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Association of placental toll-like receptor 4 and fatty acid transport protein expression with neonatal weight

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ABSTRACT: Fetal macrosomia is associated with several maternal and fetal complications. Toll-like receptors (TLRs) and fatty acid transport proteins (FATPs) are related with fetal growth and development. However, the association of the levels of TLR and FATP expression in placenta with neonatal weight is not known. Thus, we sought to evaluate the effect of blood lipid, TLRs, and FATPs on neonatal weight. According to birth weight, specimens were divided into four groups: low ($\leqslant 3000$ g), middle (3000 g $<$ and $\leqslant 3500$ g), high (3500 g $<$ and $\leqslant 4000$ g), and macrosomia (> 4000 g) groups. The blood lipid levels, TLR4 expression level in the umbilical vein serum, and TLR4, FATP2, and FATP4 mRNA and protein expression levels in placental tissue were measured. The risk factors of fetal macrosomia, which had significant difference among the low, middle, high, and macrosomia groups, included maternal blood triglyceride (TG) and high-density lipoprotein (HDL), cord blood TG and HDL, placental weight, TLR4, FATP2, and FATP4. Multivariate logistic regression analysis indicated that the risk of fetal macrosomia was positively correlated with the TLR4 (odds ratio $= 3.053$, $p = 0.018$), FATP2 (odds ratio $= 4.824$, $p = 0.001$), and FATP4 (odds ratio $= 3.201$, $p = 0.014$). Among them, FATP2 had the most significant effect on neonatal weight. Therefore, neonatal weight could be regulated by the FATP2 expression level, which could reduce the incidence of maternal and fetal complications caused by abnormal weight.

KEYWORDS: neonatal weight, lipid profile level, toll-like receptor 4, fatty acid transport protein

INTRODUCTION

Fetal macrosomia increased risk not only for the baby, including shoulder dystocia with consequent brachial plexus or facial nerve injuries, hypoxia, fracture of humerus or clavicle, and neonatal death, but also for the mother, including trauma to the birth canal, anal sphincter injuries, and cesarean section [1]. Recently, epidemiological studies have shown that fetal macrosomia is also associated with increased risks of obesity, hypertension, mental illness, and type 2 diabetes mellitus in adulthood [2, 3]. Fetal growth and development depend on many aspects, including genetic factors, hormones, maternal characteristics, and placental substrate transport capacity [4]. Hence, early assessment of fetal growth and risk factors for fetal macrosomia has attracted wide attention in the region of maternal-fetal medicine.

The fetal growth and development need a lot of nutrients. To meet the nutritional needs of the fetus, the mother will absorb more substances, resulting in the increase of maternal blood lipid levels in varying degrees [5]. To provide more nutrition for the fetus, maternal cord blood lipid levels also increased. Some studies found significant association of maternal lipid levels and neonatal outcomes [6]. For example, elevated maternal triglyceride (TG) level, higher high-density lipoprotein cholesterol (HDL-C) level in the first trimester, and lower HDL-C level in the third trimester are associated with a higher risk of macrosomia [7]. However, there is no report about the relationship of umbilical cord blood lipid level and neonatal weight.

Toll-like receptors (TLRs) as protective immune sentries can recognize pathogen-associated molecular patterns (PAMPs), including flagellin, lipoproteins, lipopolysaccharide, single-stranded RNA, and unmethylated double-stranded DNA. Subsequently, TLRs induce the secretion of inflammatory mediators, resulting in the adaptive antigen-specific immune response of lymphocytes [8]. The TLR family consists of more than 13 members, in which TLR4 is the first to be discovered and the most in-depth research [9]. TLR4 is the central component of the mammalian innate immune system and expressed in a wide variety of cells [10]. There are a significant amount of interest and vigorous studies on abnormal expression of TLR4, which is related to the development of various disorders including obesity [11]. In nondiabetic pregnancies, maternal overweight is associated with fetal macrosomia [12]. Therefore, the TLR4 level in cord blood and placenta may be related to neonatal weight, but there is no relevant report.

