Relationships Between Placental Lipid Transporters and Macrosomia in Healthy Pregnancy

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Research

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Abstract

Background: Macrosomia is a serious public health problem worldwide, the underlying etiology and mechanism of macrosomia born to healthy mothers are still unclear. Abnormal lipid transport during pregnancy exerts potential and adverse impacts on fetus, our study aimed to assess associations between macrosomia and placental expression levels of lipid transport-related genes and umbilical cord blood lipid concentrations in healthy pregnancy.

Methods: We conducted a case-control study of 38 macrosomia and 39 normal-birth-weight newborns in healthy pregnancy. Cord blood lipid levels were measured by automatic biochemical analyzer, mRNA and protein expression levels of placental lipid transport-related factors were determined by real-time polymerase chain reaction and western blot, respectively.

Results: The single factor analysis showed that placental mRNA and protein expression levels of *PPARα, PPARγ, FABPpm, LXRα, FABP3, FABP4* and *FAT/CD36* were significantly higher and cord blood total cholesterol, low-density lipoprotein cholesterol, and non-esterified fatty acid (NEFA) concentrations were significantly lower in macrosomia group. Further analysis found that placental *PPARγ*, *FABP4* and *FABP3* mRNA expression levels were positively correlated with high-density lipoprotein cholesterol, NEFA and triglycerides concentrations in macrosomia cord blood, respectively. After multivariate adjustment, the logistic regression analysis showed that high placental *PPARα* (*AOR*=3.022; 95%*CI*:1.032-8.853) and *FAT/CD36* (*AOR*=2.989; 95%*CI*:1.029-8.679) expression increased the risk of delivering macrosomia.

Conclusions: The increase of *PPARα* and *FAT/CD36* mRNA expression levels is associated with the occurrence of macrosomia, suggesting that they may be important regulators during placental lipid transport in macrosomia.

1. Background

Macrosomia is defined as a birth weight of ≥ 4,000 g in Asia and ≥ 4,500 g in Africa and Latin America [1, 2]and is a common abnormal outcome of pregnancy. Along with the rise in the incidence of macrosomia[3, 4], its resulting health problems are getting more and more attention. The delivery of a macrosomic neonate is associated with obstetric complications, including shoulder dystocia, obstetric brachial plexus injury, birth fracture of the humerus or clavicle, birth asphyxia, and other neonatal injuries. Macrosomia at birth may even increase the risk of childhood obesity, adult diabetes, and cardiovascular diseases in the future [5, 6]. Although interventions based on evidence for the management of macrosomic infants born to gestational diabetes mellitus (GDM) women have been proposed, non-GDM macrosomia is still an obstetric dilemma. There remains no clear consensus regarding the ante-partum prediction and management of them[7]. The underlying etiology and mechanism of macrosomia born to healthy mothers are still unclear, and further research is urgently needed.

Lipids are essential materials for fetal growth and development, and the fetus receives abundant lipids through maternal-fetal transport during pregnancy[8–10]. The transfer of lipids to the fetus involves

many placental transporters, such as peroxisome proliferator-activated receptors (PPARs), fatty acid-binding proteins (FABPs), fatty acid transporters (FATPs), fatty acid translocase (FAT/CD36), liver X receptor (LXR), etc. [11]. During early pregnancy fetal cholesterol mainly comes from maternal circulation[12]. This process involves PLTP, ABCA1 and other genes, and is regulated by LXR[13, 14]. The placenta acts as a bridge between the mother and the fetus and plays an important role in regulating the growth and development of the fetus. The availability of various nutrients to the fetus depends largely on the placental capacity of transporting nutrients, which will directly affect fetal growth[15, 16]. The demand for placental lipid transport increases in the third trimester when fetal fat deposition increases exponentially[16, 17]. Accordingly, the placenta responds to the nutritional requirements of the fetus by such mechanisms as regulating the placental expression of specific genes to alter its transport capacity. Changes in placental function affect its oxidative absorption, utilization of lipids, and trans-placental lipids transport[9], which in turn affect fetal growth and fat deposition. Excessive transport of lipids across the placenta may increase the fetal absorption and utilization of lipids and fetal fat deposition, and may eventually lead to the occurrence of macrosomia[18,19].

In spite of its well-documented importance, little is known about the expression levels of placental lipids transport/metabolism-related genes and umbilical cord blood lipid concentrations in non-GDM pregnancies. Therefore, in view of the important role of placental lipid transport in fetal growth and development, we hypothesized that one of the reasons for the delivery of macrosomic neonates born to healthy women may involve changes in placental lipid transport function during pregnancies. Therefore, our study was designed to analyze the associations between placental lipid transport/metabolism-related genes and macrosomia in healthy pregnancy, so as to provide new insights into the mechanism of macrosomia.

