

Odd-numbered fatty acids in type 2 diabetes mellitus

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This work deals with the study of lipid metabolism in type 2 diabetics focusing on the changes in odd-numbered fatty acid concentrations. Diabetes mellitus is a syndrome of metabolic disorders in which the concentration of insulin and its secretion are impaired. It indicates an impaired metabolism of glucose, lipids, and proteins. The main goal of this work was to measure concentrations of odd-numbered fatty acids (ONFA) in healthy individuals and in patients with type 2 diabetes mellitus and to compare the concentrations between both groups by using statistical methods. Plasma samples were deproteinized and lipids were extracted into organic solvents. Thin layer chromatography was used to separate 5 lipid fractions. All the lipid fractions were derivatized into fatty acid methyl esters and analyzed by a gas chromatograph. ONFA are minor group of the fatty acids and their concentrations are lower in type 2 diabetes mellitus. The most significant changes in concentrations were registered for tridecanoic and pentadecanoic acid.

Keywords: Diabetes mellitus; Gas chromatography; Lipid metabolism; Odd-numbered fatty acids

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from the defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with a long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1].

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Type 2 diabetes is caused by a combination of resistance to insulin action and inadequate compensatory insulin secretory response. It is the form of diabetes, which covers about 90–95 % of those with diabetes diagnosis. Most patients with this form of diabetes are obese, and the obesity itself causes some degree of insulin resistance. The increased percentage of the body fat distributed predominantly in the abdominal region and dyslipidemia, dyslipoproteinemia and hypertension usually occurs in this type. It is characterized by the elevated plasma triglyceride levels, low levels of high-density lipoprotein (HDL) cholesterol, and small, dense low-density lipoprotein (LDL) particles.

Patients with DM type 2 have an increased risk of developing atherosclerosis and other cardiovascular diseases [1,2]. The resistance to insulin with the impairment in the insulin-mediated glucose metabolism affects both hepatic and peripheral tissues, primarily that of muscles [3]. The patho-physiology of insulin resistance involves a complex network of the signalling pathways activated by the insulin receptor, which regulates the intermediary metabolism and its organization in the cells [4]. Elevated plasma free fatty acids can account for a large part of insulin resistance in obese patients with type 2 DM [5]. This leads to the question on which fatty acids cause the insulin resistance and which are directly correlated with type 2 DM.

ONFA are a minor group of fatty acids occurring in the human body. The most abundant ONFA are pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0). In the body, there is no physiological endogenous synthesis of ONFA except for a minor way by α -oxidation [6]. ONFA and branched-chain fatty acids have a frequent occurrence in milk, meat, and the fat tissue of ruminants or ruminant products, respectively, and the consumption of these products is the main source of ONFA for human. The occurrence of ONFA in ruminants is caused mainly by ruminant bacteria, producing ONFA within their metabolism and containing a lot of ONFA in their membranes. β -oxidation of ONFA in the human body terminates at propionyl-CoA, which – after transformation into the succinyl-CoA – is then utilized in the citrate cycle. Incidental patho-biochemical defects in the enzymatic transformation of propionyl-CoA are called the propionic acidemia. Such a stage leads to the accumulation of propionate in blood and to acidosis. Propionyl-CoA competes with the acetyl-CoA in the fatty-acids synthesis, giving rise to the formation of ONFA and their occurrence in the human body is increasing [6–10].

Materials and methods

Reagents and chemicals

Isopropanol p.a., *n*-hexane p.a., acetic acid (99%) were purchased from PENTA (Prague, Czech Republic). The solvents used, namely *n*-heptane p.a., diethyl ether p.a.,

methanol p.a., toluene p.a. and potassium carbonate aqua free were purchased from Lach-Ner (Neratovice, Czech Republic). Orthophosphoric acid (p.a. grade) was purchased from CHEMAPOL (Prague, Czech Republic), 2',7'-dichlorofluorescein for TLC detection then obtained from Carl Roth (Karlsruhe, Germany), acetylchloride (>99%) was purchased from Sigma-Aldrich. Finally, internal standard of *cis*-13,16,19 docosatrienoic acid was ordered from Larodan Fine Chemicals AB (Malmö, Sweden).

Samples

In total, 21 anonymous plasma samples were processed being obtained from patients diagnosed with diabetes mellitus type 2. For the control, 17 plasma samples from healthy blood donors were also used.

Sample preparation

All the plasma samples in glass tubes were deproteinized by dissolving in the mixture of isopropanol, *n*-heptane and 2M H₃PO₄ (40:20:1), then vortexed and let conditioning for 10 min at room temperature. Subsequently, all the lipids were extracted by adding the mixture of toluene:methanol (4:1) and water. After centrifugation, the organic layer was transferred into another glass tube and dried under nitrogen.

