FREE FATTY ACIDS AND HEPATIC ACTIVITY IN TYPE 2 DIABETES

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Abstract. Metabolic derangements in Type 2 diabetes mellitus (T2D) are associated primarily with the carbohydrate and lipid levels disturbances. Increased flow of free fatty acids (FFAs) into the blood that is coming from the adipocytes as well as an elevated flux of FFAs from *de novo* lipid synthesis in the liver contribute to these metabolic disturbances. Previous studies suggested a strong association of the hepatic activity of certain enzymes, such as aspartate and alanine transferase (AST, ALT), gamma glutamyl transpeptidase (GGT), and alkaline phosphatase (ALP) with the progression of T2D. In this study, the potential association of the hepatic activities of the liver enzymes and FFAs levels in T2D was examined. Analysis of the activities of ALT, AST, GGT and AP, as well levels of FFAs, fasting plasma glucose (FPG), and lipid profile was performed in 40 healthy control and 71 diabetic subjects. All participants were free of hepatitis, viral infections or active liver damage. The results showed a positive association between levels of palmitic and oleic acids with ALT activity ($p < 0.05$), while the activity of GGT was significantly associated with the levels of palmitic, stearic, and oleic fatty acids ($p < 0.01$). Interestingly, in control group, a positive correlation was found between palmitoleic acid levels with ALT activity ($p < 0.05$), and a negative correlation palmitoleic acid with ALP activity ($p < 0.05$) was observed. Obtained data suggest that an elevation of free fatty acid levels and the hepatic fat accumulation in insulin-resistant conditions affect the hepatic enzymes activities, which might contribute further to the progression of Type 2 diabetes and its complications.

Keywords: Free fatty acids, hepatic enzymes, Type 2 diabetes

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most prevalent chronic metabolic diseases around the world, with an increase in its prevalence due to genetic and environmental risk factors. According to International Diabetes Federation (IDF) more than 600 million world population will be affected till 2035. Metabolic derangements in T2D are associated primarily with the carbohydrate and lipid pathway metabolisms disturbances [1].

Previous studies reported that circulating concentrations of free fatty acids (FFAs) from adipose tissue have been proposed to be a possible link between abdominal obesity, insulin resistance (IR) metabolic syndrome (MetS), increased risk of T2D, and other obesity-related metabolic disorders [2]. Insulin resistant adipocytes release high concentration of FFAs to blood which promotes lipid accumulation and IR in key organs (e.g., liver, pancreas, heart, and skeletal muscle) and results in whole body-metabolic alterations [3, 4]. Increased flow of FFAs into the blood that is coming from the adipocytes as well as an elevated flux of FFAs from *de novo* lipid (DNL) synthesis in the liver contribute to accumulation of fat and hepatic insulin resistance [5-7].

The liver is a major organ that has a key role in carbohydrate, lipid and protein metabolisms. In clinical practice, measurement of the activity of specific

liver enzymes, such as aspartate and alanine transferase (AST, ALT), gamma glutamyl transpeptidase (GGT), and alkaline phosphatase (ALP) demonstrate the liver function [8, 9]. Several investigations have been observed the association of elevated serum hepatic enzyme levels with developing of T2D. Also, studies suggested a strong predictive function of the liver enzymes as independent markers with the progression of T2D [10-12].

Figure 1. Increased release of free fatty acids by adipose tissue and hyperglycemia/hyperinsulinemia, which promotes hepatic *de novo* lipogenesis (DNL).

Furthermore, fat accumulation with both elevated FFAs and liver enzymes activity lead to nonalcoholic fatty liver disease (NAFLD) as the most common liver disease and disorder. Recent epidemiological

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investigations reported that T2D patients have a two-fold higher risk for development of NAFLD and vice versa; the prevalence of NAFLD among patients with T2D is about 80 % [13].

The diagnosis of NAFLD in patients with or without T2D according to recommendation of the ADA is elevated ALT or nonalcoholic fatty liver (NAFL). The identification and detection of NAFLD performed by standard imaging techniques, such as ultrasonography (USG), computed tomography (CT), and magnetic resonance imaging (MRI) or the gold standard method - liver biopsy. However, these methods are insensitive, time-consuming, expensive and invasive with seriously consequences for patients, and in routine clinical laboratory often are not appropriate. Therefore, a multidisciplinary approach needed to identify T2D patients at risk for developing of NAFLD, using sensitive, accurate, reliable and cost-effective analytical tool [14, 15].

In this study, the potential association of the activities of the liver enzymes and free fatty acids levels in T2D patients was examined.

2. EXPERIMENTAL

2.1. Subjects

The study included 111 participants: 71 patients with T2D, and 40 healthy controls of both gender, aged from 35 up to 65 years. T2D was diagnosed by endocrinologists and diabetologists according to definitions of American Diabetes Assotion (ADA) [16]. Diabetic patients and healthy subjects were recruited at the Department of Internal Medicine, General Hospital in Tešanj. Patients treated with insulin, lipid-lowering drugs and patients with acute and chronic kidney disease, endocrine disorders, acute infection and/or inflammation and hormonal therapy were excluded. Individuals in control group were not taking any medication during of the study. The research was approved by the Hospital Ethics Committee and was performed in accordance with the Helsinki Declaration – Ethical Principles for Medical Research Involving Human Subjects (initiated June 1964, last amended October 2000) and each patient has given a written informed consent [17].

