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Activation of brown adipocytes by placental growth factor

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ABSTRACT

Gestational diabetes mellitus (GDM) is a type of diabetes and occurs during pregnancy. Brown adipose tissue (BAT) improves glucose homeostasis and mitigates insulin resistance, however, its activity is reduced in GDM. Placenta growth factor (PlGF) is an angiogenic factor produced by placental trophoblasts. Nevertheless, whether and how PlGF could affect BAT function in GDM are not defined. To investigate this question, 91 non-diabetic pregnant participants and 73 GDM patients were recruited to Gynaecology and Obstetrics Centre in Lu He hospital. Serum levels of PlGF were quantified by ELISA. Skin temperature was measured by far infrared thermography in the supraclavicular region where classical BATs were located. The direct effect of PlGF on BAT function was explored using the established human preadipocyte differentiation system. Thereby, we demonstrated that serum levels of PlGF were lower in GDM patients compared with controls, which was accompanied by decreased skin temperature in the supraclavicular region. By qPCR and western blot, mRNA and protein expression of UCP1 and OXPHOS were elevated in differentiated adipocytes treated with PlGF. PlGF stimulated mitochondrion transcription and increased copy number of mitochondrial. When subjected for respirometry, PlGF-treated differentiated adipocytes showed higher oxygen consumption rates than controls. PlGF induced AMPK phosphorylation and blockade of AMPK phosphorylation blunted UCP1 and OXPHOS expression in differentiated adipocytes. PlGF administration reduced cholesterol and triglyceride content in the liver and improved insulin sensitivity in db mice compared with control. In Conclusion, PlGF could activate BAT function. Downregulation of PlGF might contribute to the reduced BAT activity in GDM.

1. Introduction

Gestational diabetes mellitus (GDM) is a special type of diabetes and mostly occurs after 24th gestational week. Over the years, the prevalence of GDM is approximately 9.3–25.5% worldwide and 3.9–18.9% in China [1,2]. GDM is not only associated with pre- and perinatal complications but also exposes high risk of type 2 diabetes mellitus (T2DM) for mothers after pregnancy [3]. Although the aetiology of GDM is not yet clear, it shares common features with T2DM such as adiposity [4], insulin resistance [5,6] and inflammation [7,8]. Activation of brown adipocytes and beige cells improves glucose homeostasis and attenuates excessive nutrients-induced obesity and insulin resistance [9]. Unfortunately, BAT activity is decreased in both T2DM and GDM patients [10,11].

Norepinephrine is the classical stimulator for brown adipocyte and beige cell activation. Binding norepinephrine to β-adrenergic receptor adipocytes induce AMPK phosphorylation that in turn upregulates UCP1 expression for mitochondrial biogenesis and thermogenesis [12]. Except norepinephrine, brown fat activation could be activated by adipokines such as lipocalin 2 [13] and hormones such like incretin [14]. Placental trophoblasts secret a large amount of proteins, growth factors, cytokines and hormones by which the cross-talk between placenta and peripheral tissues is established [15]. Nevertheless, how placenta communicates with BAT remains unknown.

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Placenta growth factor (PlGF) is a member of vascular endothelial growth factor (VEGF) family and mainly produced by trophoblasts in placenta. PlGF levels are reported reduced in subjects with pregnancy complications, which is associated with early pregnant losses [16]. In line with this study, downregulation of PlGF in GDM renders the physical interaction between beta cells and endothelial cells in islets, resulting in impaired beta cells proliferation [17]. We hypothesized that PlGF could be the messenger between BAT function and placenta. To investigate this question, we compared serum PlGF levels in healthy pregnant subjects and GDM patients. We studied the effect of PlGF on BAT marker expression and function in preadipocytes isolated from human embryos [18].

2. Materials and methods

2.1. Human study

This case-control study was performed among pregnant women who admitted in the Gynaecology and Obstetrics Centre in Lu He hospital between November 2015 till December 2016. The subjects who were diagnosed preeclampsia, retinopathy or nephropathy were excluded. Finally, 91 non-diabetic pregnant participants and 73 GDM patients were analysed in the study. Our study complied with the Helsinki Declaration for investigation of human subjects. The study protocol was obtained ethical approval from the competent Institutional Review Boards of Capital Medical University. All participants provided written informed consent.

