High levels of chorionic gonadotrophin attenuate insulin sensitivity and promote inflammation in adipocytes

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Abstract

Gestational diabetes mellitus (GDM) presents with moderate inflammation, insulin resistance and impaired glucose uptake, which may result from increased maternal fat mass and increased circulation of placental hormones and adipokines. In this study, we set out to test whether the surge in chorionic gonadotrophin (CG) secretion is a cause of inflammation and impaired insulin sensitivity in GDM. We first found that LH/chorionic gonadotrophin receptors (CG/LHR) were expressed at low levels in insulin-sensitive murine 3T3-L1 adipocytes and murine C2C12 myocytes. CG treatment not only directly reduced insulin-responsive gene expression, including that of glucose transporter 4 (GLUT4), but also impaired insulin-stimulated glucose uptake in 3T3-L1 cells. Moreover, CG treatment increased the expression of the proinflammatory cytokine monocyte chemotactic protein 1 (MCP1) and upregulated nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) activity in 3T3-L1 cells. Clinically, pregnant women who had higher CG levels and elevated MCP1 developed GDM. Above all, apart from prepregnancy BMI and MCP1 level, CG level was associated with abnormal glucose tolerance. In summary, our findings confirmed that higher CG levels in pregnancy possibly played a role in GDM development partly by impairing the functions of insulin, such as glucose uptake, while promoting inflammation in adipocyte.

Key Words
- chorionic gonadotrophin (CG)
- gestational diabetes mellitus (GDM)
- 3T3-L1 cells
- insulin resistance
- proinflammation

Introduction

Gestational diabetes mellitus (GDM) affects 2–5% of pregnant women (Gilmartin et al. 2008), which is characterised by maternal peripheral insulin resistance (IR) and increased inflammation. Increased amounts of placental hormones, such as progesterone, cortisol, prolactin and placental lactogen, induce IR (Ryan & Enns 1988). Moreover, increased maternal fat mass and elevated adipokine also play a role (Catalano et al. 1998, Kirwan et al. 2002).
Results of previous studies indicated that moderate inflammation during pregnancy is correlated with IR and GDM (Wolf et al. 2004, Kinalska et al. 2005, de Castro et al. 2011).

Chorionic gonadotrophin (CG) is produced by the placenta and binds to the luteinising hormone/chorionic gonadotrophin receptor (CG/LHR), a transmembrane glycoprotein receptor that belongs to the G-protein-coupled receptor superfamily (Loosfelt et al. 1989, McFarland et al. 1989). CG is essential for the maintenance of human pregnancy by stimulating progesterone production in the corpus luteum. Blood CG levels are known to exponentially increase during the first 2 months of pregnancy (Cole 2010). Furthermore, elevated CG levels are also a useful predictor of pregnancy-related diseases, including gestational hypertension, preeclampsia, intrauterine growth restriction and GDM (Heikkila et al. 2001, Merviel et al. 2001, Androutsopoulos et al. 2009), which indicates that CG may have extragonadal functions. Accumulating evidence indicates that several non-gonadal tissues contain low levels of functional CG/LHR and that the non-gonadal actions of LH/C冈 are physiologically important (Rao 2001, Abdallah et al. 2004). Results described in previous reports have indicated that CG/LHR is expressed in human primary adipocytes and 3T3-L1 adipocytes, CG treatment influences preadipocyte differentiation and leptin secretion (Sivan et al. 1998, Dos Santos et al. 2007). However, up till now, it remains unclear whether the surge in CG secretion with GDM contributes to IR and inflammation through functional CG/LHR in non-gonadal tissues. In this study, we confirmed that CG can influence insulin sensitivity and induce inflammation by the CG/LHR expressed in adipocytes, which possibly contributes to GDM development.

Materials and methods

Patients

In this study, a standard 75 g oral glucose tolerance test (OGTT) was performed on pregnant women with fasting plasma glucose of 4.4 mmol/l or greater at 24–28 weeks of gestation. Participants who visited peripheral antenatal clinics and underwent an OGTT were selected according to the inclusion criteria as follows: i) pregnant Han Chinese women; ii) 25–35 years old; iii) primipara and iv) singleton pregnancy. Exclusion criteria included the following: i) trophoblastic diseases; ii) triploidies and iii) multiple pregnancies. The diagnosis of GDM was based on the new criteria established by the American Diabetes Association (2011). GDM was diagnosed when one or more of the following plasma levels were abnormal: fasting plasma glucose level ≥ 5.1 mmol/l; 1 h glucose level ≥ 10.0 mmol/l; or 2 h glucose level ≥ 8.5 mmol/l. A total of 42 patients with GDM and 47 controls with normal glucose tolerance (NGT) as planned were enrolled in this study. None of the GDM patients received any treatment before the test. The study protocol was approved by the Research Ethics Board of the International Peace Maternity and Child Health Hospital, and all participants provided their written informed consent.