Fatty acid transport proteins (FATPs) form a family of 6 related proteins with a highly conserved 311-amino acid signature sequence and play an important role in protein-mediated transport of long chain fatty acids (LCFA) [13]. Uptake and activation of LCFA are critical to many physiological processes, so aberrant accumulation or depletion of LCFA is the pathology

of many metabolic diseases. Many obesity related diseases are caused by abnormal influx of LCFA into heart, liver, muscle, and other tissues, which leads to aberrant accumulation of lipids [14]. As mentioned above, maternal lipid level is associated with neonatal weight [6]. Compared to the microvillous membrane, FATP2 and FATP4 expressions are higher in the basal plasma membrane, and FATP2 expression correlates with maternal body mass index (BMI) [15]. However, there is no research on the association of the FATP2 and FATP4 expressions in placenta with fetal macrosomia.

Herein, the expressions of TLR4, FATP2, and FATP4 placenta tissue were detected by reverse transcription-quantitative PCR (RT-qPCR) and Western blotting. In addition, the TLR4 level in umbilical cord was determined by ELISA, and the blood lipid levels of umbilical cord and maternal blood were compared. The associations of neonatal weight with TLR4, FATP2, and FATP4 were further discussed. The aim of the current study was to elucidate the associations of the levels of TLR and FATP expression in placenta with neonatal weight, which may provide a theoretical basis for the control of neonatal weight.

MATERIALS AND METHODS

Patients

A total of 160 pregnant women in the Department of Obstetrics and Gynecology of the North China University of Science and Technology Affiliated Hospital (Tangshan, China) was recruited in November 2017 and November 2018 and provided written informed consent for the use of their placenta, blood, and clinical information. This study was performed in accordance with the Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects and approved by the Ethics Committee of the North China University of Science and Technology Affiliated Hospital (No. 2017012).

The inclusion criteria for participants were as follows: Natural pregnancy, BMI before pregnancy from 18.5 to 23.9, gestational weeks from 37 to 41 weeks, age from 20 to 35 years, normal blood pressure and blood sugar, normal pregnancy screening (e.g., Tang screening, nuchal translucency, and ultrasound to exclude malformations), and normal liver and kidney function. The exclusion criteria were pregnant women with the following conditions: growth weight-limited fetus, premature infants, elective fetuses, premature rupture of membranes and placental abruption complication, complications such as heart and hypothyroidism, systemic or local acute, chronic infection, tumor and immune system diseases, fetal distress or neonatal asphyxia, and congenital or hereditary allergic diseases. According to the birth weight of newborns, pregnant women were divided into 4 groups. The birth weight $\leqslant$ 3000 g, 3000 g $<$ and $\leqslant$ 3500 g, 3500 g $<$ and $\leqslant$ 4000 g, and $>$ 4000 g were marked as the low, middle, high, and macrosomia [16] groups, respectively.

Clinical examination data and blood and tissue analyses

Clinical examination data

The Department of Obstetrics and Gynecology collected the general clinical examination data of pregnant women and newborns, including age, height, pregnancy time, production time, gestational day, BMI before pregnancy, BMI increase, and placental weight.

Blood analyses

The 3–5 ml of fasting cubital venous blood of pregnant woman was collected 1 week before delivery. After birth and before the umbilical cord was broken, the 5–10 ml of neonatal venous blood was extracted from the farthest end of the umbilical cord. For all blood samples, serum was subsequently separated by centrifugation (1000 *g* at 4 °C for 15 min), sealed, and stored in a refrigerator at −20 °C for further use. The blood lipid levels, including triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL), were determined with automatic biochemical detector (AU5800, Beckman Coulter, Suzhou, China) by the Clinical Laboratory of the North China University of Science and Technology Affiliated Hospital. The TLR4 level in the umbilical vein serum was detected by double antibody enzyme-linked immunosorbent assay (ELISA) with commercial ELISA kit (cat. no. JYM0274Po; Wuhan Gene Beauty Biotechnology Co., Ltd, Wuhan, China) based on the manufacturer's instruction.

Placental tissue collection

Within 5 min of placenta stripping off mother's body, about 1 cm^3 of placental tissue section was randomly taken from the surface of maternal placenta under strict aseptic conditions; the calcification and mechanicalized lesions should be avoided. After repeated washing with saline, the placental tissue sample was stored in a refrigerator at −80 °C for further analysis.

