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SHORT COMMUNICATION

Circulating betatrophin correlates with atherogenic lipid profiles but not with glucose and insulin levels in insulin-resistant individuals

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Abstract
Aims/hypothesis The newly identified liver- and fat-derived hormone, betatrophin, has recently been linked to insulin resistance and pancreatic beta cell growth in mice. These preclinical findings have suggested betatrophin as a potential candidate for novel glucose-lowering treatment concepts involving beta cell regeneration. However, the role of betatrophin in human insulin resistance and type 2 diabetes is currently unknown. Hence, the aim of this study was to investigate circulating betatrophin concentrations in two distinct cohorts with insulin resistance.
Methods Betatrophin concentrations were analysed in (1) age- and sex-matched lean (n=20) and morbidly obese individuals (n=19), and (2) age-, sex- and BMI-matched non-diabetic (n=19) and type 2 diabetic individuals (n=18).
Results Betatrophin concentrations did not differ between lean and morbidly obese or between non-diabetic and type 2 diabetic participants. No association was found with variables of beta cell function and glucose homeostasis. However, betatrophin did correlate significantly with plasma atherogenic lipids including total cholesterol, LDL-cholesterol and apolipoprotein B in morbidly obese and type 2 diabetic patients but not in controls. Insulin-resistant individuals with hypercholesterolaemia (≥5.2 mmol/l) had significantly higher betatrophin concentrations than those with normal cholesterol (<5.2 mmol/l).
Conclusions/interpretation Betatrophin is a recently identified hormone, the circulating concentrations of which are unaltered in human insulin resistance but correlate significantly with atherogenic lipid profiles in high-risk cohorts with morbid obesity or type 2 diabetes. Betatrophin could therefore be a novel pathomechanistic player in dysfunctional lipid metabolism associated with high cardiovascular risk.

Keywords ANGPTL8 · Betatrophin · Insulin resistance · Lipid metabolism · LDL-cholesterol

Abbreviations

ANGPTL	Angiopoietin-like protein
HDL-C	HDL-cholesterol
LDL-C	LDL-cholesterol

Introduction

The recent report that a newly identified hormone called betatrophin promotes pancreatic beta cell proliferation and improves glucose tolerance has created a stir in diabetes research [1–3]. Betatrophin (alternatively named angiopoietin-like protein [ANGPTL] 8, TD26, RIFL and lipasin) is predominantly expressed in liver and fat, and its hepatic expression is increased in pharmacological and genetic mouse models of insulin resistance [1, 4, 5]. Melton and his colleagues found that hepatic overexpression of betatrophin in

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mice causes an increase in the rate of pancreatic beta cell proliferation, islet size and insulin content with benefits for glucose homeostasis [1]. Hence, they concluded that betatrophin acts as a hepatic signal for compensatory beta cell growth in response to insulin resistance. On the basis of these results, betatrophin has now been suggested as a novel candidate for therapeutic approaches involving beta cell regeneration in diabetes [2, 3].

Betatrophin has previously been linked to altered lipid metabolism [4–6]. Mice deficient in the betatrophin gene (*Gm6484*) display lower serum triacylglycerol levels in response to refeeding [7], whereas adenovirus-mediated betatrophin overexpression increases circulating triacylglycerol concentrations [5]. Betatrophin has been demonstrated to affect blood lipid profiles by mechanisms involving regulation of hepatic VLDL secretion as well as altered lipoprotein lipase activity [5, 7]. Betatrophin may also act in concert with other ANGPTL family members such as ANGPTL3 [5, 7], a known regulator of cholesterol metabolism in mice and humans [8, 9]. Notably, betatrophin-deficient mice do not show any alterations in glucose homeostasis on normal chow or high-fat diet compared with wild-type animals [7].

Despite the emerging importance of betatrophin as a critical regulator of metabolic pathways in preclinical models, very little is known about its role in energy metabolism in humans. Only recently, betatrophin was reported to be increased in patients with type 1 diabetes, but no association was found between betatrophin concentrations and insulin secretion [10]. The recent discovery that betatrophin is a circulating factor secreted in response to insulin resistance raises fundamental questions about a potential link between betatrophin and glucose homeostasis as well as lipid metabolism in insulin-resistant individuals. Hence, we set out to study circulating betatrophin concentrations and their associations with glucose and lipid variables in two distinct cohorts with insulin resistance.

Methods

Study population Plasma samples were obtained from participants at clinical trials previously approved by the ethics committees of the Medical University of Vienna and Göttlicher Heiland Hospital (EK-No. 074/2008, EK-No. 963/2009, EK-No. E10-N01-01, EK-No. 488/2006, EK-No. 575/2010, EK-No. 1093/2010). The study was performed in compliance with the Declaration of Helsinki and Good Clinical Practice.

