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ASSOCIATION OF FREE FATTY ACID CONCENTRATIONS WITH GLUCOSE LEVELS IN BOSNIAN SUBJECTS

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Abstract. *Although there is considerable evidence suggesting a strong association of glucose, glycated hemoglobin and fatty acid levels with Type 2 diabetes mellitus (T2D), a limited number of studies have examined the association of individual fatty acids with disease progression. Acutely elevated plasma fatty acids stimulate insulin secretion while chronically elevated plasma fatty acids alter and disrupt insulin secretion. Furthermore, free fatty acids (FFA) are known to interfere with normal glucose homeostasis and affect pancreatic β-cell dysfunction. The study included 24 patients with newly diagnosed type 2 diabetes and 27 healthy controls, and analysis of the level of glucose and glycated hemoglobin was done by routine methods. The concentration of individual FFA was determined by gas chromatography with mass spectrometry detection. The results showed statistically significant differences in glucose, HbA1c, lipid profile, palmitic, linolenic, arachidonic, arachidonic, behenic acid as well as in DHA levels in all participants. In healthy subjects, no significant correlation was found between glucose and individual free fatty acids but a negative correlation was observed between DHA and glycated hemoglobin ($p<0.05$). Newly diagnosed diabetics showed a negative significant association between glucose and lauric acid concentrations, and also the association of glycated hemoglobin with myristic acid levels ($p<0.01$ and $p<0.05$, respectively). These data indicate the association of different types of free fatty acids with glucose levels and their control in the serum of healthy and newly diagnosed type 2 diabetics, and therefore indicate the importance of monitoring glucose levels as well as glycated hemoglobin with concentrations of individual free fatty acids in the progression of diabetes.*

Keywords: *Free fatty acids, glycemic control, Type 2 diabetes*

1. INTRODUCTION

Type 2 diabetes (T2D) as a common type of diabetes is a heterogeneous condition that is caused by insulin resistance (IR) and a progressive decrease in pancreatic β-cell function, leading to defective insulin secretion and insulin sensitivity. Fatty acids, especially elevated concentrations of free fatty acids (FFA) in the circulation and disorders of lipid metabolism are associated with an increase in the incidence of T2D. [1].

Although there is strong evidence suggesting a strong association of glucose as well as glycated hemoglobin and fatty acids with T2D, a limited number of studies have examined the association of individual fatty acids with disease progression. Acutely elevated plasma fatty acids stimulate insulin secretion, while chronically elevated plasma fatty acids alter and disrupt insulin secretion. It is also known that free fatty acids interfere with normal glucose homeostasis and affect pancreatic β-cell dysfunction (Figure 1). [2-3].

Mechanisms underlying pancreatic β-cell failure include processes induced by lipotoxicity and gluco-lipotoxicity. When insulin resistance occurs, elevated free fatty acids such as palmitate (C16:0, PA) acutely increase β-cell mass and insulin secretion to compensate for insulin insensitivity (Figure 1). Chronic increase of free fatty acid in the plasma leads to lipotoxicity, which contributes to the dysfunction and apoptosis of β-cells and, lead to the onset and development of type 2 diabetes. [4-5].

The main aim of this study was to investigate the profile and specific composition of FFA in plasma in healthy control subjects and newly diagnosed T2D patients and their association with glucose concentrations and glycemic control.

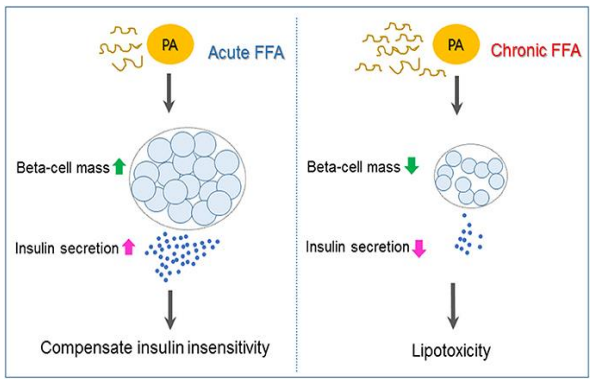

Figure 1. Free fatty acids and their role in Type-2 diabetes

2. MATERIALS AND METHODS

The study included 24 patients from Sarajevo University Clinical Centre, newly diagnosed with Type

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2 diabetes and without treatment (have not use oral hyperglycemic) and 27 healthy individuals as control, and who gave their consent to participate in the study. Classification of patients was made according to criteria used by American Diabetes Association: fasting plasma glucose (FPG) less 5.6 mmol/L for healthy individuals and 7.0 mmol/L or higher for diabetics; and glycemic control (HbA1c) less 5.7 % for healthy individuals and 6.5 % or higher for diabetes subjects. [6]. Analysis of glucose and glycated hemoglobin levels were done by IFCC methods while FFAs concentrations were determined by gas chromatography.