RT-qPCR

Total RNA was extracted from placental tissue by Trizol (Invitrogen, CA, USA), and Nano Drop (Thermo Fisher Scientific Inc., MA, USA) was used to measure the concentration of the total RNA based on the manufacturer's instruction. Next, cDNA was reverse transcribed by an All-in-one™ First-Strand cDNA Synthesis kit (cat. no. QP007; iGeneBio, Guangzhou, China), including 1 µl of total RNA, 1 µl of M-NLV RT, 4 µl of 5 × first chain synthetic buffer, 2 µl of dNTP mixture (10 mM), 2 µl of DTT (0.1 M), 1 µl of oligo $(dT)_{20}$ (50 µM) M-NLV RT, and 9 µl of RNase-free H_2O. Then, qPCR was performed using a Rotor Gene 3000 Quantitative PCR instrument (Corbett Life Science, Shanghai,

China). The primers were supplied by Sangon Biotech Co., Ltd., Shanghai, China, and their sequences were as follows: TLR4 forward, 5′-CCC TGG TGA GTG TGA CTA TTG A-3′ and reverse, 5′-TTT GAG AAC AGC AAC CTT TGA A-3′; FATP2 forward, 5′-CAT TCC GGT GGA AAG GGG AA-3′ and reverse, 5′-TCT TAG AAA CCG GGG CCT TG-3′; FATP4 forward, 5′-GGG GCC AAT AAA CTC TGC CT-3; and reverse, 5′-ACA GAT GAG GCG GGT CAA TG-3′; and β-actin forward, 5′-CGT GGA CAT CCG CAA AGA CCT-3′ and reverse, 5′-AAG AAA GGG TGT AAC GCA ACT-3′. β-actin was selected as the internal reference. The thermocycling conditions were listed as follows: initial denaturation at 95 °C for 2 min; followed by 40 cycles of 95 °C for 15 s, 56 °C for 25 s, and 72 °C for 20 s; and 72 °C for 5 min. The relative expression levels of TLR4, FATP2, and FATP4 mRNAs were normalized with the internal reference β-actin mRNA.

Western blot

The total proteins from placental tissue were first extracted with RIPA lysate (cat. no. PS0033) and then quantified with a BCA Protein assay kit (cat. no. PT0001); both reagents were from Beijing Leagene Biotech. Co., Ltd, Beijing, China, based on the manufacturer's instruction. Equal amount of proteins (10 μl per lane) was resolved using 8% SDS-PAGE gel and transferred to a PVDF membrane at 90 mV for 1 h. After 5% skimmed milk was used for blocking the nonspecific binding at 37 °C for 2 h, the PVDF membrane was incubated with one of the following primary antibodies (from Beijing Bioss Biotechnology Co., Ltd, Beijing, China): rabbit anti-human TLR4 polyclonal antibody (dilution 1:1000; cat. no. bs-20379R), rabbit anti-human FATP2 antibody (dilution1:500; cat. no. bs-3936R), rabbit anti-human FATP4 antibody (dilution1:500; cat. no. bs-11535R), or rabbit anti-human β-actin antibody (dilution1:5000; cat. no. bs-0061R) at 4 °C overnight, followed by incubation with goat anti-rabbit IgG secondary antibodies (dilution with skimmed milk at 1:1000; cat. no. ZB-2301; OriGene Technologies, Inc, Wuxi, China) at ambient temperature for 2 h. Finally, antibody binding was visualized using an ECL Chemiluminescence kit (Beyotime Institute of Biotechnology, Shanghai, China). Image Lab software (Bio-Rad Laboratories, Inc, Shanghai, China) was used for densitometry analysis of the blots, and the density of TLR4, FATP2, and FATP4 was normalized with β-actin.

Statistical analysis

Data were presented as the mean ± standard deviation and analyzed with SPSS version 17.0 (SPSS, Inc.). One-way ANOVA was used to analyze the significant differences of blood lipid, TLR4, FATP2, and FATP4 levels for continuous data. Multivariate correlation analysis was performed using a multivariate logistic regression model, and $\alpha = 0.05$ was used as the test level (bilateral). $p \leqslant 0.05$ was considered to indicate a statistically significant difference in all the experiments.

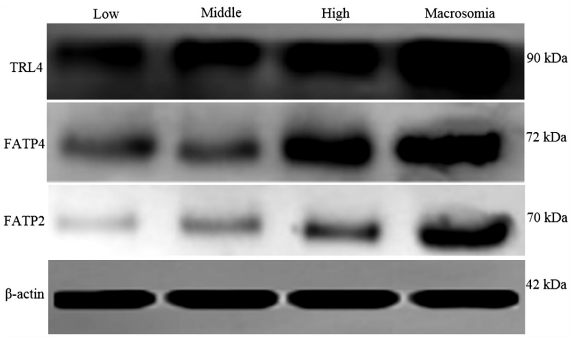

Fig. 1 Western blot analysis of TLR4, FATP2, and FATP4 protein expression levels in low, middle, high, and macrosomia groups.