2. Material And Methods

2.1 Study population

A hospital-based case-control study was performed. A case group was formed with 38 macrosomia (birth weight ≥ 4,000 g) who were identified continuously from June 2015 to June 2016 in the Obstetric Department of the Second Affiliated Hospital of Wenzhou Medical University, Wenzhou (Zhejiang), China. Thirty-nine normal-birth-weight newborns (ranging from 2,500 to 3,999 g) were randomly selected as a control group from infants delivered within 3 days of the macrosomia deliveries. All women fulfilled the following inclusion criteria: maternal age from 18 to 40 years, full-term (gestational age ≥ 37 months), and singleton pregnancy. Women with any health problems or pregnancy complications were excluded, and newborns with any health problems were excluded. Written informed consent was obtained from all participating women. The study protocol was approved by the ethics committee of Wenzhou Medical University.

2.2 Placenta and umbilical cord blood collection

In total, 77 placental tissues and 77 cord blood samples were collected. As we have described before (20), fresh placental tissue and 10 ml cord blood samples were obtained immediately after delivery. A small piece of ~ 1 × 1 × 1 cm (~ 2 g) was excised from the chorionic villus layer of placenta, washed two times with saline solution (0.9% NaCl),and immediately placed in a tube pretreated with diethyl pyrocarbonate containing 3 ml of RNAlater solution (Ambion, Austin, TX, USA). The placental tissue samples were preserved at 4°C overnight and then stored at -80°C until analysis. Umbilical cord blood samples were centrifuged at 3,000 rpm for 20 min to separate the serum and then stored in tubes at -80°C for further analysis.

The concentrations of lipids in umbilical cord blood, including total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), and triglycerides (TG), were determined with a Hitachi 7600 automatic biochemical analyzer (Hitachi, Tokyo, Japan).

2.3 Total RNA extraction and real-time quantitative polymerase chain reaction (qPCR)

Total RNA was extracted from placenta tissues with Trizol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA was prepared with a ReverTra Ace qPCR reverse transcription kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. Target genes were quantified with SYBR green dye (Life Technologies) and a CFX96 Touch real-time PCR detection system (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. The primers used for real-time qPCR were synthesized by Huada Limited Co. (Huada, Shanghai, China), and the primer sequences are as shown in Supplementary Table 1(21–25). The real-time qPCR reaction conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 10 s, 60°C for 15 s, and 72°C for 20 s. Finally, the temperature was elevated from 65°C to 99°C in 0.1°C/s increments to obtain a melting curve for confirming amplification specificity. The comparative CT method was used to calculate the relative mRNA expression levels with *GAPDH* as the reference gene and using the Eq. $2^{-\Delta\Delta Ct}$ (26).

2.4 Protein extraction and western blot analysis

Placenta tissue samples (20 mg) were washed twice with phosphate-buffered saline (PBS), homogenized in RIPA lysis buffer (Beyotime, Shanghai, China), and centrifuged at 12,000 rpm for 5 min. The supernatants were collected, and the proteins were denatured at 100°C for 15 min. Total protein concentrations were determined with a bicinchoninic acid kit (Beyotime, Shanghai, China). Equal amounts of protein were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA), and then blocked with 5% milk in Tris-buffered saline with Tween 20 (TBST) for 1 h at room temperature. The membranes were then incubated overnight at 4°C with rabbit anti-human primary antibodies (Abcam, Cambridge, UK) against GAPDH (1:5,000), PPARα (1:2,000), PPARγ (1:2,000), FABPpm (1:2,000), FABP4 (1:2,500), FAT/CD36 (1:5,000), FATP1 (1:2,000), FATP4 (1:2,000), and LXRα (1:2,000). After washing three times with TBST, the membranes were incubated for 1 h at room temperature with rabbit polyclonal secondary antibody (1:5,000; Abcam). The membranes were again washed and developed with enhanced chemiluminescence

western blotting reagents (Biological Industry, Israel) according to the manufacturer's instructions. The films were scanned and quantified with Quantity One software.

2.5 Statistical analysis

Questionnaire and experimental data were entered into an EpiData3.1 database, and statistical analyses were done using SPSS 14.0 software (SPSS Inc., Chicago, IL). Normal distribution of the data was analyzed using the Shapiro-Wilk test in order to perform the appropriate statistical analysis. Comparisons between group variables were made using a t-test or a Mann-Whitney U test for normal or not normal variable distribution, respectively. Participant characteristics were analyzed using the chi-square test or the Fisher's exact test for categorical variables. The relationship between two variables was evaluated with the Pearson linear correlation or Spearman correlation. Multivariate logistic regression analysis was used to evaluate the effects of study factors on macrosomia, and adjusted odds ratio (*AOR*) and 95% confidence interval (*CI*) were used to estimate the risk of macrosomia. Two-tailed *P*-values ≤ 0.05 were considered statistically significant.

3. Results

3.1 Subject characteristics

The maternal and infant characteristics are shown in Table 1. Maternal age and gestational weight gain were significantly higher in mothers who delivered macrosomia ($P < 0.05$), and the gender of newborns with macrosomia was more likely to be male ($P < 0.01$). There were no significant differences in gestational age, maternal weight before pregnancy, or maternal body mass index (BMI) before pregnancy between the two groups.

