

RESEARCH ARTICLE

# Increased Cord Blood Betatrophin Levels in the Offspring of Mothers with Gestational Diabetes

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## Abstract

### Aim

Exposing a fetus to hyperglycemia can increase the risk for later-life metabolic disorders. Betatrophin has been proposed as a key regulator of pancreatic beta cell proliferation and lipid regulation. Highly responsive to nutritional signals, serum betatrophin concentrations have been found to be altered by various physiological and pathological conditions. We hypothesized that betatrophin levels are increased in the cord blood in offspring exposed to intrauterine hyperglycemia.

### Methods

This was a cross-sectional study including 54 mothers who underwent uncomplicated Cesarean delivery in a university hospital. Maternal gestational glucose concentration was determined at 24–48 weeks gestation after a 75-g OGTT. Cord blood and placental tissue was collected immediately post delivery. Metabolic parameters were determined in the Clinical Laboratory. Cord blood betatrophin levels were assayed using a commercially available ELISA kit. Placental mitochondrial content was determined by real-time PCR.

### Results

Cord blood betatrophin levels were increased in the gestational diabetes mellitus (GDM) group compared with the normoglycemic group. Furthermore, betatrophin levels were positively correlated with maternal gestational 2h post-OGTT glucose, cord blood insulin, HOMA-IR, and inversely correlated with placental mitochondrial content.

**Competing Interests:** The authors have declared that no competing interests exist.

## Conclusions

Cord blood betatrophin may function as a potential biomarker of maternal intrauterine hyperglycemia and fetal insulin resistance, which may presage for long-term metabolic impact of GDM on offspring.

## Introduction

It has been well documented that early-life environment plays a substantial role in adult health [1–3]. Gestational diabetes mellitus (GDM), a common pregnancy complication, affects <1%–28% of all pregnancies, the incidence depending on the diagnostic criteria and ethnicity [4], and parallels globally the obesity pandemic [5]. GDM contributes to the long-term metabolic derangements both in mother and child [6–10]. Notably, perturbations in glucose and lipid metabolism may manifest early in children exposed to intrauterine hyperglycemia [11–14]. The mechanism underlying the long-term metabolic effect of GDM remains uncertain.

Betatrophin (aka ANGPTL8) may play a role in pancreatic beta cell proliferation and lipid regulation [15]. It is a circulating 198 amino acid protein, highly expressed in liver and adipose tissue [15, 16]. Robustly responsive to nutritional signals, betatrophin levels have been found to be altered by various physiological and pathological conditions. For example, in humans, betatrophin concentrations are increased in type 2 diabetes [17–19], obesity [17], and type 1 diabetes [20], as well as in the postprandial state [17].

Pancreatic beta cell proliferation—dictated by numerous cell cycle genes—can be induced by physiological challenges such as gestation [21] and hyperglycemia [22]. Insulin resistance is yet another inducer. Using a mouse insulin resistance model created by an insulin receptor antagonist, Yi et al. reported that betatrophin promotes a compensatory beta cell proliferation [23]. Notwithstanding this initial study, the notion that betatrophin can augment beta cell mass is now contestable: betatrophin knockout mice rendered insulin resistant can still undergo beta cell proliferation [24]. Hepatic betatrophin expression increases in parallel with the higher beta cell replication rates over the course of gestation in mice [23]. Conceivably, betatrophin concentrations are also increased in the cord blood from GDM mothers, perhaps serving as a harbinger of future metabolic dysregulation. Previous studies in mice also indicate that betatrophin may alter lipid metabolism, especially triglycerides (TG). Betatrophin knockout mice have decreased TG levels in the fed state, reduced very low density lipoprotein (VLDL) secretion, and elevated LPL activity [25]. Antithetically, betatrophin overexpression in mouse liver increased serum TG levels [26]. Type 2 diabetes [27] and GDM [28] are associated with dyslipidemia, and diabetes patients have altered circulating betatrophin levels. Thus, betatrophin may be involved in lipid dysregulation in type 2 diabetes as well as GDM.

