ORIGINAL ARTICLE

Downregulation of peroxisome proliferator-activated receptor gamma in the placenta correlates to hyperglycemia in offspring at young adulthood after exposure to gestational diabetes mellitus

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ABSTRACT

Aims/Introduction: Children who are exposed to gestational diabetes mellitus (GDM) *in utero* are at high risk of developing related illnesses, such as type 2 diabetes mellitus in young adulthood, but the underlying mechanism and related predictive biomarkers are not known.

Materials and Methods: The present study identified the related biomarkers of hyperglycemia in young adults from the relationship between fetal blood glucose and placental lipid transporters at messenger ribonucleic acid (mRNA) and protein expression levels. We recruited patients from a prospective cohort, and determined the mRNA and protein levels of placental fatty acid transporters. Diet-induced mouse models of GDM were established, and the mRNA and protein levels of the same transporters in placentas were validated.

Results: Only the mRNA levels of peroxisome proliferator-activated receptor gamma correlated with the levels of neonatal blood glucose in GDM patients using linear regression and Spearman's correlation analyses ($r = 0.774$, $P = 0.001$). The mRNA levels of peroxisome proliferator-activated receptor gamma, matrix metalloproteinase-2 and fatty acid transport protein-6 correlated with blood glucose levels in mouse offspring ($r = 0.82$, $P = 0.001$, $r = 0.737$, $P = 0.006$ and $r = -0.891$, $P = 0.001$, respectively) at young adulthood using the same analyses. Notably, we observed significantly higher blood glucose levels in GDM offspring at 12 weeks-of-age compared with the control and rosiglitazone-supplemented groups ($P < 0.05$).

Conclusions: The downregulation of peroxisome proliferator-activated receptor gamma in the placenta might predict hyperglycemia in offspring at young adulthood.

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy[1]. Children exposed to maternal GDM show an increased risk of obesity, metabolic syndrome and type 2 diabetes mellitus[2–4].

Transfer of fatty acids from the maternal circulation across the placenta to the fetal circulation is essential for proper fetal development, particularly during the latter stage of gestation[5]. The central role of the nuclear receptor, peroxisome proliferator-activated receptor-γ (PPARγ), in placental development and function is well established[5,6]. Previous studies showed that the activation of PPARγ in human placentas up- or downregulated the expression of proteins involved in fatty acid transport[5,7], including fatty acid transport protein 1-6 (FATP 1-6), which

could contribute to the developmental programming of disease susceptibility in offspring. This process might be associated with GDM lipid disorders and an inflammatory internal environment that interferes with the function of PPARγ. Activated matrix metalloproteinases (MMPs), which are zinc-dependent endopeptidases, degrade numerous extracellular matrix components. Soluble MMP2 and MMP9 are major modulators of membrane integrity, and these enzymes are responsible for membrane rupture, which can affect the transport of fatty acids[8,9].

The present study examined mRNA and protein expression levels of fatty acid transport-related factors in human placentas collected from a prospective cohort, and analyzed the correlation between mRNA expression levels and glucose levels in neonates. We validated the clinical results in a diet-induced GDM animal model, and measured blood glucose in mouse offspring at young adulthood (aged 12 weeks). We also carried out a correlation analysis between the mRNA expression levels of these factors and glucose levels, and found that the downregulation of PPAR was associated with blood glucose levels in offspring during young adulthood.

METHODS
Study population and samples
A prospective cohort was established from a population of pregnant women attending their first routine visit at an obstetric outpatient department between January 2014 and December 2016. Pregnant women who were at 24–28 weeks-of-gestation underwent blood glucose screening according to the clinical criteria of gestational diabetes mellitus after providing written informed consent. Women with fasting plasma glucose level >5.1 mmol/L or >8.5 mmol/L 2 h after an oral glucose load (75 g) were diagnosed with GDM[10,11]. All pregnancies were singleton, and women showed normal blood pressure and were without drug use, intrauterine infection or any other medical or obstetrical complication. These patients were only treated with diet (1,500 kcal and maximum 200 g of carbohydrates per day). The central area of the placentas was dissected immediately after surgery from all patients according to protocols approved by the ethics committees of Anhui Medical University (No. 20131188). The protocol for this research project conformed to the provisions of the Declaration of Helsinki.

Samples were transported to a laboratory and divided into two parts. One part of the specimen was stored in RNAlater (Life Technologies, Carlsbad, CA, USA) for quantitative reverse transcription polymerase chain reaction (PCR) analysis, and the remaining portion was snap-frozen in liquid nitrogen and stored in a −80°C freezer. Important clinical data, such as hematological examination, were obtained from each participant, and Table 1 provides a summary. All placentas selected in the present study were randomly obtained from a normal or GDM group, and samples were treated in the same manner.

To determine the mRNA and protein expression levels of PPARγ, MMP-2/-9, FAT, plasma membrane fatty acid binding protein (FABPpm) and FATP-1 to FATP-6 from placental tissues of GDM patients and the normal controls, we selected 15 GDM patients and 16 normal controls randomly from the prospective cohort with the same criteria as the others recruited in the cohort, and the clinical characteristics of 31 patients and their newborns are presented in Table S1.

Diet-induced GDM model
All procedures on animals followed the guidelines for humane treatment of the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences in Anhui Medical University.

Female C57BL/6J mice (aged 6 weeks) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China) as a joint venture with Charles River Laboratories in China. The animals were provided free access to food and water at all times, and were maintained on a 12-h light/dark cycle in a controlled temperature (20–25°C) and humidity (50 ± 5%) environment.

Female mice were randomly divided into two groups: the control ($n = 12$) and high-saturated fat diet ($n = 24$) groups. The control diet was Global Diet 2018 (18% protein and 5% fat; m/m), and the high-saturated fat diet was 18.9% protein, 32.6% total fat and 10.5% saturated fat (m/m; Table 2). The isocaloric diets for the groups were also considered during the experiments. Breeding took place overnight in a 1:2 ratio after 1 month of dietary intervention. Mating was confirmed by the presence of a vaginal mucous plug the following morning, which represented gestation day (GD)0. Mice in the high-saturated fat diet group were randomly divided into two groups on GD0: GDM group and rosiglitazone (RSG)-supplemented group.

Maternal and offspring bodyweight and blood glucose
Bodyweight was recorded using a top-loading balance. Mice were fasted for 6 h before testing. A blood sample was collected from tail venipuncture, and the blood glucose concentration was measured using a glucometer (Accu-Chek Performa; Roche Diagnostics, Mannheim, Germany). Mice were fasted for 6 h before testing. Dams were killed on GD18 by an intraperitoneal injection of ketamine (75 mg/kg bodyweight), and maternal blood was collected by cardiac puncture. Placentas were collected immediately.

