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FATTY ACID ENZYME ACTIVITIES AND RISK OF DIABETES MELLITUS

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Abstract. *The prevalence of diabetes mellitus, especially type 2 diabetes (T2D), is high worldwide and there is an ongoing challenge to find potential biomarkers that could predict the onset of diabetes and provide insight into possible mechanisms for its development. Previously published data have shown that the composition and concentrations of free fatty acids (FFA) in plasma/serum are associated with the occurrence and risk of developing diabetes. Recent evidence suggests that the composition and content of free fatty acids are influenced by many factors, such as age, sex, ethnicity, dietary habits of the subjects and their genetic predisposition. Furthermore, endogenous synthesis of FFA and the activities of the enzymes, desaturase and elongase, involved in their metabolism are also associated with the development of diabetes. The purpose of this study was to evaluate the association between different type of free fatty acids and activity of fatty acid enzymes and risk of diabetes. In 145 individuals (aged 30-70 years, both sex: 92 males and 111 females) of which 54 T2D patients, 45 prediabetes patients, 24 newly diagnosed T2D patients and 81 healthy controls, delta 6 desaturase (D6), delta 5 desaturase (D5D), stearoyl CoA-desaturase-1/2 (SCD-1/2) and elongase (ELOVL) activity were estimated from product/precursor FAs ratios (D6D=C18:3n-6/C18:2n-6; D5D=C20:4n-6/C20:3n-6; SCD-1=C16:1n-7/C16:0 and SCD-2=C18:1n-9/C18:0; and ELOVL=C18:1n-7/C16:1n-7, respectively). The clinical parameters including fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), lipid profile and hepatic enzymes were measured by standard analytical methods while the concentrations of individual FFAs were determined by gas chromatography. The results showed that higher concentrations of six FFAs in plasma were significant associated with increasing risk factors of T2D including fasting glucose levels, lipid profile and fatty acid enzymes activity (C14:0 $p<0.001$; C14:1 $p<0.01$; C16:1 $p<0.001$; C18:0 $p<0.001$; C18:2 $p<0.05$; C20:4 $p<0.01$, respectively). Furthermore, the activities of D6D, SCD-1 and ELOVL, and elevated levels of these six free fatty acids, were strongly associated with risk factors in the development of T2D in humans. Therefore, both fatty acid enzymes and individual FFAs could be used as biomarkers in the early diagnosis of T2D and subsequent treatment of future complications of the disease.*

Keywords: *Free fatty acid enzymes, biomarkers, Type 2 diabetes*

1. INTRODUCTION

The prevalence of diabetes mellitus, especially of Type 2 diabetes (T2D) is high around world, and there is a constant challenge to found risk factors and also potential biomarkers that might predict incident diabetes and provide insight possible mechanisms for its development. Previous data showed that plasma free fatty acids (FFA) concentrations and FFA composition demonstrate an association with incident of diabetes. Recent studies suggest that both, depends of many factors such as age, sex, ethnicity, endogenous synthesis of FFAs, dietary habits, but also genetic predisposition and gene-gene interaction as well gene-diet interaction [1], [2].

The concentration of different fatty acids in plasma and others tissue reflects dietary intake but is also influenced by endogenous fatty acid production mediated by various fatty acid desaturases: D5D, D6D, and stearoyl CoA desaturase encoded by the FADS1, FADS2 and SCD genes, respectively as well as different fatty acid elongase encoded by specific ELOVL genes (Figure 1) [3]. The fatty acids composition in body is not only influenced by desaturation enzymes but also lifestyle. FFAs are mainly released from adipose tissue by lipolysis, and represent an important biomolecule for providing energy to a number of organs, act as signaling molecules and as

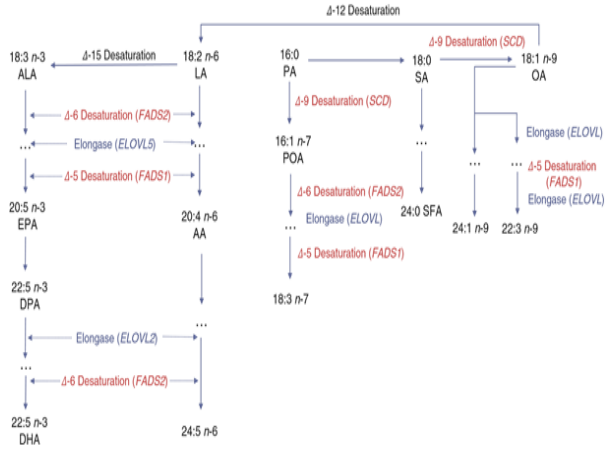

Figure 1. The role of enzymes encoded by FADS1/2 (D6D/D5D), SCD1/2 and ELOVL in the metabolism of fatty acids. (Desaturation enzymes are shown in red and elongase in blue)

major cells membrane structural components. Serum fatty acids concentration and desaturase activities have been related to obesity, insulin resistance (IR), T2D and the metabolic syndrome. Data from previous studies investigating blood fatty acid product-to-precursor ratios as estimates of desaturases or elongases activity,

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reported that lower D5D activity and higher D6D activity were associated with diabetes risk. Many of the metabolic pathways and regulators involved in an impaired insulin secretion and β-cell dysfunction is not completely understood [4], [5].