Table 1
Baseline characteristics of mothers and newborns in two groups

Parameter	Macrosomia (*n* = 38)	Control (*n* = 39)	*P*
Weight before pregnancy (kg)	54(49.5,60.0)	51.5(49.0,57.5)	0.281[a]
Height (m)	1.60(1.58,1.63)	1.60(1.58,1.63)	0.805[a]
Maternal BMI before pregnancy (kg/m²)	21.54 ± 3.14	20.76 ± 2.27	0.227[b]
Gestational weight gain (kg)	16.32 ± 5.30	14.17 ± 3.89	0.048[b]
Maternal age (yr)	29.56 ± 4.20	27.36 ± 3.48	0.015[b]
Nulliparous, *n* (%)	16(42.11)	1(2.56)	0.069[c]
Gestational age (weeks)	40(39.00,40.25)	40(38.00,40.00)	0.205[a]
Mode of delivery, *n* (%)			
Vaginal	22(57.89)	38 (97.44)	< 0.001[c]
C-section	16(42.11)	1 (2.56)	
Birth weight (g)	4210(4098,4331)	3400(3290,3575)	< 0.001[a]
Infant gender, *n* (%)			
Male	30(78.95)	19(48.72)	0.009[c]
Female	8 (21.05)	20(51.28)	
Data are expressed as the mean ± SD, median (interquartile range).			
[a] Mann-Whitney *U* test; [b] Two-sample *t*-test; [c] chi-square test.			

3.2 Lipid concentration in umbilical cord blood

Analyses of blood lipids show that the concentrations of TC, LDLC, and NEFA in cord blood of macrosomia were significantly lower than that of normal control ($P < 0.05$). No significant difference was found with regard to TG and HDLC levels in cord blood between the macrosomia and the control groups (Table 2).

Table 2
Comparison of cord blood lipid concentration between newborns in two groups

Umbilical cord blood	Macrosomia (*n* = 38)	Control (*n* = 39)	*P*
TG (mmol/L)	0.34(0.25,0.43)	0.37(0.32,0.53)	0.079[a]
TC (mmol/L)	1.45(1.18,1.70)	1.61(1.41,1.92)	0.013[a]
HDLC (mmol/L)	0.80 ± 0.18	0.88 ± 0.21	0.080[b]
LDLC (mmol/L)	0.49(0.38,0.65)	0.57(0.47,0.69)	0.027[a]
NEFA (μmol/L)	266.73 ± 142.78	366.15 ± 183.31	0.021 [b]

Data are expressed as mean ± SD or median (interquartile range). [a] Mann-Whitney *U* test; [b] Two-sample *t*-test. Key: (TG) triglycerides, (TC) total cholesterol, (HDLC) high-density lipoprotein cholesterol, (LDLC) low-density lipoprotein cholesterol, and (NEFA) non-esterified fatty acids.

3.3 Expression levels of placental lipid transport-related factors

The mRNA expression levels of *PPARα, PPARγ*, *FABP3, FABP4*, *FABPpm, FAT/CD36*, and *LXRα* were significantly higher in macrosomia placenta than in control placenta (Fig. 1). No statistical differences were observed in mRNA expression levels of other genes among the study groups.

Further analysis of protein expression levels shows that the protein expression levels of PPARα, PPARγ, FAT/CD36, and FABPpm in placenta were significantly higher in the macrosomia group than in the control group. In contrast, the protein expression levels of FABP4, FATP1, FATP4, and LXRα were not significantly different between the groups (Fig. 2).

3.4 Correlation between cord blood lipid concentration and placental mRNA expression levels

Correlation analysis was used to explore the relationship between the transcription levels of placental lipid transport-related factors and cord blood lipid concentration. We only found a weak positive correlation between the expression of *PPARγ, FABP4 and FABP3* mRNA in placenta and the HDLC(r_s = 0.439; P = 0.005),NEFA (r_s = 0.342; P = 0.041) and TG (r_s = 0.349; P = 0.034) (Fig. 3) levels in cord blood in macrosomia group respectively, but not in the control group. And no correlation was found between other cord blood lipid concentrations and detected placental lipid transport-related factors (data not shown).

3.5 Association between lipid transport-related factors and macrosomia

Logistic regression models were used to examine whether the relative expression levels of placental lipid transport-related factors or cord blood lipid concentration were associated with the likelihood of macrosomia. As presented in Tables 3 and 4, high placental *PPARα* and *FAT/CD36* mRNA expression levels increased the likelihood of macrosomia after adjusting for potential confounders (maternal age, infant gender, and gestational weight gain). In contrast, high cord blood LDLC concentration decreased the likelihood of macrosomia. No association was found with regard to expression levels of other placental lipid transport-related factors and cord blood lipid concentrations.

Table 3
Logistic regression models of association between placental lipid transporters mRNA expression levels and macrosomia.