Plasma samples

After an overnight fast, venous plasma samples were collected to determine fasting glucose levels and were collected again 1 and 2 h after oral ingestion of 75 g glucose. Plasma glucose was estimated by the glucose hexokinase method. The level of CG (Anogen, Mississauga, Ontario, Canada) and monocytechemotactic protein 1 (MCP1) (R&D Systems, Minneapolis, MN, USA) was measured by ELISA assays according to the manufacturer’s protocol.

Material

3-Isobutyl-1-methylxanthine (IBMX), dexamethasone, H89, SP600125, PD98059 and BMS345541 were obtained from Sigma. CG was purchased from Calbiochem (Darmstadt, Germany) and insulin from Eli Lilly.

Cell culture

3T3-L1 cells were grown in DMEM supplemented with 10% foetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5% CO₂. Two days after the cells reached confluence, the medium was supplemented for 2 days with 0.5 mmol/l IBMX, 1.7 µmol/l insulin and 1 µmol/l dexamethasone for adipocyte differentiation. From day 3 on, the cells were maintained with DMEM, 10% serum and 1.7 µmol/l insulin. The medium was renewed every 2 days until the end of the experiment. C2C12 cells, a mouse myoblast cell line, were cultured in DMEM with 10% FBS at 37 °C in a humidified atmosphere of 5% CO₂. When the cells approached 80–90% confluence, the growth medium was replaced with a differentiation medium consisting of DMEM supplemented with 2% horse serum to initiate differentiation. The medium was changed every other day until the cells were differentiated. After 1 h pretreatment with the protein kinase A (PKA) inhibitor H89 (20 µmol/l), MEK inhibitor PD98059 (25 µmol/l), c-Jun N-terminal kinases (JNK) inhibitor SP600125 (20 µmol/l) or I κB kinase (IKK) inhibitor BMS345541 (10 µmol/l), 3T3-L1 adipocytes were treated with 5000 mIU/ml CG for 24 h.
Western blotting analysis

3T3-L1 cells were harvested and lysed in RIPA buffer containing proteinase and phosphatase inhibitors. RIPA buffer is composed of 1× PBS, 1% NP40, 0.1% SDS, 5 mM EDTA and 0.5% sodium deoxycholate. After the protein concentration was measured by the Bradford assay, protein samples were separated on a 10% polyacrylamide gel and transferred onto a nitrocellulose membrane. The membranes were blocked using 2% (w/v) BSA and incubated overnight at 4°C with anti-GLUT4, anti-MCP1, anti-IKKβ and anti-tubulin antibodies (CST, Danvers, MA, USA), or anti-CG/LHR, anti-P65 and anti-Lamin B antibodies (Santa Cruz). The membranes were then incubated for at least 1 h at room temperature with HRP-conjugated secondary antibody (Dako, DK-2600, Glostrup, Denmark). The signal was detected using enhanced chemiluminescence (PerkinElmer, Waltham, MA, USA). Densitometry was conducted directly on the blotted membrane using a CCD camera system (LAS-4000, Fujifilm, Tokyo, Japan).

RT-PCR and real-time PCR

Total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. Samples (1 μg) of RNA were reverse transcribed to cDNA using anchored oligonucleotide (dT)15 primers and SuperScript II Reverse Transcriptase (Invitrogen). The resulting cDNA was used as a template for PCR amplification. PCR was performed with an ABI Prism 7300 instrument (Applied Biosystems) in 96-well plates using the SYBR Premix Ex Taq Kit (Takara, Otsu, Shiga, Japan) according to the manufacturer’s instructions. Gene expression was analysed by relative quantification with the 2−ΔΔCt method. Quantification was performed in quadruplicate, and the experiments were repeated independently three times. The sequences of the primers are listed in Table 1. The levels of target genes were normalised to beta actin (Actb, ID: 11461), and the results are expressed as fold changes of the threshold cycle (Ct) values relative to the controls. All of the primer sets were designed to span at least one intron to avoid amplification of genomic DNA.

