Changes of plasma fibroblast growth factor-21 (FGF-21) in oral glucose tolerance test and effects of metformin on FGF-21 levels in type 2 diabetes mellitus

Zmiany osoczowego stężenia czynnika wzrostu fibroblastów-21 (FGF-21) podczas doustnego testu tolerancji glukozy i wpływ metforminy na stężenia FGF-21 u chorych na cukrzycę typu 2

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Abstract

Introduction: The objectives of our study were to investigate whether fibroblast growth factor-21 (FGF-21) is involved in short-term regulation of glucose and the change of FGF-21 after metformin use in diabetic subjects.

Material and methods: 43 subjects were recruited in the study, including 27 new-onset type 2 diabetes patients (nT2DM). A 75 g oral glucose tolerance test (OGTT) was administered to them. Blood samples were taken at 0, 60 ,120 and 180 minute of OGTT. nT2DM subjects were invited for further investigation, metformin was administered in a dose of 1.0 g every day for 1 week.

Results: Plasma FGF-21 changed significantly in the nT2DM group during the OGTT administration but not in the control group. No gender differences were observed at different time points in FGF-21 levels ($p < 0.05$). Administration of metformin for nT2DM resulted in a significant decrease in both glucose and FGF-21 at all OGTT times and in insulin at 60 min and 180 min, indicative of a decrease in HOMA-IR.

Conclusion: FGF-21 does not seem to be involved in short-term regulation of glycaemia in human subjects, and the change in OGTT delayed in T2DM. FGF-21 may participate in the processing of metformin, improving glucose and insulin sensitivity.

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Key words: fibroblast growth factor-21, type 2 diabetes, OGTT, metformin

Streszczenie

Wstęp: Badanie przeprowadzono w celu ustalenia, czy czynnik wzrostu fibroblastów-21 (FGF-21) uczestniczy w regulacji stężenia glukozy i czy zastosowanie metforminy u chorych na cukrzycę wpływa na stężenie FGF-21.

Materiał i metody: Do badania włączono 43 osoby, w tym 27 chorych z nowo rozpoznaną cukrzycą typu 2 (nT2DM). U wszystkich przeprowadzono test doustnego obciążenia 75 g glukozy (OGTT). Próbki krwi pobrano w 0., 60.,120. i 180. minucie testu. Osobom z nT2DM zaproponowano udział w dalszych badaniach; zastosowano u nich metforminę w dawce 1,0 g/dobę przez tydzień.

Wyniki: Zmiany stężenia FGF-21 w osoczu podczas OGTT zaobserwowano tylko w grupie chorych na nT2DM; w grupie kontrolnej stężenie FGF-21 pozostało niezmienione. Nie stwierdzono, by stężenia FGF-21 w poszczególnych punktach czasowych różniły się w zależności od płci badanych ($p < 0,05$). Zastosowanie metforminy u osób z nT2DM spowodowało istotne zmniejszenie stężeń glukozy i FGF-21 we wszystkich punktach czasowych OGTT oraz zmniejszenie stężenia insuliny w 60. i 180. minucie, co wskazuje na obniżenie wskaźnika HOMA-IR.

Wnioski: FGF-21 nie uczestniczy w krótkoterminowej regulacji glikemii u ludzi, a zmiany jego stężenia podczas OGTT są opóźnione w T2DM. Być może FGF-21 bierze udział w metabolizowaniu metforminy, zwiększając wrażliwość na glukozę i insulinę.

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Słowa kluczowe: czynnik wzrostu fibroblastów-21, cukrzyca typu 2, OGTT, metformina

Introduction

Fibroblast growth factor-21, which is secreted by hepatic cells, has been proposed as a potent metabolic regulator in glucose and lipid metabolism. FGF-21 can improve glucose uptake in 3T3-L1 adipocytes, reduce blood glucose and triglyceride levels, and prevent overexpressed transgenic mice from obesity [1]. Moreover, FGF-21 can reverse hepatic steatosis and insulin resist-ance, and reduce bodyweight [2]. It has been reported that FGF-21 caused significant improvements in fasting plasma glucose and lipoprotein profiles in diabetic monkeys [3]. FGF-21 treated mice exhibited increased energy expenditure, fat utilisation and lipid excretion, reduced hepatic steatosis and ameliorated glycaemia [4]. Chen et al. [5] suggested that plasma FGF-21 levels participated in the pathogenesis of newly diagnosed patients with type 2 diabetes mellitus. A recent study