All research involving human subjects and material derived from human subjects in this study was done in accordance with ethical principles outlined in World Medical Association Declaration of Helsinki, and complied with the ethical recommendations and practices of the University Clinical Centre in Sarajevo. Written informed consents were obtained for each participant involved in the study.

2.1. Sample preparation

Blood samples collected after overnight fasting into commercially available anticoagulant-treated tubes and after centrifugation for 15 minutes at 2000 x g, plasma samples stored at – 20°C until analysis. Fatty acid analysis is described in detail elsewhere. Briefly, free fatty acids were extracted from total lipids from plasma samples, and the extracts after methylation (transesterification reaction) were separated and quantified by gas chromatography with mass spectrometry. [7-8].

2.2. Fatty acid analysis

For fatty acid analysis, lipids were extracted with chloroform-methanol 2:1 (vol/vol) (Folch et al. method) and samples of fatty acid methyl esters (FAMEs) of free fatty acids were prepared according to self-modifying method of Lepage and Roy. [7-8]. The samples were analyzed on a Shimadzu QP-5000 GC/MS gas chromatograph equipped with mass spectrometer detector (Kyoto, Japan), and capillary column Resterkorp OPTIMA® 120 (30m x 0.32 x 0.25µm film thickness) (Macherey-Nagel, Düren, Germany). The identity 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, while the concentration of individual FFAs was calculated by using known concentrations of corresponding standards.

2.3. Statistical analysis

Statistical analysis was done using SPSS 23.0 for Windows. Differences between groups was performed using Student t-test followed by the Mann-Whitney Test, while the Spearman coefficient was used to calculate the associations between FFAs and glucose and HbA1c as a parameter of glucose control. A P value ≤ 0.05 was considered statistically significant.

3. RESULTS

Table 1 shows the clinical and biochemical characteristics of all research participants, newly diagnosed T2D patients and control subjects. The values of all measured parameters were significantly different between the studied populations.

The profile and composition of the analyzed free fatty acids are shown in Table 2. The concentrations of individual free fatty acids were different between all study participants, newly diagnosed T2D patients, as well as the control group, except for palmitoleic acid (C16:1) in diabetics, dihomo-g- linolenic (DGLA) and docosahexaenoic acid (DHA) in healthy controls, and docosahexaenoic acid (DPA) for the all participants. Also, the concentration of free fatty acids in the plasma was higher in the diabetic group than in the control group, except for lauric acid (C12:0), myristic acid (C14:0), palmitoleic acid (C16:1), DGLA and DPA. It is interesting that DTA levels were the same in all examined groups (Figure 2).

Table 1. Clinical and biochemical characteristics of all study participants, patients with newly diagnosed T2D, and healthy controls.

	All participants	**Newly diagnosed T2D patients**	**Controls**
Males/Females	28/23	14/10	14/13
Age (years)	57^{c} (52-61)	64^{c} (60-68)	48^{c} (42-54)
Glucose, mmol/L	6.70^{c} (6.21-7.19)	8.20^{c} (7.59-8.81)	5.37^{c} (5.24-5.49)
Cholesterol, mmol/L	4.96^{b} (4.69-5.23)	4.54^{b} (4.12-4.94)	5.34^{b} (5.02-5.65)
HDL, mmol/L	1.22^{c} (1.08-1.36)	1.17^{c} (0.89-1.44)	1.27^{c} (1.16-1.38)
LDL, mmol/L	3.00^{c} (2.72-3.28)	2.47^{c} (2.08-2.86)	3.45^{c} (3.12-3.78)
Triglyceride, mmol/L	1.87^{b} (1.61-2.13)	2.08^{b} (1.77-2.40)	1.68^{b} (1.28-2.08)
VLDL, mmol/L	0.99^{c} (0.71-1.28)	0.95^{c} (0.80-1.10)	1.03^{c} (0.50-1.56)
HbA1c, %	6.41^{c} (6.16-6.66)	7.16^{c} (6.88-7.44)	5.74^{c} (5.57-5.90)