Parameter	*n* (%)	*OR* (95%*CI*)	*P*	*AOR* (95%*CI*)	*P*
Model A					
Maternal age (yr)[a]					
Low (< 28.5)	39(50.6)	1		1	
High (≥ 28.5)	38(49.4)	2.453(0.982–6.132)	0.055	2.308(0.824–6.464)	0.111
Infant gender					
Female	28(36.4)	1		1	
Male	49(63.6)	**3.947(1.450-10.743)**	**0.007**	4.179(1.383–12.628)	**0.011**
Gestational weight gain[b]			0.122		0.053
Low	17(22.1)	1.145(0.337–3.891)	0.828	1.205(0.314–4.627)	0.786
Moderate	29(37.7)	1		1	
High	31(40.2)	2.826(0.984–8.121)	0.054	4.016(1.233–13.083)	**0.021**
Model A + *FAT/CD36* mRNA[a]					
Low	38(49.4)	1		1	
High	39(50.4)	**3.434(1.346–8.761)**	**0.010**	**2.989(1.029–8.679)**	**0.044**
Model A + *PPARα* mRNA[a]					
Low	38(49.4)	1		**1**	
High	39(50.4)	**3.077(1.214-7.800)**	**0.018**	**3.022(1.032–8.853)**	**0.044**

[a] Maternal age and placental lipid transporters mRNA expression level were divided by the median.

[b] Maternal weight gain during pregnancy was divided according to Institute of Medicine recommendations (2009) (27). We used the ranges as the basis for the following weight-gain categories: Low (less than the lower cutoff of recommendations), Moderate (within recommended range), or High (greater than the upper cut-off of recommendations).

Key: (*OR*) odds ratio, the results of univariate analysis; (*AOR*) adjusted odds ratio, the results of adjusted for maternal age, infant gender, and gestational weight gain.

Parameter	*n* (%)	*OR* (95%*CI*)	*P*	*AOR* (95%*CI*)	*P*
Model A + *PPARγ* mRNA[a]					
Low	38(49.4)	1		1	
High	39(50.4)	1.984(0.796–4.944)	0.141	1.998(0.709–5.630)	0.190
Model A + *FABPPpm* mRNA[a]					
Low	38(49.4)	1		1	
High	39(50.4)	**3.550(1.371–9.191)**	**0.009**	2.728(0.957–7.771)	0.060

[a] Maternal age and placental lipid transporters mRNA expression level were divided by the median.

[b] Maternal weight gain during pregnancy was divided according to Institute of Medicine recommendations (2009) (27). We used the ranges as the basis for the following weight-gain categories: Low (less than the lower cutoff of recommendations), Moderate (within recommended range), or High (greater than the upper cut-off of recommendations).

Key: (*OR*) odds ratio, the results of univariate analysis; (*AOR*) adjusted odds ratio, the results of adjusted for maternal age, infant gender, and gestational weight gain.

Table 4
Logistic regression models of association between cord blood lipid concentrations and macrosomia.

Effect	***n* (%)**	***OR* (95%*CI*)**	***P***	***AOR* (95%*CI*)**	***P***
Model B					
Maternal age (yr)[a]					
Low (< 28.5)	39(50.6)	1		1	
High (≥ 28.5)	38(49.4)	2.453(0.982–6.132)	0.055	2.308(0.824–6.464)	0.111
Infant gender					
Female	28(36.4)	1		1	
Male	49(63.6)	**3.947(1.450-10.743)**	**0.007**	**4.179(1.383–12.628)**	**0.011**
Gestational weight gain[b]			0.122		0.053
Low	17(22.1)	1.145(0.337–3.891)	0.828	1.205(0.314–4.627)	0.786
Moderate	29(37.7)	1		1	
High	31(40.2)	2.826(0.984–8.121)	0.054	**4.016(1.233–13.083)**	**0.021**
Model B + LDLC[a]					
Low	38(49.4)	1		1	
High	39(50.4)	**0.331(0.130–0.840)**	**0.020**	**0.246(0.080–0.759)**	**0.015**
Model B + TG[a]					
Low	38(49.4)	1		1	
High	39(50.4)	0.707(0.290–1.727)	0.447	0.608(0.216–1.707)	0.344

[a] Maternal age and cord blood lipid concentrations were divided by the median.

[b] Maternal weight gain during pregnancy was divided according to Institute of Medicine recommendations (2009) (27). We used the ranges as the basis for the weight gain categories: Low (less than the lower cut-off of recommendations), Moderate (within recommended range), or High (greater than the upper cut-off of recommendations).

Key: (*OR*) odds ratio, the results of univariate analysis; (*AOR*) adjusted odds ratio, the results adjusted for maternal age, infant gender, and gestational weight gain.

Effect	*n* (%)	*OR* (95%*CI*)	*P*	*AOR* (95%*CI*)	*P*
Model B + HDLC[a]					
Low	38(49.4)	1		1	
High	39(50.4)	0.611(0.247–1.508)	0.611	0.976(0.340–2.803)	0.964
Model B + TC[a]					
Low	38(49.4)	1		1	
High	39(50.4)	0.592(0.239–1.467)	0.258	0.673(0.229–1.980)	0.472
Model B + NEFA[a]					
Low	38(49.4)	1		1	
High	39(50.4)	0.627(0.255–1.543)	0.310	0.531(0.183–1.537)	0.243

[a] Maternal age and cord blood lipid concentrations were divided by the median.

[b] Maternal weight gain during pregnancy was divided according to Institute of Medicine recommendations (2009) (27). We used the ranges as the basis for the weight gain categories: Low (less than the lower cut-off of recommendations), Moderate (within recommended range), or High (greater than the upper cut-off of recommendations).

