



Središnja medicinska knjižnica

Zibar K., Blaslov K., Bulum T., Knežević Ćuća J., Smirčić-Duvnjak L. (2015) *Basal and postprandial change in serum fibroblast growth factor-21 concentration in type 1 diabetic mellitus and in healthy controls.* Endocrine, 48 (3). pp. 848-55. ISSN 1355-008X

<http://www.springer.com/journal/12020>

<http://link.springer.com/journal/12020>

The final publication is available at Springer via
<http://dx.doi.org/10.1007/s12020-014-0413-9>

<http://medlib.mef.hr/2411>

University of Zagreb Medical School Repository

<http://medlib.mef.hr/>

Basal and postprandial change in serum fibroblast growth factor-21 concentration in type 1 diabetic mellitus and in healthy controls

Karin Zibar, Kristina Blaslov, Tomislav Bulum, Jadranka Knežević Ćuća, Lea Smirčić-Duvnjak

Karin Zibar, MD, Department of Endocrinology and Metabolic Diseases, Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Merkur University Hospital, Zagreb, Croatia; karinzibar@gmail.com.

Kristina Blaslov, MD, Department of Endocrinology and Metabolic Diseases, Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Merkur University Hospital, Zagreb, Croatia; kblaslov@gmail.com.

Tomislav Bulum, MD, PhD, Department of Endocrinology and Metabolic Diseases, Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Merkur University Hospital, Zagreb, Croatia; tbulum@idb.hr.

Jadranka Knežević Ćuća, PhD, Department of Clinical Chemistry and Laboratory medicine, Merkur University Hospital, Zagreb, Croatia; jcuca@idb.hr.

Lea Smirčić-Duvnjak, MD, PhD, Assistant Professor, Department of Endocrinology and Metabolic Diseases, Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Merkur University Hospital, Zagreb, Croatia; Medical School University of Zagreb, Zagreb, Croatia; lduvnjak@idb.hr.

Corresponding author: Karin Zibar, Department of Endocrinology and Metabolic Diseases, Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Merkur University Hospital, Dugi Dol 4a, Zagreb 10000, Croatia; tel.: ++385 91 171 01 95, e-mail: karinzibar@gmail.com.

Abstract

Purpose: Fibroblast growth factor-21 (FGF-21) appears to have an important role in glucose and lipid metabolism. FGF-21 secretion is mainly determined by nutritional status. The aim of this study was to measure basal and postprandial FGF-21 and postprandial change of FGF-21 concentration in type 1 diabetes mellitus (T1DM) patients and in healthy controls and to investigate the differences between the groups.

Methods: The cross-sectional study included 30 C-peptide negative T1DM patients, median age 37 years (20-59), disease duration 22 years (3-45), and 9 healthy controls, median age 30 years (27-47). Basal and postprandial FGF-21 concentrations were measured by ELISA. The associations of FGF-21 with glucose, lipids and insulin were analyzed.

Results: Individuals with T1DM showed significantly lower basal FGF-21 concentration ($P=0.046$) when compared with healthy controls (median value 28.2 vs 104 pg/mL) and had significantly different postprandial change ($\Delta 30'-0'$) of FGF-21 ($P=0.006$) in comparison with healthy controls (median value -1.1 vs -20.5 pg/mL). The glucose and lipid status did not correlate with FGF-21. In healthy controls, postprandial insulin level correlated with basal FGF-21 ($\rho=0.7$, $P=0.036$). Multiple regression analysis showed that they are independently associated after adjustment for confounding factors ($\beta=1.824$, $P=0.04$).

Conclusion: We describe the pathological pattern of basal and postprandial change of FGF-21 secretion not associated with glucose, lipid levels or insulin therapy in patients with T1DM. Since FGF-21 has numerous protective metabolic effects in the experimental model, the lower basal FGF-21 concentration in T1DM patients opens the question about the potential role of recombinant FGF-21 therapy.

Key-words: fibroblast growth factor-21, type 1 diabetes mellitus, C-peptide negative, healthy controls.

Introduction

Fibroblast growth factor-21 (FGF-21) has been recently recognized as a novel metabolic regulator with an important effect on glucose and lipid metabolism. Kharitonov *et al.* [1] first discovered that FGF-21 stimulates glucose uptake *in vitro* in primary culture of human adipocytes. The numerous positive effects of FGF-21 *in vivo* were proven somewhat later. Contrary to expectations, higher FGF-21 concentrations were found in patients with type 2 diabetes mellitus (T2DM), obesity and metabolic syndrome [2-7]. The results were explained by potential FGF-21 resistance [8,9] or compensatory protective elevation of FGF-21 [10,11]. FGF-21 is considered a metabolic protector, but its true physiological effects in humans remain unclear. FGF-21 is predominantly secreted by the liver, adipocytes and the pancreas. Circulating FGF-21 after a meal exerts a number of effects on metabolically active organs [12,13]. Metabolic crisis (starvation and overfeeding) is the greatest stimulus for FGF-21 secretion, which may be explained by excessive levels of free fatty acids that induce FGF-21 secretion [14,15].

