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RESEARCH ARTICLE | *Browning and Beiging of Adipose Tissue: Its Role in the Regulation of Energy Homeostasis and as a Potential Target for Alleviating Metabolic Diseases*

Impact of GPR1 signaling on maternal high-fat feeding and placenta metabolism in mice

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Huang B, Huang C, Zhao H, Zhu W, Wang B, Wang H, Chen J, Xiao T, Niu J, Zhang J. Impact of GPR1 signaling on maternal high-fat feeding and placenta metabolism in mice. *Am J Physiol Endocrinol Metab* 316: E987–E997, 2019. First published March 5, 2019; doi:10.1152/ajpendo.00437.2018.—Chemerin and G protein-coupled receptor 1 (GPR1) are increased in serum and placenta in mice during pregnancy. Interestingly, we observed increased serum chemerin levels and decreased GPR1 expression in placenta of high-fat-diet-fed mice compared with chow-fed mice at gestational *day 18*. GPR1 protein and gene levels were significantly decreased in gestational diabetes mellitus (GDM) patient placentas. Therefore, we hypothesized that chemerin/GPR1 signaling might participate in the pathogenic mechanism of GDM. We investigated the role of GPR1 in carbohydrate homeostasis during pregnancy using pregnant mice transfected with small interfering RNA for GPR1 or a negative control. GPR1 knockdown exacerbated glucose intolerance, disrupted lipid metabolism, and decreased β-cell proliferation and insulin levels. Glucose transport protein-3 and fatty acid binding protein-4 were downregulated with reducing GPR1 in vivo and in vitro via phosphorylated AKT pathway. Taken together, our findings first demonstrate the expression of GPR1, the characterization of its direct biological effects in humans and mice, as well as the molecular mechanism that indicates the role of GPR1 signaling in maternal metabolism during pregnancy, suggesting a novel feedback mechanism to regulate glucose balance during pregnancy, and GPR1 could be a potential target for the detection and therapy of GDM.

chemerin; GDM; GLUT-3; GPR1; placenta

INTRODUCTION

Gestational diabetes mellitus (GDM), characterized by disordered glucose metabolism, hyperglycemia, hyperinsulinemia, and insulin resistance, is defined by various degrees of impaired glucose tolerance at onset or when first recognized (21, 38). The incidence of GDM is ~10–15% of all pregnancies worldwide (20). GDM is associated with a high risk of developing type 2 diabetes in late life, as well as metabolic syndrome in offspring (31). Both clinical and animal studies have shown that it is important to transfer oxygen and nutrients to the fetus for maintaining glucose and metabolism homeostasis during pregnancy (12, 20, 27). In addition, although it has been previously reported that many genes and hormones are involved in GDM through affecting β-cell expansion and function, the development of GDM is still not understood (4). The pathogenic factors of GDM include genetic, lifestyle, and environmental factors. Therefore, a single factor cannot explain the development of GDM. Because there is no difference in the gene expression level in the initial development of GDM in humans, animal models of GDM that closely mimic the pattern seen in the clinic are significant to explore the pathophysiology of GDM.

Chemerin, also known as tazarotene-induced gene 2, and retinoic acid receptor responder protein 2, is a novel adipokine secreted by adipose tissues (37). Chemerin is often associated with carbohydrate metabolism, fat cell differentiation, inflammation, and immune responses (2, 28, 33). Chemokine-like receptor 1 (CMKLR1), chemokine (C-C motif) receptor-like 2 (CCRL2), and G protein-coupled receptor 1 (GPR1) have been identified as receptors for chemerin in vitro (3, 41). Chemerin has been shown to bind to CMKLR1 to promote the chemotaxis of leukocyte populations in dendritic and immune cells (10). Chemerin binding to CCRL2 functioned as a decoy chemokine receptor in inflammation regions, which contained cmklr1-positive immune cells (41). With respect to metabolism, there are conflicting results in which CMKLR1 knockout (KO) mice showed both normal and impaired glucose tolerance (14, 17, 33). These reports suggest that chemerin might also play a role in metabolism through receptors other than CMKLR1. At present, chemerin is the only known ligand for orphan receptor GPR1, which is highly expressed in adipose tissue and skeletal muscle. GPR1 KO mice exhibited impaired glucose tolerance and decreased insulin secretion after being fed a high-fat diet (HFD) (28). In our data, GPR1 signaling was detected in metabolic tissues and placenta during pregnancy. Furthermore, recent studies showed that circulating chemerin levels were markedly increased in obese (7) and GDM patients, and obesity was positively associated with the risk of GDM in

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the first trimester (36). However, no studies have explored the role of chemerin/GPR1 signaling in maternal metabolism during pregnancy. Thus, in this study, we investigated the role of GPR1 signaling in metabolic tissues and placenta during pregnancy, as well as potential molecular mechanism.

MATERIALS AND METHODS

Human samples. All women underwent a 75-g oral glucose tolerance test at 24–28 wk of gestation. GDM was diagnosed according to the International Association of Diabetes and Pregnancy Study Group criteria [plasma glucose: 0 h (fasting) $\geq$ 5.1 mmol/l; 1 h $\geq$ 10.0 mmol/l; 2 h $\geq$ 8.5 mmol/l]. Full-term placentas were collected from eight normal women and eight GDM patients at Shenzhen Maternity and Child Healthcare Hospital. Placental tissues were taken within 15 min of operative delivery, immediately snap-frozen in liquid nitrogen, and then stored at −80°C. Informed, written consent was obtained from the participants. All research protocols associated with clinical samples were approved by the Committee on the Use of Humans for Teaching and Research, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, and by Shenzhen Maternity and Child Healthcare Hospital, Southern Medical University.

Animal experiments. Virginal female 7- to 8-wk-old C57BL/6 mice obtained from Vitalriver (Keaoxieli, China) were acclimated to housing conditions for 1 wk, including fresh food and water ad libitum, temperature of 22.0 ± 1°C, humidity of 40–60%, and a 12:12-h light-dark cycle. Timed mating of animals was conducted by placing females with fertile males to induce pregnancy (D0 is the day of vaginal plug). Thirty-two pregnant mice were divided into two groups: chow and HFD (45% kcal fat, 35% kcal carbohydrates, and 20% kcal proteins; Research Diets). Eight pregnant mice were transfected with small interfering RNA (siRNA) with a terminal-dTT modification for GPR1 or with a negative control (NC) in the tail vein (8, 40) from gestational *day 9* (D9) to D18, respectively. Each siRNA (NC) sense, UUCUCCGAACGUGUCACGU-dTdT, and anti-sense, ACGUGACACGUUCGGAGAA-dTdT, and GPR1 sense, CCACUCUUUGGUUCCUCAA-dTdT, and anti-sense, UUGAGGAACCAAAGAGUGG-dTdT) (Genepharma), was administered at 0.1 nmol/g per injection once every other day. All research protocols associated with animal experiments were approved by the Committee on the Use of Live Animals for Teaching and Research, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences (approval no. SIAT-IRB-160226-YYS-ZHANGJ-A0198.). In addition, all animal experimental methods were conducted in accordance with the approved guidelines and regulations.

IPGTT, fasting blood glucose, body weight, and serum parameters. Intraperitoneal glucose tolerance tests (IPGTTs) were conducted in unrestrained mice that were fasted for 16 h and injected intraperitoneally with 2 g of D-(+)-glucose/kg body wt [anhydrous D-(+)-glucose; Sibopharm Chemical Regent], and blood glucose was sampled from the tail at 0, 15, 30, 60, and 120 min after the glucose injection on D16 by a glucose meter (Roche). Body weight was measured after siRNA injection. Blood was collected via retroorbital bleeding on D18. Blood was centrifuged at 3,500 rpm and 4°C for 5 min, and serum was collected from the supernatant. Serum insulin levels were measured using insulin kits (Millipore). Serum triglyceride (TG) levels were measured using kits (Dongou). Testosterone, progesterone, estradiol, and prolactin (PRL) levels in conditioned media were measured using commercial iodine-125 radioimmunoassay kits (Beijing North Biotechnology Research Institute). The sensitivity of the progesterone and estradiol radioimmunoassays was 20 ng/ml. The intra-assay and interassay errors were $<$ 10% and $<$ 15%, respectively.

Placenta weight and TG. Placentas were immediately collected and weighed, and placental wet weights were averaged per litter. Placenta TG was measured in placenta tissue homogenate that was strictly prepared according to the TG kit (Dongou) instructions.

Histopathology, immunohistochemistry, and confocal microscopy. Tissues, including pancreas and placenta samples, were then rapidly removed from the animals. Mouse tissues were fixed in Bonn's liquid, dehydrated in alcohol, embedded in paraffin, and sectioned. Morphological examination was performed on 5-μm sections of paraffin-embedded tissue with hematoxylin and eosin (H&E) stain, according to standard histological protocols. Islet areas were calculated at the tail of the pancreas, and quantification of immunohistochemistry was calculated using ImageJ software, as previously described (1). Antigens were retrieved in citrate buffer using microwave heat. Immunohistochemistry was carried out. The expression of GPR1 (Wuhan Booute Biotechnology) was determined by confocal microscopy, as described previously. JEG3 cells were probed with rabbit anti-GPR1 primary antibody to GPR1 (Wuhan Booute Biotechnology) and then incubated with goat anti-rabbit IgG Alexa 568 (Invitrogen).