Key: (*OR*) odds ratio, the results of univariate analysis; (*AOR*) adjusted odds ratio, the results adjusted for maternal age, infant gender, and gestational weight gain.

4. Discussion

In this study, we found that the mRNA expression levels of *PPARα*, *PPARγ*, *FABP3*, *FABP4*, *FAT/CD36*, *FABPpm* and *LXRα* were significantly increased in macrosomia placenta.

The lipid-transport related genes are selectively activated in macrosomia placenta, and the lipid transport function may be enhanced, which suggests that altered placental lipid transport may contribute to fetal overgrowth in healthy women without GDM. It has been reported that the expression of these genes is also increased in the placenta of GDM women (28) and the changes of placental lipid transport may be related to fetal overgrowth in obese women with GDM[29]. And a previous study found that the expression of *FATP6* and *FAT/CD36* is increased in placental microvillous plasma membrane of individuals with intrauterine growth restriction (IUGR)[30]. Animal experiments have shown that overexpression of FABPpm in mammals can increase the fatty acid rate of transportation and oxidation [31, 32]. These results are consistent with our study, indicating that changes in the expression of these genes in the placenta affect lipid maternal-fetal transport and fetal growth. With higher mRNA and protein expression levels in placenta, macrosomia have a relatively higher ability to take up and transport the

maternal fatty acids from placenta to the fetus. As a consequence, it is easy to get more body fat and have a higher birth weight when a large amount of fatty acids are deposited as fat in the fetus.

Fetal growth and development are closely related to lipid concentrations in umbilical cord blood, which can reflect the fetal status of lipid metabolism [10]. Our study showed that macrosomia had lower levels of TC, LDLC, and NEFA in cord blood. In addition,our previous non-targeted metabolomics study found a decrease of arachidonic acid content in macrosomia cord blood,which is consistent with this study to some extent[33]. However, a study by Kumar et al. [34] showing that TG level in cord blood is higher in newborns with low birth weights. Aletayeb et al. [35] found that TG, TC, LDLC, and very-low-density lipoproteins cholesterol (VLDLC) levels are higher in neonates with low or high birth weights. Taken together, all these findings further confirm that lipid metabolism is altered in newborns with abnormal birth weights.

The positive association between the expression levels of placental lipid transport-related genes(*PPARγ, FABP4 and FABP3*) and cord blood lipid levels(HDLC, NEFA and TG) only in macrosomia fetuses may possibly indicate a effect of placental lipid transport-related genes enhancing fat deposition in fetal macrosomia. Theoretically, the increased expression of placental lipid transporters will enhance the transport ability, increase the lipid transport to the fetus, and correspondingly increase the lipid concentration of the umbilical vein blood. However,because our cord blood sample is a mixture of umbilical artery blood and venous blood. Since macrosomia fetuses have more fat deposits than normal-birth-weight fetuses, the demand and utilization for fatty acids is also greater, which will significantly reduce the lipid concentration in umbilical artery blood. Although the umbilical venous blood lipids may be higher in macrosomia group, the umbilical artery blood lipid levels may be lower, and a higher lipids level in venous blood can be diluted by an arterial blood with lower lipid level in a mixed sample[36]. And this is why our research results show that the lipids level of cord blood was decreased in the macrosomia group.

If possible, follow-up studies could detect blood lipid levels in both umbilical artery and vein blood to further verify our hypothesis. In addition, fat deposition in macrosomia may be related to the leptin concentration of cord blood [37]. Our previous study found that the leptin concentration in non-GDM macrosomia cord blood is elevated beyond the normal level[38]. And it is known that high concentrations of leptin can enhance the decomposition of TG, thereby regulating fetal growth[39]. Even with these varied potential relationships and study results, the reason for the decrease of cord blood lipid level in macrosomia is still unclear, and further mechanism research needs to be initiated.

As shown by logistic regression analysis in our study, the placental higher expression levels of *PPARα* and *FAT/CD36* significantly increased the risk of macrosomia after adjusting for related confounders (including infant gender, gestational age, and gestational weight gain), whereas higher LDLC level in cord blood significantly decreased the risk of macrosomia. This finding strongly suggests that placental *PPARα* and *FAT/CD36* may play an important role in the development of non-GDM macrosomia As other studies have shown, placental *PPARA* can regulate placental fatty acid metabolism by accelerating

uptake, transport, and oxidizing long-chain polyunsaturated fatty acids [40–42]. Previous studies also showed that *FAT/CD36* can enhance fatty acid uptake [43, 44], and its expression is closely related to adipose differentiation and insulin resistance[45, 46]. Therefore, we speculate that *PPARα* and *FAT/CD36* are key regulators during fat deposition in macrosomia. We have, however, failed to determine the causality of the elevated *PPARα* and *FAT/CD36* expression and macrosomia in our observational study. In contrast, a previous study showed that the LDLC concentrations in cord blood of LGA newborns born to GDM mothers are significantly higher than that of AGA (Appropriate for Gestational Age) newborns[47]. Although these results are different from our results, both suggest that LDLC concentrations in cord blood might reflect an abnormal status of fetal growth. Further studies are still needed to validate these results and identify the mechanism involved.

