Betatrophin levels are increased in women with gestational diabetes mellitus compared to healthy pregnant controls

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Abstract

Objective: Betatrophin has recently been introduced as a novel adipokine/hepatokine, which promotes pancreatic β cell proliferation and improves glucose tolerance in several mouse models of insulin resistance. However, regulation of betatrophin in gestational diabetes mellitus (GDM), as well as its association with markers of obesity, such as glucose and lipid metabolism, inflammation, and renal function, have not been elucidated.
Design and methods: Circulating betatrophin was quantified in 74 women with GDM and 74 healthy and gestational age-matched controls by ELISA. In a subset of the study population comprising of 85 patients (41 previous controls, 44 previous women with GDM), *postpartum* betatrophin levels were measured in a follow-up study.
Results: Median (interquartile range) serum betatrophin levels were higher in women with GDM (1.79 (0.53) μg/l) as compared to non-diabetic pregnant controls (1.58 (0.44) μg/l) ($P=0.002$). In multivariate analysis, GDM status was an independent and positive predictor of circulating betatrophin ($P=0.001$). Furthermore, betatrophin levels were significantly higher during gestation (1.70 (0.53) μg/l) as compared to *postpartum* levels (1.55 (0.66) μg/l) ($P=0.028$). Moreover, *postpartum* irisin remained a positive and independent predictor of *postpartum* betatrophin concentrations.
Conclusions: Women with GDM have significantly higher betatrophin levels as compared to healthy pregnant controls and GDM status positively predicts circulating betatrophin. Furthermore, *postpartum* levels are significantly lower as compared to betatrophin concentrations during pregnancy. Moreover, irisin is a significant predictor of *postpartum* betatrophin levels.

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Introduction

Gestational diabetes mellitus (GDM) is a metabolic disorder during pregnancy leading to acute and chronic complications in both mother and newborn. Interestingly, GDM shares several risk factors with type 2 diabetes mellitus (T2DM), suggesting a potential link between these two metabolic disease states. However, the exact pathogenesis of GDM has not been fully understood, yet. In the past few years, a dysregulation of various adipocyte- and hepatocyte-derived factors, including adiponectin, leptin, fibroblast growth factor (FGF) 21, and adipocyte fatty acid-binding protein, has been reported to mediate insulin resistance and proinflammatory effects in both T2DM (1) and GDM (2, 3, 4).

Recently, betatrophin (also known as hepatocellular carcinoma-associated protein TD26, lipasin, refeeding induced fat and liver, or angiopoietin-like protein 8) has

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been introduced as a novel adipokine/hepatokine, which significantly and specifically promotes pancreatic β cell proliferation, expands β cell mass, and improves glucose tolerance in mouse models of insulin resistance (5). In their study, Yi *et al.* (5) suggested that betatrophin could potentially limit adverse metabolic status in severe insulin resistance by increasing β cell proliferation. Interestingly, serum levels of this β cell effector are doubled in patients with type 1 diabetes mellitus (T1DM) as compared to age-matched healthy controls in a very recent study (6). Furthermore, Fenzl *et al.* (7) demonstrated that the novel adipokine/hepatokine correlates significantly with an atherogenic lipid profile in small cohorts comprising of patients with morbid obesity or T2DM.

However, data on betatrophin regulation in humans so far is limited to patients with T1DM (6), morbid obesity (8), or T2DM (7, 8, 9), and there are no studies investigating betatrophin in GDM. Since betatrophin could potentially counteract impaired glucose control seen in GDM by improving β cell proliferation (5), our rationale was to investigate this novel adipokine/hepatokine and its potential associations with other metabolic markers in human GDM. Therefore, we determined circulating betatrophin concentrations in 74 well-phenotyped women with GDM during pregnancy as compared to 74 gestational age-matched, healthy pregnant controls. Furthermore, we measured *postpartum* betatrophin serum levels in a subset of these 148 women comprising 85 women. Moreover, we correlated betatrophin to clinical and biochemical measures of renal function, indices of glucose metabolism, lipid metabolism, as well as inflammation.

