Correlation between Bethatrophin and 25(OH)D Concentrations in a Group of Subjects With Normal and Impaired Glucose Metabolism

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Key words
- betatrophin
- OGTT
- vitamin D

Abstract

Aim: Inducing beta cell replication is a potential therapeutic approach for the treatment of diabetes mellitus. Recently betatrophin was suggested as a novel stimulator of beta cell proliferation in mice but its role in humans remains to be established. We aimed to investigate betatrophin concentration and its association with metabolic parameters in a group of individuals with normal glucose tolerance, pre-diabetes and diabetes mellitus who had not been previously treated.

Methods: A total of 72 subjects were recruited for this cross sectional study: 23 subjects with normal glucose tolerance (NGT), 22 subjects with prediabetes, and 27 subjects with diabetes mellitus (DM). Circulating betatrophin concentration, 75 g oral glucose tolerance test, fasting insulin, glycosylated hemoglobin, 25hydroxy vitamin D and HOMA IR were measured.

Results: The difference in betatrophin values did not reach statistical significance between the 3 groups [NGT:206 (176–297)pg/mL, Prediabetes:232 (181–254)pg/mL, DM:245 (205–526)pg/mL, p = 0.078]. Betatrophin was negatively significantly correlated with BMI and positively significantly correlated with 25(OH)vitD (p = 0.043 and p = 0.001 respectively). Multivariate linear regression showed that 25(OH) vitD (β = 0.440 p = 0.001) and fasting glucose (β = 0.003 p = 0.038) were variables associated with betatrophin concentration (R2 = 0.251).

Conclusions: In a group of subjects ranging from those with normal glucose tolerance to newly diagnosed diabetes, we found that 25(OH)D and fasting glucose were factors associated with serum betatrophin concentration.

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Introduction

Diabetes mellitus is a chronic hyperglycemic metabolic disease resulting from insulin secretion defect or insulin resistance. Type 2 diabetes encompasses individuals with insulin resistance and relative insulin deficiency [1]. Insulin resistance is the primary problem in type 2 diabetes leading to functional changes in pancreatic beta cells. Beta cells show compensatory responses to insulin resistance by increasing secretory capacity and beta cell mass [2]. After the compensatory stage of type 2 diabetes, the function of beta cells becomes inadequate and beta cell loss begins.

It was reported that beta cell mass of diabetic subjects was about 30–70 % of nondiabetic controls and in subjects with impaired fasting glucose status beta cells were also reduced to 60 % of normal [3]. Beta cell regeneration is a promising approach that may be relevant in the treatment of diabetes. However the mechanisms underlying beta cell proliferation are currently under investigation. Factors including gestation, high blood sugar, and insulin resistance were reported to induce beta cell proliferation [4–6].

Betatrophin is another factor that was reported to be associated with beta cell proliferation [7]. It is also known as hepatocellular carcinoma associated protein TD26, lipasin or refeeding-induced fat liver (RIFL) and it was initially detected in 2004 as a tumor associated antigen [8]. Previous studies revealed that betatrophin was involved in lipid regulation. Apart from its role in lipid metabolism, it was suggested to induce beta cell proliferation in mice but subsequent studies did not confirm betatrophin's role in beta cell mass expansion [9]. Also a few studies investigated betatrophin in subjects with type 1 and type 2 diabetes mellitus. Although some studies reported higher betatrophin levels in type 2

diabetes [10–12], a recent study showed that circulating betatrophin concentrations were decreased in human obesity and type 2 diabetes [13]. The function of betatrophin in glucose metabolism is not clear and there are contradictory reports on betatrophin concentration in diabetic subjects. In this study we aimed to investigate betatrophin concentration and its associations with metabolic biomarkers in a group of subjects with diabetes and normal glucose tolerance.