Table 1 Primer pairs used for quantitative-PCR

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sense</th>
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<tr>
<td>Glut4 (Slc2a4, ID: 20528)</td>
<td>GCCCCCACAGAAGGTGATTG</td>
</tr>
<tr>
<td>Akt (Akt1, ID: 11651)</td>
<td>GAAGCAGCTAGCCACTTGAAG</td>
</tr>
<tr>
<td>Ile6 (Ile6, ID: 16193)</td>
<td>TCTCTGGGAAATCGTGGAAATG</td>
</tr>
<tr>
<td>Mcp1 (Ccl2, ID: 20296)</td>
<td>TCCAGTTGAGAATCTGGAAGAT</td>
</tr>
<tr>
<td>Pai1 (Serpinel, ID: 18787)</td>
<td>AGGTCCCTGTCATGCTTCTGG</td>
</tr>
<tr>
<td>Adipoq (Adipq, ID: 11450)</td>
<td>GACTTCGAGCAGGAGATGGC</td>
</tr>
<tr>
<td>B actin (Actb, ID: 11461)</td>
<td>CTATGAGCAGCATGGGG</td>
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2-Deoxyglucose uptake assay

Glucose transport was determined by measuring uptake of [3H]2-deoxyglucose (DOG) (PerkinElmer). 3T3-L1 cells were cultured in 24-well plates until over 90% of cells displayed an adipocyte phenotype. The cells were then treated with CG or vehicle for 24 h. The transport assay was initiated by washing the cells twice with transport solution (Krebs Ringer’s phosphate buffer). The cells were

Transfections and luciferase assays

The nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) reporter plasmid was constructed using a pGL3 reporter plasmid (Promega) and an NF-κB consensus sequence (5'-GGAGCTTTCC-3'). Transient transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. Briefly, HEK293 cells were seeded into 24-well plates and transfected with 900 ng pcDNA 3.1 human LHR plasmid, 300 ng NF-κB luciferase reporter and 3 ng SV40 (Promega) at 40–50% confluence. After 24 h, the cells were treated with CG or vehicle for an additional 24 h. The cells were then collected, and luciferase assays were conducted using a Dual-Luciferase Reporter Assay System (Promega) following the manufacturer’s instructions. Data are reported as the mean value of at least three independent experiments.

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then incubated in the transport solution for another 15 min in the presence or absence of 100 nM insulin at 37 °C, followed by 0.5 μCi [3H]2-DOG for 30 min. After incubation, the cells were quickly washed three times with ice-cold PBS containing 10 mM glucose. They were then lysed in 0.1 mol/l NaOH and subsequently solubilised in scintillation fluid overnight. [3H]2-DOG was measured using a liquid scintillation counter.

### Statistical analyses

All data are expressed as the mean ± S.D. Student’s t-test or ANOVA analysis followed by Bonferroni’s post-test was used for comparison between groups, as appropriate. Stepwise multiple linear regression analysis was performed to assess the associations between CG concentrations and glucose levels. Statistical analysis was performed using a SAS system (version 9.3; SAS Institute, Inc., Cary, NC, USA). Differences with \( P < 0.05 \) were considered to be statistically significant.

### Results

#### CG/LH receptors are expressed at low levels in insulin-sensitive mouse cells

In a previous study, the CG/LHR was detected in human adipose tissue (Dos Santos et al. 2007). In our study, we first analysed the expression of the CG/LHR in insulin-sensitive cells (3T3-L1 adipocytes and C2C12 myoblasts) by RT-PCR. As shown in Fig. 1A, the \( Lhr \) gene was expressed at a low level in 3T3-L1 adipocytes and C2C12 myoblasts. Western blotting analysis further demonstrated the expression of CG/LHR protein in these cells. In mouse adipocytes and myoblasts, the molecular mass of CG/LHR was approximately 80–90 kDa, which was the same as observed in the testis. Moreover, mature 3T3-L1 adipocytes and C2C12 myoblasts expressed more CG/LHR protein than undifferentiated precursor cells (Fig. 1B).