Cell culture and treatment. JEG3 cell line was obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured as previously described (35). Stable knockdown and overexpression of GPR1 in JEG3 were established using Lipofectamine 2000 transfection reagent (Invitrogen). The targeted GPR1 short hairpin RNA (shRNA) vector (sense, CACTCTCTGATTGTCATTATAT; anti-sense, ATATAATGACAATCAGAGAGTG; Open Biosystems) contained a puromycin resistance gene (Invitrogen), and the human GPR1 (NM_001098199.1) expression vector (Stanford University) contained a G418 resistance gene. Transfections with pSM2C-GPR1-shRNA or an empty vector, and with pcDNA3.0-GPR1 or an empty vector, were carried out according to the manufacturer's protocol, and cells were selected in medium containing 2 μg/ml of puromycin and 500 μg/ml of G418 (Sigma), respectively, for 2–3 wk. The colonized cells were isolated, amplified, and used for subsequent experiments. Cell lipid accumulation was assessed in stably transfected JEG3 cells treated with 5% bovine serum albumin (Sigma) and free fatty acids (400 μM palmitic acid and 800 μM oleic acid; Sigma) for 24 h after 1 h of incubation with a phosphorylated AKT (pAKT) inhibitor (wortmannin; Selleck) or with DMSO.

Oil Red O staining. Oil Red O staining of placenta was performed in 10-μm frozen sections that were fixed in 4% paraformaldehyde and dehydrated in a gradient of sucrose. Tissue and cell staining with the Oil Red O (Sigma) method was performed as previously described (32).

Western blotting and real-time RT-PCR analysis. Protein extraction from tissues and cells and Western blotting were performed as previously described (27). Antibodies for GPR1, glucose transport protein-3 (GLUT-3; Abcam), pAKT/AKT (CST), fatty acid binding protein 4 (FABP4), and β-actin (Sigma) were used. Real-time qPCR was performed as previously described (15). Total RNA was isolated from mouse tissues or cells using RNA plus liquid kits (Takara) following the manufacturer's protocol. RNA (0.5–2 μg) was reverse transcribed into cDNA using reverse transcription kits (Toyobo). Expression levels of several genes were determined by real-time RT-PCR using a Roche LightCycler instrument (Roche) with SYBRgreen (Toyobo) detection, according to the manufacturer's protocol. The RT-PCR primers are as follows: mouse primer sequence of 18srRNA (forward, GATCCCGGCTCTTAATATTCGAAT; reverse, GCCAGAGTCTCGTTCGTTATC); chemerin (forward, TACAGGTGGCTCTGGAGGAGTTC; reverse, CTTCTCCCGTTTGGTTTGATTG); Gpr1 (forward, GGAGCTCAGCATTCATCACA; reverse, GACAGGCTCTTGGTTTCAGC); Glut1 (forward, GAGCATCTTCGAGAAGGCAGGTGT; reverse, GGCCACAATGAACCATGGAATA); Glut2 (forward, TCCTGGTCTTCACCCTGTTTAC; reverse, GGTCATCCTCACACACTCTCTG); Glut3 (forward, CTCTTCAGGTCACCCAACTACGT; reverse, CCGCGTCCTTGAAGATTCC); C/ebpα (forward, AGCAACGAGTACCGGGTACG; reverse, TGTTTGGCTTTATCTCGGCTC); Pparγ (forward, TCTGGGAGATTCTCCTGTTGA; reverse, GGTGGGCCAGAATGGCATCT); human primer sequence of 18srRNA (forward, CGAAAGCATTTGCCAAGAAT; reverse, AGTCGGCATCGTTTATGGTC); chemerin

(forward, TACAGGTGGCTCTGGAGGAGTTC; reverse, CTTCTC-CCGTTTGGTTTGATTG); Gpr1 (forward, GGAGCTCAGCATT-CATCACA; reverse, GACAGGCTCTTGGTTTCAGC).

Statistical analysis. All data are shown as means ± SE. Statistically significant differences were confirmed using Student's *t*-test or two-way ANOVA using the software GraphPad Prism trial version 7 (GraphPad Software, La Jolla, CA). $P < 0.05$ was determined to be statistically significant.

RESULTS

Chemerin and GPR1 expression in GDM patient placenta. For clinical application of these data, such as utilizing GPR1 signaling for diagnosis and therapy, we measured chemerin/GPR1 signaling expression on GDM patient placentas. Real-time RT-PCR analysis showed that chemerin (Fig. 1*A*) was significantly increased and that GPR1 (Fig. 1*B*) was significantly decreased in GDM patient full-term placentas compared with the levels in normal placentas. Next, we further confirmed that GPR1 protein (Fig. 1*C*) was also significantly decreased in GDM patient placentas (Fig. 1*E*). GLUT-3 also showed a significantly decreased trend (Fig. 1, *C* and *D*). These results from clinical data suggest that GPR1 may plays an important role in development of GDM.

Upregulation of chemerin/GPR1 signaling in the mice placenta during pregnancy. To understand the pathophysiological significance of GPR1 in GDM, we first analyzed the mouse placental expression patterns of chemerin (Fig. 2*A*) and GPR1 (Fig. 2*B*), which displayed an increase via RT-PCR test during pregnancy. Circulating chemerin (Fig. 2*C*) also showed increase via ELISA during pregnancy. In addition, to explore the effect of GPR1 signaling on GDM, a pregnant mouse model with an induced metabolism disorder was generated by feeding a HFD after mating. Glucose tolerance was remarkably impaired in the HFD-fed pregnant mice at D16 (Fig. 2*D*). Meanwhile, the circulating chemerin levels were significantly increased (Fig. 2*E*), but GPR1 mRNA expression showed a significant decrease compared with that in the chow-fed pregnant mice (Fig. 2*F*) at D18. Taken together, these results reveal that chemerin/GPR1 signaling was upregulated during pregnancy; however, GPR1 expression levels were downregulated in HFD-fed pregnant mice, which raised the question of whether chemerin/GPR1 signaling plays a role in metabolism homeostasis during pregnancy.

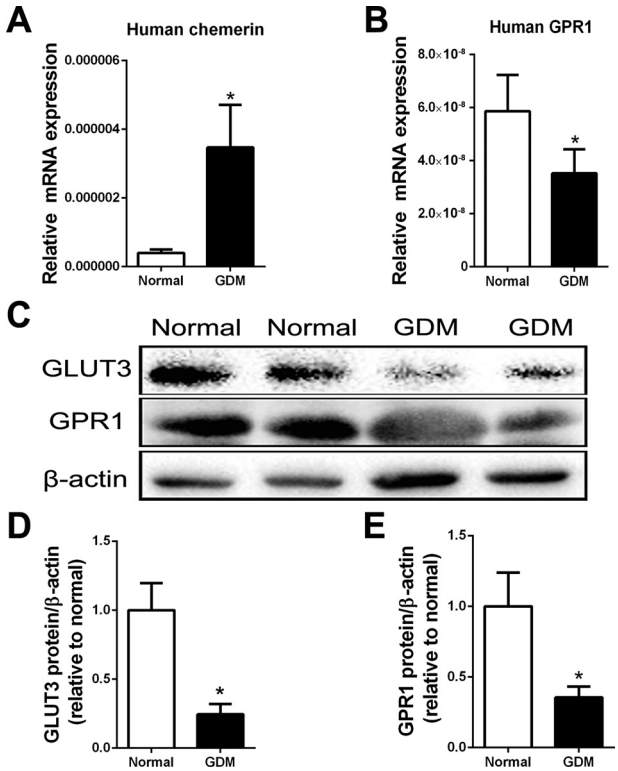

Fig. 1. Gene expression of chemerin was increased, and its receptors were decreased in gestational diabetes mellitus (GDM) patient placentas. Real-time PCR analysis of chemerin (*A*) and G protein-coupled receptor 1 (GPR1) mRNA (*B*) expression levels in the full-term placentas of normal women and GDM patients are shown. Western blotting analysis of glucose transport protein-3 (GLUT-3) and GPR1 protein expression levels (*C*) and quantification of GLUT-3 (*D*) and GPR1 protein (*E*) in the placentas of normal women and GDM patients from *C* are shown. Values are means ± SE; $n = 8$. *$P < 0.05$ compared with normal placenta.

GPR1 knockdown impairs glucose homeostasis in HFD-fed pregnant mice. To further study the function of GPR1 signaling in metabolism during pregnancy, GPR1 signaling was knocked down in chow- and HFD-fed pregnant mice via injecting siRNA-GPR1 into the tail veins. The GPR1 knockdown pregnant mice displayed normal body weights (Fig. 3, *A* and *B*). Then IPGTTs were performed to further identify the effect of GPR1 on metabolism homeostasis. Glucose tolerance was not dramatically different in the chow-fed GPR1 knockdown pregnant mice at D16 (Fig. 3*C*), whereas prominent glucose intolerance was observed in the HFD-fed GPR1 knockdown pregnant mice at D16 (Fig. 3*D*). Similar results were obtained via quantification of the IPGTT area under the curve with one-way ANOVA, showing significantly impaired glucose tolerance compared with that in the chow-fed pregnant mice injected with the NC (Fig. 3*E*). As we know, circulating insulin plays a role in regulating blood glucose homeostasis. We also measured serum insulin levels via an ELISA kit. Serum insulin was significantly increased in the HFD-fed pregnant mice compared with that in the chow-fed pregnant mice after injection of NC, and there was a dramatic decrease in serum insulin in the GPR1 knockdown group compared with that in the NC group of HFD-fed pregnant mice at D18 (Fig. 3*F*). These data indicated that the HFD induced the change of glucose intolerance, and GPR1 signaling knockdown exacerbated glucose intolerance and reduced insulin secretion, suggesting that GPR1 signaling knockdown may impact glucose metabolism during pregnancy.

