Free fatty acid profile in Type 2 diabetic subjects with different control of glycemia

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Abstract. Type 2 diabetes (T2D) as a multifactorial disease is characterized not only by chronic hyperglycaemia but also with defects in lipid and protein metabolism. These defects impact the utilization of glucose and non-esterified "free" fatty acids (NEFA) by muscle, liver, and adipose tissue. Free fatty acids (FFA) represent an important link between obesity, insulin resistance, and T2D. Eleveted plasma concentration of FFA (especially saturated FFA) is associated with impaired insulin secretion and sensitivity and glucose intolerance.

The major objective of the present study was to investigate association of plasma free fatty acid profile in Type 2 diabetic subjects with different control of glycemia. This study involved 40 patients with T2D and 40 healthy subjects. Preparation of samples for FFA analysis was done by extraction and methanolysis of plasma lipids while detection and quantification of FFA concentrations was done by gas chromatography/mass spectrometry. Other biochemical analyses, including glucose, glycated hemoglobin (HbA1c), cholesterol, and triglycerides were done according to standard IFCC methods. A significant difference between T2D and control subjects was demonstrated only for palmitic acid (C16:0). There was a significant correlation of C16:0 with HbA1c levels (p<0.001) in patients with both adequate and poor T2D control. Also, a significant correlation was obtained at a level of plasma C18:1(p<0,05) and HbA1c level, only in patients with inadequate diabetes control. Thus, our data suggested that palmitic fatty acid (C16:0) and (C18:1) could serve as a potential biomarkers in optimal T2D management.

Keywords: free fatty acid, Type 2 diabetes, control of glycemia

INTRODUCTION

Diabetes mellitus is heterogeneous metabolic disease characterized by defective production or action of insulin and high glucose and fat levels in the blood. As a result of alterations in lipid metabolic pathways, plasma free fatty acids (FFAs) concentration rises. It is well known that the long chain fatty acids (polyunsaturated fatty acid, PUFA),very important biomolecules and main constituents of cell membrane play a key role in the cell function and therefore regulate the beta cells functionally, with effects on insulin sensitivity and secretion. Eleveted concentration of FFAs (especially saturated fatty acids) in plasma are associated with both impaired insulin sensitivity and secretion, as well as glucose intolerance. Chronic elevation of FFAs can lead to beta-cell dysfunction, which in turn lead ultimately leads to hyperglycemia (1-3).

Dysregulation of free fatty acid metabolism is a key event responsible for insulin resistance (IR) and Type 2 diabetes. According to the glucose-fatty acid cycle of Randle et al. (1963), preferential oxidation of free fatty acids over glucose plays a major role in insulin sensitivity and leads to metabolic disturbances of diabetes.(4) However, other mechanisms are now being described in order to explain the molecular basis of insulin resistance. Recent studies have suggested that local accumulation of fat metabolites such as ceramides, diacylglycerol or acyl-CoA, inside skeletal muscle and liver, may activate a serine kinase cascade leading to defects in insulin signalling and glucose transport.(5-7) Finally, modulation of transcription by free fatty acids through their binding to peroxisome proliferator-activated receptors could also contribute to impaired glucose metabolism. Fat accumulation in the human body is related to release of several adipokines from adipocytes, and it is known that some of these adipokines elevate IR, cause metabolic syndrome, and promote T2D. Numerous studies indicated that FFAs might be potential biomarkers and pharmacological targets in T2D management. Although, emerging evidence suggests a strong associtaion of glucose, glycosylated hemoglobin and fatty acid levels with T2D, limited number of studies dealt with

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associaton of different types of fatty acids with progresssion and control of the disease. (8-12)

In this work,we aimed to investigate associations of plasma free fatty acid profile in T2D subjects with different level of glycemia control.