So far, only one study explored basal serum FGF-21 concentration in newly diagnosed T1DM patients, describing markedly lower FGF-21 in comparison with healthy population, T2DM and latent autoimmune diabetes in adults [16]. The authors proposed that the autoimmune process destroys pancreatic β -cells and consequently reduces FGF-21 secretion in T1DM. They also found an inverse link between serum FGF-21 concentration and titer of pancreatic islet cell antibodies, and a positive relationship between serum FGF-21 and C-peptide concentration.

Furthermore, one study reported on a decrease in postprandial FGF-21 concentration after oral fat load in healthy subjects and postulated that it was modulated by postprandial accumulation of triglyceride-rich lipoprotein fractions [17]. Another study examined changes in FGF-21 concentration, glucose, insulin and C-peptide levels after 75-g oral glucose tolerance test in healthy subjects, people with impaired glucose tolerance and patients with T2DM, respectively. They found that FGF-21 concentrations declined in the first 60 minutes and then increased until the 180th minute in all 3 examined groups, and that changes in serum FGF-21 at different time points were inversely associated with changes in insulin and C-peptide levels only in healthy subjects but not in two other groups [18].

Postprandial concentrations and changes of serum levels of FGF-21 in T1DM patients have not been studied so far. Although a previously published Chinese study [16] showed a decreased basal FGF-21 in T1DM, the basal

serum FGF-21 concentration in long standing T1DM patients without residual pancreatic β -cell function has not been studied so far.

The primary aim of the study was to measure basal FGF-21, postprandial FGF-21 and postprandial change in FGF-21 concentration in long standing T1DM C-peptide negative patients and their matched healthy controls, and to explore the difference in the FGF-21 concentrations between the two examined groups. We also examined if glucose and insulin levels or insulin therapy and lipid profile status correlated with FGF-21 concentration. The secondary aim was to investigate whether there was a gender difference in FGF-21 within the studied groups. Our results could additionally clarify the pathophysiology not only of basal, but also postprandial FGF-21 concentration and postprandial change in FGF-21 in T1DM patients. We presumed that healthy people expressed a normal pattern of FGF-21 secretion.

Materials and Methods

Study design and subjects

The cross-sectional study included 30 C-peptide negative T1DM patients and 9 healthy volunteer controls. The patients were selected from the annual review at Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Zagreb, Croatia. The diagnosis of T1DM was defined according to the World Health Organization criteria [19]. The inclusion criteria for the T1DM patients were as follows: 18-65 years of age, at least 1-year duration of T1DM, no history of cardiovascular, severe liver or chronic kidney disease, with glomerular filtration rate $> 45 \text{ mlmin}^{-1}1.73^{-1}\text{m}^2$ and without adrenal insufficiency. The patients received intensive insulin therapy (long-acting insulin in one or two doses and ultrashort-acting insulin three times daily) and took no other medication that could affect glucose or lipid metabolism. Nine age, gender and body mass index (BMI) matched people were included as the healthy control group (without diabetes mellitus, without history of cardiovascular, severe liver or chronic kidney disease, with glomerular filtration rate $> 45 \text{ mlmin}^{-1}1.73^{-1}\text{m}^2$ and without adrenal insufficiency). The Ethics Committee of University of Zagreb School of Medicine and Hospital approved the study protocol. Written informed consent was obtained from each examinee, and the study was performed in accordance with the Declaration of Helsinki.

Detailed medical history, including age at diabetes diagnosis, type of insulin therapy and other medications were obtained. Physical examination included measuring the body weight and calculation of BMI. All investigations were performed in the morning, following an overnight fast of 12 hours. All patients received long-acting insulin

the night before and ultrashort-acting insulin before breakfast. Fasting venous blood samples were drawn at 8:00 AM and 30 minutes postprandial (we wanted to examine whether serum FGF-21 concentration is responsive to acute metabolic changes after the breakfast load which consisted mostly of carbohydrates), after a standard diabetic breakfast, both in T1DM and in healthy subjects: a mixed meal of low fat milk, black bread, low fat ham and fruit. The caloric value of the meal depended on the subject's weight and consisted of 70% carbohydrates, 25% proteins and 5% fat (glycemic index of the breakfast was in the range from 185-260). Venous blood samples (plasma and serum) were collected for biochemistry panel measurement, glycated hemoglobin (HbA1c), lipid profile, C-peptide level, insulin level and basal and postprandial FGF-21 concentration. The insulin levels in T1DM patients were not analyzed as we included patients without endogenous insulin secretion, so only exogenous administration of insulin would be measured, and it varied widely among the subjects. Thus, in T1DM patients the total daily insulin requirement and short-acting insulin requirement were measured. All samples were adequately stored at -70 C, from the collection time until analysis.

Laboratory Assays

Biochemistry panel, C-peptide, insulin level, HbA1c, lipid profile, pancreatic islet cell antibodies and serum creatinine concentration were assayed using routine laboratory methods. Serum FGF-21 concentration was measured using ELISA (sandwich) commercial kit (Human FGF-21 Immunoassay Kit, ALPCO, USA); photometer STATFAX 2100. According to the manufacturer, the range of detection was 30 pg/mL -1920 pg/mL and the intra-assay coefficients of variability (CV) were 4% at 93.3 pg/mL and 5% at 547 pg/mL; inter-assay CV of 10.2% at 273.5 pg/mL and 3.5% at 335 pg/mL, with no cross-reactivity with mouse FGF-21. Sensitivity was 10.4 pg/mL. Postprandial change in hormone concentration was calculated as the difference between postprandial and basal hormone concentration, expressed as delta (Δ).