The expression of the CG/LHR in the insulin-sensitive cells indicated that CG may affect insulin sensitivity. We then observed the effect of CG on mature adipocytes. By quantitative PCR, we found that the gene expression of adiponectin (\( Adipoq \), ID: 11450), glucose transporter 4 (\( Glut4 \), Slc2a4, ID: 20528) and protein kinase B (\( Akt \), \( Akt1 \), ID: 11651) was reduced by CG. After 6 h CG incubation, expression of \( Adipoq \) decreased to approximately 80% of control levels with 5000 mIU/ml CG treatment, while expression of \( Akt \) decreased to 88% with 500 mIU/ml and 53% with 5000 mIU/ml CG treatment in 3T3-L1 cells. Notably, expression of \( Glut4 \) mRNA decreased to 72% of control levels with 500 mIU/ml and 40% with 5000 mIU/ml CG treatment (Fig. 2A). Moreover, inhibition of \( Akt \) and \( Glut4 \) sustained for 24 h (Fig. 2B). As expression of \( Glut4 \) was inhibited more significantly, we further investigated GLUT4 protein expression after incubation with different doses of CG for 24 h. Results of western blotting analysis indicated that GLUT4 protein expression was suppressed by the higher doses of CG (Fig. 2C). GLUT4 is an insulin-regulated glucose transporter found in adipose and muscle tissue (James et al. 1988, Watson et al. 2004). We thus evaluated insulin-stimulated glucose uptake by 3T3-L1 adipocytes. The results of the 2-DOG uptake assay indicated that insulin treatment stimulated a sevenfold increase in glucose uptake compared with PBS treatment; however, after pretreating with CG for 24 h, insulin increased glucose uptake by merely 5.7-fold, a decrease by approximately 20% compared with the control group (Fig. 2D). Taken together, these results indicated that CG attenuates insulin sensitivity in adipocytes in part by influencing insulin-responsive genes and impairing insulin-stimulated glucose uptake.

#### CG modulates proinflammatory cytokines in 3T3-L1 adipocytes

To our knowledge, chronic inflammation contributes to IR in pregnancy. Though CG attenuates the insulin-sensitive mouse cells.
response in 3T3-L1 adipocytes, its effects on adipocyte inflammation remain unknown. To evaluate adipocyte inflammation, we quantified proinflammatory cytokine expression in 3T3-L1 adipocytes following 6 or 24 h of CG treatment. As shown in Fig. 3, expression of plasminogen activator inhibitor 1 (Pai1, Serpine1, ID: 18787) and interleukin 6 (Il6, ID: 16193) was not altered after CG treatment. However, Mcp1 (Ccl2, ID: 20296) was induced by 500 and 5000 mIU/ml of CG, increasing by approximately 20% compared with control levels after 6 h (Fig. 3A) and 60% after 24 h (Fig. 3B) of treatment. We further investigated the effects of a 24-h CG treatment on MCP1 protein expression and found that CG treatment upregulated MCP1 protein levels, even at a lower dose (Fig. 3C). Our present results indicated that CG primarily stimulates adipocytes to produce MCP1, which further recruits macrophages to exacerbate inflammation.

**CG in 3T3-L1 adipocytes functions partly by activating the IKKB/NF-κB signalling pathway**

The classical CG signal transduction pathway involves activation of the adenylate cyclase cAMP protein kinase A (AC cAMP PKA) pathway (Segaloff & Ascoli 1993). In addition, it has been reported that the mitogen activated protein kinase (MAPK) pathway may be an important signalling pathway in non-gonadal sites expressing CG/LHR (Rao 2001). To investigate these signalling pathways in 3T3-L1 adipocytes, we quantified proinflammatory cytokine expression in 3T3-L1 adipocytes following 6 or 24 h of CG treatment.
the CG-induced decrease in GLUT4 expression, whereas the increase in MCP1 was not influenced (Fig. 4A). These results indicated that the AC cAMP PKA pathway partially mediates the CG-induced inhibition of GLUT4. In addition, we tested whether the inflammatory NF-κB or c-Jun N-terminal kinase (JNK) signalling pathways mediated the effect of CG on 3T3-L1 cells. Interestingly, as shown in Fig. 4A, pretreatment with the IκB kinase (IKK) inhibitor BMS345541 blocked CG-induced suppression of GLUT4 protein but did not block upregulation of MCP1, which indicated that NF-κB signalling is a potential mechanism for CG activity in 3T3-L1 cells. Activation of NF-κB signalling by CG was further tested by luciferase assays. When HEK293 cells were co-transfected with an NF-κB luciferase reporter and pcDNA 3.1 CG/LH receptor, the activity of NF-κB was upregulated by CG in a dose-dependent manner (Fig. 4B); however, when transfected with the NF-κB luciferase reporter alone, CG could not activate NF-κB signalling (results not shown). Moreover, we observed that IKKβ phosphorylation in 3T3-L1 adipocytes was directly promoted by 60 min of 5000 mIU/ml CG treatment (Fig. 4C). P65 nuclear translocation was also increased after 2 h of CG treatment (Fig. 4D). In summary, these results strongly indicate that, apart from the AC cAMP PKA pathway and MAPK pathway, NF-κB signalling is an important pathway for the function of CG in 3T3-L1 adipocytes.