The purpose of this work was to evaluate the association between different fatty acid and fatty acid enzymes activity and risk of development diabetes in patients.

2. Materials and methods

2.1. Subjects

In 203 individuals (aged 30-70 years, both sex: 92 males and 111 female) of which 54 T2D patients, 44 Prediabetes (PREDM) patients, 24 Non-treated (NT-T2D) patients and 81 healthy controls, delta 6 desaturase (D6D or *FADS1*), delta 5 desaturase (D5D or *FADS2*), stearoyl CoA-desaturase-1/2 (*SCD-1/2*) and elongase (*ELOVL-1/-7*) activities were estimated by product/precursor ratios as follows D6D=C18:3n-6/C18:2n-6; D5D=C20:4n-6/C20:3n-6; *SCD-1*=C16:1n-7/C16:0 and *SCD-2*=C18:1n-9/C18:0; and *ELOVL*=C18:1n-7/16:1n-7, respectively. The clinical parameters were measured including fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), lipid profile and hepatic enzymes by standard clinical methods while the concentrations of individual FFAs were determined by gas chromatography equipped with a flame ionization detector.

FPG in the clinical laboratory is most often measured by an enzymatic method based on the glucose oxidase-peroxidase (GOD-POD) method, in a reaction in which β-D-glucose is first oxidized by the GOD enzyme (this enzyme can only act on β-D-glucose), which leads to the formation of hydrogen peroxide (H_2O_2). Formed H_2O_2 reacts with a colorless chromogenic substrate in the presence of peroxidase (POD) to produce a colored product. The intensity of the color is proportional to the concentration of glucose in the sample.

For the measurement of HbA1c, boronate affinity chromatography is used as a standardized analytical method and specific for glycation, based on the binding reaction of boronate to the unique cis-diol configuration formed by the stable binding of glucose to hemoglobin (Hb). This method measures "total HbA1c" or "true HbA1c", and since only two fractions are present (glycosylated and deglycosylated), the glycosylated fraction is compared to the total amount, and the results are expressed as % HbA1c.

Lipid profile measurements include: Total cholesterol (the sum of an individual's LDL, VLDL, and HDL cholesterol levels) a common determined by Cholesterol oxidase-peroxidase (CHO-POD). This enzyme is used to hydrolyze the cholesterol esters present in serum into free cholesterol and free fatty acids. The enzyme cholesterol oxidase, in the presence of oxygen, oxidizes cholesterol to cholest-4-en-3one and hydrogen peroxide. Hydrogen peroxide (H_2O_2) oxidizes phenol and 4-aminoantipyrine to produce red color that can be measured spectrophotometrically. The intensity of the color formed is proportional to the cholesterol concentration in serum. Enzymatic colorimetric methods are still the main assay methods for serum triglycerides (TGs), and based on the glycerol-3-phosphate (GPO)-POD chromogen method. It involves a series of coupled enzymatic reactions catalyzed by LPL, glycerol kinase (GK), GPO and POD, and the absorbance measured at 500 (480-520) nm is proportional to the amount of triglycerides in the specimen. A direct method is used to measure HDL, without any sample preparation. In the first step, LDL, VLDL particles and chylomicrons generate free cholesterol, which, through an enzymatic reaction, produces hydrogen peroxide. The peroxide formed is consumed by the reaction of peroxidase with (N,N-bis-(4-sulfobutyl)-m-toluidine-disodium) to give a colorless product. During the second step, a specific detergent solubilizes the HDL cholesterol, and in conjunction with the action of cholesterol esterase and cholesterol oxidase, peroxidase + 4-aminoantipyrene develop a colored reaction that is proportional to the concentration of HDL cholesterol; and the absorbance is measured at 600 nm. The method of reference for testing LDL cholesterol involves separating lipoproteins by density-gradient ultracentrifugation, a time-consuming method that is only available in specialized laboratories. For this reason, LDL cholesterol is often estimated by measuring total cholesterol and triglycerides (using enzymatic methods), and by direct HDL cholesterol determination. Friedewald's is the most frequently used formula. Friedewald's formula for the estimation of LDL cholesterol: F-LDL-C (mmol/L) = TC - HDL-C - TG/2.2. [6].