Blood glucose could be regulated by the β-cell producing insulin. It was previously reported that PRL is required for maternal β-cell proliferation and insulin secretion during pregnancy (16). Since HFD-fed GPR1 knockdown pregnant mice displayed decreased β-cell proliferation at D18, we measured the serum PRL levels, which showed a significant decrease in the GPR1 knockdown group compared with the NC group of HFD-fed pregnant mice (Supplemental Fig. S1*A*; Supplemental data are available in the data supplement online at the *journal* website). In addition, previous clinical results have revealed that total testosterone levels were higher among women with GDM than among women without GDM in the first trimester (13). Steroid hormones, including testosterone, progesterone, and estrogen, were measured in the serum of all of the groups of pregnant mice at D18. Serum testosterone displayed a significant increase in the GPR1 knockdown mice

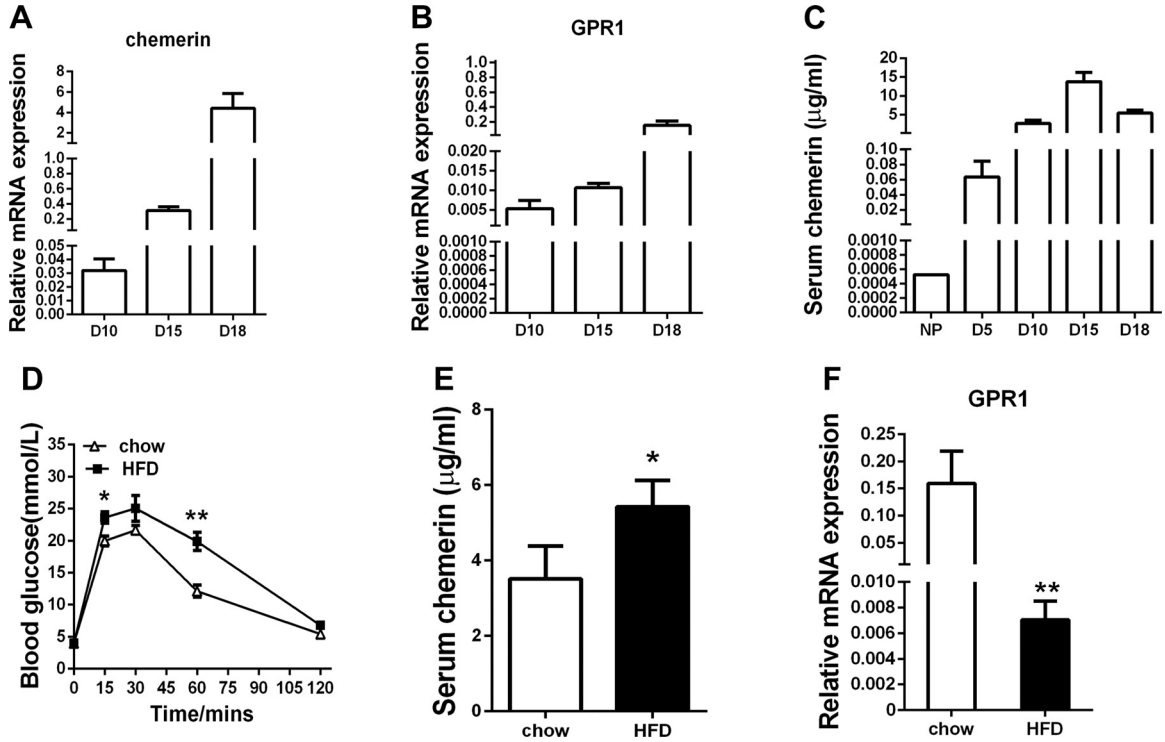

Fig. 2. Chemerin/G protein-coupled receptor 1 (GPR1) signaling pathway expression levels in the placenta during pregnancy in mice. Real-time RT-PCR analysis of chemerin (*A*) and GPR1 mRNA expression levels (*B*) in the placentas of chow-fed pregnant mice at gestational *days 10*, *15*, and *18* (D10, D15, and D18, respectively) are shown. *C*: ELISA analysis of serum chemerin levels in nonpregnant (NP) and pregnant mice at D5, D10, D15, and D18. *D*: intraperitoneal glucose tolerance tests were performed in chow- and high-fat-diet (HFD)-fed pregnant mice at D16. ELISA analysis of serum chemerin levels (*E*) and real-time RT-PCR analysis of GPR1 mRNA expression levels (*F*) in chow- and HFD-fed pregnant mice at D18 are shown. Values are means ± SE; $n = 5–8$. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with chow.

compared with the NC mice in the respective HFD-fed and chow-fed groups. However, there was no difference in serum testosterone levels in NC mice between the HFD- and chow-fed pregnant groups (Supplemental Fig. S1*B*). Interestingly, progesterone and estrogen levels were somewhat altered. Serum progesterone was significantly increased in the GPR1 knockdown mice compared with the NC mice in the chow-fed group, and it was dramatically decreased in the HFD-fed mice compared with the chow-fed mice after GPR1 knockdown (Supplemental Fig. S1*C*). Contrary to the testosterone data, serum estrogen displayed a significant decrease in the GPR1 knockdown mice compared with the NC mice in the respective HFD-fed and chow-fed groups (Supplemental Fig. S1*D*). PRL receptor expression levels were significantly decreased in HFD-feeding mice compared with GPR1 knock down (Supplemental Fig. S1*F*). Estrogen receptor-α expression levels were significantly decreased in chow-feeding mice treatment with small interfering RNA for GPR1 (siGPR1) and HFD-feeding mice treatment with small interfering RNA for NC (siNC) compared with chow-feeding mice treatment with siNC (Supplemental Fig. S1*G*). Estrogen receptor-β expression levels were only observed significantly decreased in HFD-feeding mice compared with chow-feeding mice treatment with siNC (Supplemental Fig. S1*H*). PRL could promote insulin secretion, but PRL could not recover insulin secretion in GPR1-knockdown MIN6 cells. Insulin secretion has been shown to have an increased tendency and was not significantly different (Supplemental Fig. S1, *I* and *J*).

To address whether GPR1 was important for maternal β-cell proliferation during pregnancy, we analyzed islet areas and localization of related proteins, including insulin, GPR1, and proliferating cell nuclear antigen (PCNA). According to the representative photomicrographs of pancreatic H&E-stained sections (Fig. 3*G*) that were quantified via ImageJ software (Fig. 3*H*), the islet areas were significantly increased in the HFD-fed NC pregnant mice compared with the chow-fed NC pregnant mice, and there was a dramatic decrease in the islet areas in the GPR1 knockdown group compared with the NC group of HFD-fed pregnant mice at D18. From the immunohistochemical staining (Fig. 3*I*), we can observe GPR1 protein localization on β-cells according to insulin signaling; meanwhile, siGPR1 prominently induced GPR1 protein expression compared with that in the NC group of HFD-fed pregnant mice. PCNA (Fig. 3, *J* and *K*) are proliferation markers that were decreased in the GPR1 knockdown group compared with the levels in the NC group of HFD-fed pregnant mice at D18.

These results indicate that GPR1 might contribute to GDM development. GPR1 knockdown altered insulin release and impacted β-cell proliferation in the HFD-fed mice during pregnancy.

GPR1 knockdown impairs GLUT-3 expression and lipid metabolism in HFD-fed pregnant mouse placentas. To further understand the functions and roles of the placenta, which is an important organ that regulates physiology through secreting hormones and cytokines during pregnancy, it was reported that HFD could dramatically influence placenta development (21). First, we measured expression levels via RT-PCR and Western