MATERIALS AND METHODS

Subjects: In this study, plasma levels of FFA, glucose, and glycated hemoglobin (HbA1c) were determined in 40 patients with T2D, and 40 nondiabetic subjects. Under criteria of IDF, diabetes mellitus was diagnosed when fasting plasma glucose levels were higher than 7.0 mmol/l and postprandial serum glucose was more than 11.1 mmol/. Cut of values for HbA1c values were (<5.7%) for nondiabetics, and type 2 diabetics (>6.5%) (). Accordingly, an appropriate control of T2D was defined as HbA1c < 6.5%, while HbA1c levels > 6.5% were used to classify patients as those with inadequate control of the disease. (13)

Sample Analysis: All research involving human subjects and material derived from human subjects in this study was done in accordance with the ethical recommendations and practices of the Tesanj General Hospital and complied with ethical principles outlined in World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (initiated in June 1964, last amendment in October 2000). Subjects included in this study were free of evidence of hepatitis B or C viral infection or active liver and kidney damage. All subjects underwent medical history and clinical examination. Clinical and biochemical analyses were performed by standard International Federation for Clinical Chemistry (IFCC) methods. Analysis of glucose, and HbA_{1c} levels in plasma samples were performed by employing BT PLUS 2000-Biotechnic Instruments. Preparation of samples for fatty acid analysis was done by methylation of plasma samples, followed by conversion of FFAs to corresponding fatty acid methyl esters.(14) Nineteen FFAs with medium and long-chain length, i.e. from C14 to C22 carbon atoms with different saturation and unsaturation levels, in plasma of T2D, and nondiabetic subjects were analyzed on a Shimadzu QP-5000 GC/MS gas chromatograph equipped with mass spectrometer detector. 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, and C17:0 as internal standard was used for calculation the concentration of individual FFAs.

Statistical Analysis: Calculations were done using SPSS 17.0 for Windows. Statistical significance was set as $p<0.05$.

Calculations were done using SPSS 17.0 for Windows. Comparison of groups was performed using Kruskal-Wallis followed by the Spearman's coefficient correlation. Statistical significance was set as $p<0.05$.

RESULTS

Biochemical and clinical parameters of nondiabetic subjects as controls and T2D patients are presented in Table1. As expected, significant difference were found on plasma glucose and HbA1c levels.

Table 1. Biochemical and clinical parameters of study participants.

Parameters	**Type 2 diabetic**	**Control**	**P-values**
Participants (numbers)	40	40	-
Age (years)	59	57	0.497
Fasting plasma glucose (mmol/l)	9.5±0.4	5.2±0.1	**<0.0001**
Fasting serum TGs (mmol/l)	2.6±0.2	2.2±0.2	0.542
Total cholesterol (mmol/l)	5.5±0.1	5.7±0.2	0.742
Fasting serum HDL-cholesterol (mmol/l)	1.1±0.1	1.7±0.1	0.580
Fasting serum LDL-cholesterol (mmol/l)	3.0±0.1	3.1±0.2	0.495
HbA_{1c} (% [mmol/l])	6.7±0.1 (48±2)	4.5±0.1 (30±2)	**<0.0001**

Table 2: Distribution (in percentage %) of fatty acids in controls and T2D patients

Fatty acid	Type 2 diabetic (%)	Control (%)
C14:0	2,17	1,70
C14:1 n-9	1,00	1,06
C16:0	**36,93**	**27,51**
C16:1 n-7	4,76	2,98
C18:0	**9,31**	**8,79**
C18:1 n-9	**30,12**	**22,70**
C18:2 n-6	**42,40**	**38,66**
C18:3 n-6	3,03	1,86
C20:0	-	6,76
C20:3 n-3	7,52	7,20
C22:0	-	0,75
C22:4 n-6	1,49	1,07
C22:5 n-3	2,24	1,32
C22:6 n-3	-	1,87

