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Original article

The brown-fat-secreted adipokine neuregulin 4 is decreased in gestational diabetes mellitus

S. Kralisch [a,b,1], A. Hoffmann [a,1], J. Kratzsch [c], M. Blüher [a], M. Stumvoll [a], M. Fasshauer [a,b,1], T. Ebert [a,b,*,1]

[a] Department of Endocrinology and Nephrology, University of Leipzig, 04103 Leipzig, Germany
[b] Leipzig University Medical Center, IFB Adiposity Diseases, 04103 Leipzig, Germany
[c] Institute of Laboratory Medicine, University of Leipzig, 04103 Leipzig, Germany

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ABSTRACT

Aims. – Neuregulin 4 has recently been recognized as a novel adipokine secreted by brown adipose tissue (BAT), with beneficial effects on murine insulin resistance and hepatic steatosis. Yet, thus far, neither regulation of neuregulin 4 in gestational diabetes mellitus (GDM) nor its longitudinal changes in the peripartum period have been elucidated.
Methods. – Circulating neuregulin 4 levels were measured by ELISA in 74 women with GDM and 74 healthy, gestational-age-matched controls. Also, neuregulin 4 was quantified during pregnancy and compared with postpartum levels in a follow-up study of 25 women with previous GDM and 25 healthy control women.
Results. – Women with GDM had lower median serum levels of the novel BAT-secreted adipokine neuregulin 4 (3.0 μg/L) compared with healthy (non-GDM) pregnant controls (3.5 μg/L; $P = 0.020$), and the area under the glucose curve ($AUC_{Glucose}$) was an independent and negative predictor of circulating neuregulin 4 ($P = 0.033$). Also, median postpartum serum concentrations of neuregulin 4 (3.2 μg/L) were not significantly different from prepartum levels (2.8 μg/L; $P = 0.328$). In addition, neuregulin 4 was positively and independently associated with irisin ($P = 0.009$), but not other BAT-secreted adipokines.
Conclusion/interpretation. – Women with GDM have significantly lower circulating neuregulin 4 levels compared with healthy pregnant controls, and the $AUC_{Glucose}$ is negatively and independently associated with neuregulin 4 during pregnancy. Neuregulin 4 is positively correlated with irisin during pregnancy, as well as in a longitudinal fashion. Future studies are now needed to better elucidate the precise pathomechanisms of the regulation of BAT-secreted adipokines during pregnancy.

Introduction

During the last few decades, the prevalence of gestational diabetes mellitus (GDM) has increased significantly [1]. As GDM contributes to an increased risk of acute and chronic complications in both the mother and newborn [2], the pathophysiological mechanisms behind this metabolic disorder during pregnancy are of great interest. Thus, over the past several years, a variety of cytokines derived from adipose tissue, i.e. 'adipokines', have been identified as influencing both type 2 diabetes mellitus (T2DM) and GDM including adiponectin, leptin and adipocyte fatty acid-binding protein (AFABP)[3–6]. However, most adipokines are secreted predominantly by white adipose tissue and mediate adverse metabolic systemic effects. In contrast, several adipokines derived from brown adipose tissue (BAT) have recently been suggested to beneficially influence systemic metabolism, as reviewed by Villarroya et al. [7].

Neuregulin 4 has recently been recognized as a novel and predominantly BAT-secreted adipokine that protects against diet-induced insulin resistance and hepatic steatosis in mice [8]. Wang and co-workers [8] elegantly demonstrated that mice deficient in

Abbreviations: ADA, American Diabetes Association; ANGPTL8, Angiopoietin-like protein 8; $AUC_{Glucose}$, Area under the glucose curve; AFABP, Adipocyte fatty acid-binding protein; BAT, Brown adipose tissue; BMI, Body mass index; ELISA, Enzyme-linked immunosorbent assay; FFA, Free fatty acids; FGF21, Fibroblast growth factor 21; FI, Fasting insulin; GDM, Gestational diabetes mellitus; HDL, High-density lipoprotein; HOMA-IR, Homoeostasis model assessment of insulin resistance; LDL, Low-density lipoprotein; OGTT, Oral glucose tolerance test; TG, Triglyceride.

* Corresponding author at: Liebigstr. 20, 04103 Leipzig, Germany. Fax: +49 341 9713389.
E-mail address: Thomas.ebert@medizin.uni-leipzig.de (T. Ebert).
[1] These authors equally contributed to this work.

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neuregulin 4 have increased insulin resistance and hepatic steatosis after high-fat feeding. Conversely, when mice with transgenic overexpression of neuregulin 4 were exposed to a high-fat diet, they showed less body weight gain, and improved dyslipidaemia, as well as improved insulin sensitivity [8]. Mechanistically, the authors suggested that hepatic expression of lipogenic genes is lower in transgenic mice overexpressing neuregulin 4 compared with their littermate controls [8]. Taking these data together, neuregulin 4 appears to be a novel BAT-derived adipokine with beneficial effects on both glucose homoeostasis and hepatic steatosis.

However, there are no studies investigating neuregulin 4 in GDM and in a longitudinal fashion during and after pregnancy, so far. In addition, the associations of neuregulin 4 with other BAT-secreted adipokines and markers of BAT function have not been tested in patients with GDM.