Statistical Analysis

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) ver. 17.0 for Windows. Because of the small sample size the variables were described as median and minimum-maximum range and nonparametric statistical tests were used. Descriptive statistics presented nominal variables as proportions. The difference between two independent numerical variables was tested using the Mann-Whitney test and two dependent variables using the Wilcoxon test. Correlation analysis was performed using Spearman test. Multiple linear regression analysis was performed to examine the association of basal FGF-21 with postprandial insulin level in healthy subjects after adjustment for possible confounding factors (age, gender, fasting insulin,

triglycerides and LDL concentrations). Binary logistic regression analysis was performed to examine the difference in FGF-21 concentration between the T1DM patients and healthy controls, after adjustment for possible confounding factors (age, gender, BMI and postprandial glucose), (Hosmer-Leveshow goodness-of-fit test, using OR with 95% CI). Significance level was accepted at $P < 0.05$.

Results

The basic clinical and laboratory characteristics of T1DM patients and healthy controls are summarized in Table 1. The gender distribution was almost equal, with median age of 37 (T1DM patients) and 30 years (healthy controls), and median disease duration of 22 years. The T1DM patients were C-peptide negative; the basal C-peptide concentration was 0 nmol/L (0-0.11) and postprandial C-peptide was 0 nmol/L (0-0.35). The total daily insulin requirement was 0.6 IU/kg (0.3-0.9). One patient had the value of fasting FGF-21 of 842.4 pg/mL and postprandial FGF-21 of 946 pg/mL, so those concentrations were not reported in the graph (Figure 1.), but were included in statistics (she was 25 years, 10 years of T1DM duration, BMI 23 kg/m², HbA1c value 10.3%, total daily insulin requirement 0.9 IU/kg, fasting glucose value 13.1 mmol/L and postprandial of 4.7 mmol/L). There was no statistically significant difference in fasting glucose concentration ($P=0.077$) between the groups. Besides the expected higher postprandial glucose concentration ($P=0.001$) and HbA1c level ($P < 0.001$), individuals with T1DM had significantly lower basal FGF-21 concentration ($P=0.046$) when compared with healthy controls (median value 28.2 vs 104 pg/mL) and had significantly different postprandial change ($\Delta 30' - 0'$) in FGF-21 ($P=0.006$) in comparison with healthy controls (median value -1.1 vs -20.5 pg/mL). There was no difference in postprandial FGF-21 concentration between the groups ($P=0.217$). Postprandial change, Δ FGF-21 was significantly decreased (negative) in healthy controls. There was no significant difference in other metabolic parameters between the two studied groups.

Figure 1. shows the significant difference between the basal and postprandial FGF-21 concentration in healthy controls (104 vs 66 pg/mL; $P=0.011$). The difference between basal and postprandial FGF-21 was not found in T1DM patients (28.2 vs 28.25 pg/mL, $P=0.974$) (Figure 1.). There was a big difference between the fasting and postprandial glucose level ($P < 0.001$) in T1DM patients, but no difference was found in healthy subjects ($P=0.086$) although there was a significant difference between basal and postprandial insulin levels in healthy subjects ($P=0.008$). There was no significant correlation between FGF-21 levels and HbA1c, fasting and postprandial glucose levels, total daily and short-acting insulin requirement, fasting and postprandial lipid profile status and the titer of pancreatic islet cell antibodies in T1DM patients (data not shown). Also, there was no

significant correlation between FGF-21 levels and HbA1c, fasting insulin level, fasting and postprandial glucose level and fasting and postprandial lipid profile status in healthy controls (data not shown). In healthy controls, postprandial insulin level significantly positively correlated with basal FGF-21 concentration ($\rho=0.7$, $P=0.036$). In multiple regression analysis, postprandial insulin concentration in healthy subjects remained independently associated with basal FGF-21 concentration ($\beta=1.824$, $P=0.04$), after adjustment for possible confounding factors: age, gender, fasting insulin level, fasting triglyceride and LDL concentrations.

In order to examine independent difference in basal FGF-21 concentration and postprandial change in FGF-21 between T1DM patients and healthy controls, binary logistic regression analysis with the studied group as dependent variable (T1DM patients vs healthy controls) and age, gender, BMI and postprandial glucose concentration as independent variables (possible confounders) was performed (Table 2.). Basal FGF-21 concentration did not remain significantly higher in healthy controls, but Δ FGF-21 remained independently decreased in healthy controls (OR 0.942, 95% CI 0.891-0.997, $P=0.04$) after adjustment for age, gender and BMI, but not after adjustment for all three parameters and postprandial glucose concentration ($P=0.135$).

In order to explore gender difference in FGF-21 concentration in T1DM patients and in healthy controls the Mann-Whitney test was performed. The results showed that women with T1DM had significantly higher basal FGF-21 concentration in comparison with T1DM men (51.4 pg/mL, 13.1-842.4 vs 21.1 pg/mL, 0-124.8; $P=0.042$), but only in univariate model. In healthy controls no gender-related difference in FGF-21 concentration was found (data not shown).