The limitation of this work is that it was an observational study and therefore causality of associations could not be assessed. What's more, the small sample size also results in relatively large confidence intervals. It will be very helpful to understand the relationship between placental lipid transporters and fetal umbilical cord blood lipids if the maternal blood lipid level can be detected,and this is exactly what our research team is doing now. Further studies should explore the specific mechanism of fatty acid transportation across the placenta by establishing experimental animal model to understand the function of these fatty acid transport-related proteins better.

5. Conclusion

Our results indicate that the occurrence of macrosomia in healthy pregnancy may be partially attributed to the elevated expression of placental lipid transport-related genes. The mothers would modify the expression of key genes on the placenta to affect placental lipid transport. Changes in the expression of placental lipid transport-related genes affect the function of placental lipid transport, thereby affecting fetal growth and the occurrence of macrosomia. Further research is warranted to explore precisely how placental lipid transporters affect the cord blood lipid concentration and how cord blood lipids reflect fetal growth.

List Of Abbreviations

TC, total cholesterol;

TG, triglycerides;

HDLC, high-density lipoprotein cholesterol ;

LDLC, low-density lipoprotein cholesterol;

NEFA, non-esterified fatty acid;

PPARs, peroxisome proliferator-activated receptors.

GDM, gestational diabetes mellitus

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from all participating women. The study protocol was approved by the ethics committee of Wenzhou Medical University.

Consent for publication

All authors consent to publish the present results

Availability of data and materials

The primary data for this study is available from the authors on direct

request.

Competing interests

The author declare that they have no competing interests.

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Authors' contributions:

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Miao-Miao Ding: Data collection.

Li-Fang Ni: Data analysis.

Tian Zheng: Data analysis.

Yu-Huan Wang : Data collection.

Hong-Tao Yan: Data analysis, Manuscript revising.

Xin-Jun Yang: Project development (conception and design), Manuscript revising.

