Causal relationship between obesity-related traits and TLR4-driven responses at the maternal–fetal interface

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Abstract

Aims/hypothesis—Obesity triggers complex inflammatory networks within the innate immune system. During pregnancy, the placenta amplifies the low-grade inflammation through activation of Toll-like receptor 4 (TLR4) signalling pathways. The purpose of this study was to investigate the impact of obesity on placental TLR4 expression and inflammatory signals. The secondary aim was to analyse the placental cell type responsible for TLR4 activation.

Methods—Thirty-nine women recruited at term-scheduled Caesarean section were grouped according to their pre-gravid BMI (< 25 kg/m^2 and > 30 kg/m^2). Placenta, venous maternal and cord blood were obtained at delivery for analysis. Data were analysed with linear regression and Spearman's rank correlation coefficient analysis.

Results—TLR4, IL6 and IL8 expression was increased three- to ninefold ($p < 0.001$) in the placenta of obese vs lean women. There was a positive correlation between placental TLR4 and maternal systemic and placental IL6 and IL8 concentrations. Placental TLR4 expression was correlated with maternal pre-gravid BMI, insulin resistance index, plasma insulin and C-reactive protein ($r = 0.57, 0.31, 0.35, 0.53$, respectively; $p < 0.001$) but not with plasma glucose, maternal age, gestational age and gestational weight gain ($r < 0.2$; $p > 0.1$). TLR4 was located in both trophoblast and macrovascular endothelial cells lining fetal vasculature. Lipopolysaccharide-induced TLR4 activation was more robust in trophoblasts than in endothelial vascular cells (100-fold vs tenfold; $p < 0.001$).

Conclusions/interpretation—Trophoblastic TLR4 is implicated in the propagation of placental inflammation. Placental inflammation is related to maternal metabolic conditions such as

pre-gravid BMI, whilst gestational weight gain or gestational age are not. These results implicate the pre-gravid condition as a significant contributor to metabolic inflammation in late pregnancy.

Keywords

Gestational weight gain; Inflammation; LPS; Macrovascular endothelial cells; Obesity; Placenta; Pregnancy; Toll-like receptor 4; Trophoblast

Introduction

Obesity is emerging as a trans-generational disease that propagates through an adverse programming effect in the progeny [1]. Infants of obese women develop short- and long-term health consequences [2]. Of particular concern is the increased risk to the offspring for excess neonatal adiposity and metabolic disease in adolescence and adulthood [3, 4]. Other obesity-related traits, including insulin resistance, are associated with fetal macrosomia [5–8]. However, the causal nature of the factors that result in higher fetal body adiposity remain to be elucidated. Likewise, the mechanisms underlying the in utero programming effects are not well defined.

Human and animal studies have clearly established that obesity is associated with a state of chronic low-grade inflammation [9]. During pregnancy, obese women exhibit metabolic (low-grade) inflammation characterised by elevated levels of pro-inflammatory cytokines in the systemic circulation and adipose tissue compared with lean women [10–12]. Low-grade inflammation encompasses the placenta [13], suggesting that maternal obesity exposes the developing fetus to an inflammatory environment. In this context, early-life exposure to metabolic inflammation may represent a mechanism by which metabolic dysfunction will manifest later in life. One current challenge of developmental programming is the gathering of data with which to dissect out the obesogenic stimuli that drive the increased in utero inflammation in obese women, particularly at the maternal–fetal interface.

Current literature supports a functional connection between triggers of metabolic inflammation and the innate immune system [14]. Toll-like receptor 4 (TLR4) is one isoform of pattern-recognition receptors, which trigger immune responses in response to various infectious agents and metabolic stressors [9, 15, 16]. TLR4 binds a wide spectrum of exogenous and endogenous ligands including bacterial lipopolysaccharide (LPS), its primary ligand. The sequence of events generated upon TLR4 activation leads to the release of various effectors of innate immunity, cytokines and pro-inflammatory mediators through the nuclear translocation of NFκB [17]. TLR4 is found in virtually all metabolic tissue and cells. It is overexpressed in adipose tissue of individuals who are obese or have type 2 diabetes, as well as in obese pregnant women [18]. TLR4 is expressed in the human placenta, suggesting that crosstalk may arise between immune and endocrine functions at the maternal–fetal interface. Against this background, the objective of this study was to investigate the impact of maternal obesity-related traits on placental TLR4 expression and inflammatory signals. The secondary aim was to analyse the placental cell type responsible for the propagation of TLR4-induced inflammation.

Methods

Study participants

Women with uncomplicated pregnancies were recruited at term-scheduled Caesarean delivery. Those with multiple gestation, fetal anomalies, intrauterine growth restriction and diabetes (pre-existing and gestational) were excluded. Obesity was defined as pre-gravid BMI > 30 kg/m^2 and lean body type as BMI < 25 kg/m^2. Pre-conception weight was obtained from charts as self-reported weight. Late-pregnancy weight was obtained from charts as measured at the last prenatal visit. Total gestational weight gain (GWG) was calculated as the difference between last prenatal visit and pre-conception maternal weight. Excess GWG was estimated by applying 2009 Institute Of Medicine (IOM) guidelines for each BMI category [19].