Body mass index (BMI, kg/m^2) is commonly calculated as body weight divided by the square of total subject height.

Diabetes, pre-diabetes and normoglycemia were set according to the current International Diabetes Federation and American Diabetes Association guidelines of diagnostic criteria. Normoglycemia was defined as a fasting blood glucose level \6.0 mmol/L; prediabetes was defined as a fasting blood glucose between 5.6 and 7.0 mmol/L or a non-fasting blood glucose between 7.8 and 11.1 mmol/L (when fasting samples were unavailable); type 2 diabetes was defined as a fasting blood glucose above 7.0 mmol/L, or a non-fasting blood glucose above 11.1 mmol/L (when fasting samples were unavailable), or the use of blood glucose lowering medication [7], [8].

2.2. Sample preparation for analysis

After overnight fasting collecting blood into EDTA-containing vacutainer tubes, tubes were placed in the fridge at +4°C up to 1 h, and plasma samples were separated by centrifugation at 3,000 × g for 10 min at +4°C (118). The aliquots of plasma were kept frozen at −80°C until further analyses. Plasma and samples for FAs analysis are stable for up to 4 years when stored at −80°C, but analyses were performed within 6 months.

2.3. Fatty acid analysis

The total lipid extracts from plasma were isolated by mixture of chloroform/isopropanol (7:11, v/v) by the method of Bligh and Dyer [9], with addition of 2,6-di-tert-butyl-4-methylphenol (BHT, 10 mg/100 mL) as an antioxidant. Fatty acid methyl esters (FAME) were prepared as described previously which involved

methanolysis of samples at 50°C for 90 minutes, followed by conversion of free fatty acids to corresponding fatty acid methyl esters [10]. In a screw top tubes aliquot of 100 µL plasma added 2.0 mL of mixture methanol/benzene (4:1, v/v), and samples shaken on a vortex for 5 min. Volume of 200 µl acetyl chloride was added and shaken again on a vortex for 3 min. Methanolysis carried out on the water bath and 50°C, during 90 minutes and by occasional shaking. Samples cooled to room temperature, the content neutralized by 6.0% solution of potassium carbonate, and then centrifuged (3200 o/min; 10 minutes; 20°C). Upper organic layer separated into labeled Eppendorf tubes and mixture of FAMEs stored at -20°C until analysis.

Prepared FAME were dissolved in 20 µL of hexane, and 1 µL of sample was injected into the gas–liquid chromatograph (Shimadzu GC-17A, Shimadzu Co. Ltd, Kyoto, Japan), equipped with a flame ionization detector and separation was achieved using a high polar capillary column (60 m × 0.25 mm ID, film thickness of 0.2 µm, RESTEK, Machnery-Nagel, Germany). The conditions for chromatographic analysis were: the column oven temperature profile was: 80°C initial hold for 1.0 min, then programmed to 165°C at 10°C/min, then to 200°C at 4°C/min and then to 230°C at 20°C/min with a final hold of 7 min (total run time of 95 min); the injection volume is 1µL with split ratio 7:1, split flow 13 mL/min, set press flow 3.3, and a linear velocity of 33cm/s. The carrier gas was hydrogen held at a constant flow of 1.9 mL/min using electronic flow control. Nitrogen was used as make-up gas at 40mL/min. The identification of FAME (from C:12 to C:22) was made by comparing the sample peak retention times with the certified calibration standards of corresponding FAME, 100 mg (RESTEKCORP, Germany). Finally, results of individual FAs are expressed as a µmol/L.

Table 1. Clinical and biochemical characteristics in study population

Parameter	Cases (n=122)	Control (n=81)	*p*
Glucose, mmol/L	8.67 (8.13-9.21)	5.29 (5.18-5.40)	0.001
Cholesterol, mmol/L	5.22 (5.00-5.43)	5.55 (5.33-5.77)	0.001
HDL, mmol/L	1.13 (1.04-1.22)	1.51 (1.43-1.59)	0.001
LDL, mmol/L	2.93 (2.74-3.13)	3.21 (2.99-3.44)	0.001
TG, mmol/L	2.61 (2.31-2.91)	2.00 (1.75-2.25)	0.001
AST, U/L	27.34 (24.42-30.26)	28.44 (25.76-31.13)	0.001
ALT, U/L	31.73 (27.99-35.48)	31.574 (26.14-37.01)	0.001
GGT, U/L	32.78 (22.16-43.40)	18.72 (16.06-21.39)	0.001
HbA1c, %	6.47 (6.19-6.74)	5.11 (4.94-5.27)	0.001
Age, years	61 (59-63)	45 (42-48)	0.001
Sex (M/F)	60/62	32/49	-
BMI, kg/m^2	31 (23-39)	29 (19-39)	0.076