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Figures

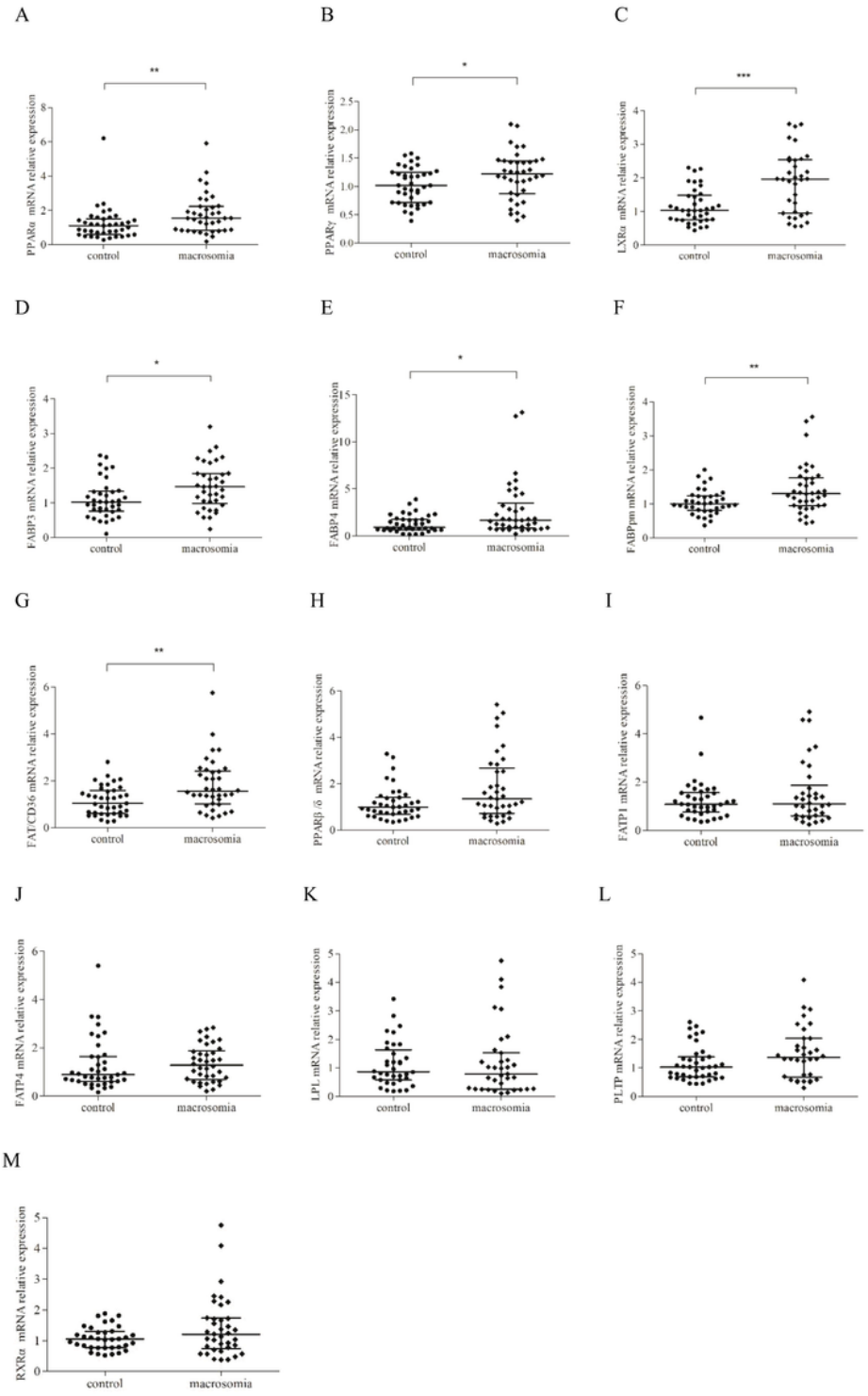

Figure 1

mRNA expression levels of placental lipid transport-related factors The placental mRNA expression levels of lipid transport-related factors in macrosomia group (n = 38) and control group (n = 39). The mRNA expression levels of (A) PPARα, (B) PPARγ, (C) LXRα, (D) FABP3, (E) FABP4, (F) FABPpm, and (G) FAT/CD36, are higher in macrosomia group (*P < 0.05, **P < 0.01). Each dot represents a single sample

(A-M), and the data are expressed according to the normality of the data as the mean ± standard deviation (SD) for (B) or as the median (interquartile range) for A and C-M.

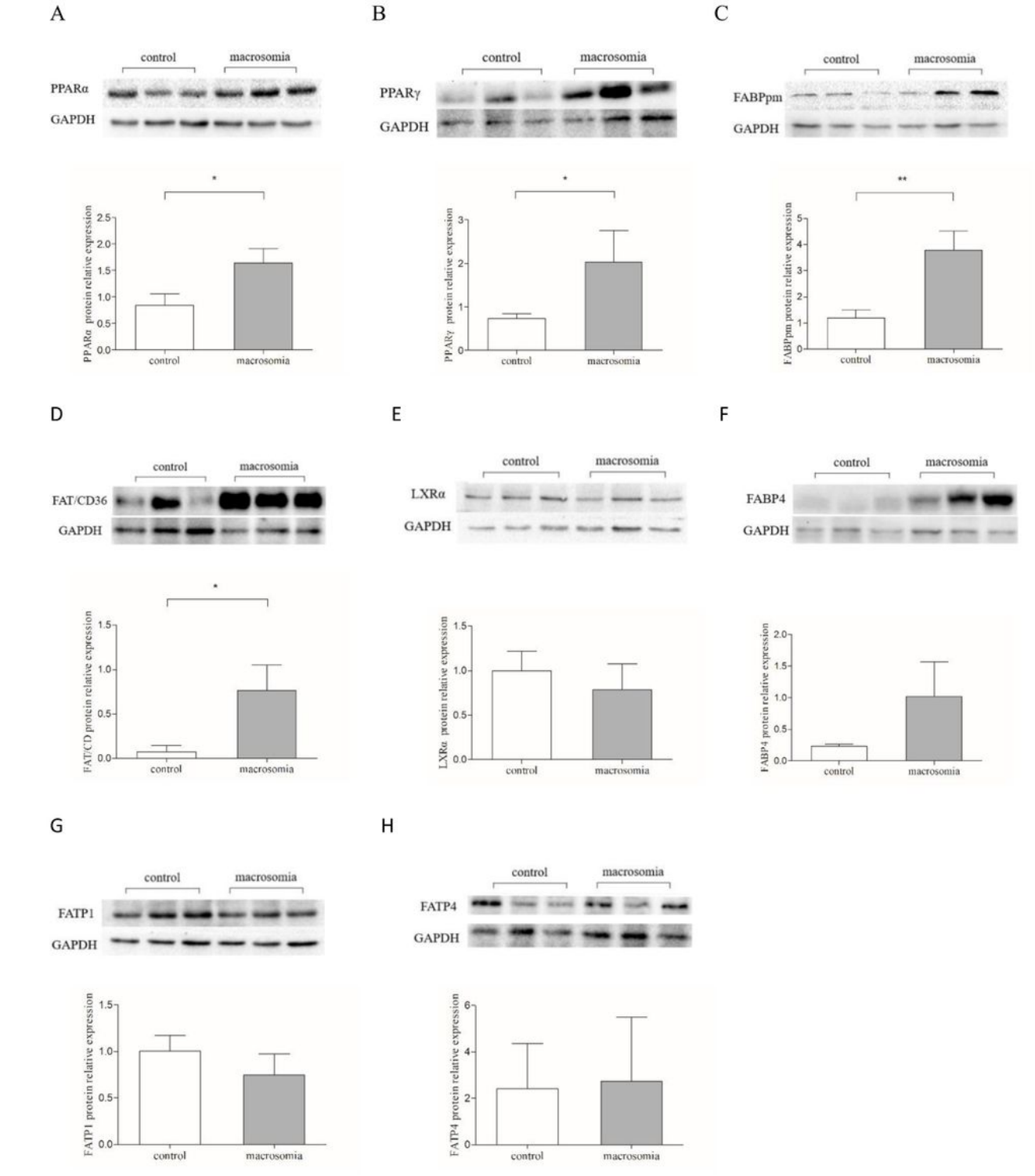

Figure 2

Protein expression levels of placental lipid transport-related factors The placental protein expression levels of (A) PPARα, (B) PPARγ, (C) FABPPpm, and (D) FAT/CD36 are significantly higher in the macrosomia group (n = 3) than in the control group (n = 3). The expression levels were normalized to the

expression of the endogenous reference gene GAPDH. The statistical differences were calculated using the two-sample t-test (*P < 0.05, **P < 0.01).