Thin-layer chromatography (TLC)

The evaporation residue was dissolved in chloroform and transferred on a TLC plate that was inserted into a chromatographic container saturated with mobile phase (160 mL *n*-hexane, 40 mL diethyl ether and 6 mL acetic acid). The plate development took around one hour, when the mobile phase reached the front. The visualisation was made with 2',7'-dichlorofluorescein at the line with a TLC standard (pool plasma) and five fractions were marked, extracted from the plate and moved to Pyrex tubes with Teflon liner screw caps. Five fractions; namely, phospholipids, diacylglycerols, free fatty acids, triacylglycerols, and cholesterol esters (all in Pyrex tubes) were mixed with an internal standard (*cis*-13,16,19 docosatrienoic acid) with 10 µg mL⁻¹ in concentration and the resultant mixture dissolved in a mixed medium of toluene:methanol (4:1).

Derivatization

All the fractions were derivatized into fatty-acid methyl esters by addition of acetylchloride and the reaction was run for one hour in a thermoblock heated to 100 °C. Pyrex tubes were left to cool down at room temperature in a fume hood. A solution of 6% (w/w) potassium carbonate was added into the pyrex tubes to neutralize the content. After centrifugation, the organic layer was transferred into new tubes and dried / evaporized under nitrogen. The residue obtained in this way was dissolved in toluene and moved into chromatographic vials.

Gas chromatography

Samples in chromatographic vials were measured in a gas chromatograph (model 7890A; Agilent Technologies; Santa Clara, CA, USA) with an autosampler and a FID detector. A column ("HP-88" type, 100 m × 0.25 mm × 0.2 μm) was used and devised for separation of fatty-acid methyl esters. Chromatograms were evaluated via integrating by GC ChemStation B.04.03 program. The quantification was done by multiple-point external standard calibration method, and the acquired data processed in Microsoft Excel 2010 and Statistica 12 using the *t*-test (where *p* value < 0.05 was considered as statistically significant).

Results and discussion

In this work, a set of 41 fatty acids were measured, from which eight were ONFA. The ONFA of interest were: undecanoic acid (C11:0), tridecanoic acid (C13:0), pentadecanoic acid (C15:0) and its isoforms 12-methylmyristic acid (12M-C14:0; anteiso C15:0) and 13-methylmyristic acid (13M-C14:0, iso C15:0), heptadecanoic acid (C17:0) and its isoform 14-methylhexadecanoic acid (14M-C16:0, anteiso C17:0) and nonadecanoic acid (C19:0). In total, our analysis comprised 38 samples, from which a collection of 17 was sampled from healthy blood donors used as control and another set of 21 from patients with type 2 DM; each sample being divided into the phospholipid (PL), diacyl-glycerol (DAG), free fatty acid (FFA) triacylglycerol (TAG), and cholesterol ester (CE) fractions.

As expected, the most abundant ONFA were C15:0 and C17:0. The reason for those higher concentrations may be due to a similarity in the carbon number with palmitic acid (C16:0) and stearic acid (C18:0) that are the most abundant evenly-numbered fatty acids and *de novo* synthesis of fatty acids ends at C16:0. ONFA are a minor group of fatty acids and their content in the control group was 1.1 % and 0.96 % in diabetics, respectively. Previous studies described a lower risk of type 2 DM with higher concentrations of ONFA [6,11,12].

This finding was also observed in our work (see Fig. 1), where diabetics have lower concentrations of ONFA in FFA, TAG, and CE fractions and thus higher risk of type 2 DM. The most significant changes were observed in concentrations of C15:0 (Fig. 2). C15:0 is slightly increased in fractions DAG and FFA in diabetics. Those fractions mostly reflect the presence of lipids and fatty acids from diet and they are being processed in liver. Fractions TAG, PL, and CE are formed primarily in liver and there is C15:0 in lower concentrations in diabetics compared with the control group.