Discussion

We found a significant difference in basal FGF-21 concentration and postprandial change in FGF-21 between T1DM patients and healthy controls. T1DM patients had significantly lower basal FGF-21 when compared with healthy controls using univariate analysis. Healthy controls had independently significant postprandial fall (Δ) in FGF-21 concentration after adjustment for age, gender and BMI in comparison with T1DM patients, but not after adjustment for postprandial glucose level. The glucose concentration did not show any correlation with FGF-21 concentration in either group of subjects, but univariate statistics showed significant difference in postprandial glucose concentration between the healthy controls and T1DM patients (6.2 vs 11.2 mmol/L). Glucose was described as one of the major factors that influenced FGF-21 secretion [14], and carbohydrates were the major constituents of the diet in our study. The postprandial FGF-21 concentration did not differ from the basal value in T1DM patients. Lipid profile status also did not show any correlation with FGF-21 level in

either group, while basal FGF-21 showed independently positive correlation with postprandial insulin level in healthy subjects in multivariate analysis.

The authors of a Chinese study reported on the basal FGF-21 concentration in T1DM patients [16]. They found a significantly decreased basal FGF-21 concentration in T1DM patients compared to healthy controls. Their group of T1DM patients (N=76) had a higher basal median FGF-21 concentration in comparison with our T1DM patients, but still significantly lower than their healthy controls. The Chinese study included mostly women of younger age and with newly diagnosed C-peptide positive T1DM (within one year). In our study, the patients were C-peptide negative, which could explain the lower basal FGF-21 concentration in our T1DM group of patients in comparison with the Chinese group. The authors of the Chinese study hypothesize that autoimmune destruction of pancreas could be the cause of impaired FGF-21 secretion in T1DM. The Chinese study also described the inverse correlation between FGF-21 level and the titer of pancreatic islet cell antibodies, while we did not find any correlation. Furthermore, in comparison with the Chinese group of patients, our patients mostly had long standing T1DM, with fewer patients positive for pancreatic islet cell antibodies, which can also be explained by the longer average duration of autoimmune diabetes in our group of patients. Xiao *et al.* did not examine the correlation between FGF-21 level and glucose and lipid profile status or insulin therapy. Although our finding of lower basal FGF-21 supports the previous Chinese report, our study is the first study of basal serum FGF-21 concentration in long standing T1DM European patients without residual pancreatic β -cell function.

The study of Matikainen *et al.* [17] described a decrease in FGF-21 serum concentration in healthy subjects 4 hours after oral fat load and regress to basal values after 8 hours. In their study FGF-21 postprandial concentrations inversely correlated with triglyceride-rich lipoprotein fractions in large VLDL, but not with plasma free fatty acids. This was the first report in humans and they hypothesized that both fasting and postprandial chylomicron and VLDL particles were better predictors of FGF-21 than fasting free fatty acids, and that suppression of FGF-21 regulates postprandial lipid metabolism and permits better clearance of triglyceride-rich lipoprotein fractions [17]. In our study, we did not find any correlation between fasting and postprandial FGF-21 with fasting and postprandial glucose and lipid status in either group studied. A positive correlation was only found between basal FGF-21 and postprandial insulin level in healthy subjects, which supports the role of FGF-21 as a potential metabolic regulator in humans. To date, little is known about FGF-21 in patients with T1DM, especially about postprandial FGF-21. Available data is not yet sufficient to explain the mechanism of the results.

The study of Lin *et al.* [18] described the dynamic change of serum FGF-21 in healthy subjects, in subjects with impaired glucose tolerance and in subjects with T2DM in response to oral glucose tolerance test, respectively. Acute metabolic change showed that FGF-21 concentrations decrease in 60 minutes in all 3 groups of subjects and progressively increase until the 180th minute. The decrease in FGF-21 was significantly smaller in the 2 groups of patients than in healthy controls at the first 60 minutes of oral glucose tolerance test. Furthermore, they found an inverse correlation between the fold changes in FGF-21 and insulin and C-peptide levels only in healthy controls, suggesting that abnormal FGF-21 production in response to glucose may be related to impaired insulin secretion or resistance in patients with impaired glucose tolerance and T2DM, respectively [18]. In our study with C-peptide negative T1DM patients we showed a lack of FGF-21 suppression after the breakfast load at 30 minutes, suggesting abnormal FGF-21 production in T1DM patients in response to acute metabolic change. One study by Yang *et al.* [20] demonstrated that elevation of insulin acutely suppressed FGF-21 production. Consequently, we measured FGF-21 postprandial concentration at 30 minutes, after an ultrashort-acting insulin application in T1DM patients, but there was no association between insulin therapy and postprandial FGF-21 secretion. Our healthy controls showed normal FGF-21 production in response to meal load at 30 minutes: FGF-21 levels decreased significantly.

The study of Kralisch *et al.* [21] demonstrated statistically positive (although weak) correlation of FGF-21 with metabolic syndrome parameters in healthy controls. They also described significantly higher FGF-21 concentration in T2DM patients in comparison with healthy controls, but did not study correlation of FGF-21 with metabolic syndrome parameters in T2DM group of patients. In our healthy controls we found no correlation of FGF-21 with glucose or lipid profile status, but we studied only 9 healthy controls compared with 670 healthy controls in their study.