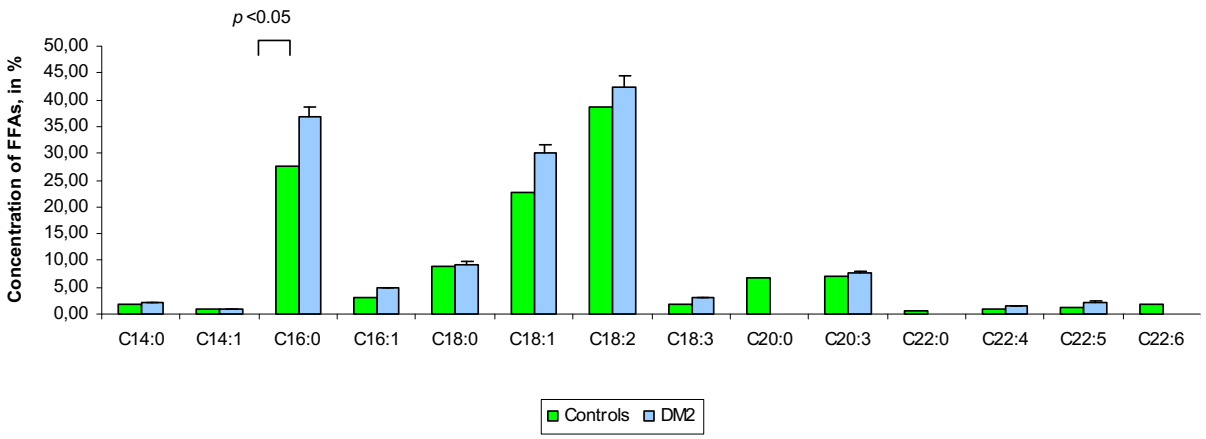

Figure 1. Concentrations and distribution of FFAs in study participants.

FFAs that were present in the highest concentration among a total of 19 FFA species in plasma of both, diabetic and control subjects participating in this study, were C16:0, C18:0, C18:1, and C18:2. (Table 2).

Statistical analysis was done for fourthteen fatty acids, i.e. clinical and metabolic important FFAs with medium and long-chain fatty acids from C14 to C22 atoms. Qualitative and quantitative analysis of FFA profile in healthy controls and T2D patients,showed significant differences in levels of individual FFAs only for palmitic acid, C16:0. (Figure 1)

Our data showed a significant difference in C16**:**0 levels between patients with poorly and well-controlled diabetes.

In Figure 3, association between C16:0 and C18:1 with HbA1c levels in patients with both, adequate and inadequate T2D control was tested.

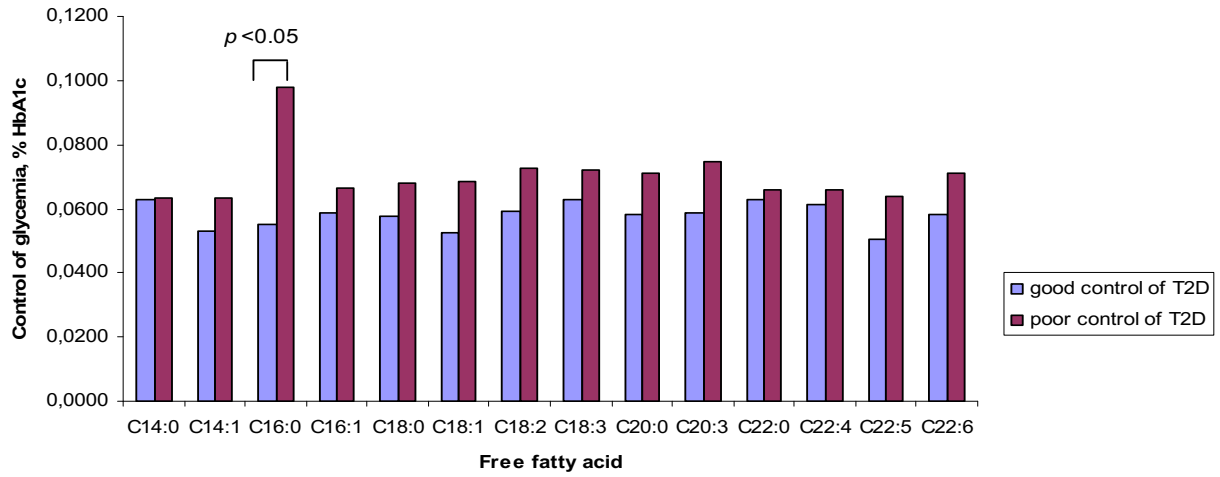

Figure 2. Difference in levels of FFA between patients with poorly and well-controlled diabetes (for palmitic acid, C16:0)

