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Rafał Maciulewski[1], Anna Zielińska-Maciulewska[1], Katarzyna Siewko[1],
Gabryela Kozłowska[1], Danuta Lipińska[1], Anna J. Milewska[2], Maria Górska[1],
Adam Krętowski[1], Małgorzata Szelachowska[1]

[1]Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Poland
[2]Department of Statistics and Medical Informatics, Medical University of Bialystok, Poland

Elevated levels of betatrophin in patients with newly diagnosed diabetes

ABSTRACT

Introduction. Betatrophin is primarily produced in the liver and regulates the metabolism of triglycerides. Its elevated concentration might be associated with an increased risk of type 2 diabetes. The aim of the study was to evaluate the impact of betatrophin on beta cell function and to compare the concentration of betatrophin in patients newly diagnosed with type 1 diabetes mellitus (T1DM including LADA), type 2 diabetes mellitus (T2DM) and a control group (CG) of healthy volunteers.

Patients and methods. The study included 210 patients with newly diagnosed diabetes (70 with T1DM, 140 with T2DM) and 70 CG. To evaluate the relationship between betatrophin and insulin secretion, a glucagon stimulation test was conducted.

Results. Serum betatrophin concentrations were significantly elevated in T1DM and T2DM in comparison to the control group (3.47 [Q_1 = 2.28, Q_3 = 4.54] in T1DM *vs.* 1.81 [Q_1 = 1.04, Q_3 = 2.67] ng/ml in CG, p < 0.001; 3.12 [Q_1 = 1.89, Q_3 = 4.48] in T2DM *vs.* 1.81 [Q_1 = 1.04, Q_3 = 2.67] ng/ml in CG, p < 0.001). No statistically significant differences in betatrophin concentration were observed between the T1DM and T2DM groups. Significant correlations were established between betatrophin, triglyceride (TG) and high-density lipoprotein (HDL) levels in all study participants, and C-peptide in the T1DM group.

Conclusions. Betatrophin concentration was significantly elevated in patients with newly diagnosed T1DM and T2DM, compared to the control group and could be a biomarker of diabetes. Our study provided evidence which supports the impact of betatrophin on lipid metabolism. The positive correlation between betatrophin and C-peptide in the T1DM group suggests that betatrophin is associated with insulin secretion in T1DM. (Clin Diabetol 2019; 8, 2: 110–115)

Key words: betatrophin, C-peptide, glucagon stimulation test, newly diagnosed diabetes

Introduction

Betatrophin is a protein encoded by the chromosome 19 open reading frame 80 (C19orf80) gene [1] and is produced primarily in the liver and adipose tissue. Despite the fact that it was discovered in 2004, its mechanism of action has not been fully elucidated. As our knowledge about betatrophin evolved, the protein was given various names including hepatocellular carcinoma-associated protein (TD26) [2], angiopoietin-like protein 8 (ANGPTL8) [3], refeeding-induced fat and liver protein (RIFL) [4], and lipasin [5]. However, the best documented and most widely known effect of betatrophin is on lipoprotein lipase (LPL). Together with ANGPTL3 and ANGPTL4, betatrophin is involved in the regulation of fasting and postprandial triglyceride (TG) levels by inhibiting LPL activity. These mechanisms also impact on the distribution of fatty acids to muscle or adipose tissue depending on the individual's nutritional intake and physical activity.

Since the liver and adipose tissue are the two key organs involved in insulin resistance, the role of betatrophin in insulin resistance and type 2 diabetes mellitus (T2DM) has been investigated by a number of researchers. In the majority of published studies in diabetic and obese patients, betatrophin concentrations have been demonstrated to be elevated [6–8]. Furthermore, Abu-Farha et al. showed betatrophin to be an independent predictor of T2DM development. The risk of developing the condition was six times higher in patients with betatrophin concentrations in the highest, third tertile after taking into account the effects of multiple confounders such as age, sex, nationality and lipid profile [7]. A subsequent study by the same research team found that betatrophin concentrations were not associated with C-peptide levels in patients with T2DM, whereas in non-diabetic individuals a positive relationship between betatrophin and C-peptide concentrations was established [9]. An association between betatrophin, insulin resistance and beta cell function has been observed in other diseases such as polycystic ovary syndrome [10].

One of the aims of the study when it was initially designed was to explore the crucial issue regarding the potential role played by betatrophin in insulin secretion. However, a publication supporting the hypothesis that betatrophin was responsible for beta cell proliferation in mice had been retracted [11] since subsequent studies produced contradictory results [12–15]. Both Yi et al. and Cox et al. established that betatrophin did not contribute to pancreatic beta cell proliferation in animals [11, 13, 14].