Materials and Methods

Study population

Subjects who were admitted to the Endocrinology and Metabolism outpatient clinic with suspicion of glucose disorders from October 2013 to January 2014 were candidates for participation. Subjects without exclusion criteria were invited to participate in the study. Local ethics committee approval was received and each participant signed informed consent. To evaluate glucose tolerance, the 75 g oral glucose tolerance test (OGTT) was performed in fasting participants. Based on American Diabetes Association diagnostic criteria, participants were classified into 3 groups: NGT, prediabetes, and T2DM. Glucose tolerance status was evaluated according to OGTT results as follows: NGT (fasting glucose < 100 mg/dL and 2-h glucose < 140 mg/dL), prediabetes (fasting glucose 100 mg/dL to 125 mg/dL or 2-h glucose 140 mg/dL to 199 mg/dL) and newly diagnosed type 2 diabetic subjects (fasting glucose > 125 mg/dL or 2-h glucose ≥ 200 mg/dL) [1]. The exclusion criteria included known diabetes, cardiovascular, gastrointestinal and kidney diseases, acute or chronic inflammatory disorders, cancer, other known major diseases, pregnancy, medication use, smokers and alcohol use.

Clinical, demographic, and laboratory parameters

In each participant, sex, age, family and medical histories were recorded. Body height and weight were measured. Body mass index was estimated using the kg/m^2(height^2) equation. Insulin resistance was measured with HOMA-IR (homeostasis model assessment of insulin resistance) method (fasting insulin (μU/ml) × fasting glucose (mg/dL)/405). Blood samples were collected from all subjects after fasting by venous puncture technique into Vacutainer® serum tubes with gel separator (BD Vacutainer, Plymouth, UK). Glucose levels were enzymatically determined with the hexokinase method on the same day by Olympus AU 5800 analyzer (Beckman Coulter Inc., Brea CA, USA). Insulin and 25-hydroxyvitamin D concentrations were measured by electrochemiluminescence immunoassay with Roche Cobas e 601 analyzer (Roche Diagnostics, Indianapolis, IN, USA). Glycated hemoglobin (HbA1c) was measured using boronate affinity high-performance liquid chromatography method (Trinity Biotech, Kansas City, MO, USA).

Serum samples collected for betatrophin quantitation were stored at −80° until analyses. Serum betatrophin levels were measured using betatrophin enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Cat. No: 201-12-5327, Sunred biological technology, Shangai, China). The intra-assay and inter-assay coefficients of variations were < 10 % and < 12 % for betatrophin (ng/L) respectively.

Statistical analyses

Statistical analyses were conducted using SPSS version 17 (SPSS Inc. Chicago, IL). Parameters with normal distribution are presented as mean ± SD and parameters with non-normal distribution are presented as median (25^{th} and 75^{th} percentiles). Betatrophin and 25(OH)D were log transformed for analyses. The 3 study groups were compared using one-way analysis-of-variance (ANOVA) with post-hoc analysis based on Games-Howell post-hoc comparisons. The association between variables was determined with Pearson correlation coefficients. Multivariate linear regression was performed to identify the independent relationship with betatrophin and other factors. $P < 0.05$ was considered statistically significant.

Results

Study population consisted of 72 subjects (23 subjects with normal glucose tolerance, 22 subjects with prediabetes, 27 subjects with diabetes mellitus). The baseline characteristics of the subjects with normal glucose tolerance, prediabetes and type 2 diabetes mellitus are presented in **Table 1**. The 3 groups were not significantly different with respect to age, BMI and 25(OH)D. Fasting glucose, 2 h glucose, insulin, HOMA IR and HbA1c values were significantly different among the groups ($p < 0.001$, $p < 0.001$, $p = 0.001$, $p < 0.001$, $p < 0.001$ respectively). Also the difference in betatrophin values did not reach statistical significance ($p = 0.078$). We did not observe a significant increasing trend in betatrophin concentrations from NGT group to T2DM group (p for trend = 0.708). There was no significant difference in