All participants provided informed consent; they were fully aware of the study procedures and made an informed decision on their participation. Our investigations were approved by the MetroHealth Medical Center institutional review board (IRB) for collection of maternal blood, placenta and venous cord blood according to the protocol (IRB05-288). Maternal pre-gravid BMI was obtained from medical records. Placentas were weighed after the umbilical cord and fetal membranes had been trimmed by research staff. Anthropometric variables of the study cohort are presented in Table 1.

Biological specimen collection

Blood and placental tissue were obtained from 39 women at term pregnancy scheduled for elective Caesarean section. Maternal venous blood samples were collected in EDTA before placement of intravenous lines for hydration. Placental and cord blood samples were obtained immediately after double-clamping of the umbilical cord. Fragments of placental villous tissue were collected for either paraffin embedding for immunohistochemistry or molecular analysis, and stored at −80°C. Adjacent placental fragments (total 50 g) were immediately processed for isolation of trophoblast cells. Placental macrovascular veins were dissected within15 min for isolation of endothelial cells.

Plasma assays

Plasma glucose was assessed by the glucose oxidase method, using YSI 2300 STAT Plus Glucose & Lactate Analyzer (YSI Incorporated, Yellow Springs, OH, USA) [11]. Plasma insulin was measured using ELISA (EMD Millipore Corporation, Billerica, MA, USA) with an intra-assay CV of 3.0–8.2%. Leptin, IL8 and IL6 were measured using ELISA kits (R&D Systems, Minneapolis, MN, USA) with intra-assay CVs of 0.11–10.7%, 0.4–4.7% and 0.1–7.2%, respectively. All plasma samples were assayed in duplicate. Plasma C-reactive protein (CRP) concentration was measured by ELISA (Alpha Diagnostics International, San Antonio, TX, USA). Insulin resistance was estimated using HOMA-IR, as published previously [20]. Plasma NEFA levels were measured by a HR series NEFA kit (Wako Diagnostics, Richmond, VA, USA).

Immunofluorescence

Paraffin blocks of villous tissue were sectioned (5 μm thick) and collected on Excell Slides (Fisher Scientific, Pittsburgh, PA, USA). The slides were deparaffinised, followed by 30 min incubation in 10 mmol/l sodium citrate with 0.05% (vol./vol.) Tween solution (pH 6.0) in a 100°C water bath for antigen retrieval. The tissue sections were blocked for non-specific staining with PBS containing 0.3% (vol./vol.) Triton X-100 (Sigma-Aldridge, St Louis, MO, USA), 5% (vol./vol.) Normal Donkey Serum, 1% (vol./vol.) IgG-free BSA (Jackson Immunoresearch, West Grove, PA, USA) for 30 min. The slides with isolated cells or villous tissue were incubated overnight at 4°C with the following antibodies: TLR4 (H-80, 1:50; Santa Cruz, Dallas, TX, USA) and CD34 (ab8536, 1:400; Abcam, Cambridge, MA, USA). For visualisation, Alexa Fluor 488 and 594 secondary antibodies (1:1000; Jackson Immunoresearch, West Grove, PA, USA) were used. The nucleus was labelled with To-Pro 3 (Sigma-Aldridge). The slides were imaged with Leica Microsystem SP2 Confocal Microscope (Leica Microsystems, Buffalo Grove, IL, USA). All antibodies were diluted in PBS and validated by negative control.

Placental cell isolation and cell culture

Trophoblast cells from obese women were isolated by sequential trypsin and DNase digestion followed by gradient centrifugation [21]. Flow cytometry analysis of isolated placental cells indicated that $86.7 \pm 6.7\%$ cells stained positive for cytokeratine 7, $1.6 \pm 0.8\%$ for placental alkaline phosphatase and $4.8 \pm 1.7\%$ for CD68 (data not shown). Cells were placed into 12-well plates at a density of 1.5×10^6 cells/well and cultured overnight in Iscoves's modified DMEM culture medium supplemented with 10% (vol./vol.) FBS (Gibco, Grand island, NY, USA) and 1% (vol./vol.) penicillin/streptomycin at 37°C under 5% CO_2. The medium was replaced with 1 ml DMEM with 1% (vol./vol.) FBS 2 h before addition of 100 ng/ml LPS (Sigma-Aldridge) and plates were left for 24 h. Medium with PBS alone was used as control.

For placental macrovascular endothelial cell (PVEC) isolation, the amnion was removed and macrovascular veins from the placental chorionic plate of obese women were cut out in lengths of 3–5 cm and washed with Hank's Balanced Salt Solution (HBSS; 2.5% (vol./vol.) penicillin/streptomycin). A cannula was inserted into the vessel and fixed with sterilised surgical thread. Following insertion of a syringe containing 0.05% (wt/vol.) collagenase/dispase solution (Sigma Aldrich) in HBSS pre-warmed to 37°C, 20 ml was slowly rinsed through the vessel by using a syringe pump (syringe diameter of 30 ml, 27.6 mm, flow rate, 2.5 ml/min). The released cells were collected in a 50 ml Falcon centrifuge tube (Corning, Corning, NY, USA) containing 5 ml FBS. The cell suspension was centrifuged at 900 rpm (200 g) for 5 min at 4°C, then resuspended in complete media (500ml Endothelial Basal Medium [EBM; Clonetics, Lonza, Walkersville, MD, USA] + one EGM-MV BulletKit [Clonetics, Lonza] + 10% (vol./vol.) FBS + 1% (vol./vol.) penicillin/streptomycin) and cultured in 12-well plates that had been pre-coated with 1% (vol./vol.) gelatin (Sigma Aldrich) in HBSS for 1 h at 37°C. After reaching 80% confluence at 21% oxygen and 37°C, cells were transferred to 25 cm² flasks and expanded for identification purposes and determination of purity [22–24]. The endothelial identity of the cells was confirmed by immunohistochemical staining, with the endothelial markers anti-von Willebrand factor