Screening of GDM was performed at 24–28 gestation weeks by an oral glucose tolerance test. The criteria of GDM diagnosis was adapted from American Diabetes Association guideline as following: 1) fasting blood glucose levels $\geq$5.3 mmol/L or 2) 1-h oral 100-g glucose tolerance test $\geq$10.0 mmol/L [19]. General information including age, disease history and medications was obtained. Body mass index (BMI) at 24–28 gestation weeks were obtained by weight in kilograms divided by the square of height in meters (kg/m^2).

2.2. Clinical measurement

At the antenatal examination, blood pressure was recorded by auscultation of the Korotkoff sounds, using a standard mercury sphygmomanometer. Fasting blood samples were collected and glucose, insulin, total cholesterol, triglyceride and HDL-cholesterol (HDL-c) and creatinine were measured in the central laboratory in the hospital [20]. LDL-cholesterol (LDL-c) was computed from serum total cholesterol and HDL-c and serum triglycerides by the Friedewald equation [21].

2.3. Far infrared thermography

Because PET-CT could not be applied to measure BAT activity in pregnant women, infrared thermography was used alternatively. As described before [22], the study subject was seated in the upright position with head positioned in a neutral position and the subject looked straight ahead. Images were acquired by a thermal imaging camera (FLIR B425, 3.1Mpixel, FLIR Systems Australia Pty Ltd, Melbourne, Vic., Australia) in the anterior neck and upper chest. Skin temperature in the supraclavicular region where classical BATs were located was analysed by two experienced investigators who were blind to the study using the software (Version 1.2, Wilsonville, OR).

2.4. Mice study

Diabetic db mice at the age of 8 weeks old were used in the study. They were randomized and blindly assigned to receive totalled 1.5 μg murine recombinant PlGF (RD, Bio-Techne, USA) for 4 weeks or equivalent volume of saline through a subcutaneous implanted osmotic minipump (Durect, Cupertino, CA, USA) as described before [23]. At sacrifice, after overnight fasting, blood was bled and liver and fat tissues were dissected.

2.5. Cell culture

Primary human embryo adipocytes were isolated from human embryos during abortion and cultivated as described before [18]. Once they reached 100% confluence, cells were induced adipogenic differentiation in the absence or presence of recombinant human PlGF (0–1 ng/mL) for 8 days. Medium was refreshed every two days. For inhibitor experiments, cells were differentiated with 1 μM AMPK phosphorylation inhibitor-Compound C. The detailed information of measurement of oxygen consumption and Phosphorylation pathways using Luminex technology were shown in Supplementary Materials and methods.

2.6. Real-time PCR and western blotting

Real-time PCR and western blotting were performed as described previously. The primer sequences are listed in Supplementary Table 1. The specific primary antibodies were shown in Supplementary Materials.

2.7. Immunofluorescence staining

Differentiated adipocytes were stained with 1 μg/ml anti-human UCP1, followed by Alexa 488-conjugated secondary antibody (Invitrogen), BODIPY (Thermo) and DAPI (Leagene) complying with the procedure. Brown adipocytes were positive for both UCP1 and BODIPY. Negative controls were stained with omission of primary antibody. Images were taken by Zeiss laser scanning confocal microscopy (LSM780, Germany).

2.8. ELISA

Serum levels of PlGF were quantified by enzyme-linked immunosorbent assay (ELISA) according to the manual (MLBio, Shanghai, China).

2.9. Statistical analysis

Data were expressed as mean ± SEM. In the experiments where there were 2 experimental groups, unpaired, 2-tailed Student's *t*-test was used for data with normal distribution. For data that did not fit normal distribution, nonparametric Mann Whitney analysis was used. In the experiments containing more than 2 experimental groups, one-way ANOVA with Dunnett was used when comparing treated groups against control and ANOVA with Bonferroni was applied when comparing all groups. Statistics analysis was performed using GraphPad Prism (GraphPad Software Inc, La Jolla, CA, U.S.A.). A P value less than 0.05 was considered significant.

3. Results

3.1. Serum PlGF levels were reduced in GDM patients

At 24–28 gestation weeks when GDM was diagnosed, BMI was already higher in GDM patients than healthy pregnant subjects (26.24 ± 0.64 kg/m^2 vs 24.38 ± 0.87 kg/m^2, $p = 0.09$). General characterization of the study subjects at the antenatal visit was listed in Table 1. Compared with non-diabetic pregnant controls, GDM

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