Table 1 Biochemical and clinical parameters of study participants

	NGT (n = 23)	Prediabetes (n = 22)	T2DM (n = 27)	P-value
Age	44 ± 10	50 ± 10	50 ± 10	0.076
BMI (kg/m^2)	31.3 ± 5.3	30.2 ± 3.1	32.1 ± 4.9	0.373
Gender	6 male (26 %)	7 male (31 %)	12 male (44 %)	
$Glucose_{0h}$ (mg/dL)	90.3 ± 5.7 [a]	113.6 ± 6.5 [b]	136 ± 12.6 [c]	<0.001
$Glucose_{2h}$ (mg/dL)	95.4 ± 20.9 [a]	160.7 ± 12.9 [b]	208.6 ± 47.3 [c]	<0.001
$Insulin_{0h}$ (μU/mL)	10.3 ± 4.2 [a]	15.3 ± 7.1 [b]	18.2 ± 9.0 [b]	0.001
HOMA IR	2.32 ± 0.99 [a]	4.32 ± 2.04 [b]	6.18 ± 3.10 [c]	<0.001
HbA1c (%)	5.4 ± 0.3 [a]	6.0 ± 0.3 [b]	6.5 ± 0.5 [c]	<0.001
25(OH)D (ng/mL)	14.4 (10.5–22.6)	10.8 (7.4–18.4)	16.5 (9.8–22.2)	0.161
Betatrophin (pg/mL)	206 (176–297)	232 (181–254)	245 (205–526)	0.078

Values in the same row not sharing the same superscript are significantly different
Log transformed bethatrophin and 25(OH)D was used in statistical analyses
Data was presented as mean ± SD and median ($25–75^{th}$ percentile)

Table 2 Correlation analysis of serum bethatrophin and other variables.

	Betatrophin	
	r	P-value
Age	−0.050	0.675
BMI (kg/m^2)	**−0.240**	**0.043**
Glucose$_0$ (mg/dL)	0.217	0.067
Glucose$_{120}$ (mg/dL)	0.133	0.265
HOMA IR	0.026	0.830
Insulin (μU/mL)	−0.044	0.712
25(OH) vit D (ng/mL)	**0.392**	**0.001**
HbA1c (%)	0.157	0.189

betatrophin values between male and female subjects (p = 0.095 for NGT, p = 0.684 for prediabetes, p = 0.742 for T2DM group).

In the Pearson correlation analysis, betatrophin was negatively significantly correlated with BMI and positively significantly correlated with 25(OH)vitD (p = 0.043 and p = 0.001 respectively). There were no significant correlations between age, fasting glucose, 2 h glucose, insulin, HOMA IR and HBA1c with serum betatrophin concentrations (**Table 2**).

We also performed multivariate regression analysis to test the independent effects of each variable. Variables with non-normal distribution were log transformed. Age, gender, BMI, HOMA IR, fasting glucose, 2 h glucose and HbA1c were selected as candidate variables in the first model. Unimportant variables were omitted one by one with backward procedure and values with a p-value < 0.2 remained in the last model. The last model demonstrated that 25(OH) vitD (β = 0.440 p = 0.001) and fasting glucose (β = 0.003 p = 0.038) were independently associated with betatrophin concentrations (R^2 = 0.251). BMI was marginally non-significant in the last model (β = −0.011 p = 0.054).

Discussion

In our study although subjects with type 2 diabetes have higher betatrophin values than those with normal glucose tolerance, the difference did not reach statistical significance possibly due to our limited sample size. The results of previous studies investigating betatrophin concentration in diabetes are very heterogeneous. However most of the studies reported higher betatrophin concentrations in type 2 diabetes mellitus [10, 11, 14]. The compensatory increase in betatrophin concentrations in response to increased glucose concentrations or insulin resistance seems to be a reasonable mechanism explaining betatrophin levels in diabetes. Our data revealed that fasting glucose was a factor associated with betatrophin levels. In a recent study by Hu et al., 2 h glucose and postprandial insulin were independently associated with betatrophin concentrations in newly diagnosed type 2 diabetic patients [10]. The mentioned study is similar to ours as we also conducted the study in newly diagnosed patients with no use of antidiabetic medications. In the study by Hu et al. patients had approximately 2 fold higher postprandial glucose concentrations than our study population. The difference between the 2 studies may be attributed to this variation in postprandial glucose levels.