We hypothesized that the adipokine/hepatokine is increased in GDM as compared to non-diabetic and healthy women.

Subjects and methods

Study participants

For this cross-sectional study, 148 pregnant patients were recruited from the outpatient care unit of the Department of Endocrinology and Nephrology, University of Leipzig between 2006 and 2011. The study design has recently been described (10, 11). In brief, a 75 g, 2 h oral glucose tolerance test (OGTT) was performed in all participants according to the criteria of the American Diabetes Association (12). GDM was diagnosed if one or more plasma glucose levels were elevated during the OGTT using the following threshold plasma glucose levels: fasting ≥5.1 mmol/l; 1 h ≥10.0 mmol/l; 2 h ≥8.5 mmol/l. Based on these thresholds, 74 pregnant subjects were classified as GDM patients. Furthermore, 74 gestational age-matched pregnant women with normal glucose tolerance served as controls. To investigate *postpartum* regulation of this novel adipokine/hepatokine, all women were invited to take part in a follow-up study in 2012. A total of 85 patients (41 previous controls, 44 previous GDM) were available for follow-up (median time after delivery: 1576 days). BMI was determined as weight before gestation divided by squared height. Furthermore, homeostasis model assessment of insulin resistance (HOMA-IR) and β-cell function (HOMA-B) were determined as described in (13). The study was approved

Table 1 Baseline characteristics of the study population during pregnancy. Values for median (interquartile range) are shown.

	Controls	GDM	*P*
n	74	74	
Betatrophin (μg/l)	1.58 (0.44)	1.79 (0.53)	0.002*
Age (years)	28.9 (4.5)	31.0 (7.5)	0.087
Gestational age at blood sampling (days)	199 (40)	202 (33)	0.568
Gestational age at delivery (days)	275 (15)	273 (14)	0.312
Infant birth weight (g)	3360 (790)	3400 (805)	0.471
BMI (kg/m^2)	22.4 (6.7)	24.5 (6.6)	0.117
SBP (mmHg)	125 (17)	120 (20)	0.338
DBP (mmHg)	75 (13)	73 (15)	0.349
HbA1c (%)	5.3 (0.6)	5.4 (0.6)	0.729
HbA1c (mmol/mol)	34.4 (6.6)	35.5 (6.6)	0.729
Glucose 0 h$_{(OGTT)}$ (mmol/l)	4.3 (0.5)	4.5 (0.9)	<0.001*
Glucose 1 h$_{(OGTT)}$ (mmol/l)	7.5 (1.6)	10.1 (1.7)	<0.001*
Glucose 2 h$_{(OGTT)}$ (mmol/l)	6.4 (1.8)	8.7 (2.3)	<0.001*
FI (pmol/l)	57.9 (38.4)	70.6 (66.7)	0.003*
HOMA-IR	1.57 (0.97)	1.99 (1.90)	<0.001*
HOMA-B (%)	204.2 (183.0)	209.6 (219.0)	0.853
GlucoseAUC	12.7 (2.4)	16.6 (2.1)	<0.001*
Cholesterol (mmol/l)	6.31 (1.84)	6.71 (1.74)	0.199
HDL cholesterol (mmol/l)	1.93 (0.51)	1.82 (0.80)	0.419
LDL cholesterol (mmol/l)	3.73 (1.57)	4.05 (1.91)	0.569
TG (mmol/l)	2.02 (1.43)	2.14 (1.31)	0.453
FFA (mmol/l)	0.47 (0.30)	0.55 (0.31)	0.047*
Creatinine (μmol/l)	49.0 (11.0)	46.0 (11.3)	0.086
hsCRP (mg/l)	4.20 (4.30)	3.99 (6.09)	0.903
Leptin (mg/l)	23.0 (11.9)	26.5 (21.6)	0.131
Adiponectin (mg/l)	7.0 (3.8)	6.7 (4.4)	0.486
Irisin (μg/l)	466.6 (178.0)	482.1 (132.1)	0.340