(A0082, 1:2000; Dako, Carpinteria, CA, USA), Vimentin (M0725, 1:100; Dako) and Desmin (M0760, 1:200; Dako). Lack of immunoreactivity with a marker for smooth muscle cells (smooth muscle actin, M0851, 1:100; Dako) confirmed cell purity (data not shown). Cells were placed into 12-well plates in 5% (vol./vol.) FBS + EBM + EGM-MV BulletKit culture medium at 37°C under 5% CO_2. After reaching 90% confluence, the medium was replaced with EBM + EGM-MV BulletKit 2 h before adding 50 ng/ml LPS and plates were left for 24 h. PBS was used as control.

IL6, IL8 and TNF-α concentrations in culture medium were measured by ELISA (HS600B (S6050), HS800 (S8000C) and, HSTA00D (STA00C), respectively; R&D System, Minneapolis, MN, USA).

RNA preparation and quantitative real-time PCR

Total RNA was extracted from 30 mg frozen placental tissue or trophoblast cells and endothelial cells (cultured on 12-well plates) using RNeasy Mini Kit (Qiagen, Valencia, CA, USA) or TRIzol (Invitrogen, Carlsbad, CA, USA). RNA was reversed-transcribed using a Superscript II RNase H-Reverse Transcriptase system (Invitrogen). Gene expression was measured in duplicates by quantitative real-time PCR (qPCR) using a Roche thermal cycler (Roche Applied Science, Indianapolis, IN, USA) with Lightcycler Fast-start DNA Sybr Green 1 master mix and primers (Integrated DNA Technologies, Coralville, IA, USA). Specific primers are shown in electronic supplementary material (ESM) Table 1. Results were normalised for *β-actin* and performed by the comparative C_t method. Data were quantified as copy number/(ng RNA) calculated against the standard curve of human term placental cDNA or expressed as fold change of treated vs control groups in cultured trophoblast cells and endothelial cells.

TLR4 protein detection

Total protein was extracted from 25 mg frozen placenta tissue, 1.5×10^6 cultured trophoblast cells or 1.5×10^6 endothelial cells. The protein lysates were prepared by homogenisation in buffer (10 mmol/l Tris pH 8, 130 mmol/l NaCl, 1% [vol./vol.] Triton X-100, 10 mmol/l sodium fluoride, 10 mmol/l sodium phosphate and 10 mmol/l sodium pyrophosphate) with protease inhibitors (P8340; Sigma-Aldridge, St Louis, MO, USA). Lysates were centrifuged at 20,000 *g* for 10 min at 4°C. Protein concentrations were measured with a BCA protein assay kit (Pierce, Carlsbad, CA, USA). Proteins were electrophoresed on a 7.5% SDS gel (Bio-Rad, Hercules, CA, USA), loaded with 100 μg total protein per well, and then transferred to a nitrocellulose filter (Invitrogen, Carlsbad, CA, USA). The membrane was blocked with 5% (wt/vol.) non-fat milk for 1 h, incubated with rabbit polyclonal TLR4 (H-80, 1:200; Santa Cruz) and β-actin (1:2000; Abcam, Cambridge, MA, USA) overnight then secondary antibodies (1:2000 and 1:6000) for 1 h. Amersham ECL Plus Western blotting Reagents (GE Healthcare, Aurora, OH, USA) was used for detection. All antibodies were diluted in PBS and validated by Precision Plus Protein Kaleidoscope Standards (BIO-RAD, Hercules, CA, USA). Densitometric data from autoradiograms were quantified by Image J (https://imagej.nih.gov/ij/download.html).

Statistical analysis

Values in Tables 1 and 2 are presented as means ± SD. Differences among dependent variables were analysed by Student's t test and χ^2 test. Associations between placental *TLR4* mRNA expression (copy number/ng RNA) and maternal variables were analysed by Spearman's rank correlation coefficient and linear regression analysis. Statistical mean differences for qPCR data were calculated by Kruskal–Wallis test for non-parametric data. Statistical significance was set at $p < 0.05$.

Results

Metabolic inflammation at the maternal–fetal interface

Metabolic variables were characterised in obese and lean women at term normal delivery. As expected, obese women were hyperinsulinaemic and hyperleptinaemic and had a higher insulin resistance index compared with their lean counterparts (Table 2). They also displayed low-grade systemic inflammation, with an increased plasma concentration of CRP, IL6 and IL8 ($p < 0.05$). Placentas of obese women displayed higher mRNA levels of *TLR4*, *IL6* and *IL8* (Fig. 1). Sex of offspring had no effect on the pattern of placental *TLR4* expression (data not shown). Placental *TLR4* mRNA levels were strongly correlated with the levels of IL6 and IL8 in maternal systemic circulation and expression of *IL6* and *IL8* in the placenta (Fig. 2).

