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Short Report: Pathophysiology

Correlation of circulating betatrophin concentrations with insulin secretion capacity, evaluated by glucagon stimulation tests

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Abstract

Aim To investigate the relationship between plasma betatrophin concentrations and insulin secretion capacity in people with Type 2 diabetes.

Methods Glucagon stimulation tests (1 mg) were performed in 70 people with Type 2 diabetes after an overnight fast. Plasma betatrophin concentrations were measured using an enzyme-linked immunosorbent assay. Insulin secretion capacity was evaluated by measuring increments of C-peptide concentration in response to glucagon stimulation, and creatinine clearance was determined by comparing creatinine concentrations in serum and 24-h urine samples.

Results Plasma betatrophin concentrations were positively correlated with duration of Type 2 diabetes ($r = 0.34$, $P = 0.003$), and negatively correlated with increments of C-peptide concentration ($r = 0.37$, $P = 0.001$) and creatinine clearance ($r = 0.37$, $P = 0.001$). The correlation with increments of C-peptide concentration remained significant after adjustment for age and duration of Type 2 diabetes ($r = 0.25$, $P = 0.037$). Multivariate analysis identified age and increments of C-peptide concentration as independent factors associated with plasma betatrophin levels.

Conclusion Plasma betatrophin levels inversely correlate with insulin secretion capacity, suggesting that betatrophin levels are regulated by insulin secretion capacity in humans.

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Introduction

Decompensation of β-cell mass and function for increased insulin demand is a central feature of Type 2 diabetes mellitus. In conditions of increased insulin resistance, such as obesity and pregnancy, enlargement of β-cell mass and increased insulin secretion are commonly observed, but the mechanisms that underlie these compensatory actions are largely unknown. Recently, Yi *et al.* [1] identified a secreted protein, termed betatrophin, which links hepatic insulin resistance to β-cell proliferation in mice. Betatrophin, also known as hepatocellular carcinoma-associated protein TD26 [2], refeeding-induced fat and liver protein [3], lipasin [4] and angiopoietin-like protein 8 [5], is a potential stimulator of β-cell mass expansion; however, according to the few available experimental study results, although betatrophin dramatically stimulates the proliferation of mouse β cells [1], its effects on the proliferation of human β cells are limited [6]. The pathophysiological role and even metabolism of betatrophin are still unclear, and the relationship between betatrophin and human β-cell function has yet to be addressed. In addition, the results of previous clinical investigations of betatrophin are inconsistent [7–10]. Recent studies showing that circulating betatrophin concentrations are significantly higher in people with Type 1 diabetes [8] or newly diagnosed Type 2 diabetes [9] than in healthy people suggest a potential connection between betatrophin concentration and insulin secretion capacity in people with Type 2 diabetes. To investigate this hypothesis, we evaluated insulin secretion capacity in people with Type 2 diabetes by

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What's new?

- Betatrophin has recently been identified as a potential stimulator of β-cell mass expansion in mice; however, the pathophysiological role of betatrophin in people with diabetes is still unknown.
- This is the first report to show that betatrophin concentrations correlate negatively with insulin secretion capacity and positively with duration of Type 2 diabetes.

measuring incremental C-peptide concentrations induced in glucagon stimulation tests, and analysed its correlation with circulating betatrophin concentrations.

Patients and methods

We recruited 70 hospitalized people with Type 2 diabetes, diagnosed on the basis of the criteria in the American Diabetes Association's 2014 guidelines. The antidiabetic therapies at admission were oral hypoglycaemic agents (n = 28), insulin (n = 6), oral hypoglycaemic agents with insulin (n = 24), and diet therapy (n = 12). Oral hypoglycaemic agents included a sulphonylurea, biguanide, α-glucosidase, and a dipeptidyl peptidase-4 inhibitor. Clinical and laboratory assessments of all patients during hospitalization included medical histories, physical examinations, 24-h urine analyses and 6-min glucagon stimulation tests. The study design was approved by the Kitano Hospital Ethics Committee. We obtained written informed consent from all patients.