Data are presented as median (interquartile range). LDL – low-density lipoprotein; HDL – high-density lipoprotein; VLDLC – very low-density lipoprotein; ALT – alanin transferase, AST – aspartat transferase, GGT – gamma glutamyl transferase; HbA1c – glycosylated hemoglobin; BMI – body mass index. *Significance of difference in Mann-Whitney test (0.001).

2.4. Calculation for fatty acid enzyme activity

The activities of *SCD1/2* (D9D), D6D, D5D and elongase were estimated from the product/precursor FAs ratios (*SCD1/2*: C16:1n-7/C16:0 and C18:1n-7/C18:0; D6D: C18:3n6/18:2n-6; D5D: C20:4n-6/C20:3n-6 and *ELOVL*: C18:1n-7/C16:1n-7). Since that C18:1n-9 and C18:0 concentrations and their ratio can be more influenced by dietary intakes, it was choosing 16:1n-7/16:0 ratio for the estimation of *SCD1* activity.

2.5. Statistical analysis

Differences between groups was performed using Student t-test followed by non-parametric Mann-Whitney Test to compare the distribution of the variables between different groups. Correlation analysis among variables was performed by Spearman coefficient to calculate the associations between different type of free fatty acids, fatty acid enzymes activity and some clinical and biochemical parameters including fasting plasma glucose, glycosylated hemoglobin, lipid profile, and hepatic enzymes activity. Statistical analyses were done using SPSS software (ver. 23.0) and statistical significance was assumed at a two-tailed $p < 0.05$.

3. Results

The baseline characteristics of diabetes patients (Type 2 diabetes, prediabetes and newly diagnosed T2D) and controls are shown in Table 1. Values of all the measured characteristics were significantly different between cases and controls, except for BMI levels.

Comparison between groups showed significant differences in glucose and glycemic control, lipid profile, liver enzyme activity, and age of study participants (Table 1). Sex differences were observed for the study population in TC, HDL, and TG levels, age, and ALT levels, as well as differences in *SCD1* and elongase activities. In the diabetic group, significant differences were shown in participant age and ALT activity, while in this group, differences in desaturase and elongase activities were not observed. Healthy participants as controls showed differences in glucose levels and glycemic control, HDL and TG levels, age, and AST activity, as well as *SCD1* and elongase activities by sex (data not shown).

The composition and concentration of free fatty acids in plasma, as well as desaturase and elongase activity in diabetic patients and controls are shown in Table 2.

As expected, differences in different types of free fatty acids (i.e. SFA, MUFA and PUFA) and activity of fatty enzymes desaturases and elongases between cases and controls were shown.

Table 2. Concentration of identified and measured of free fatty acids in plasma, and fatty acid enzymes activity (desaturases and elongase) in diabetics and controls

Fatty acid (μmol/L)	Diabetics (n=122)	Control (n=81)	*p**
Lauric acid C12:0	62.35 (21.58-103.12)	20.08 (11.61-28.55)	0.001
Myristic acid C14:0	62.17 (36.44-87.89)	155.18 (105.23-205.12)	0.000
Myristoleic acid C14:1n-5	3.66 (2.53-4.79)	67.34 (15.80-118.88)	0.004
Palmitic acid C16:0	138.20 (103.01-173.39)	106.83 (95.84-117.81)	0.000
Palmitoleic acid C16:1n-7	18.81 (6.61-31.02)	40.04 (17.40-62.68)	0.004
Stearic acid C18:0	62.73 (45.33-80.13)	43.24 (33.95-52.54)	0.000
Oleic acid C18:1n-9	84.09 (61.20-106.98)	86.48 (69.30-103.67)	0.001
Linoleic acid C18:2n-6	141.90 (110.02-173.77)	139.92 (122.44-157.41)	0.004
Linolenic acid C18:3n-6	59.91 (42.59-77.23)	65.42 (34.80-96.05)	0.001
Arachidic acid C20:0	6.16 (4.57-7.75)	11.39 (5.60-17.17)	0.000
DGLA, C20:3n-6	15.36 (13.37-17.35)	19.17 (15.44-22.90)	0.010
Arachidonic acid C20:4n-6	97.17 (63.66-130.67)	34.9 (25.42-44.49)	0.000
EPA, C20:5n-3	6.45 (4.69-8.21)	7.28 (5.37-9.20)	0.001
Behenic acid C22:0	7.05 (3.98-10.13)	15.29 (6.88-23.70)	0.000
DTA, C22:4n-6	3.41 (2.01-4.82)	0.76 (0.050-1.47)	0.000
DPA, C22:5n-3	0.79 (-1.33-2.91)	1.64 (0.12-3.15)	0.010
DHA, C22:6n-6	0.36 (0.82-0.63)	0.98 (-0.23-2.18)	0.035
Sum SFA	338.66 (6.16 to 138.20)	352.01 (11.39 to 155.18)	0.000
Sum MUFA	106.56 (3.66 to 84.09)	193.86 (40.04 to 86.48)	0.000
Sum PUFA	325.31 (0.36-141.90)	270.12 (0.76 to 139.92)	0.000
Fatty acid enzymes activity			
D6D (*FADS2*)	2.72 (2.49-2.95)	3.26 (1.81-4.71)	0.007
D5D (*FADS1*)	2.05 (0.81-3.28)	7.15 (3.42-10.89)	0.002
D9D (*SCD1/2*)	0.14 (0.05-0.23)	0.11 (0.04-0.19)	0.010
ELOVL	0.44 (0.32-0.55)	0.57 (0.40-0.74)	0.010