Association between placental TLR4 and maternal metabolic variables

Multiple correlation analyses (Table 3) indicated that placental TLR4 exhibited a strong positive correlation with maternal pre-gravid BMI and BMI at delivery ($r = 0.57$ and 0.67, respectively; $p < 0.0001$) as well as with plasma insulin, leptin and CRP ($r = 0.35$, 0.73 and 0.53, respectively; $p < 0.01$). HOMA-IR estimated at delivery showed a weaker correlation with *TLR4* and this did not quite reach statistical significance ($p < 0.06$). In contrast, maternal plasma glucose, gestational age and total GWG were not significantly associated with placental *TLR4*. Therefore, in our population, maternal pre-gravid obesity and not GWG appears to be the more important factor associated with placental innate immune response.

Regulation of placental TLR4 expression

TLR4 staining was detected by immunohistochemistry on syncytiotrophoblasts and the perivascular layer of the placental villous, including fetal vascular endothelium (Fig. 3). The presence of TLR4 was confirmed by western blot analysis on cells isolated from the same placentas (Fig. 3c). The signalling mechanisms of TLR4 activation were characterised in primary cultures of term trophoblast cells and PVECs from obese women (Fig. 4). LPS, the primary ligand of TLR4, was used to evaluate TLR4-stimulated cytokine release. The amount of IL6 and IL8 released by trophoblast cells was increased by 80-fold and 100-fold, respectively ($p < 0.01$) vs control upon 24 h treatment with 100 ng/ml LPS. Under the same stimulation conditions, the release of IL6 and IL8 was increased 10-fold and 20-fold, respectively, in PVECs, $p < 0.05$. The release of TNF-α was stimulated six- to ninefold ($p < 0.001$) in trophoblast cells whereas there was no detectable release by PVECs. IL6 and IL8

levels in culture media of LPS-treated trophoblast cells were four- to fivefold higher than those in PVEC media.

Discussion

In this prospective cohort study of healthy pregnant women at term, we observed a significant induction of TLR4-induced inflammatory signals in the placenta of obese compared with lean women. These findings suggest that factors related to the obesogenic environment of the pregnant women had triggered the propagation of an inflammatory cascade through activation of TLR4-induced pathways. The molecular mechanisms of TLR4 activation have been well described in adipose tissue, particularly in rodent models of obesity [25]. LPS and saturated fatty acids both have the ability to activate TLR4 immune responses leading to activation of NFκB and several inflammatory genes in adipocytes of non-pregnant individuals [17, 26, 27] and pregnant women [18]. Likewise, we have recently reported that saturated fatty acids promote the activation of TLR4 in primary human trophoblast cells [28]. The intense TLR4 staining detected in trophoblast cells extends the previous observation made by Powell et al, of TLR4 expression in term placenta [29]. It further supports our original hypothesis that ligand binding sites of placental TLR4 are readily accessible to factors derived from the maternal systemic circulation. An important finding is the localisation of TLR4 in the immediate perivascular region of placental–fetal vessels, which is in accordance with TLR4 expression in umbilical endothelial cells [30]. The human placental structure allows direct contact between the fetal vascular endothelial cells and fetal blood. Hence, the vascular TLR4 may be directly targeted by factors derived from the fetal circulation. This may contribute to regulation of the local cytokine environment and convey to the placenta immune signals originating from the fetus [31]. Beside LPS, glucose, NEFA and LDL are additional metabolic stimulators of TLR4-induced innate immune response [32–34]. In the context of this study, obese women did not exhibit overt hyperglycaemia or glucose intolerance (data not shown); this does not point towards a role for maternal plasma glucose in placental TLR4 activation. However, in late pregnancy the decreased ability of obese women to respond to the anti-lipolytic effect of insulin results in an increased circulating NEFA concentration under insulin-stimulated condition [35–37]. Furthermore, the placentas of obese women, compared with their lean counterparts, have an enhanced capacity for lipid storage [38]. It has been suggested that triacylglycerols and phospholipid species stored in the placenta are made available for use as fatty acids through the activation of endogenous lipases [39–41]. We have previously reported that in cultured trophoblast cells long-chain saturated fatty acids such as palmitate induce a TLR4 inflammatory cascade and cytokine release through NFκB activation [28]. Together these data suggest that in obese women the lipid overload at the maternal–fetal interface may trigger TLR4-induced placental inflammation. There is currently no data relating to the regulation of trophoblast TLR4 by other maternal lipid species such as LDL or HDL. As regards to fetal lipid substrates, HDL/ApoE has been proposed as a regulator of gene expression in human placental endothelial cells [42]. However, it is unclear whether or not specific LDL species are present in the placental perivascular milieu in proximity to the fetal circulation. If they are, this could represent a mechanism for regulation of placental TLR4 through fetal-derived stimuli and needs further examination.

In addition to nutritional adaptations, increased fat mass and insulin resistance are the hallmarks of obesity whose mechanisms have been best defined [43, 44]. In this cohort of women at term pregnancy, we observed a strong association between maternal pre-gravid BMI and placental TLR4 expression and a weaker association with maternal insulin resistance estimated in late pregnancy. Current knowledge favours metabolic inflammation as a trigger of chronic insulin resistance, rather than the reverse situation. Together, these observations suggest that it is placental inflammation that enhances insulin resistance in obese women through cytokine release rather than pre-existing maternal insulin resistance enhancing placenta TLR4 activation.