2.2. Biochemical analyses

Fasting whole blood samples were obtained from an antecubital vein and blood samples were left for 20 minutes at room temperature, and centrifuged at 3000 rpm for 4 to 5 minutes. Serum levels of fasting glucose, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol), glycated hemoglobin (HbA1c), liver enzyme activities alanine aminotransferase, aspartate aminotransferase, γ -glutamyl transpeptidase and alkaline phosphatase (ALT, AST, GGT and ALP) were determined by using VITROS auto analyzer 350 Chemistry System (Ortho-Clinical Diagnostics, Rochester, New York, USA). For analysis of all biochemical parameters, IFCC

(International Federation of Clinical Chemistry and Laboratory Medicine) standard protocols were used.

2.3. Sample preparation

Preparation of samples for fatty acid analysis was done by methylation of serum samples, followed by conversion of FFAs to corresponding fatty acid methyl esters [15]. Fourteen FFAs with medium and long-chain length, i.e. from C14 to C22 carbon atoms with different degree of unsaturation, in serum of T2D, and nondiabetic subjects were analyzed on a Shimadzu QP-5000 GC/MS gas chromatograph equipped with mass spectrometer detector (Shimadzu, Tokyo, Japan). The identification of each fatty acid peak was obtained by comparing the retention time of the peak with the retention times of referent standards with known fatty acids composition, and heptadecanoic acid C17:0 as internal standard was used for calculation the concentration of individual FFAs.

Statistical analysis was done using SPSS 17.0 for Windows (SPSS, Chicago, IL, USA). Data are presented as the mean $\pm$ standard deviation when data were found to be normally distributed or as the median if the distribution was skewed. Differences between groups were analyzed using the Student's *t*-test or the Mann–Whitney U test. Spearman correlation analysis was used to examine the correlation between serum FFA levels and biochemical and laboratory parameters. Statistical significance was set as $P < 0.05$.

3. RESULTS AND DISCUSSION

The biochemical characteristics of study participants are presented in Table 1. Values of all the measured traits were significantly different between cases and controls.

Table 1. Biochemical characteristics in study populations

Parameter	Cases N=71	Controls N=40	<i>p</i> value**
Age, years	61 (37-87)	43 (23-62)	0.000
Glucose, mmol/L	9.53 (4.97-16.60)	5.26 (4.10-6.50)	0.000
Total Cholesterol, mmol/L	5.51 (3.83-10.90)	5.77 (3.30-8.00)	0.000
Triglyceride, mmol/L	5.77 (3.30-8.00)	2.63 (0.46-12.06)	0.000
AST, U/L	32 (18-62)	29 (13-125)	0.000
ALT, U/L	37 (17-125)	31 (12-125)	0.000
GGT, U/L	24 (6-258)	17 (6-32)	0.000
ALP, U/L	75 (41-155)	65 (30-111)	0.000
HbA1c, %	6.72 (4.71-10.20)	4.52 (4.00-5.51)	0.000

*Values represent medians (lower–upper quartile).

**Significance of difference in Mann–Whitney test for data following non-normal distributed and *t*-test for normal distributed data. AST-aspartate aminotransferase; ALT-alanine aminotransferase; GGT-gamma glutamyl transpeptidase; ALP-alkaline phosphatase; HbA1c-glycated hemoglobin

Table 2. Composition and concentration of free fatty acids in study populations.

Fatty acid	Cases N=71	Controls N=40	p value**
Myristic acid, C14:0 μmol/L	2.22 (0.60-10.30)	1.73 (0.40-5.00)	0.000
Myristoleic acid, C14:1 μmol/L	1.01 (0.40-1.80)	1.09 (0.80-2.00)	0.000
Palmitic acid, C16:0 μmol/L	37.76 (2.40-158.10)	28.13 (11.40-57.30)	0.000
Palmitoleic acid, C16:1 μmol/L	4.87 (0.90-23.20)	3.04 (0.500-6.80)	0.000
Stearic acid, C18:0 μmol/L	9.52 (3.70-37.10)	8.99 (4.10-36.40)	0.000
Oleic acid, C18:1 μmol/L	30.79 (10.70-137.20)	23.21 (8.80-42.20)	0.000
Linoleic acid, C18:2 μmol/L	43.35 (16.50-140.90)	39.53 (2.50-78.50)	0.000
γ-Linolenic acid, C18:3 μmol/L	3.13 (0.60-7.70)	1.90 (1.90-1.90)	0.185
Arachidic acid, C20:0 μmol/L	LD***	6.90 (6.90-6.90)	0.000
Eicosatrienoic acid, C20:3 μmol/L	7.69 (1.60-17.50)	7.37 (3.00-14.40)	0.000
Behenic acid, C22:0 μmol/L	LD***	0.80 (0.80-0.80)	0.000
Docosatetraenoic acid, C22:4 μmol/L	LD***	1.08 (0.90-1.30)	0.001
Docosapentaenoic acid, C22:5 μmol/L	LD***	1.34 (0.60-2.30)	0.013
Docosaxaenoic acid, C22:6 μmol/L	LD***	1.92 (1.00-2.90)	0.005

*Values represent medians (lower–upper quartile).

