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ORIGINAL

Circulating betatrophin is elevated in patients with type 1 and type 2 diabetes

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Abstract. There is evidence that betatrophin, a hormone derived from adipose tissue and liver, affects the proliferation of pancreatic beta cells in mice. The aim of this study was to examine circulating betatrophin concentrations in Japanese healthy controls and patients with type 1 and type 2 diabetes. A total of 76 subjects (12 healthy controls, 34 type 1 diabetes, 30 type 2 diabetes) were enrolled in the study. Circulating betatrophin was measured with an ELISA kit and clinical parameters related to betatrophin were analyzed statistically. Circulating betatrophin (Log transformed) was significantly increased in patients with diabetes compared with healthy subjects (healthy controls, 2.29 ± 0.51; type 1 diabetes, 2.94 ± 0.44; type 2 diabetes, 3.17 ± 0.18; $p<0.001$, 4.1 to 5.4 times in pg/mL order). Age, HbA1c, fasting plasma glucose and Log triglyceride were strongly associated with Log betatrophin in all subjects ($n=76$) in correlation analysis. In type 1 diabetes, there was a correlation between Log betatrophin and Log CPR. These results provide the first evidence that circulating betatrophin is significantly elevated in Japanese patients with diabetes. The findings of this pilot study also suggest a possibility of association between the level of betatrophin and the levels of glucose and triglycerides.

Key words: Betatrophin, Beta cells proliferation, Insulin resistance, Lipid metabolism

PROGRESSIVE loss of mass and function of beta cells is an important pathophysiological feature of diabetes. Regenerative medicine, including promotion of proliferation of pancreatic beta cells, has potential as a clinical application for insulin dependent diabetic patients. Many factors are known to influence beta-cell proliferation, including serotonin, glucagon-like peptide-1, placental lactogen, and physiological effects of pregnancy, but inducing a proliferative effect in beta cells is difficult [1, 2]. In this context, Yi and colleagues found that betatrophin, a fat and liver derived hormone, had a powerful stimulatory effect on proliferation of beta cells [3]. Betatrophin is also a regulator of lipid metabolism and is alternatively referred to as TD26, RIFL, lipasin and angiopoietin-like protein 8 (ANGPTL8) [4-6]. Strong secretion of betatrophin was observed following blocking of the insulin receptor by S961 in a mouse model of insulin resistance. Elevation of betatrophin was also observed in *ob/ob* and *db/db* mice, which are models of diabetes, and transient expression of betatrophin in mouse liver significantly increased proliferation of pancreatic beta cells [3]. If this effect occurs in humans, betatrophin may offer a novel choice of diabetes treatment. However, there are some studies which showed that betatrophin was not an essential hormone for beta cells proliferation in mice [7, 8]. There are controversial results that betatrophin is increased or decreased in patients with diabetes [9-15]. Thus, the focus on betatrophin effect might move to its metabolic affection on glucose and lipid. In the present study, firstly, we examined whether circulating betatrophin was elevated or not in Japanese patients with diabetes. In addition, the relation of betatrophin with glucose and lipid was determined.

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Materials and Methods

Subjects

The subjects were 12 healthy controls (HC), 34 patients with type 1 (T1DM) diabetes and 30 patients with type 2 diabetes (T2DM). The diabetic patients and HCs were matched in age and sex. T1DM is classified into two groups: type 1A and type 1B. Type 1A

is the "autoimmune" type 1 diabetes with autoantibodies against islet antigens (islet-associated antibodies) and type 1B is the "idiopathic" type 1 diabetes without verifiable autoantibodies [16]. In patients with type 1 diabetes of this present study, only type 1A diabetes patients were enrolled. Diabetic patients with micro- and macroalbuminuria were included in the study, but those under hemodialysis treatment and those with chronic liver and kidney diseases, malignancy, or pregnancy were excluded. All patients with T1DM received insulin therapy, while patients with T2DM received oral hypoglycemic agents. Six patients with T1DM and 14 with T2DM received statins for treatment of dyslipidemia. No patient were treated with fibrates or eicosapentaenoic acid. Healthy controls had no relevant medical history.

Measurements

Blood samples were collected from the subjects after an overnight fast. The samples were centrifuged at 3,000 rpm at 4°C for 15 min. Supernatants were decanted and frozen at -80°C until assayed. Serum concentrations of normal laboratory parameters were measured using standard biochemical methods at Jichi Medical University Saitama Medical Center. Serum betatrophin was measured using an ELISA kit (Wuhan EIAab Science Co., Wuhan, China; Catalogue No. E11644h). Fasting serum C-peptide immunoreactivity (CPR) was measured by enzyme immunoassay (EIA; ST Aia-Pack C-Peptide, Toso Corp., Tokyo, Japan). Renal function was calculated as the estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease equation revised for Japanese subjects by the Japanese Society of Nephrology [17].