RESULTS

Clinical data analysis

As shown in Table 1, for the general clinical examination data, there were no significant differences in age, height, pregnancy time, production time, gestational day, BMI before pregnancy, and BMI increase among the low, middle, high, and macrosomia groups (all $p > 0.05$). The placental weight was closely associated with the neonatal weight and increased significantly with the increase of neonatal weight ($p < 0.05$). For blood lipid levels in elbow (Table 2) and umbilical vein (Table 3), there were no significant differences in TC and LDL among the 4 groups (all $p > 0.05$). The TC and HGL in elbow (Table 2) and umbilical vein (Table 3) were both closely associated with the neonatal weight, which increased significantly with the increase of TG and decrease of HGL (all $p < 0.05$), respectively.

TLR4, FATP2, and FATP4 levels

The TLR4 level in umbilical vein (Table 3) was detected by ELISA and associated with the neonatal weight, which increased significantly with the increase of TLR4 level ($p < 0.05$). RT-PCR results (Fig. 1) indicated that the relative expression levels of TLR4, FATP2, and FATP4 mRNA in placental tissue were all associated with the neonatal weight (Table 4) and increased significantly with the increase of the neonatal weight (all $p < 0.05$). According to the Western blot results (Fig. 1), the relative expression levels of TLR4, FATP2, and FATP4 proteins in placental tissue were also all associated with the neonatal weight (Table 5) and increased significantly with the increase of the neonatal weight (all $p < 0.05$). The results of Western blot and RT-PCR were consistent.

Table 1 Comparison of clinical data of the 4 groups of pregnant women.

	Low (32)	Middle 2 (49)	High (51)	Macrosomia (28)	*p*-value
Age (years)	28.67 ± 3.69	28.29 ± 3.73	29.83 ± 4.18	27.40 ± 4.37	0.261
Height (cm)	162.47 ± 5.78	163.49 ± 4.75	163.50 ± 3.92	163.73 ± 4.68	0.847
Pregnancy time (time)	1.67 ± 0.82	1.75 ± 0.99	1.51 ± 0.69	1.47 ± 0.74	0.220
Production time (time)	1.27 ± 0.46	1.27 ± 0.49	1.46 ± 0.59	1.13 ± 0.49	0.233
Gestational day (days)	277.21 ± 5.78	277.37 ± 5.06	276.71 ± 4.19	280.73+4.49	0.072
BMI before pregnancy	25.49 ± 2.42	26.98 ± 2.73	27.08 ± 2.62	27.99 ± 2.04	0.126
BMI increase	5.31 ± 1.57	5.56 ± 1.56	5.82 ± 1.65	6.02 ± 1.52	0.312
Placental weight (g)	512.00 ± 52.12[a]	619.80 ± 70.44[b]	711.25 ± 110.91[c]	723.33 ± 96.78[d]	0.001

[a] $p < 0.05$, VS. middle, high, and macrosomia groups, respectively; [b] $p < 0.05$, VS. low, high, and macrosomia groups, respectively; [c] $p < 0.05$, VS. low and middle groups, respectively; [d] $p < 0.05$, VS. low and middle groups, respectively.

Table 2 Comparison of blood lipid levels in elbow vein of the 4 groups of pregnant women.

	Low (32)	Middle 2 (49)	High (51)	Macrosomia (28)	*p*-value
TG (mmol/l)	2.59 ± 0.91[a]	2.67 ± 0.60[b]	2.79 ± 0.74	3.03 ± 1.06[c]	0.007
TC (mmol/l)	5.94 ± 1.57	6.24 ± 1.47	6.76 ± 1.44	7.60 ± 1.84	0.367
HDL (mmol/l)	2.44 ± 0.61[d]	2.19 ± 0.55	1.91 ± 0.39[e]	1.88 ± 0.51[f]	0.002
LDL (mmol/l)	3.58 ± 0.91	3.47 ± 1.03	3.18 ± 0.87	3.08 ± 0.63	0.210

[a] $p < 0.05$, VS. macrosomia group; [b] $p < 0.05$, VS. macrosomia group; [c] $p < 0.05$, VS. low and middle groups, respectively; [d] $p < 0.05$, VS. high and macrosomia groups, respectively; [e] $p < 0.05$, VS. low group; [f] $p < 0.05$, VS. low group.