Data are presented as median (interquartile range. DGLA – dihomo-γ-linolenic acid; EPA – eicosapentaenoic acid; DTA – docosatetraenoic acid; DPA – docosapentaenoic acid; DHA – docosahexaenoic acid; D6D – D6 desaturase; D5D – D5 desaturase; *ELOVL* – fatty acid elongase; *SCD1* – stearoyl Coenzyme A desaturase; NS – non significant. * All differences were tested using ANOVA test.

The association between glucose and glycemic control, lipid profile, liver enzyme activity and age with fatty acid enzyme activity is shown in Table 3. A negative association was found between fasting plasma glucose levels and elongases activity, while no association was observed between fatty acid enzyme activity and glucose control (glycosylated hemoglobin). Interestingly, in the study population, significant associations were not shown between lipid profile and desaturases and elongase activity except for HDL levels and elongases activity, as well as the association between TG levels and D6D activity and elongases activity. A strong association was observed between D6D activity and liver enzyme activities (AST, ALT and GGT), and elongases activity with AST and GGT activities. Also, a negative association was found between the age of the participants and D5D and elongase activities.

Spearman's correlation coefficient was calculated for FFAs (saturated, monounsaturated and polyunsaturated fatty acids) and D6D, D5D, *SCD1* and elongase activities (Table 4), and significant differences between diabetics and controls were observed. This association was driven by the D6D, D5D and D9D variants encoded by the *FADS2* and *FADS1* genes and *SCD1/2*, as well as various fatty acid elongases encoded mainly by the *ELOVL2/6* genes. Three of the identified SFAs, myristic, palmitic and stearic acids, were associated with D6D and *ELOVL* activities. MUFA concentrations, i.e. myristoleic, palmitoleic and oleic acids, had a significant association with *FADS1*, *SCD1/2* and *ELOVL*, respectively. Some of the PUFAs, such as linoleic, linoleic, arachidonic and docosatetraenoic acids were associated with *FADS2* and *ELOVL*, while arachidonic acid was also associated with *SCD1/2* activity. A negative association between myristic acid and myristoleic acid with BMI was found (Table 4).

4. Discussion

The role of fatty acids, especially free fatty acids with different saturation index, in the development of *diabetes mellitus* has long been known. Reported results suggests that their functions depend of many factors such as age, sex, ethnicity, endogenous synthesis of FFAs, dietary habits, but also genetic predisposition and gene-gene interaction as well as gene-diet interaction [11]-[13].

Endogenous metabolism of fatty acids and their composition in the plasma is influenced by the age and gender of the person, health status, epigenetic changes and genes. Desaturation and elongation are two key metabolic reactions in which dietary and endogenous SFA are elongated and converted into monounsaturated FA (MUFA), while highly polyunsaturated FA (PUFA), which the body cannot produce, are synthesized in the liver and adipose tissue from dietary n-3 FA (e.g. α-linolenic acid) and n-6 FA (e.g. linoleic acid). Desaturases and elongases are enzymes that activate these metabolic pathways. Desaturases add double bonds to FAs, while elongases lengthen FAs by adding two carbon atoms to the carbon chain (Figure 1). The metabolism of long-chain fatty acids with different saturation index is important because they are essential in the human body [14]-[19].