The primary aim of the study was to assess the concentration of betatrophin in patients with newly diagnosed diabetes in comparison to a control group (CG). The secondary objective was to determine the relationship between betatrophin concentration, residual beta cell secretory capacity and lipid concentration.

Materials and methods

In total, 280 subjects recruited from the Diabetology Department and the Diabetology Outpatient Clinic of the Medical University of Bialystok participated in the study — 210 with newly diagnosed diabetes and 70 controls. All participants were interviewed by a doctor and their medical history, smoking status, and alcohol and drug use were assessed. Patients with diabetes secondary to another condition (e.g. steroid use, Cushing's disease, acute and chronic pancreatitis), liver cirrhosis, advanced renal failure, cancer, advanced heart failure (NYHA III–IV) or acute inflammation (based on CRP indication) were excluded from this study. Written informed consent was obtained from all participants.

The study design was approved by the Local Ethics Committee of the Medical University of Bialystok, Poland.

Diabetes was diagnosed in accordance with the 1999 WHO criteria [16]. Patients with diabetes were divided into two groups: those with type 1 diabetes mellitus (T1DM including patients with latent autoimmune diabetes of adults [LADA]; 70 patients) and those with type 2 diabetes mellitus (T2DM; 140 patients). In the patients with T1DM and T2DM with high blood glucose levels, fasting blood sampling and a glucagon stimulation test (GST) were performed after prior metabolic adjustment (glycemic control, and fluid and electrolyte management). In the patients with T2DM without severe hyperglycaemia (i.e. those who did not require hospitalisation), blood samples were collected and a GST conducted prior to hypoglycaemic treatment. The control group was recruited (through advertising in the local community) from healthy volunteers with no family history of T1DM or other autoimmune diseases. Each candidate for the control group underwent an OGTT and routine blood tests (CRP, creatinine, and transaminase). Only individuals with normal test results were included in the control group. Fasting blood samples were collected from all study participants to determine the concentrations of betatrophin, glucose, C-peptide, total cholesterol, LDL, HDL, TGs, free fatty acids, CRP, creatinine, percentage of HbA_{1c}, activity of AST and ALT as well as titration of anti-glutamic acid decarboxylase (anti-GAD), anti-tyrosine phosphatase (anti-IA2) and anti-insulin antibodies. Subsequently, a GST was performed in which 1 mg glucagon was administered intravenously and the concentration of C-peptide was measured at 0 and 6 minutes after glucagon administration. Selected anthropometric measurements including weight (using electronic weigh scales), height, waist and hip circumference, and waist to hip ratio (WHR) were obtained from all study participants. BMI was calculated according to the standard formula (body weight in kg/height in meters squared).

Betatrophin concentrations were measured using a commercially available ELISA kit (USCN Life Science Inc., Wuhan, China) with both an intra- and inter-assay coefficient of variation (CV) of < 10%. The assessment of serum fatty acids was conducted using a calorimetric measure of non-Estrified fatty acids (NEFA) (Zenbio, Research Triangle Park, North Carolina, USA). Anti-islet antibodies (GADA, IA-2A, IAA) were evaluated using ELISA kits (Euroimmun AG, Lubeck, Germany). C-peptide levels were analysed using an enzyme-amplified sensitivity immunoassay performed on a microtiter plate (DiaSource Europe SA, Ottignies-Louvain-La-Neuve, Belgium). Glycated hemoglobin (HbA_{1c}) was assessed using high-performance liquid chromatography (HPLC;

BIO-RAD Laboratories, Munich, Germany). Plasma glucose concentration was measured using an enzymatic method with hexokinase (Cobas c111, Roche Diagnostic Ltd, Basel, Switzerland). Total cholesterol, high-density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol and TG concentrations were assayed using an enzymatic-colorimetric method (Cobas c111, Roche Diagnostic Ltd, Basel, Switzerland).

As regards the GST, the area under the curve of the C-peptide level (AUC) was calculated using the formula:

$$AUC = (fasting\ C\text{-}peptide \times C\text{-}peptide\ after\ glucagon) \times 3.$$

Statistical analysis

Data were analysed using the STATISTICA software, v. 12.5 (Stata Soft, Tulsa, USA) and STATA 12.0 software. Statistical significance was determined at $p < 0.05$. The Shapiro–Wilk normality test was used to evaluate data distribution. The data are presented as median and first–third quartiles (Q_1–Q_3) due to a lack of normal distribution of the tested parameters. To compare differences between groups, the Kruskal-Wallis test with a post-hoc analysis and the Mann-Whitney test were used. Correlations between betatrophin concentrations and biochemical variables were established using Spearman's correlations coefficient. A multivariate linear regression analysis was performed to evaluate which factors were independently associated with the serum betatrophin level.