Values represent medians (lower-upper quartile). LDL – low-density lipoprotein; HDL – high-density lipoprotein; VLDLC – very low-density lipoprotein; HbA1c – glycated hemoglobin. *Significance of difference in Mann-Whitney test (^{a}0.05, ^{b}0.01, ^{c}0.001).

Table 2. Free fatty acids of all participants, newly diagnosed T2D patients and control subjects.

Fatty acid (µmol/L)	**All participants**	**Newly diagnosed T2D patients**	**Controls**
Lauric acid C12:0	27.88^{c} (15.30-40.45)	36.23^{b} (12.27-60.19)	20.45^{b} (8.76-32.14)
Myristic acid C14:0	245.85^{c} (196.44-295.26)	239.78^{c} (172.08-307.48)	251.68^{c} (175.04-328.32)
Myristoleic acid C14:1	54.29^{a} (11.88-96.64)	5.09^{c} (2.57-7.61)	101.46^{a} (20.19-182.73)
Palmitic acid C16:0	233.58^{c} (160.76-306.41)	362.45^{c} (227.05-497.86)	115.02^{c} (93.44-136.61)

Palmitoleic acid C16:1	63. 19[c] (27.86-98.52)	49.64[NS] (-5.16-104.44)	75.70[b] (27.14-124.26)
Stearic acid C18:0	154.97[c] (123.73-186.21)	242.87[c] (204.20-281.54)	76.83[c] (55.78-97.89)
Oleic acid C18:1	137.14[c] (80.52-193.76)	187.16[b] (78.69-295.64)	92.67[b] (43.94-141.41)
Linoleic acid C18:2	259.95[c] (194.16-325.73)	363.41[c] (239.04-487.79)	167.98[c] (128.80-207.15)
Linolenic acid C18:3	65.75[c] (51.37-80.13)	103.22[c] (86.15-120.30)	29.78[c] (18.88-40.67)
Arachidic acid C20:0	4.97[c] (3.55-6.38)	7.48[c] (4.86-10.10)	2.73 (1.96-3.49)
DGLA, C20:3	12.79[a] (3.28-22.30)	11.91[b] (5.37-18.44)	13.51[NS] (-4.59-31.61)
Arachidonic acid C20:4	118.19[c] (88.03-148.35)	201.18[c] (159.97-242.39)	41.58[c] (31.77-51.39)
EPA, C20:5	7.80[c] (6.57-9.04)	8.21[c] (7.57-8.84)	7.28[b] (4.09-10.48)
Behenic acid C22:0	3.95[c] (2.32-5.58)	7.05[c] (3.98-10.13)	1.19[c] (0.75-1.64)
DTA, C22:4	0.13[c] (0.08-0.18)	0.13[b] (0.07-0.18)	0.13[a] (0.02-0.24)
DPA, C22:5	0.091[NS] (-0.003-0.184)	0.03[NS] (-0.03-0.08)	0.14[NS] (-0.04-0.32)
DHA, C22:6	0.22[c] (0.07-0.38)	0.36[a] (0.08-0.63)	0.07[NS] (-0.03-0.18)

Values represent medians (lower-upper quartile). DGLA – dihomo-γ-linolenic acid; EPA – eicosapentaenoic acid; DTA – docosatetraenoic acid; DPA – docosapentaenoic acid; DHA – docosahexaenoic acid; NS – non significant. *Significance of difference in Mann-Whitney test ([a]0.05, [b]0.01, [c]0.001).

Table 3. Spearman´s correlation coefficient for association of FFAs, glucose, and HbA1c between all participants, newly diagnosed patients with the T2D, and control subjects.