To date, there is no study describing postprandial change in FGF-21 concentration in T1DM patients and comparing it with healthy subjects. During the fasting period, in metabolic crisis, free fatty acids induce FGF-21 secretion. That is probably the cause of normally higher basal FGF-21 concentration in healthy controls. However, in the postprandial period, FGF-21 secretion is not induced by free fatty acids, which could explain lower postprandial FGF-21 concentration in healthy controls. Based on our results, we believe that healthy people expressed a physiological pattern of FGF-21 secretion, which was lost in T1DM. There was no postprandial change/fall in FGF-21 concentration in T1DM patients; the postprandial FGF-21 concentration was equal to basal. As far as we know, there is no study comparing basal and postprandial FGF-21 concentration, and dealing with postprandial FGF-21 change in T1DM patients so we are not able to compare our results. A large

reference range observed in FGF-21 concentrations was also supported by other studies among healthy subjects and patients [22,5,23]. It was suggested that the FGF-21 concentrations peak in the early morning, after an overnight fast and thereafter oscillate parallel to the changes in concentrations of free fatty acids [15].

In the present study we found a significantly higher basal FGF-21 concentration in women in comparison with men with T1DM, but in univariate model. It is possible that our findings could additionally explain why the Chinese group of patients (mostly women) had higher basal FGF-21 concentration than our group (where gender distribution was almost equal). In healthy controls there was no gender difference in FGF-21 concentration. There is no published study in T1DM patients that describes gender difference in FGF-21 concentration. Available data on gender differences in FGF-21 concentration in non-diabetic population vary widely: from equal FGF-21 concentration [24,25] to lower [21] and higher FGF-21 concentration in women [26,27].

The higher FGF-21 concentration was recently described in girls and explained it by higher triglycerides levels [27]. In our study there was no difference in triglycerides between the genders. Maybe the difference in sex hormones could explain the gender difference in FGF-21 in T1DM patients or it might be closely related to the pathology of the disease.

We have to emphasize the strengths and limitations of our study. We included T1DM patients without residual β -cell function (C-peptide negative). Our sample was therefore more homogenous and we could eliminate endogenous insulin impact on FGF-21 concentrations. All subjects were adults, of same race (and presumed eating tradition), without significant difference in gender prevalence, and all subjects were controlled in one center with standardized therapeutic approach; thus our results could be considered region specific. On the other hand, it was a cross-sectional study, which restricted the ability to establish causality. We did not measure hormones in duplicates and blood was collected only once. The results could not be generalized for the whole population of healthy individuals because of the limited number of control participants. Therefore, we were limited by relying on the resulted data.

In conclusion, we describe the pathological pattern of basal and postprandial change of FGF-21 secretion not associated with glucose, lipid levels or insulin therapy in patients with T1DM. T1DM patients had lower basal FGF-21 concentration in comparison with healthy subjects. Healthy controls had an expectedly negative postprandial change in FGF-21 in comparison with T1DM patients, which could be explained by the physiological pattern of FGF-21 secretion in healthy people. The glucose and lipid profile status, insulin therapy and fasting insulin levels did not correlate with FGF-21 concentration in either studied group. Thus we

hypothesized that autoimmune destruction of pancreas could be contributing to impaired FGF-21 secretion in T1DM. Since FGF-21 has numerous protective metabolic effects, so far confirmed in experimental models [28,29], lower FGF-21 concentration in T1DM patients opens the question about the potential role of recombinant FGF-21 therapy in patients with T1DM. Further larger studies are needed to confirm the results and clarify a potential role of FGF-21 in the pathophysiology of T1DM.

Acknowledgements

The authors would like to thank the laboratory staff at Merkur University Hospital, Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Zagreb, Croatia. The work was supported by The Ministry of Science, Education and Sports of the Republic of Croatia Grant 045-1080230-0516.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical standard

The study protocol was approved by the Ethics Committee of The University of Zagreb School of Medicine and Merkur University Hospital of Zagreb, Croatia.

References

1. Kharitononkov, A., Shiyanova, T.L., Koester, A., Ford, A.M., Micanovic, R., Galbreath, E.J., Sandusky, G.E., Hammond, L.J., Moyers, J.S., Owens, R.A., Gromada, J., Brozinick, J.T., Hawkins, E.D., Wroblewski, V.J., Li, D.S., Mehrbod, F., Jaskunas, S.R., Shanafelt, A.B.: FGF-21 as a novel metabolic regulator. *The Journal of clinical investigation* **115**(6), 1627-1635 (2005).
2. Mraz, M., Bartlova, M., Lacinova, Z., Michalsky, D., Kasalicky, M., Haluzikova, D., Matoulek, M., Dostalova, I., Humenanska, V., Haluzik, M.: Serum concentrations and tissue expression of a novel endocrine regulator fibroblast growth factor-21 in patients with type 2 diabetes and obesity. *Clinical endocrinology* **71**(3), 369-375 (2009).
3. Chen, W.W., Li, L., Yang, G.Y., Li, K., Qi, X.Y., Zhu, W., Tang, Y., Liu, H., Boden, G.: Circulating FGF-21 levels in normal subjects and in newly diagnose patients with Type 2 diabetes mellitus. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association* **116**(1), 65-68 (2008).