We report that maternal pre-conception and late BMI had the strongest association with placental TLR4 expression. In contrast, maternal GWG was not significantly correlated with TLR4. Importantly, the timing of weight gain rather than total GWG may have greater impact on fetal body composition [45]. Hence, our analysis in which we included total GWG leaves open the possibility that weight gain in early pregnancy may positively associate with placental inflammation.

Our findings suggest that in obese women, the maternal pre-gravid metabolic condition has a stronger impact on the regulation of feto–placental adaptations than gestational changes such as GWG [5]. Our observations are consistent with those made in many studies [46–48], although not all [49], relating to the importance of pre-pregnancy obesity as a risk factor for neonatal adiposity.

In conclusion, TLR4 is strongly implicated in the propagation of placental inflammation. It is not merely the increased mass of adipose and other maternal tissues accrued during gestation that is related to placental TLR4 activation but rather an excess maternal BMI at the start of pregnancy. The distribution of TLR4 in placental cell types may have important functional implications in favouring access to maternal rather than fetal obesogenic stimuli. Therefore, we propose that trophoblastic TLR4 represents an accessible target for nutritional intervention in pregnant women. Overall, these findings contribute to our understanding of the programming mechanisms of obesity and provide compelling pre-clinical evidence for a potential strategy for improving pregnancy metabolic outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

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CRP	C-reactive protein
GWG	Gestational weight gain
HBSS	Hanks' balanced salt solution
LPS	Lipopolysaccharide
PVEC	Placental macrovascular endothelial cell
TLR4	Toll-like receptor 4
qPCR	quantitative real-time PCR

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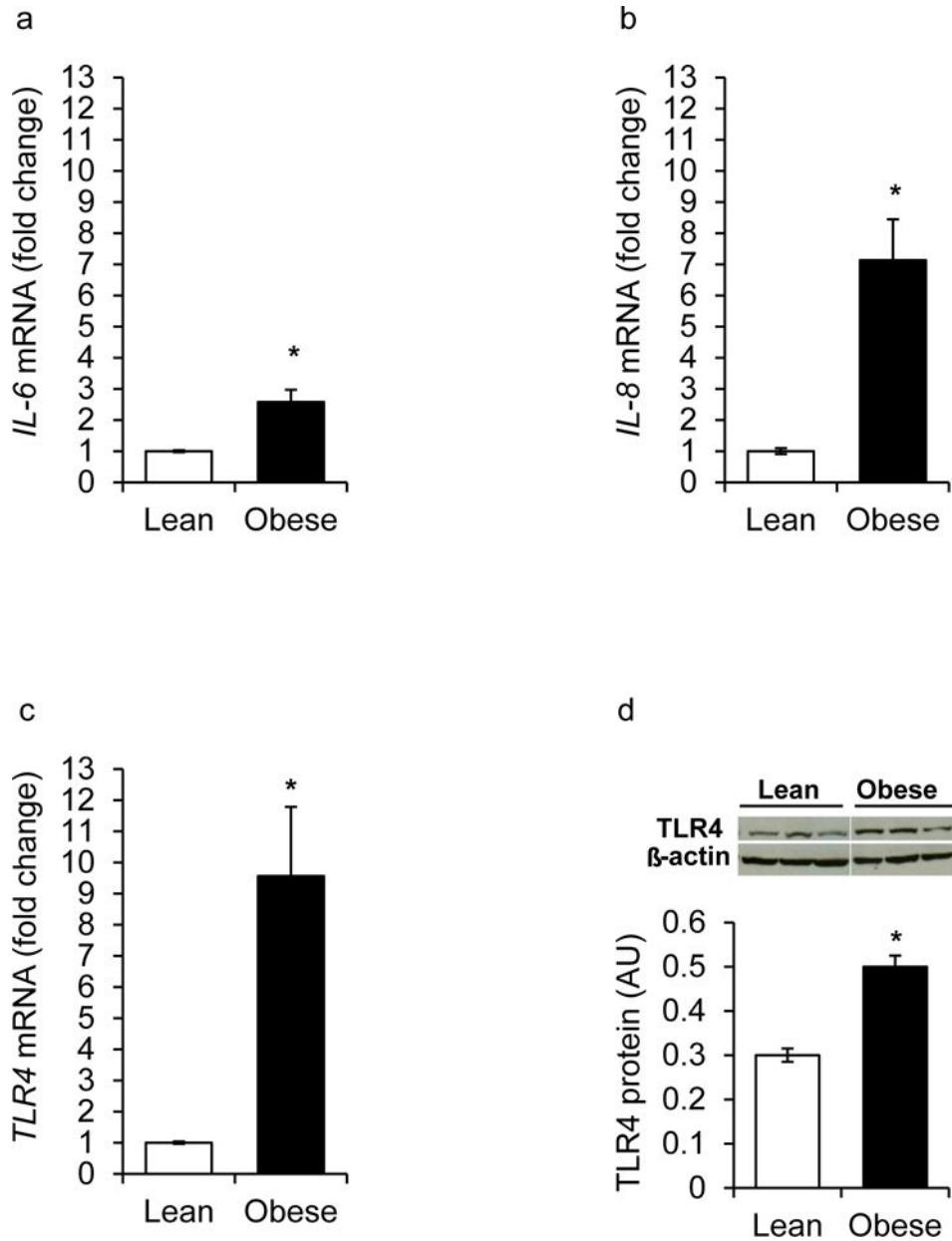
Fig. 1.