Statistical analysis

Data are expressed as mean ± S.D. In statistical analyses, serum CPR was defined as 0.2 ng/mL when present at an undetectable level. Betatrophin, CPR and triglyceride (TG) were evaluated after logarithmic transformation (Log). Clinical characteristics were compared among the three groups using one-way ANOVA as appropriate. *Post hoc* analysis was performed by Tukey test. Categorical variables were compared by Fisher exact test. Correlation analysis was performed by parametric analysis except for Log CPR, which was tested by non-parametric analysis because of a non-normal distribution. All analyses were performed by SPSS Statistics 17.0. $P < 0.05$ was considered significant in all analyses.

Ethics committee approval

This study was approved by the Ethical Committee at Jichi Medical University Saitama Medical Center and was performed in compliance with the Helsinki Declaration.

Results

Baseline characteristics of HC, T1DM and T2DM are shown in Table 1. There were no significant differences in age and sex. HbA1c and fasting plasma glucose (FPG) were higher in diabetic subjects. In lipid profile, TG level was high and HDL-C level was low in T2DM. Serum fasting CPR was low in T1DM but high in T2DM compared with HC. Serum levels of circulating betatrophin (pg/mL) were increased by 4.1 to 5.4 times in patients with T1DM and T2DM compared with healthy controls. In all subjects, correlation analysis showed positive or negative correlations of Log betatrophin with age, HbA1c, FPG, Log CPR, HDL-C and Log TG, but not with BMI, eGFR and serum total cholesterol (Table 2). Sex was not related to Log betatrophin ($p = 0.566$). In three categories of HC, T1DM and T2DM, only T1DM showed positive correlation between betatrophin and Log CPR levels ($n = 34$, $r = 0.432$, $p = 0.05$), not HC and T2DM.

Discussion

Betatrophin is a hormone derived from liver and white adipose tissue that was found to promote beta cell proliferation in a mouse model of insulin resistance induced by S961, an antagonist of the insulin receptor [3]. In this study, we provide the first evidence that circulating betatrophin is elevated in Japanese patients with diabetes and is associated with the TG level.

Several reports have shown that serum betatrophin is increased in patients with diabetes [9-12], but without a positive correlation with BMI [9-11]. We also found no correlation of circulating betatrophin with BMI. However, Fu *et al.* found that serum levels of lipasin/betatrophin were increased in overweight and obese subjects, and suggested that the conflicting results may be due to differences between ELISA/EIA kits with reactivity to the C- or N-terminus of betatrophin [13]. Gómez-Ambrosi *et al.* found that circulating betatrophin was decreased in patients with obe-

Table 1 Background characteristics of healthy controls (HC) and patients with diabetes.

	HC	T1DM	T2DM	*p*-value [1]
Age (years)	55.6±4.6	55.0±16	56±15	0.985
Sex (M/F)	12 (7/5)	34 (14/20)	30(15/15)	0.558
BMI (kg/m²)	22.7±3.0	22.5±6.2	25.7±4.6	0.040
HbA1c (%)	5.2±0.3	9.4±2.7*	8.8±1.1**	<0.001
FPG (mg/dL)	95±5	154±71*	144±34**	0.005
Log CPR	0.03±0.15	-0.34±0.41*	0.44±0.14**,#	<0.001
TC (mg/dL)	199±25	198±37	209±45	0.517
HDL-C (mg/dL)	55±13	65±17	45±11#	<0.001
Log TG	1.90±0.17	1.97±0.29	2.16±0.21**,#	0.002
LDL-C (mg/dL)	127±21	109±28	131±36#	0.010
eGFR (mL/min/1.73m²)	78±14	83±23	87±26	0.462
Betatrophin (pg/mL)	300±236	1233±865*	1614±647**,#	<0.001
Log Betatrophin	2.29±0.51	2.94±0.44*	3.17±0.18**,#	<0.001

[1] One-way ANOVA (or Kruskal-Wallis test) and Fisher exact test between HC, T1DM and T2DM. *Post hoc* analysis was performed by Tukey test. Data are expressed as mean ± S.D. *, $p < 0.05$ *vs*. HC. **, $p < 0.05$ *vs*. HC. #, $p < 0.05$ *vs*. T1DM.