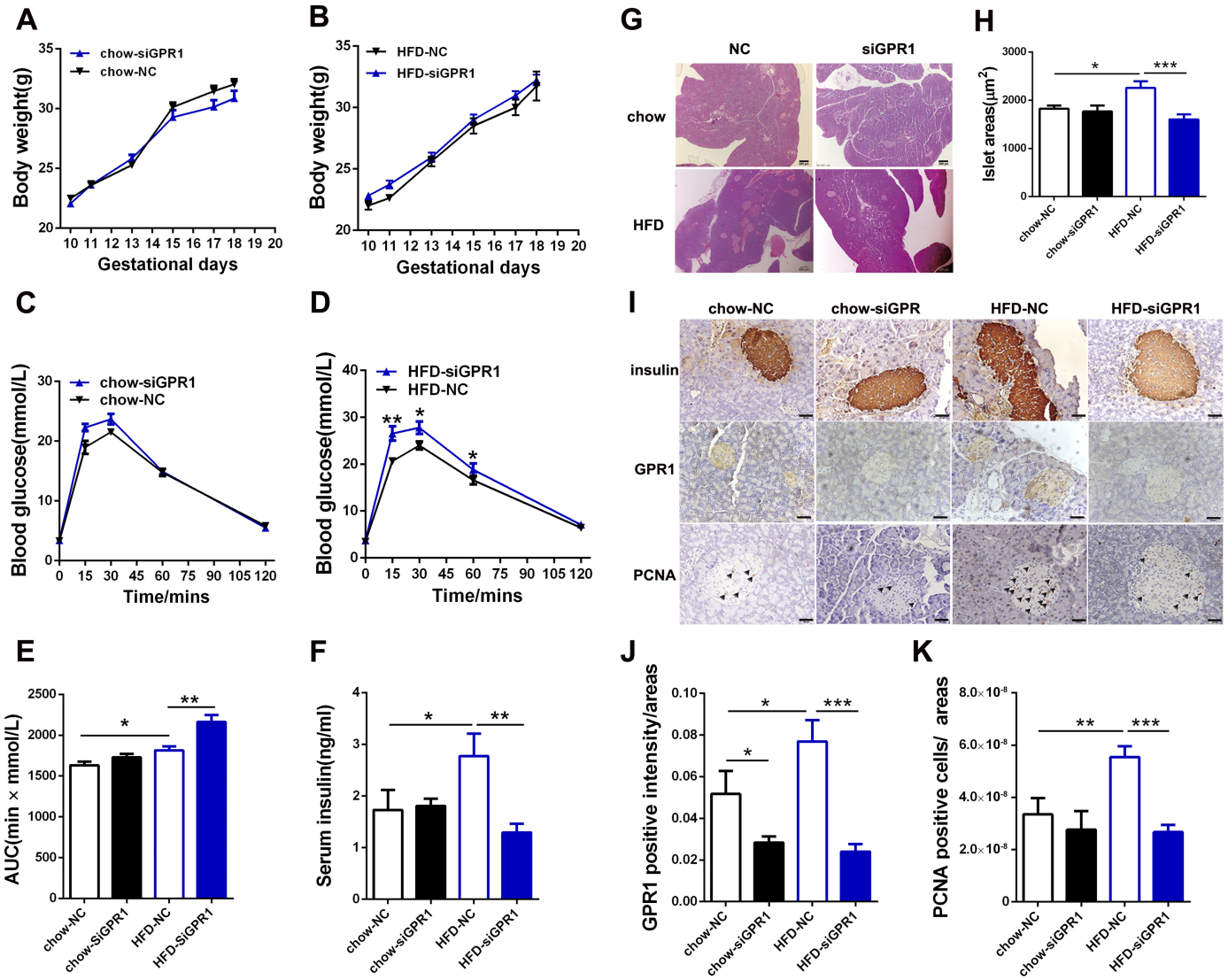

Fig. 3. G protein-coupled receptor 1 (GPR1) knockdown impaired glucose homeostasis and fetal weight, and placenta weight was decreased in high-fat-diet (HFD)-fed pregnant mice. Body weights were measured at gestational *days 10*, *11*, *13*, *15*, *17*, and *18* (D10, D11, D13, D15, D17, and D18, respectively) in chow- (*A*) and HFD-fed (*B*) pregnant mice injected with negative control (NC) or small interfering RNA for GPR1 (siGPR1). Intraperitoneal glucose tolerance tests were performed at D16 in chow- (*C*) and HFD-fed (*D*) pregnant mice injected with NC or siGPR1. *E*: area under the curve (AUC) was calculated from *C* and *D*. *F*: ELISA analysis of serum insulin levels at D18 in chow- and HFD-fed pregnant mice injected with NC or siGPR1. *G*: hematoxylin and eosin staining analysis at D18 of the pancreas and islet cell morphology in chow- and HFD-fed pregnant mice injected with NC or siGPR1. Scale bar: 200 μm. *H*: islet areas were measured at D18 by ImageJ software in chow- and HFD-fed pregnant mice injected with NC or siGPR1. *I*: immunohistochemical staining of insulin, GPR1, and proliferating cell nuclear antigen (PCNA; black arrowheads) protein. Scale bar: 20 μm. *J* and *K*: quantification (GPR1 positive intensity and PCNA positive cells, respectively) of *C* in the pancreas at D18 in chow- and HFD-fed pregnant mice injected with NC or siGPR1. Values are means ± SE; $n = 7–8$. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with chow-NC or HFD-NC.

blotting, which indicated a significant decrease in GPR1 signaling in the placenta via injection of siGPR1 (Fig. 4, *A*–*C*). We also analyzed GPR1 protein localization on D18 in the mouse placentas. GPR1 was mainly expressed in the labyrinth and junction zone, and GPR1 signaling was also reduced in the placenta via injection of siGPR1 compared with that in the NC-injected mice (Fig. 4*E*). Meanwhile, GPR1 signaling was also reduced in the fetus via injection of siGPR1 compared with that in the NC-injected mice (Supplemental Fig. S2). In addition, pAKT levels were decreased relative to the total AKT expression in the placenta in the HFD-fed GPR1 knockdown mice compared with the NC mice (Fig. 4, *B* and *D*). Nevertheless, the placenta weights were dramatically decreased in the GPR1 knockdown group compared with the NC group of the HFD-fed pregnant mice at D18 (Fig. 4*F*). The fetal weights were prominently increased in the HFD-fed group compared with the chow-fed group after injection of NC, and there was a significant decrease in the fetal weights in the GPR1 knockdown group compared with the NC group of HFD-fed pregnant mice at D18 (Fig. 4*G*). These results suggested that GPR1 may play an important role in GDM through the placenta.

The placenta carries out the function of transportation to provide nutrients and oxygen to the fetus and to eliminate waste matter into the mother. In this process, it is always accompanied with energy metabolism (27). In this study, we thoroughly explored the processes of glucose transport and

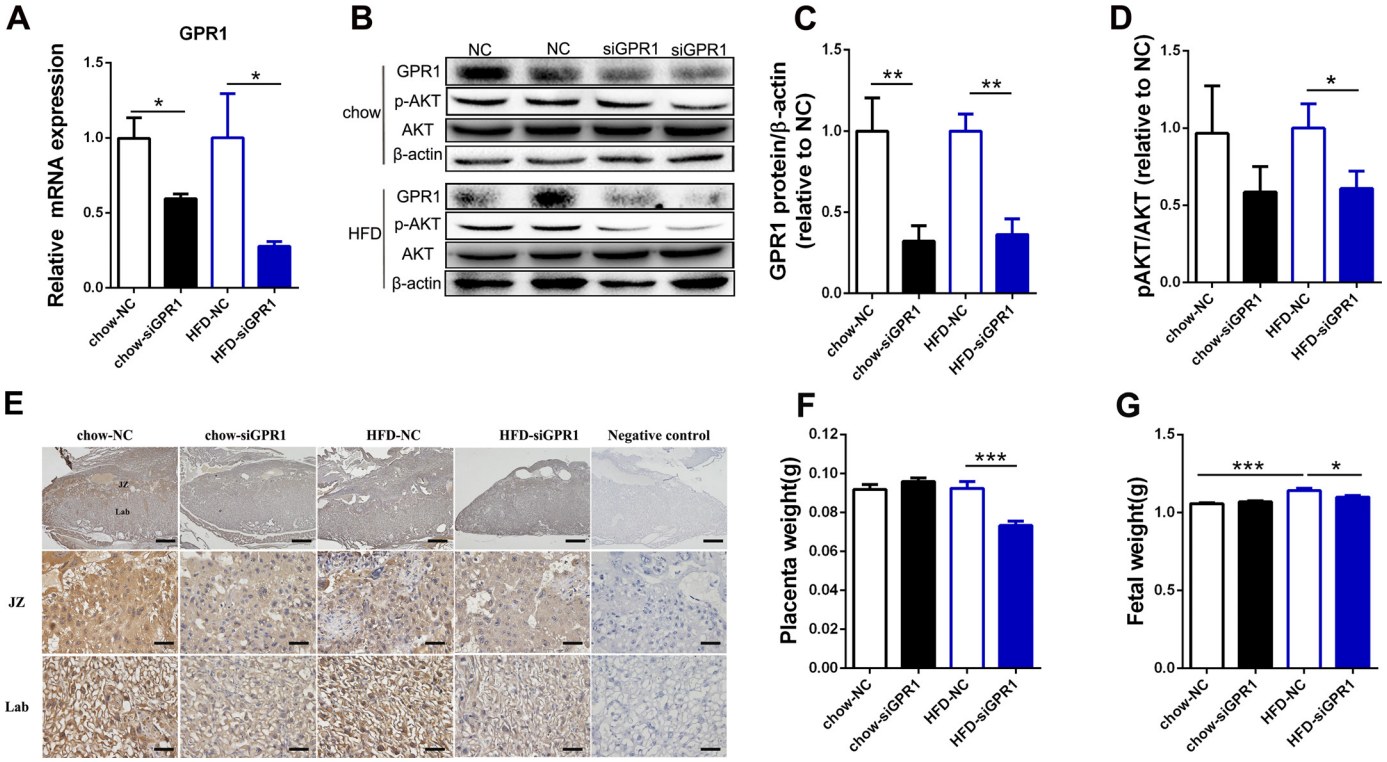

Fig. 4. Validation of G protein-coupled receptor 1 (GPR1) signaling expression levels in mouse placentas. Real-time PCR analysis of GPR1 mRNA levels (*A*) and Western blotting analysis of GPR1 protein expression (*B*) at gestational *day 18* (D18) in the placentas of chow- and high-fat-diet (HFD)-fed pregnant mice injected with negative control (NC) or small interfering RNA for GPR1 (siGPR1) are shown. Quantification of GPR1 protein (*C*) and pAKT/AKT protein (*D*) at D18 in chow- and HFD-fed pregnant mice injected with NC or siGPR1 from *B* are shown. *E*: immunohistochemical staining at D18 of GPR1 protein in the placentas of chow- and HFD-fed pregnant mice injected with NC or siGPR1. Negative control: primary antibody was substituted by PBS. Scale bar: *top*, 200 μm; *middle* and *bottom*, 20 μm. Lab, labyrinth; JZ, junction zone. Fetal (*F*) and placenta (*G*) weight were measured at D18 in chow- and HFD-fed pregnant mice injected with NC or siGPR1. Values are means ± SE; $n = 7–8$. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with chow-NC or HFD-NC.