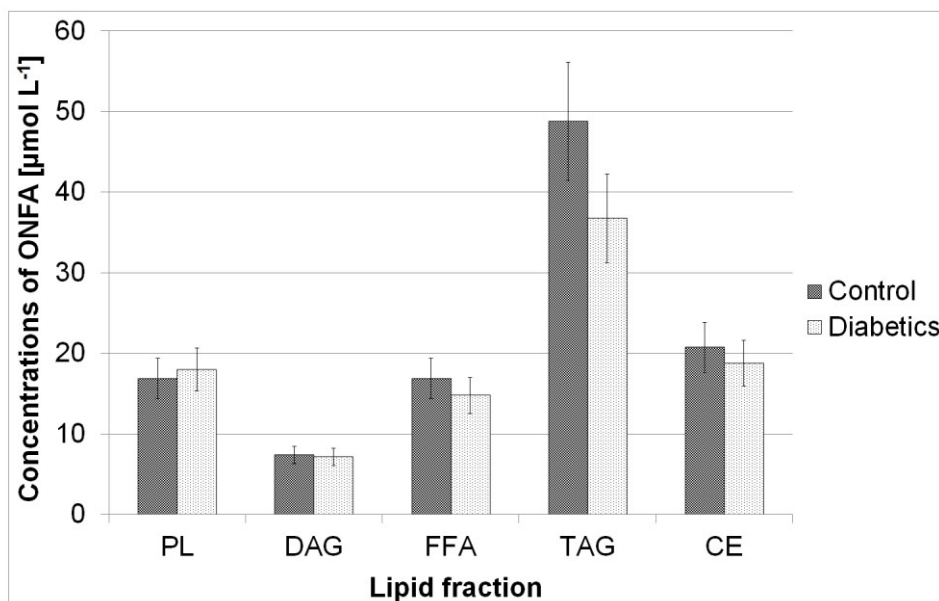


Fig. 1 Concentrations of ONFA in all lipid fractions in control group and in type 2 diabetics

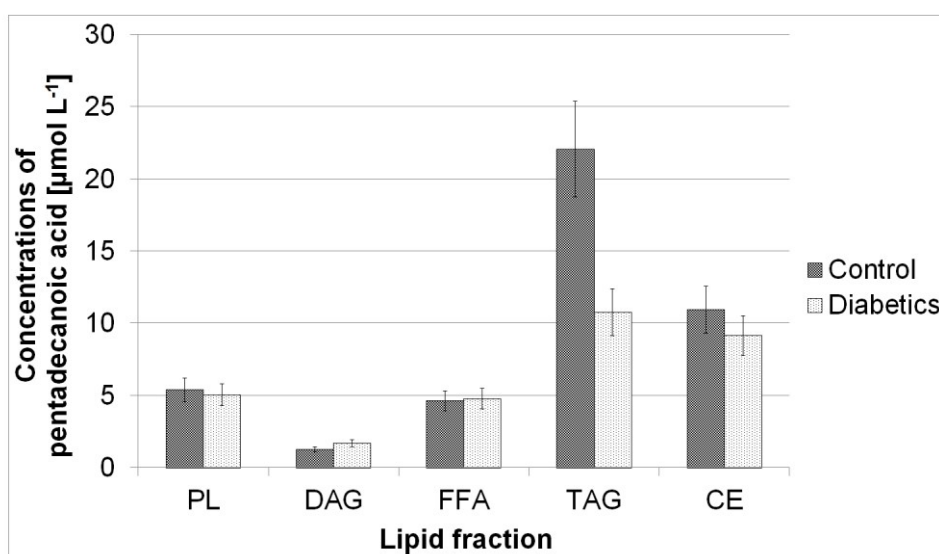


Fig. 2 Concentrations of pentadecanoic acid in all lipid fractions in the control group and in type 2 diabetics

In TAG fraction, one can find the most significant difference. According to this finding, we set up the hypothesis that C15:0 is metabolized in liver. To support such a presumption, we have analysed the activities of liver enzymes – Δ^9 -desaturase (Fig. 3) and elongase (Fig. 4). Δ^9 -desaturase forms a double bond at the 9th position from the carboxyl end and elongase adds two carbons to the fatty acid chain. Activities of both enzymes were calculated as the "product-to-substrate concentration" ratio and therefore, the resultant activity is without a unit. For example, Δ^9 -desaturase forms a double bond in stearic acid to give rise to oleic acid. The activity is calculated by dividing the concentrations of oleic acid and of stearic acid. The activity of Δ^9 -desaturase is significantly higher in all lipid fractions of type 2 diabetics except PL. On the other hand, the activity of elongase is decreased in all lipid fractions except for PL fraction. This finding confirms probable metabolism of C15:0 by the Δ^9 -desaturase, but not by elongase.

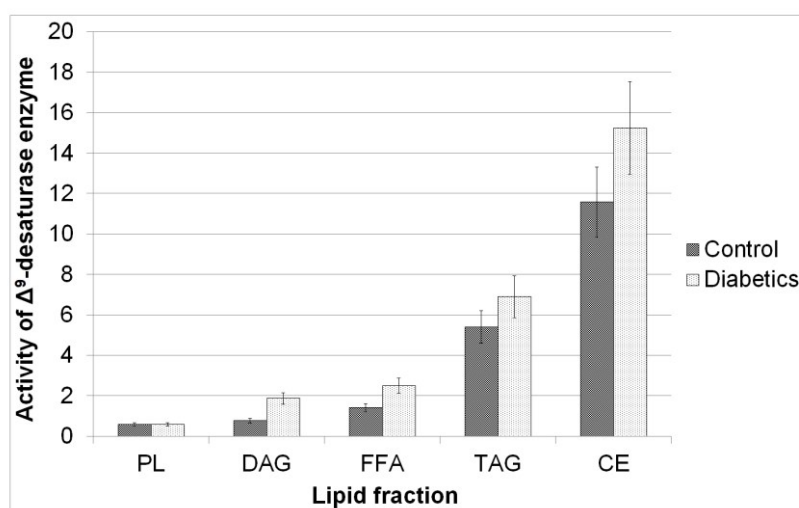


Fig. 3 Activity of Δ^9 -desaturase in all lipid fractions in the control group and in type 2 diabetics