Herein we investigated the alteration of cord blood betatrophin levels in offspring exposed to intrauterine hyperglycemia, and explored the correlation between betatrophin and various metabolic parameters including maternal gestational blood glucose, BMI, cord blood lipid profile and homeostasis model assessment of insulin resistance (HOMA-IR). We hypothesized that cord blood betatrophin levels would be increased in the offspring from GDM mothers, and significantly correlated with maternal gestational glucose levels as well as other abovementioned metabolic parameters.

## Patients and Methods

### Study design and Participants

Enrollment consisted of women with singleton pregnancy who underwent uncomplicated Cesarean delivery at Tongji Hospital in Wuhan, China, from August 2013 to October 2013. All

mothers are from Chinese Han ethnicity and had undergone a 75-g OGTT following the standard protocol at 24–28 weeks of gestation. According to the criteria set by International Association of Diabetes and Pregnancy Study Groups (IADPSG) [29, 30], GDM was diagnosed when the fasting plasma glucose  $\geq 5.1$  mmol/L or 1h post-OGTT glycemia  $\geq 10.0$  mmol/L or 2h post-OGTT glycemia  $\geq 8.5$  mmol/L. Women were excluded from the study if  $< 20$  or  $> 40$  years of age, or had been previously diagnosed with any disorder known to affect glucose metabolism including diabetes, GDM, polycystic ovarian syndrome, uncontrolled thyroid or liver disease. Mothers with a history of smoking or any substance abuse during the current pregnancy, or if there was premature delivery ( $< 37$  wk gestation) or birth weight  $< 2500$  g were likewise excluded. Furthermore, participants with pregnancies resulting from in vitro fertilization or exposure to dexamethasone during pregnancy were excluded for the potential effect on offspring glucose metabolism. Additionally, participants with unavailable maternal gestational glucose records or if a cord blood sample was not obtained were ineligible. Only women undergoing uncomplicated Cesarean deliveries were recruited to exclude the influence of different mode of delivery on cord blood glucose. In total, data from 54 enrollees was available for analysis. All mothers with GDM were treated solely with diet in this study.

Cord blood and placental tissue were collected immediately after delivery. Cord blood was collected from the umbilical vein following standard protocols. Thereafter, the cord blood was centrifuged at 4°C. Insulin and glucose were measured within 2 hours. A portion of plasma and serum were frozen and stored at -80°C immediately after the centrifugation. Placenta tissue from the fetal side were obtained as previously described [31]. The samples were rinsed in normal saline at 4°C to remove excess maternal blood. Thereafter, samples were frozen in liquid nitrogen and stored in freezer of -80°C.

Most of the patients in this report were from previously reported cohorts [31]. All study procedures were approved by the Ethic Committee of Tongji Hospital, Huazhong University of Science and Technology (Approval number: TJ-C20130711), and written informed consent was obtained from each participant in accordance with the Declaration of Helsinki as revised in 2008.

## Laboratory measurements

All parameters were determined in the Clinical Laboratory of Genetic Metabolic Disease (Tongji Hospital, Huazhong University of Science and Technology). Blood glucose was measured using a glucose-oxidase/peroxidase method (Biosino Bio-Technology and Science Inc, Beijing, China), and the inter-assay and intra-assay CV were 3% and 2%, respectively. Insulin concentrations were determined using a chemiluminescent immunoassay (Beckman Coulter Inc., Brea, CA), with the inter-assay and intra-assay CV 3.5–4.5% and 2–2.6%, respectively. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as:  $\text{HOMA-IR} = \text{fasting glucose (mmol/L)} \times \text{fasting insulin (mU/L)} / 22.5$ . Serum total cholesterol (TC) and triglyceride (TG) were assayed using an enzymatic colorimetric method (Roche Diagnostics, Penzberg, Germany). The inter-assay CVs were 1.7% (TC) and 1.9% (TG) and intra-assay CVs were 0.8% (TC) and 1.6% (TG). HDL-cholesterol (HDL-C) and LDL cholesterol (LDL-C) were determined using commercially available kits (SEKISUI MEDICAL CO., Tokyo, Japan), and the inter-assay and intra-assay CVs were  $\leq 10\%$  and  $\leq 5\%$ , respectively. Apolipoprotein A and B (Apo-A and Apo-B) were quantified by immunoturbidimetric methods (Roche Diagnostics, Penzberg, Germany). The intra-assay CV were 0.7–1.8% (Apo-A) and 1.3–2.0% (Apo-B), and the inter-assay CVs were 1.4–3.6% (Apo-A) and 2.7–2.9% (Apo-B). All the lipids were measured on a cobas 8000 modular analyzer (Roche Diagnostics, Penzberg, Germany). Cord blood betatrophin levels were determined using a commercially available ELISA kit (EIAab, Wuhan, China) according to the manufacturer's instructions [20, 32]. Each sample was measured in triplicate.