Fatty acid (μmol/L)	**All participants**		**Newly diagnosed T2D patients**		**Controls**	
	Glucose	**HbA1c**	**Glucose**	**HbA1c**	**Glucose**	**HbA1c**
Lauric acid C12:0	r=0.061 p=0.670	r=0.061 p=0.674	**r=-0.568 p=0.004**	r=-0.274 p=0.196	r=0.334 p=0.088	r=0.054 p=0.790
Myristic acid C14:0	r=0.001 p=0.994	r=0.097 p=0.507	r=0.099 p=0.646	**r=0.405 p=0.050**	r=0.232 p=0.265	r=0.254 p=0.221
Myristoleic acid C14:1	r=-0.197 p=0.176	r=-0.269 p=0.062	r=-0.046 p=0.831	r=-0.236 p=0.268	r=0.053 p=0.800	r=0.027 p=0.899
Palmitic acid C16:0	r=0.235 p=0.108	r=0.271 p=0.062	r=-0.219 p=0.315	r=0.009 p=0.968	r=-0.290 p=0.406	r=-0.290 p=0.159
Palmitoleic acid C16:1	**r=-0.284 p=0.045**	**r=-0.300 p=0.034**	r=-0.119 p=0.580	r=-0.294 p=0.164	r=-0.167 p=0.414	r=-0.062 p=0.764
Stearic acid C18:0	**r=0.722 p=0.000**	**r=0.667 p=0.000**	r=0.029 p=0.894	r=-0.173 p=0.419	r=0.080 p=0.690	r=0.030 p=0.881
Oleic acid C18:1	r=0.136 p=0.341	r=0.162 p=0.257	r=-0.028 p=0.896	r=0.114 p=0.597	r=-0.074 p=0.712	r=-0.110 p=0.584
Linoleic acid C18:2	r=0.258 p=0.068	**r=0.329 p=0.018**	r=-0.026 p=0.894	r=0.360 p=0.084	r=-0.030 p=0.882	r=-0.262 p=0.187
Linolenic acid C18:3	**r=0.674 p=0.000**	**r=0.598 p=0.000**	r=-0.233 p=0.273	r=-0.226 p=0.287	r=0.095 p=0.651	r=-0.193 p=0.356
Arachidic acid C20:0	**r=0.388 p=0.005**	**r=0.349 p=0.012**	r=-0.016 p=0.940	r=-0.202 p=0.343	r=-0.008 p=0.968	r=-0.033 p=0.869
DGLA, C20:3	r=0.239 p=0.311	r=0.295 p=0.207	r=-0.317 p=0.406	r=-0.227 p=0.557	r=-0.174 p=0.609	r=-0.184 p=0.588
Arachidonic acid C20:4	**r=0.736 p=0.000**	**r=0.646 p=0.000**	r=-0.047 p=0.826	r=-0.070 p=0.744	r=0.150 p=0.670	r=0.0610 p=0.674
EPA, C20:5	r=0.308 p=0.246	r=0.201 p=0.456	r=0.533 p=0.139	r=0.017 p=0.966	r=0.455 p=0.305	r=0.630 p=0.129
Behenic acid C22:0	**r=0.505 p=0.000**	**r=0.377 p=0.006**	r=0.029 p=0.892	r=-0.194 p=0.364	r=0.062 p=0.760	r=-0.264 p=0.183
DTA, C22:4	r=0.220 p=0.431	r=0.059 p=0.834	r=0.643 p=0.086	r=0.072 p=0.865	r=-0.126 p=0.788	r=-0.018 p=0.969
DPA, C22:5	r=-0.383 p=0.308	r=-0.552 p=0.123	r=-0.400 p=0.600	r=-0.600 p=0.400	r=-0.200 p=0.747	r=-0.700 p=0.188
DHA, C22:6	**r=0.519 p=0.048**	**r=0.513 p=0.050**	r=-0.048 p=0.911	r=0.277 p=0.506	r=-0.055 p=0.908	**r=-0.778 p=0.039**

*Significance of rho/Spearman´s correlation coefficient for association of FFAs, glucose, and HbA1c between all participants, newly diagnosed patients, and controls. DGLA – dihomo-γ-linolenic acid; EPA – eicosapentaenoic acid;

DTA – docosatetraenoic acid; DPA – docosapentaenoic acid; DHA – docosahexaenoic acid; HbA1c – glycated hemoglobin.