Two separate insulin-resistant cohorts were analysed. The first cohort included 19 non-diabetic but morbidly obese participants and 20 sex- and age-matched healthy lean controls. The second cohort included 18 obese type 2 diabetic patients and 19 sex-, age- and BMI-matched non-diabetic controls. Mean disease duration in the type 2 diabetic patients was 8.3±1.0 years. All patients with type 2 diabetes received metformin as baseline therapy, and five had sulfonylurea in addition. Controls had no current or previous medication.

Laboratory analysis Blood samples were collected after overnight fasting, and serum and EDTA plasma was stored at −20°C. Serum and plasma variables were analysed at the Department of Medical and Chemical Laboratory Diagnostics at the Medical University of Vienna using routine procedures. Plasma betatrophin concentrations were quantified using a commercially available ELISA kit (Wuhan Eiaab Science, Wuhan, China; catalogue No. E11644h) according to the manufacturer's instructions.

Statistical analysis Differences between groups were compared by unpaired two-tailed Student's *t* test for normally distributed data, otherwise by Mann–Whitney *U* test. Correlation coefficients were analysed using Pearson's correlation (normally distributed data) or Spearman's rank correlation (data not normally distributed). To determine the correlation independently of age, partial correlation with age as the control variable was used.

Data are given as mean ± SEM. A p value <0.05 was considered significant. All statistical analyses were performed using SPSS 21.0 software (Chicago, IL, USA).

Results

Circulating betatrophin concentrations do not differ between lean and morbidly obese participants or type 2 diabetic patients and non-diabetic controls Based on a recent study in mice suggesting betatrophin to be a circulating factor secreted in response to insulin resistance in order to stimulate compensatory beta cell growth [1], we investigated plasma concentrations of betatrophin in two independent insulin-resistant cohorts. We studied betatrophin concentrations in age- and sex-matched healthy lean and morbidly obese individuals as well as in type 2 diabetic patients and sex-, age- and BMI-matched non-diabetic controls. Baseline characteristics of all participants are shown in Table 1. In contrast with recently published preclinical findings [1], plasma betatrophin concentrations did not differ between healthy lean and insulin-resistant obese participants or between type 2 diabetic patients and non-diabetic controls (Table 1).

Betatrophin correlates with atherogenic lipid profiles in morbidly obese and type 2 diabetic individuals Next we studied correlations between circulating betatrophin and variables of glucose homeostasis. Notably, betatrophin was not related to

Table 1 Baseline characteristics of two distinct insulin-resistant cohorts: (1) lean and morbidly obese individuals and (2) non-diabetic and type 2 diabetic individuals

Variable	Lean (n=20)	Obese (n=19)	p value	Non-diabetic (n=19)	Type 2 diabetic (n=18)	p value
Age (years)	45.5±2.4	40.6±2.8	0.192	56.9±2.5	59.9±2.7	0.425
Sex (female:male)	13:7	14:5	0.569	9:10	8:10	0.863
Betatrophin (pg/ml)	1203.3±121.1	973.0±92.3	0.142	1643.4±229.9[a]	1646.5±129.9[a]	0.358
BMI (kg/m^2)	24.6±0.6	46.9±1.4	0.000	35.2±1.9[a]	32.2±1.2	0.371
Glucose (mmol/l)	5.28±0.19[a]	5.18±0.11	0.749	5.19±0.13[a]	7.29±0.39	0.000
Insulin (pmol/l)	51.7±10.9[a]	114.5±17.7[a]	0.001	70.2±8.4	70.1±15.2[a]	0.641
C-peptide (nmol/l)	0.19±0.05[a]	1.27±0.12[a]	0.000	1.02±0.16[a]	1.09±0.10	0.258
HOMA-IR	2.10±0.43[a]	4.34±0.65[a]	0.001	2.72±0.34	3.72±0.80[a]	0.538
HbA_{1c} (%)		5.49±0.10		5.73±0.08	7.72±0.27	0.000
HbA_{1c} (mmol/mol)		36.56±1.08		39.1±0.9	60.8±3.0	0.000
Triacylglycerol (mmol/l)	1.71±0.20[a]	1.76±0.12	0.283	1.37±0.07	1.96±0.20	0.010
Total cholesterol (mmol/l)	4.81±0.19	5.38±0.2	0.047	3.95±0.18[a]	5.48±0.32	0.000
LDL-C (mmol/l)	2.96±0.14	3.41±0.19	0.070	2.55±0.22	3.22±0.28	0.086
HDL-C (mmol/l)	1.06±0.05[a]	1.17±0.05	0.084	1.73±0.08	1.33±0.06[a]	0.000
Apolipoprotein B (g/l)	0.98±0.06	1.09±0.06[a]	0.365			