**Significance of difference in Mann–Whitney test for data following non-normal distributed and t-test for normal distributed data.

***LD-limit detection, concentration below of detection limit.

Composition and concentrations of individual FFAs are summarized in Table 2, and there were significantly differ between two groups, except for γ-linolenic acid (γ-C18:3) concentrations [18]. T2D and state of hyperinsulinemia in combination with a high FFA flux as well as hyperglycemia are known to up-regulate lipogenic transcription factors. The fatty acids overload and the hepatic mitochondrial oxidation system leading to accumulation of FFAs in the liver. In the most of cases, liver function in Type 2 diabetes patients contribute alteration of liver enzyme levels i.e. ALT, AST, AP and GGT. Of these liver enzymes, ALT is most closely related to liver fat accumulation and consequently ALT have been used as a marker of fatty liver and its dysfunction [19].

The results of Spearman's analysis showed a positive association between levels of palmitic and stearic acids with ALT activity ($p < 0.05$), while the activity of GGT was significantly associated with the levels of palmitic, stearic, and oleic acids ($p < 0.01$). Negative correlation of ALP activity with myristoleic and dihomo-γ-linolenic acids was observed ($p < 0.05$). In addition, the one of the main fatty acids in DNL, palmitoleic acid (C16:1) negatively associated with ALT activity but only in controls, while the same fatty acid was negative correlated with ALP in diabetic patients. Interestingly, AST showed no significant association with detected FFAs (Table 3).

Table 3. Spearman's correlation coefficient (rho) between serum free fatty acid concentrations (μmol/L) and liver enzymes (AST, ALT, GGT, and ALP) in populations.

Enzymes FFA	AST	ALT	GGT	ALP
C 14:1	n.d.	n.d.	n.d.	$r = -0.539$ $p = 0.026$
C 16:0	n.d.	$r = 0.217$ $p = 0.021$	$r = 0.196$ $p = 0.015$	n.d.
C 16:1	n.d.	$r = 0.402$ $p = 0.034$ (only in controls)	n.d.	$r = -0.304$ $p = 0.025$ (only in cases)
C 18:0	n.d.	n.d.	$r = 0.213$ $p = 0.025$	n.d.
C 18:1	n.d.	$r = 0.220$ $p = 0.021$	$r = 0.220$ $p = 0.006$	n.d.
C 20:3	n.d.	n.d.	n.d.	$r = -0.198$ $p = 0.039$

n.d.-not detected

De novo lipogenesis is the primary metabolic pathway involved in the endogenous synthesis of specific fatty acids, such as C16:0, C16:1n-7, C18:0, and C18:1n9, and it is linked to the pathophysiology of many metabolic diseases including incidence of T2D [20]. Chronic hyperglycemia and IR together with hyperinsulinemia cause biochemical reactions that lead to increase in lipogenesis and its associated fatty acid changes. Accumulation of FFAs is known to be toxic to hepatocytes engendering disruption of cell membrane, mitochondrial dysfunction, oxidative stress and increase in production of proinflammatory cytokines [21]. Fat accumulation in hepatocytes lead to liver injury which shown with slightly rise in ALT, AST, and GGT activities, and normal function with or without ALP elevation. These findings consistent and in line with previously researches which confirmed suggested potential pathophysiological and metabolic pathway mechanisms [22, 23].

Recent studies showed that patients with T2D have identified at independent and high risk factor for development of NAFLD. NAFLD is characterized by increased lipids accumulation and fatty infiltration in the liver but still underlying metabolic pathways unknown. Researchers are proposed two possible mechanisms that may explain this chronic metabolic state: i) elevated concentrations of FFA to the liver cause fatty infiltration in the hepatic cells, and ii) increase of DNL as an important source of FFAs and triglycerides in the liver. In present study, although do not performed NAFLD test neither noninvasive nor invasive methods, significant association of elevated ALT, GGT, and ALP especially in cases (Table 3), suggested that Type 2 diabetes patients included in this study have NAFLD [24, 25].

However, the possible explanation of different profile and composition of FFAs and hepatic enzyme activities in serum might be due to influence of gender, age, and ethnicity as well dietary habit and physical activity in study population.

5. CONCLUSION

In summary, obtained data suggest that an elevation of free fatty acid levels and the hepatic fat accumulation in insulin-resistant conditions affect the hepatic enzymes activities, which might contribute further to the progression of Type 2 diabetes and developing of its complications.

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