Fatty acid desaturases and elongases have long been recognized as an important enzyme for fatty acids regulation by hormones and nutrients, as well as for the formation of specific unsaturated fatty acids. Previously data have shown that the activities of these enzymes, involved in the metabolism and composition of fatty acids in blood cells and different plasma lipids (phospholipids, cholesterol-esters, triglycerides and free

fatty acids) are influenced by various factors: genetics, sex, ethnicity, age, hormonal status, dietary habits and lifestyle. Some of metabolic conditions such as obesity, dyslipidemia, metabolic syndrome, fatty liver disease and diabetes, as well as the use of some antilipemic drugs (e.g. statins, fibrate, etc.) can also affect their activity [20]. In this study, the association between different types of free fatty acids and activity of fatty acid enzymes and the risk of diabetes was investigated. The method of estimating the activities of desaturases (D5D, D6D and D9D) and elongases was used for the reason that it is

Table 3. Spearman's correlation coefficient between FPG, HbA1c, lipid profile, hepatic enzymes activity, BMI and age with desaturases (D6D, D5D, D9D), and elongase activity in diabetics and controls

Fatty acid enzymes	D6D	D5D	D9D	Elongase
Fasting plasma glucose, mmol/L	rho=0.111 p= 0.142	rho=-0.160 p= 0.150	rho=0.035 p= 0.647	rho=-0.217 *p*= 0.004
Total cholesterol, mmol/L	rho=0.014 p= 0.358	rho=-0.103 p= 0.358	rho=0.052 p= 0.495	rho=0.005 *p*= 0.946
HDL, mmol/L	rho=-0.119 p= 0.121	rho=0.130 p= 0.241	rho=-0.115 p= 0.130	rho=0.271 p= 0.000
LDL, mmol/L	rho=-0.019 p= 0.806	rho=-0.052 p= 0.641	rho=0.093 p= 0.230	rho=0.000 p= 0.993
TG, mmol/L	rho=0.238 p= 0.001	rho=0.024 p= 0.830	rho=0.029 p= 0.705	rho=-0.241 *p*= 0.001
AST, U/L	rho=0.251 p= 0.001	rho=-0.034 p= 0.759	rho=0.054 p= 0.477	rho=-0.214 p= 0.005
ALT, U/L	rho=0.194 p= 0.010	rho=0.077 p= 0.492	rho=-0.013 p= 0.867	rho=-0.112 p= 0.142
GGT, U/L	rho=-0.173 p= 0.025	rho=0.136 p= 0.222	rho=-0.025 p= 0.750	rho=0.174 p= 0.024
HbA1c, %	rho=-0.074 p= 0.333	rho=0.020 p= 0.855	rho=0.000 p= 0.996	rho=-0.041 p= 0.592
Age, years	rho=0.042 p= 0.595	rho=-0.331 *p*= 0.003	rho=0.086 p= 0.272	rho=-0.163 *p*= 0.037
BMI, kg/m^2	rho=0.007 p= 0.922	rho=0.016 p= 0.883	rho=0.111 p= 0.142	rho=-0.032 p= 0.654

Table 4. Spearman correlation between fatty acids with desaturases (D6D, D5D, SCD-1), elongase activity and BMI in diabetics and controls