Table 3 Comparison of blood lipid and TLR4 levels in umbilical vein of the 4 groups of pregnant women.

	Low (32)	Middle 2 (49)	High (51)	Macrosomia (28)	*p*-value
TG (mmol/l)	1.46 ± 0.35[a]	1.61 ± 0.29	1.74 ± 0.28[b]	1.90 ± 0.40[c]	0.001
TC (mmol/l)	0.25 ± 0.09	0.22 ± 0.04	0.28 ± 0.11	0.29 ± 0.12	0.131
HDL (mmol/l)	0.82 ± 0.12[d]	0.76 ± 0.10	0.72 ± 0.12[e]	0.70 ± 0.15[f]	0.030
LDL (mmol/l)	0.63 ± 0.21	0.64 ± 0.16	0.62 ± 0.13	0.65 ± 0.20	0.648
TLR4 (pg/ml)	3632.37 ± 558.44[g]	3855.21 ± 774.48[h]	4089.24 ± 437.63[i]	4204.38 ± 841.63[j]	0.003

[a] $p < 0.05$, VS. high and macrosomia groups, respectively; [b] $p < 0.05$, VS. low group; [c] $p < 0.05$, VS. low group; [d] $p < 0.05$, VS. high and macrosomia groups, respectively; [e] $p < 0.05$, VS. low group; [f] $p < 0.05$, VS. low group; [g] $p < 0.05$, VS. middle, high, and macrosomia groups, respectively; [h] $p < 0.05$, VS. low and macrosomia groups, respectively; [i] $p < 0.05$, VS. low and macrosomia groups, respectively; [j] $p < 0.05$, VS. low, middle, and high groups, respectively.

Table 4 Comparison of TLR4, FATP2, and FATP4 mRNA levels in placental tissue of the 4 groups of pregnant women.

	Low (32)	Middle 2 (49)	High (51)	Macrosomia (28)	*p*-value
TLR4	136.11 ± 38.02[a]	162.45 ± 36.01[b]	183.35 ± 52.28[c]	202.90 ± 42.77[d]	0.001
FATP2	81.21 ± 25.71[e]	105.75 ± 37.09[f]	113.70 ± 41.73[g]	146.65 ± 51.86[h]	0.001
FATP4	93.89 ± 51.05[i]	112.10 ± 50.25[j]	138.15 ± 53.95[k]	151.80 ± 67.67[l]	0.009

[a] $p < 0.05$, VS. high and macrosomia groups, respectively; [b] $p < 0.05$, VS. macrosomia group; [c] $p < 0.05$, VS. low group; [d] $p < 0.05$, VS. low and middle groups, respectively; [e] $p < 0.05$, VS. high and macrosomia groups, respectively; [f] $p < 0.05$, VS. macrosomia group; [g] $p < 0.05$, VS. low and macrosomia groups, respectively; [h] $p < 0.05$, VS. low, middle, and high groups, respectively; [i] $p < 0.05$, VS. high and macrosomia groups, respectively; [j] $p < 0.05$, VS. macrosomia group; [k] $p < 0.05$, VS low group; [l] $p < 0.05$, VS. low and middle groups, respectively.

Table 5 Comparison of TLR4, FATP2, and FATP4 protein levels in placental tissue of the 4 groups of pregnant women.

	Low (32)	Middle 2 (49)	High (51)	Macrosomia (28)	*p*-value
TLR4	23.57 ± 5.41[a]	30.83 ± 8.89[b]	47.79 ± 10.68[c]	48.90 ± 10.94[d]	0.001
FATP2	101.84 ± 10.64[e]	138.55 ± 23.31[f]	180.90 ± 31.08[g]	229.60 ± 26.50[h]	0.001
FATP4	128.79 ± 66.03[i]	157.60 ± 56.70[j]	169.65 ± 57.68[k]	195.65 ± 47.93[l]	0.005

[a] $p < 0.05$, VS. middle, high, and macrosomia groups, respectively; [b] $p < 0.05$, VS. low and macrosomia groups, respectively; [c] $p < 0.05$, VS. low and macrosomia group, respectively; [d] $p < 0.05$, VS. low, middle, and high groups, respectively; [e] $p < 0.05$, VS. middle, high, and macrosomia groups, respectively; [f] $p < 0.05$, VS. low, high, and macrosomia groups, respectively; [g] $p < 0.05$, VS. low, middle, and macrosomia groups, respectively; [h] $p < 0.05$, VS. low, middle, and high groups, respectively; [i] $p < 0.05$, VS. high and macrosomia groups, respectively; [j] $p < 0.05$, VS. macrosomia group; [k] $p < 0.05$, VS. low group; [l] $p < 0.05$, VS. low and middle groups, respectively.