In crude analysis BMI was significantly associated with betatrophin concentrations but after controlling for glucose and vitamin D the association between BMI and betatrophin was not significant. Fu et al. reported positive correlation of BMI with betatrophin [14], conversely Ambrosi et al. reported negative correlation between BMI and betatrophin values [13]. Different study populations may be responsible for these contradictory results. It was also reported that using different Elisa kits results in different circulating levels of betatrophin [15]. There may also be some factors affecting betatrophin concentrations that we do not know yet. Our study reveals vitamin D as a factor associated with betatrophin concentrations.

In recent years the effects of vitamin D on glucose metabolism has been investigated by several observational and interventional studies. Observational studies suggest that higher 25 hydroxyvitamin D concentrations are associated with lower risk of developing type 2 diabetes [16, 17]. Particularly vitamin D was reported to be associated with beta cell function [18]. In addition to observational studies a few randomized controlled trials showed that vitamin D supplementation has beneficial effects on glycemic control by improving beta cell function and insulin secretion [19, 20]. Another interventional study suggested that vitamin D supplementation shows its effects by reducing insulin resistance [21]. The mechanism of action responsible for the beneficial effects of vitamin D on glucose metabolism is not clear. The presence of vitamin D receptors on beta cells and the presence of vitamin D response element in the human insulin gene promoter were previously discussed as potential mechanisms of effect [21–23]. Also it was proposed that vitamin D may show its effects on glycemic status by normalizing calcium concentrations and ensuring adequate intracellular calcium [23]. Our study revealed a positive correlation between betatrophin and 25(OH)D concentrations. This association may be another mechanism of action of vitamin D on pancreatic beta cells. However the question of whether human beta cells are responsive to human betatrophin should be clarified to confirm this hypothesis. Yi et al. showed that delivering betatrophin with hydrodynamic injection to mice leads to a 17 fold increased beta cell proliferation rate [7], but this effect could not be observed for human beta cells in the transplant setting [9]. Although Cox et al. reported that no beta cell mitogenic effect was detected following a 26 fold increased betatrophin expression in mice [24], Chen et al. reported that delivering betatrophin to adult rats with ultrasound-targeted microbubble destruction induced adult and aged beta cell regeneration [25]. The study by Gusarova et al. showed that Angptl8−/− mice undergo entirely normal beta cell expansion in response to insulin resistance and the authors concluded that betatrophin does not control pancreatic beta cell expansion [26]. After these negative studies and their subsequent experiments, Yi et al. modified their conclusion and reported that betatrophin alone is not capable of inducing beta cell proliferation [27]. Further studies are needed to elucidate these contradictory reports.

The current study does not add a significant contribution to the understanding of betatrophin's effect on beta cells. However our results may help to understand the regulation of betatrophin levels in human subjects. Our statistical analysis showed that the association between betatrophin and 25(OH) is stronger than for previously reported factors such as glucose, insulin and BMI. Studies investigating betatrophin levels in circulation should consider 25(OH)D as a possible agent that may be associated with betatrophin. Since this is the first study linking betatrophin and vitamin D, there are very limited data to support a direct relationship between these parameters. There may be a direct link or there may also be another factor affecting both of these parameters such as diet or physical activity.

The limitations of the current study come from its design. Our study is a cross-sectional study and cannot infer causality. The samples reflect only one time point and do not provide temporal variations in betatrophin levels. Also HOMA-IR is not the gold standard method for measuring insulin sensitivity. The strength of the study is that it was done in subjects with no use of anti-diabetic medications.

In conclusion the current study evaluated betatrophin concentrations in a group of subjects ranging from those with normal glucose tolerance to newly diagnosed diabetes and for the first time showed that 25(OH)D may be associated with serum betatrophin concentrations.

Disclosure: The authors declare that there is no conflict of interest associated with this manuscript.

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