Real-time PCR
Fresh placenta samples (100 mg) were homogenized, and total RNA was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA concentration and purity were examined using a spectrophotometer (NanoDrop One; Thermo Scientific, Carlsbad, CA, USA). RNA samples that showed 260/280 and 260/230-nm absorbance ratios >1.9 were used. The integrity of total RNA was measured using 0.8% agarose gel electrophoresis. High integrity of RNA with a 28s : 18s ratio of 2.0 was included for the next primer efficacy tests, and >90% primer efficiency was

Table 1 | Clinical characteristics of patients and newborns

	Normal (n =378)	GDM (n = 177)	P-value
Maternal variables			
Age (years)	26.98 ± 4.02	27.98 ± 8.21	0.053
Height (cm)	161.03 ± 4.68	160.73 ± 4.60	0.473
Bodyweight (kg)	52.44 ± 7.23	53.33 ± 7.04	0.175
BMI (kg/m^2)	20.20 ± 2.45	20.63 ± 2.53	0.054
Gestational weight gain (kg)	13.49 ± 5.48	12.68 ± 5.35	0.128
Delivery pregnancy age (weeks)	39.73 ± 1.20	39.71 ± 1.27	0.883
Early gestation			
Cigarette smokers			
Every day	1	0	0.787
Quit after gestation	7	2	
Quit ≥3 months before gestation	8	2	
Never	362	173	
Alcohol drinkers			
Once per week	1	1	0.806
1–2 times per month	26	11	
Never	351	165	
Bodyweight (kg)	53.03 ± 8.84	53.11 ± 8.70	0.182
Systolic blood pressure (mm Hg)	101.57 ± 8.94	103.11 ± 10.07	0.071
Diastolic blood pressure (mm Hg)	65.29 ± 7.58	66.62 ± 9.05	0.073
FBG (mmol/L)	5.03 ± 0.42	5.15 ± 0.68	0.011*
Hb (g/L)	121.30 ± 10.73	123.49 ± 8.81	0.02*
WBC (×10^9/L)	7.57 ± 1.87	7.87 ± 2.02	0.099
Blood platelet (×10^9/L)	164.69 ± 43.94	159.53 ± 42.67	0.216
TG (mmol/L)	1.31 ± 0.50	1.53 ± 0.68	<0.001*
TC (mmol/L)	3.90 ± 0.75	4.06 ± 0.93	0.044*
HDL (mmol/L)	1.52 ± 0.37	1.62 ± 0.42	0.005*
LDL (mmol/L)	2.10 ± 0.44	2.21 ± 0.48	0.013*
Midgestation			
Bodyweight (kg)	60.48 ± 9.02	61.56 ± 7.80	0.214
Systolic blood pressure (mm Hg)	104.59 ± 10.47	105.64 ± 8.29	0.873
Diastolic blood pressure (mm Hg)	66.17 ± 6.74	66.79 ± 6.89	0.604
FBG (mmol/L)	4.55 ± 0.27	5.17 ± 0.46	<0.001*
OGTT 1-h (mmol/L)	7.23 ± 1.40	9.83 ± 1.67	<0.001*
OGTT 2-h (mmol/L)	6.00 ± 1.01	7.54 ± 1.39	<0.001*
HbA1c (mmol/mol)	5.78 ± 0.48	5.72 ± 0.43	0.203
TG (mmol/L)	2.48 ± 0.87	2.56 ± 1.19	0.44
TC (mmol/L)	6.26 ± 0.94	5.75 ± 1.27	<0.001*
HDL (mmol/L)	1.87 ± 0.38	1.61 ± 0.49	<0.001*
LDL (mmol/L)	3.21 ± 0.84	2.84 ± 0.96	<0.001*
Insulin (mU/L)	10.10 ± 4.55	11.95 ± 6.31	0.002*
Late gestation			
Bodyweight (kg)	65.80 ± 9.21	66.50 ± 8.56	0.425
Systolic blood pressure (mm Hg)	108.02 ± 11.93	108.14 ± 8.47	0.975
Diastolic blood pressure (mm Hg)	69.99 ± 7.71	69.89 ± 7.70	0.687
FBG (mmol/L)	4.59 ± 0.37	4.96 ± 0.54	<0.001*
Hb (g/L)	110.87 ± 14.01	112.50 ± 10.32	0.188
WBC (×10^9/L)	9.73 ± 2.11	9.49 ± 2.18	0.249
Blood platelet (×10^9/L)	156.60 ± 45.98	149.12 ± 45.19	0.088
TG (mmol/L)	3.26 ± 0.94	3.85 ± 1.57	<0.001*
TC (mmol/L)	6.43 ± 1.33	6.38 ± 1.53	0.728
HDL (mmol/L)	1.96 ± 0.41	1.92 ± 0.45	0.313
LDL (mmol/L)	3.49 ± 0.66	3.40 ± 0.88	0.254
Insulin (mU/L)	9.77 ± 4.89	9.89 ± 5.36	0.852

Table 1 (*Continued*)

	Normal (*n* =378)	GDM (*n* = 177)	*P*-value
Newborn variables			
Length of placenta (cm)	20.30 ± 2.37	20.52 ± 2.92	0.472
Width of placenta (cm)	18.89 ± 2.25	18.99 ± 2.23	0.679
Thickness of placenta (cm)	2.06 ± 0.34	2.16 ± 0.42	0.027*
Weight of placenta (g)	556.05 ± 93.15	573.43 ± 92.22	0.153
Fetal weight (kg)	3.37 ± 0.43	3.46 ± 0.47	0.037*
Fetal sex (male/female)	177/201	85/92	0.792
Blood glucose (mmol/L)	4.65 ± 0.81	4.09 ± 0.84	0.005*

*$P < 0.05$. BMI, body mass index; FBG, fasting blood glucose; GDM, gestational diabetes mellitus; Hb, hemoglobin; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test; TC, total cholesterol; TG, triglyceride; WBC, white blood cells.

Table 2 | Composition of the high-saturated fat diet

High-saturated fat diet	g%	kcal%
Protein	18.9	15.9
Fat	32.6	61.8
Carbohydrate	26.3	22.3
Fat composition		%
Saturated		32.1
Monounsaturated		43
Polyunsaturated		16.9

acceptable for reverse transcription and quantitative PCR (Q-PCR). Reverse transcription was carried out using the ImProm-II RTSystem (Promega, Madison, WI, USA) according to the manufacturer's protocol. Q-PCRs were carried out in a Light-Cycler 96 (Roche Diagnostics) using SYBR Green Mix (Roche) following the manufacturer's instructions. PCR was carried out using complementary deoxyribonucleic acid samples (5 ng) in triplicate under the following conditions: 95°C for 5 min, followed by 35 cycles of 95°C for 15 s, 60°C for 15 s and 72°C for 15 s. The primers were designed following criteria for real-time PCR and synthesized by Sangon Biotech (Shanghai, China). Table 3 lists the primer sequences.