lipid metabolism. First, we measured GLUT expression, both Glut1 and Glut2 mRNA showed no difference (Supplemental Fig. S3), and GLUT-3 showed significant decrease in the GPR1 knockdown mice compared with the NC mice via Western blotting and RT-PCR (Fig. 5, *A* and *B*, and Supplemental Fig. S2). To characterize the effect of GPR1 signaling on lipid metabolism, we also measured metabolic parameters in the serum and placenta. Maternal serum TG levels in the HFD-fed GPR1 knockdown mice were significantly decreased compared with those in the NC mice (Fig. 5*C*). Placenta TG levels in the HFD-fed GPR1 knockdown mice were remarkably lower than those in the NC mice (Fig. 5*D*). Of note, lipid accumulation in the placenta tissue from the HFD-fed GPR1 knockdown mice was decreased compared with that from the NC mice by Oil Red O staining (Fig. 5*E*). Next, we measured related markers of lipid metabolism. FABP4 protein (Fig. 5*F*) was also dramatically decreased via quantification of Western blotting (Fig. 5*G*) in the HFD-fed GPR1 knockdown mice compared with the NC mice. Meanwhile, RT-PCR analysis of peroxisome proliferator-activated receptor-γ (PPAR-γ) (Fig. 5*H*) and CCAAT-enhancer binding protein-α (C/EBP-α) (Fig. 5*I*) showed significantly decreased mRNA expression levels in the HFD-fed GPR1 knockdown mice compared with the NC mice. Taken together, decreased GLUT-3 protein, C/EBP-α and PPAR-γ mRNA levels, low serum and placenta content, and reduced placenta lipid accumulation support the hypothesis that GPR1 knockdown may impair nutrient transport and metabolism, leading to a disruption in metabolism homeostasis and to the development of GDM.

GPR1 promotes GLUT-3 and lipid accumulation in trophoblasts. To understand the mechanism of GPR1 in maternal metabolism homeostasis, we explored the impact of GPR1 signaling on trophoblasts. First, we constructed stable transfection cell lines with GPR1 knockdown and overexpression via an shRNA vector and an expression vector, respectively. Immunostaining analysis revealed that GPR1 expression in the stably transfected GPR1 knockdown cell line was barely detectable compared with that of the NC. Meanwhile, GPR1 localized on the surface and nucleus of the cell line, which may be associated with GPR1 internalization (Fig. 6*A*). In the stably transfected GPR1 knockdown cell line (Fig. 6*B*), GPR1 protein expression was lower than that in the cells transfected with an empty vector. Interestingly, GLUT-3 and FABP4 protein levels were decreased when GPR1 signaling was reduced. We assessed lipid accumulation (Fig. 6*C*) and showed that the GPR1 knockdown significantly decreased lipid accumulation via quantification of Oil Red O staining (Fig. 6*D*). In the GPR1-overexpressed cell line, GPR1 protein expression was higher than that in the cells transfected with an empty vector (Fig. 6*E*). In contrast to their expression in the GPR1 knockdown trophoblasts, GLUT-3 and FABP4 protein levels were increased. Lipid accumulation (Fig. 6*F*) in the GPR1-overexpressed cell line was significantly decreased via Oil Red O staining (Fig. 6*G*). It was noted that pAKT was also associated with GPR1

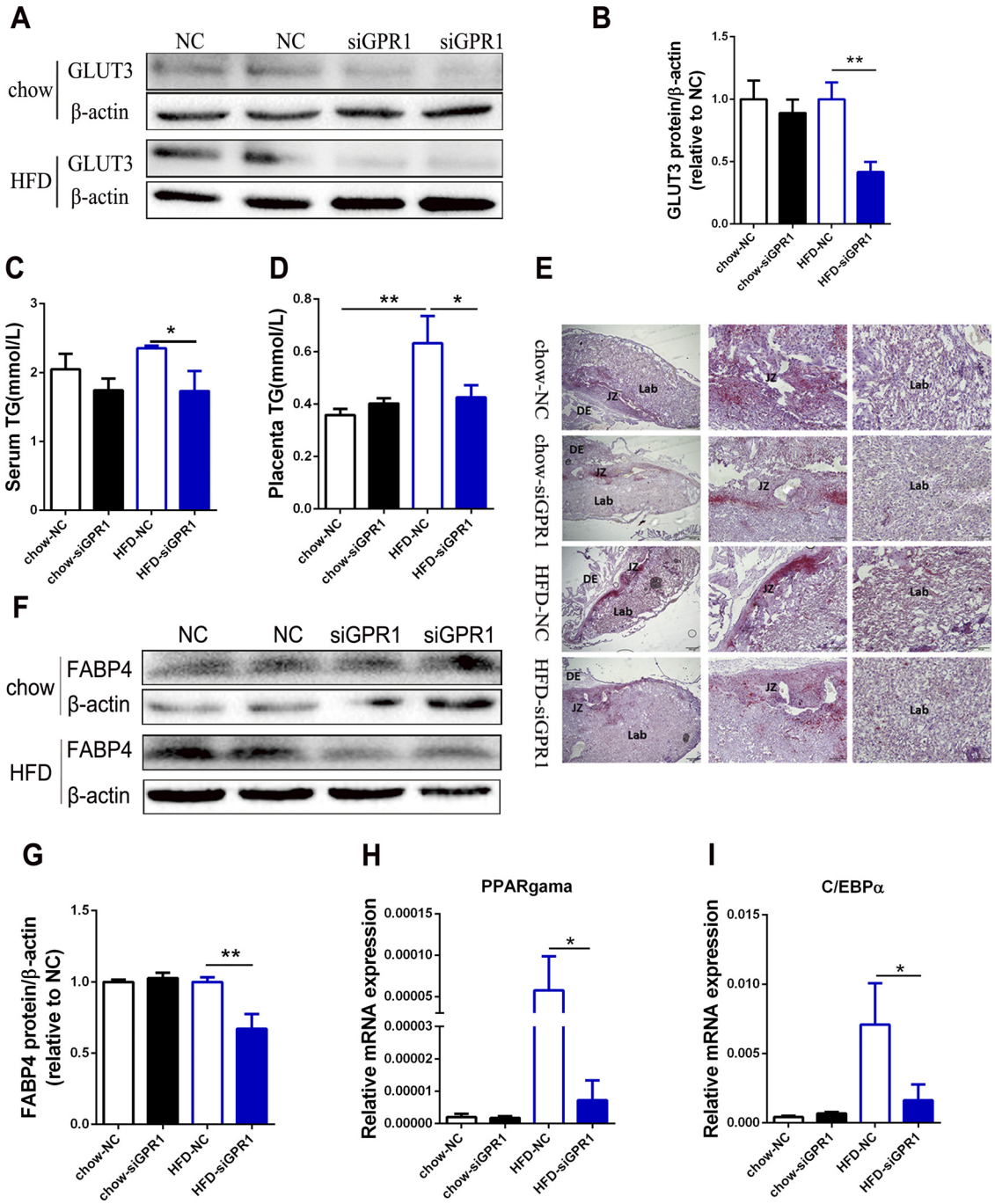

Fig. 5. G protein-coupled receptor 1 (GPR1) knockdown reduced glucose transport protein-3 (GLUT-3) expression and lipid metabolism in the placentas of high-fat-diet (HFD)-fed pregnant mice. Western blotting analysis of GLUT-3 protein expression levels in the placenta (*A*) and quantification of GLUT-3 protein at gestational *day 18* (D18) (*B*) in chow- and HFD-fed pregnant mice injected with negative control (NC) or small interfering RNA for GPR1 (siGPR1) from *A* are shown. Serum (*C*) and placenta (*D*) triglyceride (TG) levels were measured at D18 using a kit in chow- and HFD-fed pregnant mice injected with NC or siGPR1. *E*: Oil Red O staining analysis of lipid accumulation in the placenta at D18 in chow- and HFD-fed pregnant mice injected with NC or siGPR1. Scale bar: *left*, 200 μm; *middle* and *right*, 100 μm. Lab, labyrinth; JZ, junction zone. Western blotting analysis of fatty acid binding protein 4 (FABP4) protein expression levels in the placenta (*F*), and quantification of FABP4 protein (*G*) at D18 in chow- and HFD-fed pregnant mice injected with NC or siGPR1 from *F* are shown. Real-time PCR analysis of peroxisome proliferator-activated receptor-γ (PPAR-γ; *H*) and CCAAT-enhancer binding protein-α (C/EBP-α; *I*) mRNA at D18 in the placentas of chow- and HFD-fed pregnant mice injected with NC or siGPR1 are shown. Values are means ± SE; $n = 7–8$. $^*P < 0.05$ and $^{**}P < 0.01$ compared with chow-NC or HFD-NC.

signaling expression (Fig. 6, *B* and *E*). These results indicate that GPR1 may regulate glucose transport through GLUT-3 and regulate lipid metabolism in trophoblasts. Therefore, we hypothesize that decreased GPR1 signaling through inhibiting the pAKT signaling pathway mediates the placental expression of GLUT-3 in HFD-fed mice, leading to glucose homeostasis, and decreased GPR1 signaling disrupts lipid metabolism by reducing FABP4 expression.