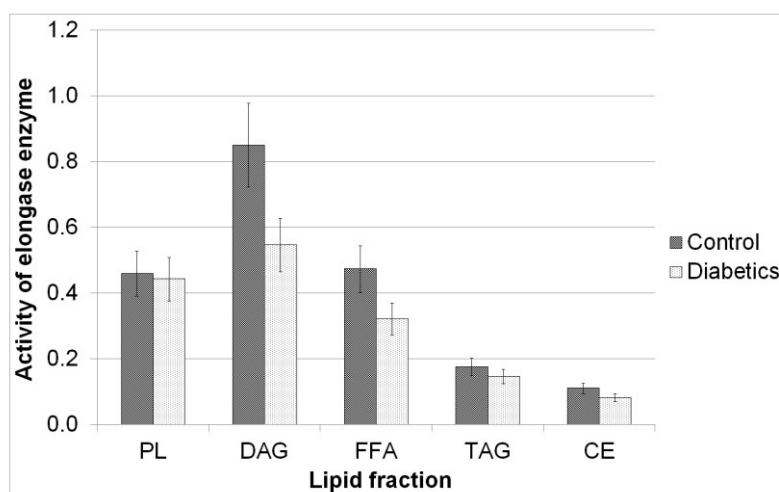


Fig. 4 Activity of elongase in all lipid fractions in the control group and in type 2 diabetics

Another ONFA exhibiting significant changes in all lipid fractions is C13:0 (Fig. 5). This fatty acid is positively correlated with type 2 DM and has increased the concentrations in all lipid fractions despite the fact that most of ONFA revealed the negative association with type 2 DM. C13:0 could be used as the marker of type 2 DM. All other ONFA have had less or no significant correlation with type 2 DM.

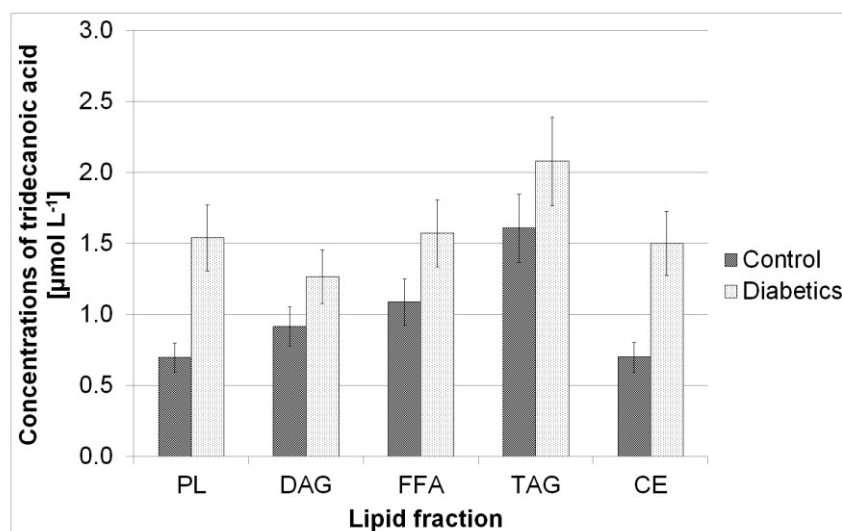


Fig. 5 Concentrations of tridecanoic acid in all lipid fractions in the control group and in type 2 diabetics

Conclusions

Nowadays, the number of patients with type 2 DM is increasing. To avoid chronic complications connected with hyperglycemia and type 2 DM, there should be the proper diagnostic tools for earlier diagnosis of DM. Because of the impaired lipid metabolism in type 2 diabetics, this work has dealt with the monitoring of constellation of the fatty acid content in diabetics (21 samples) comparing the respective concentrations with those in the control group (17 samples). ONFA were the central point of interest, allowing us to define the changes in concentrations of ONFA between both groups. As ascertained, ONFA concentrations are lower in diabetics except for tridecanoic acid. This acid is at significantly higher concentrations in all lipid fractions in diabetic group and therefore, it can be used as a marker for diagnosis of type 2 DM. Concentrations of pentadecanoic acid have been changing between FFA, DAG fractions and TAG, CE fractions and this phenomenon is probably due to the desaturation of pentadecanoic acid in liver.

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