Chemistry and Physics of Lipids 178 (2014) 11–17

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

# Comparison of plasma and erythrocyte membrane fatty acid compositions in patients with end-stage renal disease and type 2 diabetes mellitus

Erdim Sertoglu[a,*], Ismail Kurt[b], Serkan Tapan[b], Metin Uyanik[b], Muhittin A. Serdar[c], Huseyin Kayadibi[d], Saad El-Fawaeir[b]

[a] Ankara Mevki Military Hospital, Anittepe Dispensary, Ankara, Turkey
[b] Gulhane School of Medicine, Department of Medical Biochemistry, Ankara, Turkey
[c] Acıbadem University School of Medicine, Department of Medical Biochemistry, Istanbul, Turkey
[d] Adana Military Hospital, Department of Medical Biochemistry, Adana, Turkey

ARTICLE INFO

*Article history:*
Received 14 November 2013
Received in revised form
22 December 2013
Accepted 23 December 2013
Available online 31 December 2013

*Keywords:*
End-stage renal disease
Erythrocyte membrane
Fatty acids
Plasma
Type 2 diabetes mellitus

ABSTRACT

*Background:* In this study, we aimed to compare the serum lipid profile and fatty acid (FA) compositions of erythrocyte membrane (EM) and plasma in three different patient groups (group 1: type 2 diabetes mellitus (T2DM) + end-stage renal disease (ESRD), group 2: ESRD, group 4: T2DM) and healthy controls (group 3) simultaneously.
*Methods:* 40 ESRD patients treated with hemodialysis (HD) in Gulhane School of Medicine (20 with T2DM) and 32 controls (17 with T2DM, 15 healthy controls) were included in the study. Plasma and EM FA concentrations were measured by gas chromatography-flame ionization detector (GC-FID).
*Results:* Plasma and EM palmitic acid (PA) and stearic acid (SA) levels were significantly higher in T2DM patients compared to controls ($p = 0.040$ and $p = 0.002$ for plasma, $p = 0.001$ and $p = 0.010$ for EM, respectively). EM docosahexaenoic acid (DHA) levels were also significantly lower in patients with ESRD + T2DM and ESRD compared to controls ($p = 0.004$ and $p = 0.037$, respectively).
*Conclusions:* Patients with insulin resistance display a pattern of high long chain saturated FAs (PA, SA and arachidic acids). However, while there are no recognized standards for normal EM DHA content, decreased levels of EM DHA in ESRD patient groups (groups 1 and 2) suggest that there may be reduced endogenous synthesis of DHA in HD subjects, due to the decreased functionality of desaturase and elongase enzymes. Because membrane PUFA content affects membrane fluidity and cell signaling, these findings are worthy of further investigation.



## 1. Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. A consequence of this is the chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. Type 2 DM (T2DM) is the fourth leading cause of the death in developed countries with a two to four fold increased risk of cardiovascular disease (CVD) and stroke (Hartweg et al., 2009). The high cardiovascular mortality rate in T2DM has been partly attributed to an unfavorable lipoprotein profile which is the combination of hypertriglyceridemia, low levels of high density lipoprotein cholesterol (HDL-C) and high levels of low density lipoprotein cholesterol (LDL-C). In addition, fatty acid (FA) profile of peripheral tissue membranes influences the sensitivity of the tissue to insulin (Storlien et al., 1997). Epidemiologic data support a positive association between intake of saturated fatty acids (SFAs) and risk of impaired glucose tolerance, insulin resistance (IR), and diabetes. The association for polyunsaturated fatty acids (PUFAs) is controversial: both positive and inverse associations have been reported (Shearer et al., 2012).

T2DM is the leading cause of the end-stage renal disease (ESRD) worldwide. Patients with ESRD treated by hemodialysis (HD) are characterized by a number of biochemical abnormalities including hyperlipidemia. The importance of CVD as the cause of death in these patients makes it considered as an important risk factor (United States Renal Data System, 2008). Additionally, patients with ESRD exhibit plasma FA patterns which are indicative of essential fatty acids (EFAs) deficiency (Elshamaa et al., 2010). Same changes in the lipid layer of red blood cell membranes which alter their fluidity may contribute to clinical problems such as susceptibility to infection, delayed wound healing, anemia and even

* Corresponding author. Tel.: +90 539 206 2486; fax: +90 312 304 3300.
*E-mail address:* erdimsertoglu@gmail.com (E. Sertoglu).

0009-3084/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemphyslip.2013.12.011

increased risk of CVD (Kwiatkowska et al., 2007; Pilz and März, 2008). The implications of circulating EFAs, as a reflection of long-term dietary intake, on the inflammatory risk profile and clinical outcome of ESRD subjects are still unclear (Shearer et al., 2012).

FAs, particularly omega-3 (ω-3) and ω-6 PUFAs, mediate a number of key biologic processes including eicosanoid production, cell membrane physiology, signaling pathways, inflammation, gene regulation and expression (Friedman et al., 2012). Therefore, accurate measurement of plasma free fatty acids (FFAs) has important physiological and clinical implications.

A better understanding of the distribution of FAs in the plasma and erythrocyte membrane (EM) of HD patients will provide important insights into the lipid metabolism of that population as well as guide for future biomarker selection. The beneficial effect of ω-3 PUFA on lowering cardiovascular mortality in the general population has previously been reported (Hooper et al., 2004). Moreover, the effects of dietary ω-3 PUFA supplementation on the prevention and treatment of a variety of complications due to diabetes and ESRD have previously been studied many times (Friedberg et al., 1998; Noori et al., 2011). There are studies which are demonstrating lower ω-3 and ω-6 PUFA, arachidonic acid (AA) and eicosapentaenoic acid (EPA) levels in HD patients than in healthy controls (Peck et al., 1996; Varga et al., 1997; Koorts et al., 2002). In addition, there are also studies showing decreased levels of EPA in EM (Koorts et al., 2002; An et al., 2009). However, there is no study determining and comparing the plasma and EM FA levels in patients with ESRD and T2DM.