Table 6 Multivariate logistic regression analysis of influencing neonatal weight.

	β	Waldc^2	*p*-value	OR	95% CI
Maternal blood TG	0.766	2.616	0.106	2.152	0.850–5.447
Maternal blood HDL	0.230	0.145	0.703	1.258	0.385–4.109
Cord blood TG	−0.238	0.068	0.795	0.789	0.132–4.726
Cord blood HDL	0.182	0.097	0.755	1.200	0.381–3.780
Placental weight	1.084	5.260	0.022	2.957	1.171–7.467
TLR4	1.116	5.635	0.018	3.053	1.215–7.672
FATP2	1.574	11.419	0.001	4.824	1.937–12.016
FATP4	1.163	6.022	0.014	3.201	1.264–8.105

Relationship analysis

To investigate the risk factors of fetal macrosomia, the indicators with significant difference among the low, middle, high, and macrosomia groups, including maternal blood TG and HDL, cord blood TG and HDL, placental weight, TLR4, FATP2, and FATP4, were selected as independent variables. Their averages were 2.93 mmol/l for maternal blood TG, 2.39 mmol/l for maternal blood HDL, 0.39 mmol/l for cord blood TG, 0.66 mmol/l for cord blood TG HDL, 665 g for placental weight, 161 for TLR4 in placental tissue, 169 for FATP2 in placental tissue, and 157.5 for FATP4 in placental tissue and used as the boundary of high and low levels. As shown in Table 6, multivariate logistic regression analysis indicated that maternal blood TG and HDL, cord blood TG and HDL, and placental weight were not statistically significant (all $p > 0.05$). On the contrary, TLR4, FATP2, and FATP4 were statistically significant (all $p > 0.05$), and odds ratios were 3.053, 4.824 and 3.201, respectively. Their corresponding 95% *CIs* were (1.215, 7.672), (1.937, 12.016), and (1.264, 8.105), respectively.

DISCUSSION

The nutrients for the growth and development of the fetus all come from the mother. Although glucose is the main energy source, blood lipids are also involved in its nutritional supply. Therefore, to meet the nutritional needs of the fetus, the intestinal absorption capacity of pregnant women to various substances is enhanced, resulting in the increase of maternal and umbilical blood lipid levels, which may affect the neonatal weight [17]. Our result showed that the levels of TG in elbow vein and umbilical cord blood for 4 groups were statistically different, and the TG level of macrosomia group was significantly increased. TG cannot be directly used by the fetus through the placental barrier, so it needs to be hydrolyzed into small molecular substances by lipase and then transported through the placenta into the umbilical vein blood, which is absorbed by the fetus, resulting in the increase of neonatal weight [18]. In addition, the levels of HDL in elbow vein and umbilical cord blood for 4 groups were also statistically different, and the HDL level of macrosomia group was significantly decreased. Paradoxically, multivariate logistic regression analysis showed that TG and HDL levels in elbow vein and umbilical cord blood were not significantly correlated with neonatal weight. Yu's report exhibited similar result that the serum TC and LDL-C levels were not significantly different of the macrosomia and control groups, but maternal TG/HDL levels were positively associated with neonatal weight [19]. However, maternal blood lipid levels during pregnancy significantly increased, and hyperlipidemia may participate in the circulation and metabolism of other nutrients, indirectly affecting the growth and development of the fetus [20]. Therefore, it is important to maintain maternal lipid levels to the appropriate extent in order to avoid fetal overgrowth and perform primary prevention of macrosomia.