### Elevated CG levels in pregnant women with GDM are associated with abnormal glucose tolerance

A total of 89 pregnant women were included in our final study: 42 patients were GDM and 47 patients had NGT. As listed in Table 2, glucose levels, including fasting and 1- and 2-h post glucose load, were higher in GDM. However, there was no significant difference in the age of the two groups (28.85 ± 2.37 versus 29.25 ± 2.26). No significant association was found between age and fasting, 1- and 2-h glucose levels. Moreover, prepregnancy BMI in GDM subjects was higher than that of NGT subjects (25.83 ± 2.7 versus 23.85 ± 2.62), and prepregnancy BMI was associated with fasting (P < 0.0001) and 1-h (P = 0.002) glucose levels (Table 3). Results of ELISA assays also indicated that MCP1, a key factor in the recruitment and activation of peripheral blood leukocytes, was elevated in GDM woman compared with healthy pregnant woman (83.71 ± 21.1 versus 100.72 ± 32.82 pg/ml), which was consistent with previous report (Klein et al. 2008). Furthermore, MCP1 levels were associated with abnormal 1- (P = 0.025) and 2-h (P = 0.008) post glucose load (Table 3). Interestingly, the elevated CG levels in pregnancy were notably higher in pathways in adipocytes, 3T3-L1 cells were pretreated with the PKA inhibitor H89 or the mitogen-activated protein kinase kinase (MEK) inhibitor PD98059 before 24 h of treatment with 5000 mIU/ml CG. Results of western blot analysis indicated that preincubation with H89 blocked

Figure 3

CG modulates pro-inflammatory cytokines in 3T3-L1 adipocytes. (A and B) Gene expression of Mcp1, Papi and Ile in mature 3T3-L1 adipocytes was measured by quantitative PCR after incubation with 500 or 5000 mIU/ml CG for 6 h (A) and 24 h (B). *P < 0.05; **P < 0.01. Relative mRNA levels were normalised to β actin and compared with those for the untreated control. Data are presented as means ± S.D. of three independent experiments. (C) MCP1 protein expression was stimulated by CG in a dose-dependent manner. After incubation with 100 mIU/ml and 10,000 mIU/ml CG, MCP1 protein levels were evaluated by western blotting. MCP1 expression was normalised to tubulin. Bands were quantified by densitometry using the Quantity One system. Protein levels are expressed as percentages relative to the control value. Data are presented as means ± S.D. of three independent experiments, *P < 0.05; **P < 0.01, compared with control.
the GDM than in the NGT group (144 668.8 versus 63 999.04 G vs. 63 999.04 G vs. 54 871.3 mIU/ml), in particular, CG levels were associated with abnormal 1-h post glucose load (P<0.006) (Table 3). These clinical data further supported the hypothesis that higher CG may contribute to inflammation and IR in GDM.

Discussion

CG is indispensable for maintenance of human pregnancy as well as gametogenesis and production of gonadal sex steroids (Pierce & Parsons 1981, Cole 2010). Results described in recent publications have indicated that CG/LHR is expressed in a variety of extragonadal tissues and that CG has extragonadal functions (Rao 2001, Abdallah et al. 2004). In particular, CG may either stimulate (Czerwiec et al. 1989, Horiuchi et al. 2000) or inhibit (Ku et al. 2002, Rao et al. 2004) the growth of different cell types. CG has also been characterised as a novel angiogenic factor in human endothelial cells (Zygmont et al. 2002). Dos Santos et al. (2007) reported that CG/LHR is expressed in human primary adipocytes and that the proadipogenic effect of CG in human preadipocytes leads to the increased fat storage that occurs during the first trimester of pregnancy. In this study, we first confirmed that mature 3T3-L1 adipocytes and C2C12
cells express CG/LHR, which indicates a functional role for CG in these insulin-sensitive cells. Furthermore, we observed that the expression of insulin-responsive genes, including ADIPOQ, AKT and GLUT4, was suppressed by CG treatment of mature 3T3-L1 cells. ADIPOQ, an important adipokine, is specifically enriched in adipose tissue and directly sensitises the body to insulin (Kadowaki et al. 2006). In GDM pregnancies, ADIPOQ levels decrease independently from maternal BMI or insulin sensitivity (Kinalski et al. 2005, Ategbo et al. 2006). Our results indicate that higher levels of CG might be an important reason for the decreased ADIPOQ in patients with GDM. Moreover, previous results confirm that decreased expression of GLUT4, an insulin-regulated glucose transporter, results in impaired insulin-stimulated glucose transport (James et al. 1988, Watson et al. 2004). In our study, CG decreased GLUT4 levels so as to influence insulin-stimulated glucose uptake in 3T3-L1 adipocytes. In addition, AKT, a modulator of insulin signalling that is important for the maintenance of normal glucose homeostasis (Cho et al. 2001) was also downregulated by CG. Taking these results together, higher level of CG decreased the expression of insulin-responsive genes and reduced insulin-stimulated glucose uptake in 3T3-L1 adipocytes, which indicated that CG may impair insulin sensitivity in vivo to induce the development of GDM, in view of higher CG in pregnancy and GDM.