Therefore, our present study has quantified circulating neuregulin 4 concentrations in 74 well-phenotyped women with GDM during pregnancy and compared them with 74 gestational-age-matched, healthy pregnant controls. Neuregulin 4 was also correlated with clinical and biochemical measures of obesity, hypertension, indices of glucose metabolism, lipid metabolism, renal function and inflammation, as well as other markers of BAT function. Furthermore, neuregulin 4 was assessed longitudinally in 25 healthy (non-GDM) and 25 GDM women during pregnancy and postpartum in a follow-up study.

Our hypothesis was that:

- women with GDM have decreased neuregulin 4 levels during pregnancy compared with non-diabetic, healthy pregnant women;
- circulating postpartum neuregulin 4 is increased because of reduced insulin resistance postpartum;
- and neuregulin 4 correlates with other markers of BAT function, including irisin, adiponectin, fibroblast growth factor (FGF)-21 and angiopoietin-like protein 8 (ANGPTL8) [7].

Methods

Study participants

The design of the present study has been described previously [9–14]. In brief, 148 pregnant women were consecutively recruited from the outpatient care unit of the Department of Endocrinology and Nephrology, University of Leipzig, between 2006 and 2011. Based on the 2012 guidelines of the American Diabetes Association (ADA) [15], a 75-g 2-h oral glucose tolerance test (OGTT) was performed in all participants. As per ADA criteria, GDM was defined as having one or more elevated plasma glucose levels during OGTT, using the following thresholds: fasting plasma glucose (FPG) $\geq$ 5.1 mmol/L; 1-h FPG $\geq$ 10.0 mmol/L; and 2-h FPG $\geq$ 8.5 mmol/L. Based on these levels, 74 women were classified as patients with GDM, whereas 74 gestational-age-matched pregnant women with normal glucose tolerance served as controls. Anthropometric measures included body mass index (BMI), determined by weight before pregnancy divided by squared height (kg/m^2). In addition, the homoeostasis model assessment of insulin resistance (HOMA-IR) score and area under the glucose curve ($AUC_{Glucose}$) were calculated, as previously described [16,17]. To investigate postpartum regulation of the novel BAT-secreted factor, follow-up examination was carried out in 2012. For this postpartum subcohort, 25 women with previous GDM and 25 healthy former control women were included in the analysis. Relative changes (postpartum-to-prepartum ratios) were calculated for all adipokines and BAT markers as follows: parameter (ratio) = $parameter_{postpartum}/parameter_{prepartum}$ [18].

The present study was approved by the local ethics committee, and all subjects gave their written informed consent before taking part.

Assays

All blood samples were obtained after a fasting period of at least 8 h. Serum levels of neuregulin 4 (Phoenix Pharmaceuticals, Burlingame, CA, USA) were quantified using enzyme-linked immunosorbent assays (ELISAs) as per the manufacturer's instructions. All other adipokines were quantified by ELISA kits as previously reported [9,11,19]. Fasting insulin (FI) was determined by two-site chemiluminescent enzyme immunometric assay, using a LIAISON automated analyzer (DiaSorin, Saluggia, Italy). All other parameters were measured by standard laboratory methods at a certified laboratory, as previously described [9–14].

Statistical analysis

SPSS version 24.0 software (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Differences between women with GDM and the controls were assessed by non-parametric Mann–Whitney U test. Univariate correlations were performed using non-parametric Spearman's rank-correlation method. Multivariate linear-regression analysis was subsequently performed to identify independent relationships. Before the multivariate correlation analyses were calculated, distribution was tested for normality, using the Shapiro–Wilk W test, and non-normally distributed parameters were logarithmically transformed. Longitudinal changes in neuregulin 4 during pregnancy compared with postpartum levels were analyzed by non-parametric Wilcoxon signed-rank test. A P-value < 0.05 was considered statistically significant in all analyses.

Results

Baseline characteristics

The median [interquartile range] serum level of neuregulin 4 for the total sample during pregnancy was 3.3 [1.7] μg/L. Clinical characteristics of both subgroups (controls and GDM) are shown in Table 1. Median circulating neuregulin 4 levels were significantly lower in women with GDM (3.0 [1.2] μg/L) compared with the non-GDM pregnant controls (3.5 [1.9] μg/L; $P = 0.020$; Table 1). At the first time point during pregnancy, markers of glucose homoeostasis, including plasma glucose levels during OGTT, $AUC_{Glucose}$, FI and HOMA-IR, as well as free fatty acids (FFAs), were significantly higher in women with GDM compared with the controls ($P < 0.05$; Table 1). In contrast, there were no significant differences in age, gestational age at blood sampling, gestational age at delivery, birth weight or markers of obesity, hypertension, dyslipidaemia, renal function or inflammation.

Univariate correlations

On univariate correlation analysis of the entire cohort during pregnancy, neuregulin 4 positively correlated with systolic and diastolic blood pressure ($P < 0.05$) (Table 2). Also, the novel adipokine was negatively correlated with glycated haemoglobin (HbA_{1c}), 1-h and 2-h glucose during OGTT, and $AUC_{Glucose}$ ($P < 0.05$; Table 2). In contrast, no significant correlations were established between circulating neuregulin 4 and markers of gestational outcome, obesity, dyslipidaemia, renal function or inflammation in the total study population (Table 2).

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