## Measurement of mitochondria content

The mitochondria content was presented as the mitochondrial/nuclear DNA ratio (mtDNA/nDNA) and determined as previously described [33–35]. A fragment located in MT-ND1 (NC\_012920.1) was amplified with the primers forward: TGGGCCATACGGTAGTATTTAG TTGG and reverse: TTACCCTATAGCACCCCCTCTAC for mitochondrial DNA; while a fragment in HBB (NG\_000007.3) sequence were amplified with the primers forward: TTTTCC CACCCTTAGGCTG and reverse: CTCACTCAGTGTGGCAAAG for nuclear DNA [35]. The amplifications were performed in an ABI 7500 real-time PCR system (Life Technologies, Carlsbad, CA) with a 20  $\mu$ l reaction mixture containing 80ng of purified DNA, 1 x SYBR Green/ROX qPCR Master Mix (Thermo Scientific, Waltham, MA) and 0.3  $\mu$ M of each primer. The specificity of amplification was confirmed by melting curve analysis and agarose gel electrophoresis of the products. Each sample was measured in triplicate.

## Statistical analysis

A Kolmogorov-Smirnov analysis assessed the probability distributions of parameters except offspring parity and sex, which were analyzed by chi-square distribution. Normally distributed data are presented as mean  $\pm$  SEM, and non-normally distributed data are presented as median (interquartile range). Comparisons between groups were compared by independent-sample t-test (normally distributed data) or Mann-Whitney U-test (non-normally distributed data). Differences in offspring parity and sex distribution were examined by chi-square test. Correlations between cord blood betatrophin and the normally distributed variables of interest (maternal gestational glycemic results, pre-pregnant BMI, gestational weight gain, cord blood HOMA-IR, TC, TG, LDL-C, Apo-A and Apo-B, placental mitochondrial content) were examined using Pearson's correlation coefficients. Association between betatrophin and non-normally distributed variables including HDL were analysed by Spearman's rank correlation coefficients. When appropriate, potential confounders (maternal gestational weight gain, pre-gestational BMI, age, offspring sex, birth weight, gestational age at delivery, parity, as well as cord blood HOMA-IR and lipid profile) were incorporated into the correlation analysis. The diagnostic value of betatrophin for GDM group was assessed using the area under the receiver operating characteristic (ROC) curve. A  $P < 0.05$  (two-tailed) was considered statistically significant. All statistical analyses were performed using SPSS software (version 13.0, Chicago, IL, USA).

## Results

Participants were divided into the GDM group and the Normoglycemia groups according to the IADPSG criteria [29, 30]. Eighty-two women (36 GDM and 46 Normoglycemia) with singleton pregnancy and underwent uncomplicated Cesarean delivery at our University Hospital were enrolled. Fifteen participants (6 GDM and 9 Normoglycemia) were excluded from the study since they did not meet the study criteria abovementioned. Finally, after excluding 13 participants (7 GDM and 6 Normoglycemia) because of unavailable maternal gestational glucose records or cord blood samples, data from 54 enrollees (23 GDM and 31 Normoglycemia) was eligible for analysis. Maternal and newborn descriptive information is presented in [Table 1](#). The average glucose concentrations (fasting, 1h and 2h post-OGTT) at 24 to 28 weeks' gestation and cord blood glucose levels were higher in GDM group ( $P < 0.05$ , [Table 1](#)). In addition, cord blood insulin also was higher in GDM trend ( $P < 0.1$ ). Consistently, cord blood HOMA-IR was greater in the GDM group ( $P < 0.05$ , [Table 1](#)). Moreover, the cord blood TG also revealed a similar trend ( $P < 0.1$ , [Table 1](#)).