Inflammatory cytokine and TLR4 expression in placenta of lean and obese women. (**a–c**) *IL6* (**a**), *IL8* (**b**) and *TLR4* (**c**) mRNA expression in placental tissue from lean (*n*=20) and obese (*n*=19) women was measured by qPCR. Results were normalised to *β-actin* and presented as fold change of obese vs lean group. (**d**) TLR4 protein in placental tissue was measured by western blot. Results were normalised to β-actin, *n*=8. AU, arbitrary units. Data are shown as means±SEM; *$p<0.05$ obese vs lean
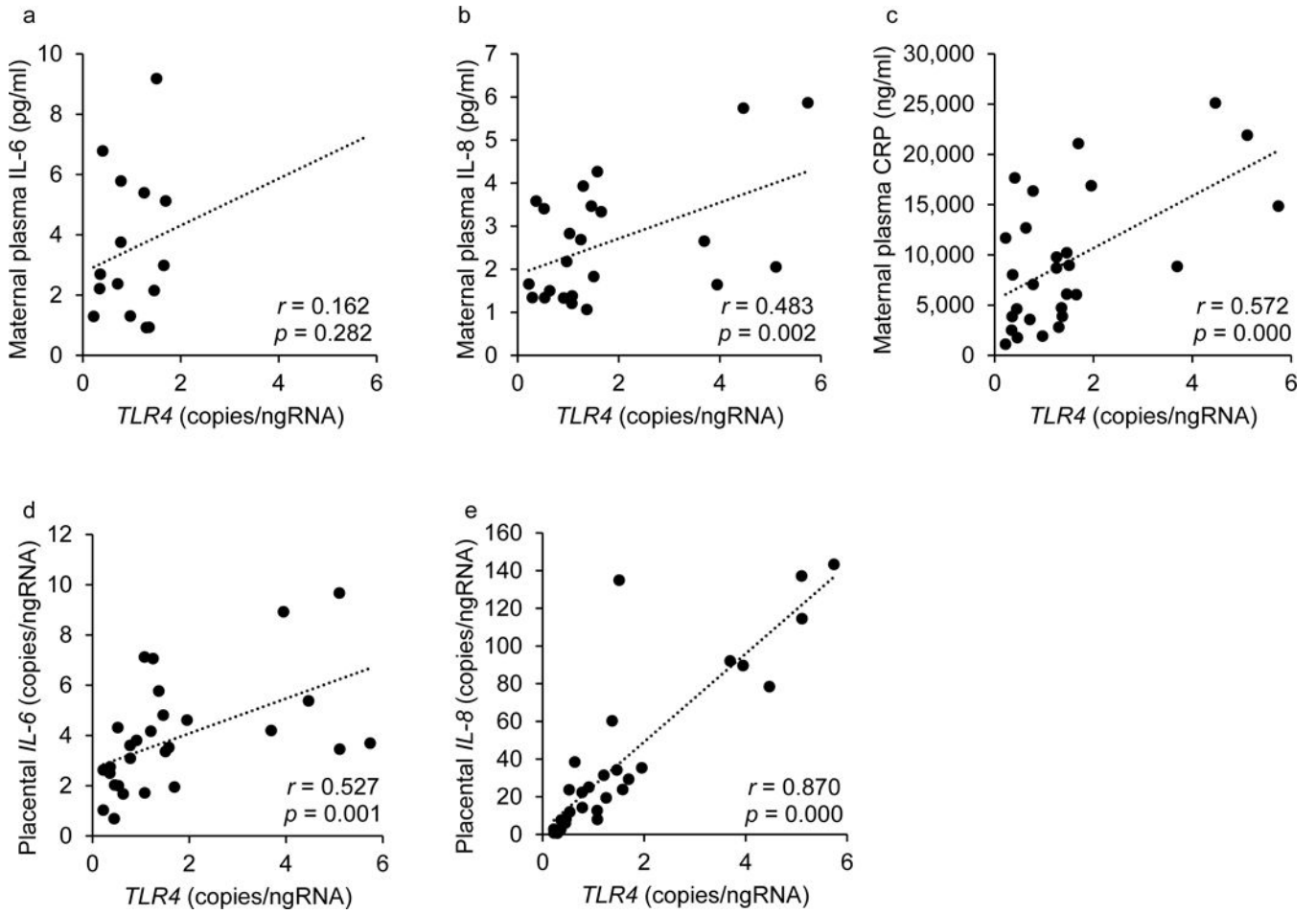
Fig. 2.
Correlation between placental *TLR4* expression and systemic or placental inflammation. The correlation between placental *TLR4* and pre-gravid maternal plasma IL6 (**a**), IL8 (**b**) and CRP (**c**) levels orplacental *IL6* (**d**) and *IL8* (**e**) expression was analysed by simple linear regression analysis. Statistical significance was set at $p<0.05$
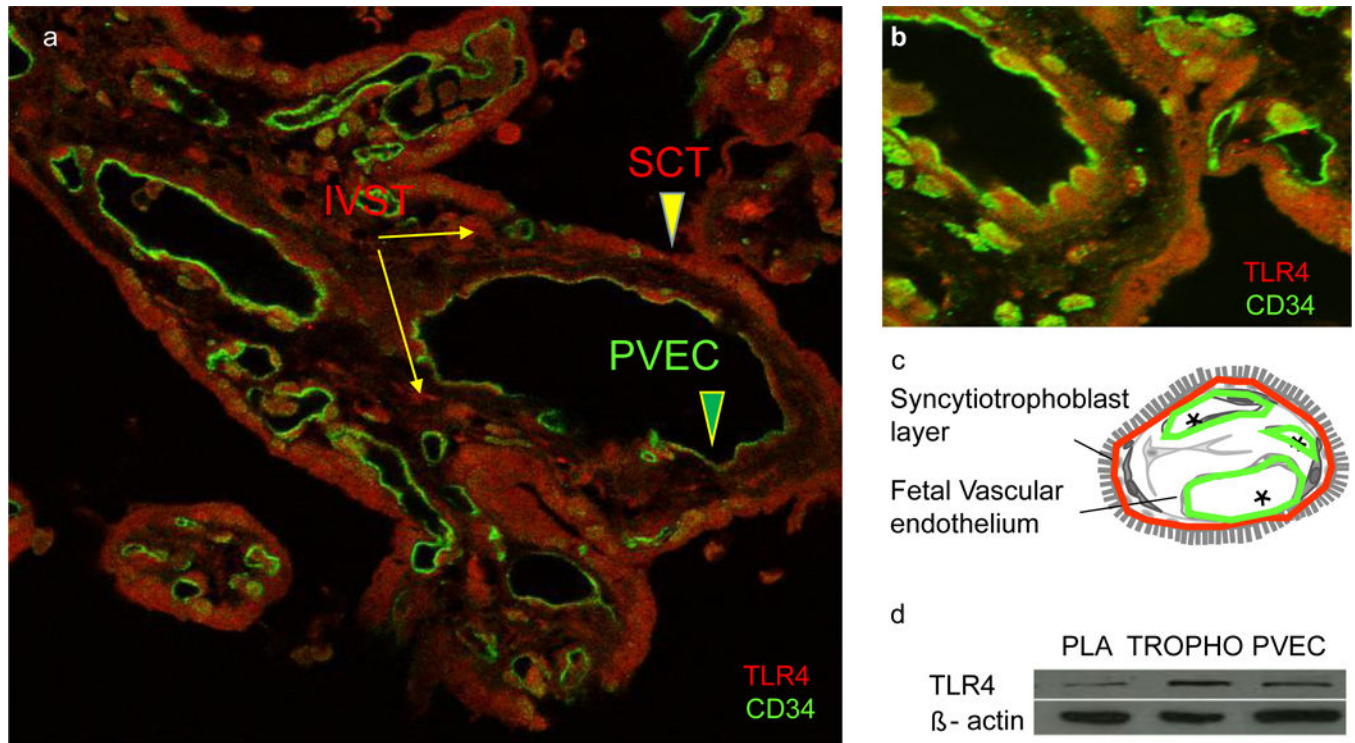
Fig. 3.