Western blot analysis
Total lysates from placentas were prepared by homogenization of 50 mg of placental tissue in ice-cold 300-μL T-PER (Tissue Protein Extraction Reagent; Thermo Scientific) and Halt Protease & Phosphatase Inhibitor Single-use Cocktail (Thermo Scientific; 99:1, v/v). Total lysates from placentas were suspended in hypotonic buffer and incubated on ice for 15 min for nuclear protein extraction. The suspension was mixed with detergent and centrifuged for 30 s at 14,000 *g*. The nuclear pellet obtained was resuspended in complete lysis buffer in the presence of the protease and phosphatase inhibitor cocktail, incubated for 30 min on ice, and centrifuged for 10 min at 14,000 *g*. Protein concentrations were determined using bicinchoninic acid protein assay reagents (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. The same amount of protein (40–80 μg) was separated electrophoretically using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to a polyvinylidene fluoride membrane. The membranes were incubated with the following antibodies for 2 h: PPARγ, FATP-1/-6, β-actin and α-tubulin (all purchased from Abcam, Cambridge, UK). Membranes were washed four times for 10 min in Dulbecco's phosphate-buffered saline containing 0.05% Tween-20, and incubated with goat anti-rabbit immunoglobulin G or a goat anti-mouse antibody for 2 h. Membranes were washed four times in Dulbecco's phosphate-buffered saline containing 0.05% Tween-20 for 10 min, and signals were developed using chemiluminescent detection with a Thermo Scientific SuperSignal West Pico PLUS Substrate.

Statistical analysis
Data are presented as the mean ± standard error of the mean or frequency. Independent sample *t*-test or one-way analysis of variance (ANOVA) followed by the least significant difference test were used for comparisons between groups. Linear regression analysis and Spearman's correlation were used to assess the relationship between two quantitative variables. Statistical significance was defined as $P < 0.05$. Statistical analyses were carried out using SPSS (version 16.0; IBM, Armonk, NY, USA) and GraphPad Prism Software (version 7.0; San Diego, CA, USA).

RESULTS
Patients
Women were prospectively recruited into the present study and retrospectively stratified into normal pregnancy and GDM. A total of 555 women with normal glucose tolerance (*n* = 378) or GDM (*n* = 177) were included in this study. Women were matched for age, height, bodyweight and BMI (Table 1). Almost

Table 3 | Primer sequence of human and mice fatty acid transport-related genes

Gene	Primers (5′→3′)	GenBank accession	Species
pparγ	Fwd TCGAGGACACCGGAGAGG	NM_001330615.1	Human
	Rev CACGGAGCTGATCCCAAAGT		
mmp2	Fwd GTCTGTGTTGTCCAGAGGCA	NM_001127891.2	Human
	Rev CTAGGCCAGCTGGTTGGTTC		
mmp9	Fwd CCTGGGCAGATTCCAAACCT	NM_004994.2	Human
	Rev GTACACGCGAGTGAAGGTGA		
cd36	Fwd GCAACAAACCACACACTGGG	NM_000072.3	Human
	Rev AGTCCTACACTGCAGTCCTCA		
fabppm	Fwd CGTCCGCAAGTTTGTCACTG	NM_001286220.1	Human
	Rev GGCAGAAAGACATCTCGGCT		
fatp1	Fwd CTCTCTCTGCTTCCCCAGGA	NM_198580.2	Human
	Rev GCACAGAGAGACCGAAGAGG		
fatp2	Fwd TGGAACCACAGGTGCTACTC	NM_001159629.1	Human
	Rev CCCAGTGCCAGTCTCACTTT		
fatp3	Fwd CCCACTCTACCACATGTCCG	NM_001317929.2	Human
	Rev ACAAGGTATCGGCACAGCTC		
fatp4	Fwd ATGGCATGACGGTGGTGATT	NM_005094.3	Human
	Rev TAGCGGCACAGTTCACCAAT		
fatp5	Fwd GGTTGTGAGGGTAAGGTGGG	NM_001321196.1	Human
	Rev CACCAACCGGGTCTTCATCA		
fatp6	Fwd GGAAAGTCCTGGGGAACTCG	NM_001017372.2	Human
	Rev CCCCAAGTGGTGCTGAAGAT		
gapdh	Fwd CGGCTACCACATCCAAGGAA	NM_001256799.2	Human
	Rev GCTGGAATTACCGCGGCT		
pparγ	Fwd GGGCTGAGGAGAAGTCACAC	NM_001127330.2	Mouse
	Rev TCAGTGGTTCACCGCTTCTT		
mmp2	Fwd ACAAGTGGTCCGCGTAAAGT	NM_008610.3	Mouse
	Rev GTAAACAAGGCTTCATGGGGG		
mmp9	Fwd GCCGACTTTTGTGGTCTTCC	NM_013599.4	Mouse
	Rev CTTCTCTCCCATCATCTGGGC		
cd36	Fwd TTCTTCACAGCTGCCTTCTGA	NM_001159555.1	Mouse
	Rev TGGCCCGGTTCTAATTCATGC		
fabppm	Fwd GTGGAAGGAGATAGCGTCCG	NM_010325.2	Mouse
	Rev AGAGGCAGACATTGATGCCC		
fatp1	Fwd GAGCTCAGAACTTCCCAGTCCA	NM_011977.3	Mouse
	Rev TGTGCTGGAGCTTGCCTGAT		
fatp2	Fwd CCTCCTGATGATCGACCGTG	NM_011978.2	Mouse
	Rev AGGCACGCCATACACATTCA		
fatp3	Fwd GTGCTGGCCACAGAGTTCCT	NM_001316688.1	Mouse
	Rev TGGTCTGCTGCTTCCGATAG		
fatp4	Fwd CGCTGGAAAGGGGAGAATGT	NM_011989.4	Mouse
	Rev TCTGTGCAAAGCTCTCCAGG		
fatp5	Fwd TTTCTGGGGTTGGCCAAGTT	NM_009512.2	Mouse
	Rev TGGCCAAGGTAGAAGCAGTG		
fatp6	Fwd ATCGGGTACGTTTGGCAGTT	NM_001081072.1	Mouse
	Rev TCCTTCGGTAGCTCCGTACA		
gapdh	Fwd CCCTTAAGAGGGATGCTGCC	NM_001289726.1	Mouse
	Rev TACGGCCAAATCCGTTCACA		
pparγ	Fwd GGGCTGAGGAGAAGTCACAC	NM_001127330.2	Mouse
	Rev TCAGTGGTTCACCGCTTCTT		

one-third of the GDM patients were diagnosed before 24 weeks-of-gestation (31.89%), which is early GDM. The mean triglyceride, total cholesterol, high-density lipoprotein and low-density lipoprotein were significantly higher at early gestation (11–14 weeks) than the normal group ($P < 0.001$, $P = 0.044$, $P = 0.005$ and $P = 0.013$, respectively). The mean total

cholesterol, high-density lipoprotein and low-density lipoprotein were lower at middle gestation (22–24 weeks; all $P < 0.001$). Fasting blood glucose, oral glucose tolerance test and insulin were significantly higher than the normal group ($P < 0.001$, $P < 0.001$ and $P = 0.002$, respectively). Only triglyceride and fasting blood glucose were significantly higher at late gestation (32–36 weeks) than the control group (both $P < 0.001$).