In humans, circulating betatrophin is elevated in type 2 diabetes [17–19], obesity [17], and type 1 diabetes [20]. Notably, in this study, cord blood betatrophin was significantly elevated in

the GDM group compared with those who were euglycemic ( $p < 0.01$ , Fig 1). The ROC curve had an area under the curve (AUC) of 0.74, 95% confidence interval [CI] = 0.60 to 0.88,  $p = 0.003$ . Based on the ROC curve, a cutoff value of 5.3 ng/ml was chosen to predict the absence (cut-off value  $\leq 5.3$  ng/ml) or presence (cut-off value  $> 5.3$  ng/ml) of GDM (S1 Fig). Twenty-six of 35 (74.3%) normoglycemic mothers had a cord blood betatrophin values of 5.3 ng/ml or less; meanwhile, 14 of 19 (73.7%) GDM were identified by this cut-off value correctly, indicating that the positive predictive value was 0.737. Nine (39.1%) of the 23 GDM mothers had a cord blood betatrophin value less than 5.3 ng/ml, representing 60.9% sensitivity. The sensitivity and specificity were 0.609 and 0.839, respectively. Collectively, these data indicates that 40 of 54 (74.1%) could be diagnosed correctly as either with or without GDM by using this predictive model at cut-off value (5.3 ng/ml) (S1 Table).

Cord blood betatrophin levels were also positively correlated with maternal gestational fasting, 1h and 2h post-OGTT glucose ( $p < 0.05$ ), however, after adjusting for the potential confounders including cord blood HOMA-IR, lipid profile, maternal ages, pre-pregnant BMI, prenatal BMI, gestational weight gain, offspring parity, sex, birth weight and gestational age at delivery, only the 2h post-OGTT glycemia level significantly correlated with betatrophin level (Table 2, Fig 2).

Betatrophin levels were significantly associated with insulin as well as HOMA-IR in cord blood ( $r = 0.556$ ,  $p = 0.000$  for insulin and  $r = 0.598$ ,  $p = 0.000$  for HOMA-IR), and the correlation remained significant with adjustment for the above-listed potential confounders ( $r = 0.457$ ,  $p = 0.003$  for insulin and  $r = 0.440$ ,  $p = 0.005$  for HOMA-IR, Fig 3).

**Table 1. Maternal and newborn characteristics.**

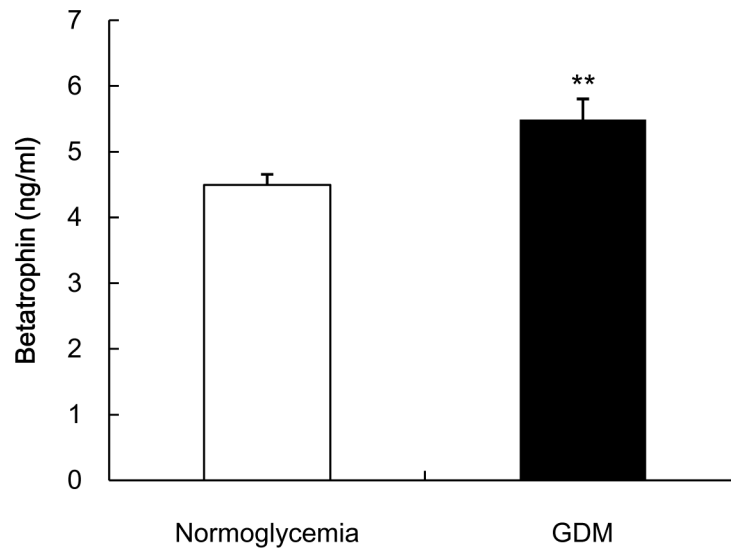
	GDM	Normoglycemia
n	23	31
Mother's age (years)	32 (28, 34)	30 (26, 32)
Pre-pregnant weight (kg)	57.1±1.86	54.1±1.39
Pre-pregnant BMI (kg/m <sup>2</sup> )	21.6±0.59	20.5±0.47
Gestational weight gain (kg)	15.8±1.1	17.5±1.0
Fasting plasma glucose (mmol/L) at 24 to 28 weeks' gestation	5.0±0.09	<b>4.3±0.08**</b>
1-h post-OGTT glucose (mmol/L) at 24 to 28 weeks' gestation	10.4±0.27	<b>7.8±0.21**</b>
2-h post-OGTT glucose (mmol/L) at 24 to 28 weeks' gestation	8.7±0.30	<b>6.5±0.15**</b>
Cord blood insulin levels (mU/L)	6.7±0.60	<b>5.1±0.43*</b>
Cord blood glucose levels (mmol/L)	4.05±0.18	<b>3.5±0.12*</b>
Cord blood HOMA-IR	1.3±0.13	<b>0.80±0.07*</b>
Cord blood TC (mmol/l)	1.75±0.10	1.71±0.07
Cord blood TG (mmol/L)	0.20(0.12, 0.28)	0.15(0.11, 0.20)
Cord blood HDL-C (mmol/l)	0.75(0.61, 1.06)	0.71(0.58, 0.96)
Cord blood LDL-C (mmol/l)	0.55±0.04	0.58±0.03
Cord blood Apo-A (g/l)	0.87±0.04	0.80±0.02
Cord blood Apo-B (g/l)	0.18±0.01	0.18±0.01
Birth weight (kg)	3.3±0.10	3.2±0.06
Offspring sex (males/females)	9/14	13/18
Gestational age at delivery (days)	271.0±1.33	272.5±1.24
Parity (% primiparous)	774	78