4. Chavez, A.O., Molina-Carrion, M., Abdul-Ghani, M.A., Folli, F., Defronzo, R.A., Tripathy, D.: Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. *Diabetes care* **32**(8), 1542-1546 (2009).
5. Jian, W.X., Peng, W.H., Jin, J., Chen, X.R., Fang, W.J., Wang, W.X., Qin, L., Dong, Y., Su, Q.: Association between serum fibroblast growth factor 21 and diabetic nephropathy. *Metabolism: clinical and experimental* **61**(6), 853-859 (2012).
6. An, S.Y., Lee, M.S., Yi, S.A., Ha, E.S., Han, S.J., Kim, H.J., Kim, D.J., Lee, K.W.: Serum fibroblast growth factor 21 was elevated in subjects with type 2 diabetes mellitus and was associated with the presence of carotid artery plaques. *Diabetes research and clinical practice* **96**(2), 196-203 (2012).
7. Mashili, F.L., Austin, R.L., Deshmukh, A.S., Fritz, T., Caidahl, K., Bergdahl, K., Zierath, J.R., Chibalin, A.V., Moller, D.E., Kharitononkov, A., Krook, A.: Direct effects of FGF21 on glucose uptake in human skeletal muscle: implications for type 2 diabetes and obesity. *Diabetes/metabolism research and reviews* **27**(3), 286-297 (2011).
8. Reinehr, T., Woelfle, J., Wunsch, R., Roth, C.L.: Fibroblast growth factor 21 (FGF-21) and its relation to obesity, metabolic syndrome, and nonalcoholic fatty liver in children: a longitudinal analysis. *The Journal of clinical endocrinology and metabolism* **97**(6), 2143-2150 (2012).
9. Hale, C., Chen, M.M., Stanislaus, S., Chinookoswong, N., Hager, T., Wang, M., Veniant, M.M., Xu, J.: Lack of overt FGF21 resistance in two mouse models of obesity and insulin resistance. *Endocrinology* **153**(1), 69-80 (2012).
10. Murata, Y., Konishi, M., Itoh, N.: FGF21 as an Endocrine Regulator in Lipid Metabolism: From Molecular Evolution to Physiology and Pathophysiology. *Journal of nutrition and metabolism* **2011**, 981315 (2011).
11. Fisher, F.M., Chui, P.C., Antonellis, P.J., Bina, H.A., Kharitononkov, A., Flier, J.S., Maratos-Flier, E.: Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. *Diabetes* **59**(11), 2781-2789 (2010).
12. Kharitononkov, A., Dunbar, J.D., Bina, H.A., Bright, S., Moyers, J.S., Zhang, C., Ding, L., Micanovic, R., Mehrbod, S.F., Knierman, M.D., Hale, J.E., Coskun, T., Shanafelt, A.B.: FGF-21/FGF-21 receptor interaction and activation is determined by betaKlotho. *Journal of cellular physiology* **215**(1), 1-7 (2008).

13. Hojman, P., Pedersen, M., Nielsen, A.R., Krogh-Madsen, R., Yfanti, C., Akerstrom, T., Nielsen, S., Pedersen, B.K.: Fibroblast growth factor-21 is induced in human skeletal muscles by hyperinsulinemia. *Diabetes* **58**(12), 2797-2801 (2009).
14. Uebanso, T., Taketani, Y., Yamamoto, H., Amo, K., Ominami, H., Arai, H., Takei, Y., Masuda, M., Tanimura, A., Harada, N., Yamanaka-Okumura, H., Takeda, E.: Paradoxical regulation of human FGF21 by both fasting and feeding signals: is FGF21 a nutritional adaptation factor? *PloS one* **6**(8), e22976 (2011).
15. Yu, H., Xia, F., Lam, K.S., Wang, Y., Bao, Y., Zhang, J., Gu, Y., Zhou, P., Lu, J., Jia, W., Xu, A.: Circadian rhythm of circulating fibroblast growth factor 21 is related to diurnal changes in fatty acids in humans. *Clinical chemistry* **57**(5), 691-700 (2011).
16. Xiao, Y., Xu, A., Law, L.S., Chen, C., Li, H., Li, X., Yang, L., Liu, S., Zhou, Z., Lam, K.S.: Distinct changes in serum fibroblast growth factor 21 levels in different subtypes of diabetes. *The Journal of clinical endocrinology and metabolism* **97**(1), E54-58 (2012).
17. Matikainen, N., Taskinen, M-R., Stenabb, S., Lundbom, N., Hakkarainen, A., Vaaralahti, K., Raivio, T.: Decrease in circulating fibroblast growth factor 21 after an oral fat load is related to postprandial triglyceride-rich lipoproteins and liver fat. *European Journal of Endocrinology* **166**, 487-492 (2012).
18. Lin, Z., Gong, Q., Wu, C., Yu, L., Pan, X., Lin, S., Li, X.: Dynamic change of serum FGF21 levels in response to glucose challenge in human. *Journal of Clinical Endocrinology and Metabolism* **97**, E1224-E1228 (2012).
19. World Health Organization, G., Department of Noncommunicable Disease Surveillance: Definition, diagnosis and classification of diabetes and its complications. Part 1: diagnosis and classification of diabetes mellitus. (1999).
20. Yang, M., Dong, J., Liu, H., Li, L., Yang, G.: Effects of short-term continuous subcutaneous insulin infusion on fasting plasma fibroblast growth factor-21 levels in patients with newly diagnosed type 2 diabetes mellitus. *PLoS One* **6**, e26359.
21. Kralisch, S., Tonjes, A., Krause, K., Richter, J., Lossner, U., Kovacs, P., Ebert, T., Bluher, M., Stumvoll, M., Fasshauer, M.: Fibroblast growth factor-21 serum concentrations are associated with metabolic and hepatic markers in humans. *The Journal of endocrinology* **216**(2), 135-143 (2013).