Laboratory tests

All antidiabetic agents were discontinued 24 h before the glucagon stimulation tests, which were performed on all patients after an overnight fast. Glucagon (1 mg) was injected intravenously; blood samples before (0 min) and 6 min after injection were collected. Endogenous insulin secretion capacity was estimated according to the change in C-peptide concentration between 0 and 6 min. Plasma betatrophin concentrations of blood samples obtained before injection in P800 tubes (Becton Dickinson, Franklin Lakes, NJ, USA) were measured in duplicate using an enzyme-linked immunosorbent assay kit (Wuhan Eiaab Science, Wuhan, China; Catalogue No.E11644 h). All duplicates with a coefficient of variation > 15% were excluded. Twenty-four-hour urine samples were collected from all participants, and creatinine clearance was calculated using the formula:

$$\mathrm{UcrV/Scr} \times 1.73/\mathrm{BSA},$$

where Ucr is urine creatinine concentration, V is urine volume per minute, Scr is serum creatinine concentration and BSA is body surface area. Estimated GFR was calculated using a revised Japanese equation:

$$\text{estimated GFR (ml/min/1.73 m}^2\text{)} = 194 \times \text{serum creatinine}^{1.094} \times \text{age}^{0.287} \times 0.739(\text{if female}).$$

LDL cholesterol concentrations were calculated using the Friedewald equation. The concentrations of other serum biomarkers were determined in the laboratory at our hospital.

Statistical analysis

Statistical analyses were performed using SPSS software, version 22.0 (SPSS Inc., Chicago, IL, USA). Pearson's correlation coefficient was used to examine the association of betatrophin with other variables. Partial correlations were used for the adjustment of age and duration of Type 2 diabetes. Multivariate regression analysis was performed with betatrophin as the dependent variable and the variables of interest as independent variables. All data are presented as mean ± SD values. A P value < 0.05 was taken to indicate statistical significance.

Results

The clinical and laboratory characteristics of the participants are shown in Table 1. Circulating betatrophin concentrations correlated positively with age (r = 0.56, P < 0.001; Fig. 1a) and duration of Type 2 diabetes (r = 0.34, P = 0.003) and negatively

Table 1 Clinical and biochemical characteristics of the study population

Variable	People with Type 2 diabetes (n = 70)
Age, years	60.3 ± 11.9
Sex: men/women	45/25
BMI, kg/m^2	25.9 ± 4.0
Duration of Type 2 diabetes, years	12.6 ± 9.9
HbA_{1c}, mmol/mol	74.9 ± 20.7
HbA_{1c}, %	9.0 ± 1.9
Fasting plasma glucose, mg/dl	166.3 ± 48.3
Fasting C-peptide concentration ng/ml	1.45 ± 1.54
Increment of C-peptide concentration ng/ml	1.23 ± 0.71
Estimated GFR, ml/min/1.73 m^2	70.95 ± 19.03
Creatinine clearance, ml/min/1.73 m^2	98.21 ± 33.22
Total cholesterol, mg/dl	194.8 ± 42.0
Triacylglycerol, mg/dl	171.5 ± 124.6
HDL cholesterol, mg/dl	53.6 ± 18.9
LDL cholesterol, mg/dl	106.7 ± 30.3
Betatrophin, pg/ml	1367.0 ± 745.2
Diabetes medications, n (%)	
Metformin	30 (42.8)
Sulphonylurea	32 (45.7)
Dipeptidyl peptidase-4 inhibitors	32 (45.7)
Insulin	6 (8.5)
Insulin therapy + oral hypoglycaemic agents	24 (34.2)

Data are means ± SD, unless otherwise indicated.

