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Original article

Levels of betatrophin decrease during pregnancy despite increased insulin resistance, beta-cell function and triglyceride levels

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Abstract

Aim. – Evidence in support of an association between betatrophin and insulin resistance (IR) is mounting, with studies demonstrating that betatrophin is elevated in patients with type 2 diabetes, obesity and gestational diabetes. The aim of this study was to evaluate the role of betatrophin in IR and physiological proliferation of beta cells during pregnancy in healthy women.

Methods. – Eighty healthy pregnant women were examined at each trimester [T1 (first), T2 (second), T3 (third)], with a subgroup ($n = 45$) that was also examined at 3 months postpartum (3MPP). The controls comprised 30 non-pregnant healthy women (HW) of reproductive age. Also measured were levels of betatrophin (ELISA), glucose (enzymatic method with hexokinase), insulin (IRMA), C-peptide (EASIA) and HbA_{1c} (HPLC), while HOMA-IR and HOMA-β scores were calculated.

Results. – Betatrophin concentration was highest at T1, and differed significantly from T2 and T3 (1.84 [$Q_1 = 1.16$, $Q_3 = 2.67$] ng/mL *vs* 1.46 [$Q_1 = 0.96$, $Q_3 = 2.21$] ng/mL; $P < 0.05$ and 1.23 [$Q_1 = 0.85$, $Q_3 = 2.14$] ng/mL; $P < 0.01$, respectively). The T3 median concentration of betatrophin was the lowest of all trimesters, and significantly lower than at 3MPP (1.23 [$Q_1 = 0.85$, $Q_3 = 2.14$] ng/mL *vs* 1.49 [$Q_1 = 1.06$, $Q_3 = 2.60$] ng/mL; $P < 0.01$, respectively). At 3MPP, the level of betatrophin was similar to that of HW (1.47 [$Q_1 = 0.89$, $Q_3 = 2.67$] ng/mL). HOMA-IR and HOMA-%β index scores increased during gestation, peaking at T3 (2.3 [$Q_1 = 1.66$, $Q_3 = 2.72$] and 227.7 [$Q_1 = 185.49$, $Q_3 = 326.31$], respectively) and returning to levels similar to those of HW at 3MPP (1.53 [$Q_1 = 1.12$, $Q_3 = 2.41$] and 88.86 [$Q_1 = 62.73$, $Q_3 = 130.45$] *vs* 1.35 [$Q_1 = 1.02$, $Q_3 = 1.62$] and 92.5 [$Q_1 = 74.20$, $Q_3 = 111.47$], respectively).

Conclusion. – Concentrations of betatrophin decrease during pregnancy, suggesting that the hormone does not play a significant role in the expansion of beta-cell mass and IR during pregnancy.

Keywords: Betatrophin; Insulin resistance; Pregnancy

1. Introduction

Betatrophin, a member of the angiopoietin-like protein family (ANGPTL), is also known as ANGPTL8, lipasin, RIFL (refeeding-induced fat and liver) and hepatocellular carcinoma-associated protein TD26 [1–3]. In humans, betatrophin is predominantly produced in the liver, with smaller amounts made in adipose tissue. In mice, betatrophin has been shown to significantly improve beta-cell function and glucose tolerance [4], and there is evidence to support a relationship between betatrophin and insulin resistance (IR) [5]. Increased betatrophin concentrations have been found in type 2 diabetes (T2D), obesity and gestational diabetes mellitus (GDM) [6–11].

During pregnancy, IR is known to increase due to the antagonistic effects of hormones produced by the placenta on insulin action; these hormones include chorionic gonadotropin, growth hormone, adrenocorticotropic hormone, placental lactogen, prolactin, oestrogens and progestogens. Hyperplasia of beta cells prevents such IR from leading to the development of T2D [12]. Pregnancy is also a period of remarkably impaired lipid profiles;

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specifically, in both human and animal models, betatrophin has been shown to be involved in the regulation of lipid metabolism, especially triglycerides (TG) [13–16]. In the biggest human study — of 1047 non-diabetic patients — conducted to date, betatrophin was positively correlated with age, body mass index (BMI), waist-to-hip ratio, fasting plasma glucose (FPG), HbA_{1c}, homoeostasis model assessment of insulin resistance (HOMA-IR) scores and TG whereas, in 556 T2D patients, these correlations were not observed [7]. Despite numerous human studies, at present there is still no evidence in support of betatrophin positively influencing beta-cell proliferation. Gusarova et al. [17] showed that, in mice, the lack of betatrophin does not perturb beta-cell function in an IR state, but is important for TG metabolism; in addition, they demonstrated that an absence of betatrophin resulted in a reduction of the level of TG in plasma.

The aim of the present study was to investigate the relationship between betatrophin concentration and IR during each trimester of pregnancy, as well as in the postpartum period, in healthy pregnant women.