TLR4, as a pattern recognition receptor, can recognize various pathogen-associated molecular patterns, which uniquely expressed on the surface of pathogens [9]. Placenta is an immunological site in maternal-fetal interface and expresses TLR4 [21]. Under normal circumstances, the maternal immune system is in a state of dynamic balance. TLR4 in placenta can recognize the invading pathogens and some inflammatory mediators. TLR4 can activate the cellular immune system after binding with inflammatory ligands, induce the mother to produce various inflammatory factors, activate the mother's innate immune system, resist the damage of harmful inflammatory products to the body, and provide a good environment for the growth and development of the fetus [22]. If this balance is broken, severe infection of placenta and cord blood may occur, which will affect the normal development of fetus. Our study demonstrated that TLR4 could be detected in cord blood serum of the 4 groups, and the neonatal weight increased with the increase of TLR4 level. Similarly, TLR4 protein and mRNA were expressed in placentas of the 4 groups, and their expression levels increased with the increase of neonatal weight. With the slow increase of fetal weight during pregnancy, the level of TLR4 gradually increased. The maternal immune system gradually adapted to this situation and could achieve dynamic balance, so there was no obvious inflammation in the fetus. After the delivery of the fetus, the newborn lost the protection of the mother; the dynamic balance of the immune system would be broken. Therefore,

the higher the weight of the newborn, the higher the TLR4 level in the body, which might lead to more inflammatory reaction and affect the healthy growth of the infant in the future.

FATP2 possesses dual functions of the transport of exogenous LCFA and the activation of very long chain fatty acids (VLCFA) [23] and promotes the metabolic activity of adipocytes [24]. At high glucose level, the FATP2 level in human islet cells increased significantly, which indicated that FATP2 was the basis of fatty acid transport in β cells and suggested that FATP2 might be an ideal marker for the development of overweight [25]. For overweight pregnant women, the increase of FATP2 expression could lead to the enhanced ability to deliver fatty acids to the fetus during pregnancy, which could lead to fetal macrosomia and obesity [15]. The expression of FATP2 in placenta was regulated by many factors. For example, under hypoxia, the FATP2 protein level in placental tissue showed a significant upward trend, indicating that the fetal essential fatty acid supply via the placenta was protected under hypoxia [26]. Our results showed that the FATP2 protein and mRNA were both expressed in placenta of the 4 groups, and neonatal weight increased with the increase of FATP2 mRNA and protein levels. The higher the expression level of FATP2 protein and mRNA in placenta, the stronger the ability of placenta to transport fatty acids and accumulate more fat. This not only increases the weight of the placenta, but also promotes the growth and development of the fetus in the uterus, resulting in an increase in the neonatal weight. Therefore, fetal weight could be indirectly controlled by regulating the FATP2 protein and mRNA expression levels, which would avoid the influence of fat accumulation on organ development.

FATP4 is the primary FATP expressed in intestinal epithelial cells and mainly located at the top of the intestinal epithelial cells [27]. Overexpression of FATP4 resulted in the increase of LCFA uptake, while knockdown of FATP4 in intestinal epithelial cells led to the decrease of LCFA uptake [27]. FATP4 could accelerate the transport of fatty acids, especially LCFA and VLCFA, and FATP4 had stronger esterification effect on VLCFA [28]. FATP4 is also expressed in trophoblasts of the placenta [29], so it is related to fetal growth. For example, the activation of peroxisome proliferator-activated receptor-gamma and retinoid X receptor enhanced the FATP4 expression, which facilitated the fatty acid transport and storage in human placental trophoblasts [30]. Our results demonstrated that the protein and mRNA levels of FATP4 in placenta of the 4 groups were consistent with these of FATP2, and neonatal weights were also positively correlated with FATP4 mRNA and protein levels. The up-regulation of mRNA and protein expression of FATP4 in human adipose tissue led to the increase of lipid transport and metabolic rate [31]. Accordingly, the increased levels of FATP4 protein and mRNA in placenta will increase the concentration of fatty acids transported from placenta to fetus, resulting in fetal weight gain. Therefore, neonatal weight can also be controlled by regulating the expression level of FATP4 in placenta.

In conclusion, the levels of TG and HDL in maternal elbow venous blood and umbilical cord blood, TLR4 expression in cord blood and placenta, and protein and mRNA of FATP2 and FATP4 in placenta were all related to neonatal weight. Logistic regression analysis showed that the weight of placenta and the levels of TLR4, FATP2, and FATP4 expression in placenta had significant effects on the neonatal weight. The expression level of FATP2 increased, and the weight of the newborn increased more significantly. Therefore, we can regulate the weight of newborns by inducing or inhibiting the level of FATP2 and reduce the incidence of maternal, fetal, and neonatal diseases caused by abnormal weight. The limitation of this study is that there is no study on the molecular mechanism of fetal weight regulation and the establishment of neonatal weight evaluation model, which will be carried out in the future clinical work.

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