TLR4 localisation in human term placenta. (**a**, **b**) Immunofluorescence analysis showing TLR4 (red) localised in the syncytiotrophoblast (SCT) layer (**a**) and perivascular layer (**b**) with an endothelial cell marker CD34 (green). (**c**) TLR4 protein was examined by western blot in placental tissue (PLA), term human trophoblast cells (TROPHO) and PVECS, n=3. IVST, intravillous stromal cells
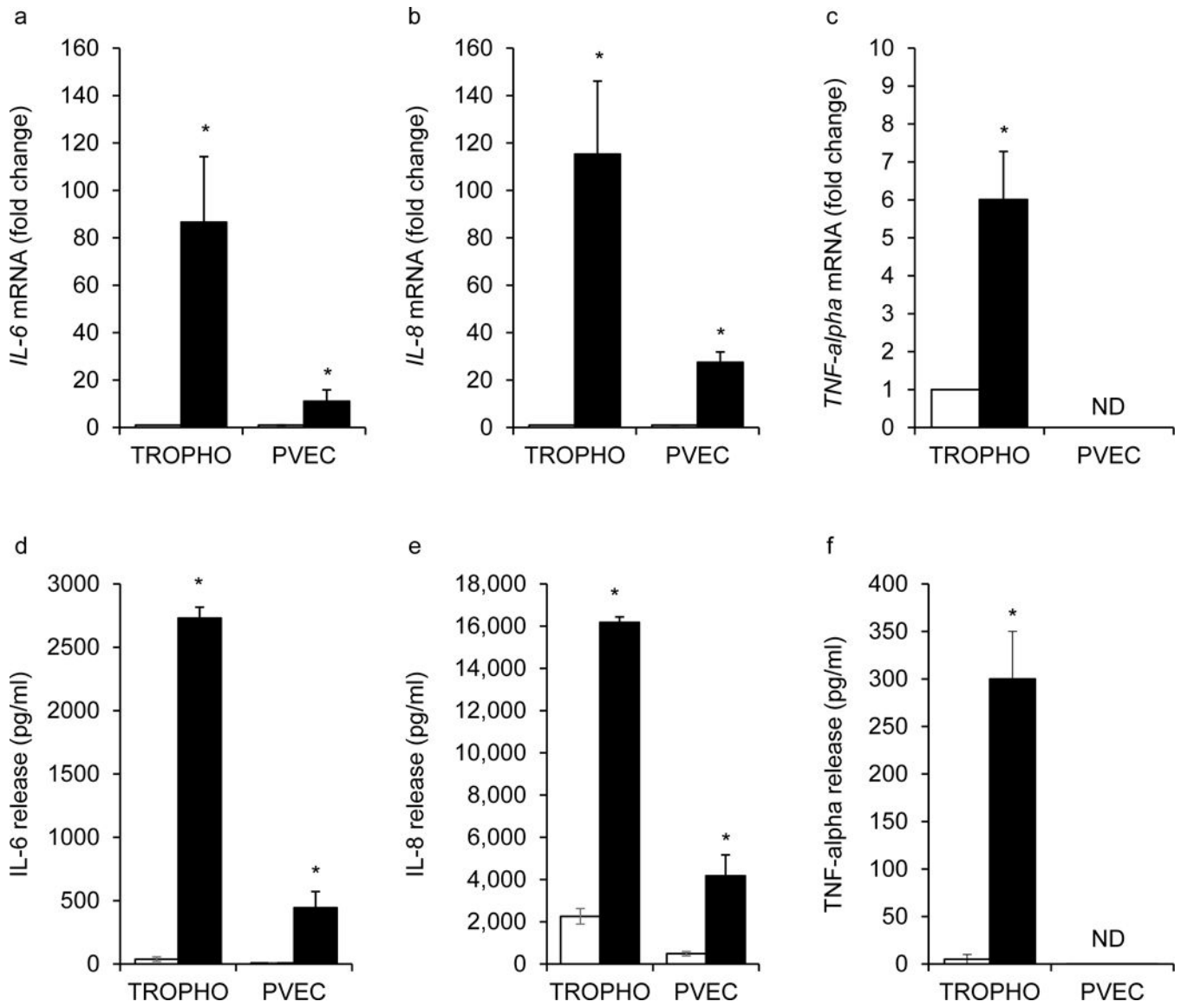
Fig. 4.
Regulation of inflammatory cytokines in cultured human trophoblast cells and placental venous endothelial cells. Primary human trophoblast cells (TROPHO) and PVECs from obese placentas were treated with LPS. *IL6, IL8* and *TNF-α* (**a–c**) mRNA was detected by qPCR and (**d–f**) protein levels by ELISA. TNF-α expression in endothelial cells was below the detection threshold. qPCR results were normalised to *β-actin* and expressed as fold change vs control (no LPS). Data are shown as means±SEM for 3–6 independent experiments; *$p<0.05$ vs control. White bars, control; black bars, LPS. CTL, control; ND, non-detectable