Table 2 Association of variables with circulating betatrophin in correlation analyses (n=76)

	Correlation analysis	
	r	*p*-value
Age (years)	0.360	0.001
BMI (kg/m²)	0.140	0.226
HbA1c (%)	0.549	<0.001
FPG (mg/dL)	0.462	<0.001
Log CPR	0.320	0.005
TC (mg/dL)	0.167	0.149
HDL-C (mg/dL)	-0.247	0.032
Log TG	0.402	<0.001
LDL-C (mg/dL)	0.122	0.293
eGFR (mL/min/1.73m²)	-0.170	0.142

Correlation coefficients were analyzed using Pearson's correlation or Spearman's rank correlation by SPSS.

sity and T2DM [14], based on measurements using a human betatrophin ELISA kit. Recently, Fu *et al*. compared two other commercial betatrophin kits (EIAab, Wuhan, China; Catalogue No. E11644h; and Phoenix Pharmaceuticals, Burlingame, CA, USA, Catalogue No. EK-051-55) in 30 males [15]. The antibody in the EIAab kit recognizes the N-terminus and that in the Phoenix kit recognizes the C-terminus of betatrophin. In measurement of a human recombinant betatrophin (EIAab), these two kits both showed linear regression ($r = 0.99$), but arrived at a different result in measurement of serum samples. Serum betatrophin and BMI were negatively correlated in a former kit and positively correlated in a latter kit. They pointed out the possibility that proteolytic cleavage of betatrophin may affect the difference determined by ELISA kits that depend on the particular antibodies used against the N- or the C-terminus [15]. Our results support an elevation of serum betatrophin in patients with diabetes, but a further study is needed to determine the exact changes in serum betatrophin [9-12, 15].

In a study of the role of betatrophin introduced (under the kidney capsule) into C57BL6/J mice islets and human beta cells in immunodeficient NOD-Scid mice, Jiao *et al*. found a marked increase in DNA replication in murine beta cells, whereas the human beta cells did not respond [7]. This suggests that betatrophin from mice may not induce proliferation of human beta cells. Moreover, a recent study showed that betatrophin did not affect mouse beta cell proliferation in betatrophin-knockout mice, in mice with overexpression of betatrophin in the liver, and in mice administered S961, an insulin receptor antagonist [8]. These controversial results show that betatrophin may not be an essential hormone for beta cells proliferation in mice. In our retrospective study, circulating betatrophin was correlated with log CPR in linear regression. When the relation was analyzed in three groups, only T1DM showed positive correlation. Espes *et al.* revealed that circulating betatrophin was elevated in T1DM, and that it had no correlation C-peptide concentration [10]. Among the study subjects of this study had 18 had completely insulin dependent T1DM and the other 16 patients had detectable levels of serum C-peptide who were classified as slowly progressive insulin-dependent diabetes mellitus. The positive correlation of serum betatrophin with log CPR was found, but we could not suggest this was the direct affect of betatrophin on pancreatic beta cells in T1DM because of since this was a retrospective study based on a small number of patients. A prospective study enrolled a large number of patients is needed to examine the potential protective effects of betatrophin on pancreatic beta cells in humans.

Age, glucose markers (HbA1c, FPG), and TG strongly associated with betatrophin in correlation analysis. Espes *et al.* found a positive correlation between betatrophin and age in non-diabetic controls and a positive correlation between betatrophin and HbA1c in patients with T2DM [11]. Factors contributing to the concentration of betatrophin may differ between these groups and larger studies are needed to determine the relationships of circulating betatrophin

with background factors.

Betatrophin is a novel lipid metabolism regulator that inhibits lipoprotein lipase (LPL) activity and increases the TG level [4-6]. Fenzl *et al.* showed that betatrophin was associated with plasma atherogenic lipids in obesity and type 2 diabetes, and not associated with beta cell function and glucose homeostasis [9]. In *Angptl8* (*betatrophin*) knockout mice, serum TG was reduced after refeeding and low TG was associated with reduced very low density lipoprotein secretion and increased LPL activity, but not with glucose impairment in glucose tolerance tests [18]. Betatrophin (Angptl8) inhibits LPL activity directly or indirectly by promoting Angptl3 cleavage, since cleavage of Angptl3 inhibits LPL activity directly [19-22]. Gusarvoa *et al.* found that *Angptl8* (*betatrophin*) knockout mice have a low plasma TG level and that mice overexpressing betatrophin have an increased plasma TG level [6]. These results suggest that betatrophin may be an essential hormone for maintenance of the TG level. Indeed, a low-frequency and rare coding-sequence variant of *Angptl8* (*betatrophin*) in humans results in low levels of TG [23]. Thus, betatrophin may be an important hormone that explains high TG level in diabetes pathophysiologically. If betatrophin increases the TG level in diabetes, an intervention for hyperbetatrophinemia may not be a new therapeutic target of insulin dependent diabetes, but a target in hypertriglyceridemia. However, we could not examine lipid profiles and LPL in detail, and so further studies are needed to answer this question.

The results of the current study show that circulating betatrophin is significantly elevated in Japanese patients with diabetes. These findings suggest a possibility of association between betatrophin and the levels of glucose and TG.

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Disclosure

The authors declare that there is no conflicts of interest that could be perceived as prejudicing the impartiality of the reported research.

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