In the present study, we aimed to determine and compare the serum lipid profile and FAs compositions of EM and plasma in three different patient groups (T2DM, ESRD and T2DM + ESRD) and healthy controls simultaneously by gas chromatography-flame ionization detector (GC-FID).

## 2. Materials and methods

The study was approved by the local ethics committee of Gulhane School of Medicine (2011), which was conducted according to the Helsinki Declaration.

### 2.1. Study population

40 ESRD patients undergoing HD treatment in Gulhane School of Medicine (20 with T2DM) and 32 controls (17 with T2DM, 15 healthy controls with no chronic disease) were included in the study.

### 2.2. Reagents and chemicals

Methyl esters are used almost universally for GC-FID analysis of fatty acids (Shantha and Napolitano, 1992). The most common procedure used for measuring plasma and EM FFA concentrations and FA enrichment involves the (a) extraction of lipid from plasma and EM and (b) derivatization of FFA to fatty acid methyl esters (FAMEs) steps.

1. Heptadecanoic acid (98% C 17:0, C17H34O2, Sigma–Aldrich, USA) was used as an internal standard (Blau et al., 2008). For this purpose 50 mg of the C 17:0 was weighed and dissolved in 10 ml of chloroform (approximately 5000 μg/mL, stored at 4 °C), then stock solution was diluted to the final concentration of 500 μg/mL to use as an internal standard working solution.
2. 3 N methanolic HCl was prepared as methylation solution by adding 3 mL HCl 37% (Merck, Germany) dropwise to 9 mL methanol (Sigma–Aldrich, USA) under the fume hood.
3. Eppendorf tubes containing BHT (Butil Hidroksi Toluen): 1 g of BHT was dissolved in 100 ml of ethanol (Sigma–Aldrich, USA) and stored in a dark bottle at 4 °C. Immediately prior to handling of the erythrocytes, 100 μl of BHT was put into the Eppendorf tube and dried under a stream of nitrogen. Plasma and EM samples were stored in these BHT treated tubes at −80 °C.
4. Supelco™ 37 Component FAME Mix (Sigma–Aldrich, USA), was used to identify key FAMEs and chromatogram was shown in Fig. 1.

### 2.3. Sample collection and analysis

A minimum of 50 μl of plasma or red-cell haemolysate was needed. Venous blood samples were taken to $K_2$-EDTA-containing tubes. Hematocrit levels were determined with ABX Pentra XL 80 (Horiba Medical – USA) for all samples. Afterwards, the erythrocytes were separated from the plasma by centrifugation (3000 rpm, 1500 × *g*, for 10 min) and washed with an equal volume of saline. Following the removal of the saline, the cells were resuspended with saline to Hct of 45%. These erythrocyte suspensions (ES) and plasma were stored in a freshly BHT-treated Eppendorf vials at −80 °C.

### 2.4. Instrumentation

TRACE GC ultra gas chromatograph with FID was used (Thermo Scientific™, USA). SPTM-2560 capillary column, 100 m × 0.25 mm × 0.2 μm (Catalog no. 23362-U), was installed and conditioning process was carried out as recommended in the manual.

### 2.5. Procedure

ES and plasma were thawed at 4 °C for one night before the study. 100 μl of IS working solution, 50 μl of plasma or ES and 1 ml of 3 N methanolic HCl were added in to the 4-ml of glass vial in this order. The vial was closed with a screw cap and allowed the transmethylation to proceed at 90 °C for 4 h. After cooling the vials to room temperature, 2 ml hexane was added, the vial was closed again and vortexed for 10 s. The upper (hexane) layer was transferred to a glass tube and carefully evaporated the hexane with a gentle stream of nitrogen at room temperature. Finally, the residue was dissolved in 100 μl (plasma) or 80 μl (erythrocytes) of hexane and the sample was transferred to a GC injection vial with a screw cap. Hexane was used due to being immiscible with methanol and not shortening our column lifetime. 1 μl of sample was injected in split mode at 240 °C with a 50:1 split ratio. Helium was used as the carrier gas with an inlet pressure of 250 kPa. The temperature program was started at 50 °C and maintained for 1.5 min, followed by an increase to 190 °C at a rate of 30 °C/min. After holding the temperature at this level for 5 min, the gradient was continued at a rate of 8 °C/min until a final level of 230 °C. This was maintained to allow all high-boiling substances, cholesterol, to be eluted. The total analysis time was approximately 43 min. The FAs peaks were observed between 17.96 and 41.83 min. Linearity was obtained between 2.5 μg/ml and 1000 μg/ml ($r^2$ = 0.9579–0.9973), recovery rate was 87–107%, LOD was 0.3–4.2 μg/ml and the reproducibility was between 89.6 and 95.8%.

SFAs [C 14:0 (myristic acid – MA), C 16:0 (palmitic acid – PA), C 18:0 (stearic acid – SA), C 20:0 (arachidic acid), C 22:0 (behenic acid), C 24:0 (lignoceric acid)], monounsaturated fatty acids (MUFAs) [C 16:1, C 24:1], ω-3 PUFAs [C 20:5 ω3 (EPA), C 22:6 ω3 (docosahexaenoic acid-DHA)], ω-6 FAs [C 18:2 ω6 (linoleic acid – LA), C 20:4 ω6 (AA), C 20:3 ω6], ω-9 FAs [C 18:1 ω9 (oleic acid – OA), C 22:1 ω9 (erucic acid)] levels and EPA/AA and ω3/ω6 ratios of both plasma and EM were evaluated in our study.