Table 1

Anthropometrics of study cohort

Variable	Lean (n=20)	Obese (n=19)	p value
Maternal age (years)	28.0±6.4	29.7±7.4	0.225[a]
Gestational age (weeks)	38.9±0.6	39.1±0.4	0.114[a]
Pre-gravid BMI (kg/m²)	21.1±2.4	40.3±7.7	0.000[a]
BMI at delivery (kg/m²)	27.4±2.5	46.7±7.1	0.000[a]
GWG (kg)	16.8±5.5	17.3±9.5	0.421[a]
Excess GWG (%)	65.0	73.7	0.557[b]
Race (%)			
White	70	47.4	0.151[b]
African-American	30	42.1	0.431[b]
Hispanic	0	10.5	0.136[b]
Smoke (%)	25.0	10.5	0.239[b]
Neonatal sex (%)			
Female	55	26	0.069[b]
Male	45	74	
Plasma NEFA (mmol/l)			
Maternal	0.7±0.2	0.7±0.1	0.292[a]
Cord	0.2±0.0	0.3±0.1	0.195[a]

Data are presented as means±SD or as percentage

[a]Student's t test

[b]χ^2 test

Table 2

Metabolic and inflammatory variables

Maternal variable at delivery	Lean (*n*=20)	Obese (*n*=19)	*p* value
HOMA-IR	2.2±1.1	4.1±1.0	0.000
Plasma glucose (mmol/l)	4.1±0.5	4.4±0.4	0.026
Plasma insulin (pmol/l)	80.6±8.3	161.8±73.6	0.000
Plasma leptin (ng/ml)	23.6±4.4	81.8±4.7	0.000
Plasma IL6 (pg/ml)	2.1±1.0	5.1±2.7	0.007
Plasma IL8 (pg/ml)	1.9±0.9	3.2±1.5	0.011
Plasma CRP (nmol/l)	49.9±34.6	122.3±64.1	0.001

Data are presented as means±SD

Table 3

Correlation between placental *TLR4* mRNA and body composition or metabolic status in pregnant women

Variable	Correlation with placental *TLR4* mRNA (copies/ng RNA)	
	Spearman *r*	***p* value**
Maternal age	0.007	0.967
Gestational age	0.080	0.630
Pre-gravid BMI	0.570	0.000
BMI at delivery	0.667	0.000
Total GWG	0.178	0.278
HOMA-IR	0.309	0.062
Plasma glucose	0.007	0.969
Plasma insulin	0.350	0.034
Plasma leptin	0.729	0.000
Plasma CRP	0.532	0.004