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showed that FGF-21 was associated with abdominal obesity [6], correlated to serum adiponectin levels in subjects with type 2 diabetes [7], and influenced by BMI, fasting glycaemia, uric acid, and physical activity in healthy blue-collar workers [8]. But common to all these studies was that plasma FGF-21 levels were only assessed at one time-point. To date, there have been no published studies on the change of FGF-21 after glucose load. The primary aim of our study is to determine whether FGF-21 levels change after glucose load.

Metformin is currently the most widely used drug worldwide in the treatment of diabetes and reducing insulin resistance in humans. However, it is not clear whether the mechanisms of treatment include the FGF-21 signalling pathway. Therefore we have also endeavoured to find out the reflection of FGF-21 levels after metformin therapy for diabetes.

Material and methods

Fourty three subjects were involved in our study and divided into two groups. The first group of 27 subjects (16 females, 11 males) was new-onset type 2 diabetes patients (nT2DM), who were recruited from the diabetes specialist clinics (West China Hospital), aged 49.9 ± ± 10.9 years, BMI 24.62 ± 2.04 kg/m². The second group of 16 healthy volunteers (seven males, nine females) was recruited from among medical staff at West China Hospital, aged 46.75 ± 8.52 years, BMI 22.71 ± 3.00 kg/m². The diagnosis of diabetes was based on 1999 World Health Organisation criteria (fasting plasma glucose level ≥ 7.0 mmol/L or 2-h OGTT plasma glucose level ≥ 11.1 mmol/L). A 75 g oral glucose tolerance test (OGTT) was performed for all subjects, plasma samples were taken before (0 minute) and at 60, 120, and 180 minutes after OGTT to detect glucose, insulin, and FGF-21.

The nT2DM group was administered metformin (glucophage) (0.5 g, twice a day) for one week. Plasma concentrations of glucose, insulin, and FGF-21 were measured.

All studies were approved by the Ethics Committee at West China Hospital, Sichuan University. Informed consent was obtained from all subjects before participation.
Height, weight, and waist circumference were measured after fasting by the same physician. Plasma glucose was determined by Roche DDP. Plasma insulin was measured by electro-chemiluminescence immunoassay (Roche E170). Plasma FGF-21 levels were determined by RIA (Phoenix Pharmaceuticals, Inc, USA) using ^{125}I-labelled FGF-21 as tracer. Blood samples were collected with aprotinin (0.6 TIU/ml of blood) and detection was centralised.

FGF-21 antibody was rabbit anti-human FGF-21 IgG, which is specific for human FGF-21. There are no cross-reactions between FGF-21 antibody and human FGF-6, FGF-10, FGF-18, FGF-19, FGF-20, adiponectin, visfatin, leptin, or retinol-binding protein –4 resistin. The linear range is 0.5~8.5 ng/mL, intra-assay coefficient of variation (CV) < 5%, and inter-CV < 14%.

The formula for the homeostasis model assessment is as follows: homeostasis model assessment insulin resistance (HOMA-IR) = {(fasting insulin [μU/mL] × fasting glucose [mmol/L]}/22.5 [9].

Statistical analysis

The normal distribution of data was expressed as mean ± standard deviation, and the skewed distribution of information was expressed as median (quartile). T-tests were used to compare continuous variables normal distribution of data, while skewed distribution of information was compared using Mann-Whitney-U tests. Repeat measure analysis was used to compare the data at different times of OGTT. Pair sample t-test or 2-related sample signed ranks test was used to compare differences before and after metformin. All statistical analyses were performed using SPSS v. 16.0 package for Windows. $p < 0.05$ was considered statistically significant.

Results

FGF-21 level changes in OGTT
No significant differences in FGF-21 levels were observed between the control and nT2DM group at any OGTT time, whereas glucose levels differed at all time points, and insulin levels at 120 minutes and 180 minutes (Table I). Plasma FGF-21 changed significantly in the nT2DM group during the OGTT administration, but not in the control group. No gender differences were observed at any OGTT time point in control or patient groups.