Baseline data are given as mean ± SEM

[a] Data not normally distributed

BMI, glucose, insulin, C-peptide or HOMA-IR or the oral glucose insulin sensitivity index derived from the OGTT (the latter was only studied in morbidly obese participants; electronic supplementary material [ESM] Table 1). These data suggest no association between systemic betatrophin levels and insulin resistance in humans. However, betatrophin concentrations did correlate significantly with age in the lean and obese cohort. Strikingly, betatrophin also correlated with total cholesterol and LDL-cholesterol (LDL-C) in morbidly obese (Fig. 1a, b) and type 2 diabetic patients (Fig. 1e, f), but not in controls (ESM Tables 1 and 2). In morbidly obese participants, these correlations were slightly attenuated but remained significant after adjustment for age (ESM Table 1). Based on their total cholesterol levels, we then divided both morbidly obese and type 2 diabetic participants into two groups, with the clinically relevant plasma concentration of 5.2 mmol/l as cut-off. Betatrophin concentrations were significantly elevated in both morbidly obese and type 2 diabetic individuals with hypercholesterolaemia (≥5.2 mmol/l) compared with those with cholesterol in the normal range (<5.2 mmol/l) (Fig. 1c and g). Likewise, betatrophin concentrations were significantly increased in obese participants with high LDL-C levels (≥3.5 mmol/l) vs those with LDL-C below 3.5 mmol/l (Fig. 1d), whereas betatrophin was not significantly elevated (p=0.076) in type 2 diabetic patients with LDL-C levels ≥3.5 mmol/l (Fig. 1h). Together, these results emphasise that betatrophin concentrations are associated with atherogenic lipid profiles in people with insulin resistance.

Discussion

Betatrophin is a recently discovered liver- and fat-derived hormone. Previous studies in vitro and in mouse models suggested an important role for betatrophin in the regulation of lipid metabolism [4, 5, 7] and most recently in glucose homeostasis [1]. The preclinical observation that betatrophin may act as a circulating factor that promotes beta cell proliferation in response to insulin resistance has lately received considerable attention given the potential therapeutic implication for the treatment of diabetes [1–3]. However, in humans, it is completely unknown whether betatrophin is induced by insulin resistance and how this hormone is linked to glucose homeostasis. We show here that circulating betatrophin is unaltered in two distinct human cohorts with insulin resistance and does not correlate with variables of glucose homeostasis (Table 1, ESM Tables 1 and 2). A recent study in type 1 diabetic patients and healthy controls did not find an association between betatrophin and variables of glucose homeostasis despite increased betatrophin concentrations in patients with type 1 diabetes [10].

It is well established that insulin resistance and type 2 diabetes are associated with deleterious lipid profiles and increased cardiovascular risk. Hence, we studied a potential relationship between betatrophin and blood lipid variables in insulin-resistant individuals. Notably, circulating betatrophin correlated significantly with total cholesterol and LDL-C in morbidly obese and type 2 diabetic patients but not in controls (Fig. 1 and ESM Tables 1 and 2). Betatrophin concentrations