2. Patients and methods

A total of 80 healthy pregnant women were recruited from outpatients' clinics (based on anamnesis and laboratory tests to exclude GDM and thyroid disorders) as well as 30 healthy non-pregnant women (HW), matched for age and BMI (mean age: 32.3 ± 8.1 years; mean BMI: 22.5 ± 3.1 kg/m^2), to serve as the control group in the present study. Additional data for the HW are presented in Table S1 (see supplementary material associated with this article online). The pregnant women were examined at each trimester (T1: first, T2: second, T3: third), and a subset of women ($n = 45$) was also examined at 3 months postpartum (3MPP). Participants with multiple pregnancies were excluded. Before enrolment, all women were interviewed by a doctor, and their medical history, family history, smoking and alcohol status were recorded. Anthropometric parameters were also measured, including height and weight. All participants had previously undergone a 75 g oral glucose tolerance test (OGTT), according to current World Health Organization criteria, and only women with normal test results were enrolled. Pregnant women performed the OGTT twice: between weeks 24 and 28 of gestation and at 3MPP. Written informed consent was obtained from all participants before commencing the study. The study protocol was approved by the local ethics committee of the Medical University of Bialystok.

Blood samples were collected after overnight fasting. Betatrophin concentrations were measured using the enzyme-linked immunosorbent assay (ELISA) method (USCN Life Science Inc., Wuhan, China), with an intra- and interassay coefficient of variation (CV) < 10%. FPG concentrations were determined by an enzymatic method with hexokinase (cobas c111, Roche Diagnostic Ltd, Basel, Switzerland). Serum fasting insulin concentrations were analyzed by immunoradiometric assay (IRMA), and C-peptide concentrations by an enzyme-amplified sensitivity immunoassay (EASIA) method (DIAsource ImmunoAssays SA, Louvain-la-Neuve, Belgium). Glycated haemoglobin (HbA_{1c}) levels were assessed using high-performance liquid chromatography (HPLC; Bio-Rad Laboratories, Hercules, CA, USA). Total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, TG, thyroid-stimulating hormone (TSH), free triiodothyronine (FT3) and free thyroxine (FT4) concentrations were assayed using a clinical chemistry analyzer (ARCHITECT, Abbott Diagnostics, Abbott Park, IL, USA). HOMA-IR and HOMA-%β (for steady-state beta-cell function) were calculated using the following formulas: HOMA-IR = [FPG mg/dL × fasting plasma insulin (FPI) mIU/L]/405; and HOMA-%β = (360 × FPI mIU/L)/(FPG mg/dL–63). Every woman's BMI was calculated using the formula: body weight (kg)/height-squared (m^2). The Matsuda index (IS_{OGTT}) was calculated using the formula: $IS = 10{,}000/\sqrt{[(FPG \times FPI) \times (G \times I)]}$, where G is the mean glucose value and I is the mean insulin on OGTT [18]. The disposition index (DI_{120}) was calculated as: $DI_{120} = IS_{OGTT} \times AUC_{Ins120}/AUC_{Glu120}$, where AUC_{Ins120} is the area under the curve of insulin concentration and AUC_{Glu120} is the area under the curve of glucose concentration on OGTT [19].

For all statistical calculations, STATISTICA version 10.0 software for Windows (StatSoft, Tulsa, OK, USA) was used. Statistical significance was defined as $P \leq 0.05$. The Shapiro–Wilk test was used to evaluate data distribution. As all analyzed variables did not follow a normal distribution, the data are presented as medians and first–third quartiles (Q_1–Q_3). To compare differences between the postpartum women and HW, the Mann–Whitney U test was used. Friedman's non-parametric test with the adjusted post-hoc Conover test was used to compare differences between trimesters and also those between trimesters and the postpartum period. Correlations between betatrophin concentrations and biochemical variables were estimated using Spearman's correlation coefficient.

3. Results

During pregnancy, serum concentrations of betatrophin were decreased significantly (Fig. 1). The statistical significance established for the global Friedman test was a P value < 0.0001. At T1, the level of betatrophin was highest, and significantly higher than T2 (1.84 [$Q_1 = 1.16$, $Q_3 = 2.67$] ng/mL *vs* 1.46 [$Q_1 = 0.96$, $Q_3 = 2.21$] ng/mL; $P < 0.05$) and T3 (1.84 [$Q_1 = 1.16$, $Q_3 = 2.67$] ng/mL *vs* 1.23 [$Q_1 = 0.85$, $Q_3 = 2.14$] ng/mL; $P < 0.01$). At T3, the median value of betatrophin was the lowest compared with T1 and T2. A significant increase in betatrophin concentration was also observed at 3MPP compared with T3 (1.49 [$Q_1 = 1.06$, $Q_3 = 2.60$] ng/mL *vs* 1.23 [$Q_1 = 0.85$, $Q_3 = 2.14$] ng/mL; $P < 0.01$, respectively). However, concentrations were similar to those observed in HW (1.47 [$Q_1 = 0.89$, $Q_3 = 2.67$] ng/mL). Women at 3MPP showed no significant differences in betatrophin, lipid profiles, glucose, HbA_{1c}, fasting insulin and IR parameters in comparison to HW. One difference, however, was observed between the two groups for fasting C-peptide concentrations, which were higher in the HW (0.38 [$Q_1 = 0.33$, $Q_3 = 0.48$] pmol/L *vs* 0.48 [$Q_1 = 0.40$, $Q_3 = 0.50$] pmol/L, respectively; $P = 0.001$).