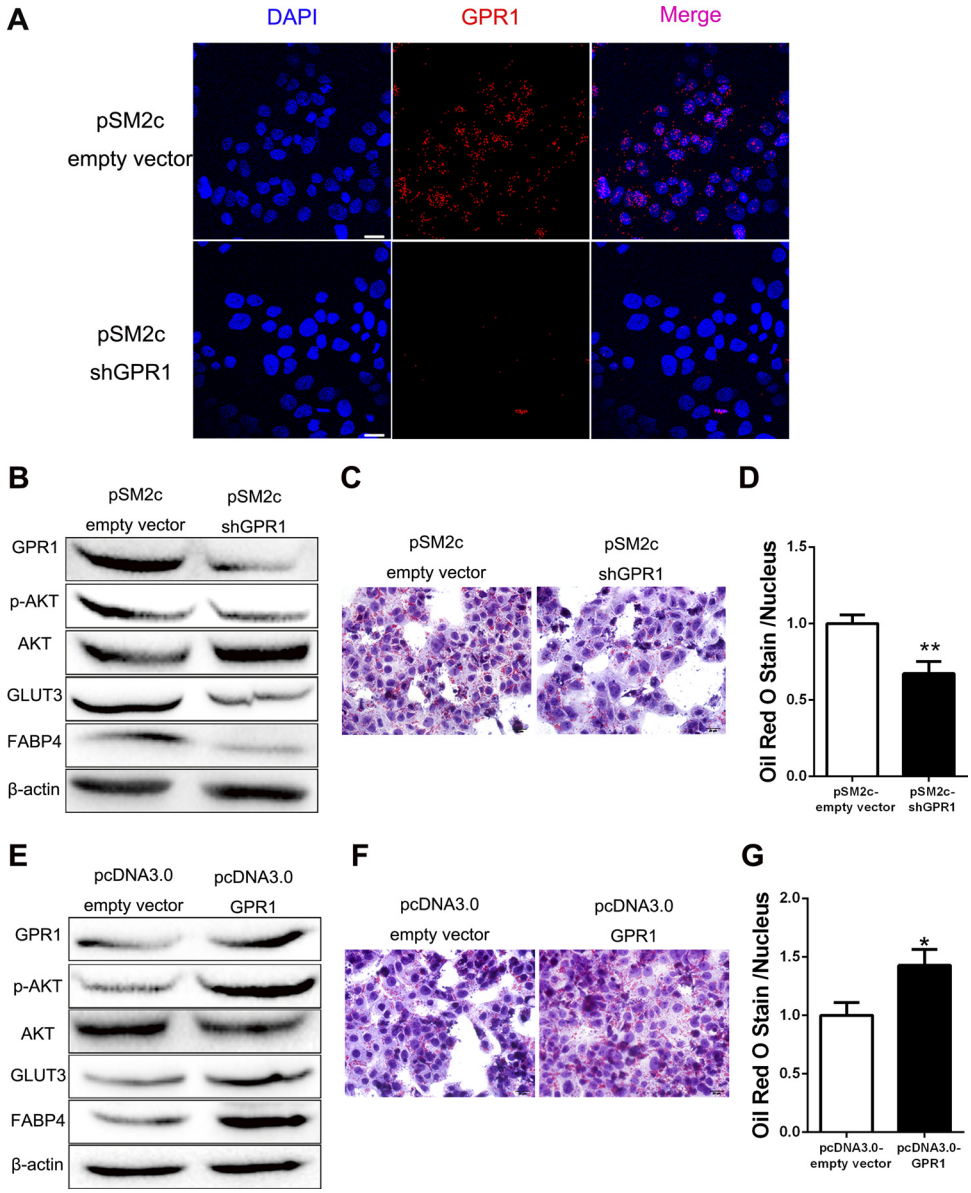

Fig. 6. G protein-coupled receptor 1 (GPR1) improved glucose transport and lipid accumulation in trophoblasts. *A*: the localization of GPR1 was analyzed in GPR1 knockdown JEG3 cell by confocal microscopy. Results are representative of three independent experiments. Scale bar, 25 μm. shGPR1, GPR1 short hairpin RNA. *B*: Western blotting analysis of GPR1, pAKT/AKT, glucose transport protein-3 (GLUT-3), and fatty acid binding protein 4 (FABP4) protein levels. Oil Red O staining analysis of lipid accumulation (*C*) and quantification (*D*) in the GPR1 knockdown JEG3 cells are shown. Scale bar: 20 μm. *E*: Western blotting analysis of GPR1, pAKT/AKT, GLUT-3, and FABP4 protein levels. Oil Red O staining analysis of lipid accumulation (*F*) and quantification (*G*) in the GPR1-overexpressed JEG3 cells are shown. Values are means ± SE from five independent experiments. $*P < 0.05$ and $**P < 0.01$ compared with controls.

GPR1 regulates glucose and lipid homeostasis in trophoblasts through pAKT. To further explore the GPR1 signaling pathway in energy homeostasis in trophoblasts, we studied the effect of AKT phosphorylation on this process by utilizing a pAKT inhibitor. We confirmed the efficiency of the inhibitor, and the AKT phosphorylation level was decreased with inhibitor treatment for 30 and 60 min in the GPR1-overexpressed cell line (Fig. 7*A*). GLUT-3 protein was decreased after the GPR1-overexpressed trophoblasts were supplemented with the inhibitor for 24 h compared with in the cells supplemented with DMSO (Fig. 7*B*). Lipid accumulation (Fig. 7*C*), similar to GLUT-3 protein expression, showed a significant decrease with inhibitor treatment compared with DMSO treatment via quantification of Oil Red O staining (Fig. 7*D*). These results suggested that GPR1 signaling may stimulate the pAKT pathway to regulate glucose and lipid homeostasis in trophoblast cells. Together, these results also supported our hypothesis that GPR1 signaling knockdown decreases AKT phosphorylation-controlled glucose homeostasis by reducing GLUT-3 expression and disrupts lipid metabolism by reducing FABP4 expression in the placenta.

DISCUSSION

In the present study, we observed that both chemerin and GPR1 displayed increasing trends in mouse placental tissue during pregnancy, and we used an HFD-fed maternal mouse model to study the effect of GPR1 on GDM. Meanwhile, chemerin mRNA expression levels were significantly increased in GDM patients compared with those with normal placentas, which is consistent with previously reported data that chemerin was significantly increased in serum from GDM women (26, 36). In addition, we found that GPR1 protein was decreased in GDM patient placentas. Furthermore, recent studies have revealed that GPR1 loss reduced insulin secretion and exacerbated glucose intolerance when using an HFD (28). These data suggested that chemerin/GPR1 signaling might participate in the pathogenic mechanism of GDM.

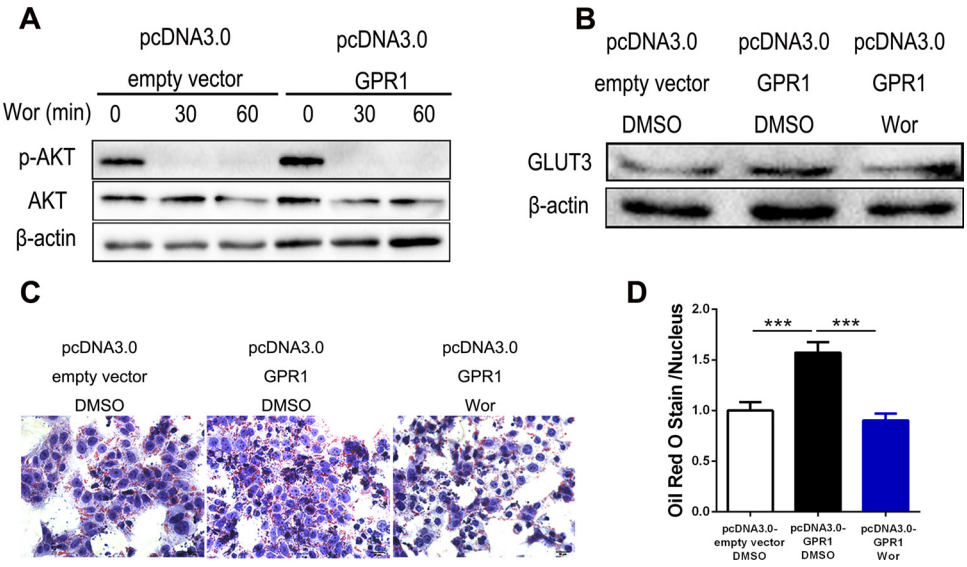

Fig. 7. G protein-coupled receptor 1 (GPR1) improved glucose transport and lipid accumulation in trophoblasts via pAKT. Western blotting analysis of pAKT/AKT (*A*) and glucose transport protein-3 (GLUT-3) protein levels (*B*) in GPR1-overexpressed JEG3 cells treated with wortmannin (Wor), a pAKT inhibitor, are shown. Oil Red O staining analysis of lipid accumulation (*C*) and quantification (*D*) in GPR1-overexpressed JEG3 cells treated with Wor are shown. Scale bar: 20 μm. Values are means ± SE from five independent experiments. ***$P < 0.001$ compared with controls.