Fatty acid enzymes Fatty acid	D6D	D5D	SCD-1	Elongase	BMI
Lauric acid, C12:0	rho=-0.233 *p*= 0.154	rho=0.265 *p*= 0.191	rho=-0.226 *p*= 0.166	rho=0.292 *p*= 0.071	rho=0.171 *p*= 0.169
Myristic acid, C14:0	rho=-0.372 *p*= 0.000	rho=0.303 *p*= 0.010	rho=-0.081 *p*= 0.356	rho=0.409 *p*= 0.000	rho=-0.280 *p*= 0.000
Myristoleic acid, C14:1n-5	rho=0.040 *p*= 0.739	rho= -0.372 *p*= 0.019	rho=0.107 *p*= 0.373	rho=-0.102 *p*= 0.397	rho=-0.337 *p*= 0.001
Palmitic acid, C16:0	rho= -0.167 *p*= 0.027	rho=0.095 *p*= 0.393	rho=0.026 *p*= 0.730	rho=0.226 *p*= 0.003	rho=-0.058 *p*= 0.418
Palmitoleic acid, C16:1n-7	rho=0.087 *p*= 0.308	rho=-0.152 *p*= 0.217	rho=0.205 *p*= 0.016	rho=-0.065 *p*= 0.446	rho=-0.073 *p*= 0.349
Stearic acid, C18:0	rho= -0.309 *p*= 0.000	rho=-0.062 *p*= 0.579	rho=-0.063 *p*= 0.405	rho=0.337 *p*= 0.000	rho=-0.039 *p*= 0.582
Oleic acid, C18:1n-9	rho=-0.072 *p*= 0.342	rho=0.144 *p*= 0.195	rho=-0.011 *p*= 0.884	rho=0.200 *p*= 0.008	rho=-0.072 *p*= 0.304
Linoleic acid, C18:2n-6	rho= -0.205 *p*= 0.006	rho=-0.078 *p*= 0.486	rho=-0.085 *p*= 0.261	rho=0.286 *p*= 0.000	rho=-0.068 *p*= 0.332
Linolenic acid, C18:3n-6	rho= -0.455 *p*= 0.001	rho=-0.019 *p*= 0.901	rho=-0.054 *p*= 0.703	rho=0.452 *p*= 0.001	rho=0.100 *p*= 0.383
Arachidic acid, C20:0	rho=-0.145 *p*= 0.316	rho=0.017 *p*= 0.910	rho=-0.053 *p*= 0.713	rho=0.098 *p*= 0.502	rho=0.188 *p*= 0.102
DGLA, C20:3n-6	rho=-0.057 *p*= 0.475	rho=0.048 *p*= 0.697	rho=0.039 *p*= 0.622	rho=0.130 *p*= 0.102	rho=-0.033 *p*= 0.671
Arachidonic acid, C20:4n-6	rho=-0.473 *p*= 0.000	rho=-0.203 *p*= 0.121	rho=-0.359 *p*= 0.005	rho=0.374 *p*= 0.004	rho=-0.038 *p*= 0.731
EPA, C20:5n-3	rho=-0.159 *p*= 0.502	rho=0.470 *p*= 0.105	rho=-0.023 *p*= 0.922	rho=0.275 *p*= 0.240	rho=-0.196 *p*= 0.327
Behenic acid, C22:0	rho=-0.110	rho=-0.096	rho=0.305	rho=0.041	rho=0.222

	p= 0.518	p= 0.578	p= 0.067	p= 0.813	p= 0.078
DTA, C22:4n-6	rho=0.585	rho=-0.247	rho=0.273	rho=-0.463	rho=0.058
	p= 0.001	p= 0.205	p= 0.144	p= 0.010	p= 0.732
DPA, C22:5n-3	rho=0.266	rho=-0.463	rho=-0.028	rho=-0.203	rho=0.038
	p= 0.404	p= 0.178	p= 0.931	p= 0.507	p= 0.885
DHA, C22:6n-6	rho=0.482	rho=-0.376	rho=-0.302	rho=-0.325	rho=0.061
	p= 0.108	p= 0.206	p= 0.274	p= 0.237	p= 0.788

more practical than measuring their activities directly using stable isotopes in liver biopsies, which is invasive for patients and expensive. Unlike desaturases, fatty acid elongases have only recently been recognized as transcriptionally regulated proteins. Seven different subtypes of fatty acid elongases (fatty acid elongase from *ELOVL-1* to *ELOVL-7*) are present in human genomes, and a significant association of *ELOVL-2/5/6* with the highest risk factors for the development of diabetes has been demonstrated [21].

As expected, significant differences were found between patients and controls in glucose, control of glycaemia, lipid profile (TC, HDL, LDL, and TG), liver enzymes activity as well as age of participants (Table 1). Also, significant differences between FFA concentrations and the activity of fatty acid desaturases and elongases were shown in diabetics and controls (Table 2). This is consistent with previous evidence that higher plasma levels of free fatty acids, particularly SFA and some PUFA, have shown a positive association with insulin resistance and increased risk of development diabetes. It was found that elevated plasma concentrations of myristic, palmitic, stearic, and oleic fatty acids were strongly correlated with elevated fasting plasma glucose and HbA1c levels, while LC-SFAs (myristic, palmitic, and stearic acid) are associated with the increasing incidence of T2D [16], [22]-[24].