Results

Serum betatrophin concentration was highest in patients with T1DM, with the levels being significantly higher than those in the CG (3.47 [Q_1 = 2.28, Q_3 = 4.54] ng/ml *vs.* 1.81 [Q_1 = 1.04, Q_3 = 2.67] ng/ml, respectively; $p < 0.001$), but not those in the T2DM group (3.12 [Q_1 = 1.89, Q_3 = 4.48] ng/ml). Similarly, in patients with T2DM, the concentration of betatrophin was significantly higher compared to that of the CG (Fig. 1). Despite the fact that patients with T1DM and the controls did not differ in terms of age and BMI, those with T2DM were older and had a higher BMI than those in the CG (see Table 1). Following adjustment for sex, age and BMI, differences in betatrophin concentration between the groups were still statistically significant ($p < 0.001$). There were no statistically significant differences in the concentration of betatrophin in smokers compared to non-smokers in either study group.

Table 1 presents the clinical and biochemical characteristics of the three study groups. In the T1DM group, a significant positive correlation between betatrophin levels and C-peptide following the GST was observed (Δ C-peptide; Table 2). A statistically signifi-
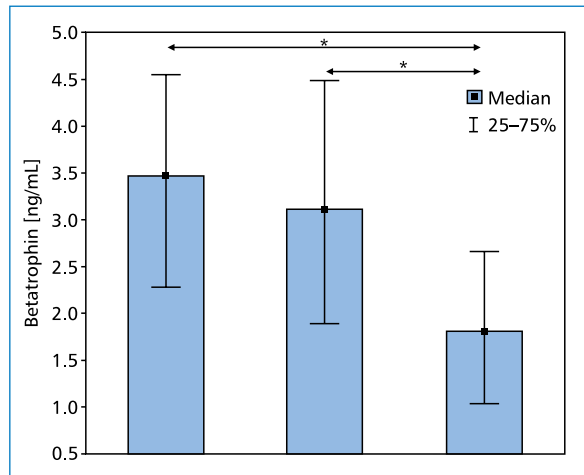
Figure 1. Significantly higher betatrophin concentrations were observed in patients with type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), compared to patients in the control group (CG). *p < 0.001 (*post hoc* analysis, Kruskal-Wallis test). T1DM: n = 70; T2DM: n = 140; CG: n = 70

cant positive correlation between the concentration of betatrophin and fasting C-peptide at the beginning of the GST (C-peptide 0') and 6 min after stimulation (C-peptide 6'), as well as the AUC of C-peptide in the GST (AUC GST; Table 3) was observed in the CG.

In all subjects, a significant positive correlation between serum betatrophin and TG concentrations, as well as a significant negative correlation with HDL cholesterol, were observed (Table 4). In the T1DM group, a significant positive correlation between betatrophin and total cholesterol levels was observed (Table 2). In the T2DM group, the concentration of betatrophin was positively correlated with the concentration of LDL cholesterol (Table 5).

Using multivariate linear regression, independent predictors of betatrophin concentration in the T2DM group were demonstrated to include: fasting glucose ($\beta = -0.01$, $p < 0.001$), BMI ($\beta = -0.06$, $p = 0.04$) and total cholesterol ($\beta = 0.01$, $p = 0.016$); $R^2_A = 0.11$. In the T1DM group, multiple linear regression revealed that independent predictors of betatrophin concentration included C-peptide AUC ($\beta = 0.13$, $p = 0.029$), gender ($\beta = 0.86$, $p = 0.019$), and LDL-cholesterol ($\beta = 0.01$, $p = 0.039$); $R^2_A = 0.17$.

C-reactive protein was within the normal range in all the subjects since individuals with acute infections were excluded from the study. We found no statistically significant differences in total and LDL cholesterol between the groups, but we established significantly higher TG levels in T2D in comparison with T1D and CG. HDL cholesterol was significantly lower in T1D and T2D in comparison with CG.