Although, there are several animal models, including genetic- and chemical-induced models, GDM animal models are generated through altering the expression of several genes, including the leptin receptor, the PRL receptor, c-Met, and menin (25). However, GDM patients do not have these KO strain alterations that cause hyperglycemia (21). In general, the chemical regent streptozotocin can disrupt islet β-cell to result in a reduced level of insulin, which more closely approximates type 1 diabetes mellitus (30). Insulin resistance develops and results in a physiological compensation mechanism that proliferates β-cells and enhances insulin secretion during pregnancy (11). So, to simulate clinical phenotype, we studied the effect of GPR1 on GDM development combined diet (HFD) via using siGPR1 knockdown to replaced GPR1 KO in mice. Our results from the HFD-fed pregnant mouse model have shown notably impaired glucose tolerance and high serum insulin levels, which are consistent with clinical data and previously literature (21, 34). In addition, high circulating chemerin levels and decreased GPR1 mRNA levels were observed in the placentas of HFD-fed mice, also consistent with clinical studies.

Previously, studies revealed that several hormones, such as estrogen, PRL, and tumor necrosis factor-α, can regulate β-cell proliferation and insulin sensitivity (5, 11, 23). Interestingly, in our data, the mice with silenced GPR1 displayed decreased circulating estradiol (E2), and only those GPR1 knockdown mice that were fed with HFD showed decreased circulating PRL during pregnancy. Together, maternal HFD feeding could also increase PRL levels, which are secreted from the placenta, the necessary organ secreting hormones and maintaining fetal growth and development during pregnancy (24, 42). To thoroughly explore the role of GPR1 on GDM, we performed GPR1 mRNA knockdown through transfecting siRNA in vivo. Pregnant HFD-fed mice with silenced GPR1 expression showed markedly exacerbated glucose intolerance that resulted from decreased β-cell size and reduced expression of proliferation-related marker PCNA, leading to diminished β-cell proliferation and insulin secretion in the pancreas. However, pregnant chow-fed mice with silenced GPR1 expression did not show altered glucose homeostasis. These data suggested that GPR1 signaling plays a role in glucose homeostasis via cooperation with other organs or tissues.

Recently, significantly different gene profiles were found in GDM patient placenta tissue (22) as an alternative target organ for analysis of GDM (29). In this study, knockdown of GPR1 did not alter the mouse placental morphology; however, a HFD may impair placental development, as shown by a decrease in the labyrinth layer, which is consistent with previous reports that a HFD impacted placental development in mice (21, 27). Macrosomia is one of the risks of GDM development (11, 27), which is consistent with a significant increase in fetal weight shown in HFD-fed dams compared with that of chow-fed dams. Interestingly, the risks of GDM include not only macrosomia, but also intrauterine growth restriction, according to alterations in placenta glucose transport protein, which cause intrauterine growth restriction and increased placental expression of GLUT-3 (6, 19). In this study, we provided our insight about GLUT-3. Glucose is transported into cells by glucose transport proteins; therefore, we also detected the expression of GLUT-3 from the glucose transport protein family, and we found that placental GLUT-3 was significantly decreased when GPR1 was knocked down. Although there is no influence in chow-fed GPR1 knockdown mice, glucose intolerance was exacerbated, and decreased fetal weights were apparent in HFD-fed GPR1 knockdown mice. It was previously reported that GPR1 is involved in lipogenesis (18), which is consistent with lipid metabolism-related markers C/EBP-α, PPAR-γ, and FABP4 and with decreased serum and placental TG measures in GPR1 knockdown HFD-fed dams. The above results suggest that GPR1 plays a role in carbohydrate homeostasis during pregnancy. Previous studies showed that AKT regulates GLUT-3 regulation expression (9, 39). In vivo, we found that pAKT was decreased, which is consistent with the ability of GPR1 to increase pAKT expression in vitro. According to our study, GPR1 impacted GLUT-3 and FABP4 expression, leading to lipid accumulation via the pAKT/AKT signaling pathway. In summary, we provide the first direct evidence that chemerin/GPR1 signaling plays a role in carbohydrate metabolism by evaluating GPR1 knockdown in pregnant mice. The

above results suggest that a novel feedback mechanism may regulate the carbohydrate balance during pregnancy. With a thorough study of the mechanisms, GPR1 could be a potential target for the diagnosis and therapy of GDM.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

B.H., C.H., H.Z., W.Z., B.W., H.W., J.C., and T.X. performed experiments; B.H., C.H., H.W., J.C., T.X., J.N., and J.Z. analyzed data; B.H., H.Z., J.C., T.X., J.N., and J.Z. interpreted results of experiments; B.H. prepared figures; B.H., T.X., J.N., and J.Z. drafted manuscript; B.H., H.Z., H.W., J.C., T.X., J.N., and J.Z. edited and revised manuscript; B.H. approved final version of manuscript; J.N. and J.Z. conceived and designed research.