BMI, body mass index; DBP, diastolic blood pressure; FFA, free fatty acids; FI, fasting insulin; GDM, gestational diabetes mellitus; GlucoseAUC, area under the glucose curve; HOMA-B, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high sensitivity C reactive protein; OGTT, oral glucose tolerance test; SBP, systolic blood pressure; TG, triglycerides. *P<0.05 as assessed by Mann–Whitney *U* test.

by the local Ethics Committee and all subjects gave written informed consent before taking part.

Assays

All blood samples at both time points, i.e. during pregnancy and *postpartum*, were obtained in a post-absorptive state after an at least 8 h fasting period in the morning. Betatrophin and irisin (Phoenix Pharmaceuticals, Burlingame, CA, USA), as well as adiponectin and leptin (Mediagnost, Reutlingen, Germany), serum concentrations were determined with an ELISA according to the manufacturers' instructions. Fasting insulin (FI) was determined with a two-site chemiluminescent enzyme immunometric assay for the LIAISON automated analyzer (DiaSorin, Saluggia, Italy). Furthermore, HbA1c, glucose levels during OGTT, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides (TG), free fatty acids (FFA), creatinine, and high sensitivity C reactive protein (hsCRP) were measured by standard laboratory methods in a certified laboratory.

Statistical analysis

SPSS Software version 20.0 (IBM, Armonk, NY, USA) was used in all statistical analyses. Differences between women with GDM and controls during and after pregnancy were assessed by non-parametric Mann–Whitney *U* test. Differences in circulating betatrophin during pregnancy and *postpartum* were assessed by non-parametric Wilcoxon signed rank test. Univariate correlations were performed using non-parametric Spearman's rank correlation method. Afterward, multivariate linear regression analysis was performed to identify independent relationships. Before multivariate correlation analyses were calculated, distribution of the respective variables was tested for normality using Shapiro–Wilk *W* test and non-normally distributed parameters were logarithmically transformed.

Table 2 Univariate correlations and multivariate regression analysis during pregnancy. Univariate correlations with betatrophin, as well as multivariate regression analysis between betatrophin (lg) (dependent variable) and age (lg), GDM status, HDL cholesterol, and leptin (lg) in all women during pregnancy. Non-normally distributed variables were logarithmically transformed (lg) prior to multivariate testing. *r* and *P* values, as well as standardized β and *P* values are given, respectively.

	Univariate correlations		Multivariate analysis	
	r	*P*	β	*P*
Age (years)	−0.090	0.278	−0.189	0.017*
GDM status	–	–	0.263	0.001*
Gestational age at blood sampling (days)	−0.033	0.693	–	–
Gestational age at delivery (days)	−0.009	0.917	–	–
Infant birth weight (g)	−0.039	0.652	–	–
BMI (kg/m^2)	−0.004	0.958	–	–
SBP (mmHg)	0.059	0.484	–	–
DBP (mmHg)	0.118	0.166	–	–
HbA1c (%)	−0.022	0.792	–	–
HbA1c (mmol/mol)	−0.022	0.792	–	–
Glucose 0 h$_{(OGTT)}$ (mmol/l)	0.116	0.159	–	–
Glucose 1 h$_{(OGTT)}$ (mmol/l)	0.146	0.086	–	–
Glucose 2 h$_{(OGTT)}$ (mmol/l)	0.135	0.114	–	–
FI (pmol/l)	0.072	0.384	–	–
HOMA-IR	0.087	0.295	–	–
HOMA-B (%)	0.022	0.794	–	–
GlucoseAUC	0.159	0.061	–	–
Cholesterol (mmol/l)	0.103	0.214	–	–
HDL cholesterol (mmol/l)	0.272	0.001*	0.279	<0.001*
LDL cholesterol (mmol/l)	0.070	0.398	–	–
TG (mmol/l)	−0.114	0.166	–	–
FFA (mmol/l)	−0.019	0.820	–	–
Creatinine (μmol/l)	−0.018	0.825	–	–
hsCRP (mg/l)	0.011	0.892	–	–
Leptin (mg/l)	0.256	0.002*	0.150	0.052
Adiponectin (mg/l)	0.057	0.495	–	–
Irisin (μg/l)	0.091	0.272	–	–