Research data show that desaturases are well-regulated enzymes that play a major role in cellular and whole body lipid composition and glycaemia characteristics in patients with type 2 diabetes [14]-[17]. A strong association between HDL and TG levels was shown, while no association was observed between total cholesterol and LDL levels (Table 3). These results suggest that TG and HDL level may be mediators that connect intake and metabolism of fatty acids to metabolic risk. Interestingly, glucose levels, but not HbA1c, were found in study participants as a marker of glycemic control (Table 3), and this in an agreement with previous findings that D6D activity effects on glucose metabolism [18], [19]. In the analysis of the relationship between activities of liver enzymes (i.e. AST, ALT, and GGT) and fatty acid enzymes, the significant association in D6D and elongases was demonstrated. This is not surprising because the *FADS2* gene encodes enzymes that play an important role in fatty acid metabolism and lipogenesis [23]. Also, specific hepatic fatty acid elongases, *ELOVL-5* and *ELOVL-6*, are regulated in the liver by glucose and lipids and insulin, which control both elongase and desaturase activities. Metabolic conditions such as diabetes and obesity cause disturbances in the composition of liver lipids by controlling the main transcription factors that affect the activities of elongase and desaturase. Thus, regulation of fatty acid enzymes activity may play a key role in managing liver lipid composition in response to changes in dietary habits and hormonal status [18], [21], [25], [26]. Obesity, primarily as a consequence of a poor lifestyle, is one of the most important risk factors for the development of type 2 diabetes [27]. According to BMI values, control participants in this study were overweight (preobese) while diabetics were obese, and no significant difference was found between these two groups (Table 1).

In this study, Spearman's correlation coefficient was examined between FPG, HbA1c, lipid profile, liver enzyme activity, BMI and age, as well as analyzed free fatty acids with desaturases activity (D6D, D5D, D9D) and elongases in diabetics and control groups. An inverse and positive strong association was found mainly between D6D and elongases for different FFAs and subject age, except for lauric acid, C20:0, DGLA, EPA, DPA and DHA (Tables 3 and 4). The strong association of *FADS1/2* with fatty acid concentrations is not surprising because *FADS1* and *FADS2* encode D-5 desaturase and D-6 desaturase, respectively, which are the major rate-limiting enzymes in the metabolism of fatty acids (Figure 1) [3]. In addition, *de novo* synthesis of fatty acids from glucose occurs in the liver, a process in which the structure of fatty acids is altered through metabolic pathways that include desaturation, elongation, oxidation, and β-oxidation. These and similar changes occur in fatty acids that come from the diet [14]. Previously studies showed that ratios of fatty acid products/precursors reflecting D-5 desaturase and D-6 desaturase activity were inversely and positively associated with incident of type 2 diabetes [28]-[30]. Interestingly, D-9 desaturase activity was estimated only with palmitoleic and arachidonic acids (Table 4). This enzyme, D9D (encoded by *SCD1*, stearoyl-CoA desaturase-1), is an important enzyme in controlling the lipid composition of the whole body. Recent data have shown that higher *SCD-1* activity is positively associated with the risk of diabetes, while deficiency of the *SCD* gene prevents diet-induced obesity and, therefore, can be used as a target for anti-obesity drugs [31]. The pathway of mechanisms mediating the association of estimated D5D, D6D and D9D as well as *ELOVLs* activity with the main risk factors, genetic and environmental for the development of T2D is still not well evaluated [18], [19], [24].

The activity of enzymes (desaturases, elongases) involved in the metabolism of both, endogenous fatty acids and dietary fatty acids, as well as their concentrations and composition, are strongly influenced by the level of obesity (BMI) and dietary habits, which represent one of the risk factors for the development of T2D [27], [32]. Thus, differences in fatty acid profiles and enzyme activities may be

consequences of obesity and diet. However, dietary habits and food intake were not investigated in this study. No correlation between fatty acid enzyme activity and BMI was observed (Table 3), while a negative correlation between myristic acid and myristoleic acid with BMI was established (Table 4). This is important because the effects of myristic and myristoleic acids have been examined in previous studies. It was found that myristic acid administration improves hyperglycemia in T2D, or that the risk of developing diabetes, which is associated with fatty acid metabolism in the liver (both of these acids participate in the DNL pathways), was independent of insulin sensitivity and response [12], [16], [28], [33]. These results as well as the published data of other authors provide insight into the roles of fatty acid desaturases and specific elongases on human health and diseases, that inhibition of these pathways may provide a new strategy to prevent diabetes. In the future, this could lead to the development of fatty acid desaturases and elongases activity-based personalized therapeutic approaches as effective and non-toxic inhibitors.

5. Conclusion

In conclusion, the assessment of the activities of *FADS1*, *FADS2*, *SCD-1/2* and elongases, as well as their altered activities in the control of long-chain fatty acids metabolism, have a strong association with the development of diabetes. Also, including known risk factors for T2D, the obtained results indicate the possible roles of these fatty acid enzymes, and in particular myristic acid (C14:0), palmitic acid (C16:0), *cis*-palmitoleic acid (C16:1n-7), *cis*-myristoleic acid (C14:1n-5), stearic acid (18:0) and *cis*-oleic acid (c18:1 n-9) in the *de novo* lipogenesis (DNL) pathway in the development of *diabetes mellitus* in humans, and represent potential biomarkers for diagnosis and individual therapeutic treatment in patients with type 2 diabetes.

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