REFERENCES

1. **Alonso LC, Yokoe T, Zhang P, Scott DK, Kim SK, O'Donnell CP, Garcia-Ocaña A.** Glucose infusion in mice: a new model to induce beta-cell replication. *Diabetes* 56: 1792–1801, 2007. doi:10.2337/db06-1513.
2. **Banas M, Zegar A, Kwitniewski M, Zabieglo K, Marczynska J, Kapinska-Mrowiecka M, LaJevic M, Zabel BA, Cichy J.** The expression and regulation of chemerin in the epidermis. *PLoS One* 10: e0117830, 2015. doi:10.1371/journal.pone.0117830.
3. **Barnea G, Strapps W, Herrada G, Berman Y, Ong J, Kloss B, Axel R, Lee KJ.** The genetic design of signaling cascades to record receptor activation. *Proc Natl Acad Sci USA* 105: 64–69, 2008. doi:10.1073/pnas.0710487105.
4. **Bo S, Menato G, Lezo A, Signorile A, Bardelli C, De Michieli F, Massobrio M, Pagano G.** Dietary fat and gestational hyperglycaemia. *Diabetologia* 44: 972–978, 2001. doi:10.1007/s001250100590.
5. **Brelje TC, Stout LE, Bhagroo NV, Sorenson RL.** Distinctive roles for prolactin and growth hormone in the activation of signal transducer and activator of transcription 5 in pancreatic islets of langerhans. *Endocrinology* 145: 4162–4175, 2004. doi:10.1210/en.2004-0201.
6. **Brown K, Heller DS, Zamudio S, Illsley NP.** Glucose transporter 3 (GLUT3) protein expression in human placenta across gestation. *Placenta* 32: 1041–1049, 2011. doi:10.1016/j.placenta.2011.09.014.
7. **Chang SS, Eisenberg D, Zhao L, Adams C, Leib R, Morser J, Leung L.** Chemerin activation in human obesity. *Obesity (Silver Spring)* 24: 1522–1529, 2016. doi:10.1002/oby.21534.
8. **Chu X, Liu L, Na L, Lu H, Li S, Li Y, Sun C.** Sterol regulatory element-binding protein-1c mediates increase of postprandial stearic acid, a potential target for improving insulin resistance, in hyperlipidemia. *Diabetes* 62: 561–571, 2013. doi:10.2337/db12-0139.
9. **Dasgupta S, Rai RC.** PPAR-γ and Akt regulate GLUT1 and GLUT3 surface localization during Mycobacterium tuberculosis infection. *Mol Cell Biochem* 440: 127–138, 2018. doi:10.1007/s11010-017-3161-3.
10. **De Henau O, Degroot GN, Imbault V, Robert V, De Poorter C, Mcheik S, Galés C, Parmentier M, Springael JY.** Signaling properties of chemerin receptors CMKLR1, GPR1 and CCRL2. *PLoS One* 11: e0164179, 2016. doi:10.1371/journal.pone.0164179.
11. **Demirci C, Ernst S, Alvarez-Perez JC, Rosa T, Valle S, Shridhar V, Casinelli GP, Alonso LC, Vasavada RC, García-Ocana A.** Loss of HGF/c-Met signaling in pancreatic β-cells leads to incomplete maternal β-cell adaptation and gestational diabetes mellitus. *Diabetes* 61: 1143–1152, 2012. doi:10.2337/db11-1154.
12. **García-Patterson A, Erdozain L, Ginovart G, Adelantado JM, Cubero JM, Gallo G, de Leiva A, Corcoy R.** In human gestational diabetes mellitus congenital malformations are related to pre-pregnancy body mass index and to severity of diabetes. *Diabetologia* 47: 509–514, 2004. doi:10.1007/s00125-004-1337-3.
13. **Gözükara YM, Aytan H, Ertunc D, Tok EC, Demirtürk F, Şahin Ş, Aytan P.** Role of first trimester total testosterone in prediction of subsequent gestational diabetes mellitus. *J Obstet Gynaecol Res* 41: 193–198, 2015. doi:10.1111/jog.12525.
14. **Gruben N, Aparicio Vergara M, Kloosterhuis NJ, van der Molen H, Stoelwinder S, Youssef S, de Bruin A, Delsing DJ, Kuivenhoven JA, van de Sluis B, Hofker MH, Koonen DP.** Chemokine-like receptor 1 deficiency does not affect the development of insulin resistance and nonalcoholic fatty liver disease in mice. *PLoS One* 9: e96345, 2014. doi:10.1371/journal.pone.0096345.
15. **Huang BB, Liu XC, Qin XY, Chen J, Ren PG, Deng WF, Zhang J.** Effect of high-fat diet on immature female mice and messenger and noncoding RNA expression profiling in ovary and white adipose tissue. *Reprod Sci*. In press. doi:10.1177/1933719118765966.
16. **Huang C, Snider F, Cross JC.** Prolactin receptor is required for normal glucose homeostasis and modulation of beta-cell mass during pregnancy. *Endocrinology* 150: 1618–1626, 2009. doi:10.1210/en.2008-1003.
17. **Huang C, Wang M, Ren L, Xiang L, Chen J, Li M, Xiao T, Ren P, Xiong L, Zhang JV.** CMKLR1 deficiency influences glucose tolerance and thermogenesis in mice on high fat diet. *Biochem Biophys Res Commun* 473: 435–441, 2016. doi:10.1016/j.bbrc.2016.03.026.
18. **Huang J, Zhang J, Lei T, Chen X, Zhang Y, Zhou L, Yu A, Chen Z, Zhou R, Yang Z.** Cloning of porcine chemerin, ChemR23 and GPR1 and their involvement in regulation of lipogenesis. *BMB Rep* 43: 491–498, 2010. doi:10.5483/BMBRep.2010.43.7.491.
19. **Janzen C, Lei MYY, Cho J, Sullivan P, Shin BC, Devaskar SU.** Placental glucose transporter 3 (GLUT3) is up-regulated in human pregnancies complicated by late-onset intrauterine growth restriction. *Placenta* 34: 1072–1078, 2013. doi:10.1016/j.placenta.2013.08.010.
20. **Li Y, Cooper A, Odibo IN, Ahmed A, Murphy P, Koonce R, Dajani NK, Lowery CL, Roberts DJ, Maroteaux L, Kilic F.** Discrepancy in Insulin Regulation between Gestational Diabetes Mellitus (GDM) Platelets and Placenta. *J Biol Chem* 291: 9657–9665, 2016. doi:10.1074/jbc.M116.713693.
21. **Liang C, DeCourcy K, Prater MR.** High-saturated-fat diet induces gestational diabetes and placental vasculopathy in C57BL/6 mice. *Metabolism* 59: 943–950, 2010. doi:10.1016/j.metabol.2009.10.015.
22. **Magee TR, Ross MG, Wedekind L, Desai M, Kjos S, Belkacemi L.** Gestational diabetes mellitus alters apoptotic and inflammatory gene expression of trophobasts from human term placenta. *J Diabetes Complications* 28: 448–459, 2014. doi:10.1016/j.jdiacomp.2014.03.010.
23. **Nadal A, Alonso-Magdalena P, Soriano S, Ropero AB, Quesada I.** The role of oestrogens in the adaptation of islets to insulin resistance. *J Physiol* 587: 5031–5037, 2009. doi:10.1113/jphysiol.2009.177188.
24. **O'Neil DS, Stewart CJ, Chu DM, Goodspeed DM, Gonzalez-Rodriguez PJ, Shope CD, Aagaard KM.** Conditional postnatal deletion of the neonatal murine hepatic circadian gene, Npas2, alters the gut microbiome following restricted feeding. *Am J Obstet Gynecol* 217: 218.e1–218.e15, 2017. doi:10.1016/j.ajog.2017.03.024.
25. **Pasek RC, Gannon M.** Advancements and challenges in generating accurate animal models of gestational diabetes mellitus. *Am J Physiol Endocrinol Metab* 305: E1327–E1338, 2013. doi:10.1152/ajpendo.00425.2013.
26. **Pfau D, Stepan H, Kratzsch J, Verlohren M, Verlohren HJ, Drynda K, Lössner U, Blüher M, Stumvoll M, Fasshauer M.** Circulating levels of the adipokine chemerin in gestational diabetes mellitus. *Horm Res Paediatr* 74: 56–61, 2010. doi:10.1159/000282114.
27. **Qiao L, Guo Z, Bosco C, Guidotti S, Wang Y, Wang M, Parast M, Schaack J, Hay WW Jr, Moore TR, Shao J.** Maternal high-fat feeding increases placental lipoprotein lipase activity by reducing SIRT1 expression in mice. *Diabetes* 64: 3111–3120, 2015. doi:10.2337/db14-1627.
28. **Rourke JL, Muruganandan S, Dranse HJ, McMullen NM, Sinal CJ.** Gpr1 is an active chemerin receptor influencing glucose homeostasis in obese mice. *J Endocrinol* 222: 201–215, 2014. doi:10.1530/JOE-14-0069.
29. **Roverso M, Berté C, Di Marco V, Lapolla A, Badocco D, Pastore P, Visentin S, Cosmi E.** The metallome of the human placenta in gestational diabetes mellitus. *Metallomics* 7: 1146–1154, 2015. doi:10.1039/C5MT00050E.

30. **Ryan EA, Tobin BW, Tang J, Finegood DT.** A new model for the study of mild diabetes during pregnancy. Syngeneic islet-transplanted STZ-induced diabetic rats. *Diabetes* 42: 316–323, 1993. doi:10.2337/diab.42.2.316.
31. **Shao J, Yamashita H, Qiao L, Draznin B, Friedman JE.** Phosphatidylinositol 3-kinase redistribution is associated with skeletal muscle insulin resistance in gestational diabetes mellitus. *Diabetes* 51: 19–29, 2002. doi:10.2337/diabetes.51.1.19.
32. **Strakovsky RS, Pan YX.** A decrease in DKK1, a WNT inhibitor, contributes to placental lipid accumulation in an obesity-prone rat model. *Biol Reprod* 86: 81, 2012. doi:10.1095/biolreprod.111.094482.
33. **Takahashi M, Okimura Y, Iguchi G, Nishizawa H, Yamamoto M, Suda K, Kitazawa R, Fujimoto W, Takahashi K, Zolotaryov FN, Hong KS, Kiyonari H, Abe T, Kaji H, Kitazawa S, Kasuga M, Chihara K, Takahashi Y.** Chemerin regulates β-cell function in mice. *Sci Rep* 1: 123, 2011. doi:10.1038/srep00123.
34. **Wang C, Wei Y, Zhang X, Zhang Y, Xu Q, Sun Y, Su S, Zhang L, Liu C, Feng Y, Shou C, Guelfi KJ, Newnham JP, Yang H.** A randomized clinical trial of exercise during pregnancy to prevent gestational diabetes mellitus and improve pregnancy outcome in overweight and obese pregnant women. *Am J Obstet Gynecol* 216: 340–351, 2017. doi:10.1016/j.ajog.2017.01.037.
35. **Yang W, Wagener J, Wolf N, Schmidt M, Kimmig R, Winterhager E, Gellhaus A.** Impact of CCN3 (NOV) glycosylation on migration/invasion properties and cell growth of the choriocarcinoma cell line Jeg3. *Hum Reprod* 26: 2850–2860, 2011. doi:10.1093/humrep/der239.
36. **Yang X, Quan X, Lan Y, Ye J, Wei Q, Yin X, Fan F, Xing H.** Serum chemerin level during the first trimester of pregnancy and the risk of gestational diabetes mellitus. *Gynecol Endocrinol* 33: 770–773, 2017. doi:10.1080/09513590.2017.1320382.
37. **Yang YL, Ren LR, Sun LF, Huang C, Xiao TX, Wang BB, Chen J, Zabel BA, Ren P, Zhang JV.** The role of GPR1 signaling in mice corpus luteum. *J Endocrinol* 230: 55–65, 2016. doi:10.1530/JOE-15-0521.
38. **Yao G, Zhang Y, Wang D, Yang R, Sang H, Han L, Zhu Y, Lu Y, Tan Y, Shang Z.** GDM-induced macrosomia is reversed by Cav-1 via AMPK-mediated fatty acid transport and GLUT1-mediated glucose transport in placenta. *PLoS One* 12: e0170490, 2017. doi:10.1371/journal.pone.0170490.
39. **Yu J, Li J, Zhang S, Xu X, Zheng M, Jiang G, Li F.** IGF-1 induces hypoxia-inducible factor 1α-mediated GLUT3 expression through PI3K/Akt/mTOR dependent pathways in PC12 cells. *Brain Res* 1430: 18–24, 2012. doi:10.1016/j.brainres.2011.10.046.
40. **Yu Q, Qiu Y, Wang X, Tang J, Liu Y, Mei L, Li M, Yang M, Tang L, Gao H, Zhang Z, Xu W, He Q.** Efficient siRNA transfer to knockdown a placenta specific lncRNA using RGD-modified nano-liposome: a new preeclampsia-like mouse model. *Int J Pharm* 546: 115–124, 2018. doi:10.1016/j.ijpharm.2018.05.001.
41. **Zabel BA, Nakae S, Zúñiga L, Kim JY, Ohyama T, Alt C, Pan J, Suto H, Soler D, Allen SJ, Handel TM, Song CH, Galli SJ, Butcher EC.** Mast cell-expressed orphan receptor CCRL2 binds chemerin and is required for optimal induction of IgE-mediated passive cutaneous anaphylaxis. *J Exp Med* 205: 2207–2220, 2008. doi:10.1084/jem.20080300.
42. **Zhang H, Zhang J, Pope CF, Crawford LA, Vasavada RC, Jagasia SM, Gannon M.** Gestational diabetes mellitus resulting from impaired beta-cell compensation in the absence of FoxM1, a novel downstream effector of placental lactogen. *Diabetes* 59: 143–152, 2010. doi:10.2337/db09-0050.