Inflammation in pregnancy is correlated with IR in GDM (Wolf et al. 2004, Korkmazer & Solak 2014). As inflammatory cytokines, such as TNF-α (Hotamisligil et al. 1993), IL6 (Rotter et al. 2003) and PAI1 (Shimomura et al. 1996) and chemokines such as MCP1 (Sartipy & Loskutoff 2003, Kanda et al. 2006) are expressed in adipose tissue, we further assessed changes in cytokine expression after incubation 3T3-L1 adipocytes with CG. Among the cytokines we tested, MCP1 was significantly upregulated by CG. MCP1 is predominantly produced by macrophages and endothelial cells and is a potent chemotactic factor for monocytes (Takahashi et al. 2003). Increased MCP1 contributes to macrophage infiltration and IR (Sartipy & Loskutoff 2003, Kanda et al. 2006). Clinically, results from previous studies have also indicated that MCP1 is elevated during pregnancy and further augmented in GDM compared with NGT. Consistent with the results reported by Klein et al. (2008), our clinical detection of MCP1 also found elevated MCP1 concentration in GDM women; moreover, elevated MCP1 is associated with abnormal glucose tolerance. Although it has been proposed that this inflammation is mediated by the placenta (Klein et al. 2008), our results confirmed that elevated MCP1 levels in pregnancy were produced by increased fat mass under surged CG stimulation.

After binding to CG/LHR, CG activated multiple signal transduction effector systems, including, importantly, the AC cAMP PKA and MAPK pathways (Cole 2010). We found that the AC cAMP PKA pathway partially mediated CG-induced GLUT4 suppression. Interestingly, CG can activate NF-κB in adipocytes. NF-κB is a
transcription factor that plays a key role in inflammatory and immune responses. The IKKB/NF-κB signalling pathway is activated by inflammatory stressors and regulates the expression of cytokines and growth factors that regulate inflammatory pathways (Karin & Delhase 2000). NF-κB has been implicated in obesity-induced IR and deranged glucose metabolism by both pharmacological and genetic approaches (Yuan et al. 2001). CG activation of NF-κB signalling further confirmed that CG plays a role in inflammation and IR.

In addition, using stepwise multiple linear regression analysis, we found that prepregnancy BMI is associated with disturbed glucose tolerance, which is consistent with results described in previous reports (Gomez-Ambrosi et al. 2011, Arjmandi Far et al. 2012). More importantly, apart from prepregnancy BMI and MCP1 levels, CG level is associated with elevated 1-h post glucose load of pregnant women. The analysis of clinical data strongly supported the hypothesis that CG attenuates insulin sensitivity and contributes to disturbed glucose tolerance.

Although persistent CG stimulation of Leydig cells has been reported to induce oxidative stress (Aggarwal et al. 2009), which is a contributor to IR (Furukawa et al. 2009), the expression of oxidative-stress-related genes was not influenced by CG in adipocytes (data not shown). In our study, we found that CG/LHR was also expressed in C2C12 cells, another important type of insulin-responsive cells, which indicated that CG possibly plays a role in these cells, this will be clarified in the future.

In conclusion, through CG/LHR in adipocytes, the higher CG in GDM directly impairs insulin sensitivity and induces inflammation to exacerbate IR, which contributes to GDM development to some extent.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
G N and Q M designed the studies. Q M, J W and Q L performed all the experimental procedures and experimental data analyses. J F, S Y and Q C collected and analysed all the clinical data. Q M and R L wrote the manuscript. All authors contributed to the discussion and revised the manuscript and all approved the final version. G N is responsible for the integrity of the work as a whole.

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