Normally distributed data are presented as mean ± SEM, and non-normally distributed data are presented as median (interquartile range) except the offspring sex and parity are expressed as ratio.

\*  $P < 0.05$

\*\*  $P < 0.01$

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**Fig 1. Cord blood betatrophin is elevated in GDM group.** Data is presented as mean ± SEM, \*\*P < 0.01 compared with the normoglycemic group.

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The ratio of mtDNA per nucleus DNA in fetal placenta of GDM group was reduced (Fig 4A), indicative of attenuated mitochondrial content. Furthermore, cord blood betatrophin levels inversely correlated with the placental mtDNA/nDNA ratio, and the correlation was still significant after adjusting for the same potential confounders ( $r = -0.463, p = 0.001$ , Fig 4B).

Circulating betatrophin reportedly correlates with cholesterol and LDL-C levels [32], as well as HDL-C and triglyceride concentrations in human [36]. Interestingly, in this study, cord blood betatrophin was positively associated with Apo-A ( $r = 0.353, p = 0.009$ ) and TG ( $r = 0.292, p = 0.032$ ). No correlation was observed between betatrophin and LDL-C and HDL-C. However, after adjusting for potential confounders (as above), betatrophin did not significantly correlate with the lipid profiles except there remained a trend between betatrophin and Apo-A ( $r = 0.289, p = 0.051$ ). In addition, cord blood betatrophin did not correlate with maternal pre-pregnant BMI or gestational weight gain ( $p > 0.05$ , data not shown).

## Discussion

Exposure of the fetus to intrauterine hyperglycemia may forebode the metabolic syndrome and diabetes in later life [6–8]. And, disturbingly, this may manifest early in childhood [11–13]. The secretion of betatrophin from liver, and possibly adipose, spurs pancreatic beta cell

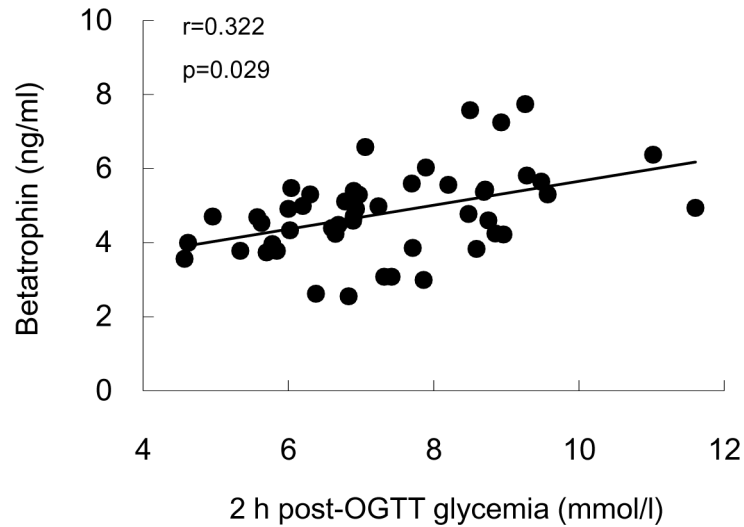
**Table 2. Pearson and Partial correlation coefficient between maternal gestational OGTT glucose concentration and cord blood betatrophin level in all participants.**