The results of examination of Spearman´s correlation showed significant association between glucose levels and palmitoleic, stearic, linolenic, arachidic, arachidonic, behenic and docosahexaenoic acids in study populations (Table 3). Also, significant association between glycated hemoglobin and palmitoleic, stearic, linoleic, linolenic, arachidic, arachidonic, behenic and docosahexaenoic acids was observed in all participants (Table 3). In healthy subjects, significant correlation was not found between glucose and individual FFAs but a negative association between DHA and glycated hemoglobin was observed ($p<0.05$) (Table 3). Newly diagnosed diabetics demonstrated a negative significant association between glucose and lauric acid concentrations and also, glycated hemoglobin was correlated with myristic acid levels ($p<0.01$ and $p<0.05$, respectively) (Table 3).

4. Discussion

The plasma FFAs are involved in impaired glucose and lipid metabolism, inflammation and IR, thus represent potential biomarkers for the T2D development. This study was examining the association of individual and specific FFAs and glucose levels, as well control of glycaemia in Bosnia newly diagnosed diabetes subjects. The obtained results showed a significant relationship between glucose and glycated hemoglobin as a marker of its control and different individual FFA species, which is in line with previously reported data.

Although the temporally elevated FFAs concentration show positively effects on both, insulin action and secretion in T2D, chronic elevation in plasma concentrations of FFAs have play an important role in the progression from normal glucose tolerance to hyperglycemia and perturbation in glycemic controls in patients. So far, only a few studies have investigated relationship between glucose and individual FFAs in newly diagnosed Type 2 diabetes patients. In this study, is demonstrated significant association between glucose levels and concertation of various FFAs i.e. saturated (SFA) and unsaturated; mono- and polyunsaturated (MUFA, and PUFA) in all participants ($p<0.0001$, $p<0.001$, and $p<0.05$, respectively). In group of newly diagnosed diabetes patients it the same results found for association between glucose levels and FFAs mentioned in above. Interesting, in healthy controls, it no showed relationship between the glucose levels and the concentration of individual FFAs. [9-13]. In previous work, the author showed significant correlation of glucose and glycated hemoglobin with C16:0 ($p<0.05$). [14]. Number of earlier researches demonstrated detrimental effects of saturated FFAs, especially palmitic acid (C16:0) and stearic acid (C18:0) on glucose metabolism and its effectiveness. Also, negative effects and toxicity of SFAs depends of the number of carbons, and fatty acids with less C atoms (e.g. lauric acid, C12:0 and myristic acid, C14:0) have lower detrimental impact of for example, C16:0, and C18:0. However, recent findings indicate that higher concentrations of circulating saturated FFAs and with carbons more than 20, as arachidic acid (C20:0), behenic acid (22:0), and lignoceric acid (24:0) are each associated with a lower risk of diabetes. [15-18].

Control of glucose levels in diabetics is a very important parameter for patient monitoring and treatment. The glycated hemoglobin (HbA1c) concentration reflects the degree of control of plasma glucose concentration, which on a direct or indirect effects of a wide range of metabolic processes including lipolysis and lipogenesis. Therefore, it is unsurprising to find association the HbA1c concentration with the concentration of the most of the main FFAs in plasma. [19-23].

In this study, significant associations of HbA1c concentration and all of major FFAs (SFA, MUFA and PUFA) i.e. C16:1, C18:0, C18:3, C20:0, C20:4, C22:0, and DHA were showed. In the control group, the only negative association between HbA1c and DHA concentrations was found ($p<0.05$), while the only positive association for HbA1c and C14:0 was demonstrated ($p<0.05$). Therefore, reducing elevated circulating FFA should be an important goal when trying to delay or prevent the onset and development of diabetes.

These findings suggest that the inhibitory effects of FFA on glucose effectiveness contribute to increasing glucose levels and worsening control of glucose. This work had a limitation and larger population numbers are needed in the future to clarify the role of specific FFA and glucose levels in T2D. Also, these results as well as published data from other studies are still inconsistent and controversial due to the fact that plasma free fatty acid concentration and profile are influenced by factors such as age, gender, ethnicity and dietary habits or duration of diabetes.

5. Conclusion

These data point out associations of different types of FFAs with glucose levels and its control in plasma of healthy and newly diagnosed type 2 diabetic subjects, and therefore, suggest the importance of monitoring glucose levels as well as glycated hemoglobin with concentrations of individual free fatty acid in the progression of diabetes.

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