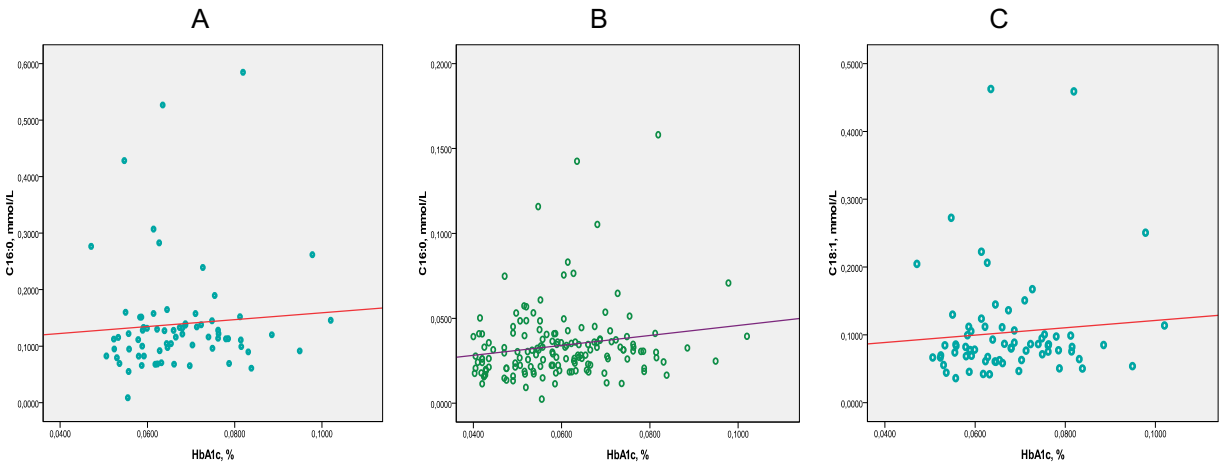

Figure 3. Correlations of palmitic acid with HbA1c levels in patients with (A) an adequate T2D control and (B) inadequate T2D control; and Correlation of oleic acid with HbA1c levels in patients with inadequate T2D control (C) (Spearman's coefficient correlation ρ= 0,704 p<0.001; ρ= 0,190 p<0.05; ρ= 0,230 p<0.05 , respectively)

DISCUSSION

The major objective of the present study was to investigate association of plasma NEFA concentrations with glucose and glycated hemoglobin levels as potential markers in both progression and control of the disase. In our study, the most prominent difference between control and T2D group was observed was observed at the levels of palmitic acid (C16:0). (15-18) Furthermore, determination of individual FFAs concentration, demonstrated that plasma palmitic acid levels significantly correlated with glucose and HbA1c levels.(19-21) Same results were obtained in a comprehensive analysis related to changes in lipid composition over time in plasma, performed by Clore et al. (22, 23), which showed that SFA levels increased in plasma, whereas the content of PUFAs decreased significantly, suggesting that the ratio of saturated to unsaturated FFAs may indeed be an important indicator for the observed development of glucose intolerance.

CONCLUSION

In present study, we investigated associations of plasma free fatty acid profile in T2D subjects with different control of glycemia. FFA that were present in the highest concentration among a total of 19 FFA were C16:0, C18:0, C18:1, and C18:2 in both groups. A significant difference between T2D and control subjects was demonstrated only for C16:0. Our data showed a significant difference in C16**:**0 levels between patients with poorly and well-controlled diabetes. There was a significant correlation of C16:0 with HbA1c levels (p<0.001) in patients with adequate and poor T2D control. Values of HbA1c correleted with plasma concentration of C18:1 (p<0.05)only in patients with an inadequate diabetes control. Thus, our data suggested that palmitic fatty acid (C16:0) and C18:1 could serve as a potential biomarkers in optimal T2D management, indicating that an individual FFAs plasma profile could be used also to predict a hallmark of the pre-diabetic state and T2D development.

A conflict of interest declaration
No conflict to declare.

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