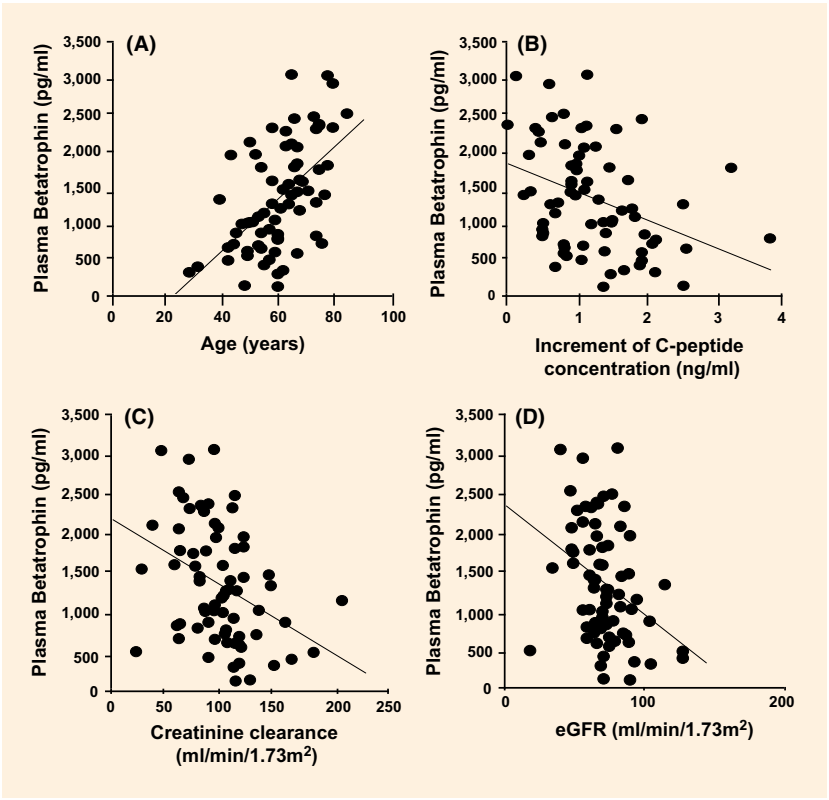

FIGURE 1 Plasma betatrophin concentrations significantly correlated with age, increment of C-peptide concentration creatinine clearance and estimated GFR. (A) Positive correlation between plasma betatrophin levels and age ($r = 0.56$, $P < 0.001$). (B) Negative correlation between plasma betatrophin levels and increment of C-peptide concentration ($r = 0.37$, $P = 0.001$). (C) Creatinine clearance ($r = 0.37$, $P = 0.001$) and (D) estimated GFR ($r = 0.35$, $P = 0.003$) correlated inversely with plasma betatrophin levels. Correlations were determined using Pearson's correlation.

with increments of C-peptide concentration ($r = 0.37$, $P = 0.001$; Fig. 1b), creatinine clearance ($r = 0.37$, $P = 0.001$; Fig. 1c) and estimated GFR ($r = 0.35$, $P = 0.003$; Fig. 1d). After adjustment for age and duration of Type 2 diabetes, only the correlation of betatrophin levels with increment of C-peptide concentration remained significant ($r = 0.25$, $P = 0.037$). There was no correlation, however, between circulating betatrophin concentrations and other clinical variables, including BMI ($r = 0.15$, $P = 0.195$) and concentrations of fasting plasma glucose ($r = 0.02$, $P = 0.862$), HbA_{1c} ($r = 0.06$, $P = 0.570$), triacylglycerol ($r = 0.06$, $P = 0.615$), HDL cholesterol ($r = 0.13$, $P = 0.261$), LDL cholesterol ($r = 0.12$, $P = 0.318$) and total cholesterol ($r = 0.06$, $P = 0.606$). Multivariate regression analysis showed that age ($\beta = 0.50$, $P < 0.001$) and increment of C-peptide concentration ($\beta = -0.25$, $P = 0.013$) were the variables significantly associated with betatrophin concentrations ($R^2 = 0.36$, $P < 0.001$).