Abbreviations are indicated in Table 1. *$P<0.05$ in univariate, as well as multivariate analysis, respectively.

A P value of <0.05 was considered as statistically significant in all analyses.

Results

Baseline characteristics of the entire cohort during pregnancy

Median (interquartile range) serum betatrophin in the total sample during pregnancy was 1.69 (0.52) μg/l. Clinical characteristics of the subgroups studied (controls, GDM) during pregnancy are shown in Table 1. Median serum betatrophin levels during pregnancy were significantly higher in women with GDM (1.79 (0.53) μg/l) compared to controls (1.58 (0.44) μg/l) ($P=0.002$) (Table 1). Furthermore, plasma glucose levels during OGTT, FI, HOMA-IR, area under the glucose curve, and FFA were significantly higher in women with GDM as compared to controls ($P<0.05$) (Table 1). In matched *prepartum* and *postpartum* samples, circulating betatrophin during pregnancy (1.70 (0.53) μg/l) was significantly higher compared to *postpartum* levels (1.55 (0.66) μg/l) ($P=0.028$).

Table 3 Baseline characteristics in the follow-up study (*postpartum*). Values for median (interquartile range) are shown.

	Controls	GDM	*P*
n	41	44	
Betatrophin (μg/l)	1.52 (0.60)	1.61 (0.74)	0.077
Age (years)	34.5 (8.8)	36.1 (7.5)	0.791
Time after delivery (days)	1606.5 (207)	1513 (709)	0.092
WHR	0.88 (0.08)	0.85 (0.11)	0.114
BMI (kg/m^2)	23.5 (4.9)	25.2 (5.5)	0.645
SBP (mmHg)	115 (18)	120 (15)	0.125
DBP (mmHg)	75 (18)	80 (15)	0.081
HbA1c (%)	5.0 (0.2)	5.0 (0.4)	0.329
HbA1c (mmol/mol)	30.9 (2.3)	31.0 (3.8)	0.329
Fasting glucose (mmol/l)	4.8 (0.4)	4.9 (0.6)	0.138
FI (pmol/l)	54.4 (63.9)	64.3 (41.9)	0.705
HOMA-IR	1.56 (1.94)	1.85 (1.30)	0.627
HOMA-B (%)	131.0 (134.4)	109.4 (110.4)	0.710
Cholesterol (mmol/l)	4.92 (1.01)	5.20 (1.37)	0.062
HDL cholesterol (mmol/l)	1.60 (0.42)	1.61 (0.52)	0.859
LDL cholesterol (mmol/l)	2.91 (0.92)	3.20 (1.32)	0.117
TG (mmol/l)	0.89 (0.60)	1.11 (0.70)	0.206
FFA (mmol/l)	0.42 (0.28)	0.55 (0.34)	0.195
Creatinine (μmol/l)	67.0 (16.5)	65.0 (14.5)	0.956
hsCRP (mg/l)	1.56 (3.60)	1.11 (4.71)	0.718
Leptin (mg/l)	10.9 (14.4)	13.6 (21.6)	0.725
Adiponectin (mg/l)	6.6 (3.4)	7.4 (3.4)	0.250
Irisin (μg/l)	378.0 (111.4)	446.3 (146.9)	0.001*

Abbreviations are indicated in Table 1. *$P<0.05$ as assessed by Mann–Whitney U test.