Human placenta length, width and weight were not significantly different between the two groups at birth, except the placenta was thicker in GDM patients compared with the normal group ($P < 0.05$). However, fetal weight in the GDM group was greater than the normal group, and their blood glucose was noticeably lower (both $P < 0.05$; Table 1).

MRNA and protein expression of human placental fatty acid transporters and their related regulators in human placentas

The mRNA and protein expression levels of PPARγ, MMP-2/-9, fatty acid translocase, FABPpm and FATP-1 to FATP-6 from 31 placental tissues of GDM patients ($n = 15$) and the normal controls ($n = 16$), which were randomly selected from the biosamples bank of the prospective cohort with the same criteria, were analyzed. The mRNA expression of all measured fatty acid transporters was altered significantly in the tissues of GDM patients compared with the normal controls. The mRNA expression of PPARγ and FATP-1 to FATP-6 decreased, and MMP-2/-9 increased noticeably in the tissues of GDM patients compared with the normal controls. The results are presented in Figure 1a. Notably, we found that PPARγ, FATP-1 and FATP-2 at the protein expression levels decreased noticeably in the tissues of GDM patients compared with the normal controls (Figure 1b, Figure S1).

MRNA and protein expression of placental fatty acid transporters and their related regulators in animal models

The mRNA and protein expression levels of PPARγ, MMP-2/-9, FAT, FABPpm and FATP-1 to FATP-6 from placental tissues of GDM mice and normal controls were also analyzed ($n = 12$ per group).

A similar pattern of mRNA expression in mouse placental tissues was observed (Figure 2a). The mRNA expression of PPARγ and FATP-1 to FATP-6 decreased, and MMP-2/-9 increased noticeably compared with the normal mouse placental tissues. RSG intervention altered MMP-2/-9, FABPpm and FATP-2/-4 mRNA expression, but the other placental fatty acid transport regulators were not affected. All of these changes were statistically significant.

PPARγ, FATP-1 and FATP-2 protein levels decreased noticeably in mouse placental tissues compared with the normal controls. RSG treatment recovered the expression of PPARγ, FATP-1 and FATP-2 proteins (Figures 2b and S2).

Maternal and offspring mouse bodyweight and blood glucose

Maternal mouse bodyweight and blood glucose were measured on GD0, 10, 12, 14, 16 and 18 in all groups. Control maternal mouse bodyweight increased moderately throughout pregnancy, from a mean of 19.8 g at GD0 to 32.2 g at GD18. Conversely, GDM dam weight increased significantly compared with controls (all $P < 0.001$ at different time-points). Maternal bodyweight in the GDM + RSG group increased slightly, but significantly, compared with controls (all $P < 0.05$ at different time-points; Figure 3a). Blood glucose levels peaked at a mean of 8.7 mmol/L on GD12 in the GDM group, 5.8 mmol/L on GD12 in the control group and 6.3 mmol/L on GD12 in the GDM + RSG group. The blood glucose levels of the GDM group were significantly higher than the control group at GD0, GD10, GD12, GD14, GD16 and GD18. The blood glucose of GDM + RSG group began to decline at GD12, which was similar to the control group, and these levels were significantly higher than the controls on GD10 and GD12 only (Figure 3b).

Mouse fetal bodyweight, crown–rump length and placental weight were obviously lower in the GDM group compared with the controls ($P < 0.001$, $P < 0.001$ and $P = 0.014$, respectively; Figure 3c,d,e), but there was no statistically significant difference in placental diameter (Figure 3f). Fetal bodyweight and crown–rump length remained lower in the GDM + RSG group compared with the controls ($P < 0.001$ and $P < 0.05$, respectively), but there was no statistically significant difference in placental weight or diameter. Notably, blood glucose levels in the young adult mice that were exposed to GDM (at 12 weeks-of-age) showed a mean blood glucose of 6.50 mmol/L, which was significantly higher than the controls and the GDM + RSG group ($P < 0.05$; Figure 3g).

Correlation of newborn plasma glucose levels and the MRNA expression levels of placental fatty acid transporters and their related regulators

Linear regression and Pearson correlation analyses were used to analyze the relationship between placental fatty acid transport regulator mRNA levels and neonatal blood glucose. Figure 4 shows the results.

There were no significant correlations between the mRNA levels of these regulators and neonatal blood glucose in the normal controls. However, PPARγ levels correlated to neonatal blood glucose levels in the GDM patients ($P = 0.001$). Therefore, PPARγ might be the primary contributor to the subsequent changes in neonatal blood glucose.

Correlation of mouse offspring plasma glucose levels and the MRNA expression levels of placental fatty acid transporters and their related regulators at young adulthood

We carried out the same analyses in the diet-induced GDM mouse model to validate the results of placental fatty acid transport regulators and neonatal blood glucose observed in GDM patients and normal controls. The results are shown in Figure 5.