FGF-21 level changes before and after metformin administration in nT2DM
Administration of metformin for one week resulted in a significant decrease in both glucose and FGF-21 at all OGTT times and in insulin at 60 and 180 minutes OGTT, indicative of a decrease in HOMA-IR, as shown in Table II. Plasma FGF-21 decreased after one week of metformin treatment, although not in all individuals. When the subjects were divided into a male group and a female group, serum FGF-21 levels decreased after one week of metformin treatment in the female group at different times of OGTT ($p < 0.05$), but differences were not noted in the male group (data not shown).

Table I. *Descriptive statistics for the levels of glucose, insulin and FGF-21 during OGTT in T2DM and control group*

Tabela I. *Statystyki opisowe stężeń glukozy, insuliny i czynnika wzrostu fibroblastów-21 podczas doustnego testu tolerancji glukozy u chorych na cukrzycę typu 2 oraz w grupie kontrolnej*

		0′	60′	120′	180′	p value*
Glucose [mmol/L]	T2DM	8.51 ± 2.14	15.23 ± 2.97	16.61 ± 3.17	14.43 ± 3.21	0.000
	Control	4.60 ± 0.50	6.30 ± 0.74	5.09 ± 0.60	4.58 ± 0.49	0.000
	P value#	0.000	0.000	0.000	0.000	
Insulin [uU/mL]	T2DM	3.80 (2.60, 5.60)	19.70 (14.10, 26.20)	37.00 (20.28, 57.75)	13.50 (7.70, 22.20)	0.000
	Control	3.45 (2.58, 4.50)	17.40 (8.40, 29.80)	16.00 (10.00, 29.60)	5.95 (4.13, 10.15)	0.000
	P value#	0.359	0.407	0.000	0.002	
FGF-21 [ng/mL]	T2DM	2.27 ± 0.60	2.09 ± 0.55	2.01 ± 0.48	2.03 ± 0.52	0.000
	Control	2.06 ± 0.39	2.00 ± 0.31	2.06 ± 0.37	2.17 ± 0.46	0.159
	P value#	0.217	0.557	0.711	0.399	

*p value represents the significance level of the repeated measure analysis for comparison of distributions of these characteristics in different OGTT time intervals; #p value represents the significance level of the Wilcoxon matched-paired test for comparison of distributions of these characteristics between T2DM group and control group; FGF-21 — fibroblast growth factor-21; OGTT — oral glucose tolerance test; T2DM — type 2 diabetes patients

Table II. *Glucose, insulin and FGF-21 levels in OGTT before and after metformin*

Tabela II. *Stężenia glukozy, insuliny i FGF-21 podczas doustnego testu tolerancji glukozy przed i po podaniu metforminy*

		M0	M1	p value
Glucose [mmol/L]	0′	8.51 ± 2.14	6.34 ± 1.46	0.000
	60′	15.23 ± 2.97	11.43 ± 2.85	0.000
	120′	16.61 ± 3.17	12.60 ± 3.13	0.000
	180′	14.43 ± 3.21	10.99 ± 3.46	0.000
Insulin [uU/mL]	0′	3.80 (2.60, 5.60)	3.06 (1.98, 5.67)	0.121
	60′	17.40 (8.40, 29.80)	11.79 (4.59, 19.08)	0.013
	120′	16.00 (10.00, 29.60)	12.24 (9.36, 18.90)	0.107
	180′	13.50 (7.70, 22.20)	9.36 (5.85, 14.04)	0.007
FGF-21 [ng/mL]	0′	2.27 ± 0.60	2.08 ± 0.54	0.006
	60′	2.09 ± 0.55	1.92 ± 0.50	0.016
	120′	2.01 ± 0.48	1.86 ± 0.50	0.010
	180′	2.03 ± 0.52	1.89 ± 0.50	0.007
HOMA-IR		1.35 (1.02, 2.01)	0.87 (0.58, 1.43)	0.001

FGF-21 — fibroblast growth factor-21; OGTT — oral glucose tolerance test

Discussion

Fibroblast growth factor-21 is a novel metabolic regulator with multiple beneficial effects on glucose and insulin resistance in animal models and humans [10–12]. A significant positive correlation has been observed with obesity, metabolic syndrome [13] and type 2 diabetes [14], but the mechanism of FGF-21 is unclear. Here, we have sought to address whether FGF-21 might be involved in short-term (i.e. postprandial) regulation of glycaemia in humans and whether metformin affects FGF-21 levels.