Discussion

The present study is the first to show that betatrophin concentrations negatively correlate with increments of C-peptide concentration in response to glucagon stimulation. Previous reports have shown that age affects circulating betatrophin concentrations [7–9]. In the present study, we found that betatrophin concentrations inversely correlated with the duration of Type 2 diabetes. Even after adjustment for age and duration of Type 2 diabetes, the correlation between betatrophin and increments of C-peptide concentration was still statistically significant, which suggests that insulin secretion deficiency is one of the factors that regulate betatrophin concentrations in humans. In contrast to previous results [7,9,10], we did not find a relationship between circulating betatrophin concentrations and BMI, HbA_{1c} or levels of blood lipids such as triglycerides and HDL cholesterol.

Diminished insulin sensitivity induced by insulin receptor antagonists increases hepatic betatrophin expression in mouse models [1] and serum betatrophin concentrations are decreased in obesity and are negatively associated with insulin resistance [10]. These results support the premise that betatrophin levels are regulated by insulin resistance and not by insulin deficiency *per se*. In contrast, elevated circulating betatrophin levels have been reported in people with Type 1 [8] and Type 2 diabetes [9], suggesting that impaired insulin secretion potentially increases circulating betatrophin levels. To measure endogenous insulin secretion capacity, we used glucagon stimulation tests in which glucagon stimulates insulin release via the production of intracellular cyclic AMP, which amplifies insulin secretion [11]. Since impaired insulin secretion in response to glucose stimulation is the central feature of β-cell dysfunction in Type 2

diabetes, glucagon-stimulated insulin secretion more likely represents the functional mass of β cells rather than function of β cell when compared with insulin secretion in an oral glucose tolerance test or a meal test. Japanese people with Type 2 diabetes are relatively lean, and insulin deficiency is predominant over insulin resistance in their aetiology [12]. Moreover, a cross-sectional study showed that long exposure to Type 2 diabetes was associated with a linear decline in endogenous insulin secretion in Japanese people with Type 2 diabetes [13]. The present data also showed that Type 2 diabetes duration was negatively associated with increments of C-peptide concentration (data not shown); therefore, the higher betatrophin concentrations in participants with lower insulin secretion capacity and longer duration of Type 2 diabetes observed in the present study might reflect a greater need for enhancement of β-cell functional mass in Japanese people with Type 2 diabetes.

Consistent with other studies [8,9], age was positively associated with plasma betatrophin concentrations in the present study. Our data also showed that circulating betatrophin concentrations negatively correlated with creatinine clearance and estimated GFR, although adjustment for age and duration of Type 2 diabetes eliminated these correlations. Aging is accompanied by the deterioration of renal function [14], and diabetes exacerbates renal dysfunction in elderly individuals [15]. Indeed, age showed a strong negative correlation with creatinine clearance and estimated GFR in the present study (data not shown), therefore, the negative relationship between circulating betatrophin concentrations and creatinine clearance could be indirect because of confounding by age.

The present study has several limitations. First, because we did not examine age-matched or BMI-matched healthy people, we could not address the physiological metabolism of betatrophin. Second, we cannot exclude other potential confounding factors, which would affect the results because we investigated the relationship of betatrophin with limited variables. Third, although we found a strong association of betatrophin concentrations and insulin secretion capacity, it was not clear whether the relationship between betatrophin levels and insulin secretion capacity was direct or indirect. Fourth, the statistical power may be insufficient because the present study included only a small number of participants in a single hospital. Finally, the potential influence of poor glycaemic control on betatrophin levels cannot be precluded because of the high HbA_{1c} concentrations at baseline.

In conclusion, our data suggest an association between plasma betatrophin concentrations and endogenous insulin secretion capacity in people with Type 2 diabetes. Further research on the regulation and metabolism of betatrophin is needed to elucidate its pathophysiological role in Type 2 diabetes.

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None.

Competing interests

None declared.

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