		Fasting-OGTT glucose level (n = 54)		1h post-OGTT glucose levels (n = 54)		2h post-OGTT glucose level (n = 54)	
		*		*		*	
Betatrophin	$r_s$	<b>0.281</b>	0.273	<b>0.323</b>	<b>0.119</b>	<b>0.500</b>	<b>0.322</b>
	$p$	<b>0.039</b>	0.066	<b>0.017</b>	<b>0.429</b>	<b>0.000</b>	<b>0.029</b>

\* Correlation were adjusted for pregestational BMI, gestational weight gain, cord blood HOMA-IR, newborn sex, birth weight, gestational age at delivery, parity and maternal age; correlations with  $P < 0.05$  are in bold.

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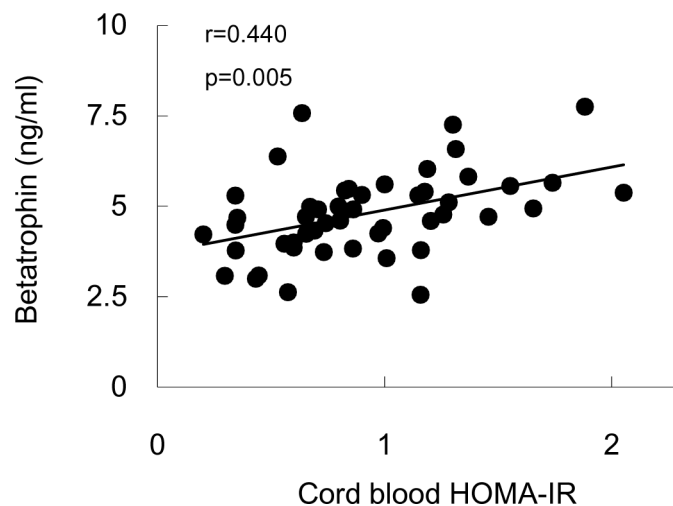


**Fig 2. Correlation analysis between maternal gestational OGTT glycemia and cord blood betatrophin.** Correlation was adjusted for pregestational BMI, gestational weight gain, cord blood HOMA-IR, newborn birth weight, sex, parity, and gestational age at delivery and maternal age. Data represents both cohorts (n = 54).

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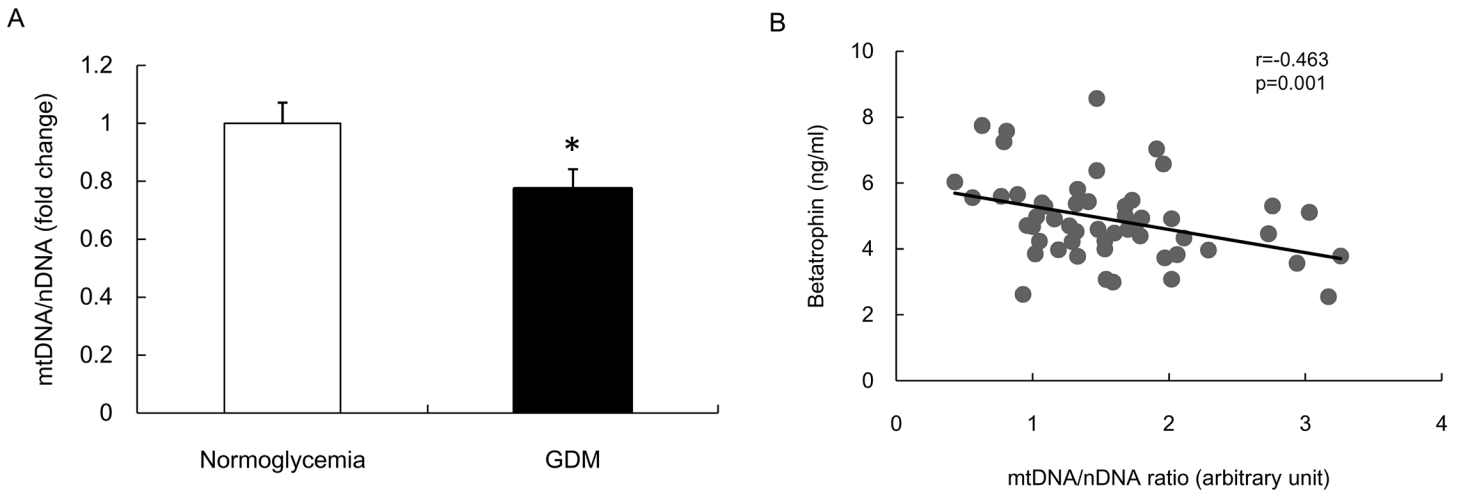
proliferation in response to insulin resistance, conceivably, in an attempt to restore glucose tolerance and mitigate lipid disturbances [15]. In concordance with this tenet, we found that cord blood betatrophin and HOMA-IR were significantly increased in the GDM group compared with the normoglycemia group. Moreover, cord betatrophin was positively correlated with maternal gestational glycemia and cord blood HOMA-IR, inferring that betatrophin could be a potential biomarker of intrauterine hyperglycemia and an indicator of insulin resistance in the offspring from GDM mothers. Admittedly, at this juncture, there is no proof that assessing betatrophin in cord blood at birth is a superior metric of fetal insult than serum glucose and insulin.