22. Semba, R.D., Sun, K., Egan, J.M., Crasto, C., Carlson, O.D., Ferrucci, L.: Relationship of serum fibroblast growth factor 21 with abnormal glucose metabolism and insulin resistance: the Baltimore Longitudinal Study of Aging. *The Journal of clinical endocrinology and metabolism* **97**(4), 1375-1382 (2012).
23. Galman, C., Lundasen, T., Kharitononkov, A., Bina, H.A., Eriksson, M., Hafstrom, I., Dahlin, M., Amark, P., Angelin, B., Rudling, M.: The circulating metabolic regulator FGF21 is induced by prolonged fasting and PPARalpha activation in man. *Cell metabolism* **8**(2), 169-174 (2008).
24. Shen, Y., Ma, X., Zhou, J., Pan, X., Hao, Y., Zhou, M., Lu, Z., Gao, M., Bao, Y., Jia, W.: Additive relationship between serum fibroblast growth factor 21 level and coronary artery disease. *Cardiovascular diabetology* **12**, 124 (2013).
25. Lee, P., Linderman, J., Smith, S., Brychta, R.J., Perron, R., Idelson, C., Werner, C.D., Chen, K.Y., Celi, F.S.: Fibroblast growth factor 21 (FGF21) and bone: is there a relationship in humans? *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* **24**(12), 3053-3057 (2013).
26. Heilbronn, L.K., Campbell, L.V., Xu, A., Samocha-Bonet, D.: Metabolically protective cytokines adiponectin and fibroblast growth factor-21 are increased by acute overfeeding in healthy humans. *PloS one* **8**(10), e78864 (2013).
27. Bisgaard, A., Sørensen, K., Johannsen, TH., Helge, JW., Andersson, A-M., Juul, A.: Significant gender difference in serum levels of fibroblast growth factor 21 in Danish children and adolescents. *International Journal of Pediatric Endocrinology* **2014**, 7 (2014).
28. Coskun, T., Bina, H.A., Schneider, M.A., Dunbar, J.D., Hu, C.C., Chen, Y., Moller, D.E., Kharitononkov, A.: Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology* **149**(12), 6018-6027 (2008).
29. Xu, J., Lloyd, D.J., Hale, C., Stanislaus, S., Chen, M., Sivits, G., Vonderfecht, S., Hecht, R., Li, Y.S., Lindberg, R.A., Chen, J.L., Jung, D.Y., Zhang, Z., Ko, H.J., Kim, J.K., Veniant, M.M.: Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. *Diabetes* **58**(1), 250-259 (2009).