Table 1. Characteristics of the study groups

	Type 1 diabetes	Type 2 diabetes	Control group	p value Kruskall-Wallis
N	70	140	70	
Age (years)	34.0 (26.0–43.0)[a]	54.0 (44.5–63.0)[c]	33.5 (27.0–50.0)	< 0.001
BMI [kg/m^2]	22.5 (20.8–25.8)[a]	29.8 (26.5–33.6)[c]	24.0 (22.0–27.9)	< 0.001
WHR	0.9 (0.8–0.9)[a]	1.0 (0.9–1.0)[c]	0.9 (0.8–0.9)	< 0.001
HbA$_{1c}$ (%)	11.0 (9.4–12.9)[a, b]	8.0 (6.5–10.9)[c]	5.3 (5.0–5.4)	< 0.001
C-peptide 0' [pmol/l]	0.3 (0.2–0.5)[a, b]	0.9 (0.6–1.3)[c]	0.5 (0.4–0.7)	< 0.001
C-peptide 6'[pmol/l]	0.5 (0.3–0.9)[a, b]	1.6 (1.2–2.3)	1.4 (1.0–1.8)	< 0.001
Δ C-peptide	0.2 (0.1–0.4)[a, b]	0.7 (0.4–1.0)	0.7 (0.5–1.1)	< 0.001
AUC C-peptide	0.5 (0.2–1.3)[a, b]	4.6 (2.3–9.3)[c]	2.1 (1.3–4.0)	< 0.001
Glucose 0' [mg/dl]	146.5 (122.5–176.5)[b]	140.5 (115.5–163.5)[c]	88.0 (83.0–93.0)	< 0.001
Glucose 6' [mg/dl]	164.0 (135.5–195.5)[b]	156.5 (131.5–179.0)[c]	106.0 (101.0–117.0)	< 0.001
Total cholesterol [mg/dl]	177.0 (154.0–208.0)	185.0 (161.0–223.0)	192.0 (170.0–222.0)	0.106
HDL cholesterol [mg/dl]	46.0 (36.0–60.0)[b]	44.0 (34.0–51.0)[c]	60.0 (51.0–74.0)	< 0.001
LDL cholesterol [mg/dl]	107.0 (83.1–131.1)	116.0 (88.0–148.0)	122.0 (91.6–140.0)	0.186
TG [mg/dl]	89.0 (66.0–145.0)[a]	148.0 (110.0–196.0)[c]	90.5 (61.0–117.0)	< 0.001
Free fatty acids [μM/ml]	1073.8 (849.3–1503.8)[b]	1174.4 (916.8–1565.9)[c]	893.7 (553.2–1070.2)	< 0.001
CRP [mg/dl]	0.9 (0.4–2.0)[a]	2.4 (1.0–5.6)[c]	0.6 (0.4–1.0)	< 0.001

Note: Values for median (first–third quartile) are presented. AUC — area under the curve; BMI — body mass index; CRP — C-reactive protein; HbA$_{1c}$ — glycated hemoglobin; HDL — high-density lipoprotein; LDL — low-density lipoprotein; WHR — waist–hip ratio. Conversion factors to SI units are as follows: glucose, 0.05551; total cholesterol, 0.02586; LDL cholesterol, 0.02586; HDL cholesterol, 0.02586; triglycerides, 0.0114; Superscript letters indicate statistically significant (p < 0.05) differences between: [a]T1DM and T2DM; [b]T1DM and control group; [c]T2DM and control group

Table 2. Variables significantly correlated with betatrophin concentration in the T1DM group

	R	p value
Waist circumference	0.28	0.021
WHR	0.27	0.029
BMI	0.27	0.022
C-peptide 6'	0.24	0.049
Δ C-peptide	0.28	0.020
Total cholesterol	0.26	0.031

BMI — body mass index; WHR — waist–hip ratio

Table 3. Variables significantly correlated with betatrophin concentration in the control group

	R	p value
C-peptide 0'	0.39	< 0.001
C-peptide 6'	0.32	0.007
AUC C-peptide	0.37	0.002

AUC — area under the curve

Table 4. Variables significantly correlated with betatrophin concentration in the whole group

	R	p value
Age	0.12	0.040
Waist circumference	0.12	0.042
WHR	0.15	0.011
HbA$_{1c}$	0.24	< 0.001
Glucose 0'	0.19	0.001
Glucose 6'	0.17	0.004
HDL cholesterol	−0.17	0.006
TG	0.20	0.001

HbA$_{1c}$ — glycated hemoglobin; HDL — high-density lipoprotein; TG — triglycerides; WHR — waist–hip ratio

Table 5. Variables significantly correlated with betatrophin concentration in the T2DM group

	R	p value
HbA$_{1c}$	−0.19	0.023
Glucose 0'	−0.23	0.005
Glucose 6'	−0.24	0.005
LDL cholesterol	0.20	0.019

HbA$_{1c}$ — glycated hemoglobin; HDL — low-density lipoprotein

Discussion

To our knowledge, no comprehensive analysis of the concentration of betatrophin in patients with newly diagnosed T1DM and T2DM has been conducted to date. The majority of published papers on betatrophin are related to T2DM. No study evaluating the concentration of betatrophin in newly diagnosed T1DM is available in the literature and only one study

on the subject in newly diagnosed patients with T2DM has been published [14]. In our study, betatrophin concentration was demonstrated to be significantly higher in patients with newly diagnosed T1DM and T2DM compared to the CG.

