lipid extraction from the blood and serum by using several extraction methods.

In this study, we examined and compared the quantities of lipid extraction with three methods, namely, accelerated solvent extraction (ASE) with the solvent of acetone/n-hexane, and solvent systems of acetone/n-hexane and ethanol/n-hexane which were basically the same as the methods of Todaka et al. [22], Schecter and Ryan [23] and Patterson et al. [24], respectively.

Materials and Methods

Peripheral blood was taken by venipuncture from three young persons aged 22 years (two males and one female) with heparin for whole blood samples and without heparin for serum samples. Three extraction methods of lipid from whole blood (10g) and serum (5g) samples are simply summarized as follows;

Method A (accelerated solvent extraction with acetone/n-hexane): Each sample was mixed with 4g Isolute (International Sorbent Technology Ltd., Hengoed, Mid Glamorgen, UK). After the mixed sample was loaded into the extraction cell of the accelerated solvent extractor (ASE-200, Dionex, Sunnyvale, CA), the following programmed parameters were used for the extraction of lipid: a pressure of 2000 psi and a temperature of 150^{o}C, with a static time of 10 minutes, a flushing volume of 50ml, 90 seconds purging, a 60% flushing volume for two cycles, and acetone/n-hexane (3:7 v/v) as the extraction solvent. The extract was evaporated near to dryness and the amount of lipid was determined gravimetrically.

Method B (solvent system of acetone/n-hexane): Twenty ml acetone/n-hexane (2:1 v/v) was added to each sample. The sample was homogenized with a Polytron for 40 seconds and then centrifuged 2800 r.p.m. for 10 minutes. The upper hexane phase was transferred to a separate container and the lower aqueous-acetone phase was extracted anew with 10ml n-hexane plus Polytron for 40 seconds and centrifuged 2800 r.p.m. for 10 minutes. This process was repeated once more. The combined hexane extracts were washed 50ml water twice and treated with anhydrous sodium sulfate. The extract was evaporated near to dryness and the amount of lipid was determined gravimetrically.

Method C (solvent system of ethanol/n-hexane): Five ml saturated ammonium sulfate and 20ml ethanol/ n-hexane (1:3 v/v) were added to each sample and extracted for 30 minutes. The upper hexane phase was transferred to a separate container and the lower aqueous-ethanol phase was extracted anew with 20ml n-hexane for 10 minutes and this was repeated again. The combined hexane extracts were concentrated near to 5ml, washed 15ml water three times and treated with anhydrous sodium sulfate. The extract was evaporated near to dryness and the amount of lipid was determined gravimetrically.

Estimates of total lipids of the whole blood and serum were calculated by summation of the individual two and/or four lipid components of the serum by the following formulas [25)].

Two components: $TL = 2.27\ TC + TG + 0.623$

Four components: $TL = 1.677\ (TC - FC) + FC + TG + PL$

Where TL is total lipids, TC is total cholesterol, FC is free cholesterol, TG is triglycerides and PL is phospholipids, all expressed in units of mg/dl. Concentrations of TC, FC, TG and PL of the serum were measured using standard enzymatic and automatic clinical procedures on a Hitachi 7600-300s (Hitachi High-Tech. Corp., Tokyo, Japan) for TC and TG and on a BM 9130 (JEOL LtD., Tokyo, Japan) for FC and PL in SRL Inc. (Tokyo, Japan), the biggest center of clinical examination, in Japan.

Correlation coefficients between the measured and estimated concentrations of total lipids in the serum and whole blood were statistically examined using Student's T test or Fisher's Z transformation, if necessary.

Results and Discussion

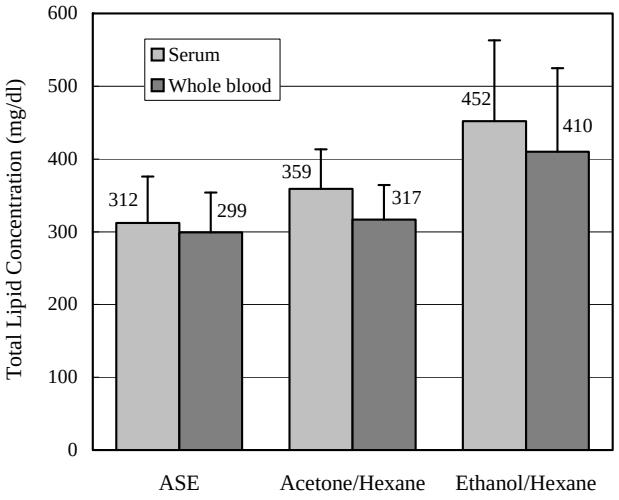
As indicated in Fig. 1, concentrations of total lipids extracted from the serum were 312±63.9 mg/dl, 359±54.4 mg/dl and 452±111 mg/dl by the methods of A, B and C, respectively. Concentrations of total lipid extracted by method C, namely, solvent system of ethanol/n-hexane were significantly higher than those of methods B

(solvent system of acetone/n-hexane) and A (ASE with acetone/n-hexane). Fig. 1 also showed the concentrations of total lipids extracted from the whole blood and the respective lipid concentrations by methods A, B and C were 299±54.8 mg/dl, 317±47.5 mg/dl and 410±115 mg/dl, which were somewhat lower than those obtained from the serum by the respective methods. Again, however, the concentrations of total lipids obtained by method C were significantly greater than those obtained by methods A and B. Accordingly, method C (solvent system of ethanol/n-hexane) was considered the best extraction method among these three methods.

We calculated the concentrations of total lipids by the summation of the individual two and four lipid components of the serum, as mentioned in Materials and Methods and obtained 475±50.1 mg/dl and 538±63.6 mg/dl, respectively. Estimated mean concentration of total lipids obtained from the four components was significantly higher than that obtained from the two components (p=0.05).