Notably, no significant correlations between the mRNA levels of these regulators' mRNA levels and mouse offspring blood glucose levels were observed in the normal controls. The same
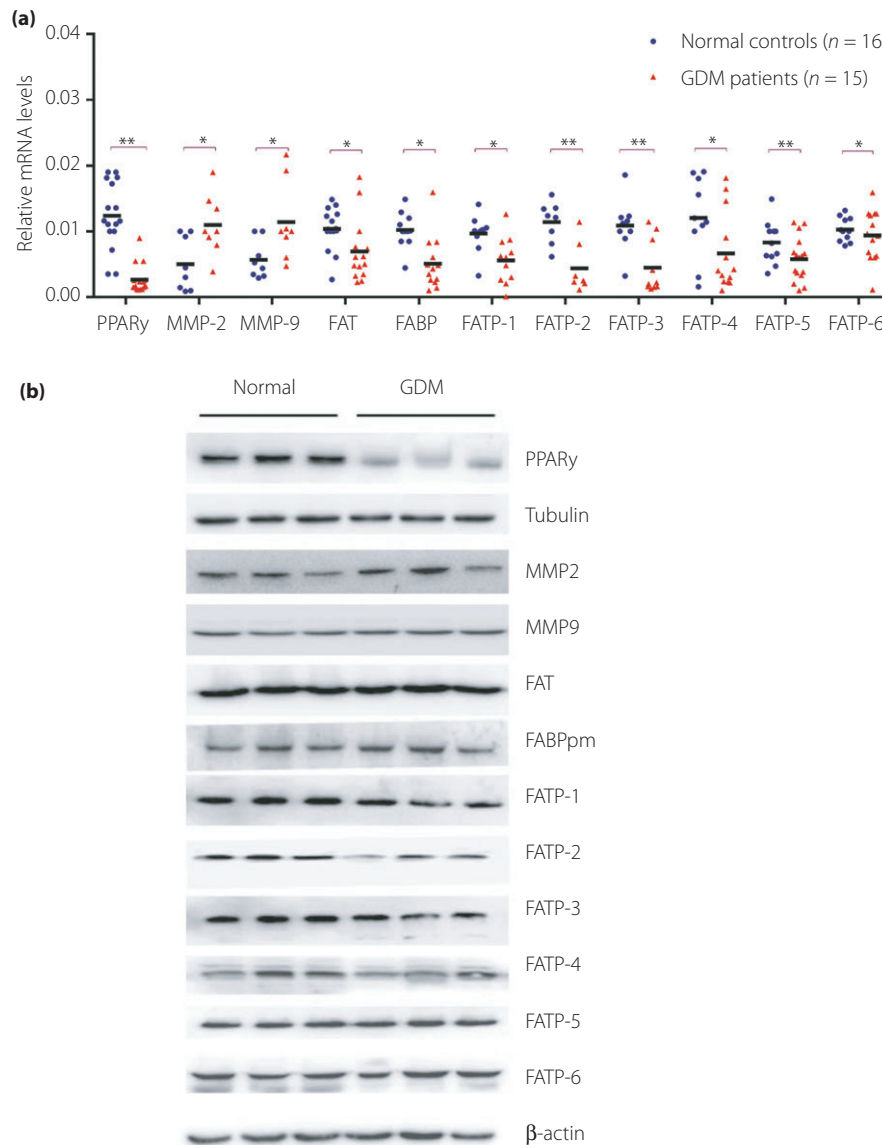
Figure 1 | The messenger ribonucleic acid (mRNA) and protein expression of human placental fatty acid transport regulators. (a) The mRNA expression levels were detected using real-time polymerase chain reaction. (b) The protein expression levels were detected using western blot analysis, and representative images from three independent experiments are shown **$P < 0.01$; *$P < 0.05$. FABPpm, plasma membrane fatty acid binding protein; FAT, fatty acid translocase; FATP, fatty acid transport protein; GDM, gestational diabetes mellitus; MMP, metalloproteinase; PPARγ, peroxisome proliferator-activated receptor-γ.

results were also found in the RSG intervention group. However, the mRNA levels of PPARγ, MMP2 and FATP-6 correlated to blood glucose levels of mouse offspring in the diet-induced GDM mice ($P = 0.001$, $P < 0.004$ and $P < 0.001$, respectively). No changes in the protein expression levels of MMP2 and FATP-6 were observed between groups. Therefore, PPARγ was likely the primary contributor to the subsequent changes in blood glucose levels in mouse offspring.

We analyzed the correlation of plasma glucose levels in mouse offspring and the mRNA expression levels of placental fatty acid transporters and their related regulators at birth, and noted a very similar trend at young adulthood (Figure S3).

DISCUSSION
PPARγ is an isoform of peroxisome proliferator-activated receptor (PPAR) that belongs to a super family of nuclear receptors. PPARγ plays a crucial role in the regulation of lipid and glucose homeostasis. The proper transfer of fatty acids from the maternal circulation across the placenta to the fetal circulation is essential for lipid homeostasis and the control of
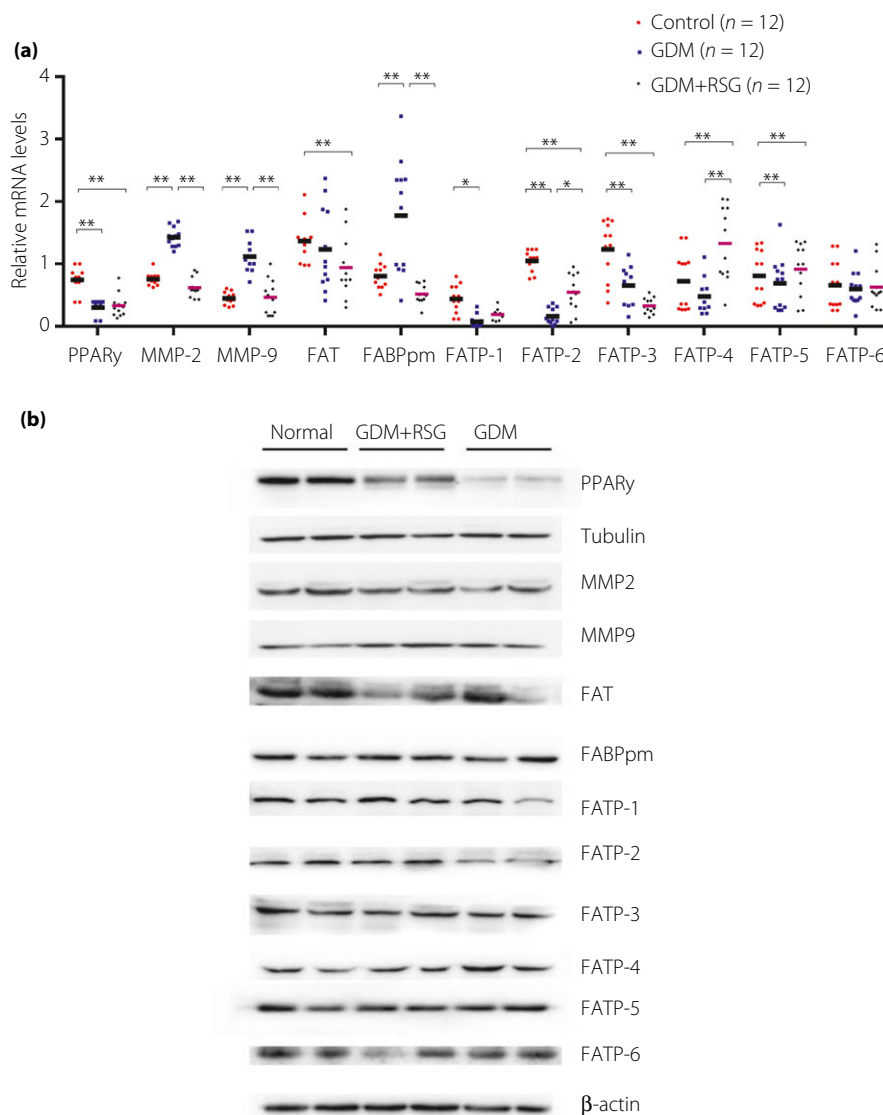
Figure 2 | The messenger ribonucleic acid (mRNA) and protein expression of placental fatty acid transport regulators in the animal model. (a) The mRNA expression levels were detected using real-time polymerase chain reaction. (b) The protein expression levels were detected using Western blot analysis, and representative images from three independent experiments are shown. **$P < 0.01$; *$P < 0.05$. FABPpm, plasma membrane fatty acid binding protein; FAT, fatty acid translocase; FATP, fatty acid transport protein; GDM, gestational diabetes mellitus; MMP, metalloproteinase; PPARγ, peroxisome proliferator-activated receptor-γ; RSG, rosiglitazone.