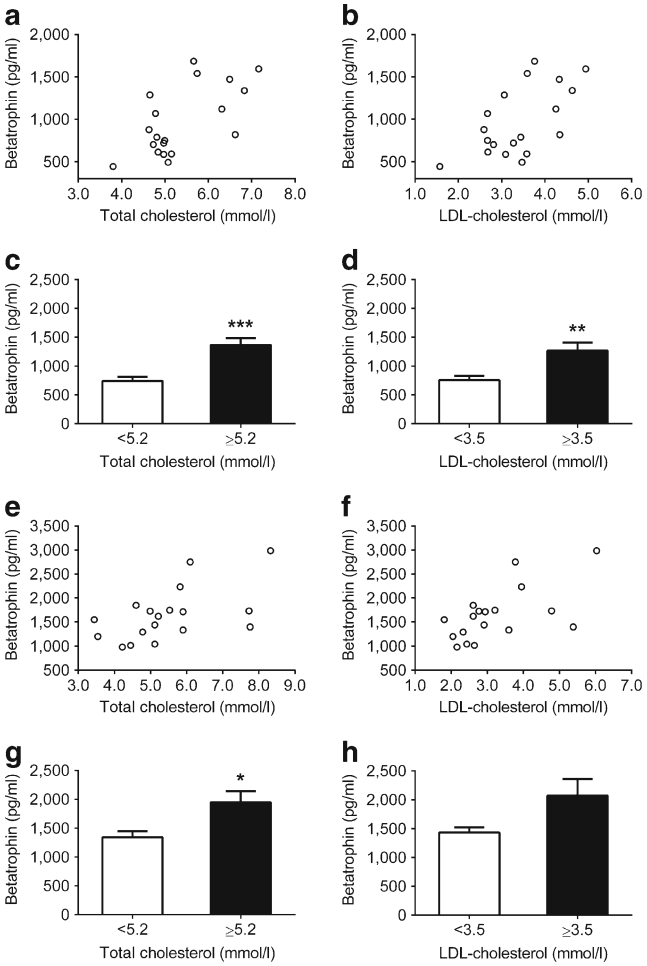

Fig. 1 Circulating betatrophin is associated with atherogenic lipids in insulin-resistant morbidly obese individuals (**a**–**d**) and type 2 diabetic patients (**e**–**h**). Scatter plots illustrate Pearson's correlation (r) between betatrophin and (**a**) total cholesterol (r=0.65, p=0.003) and (**b**) LDL-C (r=0.61, p=0.006) in morbidly obese patients. Bar graphs show betatrophin concentrations in morbidly obese participants with (**c**) normal cholesterol (<5.2 mmol/l, n=12, white bars) vs participants with hypercholesterolaemia (≥5.2 mmol/l, n=7, black bars) and (**d**) with LDL-C <3.5 mmol/l (n=11, white bars) vs those with LDL-C ≥3.5 mmol/l (n=8, black bars). Scatter plots illustrate Spearman's correlation (ρ) between betatrophin and (**e**) total cholesterol (ρ=0.55, p=0.017) and (**f**) LDL-C (ρ=0.61, p=0.008) in patients with type 2 diabetes. Bar graphs show betatrophin concentrations in type 2 diabetic patients with (**g**) normal cholesterol (<5.2 mmol/l, n=9, white bars) vs those with hypercholesterolaemia (≥5.2 mmol/l, n=9, black bars) and (**h**) with LDL-C <3.5 mmol/l (n=12, white bars) vs those with LDL-C ≥3.5 mmol/l (n=6, p=0.076, black bars). Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001

were higher in insulin-resistant participants with hypercholesterolaemia than in those with normal cholesterol (Fig. 1). Previously, ANGPTL family members, including betatrophin, have been linked to triacylglycerol and also cholesterol metabolism. A single-nucleotide polymorphism in the betatrophin gene (R59W)—a putative loss-of-function mutation—was associated with decreased LDL-C and HDL-C in genome-wide association studies [11]. This observation is completely in line with the positive correlation between betatrophin and LDL-C in insulin-resistant participants reported here (Fig. 1). Together these data may point towards a novel role for betatrophin in the pathophysiology of lipid metabolism in high-risk populations such as morbidly obese with severe metabolic dysregulation and type 2 diabetic patients, whereas in metabolically relatively healthy individuals the potential deleterious effects of betatrophin could still be compensated. It is possible that, during advanced metabolic dysregulation and insulin resistance, betatrophin signalling is altered and may thereby exert its unfavourable effects on lipid metabolism. However, the exact molecular mechanisms of betatrophin action in metabolic disease have yet to be studied.

In addition, betatrophin was found to be significantly related to age in the lean/obese cohort presented here (ESM Table 1) and in healthy controls in the study by Espes et al [10]. This association with age also explains the relatively large differences in betatrophin concentrations between the two cohorts in the present study as well as the cohort in the Espes study [10]. Obese individuals aged 50 or older had significantly higher betatrophin concentrations than those under 50 years (ESM Fig. 1a). In contrast, when age-matched obese individuals from the two cohorts were compared, no differences in betatrophin concentrations were found (ESM Fig. 1b). While the relationship between age and lipid variables such as total cholesterol, LDL-C and apolipoprotein B is well established, our data suggest that betatrophin could be a novel hormone that confers a higher risk of detrimental plasma lipids in the elderly.

In summary, we have shown here that the recently identified hormone, betatrophin, is strongly associated with atherogenic lipid profiles but not with glucose homeostasis in insulin-resistant individuals. Hence, any conclusions about the potential therapeutic relevance of betatrophin in novel treatment concepts for diabetes should be made with great caution.

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Duality of interest The authors confirm that there is no duality of interest associated with this manuscript.

Contribution statement AF, BKI, LK, MF-S, AK-W, TMS and FWK contributed to study design, data acquisition, interpretation of data, and revision of the manuscript critically for important intellectual content and gave final approval of the version to be published. AF and FWK wrote the manuscript. FWK is the guarantor of this work.

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