Similar observations were made by Hu et al., who showed that patients with newly diagnosed T2DM had higher betatrophin concentrations in comparison with the control group [17]. Despite the fact that a number of studies have demonstrated betatrophin concentrations to be higher in patients with T2DM [6, 18–20], conflicting data exist on the subject [21]. A study conducted by Yamada and colleagues [22], for instance, which included 34 patients with T1DM and 30 patients with T2DM, demonstrated that betatrophin levels were significantly higher in diabetic patients compared to the control group (12 individuals). The authors also reported that the concentration of fasting C-peptide correlated significantly with betatrophin levels in patients with T1DM but not in those with T2DM, which was also confirmed by our results. Our study revealed positive correlations between C-peptide and betatrophin levels in T1DM and the CG but no such correlations were observed in the study participants with T2DM. A study conducted by Abu-Farha and colleagues, which included a larger group of patients with T2DM (556 subjects), did not establish a correlation between betatrophin levels and fasting C-peptide [7]. Interestingly, and in contrast to the findings presented above, Tokumoto and colleagues [23] observed a negative correlation between betatrophin and C-peptide concentration in the GST in patients with T2DM. Unlike the study by Tokumoto et al., we performed the test in patients with T1DM and in the CG. To the best of our knowledge, the present study is the first to use glucagon to assess beta cell reserve and compare it to betatrophin concentration in T1DM patients. We demonstrated a significant correlation between betatrophin and C-peptide 6′ and ΔC-peptide in those patients and observed similar correlations in the CG. The mechanism responsible for this effect has not yet been elucidated. The results of our research alone do not allow us to determine why the concentration of betatrophin is not associated with beta cell reserve in patients with T2DM, unlike in patients with T1DM and the CG. To date, no evidence concerning the impact of betatrophin on the number of pancreatic beta cells in patients with T1DM, T2DM or healthy individuals has been published. Therefore, studies utilising the GST assessing beta cell function in patients with T1DM are needed.

Another aspect of our research was the contribution of betatrophin to lipid metabolism. The results of a number of recent studies confirm that betatrophin,

together with ANGPTL3 and ANGPTL4, is involved in the regulation of TG and HDL cholesterol levels by affecting lipoprotein lipase (LPL) [6, 24–26]. During fasting LPL activity is inhibited in white adipose tissue and increased in the myocardium and skeletal muscles to provide energy in the form of fatty acids. Following a meal, LPL activity in white adipose tissue increases, allowing fatty acids to be stored in adipocytes. By contrast, in muscles, under the influence of betatrophin and ANGPLT3, LPL activity is inhibited. ANGPTL4 is responsible for the inhibition of LPL activity in white adipose tissue during times of fasting and exercise. An increase in the amount of the above ANGPTLs (3, 4, 8) results in an increase in the concentration of TG in the serum while a reduction in any of these ANGPTLs decreases the levels of TG in the blood. Understandably, the distribution of fatty acids in the corresponding tissues is different, but each of these ANGPTLs is an inhibitor of LPL and therefore, the effect on the concentration of TG in the blood is the same [25]. The present study demonstrated a positive correlation between betatrophin and TG, and a negative correlation between betatrophin and HDL cholesterol, which may be evidence in support of the impact of betatrophin on lipid metabolism described above.

The situation is different in the case of patients with T1DM. In our study, this group was observed to have significantly higher concentrations of betatrophin in comparison with the CG, despite having similar levels of lipids. A potential explanation for this could be the effect of insulin deficiency in patients with T1DM. The results of a study conducted by Haridas and colleagues demonstrated that insulin reduced the concentration of circulating ANGPTL3 while increasing betatrophin expression in white adipose tissue, but not in the blood [27].

In conclusion, our study demonstrated betatrophin concentration to be significantly higher in patients with newly diagnosed T1DM and T2DM in comparison with the control group, which could potentially make it a biomarker of diabetes. Furthermore, it produced evidence supporting the impact of betatrophin on lipid metabolism. It also established that betatrophin concentration was associated with insulin secretion in T1DM, unlike in T2DM. Further research on the impact of betatrophin on insulin secretion in T1DM is needed.

Conflict of interest

The authors declare no competing interests.

Acknowledgments

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