Univariate correlations during pregnancy

Betatrophin positively correlated with HDL cholesterol and leptin in univariate analysis ($P<0.05$; Table 2). In contrast, no significant correlations could be established between serum betatrophin and blood pressure, markers of glucose tolerance including FI, fasting glucose, HOMA-IR, HOMA-B, area under the glucose curve, renal function, inflammation, as well as the adipokines adiponectin and irisin, in the total sample (Table 2).

Multivariate regression analysis during pregnancy

To verify independent associations, multiple linear regression analysis was performed. Here, GDM status remained a positive, independent, and significant predictor of betatrophin serum levels ($P=0.001$; Table 2). Furthermore, the adipokine/hepatokine was significantly and positively associated with HDL cholesterol ($P<0.001$) whereas a negative association was observed with patient age ($P=0.017$) (Table 2).

Baseline characteristics of the *postpartum* follow-up cohort

Median (interquartile range) serum betatrophin in the *postpartum* follow-up cohort was 1.55 (0.66) μg/l. Clinical characteristics of the follow-up subcohort are shown in Table 3. *Postpartum* betatrophin concentrations were not significantly different in women with prior GDM (1.61 (0.74) μg/l) compared to the control group (1.52 (0.60) μg/l) ($P=0.077$) (Table 3).

Univariate correlations and multivariate regression analysis in the *postpartum* follow-up cohort

Betatrophin positively correlated with time after delivery, HDL cholesterol, hsCRP, and irisin in univariate analysis ($P<0.05$; Table 4). Multiple linear regression analysis revealed that irisin remained a positive, independent, and significant predictor of betatrophin serum levels ($P=0.047$; Table 4). In contrast, betatrophin was not significantly associated with markers of glucose tolerance including FI, fasting glucose, HOMA-IR, and HOMA-B (Table 4).

Discussion

In the current study, we show for the first time that patients with GDM have increased betatrophin serum

Table 4 Univariate correlations and multivariate regression analysis in the follow-up study (*postpartum*). Univariate correlations with betatrophin, as well as multivariate regression analysis between betatrophin (lg) (dependent variable) and age, GDM status, time after delivery (lg), HDL cholesterol, hsCRP (lg), and irisin (lg), in the follow-up study (*postpartum*). Non-normally distributed variables were logarithmically transformed (lg) prior to multivariate testing. *r* and *P* values, as well as standardized β and *P* values, are given, respectively.

	Univariate correlations		Multivariate analysis	
	r	*P*	β	*P*
Age (years)	0.032	0.773	0.006	0.955
GDM status	–	–	0.197	0.089
Time after delivery (days)	0.245	0.024*	0.196	0.128
WHR	0.086	0.435	–	–
BMI (kg/m^2)	0.118	0.288	–	–
SBP (mmHg)	0.005	0.962	–	–
DBP (mmHg)	0.041	0.712	–	–
HbA1c (%)	−0.152	0.166	–	–
HbA1c (mmol/mol)	−0.152	0.166	–	–
Fasting glucose (mmol/l)	−0.089	0.420	–	–
FI (pmol/l)	0.069	0.532	–	–
HOMA-IR	0.048	0.664	–	–
HOMA-B (%)	0.106	0.333	–	–
Cholesterol (mmol/l)	0.086	0.431	–	–
HDL cholesterol (mmol/l)	0.226	0.038*	0.165	0.135
LDL cholesterol (mmol/l)	−0.029	0.796	–	–
TG (mmol/l)	0.190	0.082	–	–
FFA (mmol/l)	0.037	0.739	–	–
Creatinine (μmol/l)	−0.138	0.207	–	–
hsCRP (mg/l)	0.241	0.026*	0.123	0.305
Leptin (mg/l)	0.157	0.151	–	–
Adiponectin (mg/l)	0.056	0.736	–	–
Irisin (μg/l)	0.323	0.003*	0.262	0.047*

Abbreviations are indicated in Table 1. *$P<0.05$ in univariate, as well as multivariate analysis, respectively.