Our study demonstrated that the circulating FGF-21 was opposite to insulin and glucose during the oral glucose tolerance test in humans. The mode of action for FGF-21 requires glucose transporters 1 (GLUT1) transcriptional activation. In contrast to the rapid response elicited by insulin, the predominant effect of FGF-21 on glucose uptake required at least 2–4 hours of cell treatment [1].We found FGF-21 increased at 120 and 180 minutes of OGTT in control by trend, and

increased at 180 minutes in T2DM. This implies that FGF-21 does not seem to be involved in the short-term regulation of glycaemia in humans, and the change in OGTT is delayed in nT2DM.

In some studies measuring plasma FGF-21, an ELISA kit has been used [13, 15]. It is the case that FGF-21 values differ when measured by ELISA and by radio immunoassay, and the level of serum FGF-21 seems to be higher with radio immunoassay than with ELISA. This is probably due to the different methods. It might be that the antibody to FGF-21 is different, and that the antibody is specific for human FGF-21. Li et al. [16] evaluated FGF-21 levels in type 2 diabetic patients using radio immunoassay: FGF-21 concentrations were 1.81 ± 0.63 μg/L in newly diagnosed diabetic subjects and 1.52 ± 0.61 μg/L in a normal glycaemic control group when fasting. This is close to our results, although a difference exists.

Metformin is the most widely used drug in the treatment of type 2 diabetes. However, the mechanism by which metformin acts is poorly understood. Metformin improves glucose and reduces insulin resistance perhaps via the following mechanism: first, metformin activates AMP-activated protein kinase (AMPK), to reduce aggregation of lipid [17]. Second, recent findings indicate that metformin treatment speeds up the transportation of GLUT1 [18]. Moreover, AMPK phosphorylation levels were increased by FGF-21 treatment in adipocytes as well as in white adipose tissue from ob/ob mice [19]. The glucose uptake assay in 3T3-L1 adipocytes indicated that the purified human FGF-21 could stimulate glucose uptake, and GLUT1 is the functional unit [20]. It has been reported that FGF-21 treatment (1 μg/mL) led to a significant increase in GLUT1 mRNA and protein 3T3-L1 adipocytes [1]. Many studies have shown that HOMA-IR were positively associated with inflammatory parameters and metabolism characteristics [21, 22]. In our study, after metformin, improved HOMA-IR was observed, and plasma FGF-21 levels decreased. No relations were found between FGF-21 and HOMA-IR (data not shown). Thus, metformin may improve FGF-21 action through the AMPK pathway and transportation of GLUT1, and consequently alleviate FGF-21 resistance and improve glucose and insulin sensitivity, and reduce plasma FGF-21 levels.

Our findings suggest that insulin might be an important factor for FGF-21 level, and the FGF-21 pathway might be a compensatory mechanism for impaired insulin action, but this needs further investigation.

Our results showed that no gender differences were observed at different time points in the control and the nT2DM group before the use of metformin, and this is in line with other reports [13, 23], But, in the nT2DM group, after one week of metformin treatment and with the sub-jects divided into male and female groups, serum FGF-21 levels decreased in the female group at different times of OGTT, differences that were not noted in the male group. It appears that metformin treatment influences FGF-21 concentration solely in females, but the number of males was small, and this needs further investigation.

Conclusions

In summary, our study shows that FGF-21 does not increase in the short term after glucose load and the magnitude of the plasma FGF-21 was blunted in the T2DM group more than in the control group. Administration of metformin for one week resulted in a significant decrease in FGF-21 at all OGTT times. Whether the change of plasma FGF-21 represents a compensatory effect, or a causative factor, in the development of glucose and insulin resistance is yet to be determined.

Acknowledgements

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