fetal development, particularly during the latter stage of gestation[5,12]. PPARγ is also a ligand-activated transcription factor that binds to specific deoxyribonucleic acid sequences, known as peroxisome proliferator response elements, in the promoter of target genes only as a heterodimer with RXR. A peroxisome proliferator response element generally consists of an almost perfect direct repeat of the sequence TGACCT spaced by a single base pair, and this sequence was identified primarily in the upstream regulatory sequences of genes related to metabolic pathways. Recent studies showed that PPARγ regulated gene expression independently of peroxisome proliferator response elements. Embryos of PPARγ knockout mice cannot survive. PPARγ agonists, such as RSG, exert profound effects on glucose and lipid metabolism. This class of drugs improved insulin sensitivity in various animal models[13]. RSG reduced whole-body insulin resistance in humans through its insulin-sensitizing effects on the liver, skeletal muscle and adipose tissue[14]. Whether RSG supplementation in GDM mice prevented GDM placental damage and promoted fetal growth and development requires further assessment.
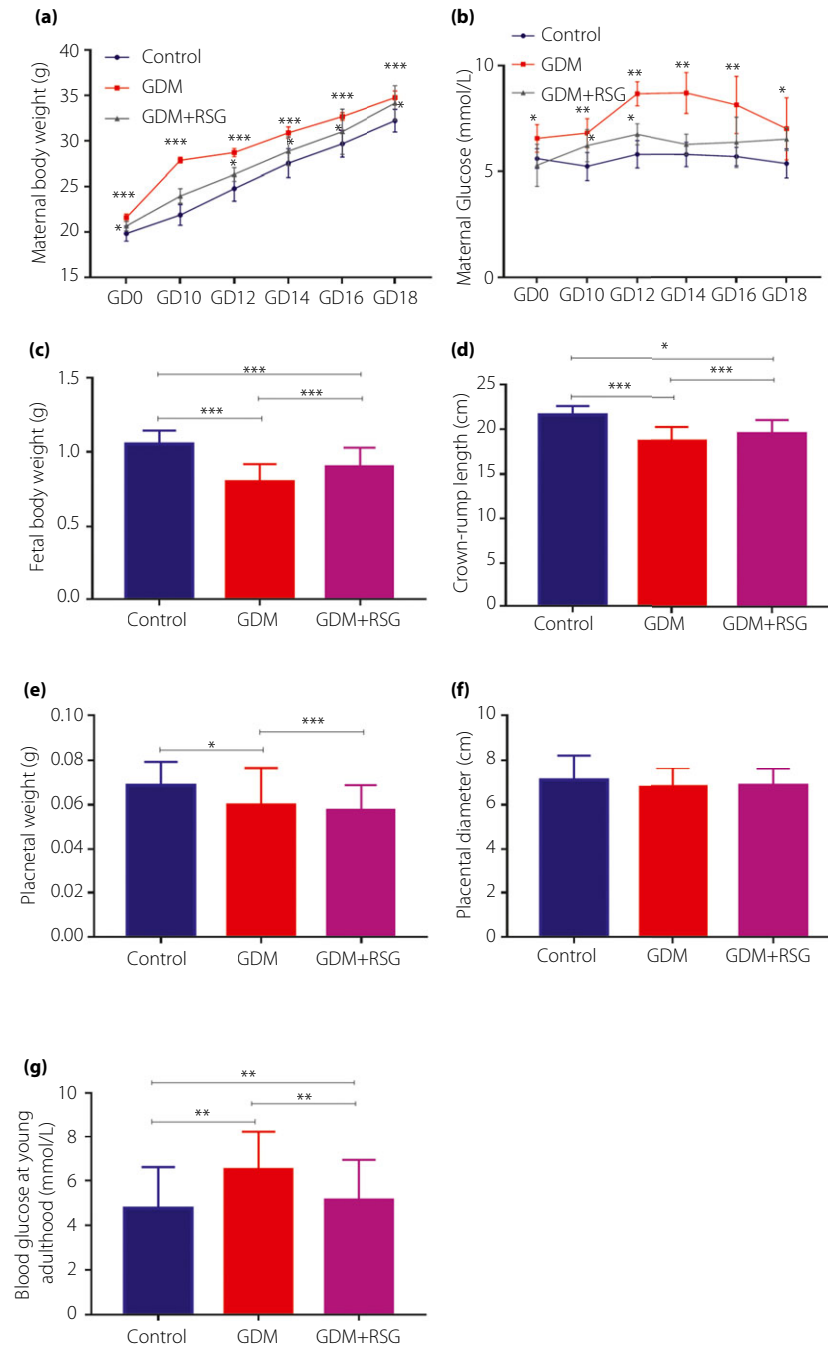
Figure 3 | (a) Maternal bodyweights of C57BL/6 mice in different groups. Bodyweights were measured during pregnancy (gestation day [GD]0, GD10, GD12, GD14, GD16 and GD18). The diet-induced gestational diabetes mellitus (GDM) dam weight increased significantly compared with the controls at different time-points. Maternal bodyweights increased slightly, but significantly, in the GDM + rosiglitazone (RSG) group compared with the controls at different time-points. (b) Maternal blood glucose levels of C57BL/6 mice in different groups. Blood glucose concentrations were measured during pregnancy (GD0, GD10, GD12, GD14, GD16 and GD18). The GDM group showed significantly higher blood glucose levels compared with the control dams. Blood glucose levels decreased obviously after RSG intervention to a level that was not significantly different compared with the control dams after GD12. The (c) fetal bodyweight, (d) crown-rump length, the (e) placental weight and (f) placental diameter of C57BL/6 mice in different groups. The fetal bodyweight, crown-rump length and placental weight in the GDM and GDM + RSG group decreased significantly compared with the controls, but the placental diameter was not different. (g) Blood glucose levels of C57BL/6 mice at young adulthood in different groups (*n* = 12 per group). Values are presented as the mean ± standard error of the mean. ***P < 0.001; **P < 0.01; *P < 0.05.
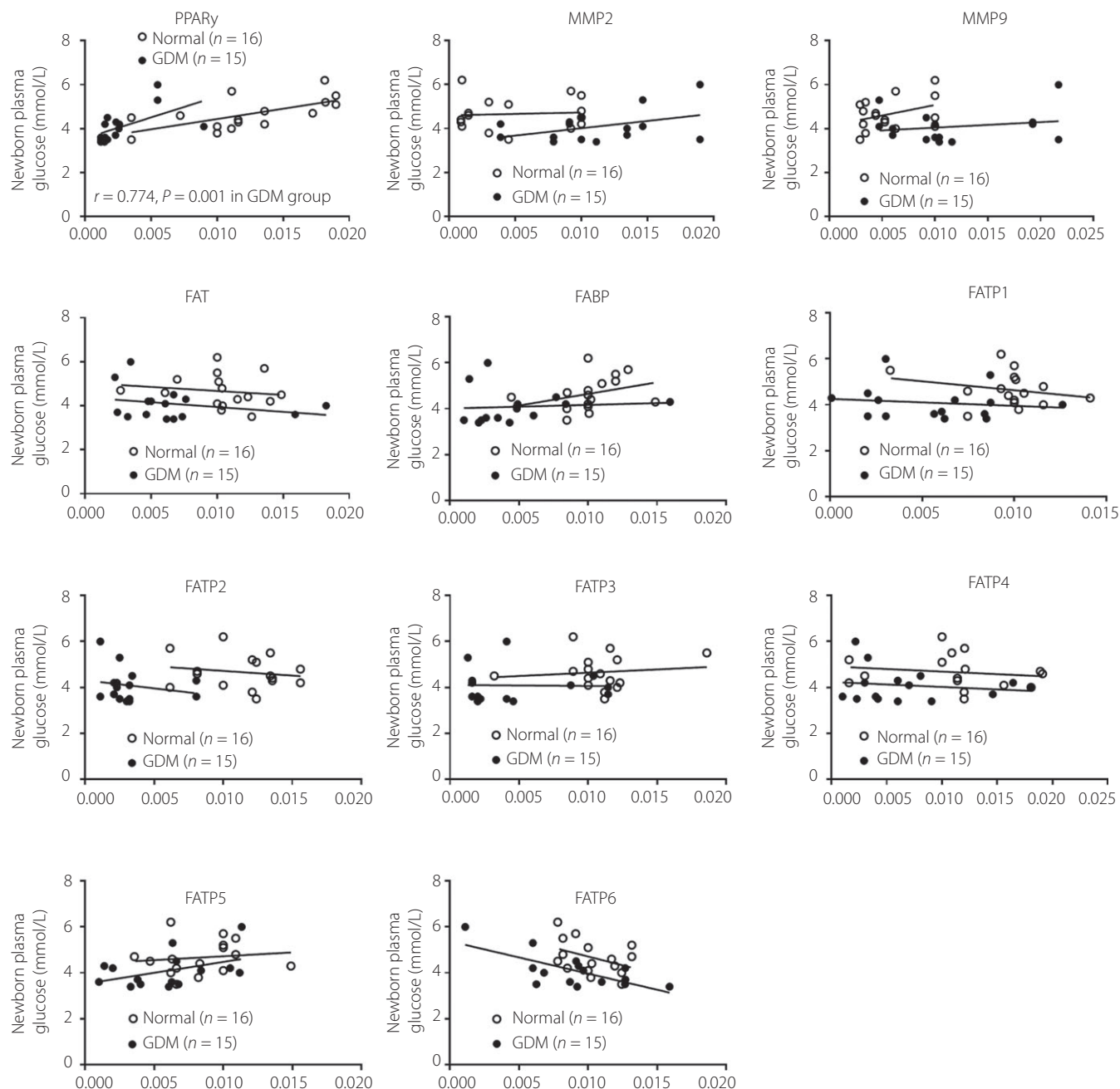
Figure 4 | Relationship between newborn plasma glucose levels and the messenger ribonucleic acid expression levels of placental fatty acid transporters and their related regulators. Linear regression and Spearman's correlation analyses were used to evaluate the relationship. Only the messenger ribonucleic acid of peroxisome proliferator-activated receptor-γ (PPARγ) in gestational diabetes mellitus (GDM) patients correlated to the levels of neonatal blood glucose ($r = 0.774$, $P = 0.001$). FABPpm, plasma membrane fatty acid binding protein; FAT, fatty acid translocase; FATP, fatty acid transport protein; MMP, metalloproteinase.