Numerous studies have found that exposure to intrauterine hyperglycemia can increase the future risk of obesity and type 2 diabetes early in life [11, 12, 14]. For example, a diabetic



**Fig 3. Correlation analysis between cord blood HOMA-IR and betatrophin.** Correlation was adjusted for cord blood lipid profile, maternal gestational 2h post-OGTT glucose, ages, pre-pregnant BMI, prenatal BMI, gestational weight gain, offspring parity, sex, birth weight and gestational age at delivery. Data represents both cohorts (n = 54).

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**Fig 4. The placental mitochondrial content and the correlation analysis between cord blood betatrophin and placental mtDNA content.** A. Reduced mitochondrial content (mtDNA/nDNA ratio) in GDM group. B. The correlation was adjusted for maternal age, pregestational BMI, gestational weight gain, cord blood HOMA-IR, newborn birth weight, sex, gestational age at delivery and parity.

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intrauterine milieu has been linked to adiposity and higher glucose and insulin levels at 5 years [11]. Additionally, in utero hyperglycemia may foretell an elevated systolic blood pressure juncture and HbA1c in childhood, posing a higher risk for cardiovascular disease and T2DM in later life [14]. A recent study found that maternal gestational glycemia inversely correlates with a child’s insulin sensitivity and beta cell response [12]—the implication being that intra-uterine hyperglycemia programs for dysfunctional beta cell secretion as well as peripheral insulin target tissues [12]. GDM may predispose to fetal hyperinsulinemia [37] and, in our study, cord blood insulin did suggest such an association. Furthermore, in concert with other studies [38, 39], we found that the GDM newborn has reduced insulin sensitivity as evidenced by an increased cord blood HOMA-IR.

Betatrophin is expressed predominantly in adipose tissue and liver, and highly responsive to metabolic cues [15, 16]. Circulating betatrophin is increased with insulin resistance, such as type 2 diabetes [17–20] and obesity [17]. Interestingly, Fenzl et al. reported that betatrophin levels were similar between type 2 diabetic and non-diabetic cohort [32] while Gómez-Ambrosi et al. found that betatrophin was decreased in T2DM patients [40].

The placenta, serving as nutrient transporter, and gas exchanger for the fetus, plays a crucial role in fetal growth, development and future metabolic outcome [41]. The placenta is also recognized as an endocrine organ since it produces essential hormones for gestation. Insulin signaling in the placenta was reported to be impaired in pregnancies complicated by obesity and diabetes [42]. Moreover, placental function may be associated with pancreatic beta cell proliferation. For example, it can secrete placental lactogen, which upregulates beta cell mass during gestation [43]. Conceivably, placenta may also produce betatrophin to regulate beta cell replication. In the current study, the cord betatrophin concentrations from GDM mothers were significantly increased, corroborating the very recent findings [44–47], and suggesting that intrauterine hyperglycemia may promote placental betatrophin secretion. Interestingly, a previous study found that placental betatrophin mRNA expression did not differ significantly between the GDM group and the control group [44].