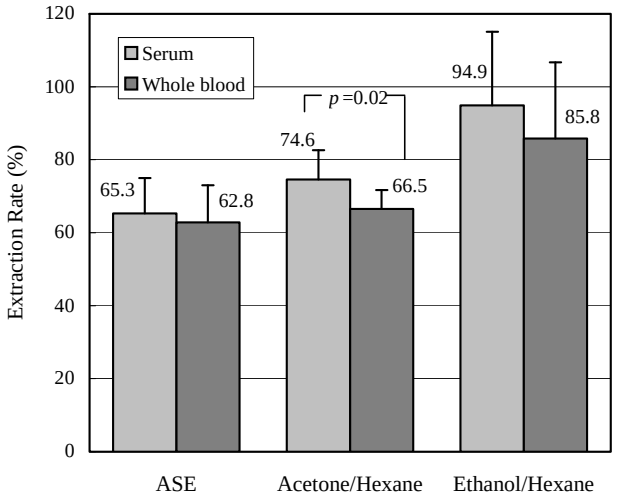
Fig. 2 showed the extraction rates in the comparison of concentrations of total lipids measured with those of total lipids estimated by two components, that is, 475±50.1 mg/dl. In agreement with the results as shown in Fig. 1, extraction rates increased with the methods A to C, namely, 65.3±9.7%, 74.6±8.0% and 94.9±20.2% in the serum and 62.8±10.2%, 66.5±5.2% and 85.8±20.9% in the whole blood. Extraction rates of method C (ethanol/n-hexane) were significantly greater than those of methods A (ASE with acetone/n-hexane) and B (acetone/n-hexane).

Same kinds but poorer extraction rates were seen in the three extraction methods when we calculated the extraction rates using the total



Serum ; ASE vs Ethanol/Hexane, p =0.005
Acetone/Hexane vs Ethanol/Hexane, p =0.04
Whole blood ; ASE vs Ethanol/Hexane, p =0.02
Acetone/Hexane vs Ethanol/Hexane, p =0.05

Fig. 1. Comparison in measured total lipid concentrations



Serum ; ASE vs Acetone/Hexane, p =0.04
ASE vs Ethanol/Hexane, p =0.001
Acetone/Hexane vs Ethanol/Hexane, p =0.02
Whole Blood ; ASE vs Ethanol/Hexane, p =0.009
Acetone/Hexane vs Ethanol/Hexane, p =0.02

Fig. 2. Extraction rates in comparison of total lipids measured with total lipids estimated by two components (TC and TG), 475 ± 50.1 mg/dl

lipids estimated by four components, that is, 538±63.6 mg/dl. These results indicate that the methods A and B are very poor ability of lipid extraction from both of the serum and whole blood.

The relationship between the concentrations of total lipids measured and those estimated by two components, namely, TC and TG in the methods A, B and C are indicated in Fig. 3. Even though we observed poor ability of lipid extraction by the methods A and B, in particular in method B highly significant correlations were observed in both of the serum and whole blood. Therefore, lipids in the serum and whole blood were considered extracted quantitatively by these three methods, but with poor ability of extraction.

We are now further investigating the lipid extraction from the serum much more quantitatively than this study with these three methods and also the solvent system of chloroform/methanol.

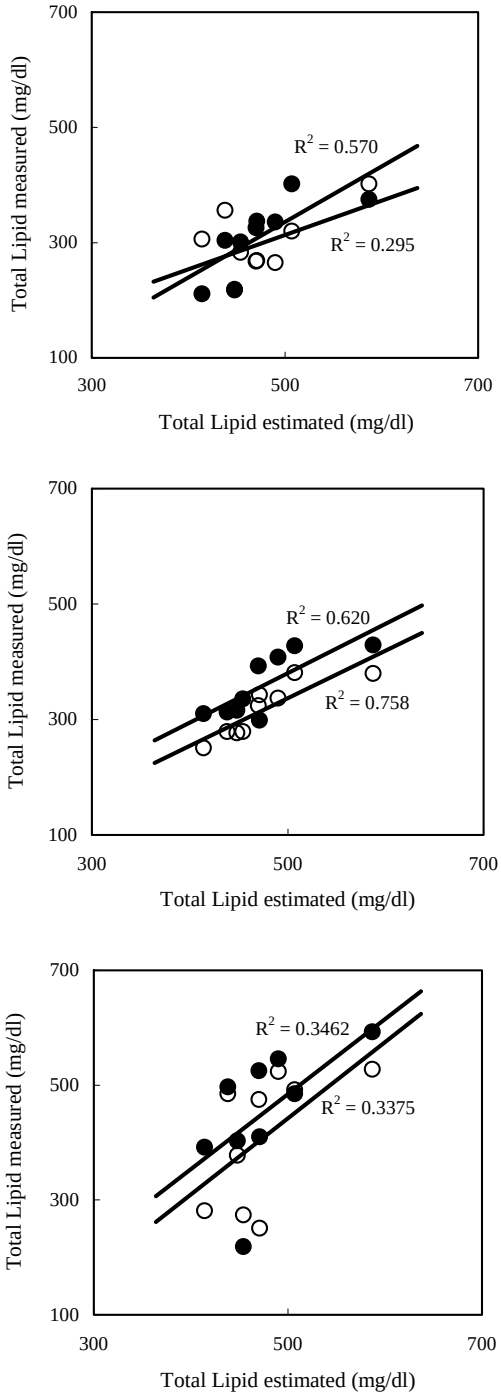


Fig. 3. Relationships between the concentrations of total lipids measured and those estimated by two components (TC and TG) in methods A (upper), B (middle) and C (lower)

○: Whole blood, ●: Serum

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