levels as compared to healthy pregnant controls. Furthermore, GDM status remains a positive and independent predictor of circulating betatrophin during pregnancy. Moreover, we also show for the first time that the *postpartum* betatrophin is significantly lower as compared to prepartal concentrations. In addition, irisin is an independent and positive predictor of betatrophin in the *postpartum* state. This is in accordance with results from Zhang *et al*. (14) demonstrating that treatment of adipocytes with recombinant irisin significantly increases expression of betatrophin in 3T3-L1 adipocytes *in vitro*. The major source of increased circulating betatrophin concentrations in women with GDM needs to be established in future experiments. In this context, it is interesting to note that betatrophin mRNA expression in the liver is upregulated three- to sixfold in S961-treated animals, ob/ob, as well as db/db mice (5). Furthermore, the same group convincingly demonstrates that hepatic betatrophin mRNA production in mice increases during pregnancy (5). Moreover, two recent papers reveal that betatrophin is only minimally expressed in human and mouse placenta tissue (5, 15). Taking these findings and our data into consideration, a hepatic rather than a placental origin seems to be responsible for the observed and transiently upregulation of betatrophin in GDM in our present cohort.

The physiological significance of increased betatrophin levels in patients with GDM remains to be determined. In this context, it is interesting to note that mice injected with plasmids encoding betatrophin have a 17-fold increase in β cell replication as compared to control animals (5). Yi *et al*. (5) suggest that this effect is mediated by altered expression of several cell cycle regulators in islets, including several cyclins, cyclin-dependent kinases, E2F transcription factors, as well as several cell-cycle inhibitors. Taking these results into consideration, it is tempting to speculate that increased circulating betatrophin in GDM counteracts metabolic dysfunction. Alternatively, GDM might be a betatrophin-resistant state with concomitant upregulation of the adipokine/hepatokine. In this context, it is interesting to note that for other adipokines/hepatokines, including

leptin (16) and FGF-21 (17) resistance has been shown in obesity-associated disease states. Clearly, these hypotheses need to be addressed in future studies. Furthermore, the physiological role and regulation of betatrophin in normal pregnancy should be established.

Besides GDM status, betatrophin is directly and independently associated with HDL cholesterol in multivariate analysis in the present study. In this context, it is interesting to note that a nonsynonymous variant in the betatrophin gene affects plasma levels of LDL and HDL cholesterol without influencing TG (15). Furthermore, a trend ($P=0.052$) towards a positive association between betatrophin and leptin is shown in multivariate analysis in the present study. In contrast to our findings, Fenzl *et al.* (7) demonstrate a correlation between the novel adipokine/hepatokine and an atherogenic lipid profile including total and LDL cholesterol, as well as apo-lipoprotein B, in patients with morbid obesity and T2DM but not in controls. Moreover, no association between circulating betatrophin on one hand and HDL cholesterol or leptin on the other hand has been found in 33 non-pregnant patients with T1DM (6). Clearly, additional work in larger pregnant and non-pregnant patient samples is necessary before more definite conclusions can be reached.

In conclusion, women with GDM have significantly higher betatrophin levels as compared to healthy pregnant controls and GDM status positively predicts circulating betatrophin. Furthermore, *postpartum* levels are significantly lower as compared to betatrophin concentrations during pregnancy, suggesting a hepatic upregulation of this novel adipokine/hepatokine in pregnancy. Moreover, irisin is a significant predictor of *postpartum* betatrophin. Further studies need to elucidate factors contributing to betatrophin regulation in humans as well as the pathophysiological significance of betatrophin upregulation in GDM.

Declaration of interest

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

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Author contribution statement

T Ebert, S Kralisch, and M Fasshauer wrote the manuscript and researched data. U Wurst and J Kratzsch researched data and reviewed/edited the manuscript. U Lössner researched data. M Blüher, M Stumvoll, and A Tönjes contributed to the discussion and reviewed/edited the manuscript.

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