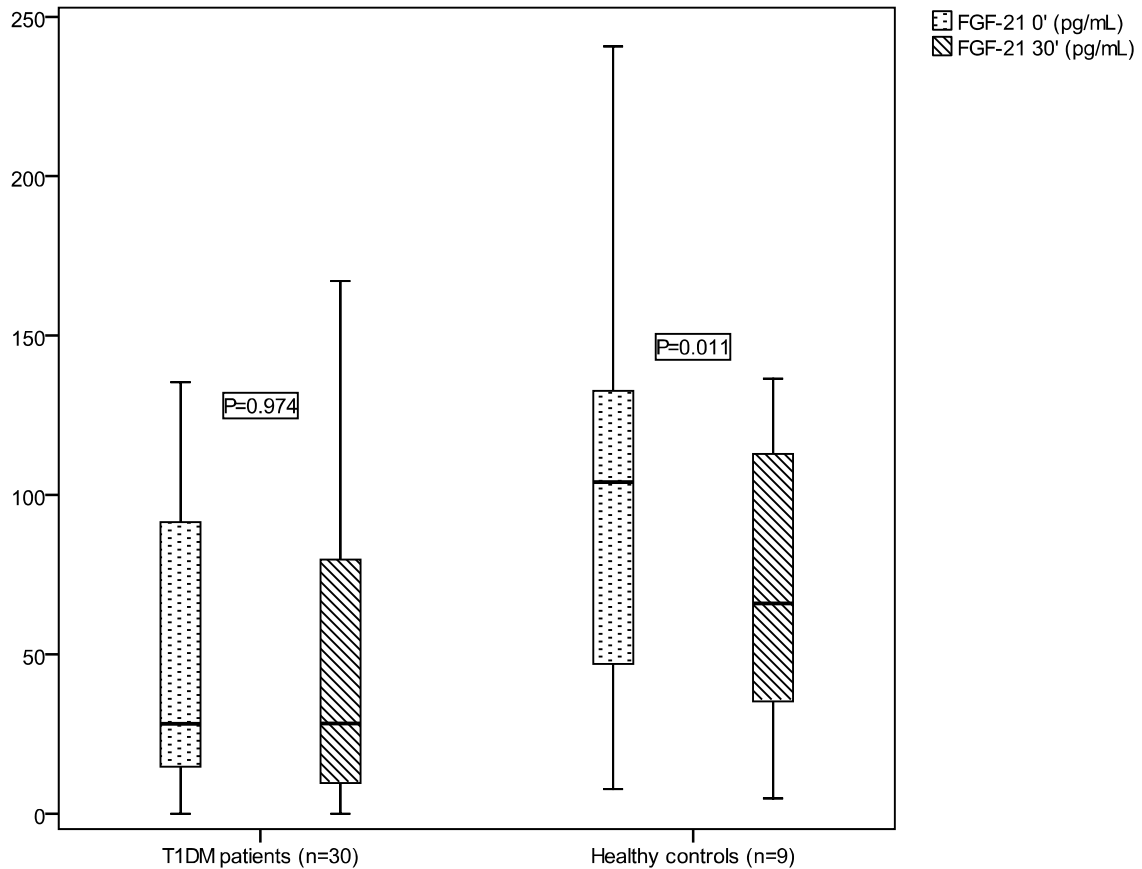


Figure 1. Basal and postprandial fibroblast growth factor-21 (FGF-21) concentrations in type 1 diabetic mellitus patients and healthy controls (Wilcoxon test). One patient had value of fasting FGF-21 of 842.4 pg/mL and postprandial FGF-21 of 946 pg/mL, so those concentrations were not reported in the graph, but were included in statistics.

Table 1. Demographics and biochemical parameters of type 1 diabetes mellitus patients (T1DM) and healthy controls.

Variable	T1DM patients (n=30)	Healthy controls (n=9)
No. of females/males	14/16	5/4
Age (years)	37 (20-59)	30 (27-47)
Disease duration (years)	22 (3-45)	
Body mass index (kg/m²)	24 (20-30)	25 (21-27)
Glucose (mmol/L)		
0' (basal)	6.9 (2.5-13.1)	5.2 (4.3-5.8)
30' (postprandial)	11.2 (3-19.5)*	6.2 (3.3-8.7)
Insulin level (pmol/L)		
0' (basal)		54.1 (17.8-116.8)
30' (postprandial)		356.6 (268.5-992.3)
Hemoglobin A1c (%)	7.2 (5.1-12.4)*	5.2 (4.8-5.8)
Total insulin requirement (IU/kg)	0.6 (0.3-0.9)	
Short-acting insulin requirement (IU/kg)	0.3 (0.2-0.6)	
Creatinine (μmol/L)	66 (43-88)	71 (43-114)
LDL (mmol/L)		
0' (basal)	2.9 (1.9-5)	3.1 (2.2-4.4)
30' (postprandial)	2.9 (2-4.7)	2.6 (2.1-3.7)

HDL (mmol/L)		
0' (basal)	1.6 (1-2.6)	1.5 (1-2.2)
30' (postprandial)	1.4 (0.9-2.5)	1.5 (1-2.1)
VLDL (mmol/L)		
0' (basal)	0.4 (0.2-1)	0.4 (0.3-0.9)
30' (postprandial)	0.4 (0.2-1)	0.7 (0.3-0.8)
Tryglicerides (mmol/L)		
0' (basal)	0.9 (0.4-2.2)	0.8 (0.6-2.1)
30' (postprandial)	1 (0.5-2.2)	1.3 (0.7-1.7)
FGF-21 (pg/mL)		
0' (basal)	28.2 (0-842.4)*	104 (7.7-240.7)
30' (postprandial)	28.25 (0-946)	66 (4.8-136.5)
Δ 30'-0' (postprandial change)	-1.1 (-53.9-103.6)*	-20.5 (-104.2-0.7)

Data are presented as medians (minimum-maximum). * $P < 0.05$ vs healthy controls by Mann-Whitney U test.

† LDL= low density lipoprotein cholesterol, HDL= high density lipoprotein cholesterol, VLDL= very low density lipoprotein cholesterol, FGF-21= fibroblast growth factor-21.

Table 2. Binary logistic regression of independent variable fibroblast growth factor-21 (FGF-21) in relation to studied group (healthy controls vs type 1 diabetes mellitus patients) as dependent variable.

Independent variable	Model A	Model B	Model C
	OR (95%CI)	OR (95% CI)	OR (95% CI)
FGF-21 (pg/mL)			
0' (basal)	1,001 (0.996-1.006)	1 (0.996-1.006)	1.002 (0.996-1.009)
30' (postprandial)	1 (0.995-1.005)	0.999 (0.994-1.004)	1.001 (0.995-1.007)
Δ 30'-0' (postprandial change)	0.945 (0.898-0.995)*	0.942 (0.891-0.997)*	0.827 (0.644-1.061)

Model A= crude model. Model B= adjusted by age, gender and body mass index. Model C= adjusted by age, gender, body mass index and postprandial glucose. * $P < 0.05$