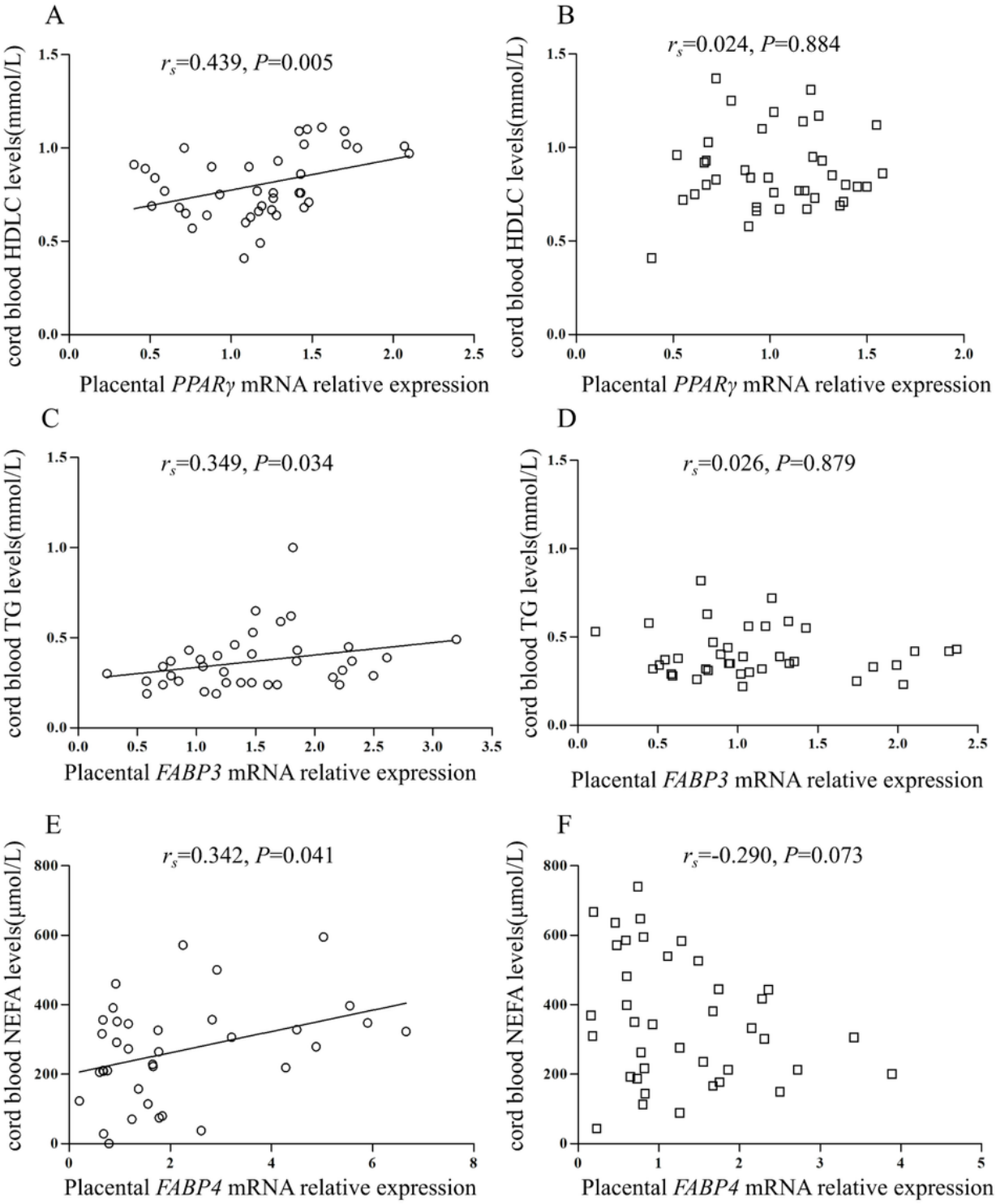

Figure 3

Correlation between cord blood lipid concentration and placental mRNA expression levels A, B Correlation between umbilical cord blood HDLC concentration and placental PPARγ mRNA expression in macrosomia and control groups. C, D Correlation between umbilical cord blood TG concentration and placental FABP3

mRNA expression in control and macrosomia groups. E, F Correlation between umbilical cord blood NEFA concentration and placental FABP4 mRNA expression in control and macrosomia groups. Key: (●) macrosomia group, (□) control group, (rs) Spearman's correlation coefficient.

Supplementary Files

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- SupplementaryTableS1.pdf