PPARγ activation increased fetal bodyweight and mRNA expression of FATP 1-6[15–17]. The mRNA expression of PPARγ and fatty acid transporters was significantly lower in GDM patients compared with normal individuals. Only the mRNA expression of soluble MMP-2/-9 was increased. These data suggest that factors associated with fatty acid transport are attenuated at the mRNA level, which might affect protein expression and function after post-transcriptional modification. Only PPARγ, FATP-1 and FATP-2 protein expression was reduced in GDM placentas, and the function of PPARγ,
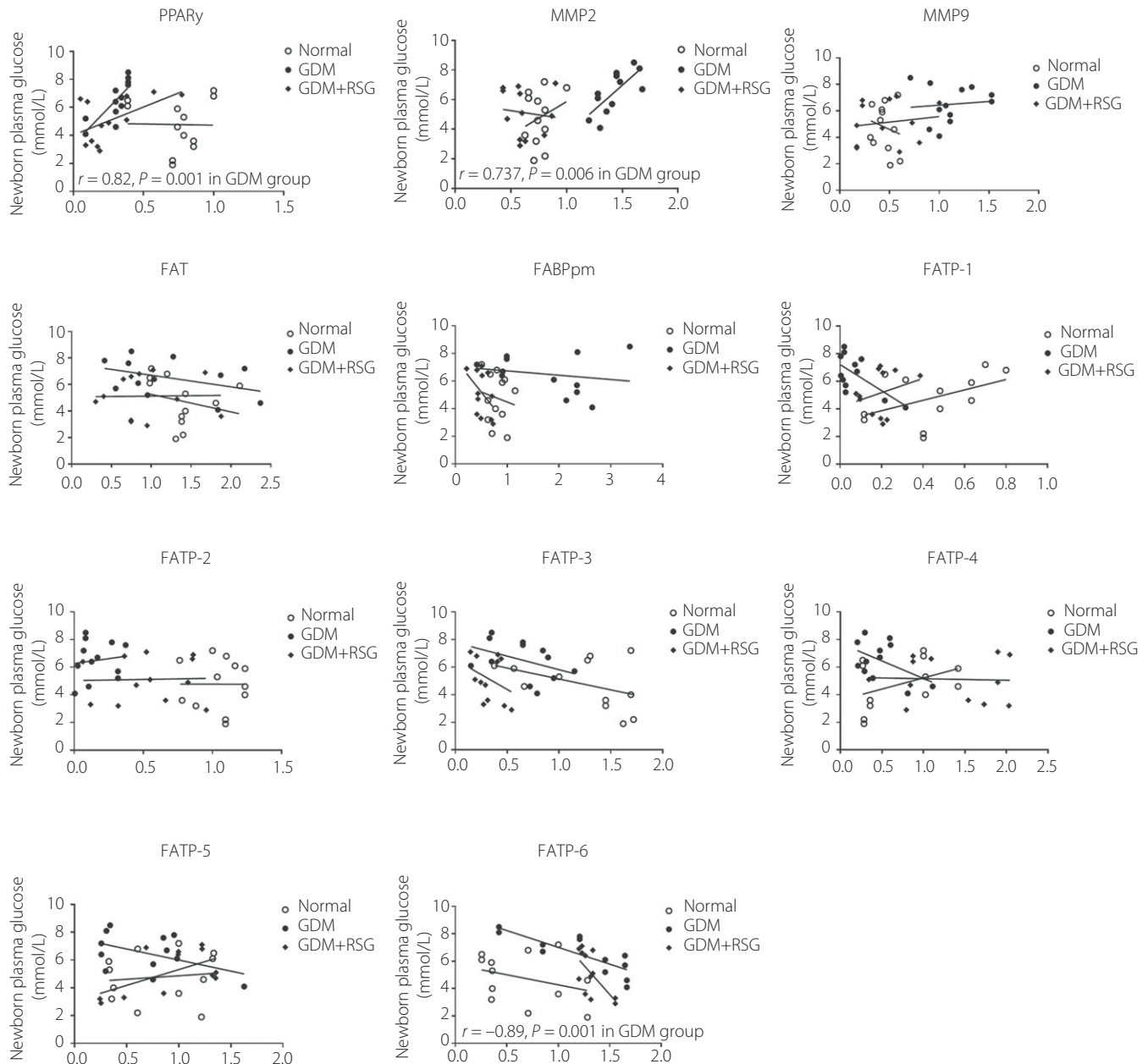
Figure 5 | Relationship between mouse offspring plasma glucose levels and the messenger ribonucleic acid expression levels of placental fatty acid transporters and their related regulators at young adulthood ($n = 12$ per group). Linear regression and Spearman's correlation analyses were used to evaluate the relationship. The messenger ribonucleic acid levels of peroxisome proliferator-activated receptor-γ (PPARγ), metalloproteinase (MMP)2 and fatty acid transport protein (FATP)-6 correlated to the levels of mouse offspring blood glucose ($r = 0.82$, $P = 0.001$, $r = 0.737$, $P = 0.006$ and $r = -0.89$, $P = 0.001$, respectively). FABPpm, plasma membrane fatty acid binding protein; FAT, fatty acid translocase; RSG, rosiglitazone.