Moreover, we observed that betatrophin levels were positively associated with maternal gestational glycemia. In humans, islet development and remodeling occur throughout the second trimester to early childhood [48]. Circulating betatrophin in humans is elevated 2 hours after a



defined meal [17]. It is of note that only the 2h post-OGTT glycemia correlated with betatrophin levels, hence, this measurement may be a superior indicator of the fetal insulin resistance than the fasting or 1h post-OGTT glycemia. Moreover, cord blood betatrophin levels correlated positively with HOMA-IR, inferring an association between betatrophin and insulin sensitivity. Previous studies reported that betatrophin levels were increased significantly in mothers with GDM compared with the control group [44–46, 49]. Moreover, in the GDM group, there was a positive association between cord blood and maternal betatrophin levels [44]. Conceivably, the betatrophin levels were also elevated in the GDM mothers participating in this study, and correlated positively with the cord blood betatrophin levels.

Mitochondrial dysfunction is closely associated with impaired insulin sensitivity and diabetes [34, 50, 51], and mitochondrial deficiency contributes to mitochondrial dysfunction [34]. Similarly, mitochondria play a major role in placental function [52], and placental mitochondrial dysfunction may be a molecular mechanism which effectuates nutritional programming of insulin resistance [53]. We observed that the mtDNA/nDNA ratio was decreased in placenta of GDM group, indicative of reduced mitochondrial content and impaired mitochondrial function. Moreover, the mtDNA/nDNA ratio inversely correlated with cord blood betatrophin levels. Although we found a correlation between betatrophin and placental mitochondrial content, there is no evidence that the alteration in circulating betatrophin levels leads to the change in mitochondrial content, both of which are likely a resultant of intrauterine hyperglycemia.

Dyslipidemia is a common morbidity associated with GDM and type 2 diabetes. Given the reports that betatrophin correlates positively with serum TG, we found a similar trend in the cord blood, namely, increased concentrations of both TG and betatrophin in the GDM cohort.

Reported betatrophin levels in obesity are inconsistent. Some reports found that betatrophin was increased [17], whereas in other studies it was reduced [40] or unaltered [32]. We found no correlation between betatrophin and maternal pre-pregnant BMI or gestational weight gain.

All statistical correlations analyzed herein have been adjusted for potential confounders to avoid misleading conclusions. However, some limitations must be acknowledged. First, since the current study was carried out in a homogeneous population of Chinese Han ethnicity, the findings may not be generalizable to other populations. Besides, the sample size was relatively small which may impede the detection of weak correlations between betatrophin levels and some metabolic parameters such as lipid profile. Although we observed positive correlations between fetal betatrophin and maternal gestational glycemia, as well as fetal cord HOMA-IR, we cannot conclude that maternal gestational hyperglycemia begets fetal betatrophin hypersecretion given the limitations of a cross-sectional study design. Additionally, the gestational glycemia data from a sole OGTT at 24–28 week of gestation may not correctly represent the glycemic status throughout the entire gestation. Also, the long term maternal glucose control and the child's outcomes are unknown and here we can not make cause and effect incontrovertible conclusions based on a cross sectional study design and correlation analysis. A future prospective study for confirmation is warranted.

In conclusion, we found that cord blood betatrophin was increased in GDM mothers at delivery, and betatrophin concentrations correlated significantly with HOMA-IR and maternal gestational glycemia. The inference is that cord blood betatrophin may be a potential biomarker of maternal intrauterine hyperglycemia and fetal insulin resistance which, ultimately, may portend later metabolic disturbances, even as early in childhood.

## Supporting Information

**S1 Fig. The diagnostic value of betatrophin for GDM was assessed by an ROC curve, with an area under the curve (AUC) of 0.74, 95% confidence interval [CI] = 0.60 to 0.88,**

$P = 0.003$ . Cut-off value (5.3 ng/ml) is depicted.  
(TIF)

**S1 Table. The diagnostic value of betatrophin for GDM and normoglycemia mothers.**  
(DOCX)

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We are grateful to the mothers who agreed to participate in this study. The authors declare that there is no conflict of interest.

## Author Contributions

Conceived and designed the experiments: HG XX XL. Performed the experiments: HG SW YZ CD. Analyzed the data: XL XX HG GY. Contributed reagents/materials/analysis tools: XX HG SW XL KM QN. Wrote the paper: XX HG XL.

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