FATP-1 and FATP-2 was impaired. No other fatty acid transport-related molecules were altered in the present study. The correlation analyses between newborn plasma glucose levels and the mRNA expression levels of placental fatty acid transport regulators showed that the mRNA level of PPARγ was related to newborn blood glucose levels. These results suggest that the PPARγ expression level might predict the blood glucose levels of the next generation. In further studies, more reference genes, besides glyceraldehyde 3-phosphate dehydrogenase, were required for more accuracy[18,19], such as adenosine

triphosphate synthase, beta-actin, ubiquitin C, 18s ribosomal ribonucleic acid and so on. Previous studies showed that PPARγ expression in placentas from born appropriate for gestational age (AGA) and large for gestational age (LGA) infants was nearly twofold higher than placentas from born small for gestational age (SGA) infants. Placental PPARγ expression is positively associated with placental and fetal weight at birth, particularly within the SGA subpopulation[20]. A higher mean fetal weight was observed in GDM patients compared with the normal group in the present study, after matching for age, height, bodyweight and BMI ($P < 0.05$). PPARγ expression at the mRNA and protein levels was impaired. The downregulation of PPARγ expression at mRNA and protein levels might predict the adverse pregnancy outcome of the fetuses exposed to maternal GDM. We also observed similar results of a downregulation of PPARγ expression at mRNA and protein levels in diet-induced GDM animal models. The mRNA and protein expression of PPARγ was upregulated after RSG administration to GDM group mice, but expression remained lower than the control group. Upregulated PPARγ was accompanied by an obvious decrease in maternal and offspring blood glucose after treatment.

Fatty acid uptake and transport primarily rely on autocrine actions, and PPARγ strictly controls this type of autocrine activity[21]. FATP-4 is the only FATP expressed in the small intestine, and it is localized on the apical brush-border membrane of intestinal enterocytes. FATP-4 is responsible for the uptake and transport of dietary fatty acids[22,23]. FATP-4 also localizes to the apical brush-border membrane of yolk sac trophoblasts in E8 murine embryos[7]. FATP-1/-4 expression was upregulated after PPAR/RXR activation; conversely, FATP-2 expression was downregulated, and FATP-3/-6 expression was not altered[20]. FATP-1 and FATP-4 were enhanced in the placentas of RSG-treated embryos, and FATP-2/-3 and FATP-6 expression decreased[24]. FATPs might show different levels of mRNA and protein expression under different physiological conditions, and play different physiological roles in the fatty acid transport. The results of the correlation analysis in the present study showed no correlation of FATP mRNA expression with neonatal blood glucose in the population study. Only the expression level of FATP-6 mRNA correlated with blood glucose during the neonatal period and young adulthood in GDM animals, but the protein expression of FATP-6 was not significantly different between groups. The mRNA expression of PPARγ was consistent with the neonatal blood glucose in the population study, and the blood glucose in the neonatal period and young adulthood in GDM animal tests.

Drug intervention is a very important step for patients who engaged in a reasonable diet and appropriate exercise to control blood glucose but failed. The present study observed an effect of RSG on the blood glucose of GDM mice. Previous studies showed that PPARγ activation altered gene expression *in vivo*[25–29]. Exposing the placenta to a PPARγ agonist for a limited time earlier in pregnancy might have affected placental development without depleting the trophoblastic stem cell pool, which could have occurred when the placenta was exposed to RSG for an extended period. Sevillano *et al.*[30] reported a decrease in neonatal, but not fetal, mass in rats given englitazone (50 mg/kg/day) between E16 and E21. In contrast, exposure of pregnant rats to the PPARγ agonist troglitazone (20 mg/kg/day) on E9–E11 was associated with reduced fetal mortality and no change in fetoplacental weight[31].

Taken together, the identification of the mRNA and protein expression of the fatty acid transport-related factors of placentas from human patients and GDM mice showed that the mRNA levels of PPARγ correlated with the levels of neonatal blood glucose exposed to maternal GDM in humans and mice using linear regression and Spearman's correlation analyses. A similar expression pattern of PPARγ protein was detected. The mRNA levels of MMP-2 and FTAP-6 also correlated with the levels of offspring blood glucose in young adulthood in GDM. MMP-2 and FTAP-6 protein expression levels were not different between groups. Therefore, the downregulation of PPARγ in the placenta might predict hyperglycemia in offspring at young adulthood.

DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1| The density ratio of the protein expression levels of human placental fatty acid transporters and their related regulators to their loading controls.

Figure S2| The density ratio of the protein expression levels of gestational diabetes mellitus mouse placental fatty acid transporters and their related regulators to their loading controls.

Figure S3| Relationship between mice offspring plasma glucose levels and the messenger ribonucleic acid expression levels of placental fatty acid transporters and their related regulators at birth ($n = 12$ per group).

Table S1| Clinical characteristics of 31 participants and newborns.