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ORIGINAL ARTICLE

Cross-talk between fetal membranes and visceral adipose tissue involves HMGB1–RAGE and VIP–VPAC2 pathways in human gestational diabetes mellitus

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Abstract

Aims Gestational diabetes mellitus (GDM) is defined as glucose intolerance that is first diagnosed during pregnancy. Maternal adipose tissue and fetal membranes secrete various molecules that are relevant players in the pathogenesis of GDM. This pilot study aimed to examine whether the expression of the high mobility group box 1 protein (HMGB1) and its receptor for advanced glycation end products (RAGE), and the vasoactive intestinal peptide (VIP) and its receptors (VPAC-1,-2) were modified in pregnant women with GDM.

Methods Fetal membranes (FMs), omental adipose tissue (VAT) explants, and serum samples were obtained from 12 women with GDM and 12 with normal glucose tolerance (NGT) at delivery. The expression of HMGB1, RAGE and VIP, VPAC-1,-2 was detected by Western Blotting in explants; circulating levels and "in vitro" release of HMGB1 and VIP were measured by ELISA tests.

Results HMGB1 tissue expression was higher in FMs obtained from GDM women ($p=0.02$) than in FMs from NGT women. VPAC2 ($p=0.03$) and RAGE ($p=0.03$) tissue expressions were significantly increased in VAT from GDM subjects. Only FMs of NGT released detectable levels of HMGB1, which was not observed in samples obtained from GDM. VAT of GDM released lower levels of VIP ($p=0.05$) than NGT samples.

Conclusions This study indicates that a fine tuned regulation exists between FMs and VAT throughout pregnancy to maintain immune metabolic homeostasis. In GDM a balance between inflammatory and anti-inflammatory mediators has been observed. Further studies are needed to establish their exact role on fetal and maternal outcomes in GDM.

Keywords Gestational diabetes mellitus · Fetal membranes · Visceral adipose tissue · High mobility group box 1 protein · Vasoactive intestinal peptide · Inflammation

Introduction

Gestational diabetes mellitus (GDM) is one of the most common metabolic disorders in pregnancy, with possible deleterious effects both on the mother and on the offspring [1, 2]. Its worldwide prevalence is rising (1–36% affected pregnancies) probably because of growing maternal obesity [3]. Excess adiposity and obesity are important risk factors for GDM; both conditions contribute to the increased risk of type 2 diabetes (T2D) in women with a history of GDM [4, 5] and of childhood obesity and metabolic diseases in children born to GDM mothers, later in life [6]. Despite the considerable public health relevance of GDM and the conspicuous economic impact, its pathophysiological mechanisms are not completely understood.

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During pregnancy, both maternal and fetal tissues are actively involved in maintaining metabolic and immune homeostasis to support maternal modifications and fetal growth until delivery [7, 8]. Metabolic and inflammatory changes occurring in physiological pregnancy appear to be exacerbated in GDM. Several factors, whose functions are not completely clear, might contribute to maintain the diabetic status [9]. In support, adipose tissue [9] and fetal membranes synthesize and secrete a large array of molecules at different stages of gestation [8] and exert immune, metabolic and mechanic effects, which might promote pregnancy complications as well. Among the known pro-inflammatory factors, the high mobility group box 1 (HMGB1) molecule has recently received attention because circulating levels of HMGB1 were found to be increased in patients with T2D [10] and in plasma of GDM women in the third trimester of pregnancy [11]. HMGB1 is a well-known alarming molecule, which can activate pro-inflammatory response by interacting with multiple receptors, such as receptor for advanced glycation end products (RAGE). It can be actively secreted by immune and nonimmune cells into the extracellular space, or passively released by necrotic cells. Thus, HMGB1 appears to be a critical molecular target in multiple human diseases, including infections, ischemia, immune disorders, neurodegenerative diseases, metabolic disorders, and cancer [12]. Furthermore, the high levels of HMGB1 found in spontaneous preterm birth (PTB) indicate its involvement in sterile inflammation [13]. Increasing evidence suggests a role of HMGB1 pathway in the regulation of metabolic processes. Obesity alters HMGB1 expression and secretion by adipose tissue [14] and RAGE overexpression induces adipocyte hypertrophy in 3T3-L1 cells, in association with reduced levels of glucose transporter type 4 (GLUT-4), and attenuation of insulin sensitivity [15]. Recent data showed that the neuropeptide vasoactive intestinal peptide (VIP) participates in maintaining immune homeostasis at an early stage of pregnancy with anti-inflammatory and tolerogenic effects [16]. VIP is produced by cells of the immune system and expressed in both central and peripheral nervous systems. It acts as a neurotransmitter and a neuromodulator in nearly all tissues, exerting a wide variety of biologic effects [17]. VIP mediates anti-inflammatory, immunomodulatory, neuroprotective and antidiabetic effects by binding its specific and widespread membrane receptors VPAC1 and VPAC2 [18]. VIP–VPACs pathway influences the production of inflammatory cytokines and chemokines by macrophages [19], and promotes Th2 differentiation [20]. Of interest, VIP contributes to the control of feeding behavior [21], and VPAC2 agonists can stimulate glucose-dependent insulin secretion [18].

In this pilot study, we examined the hypothesis that the expression of HMGB1/RAGE and VIP/VAPC1-2 in fetal membranes and visceral adipose tissue could be altered in pregnant women with GDM. Moreover, any association between these mediators of inflammation and metabolic parameters in GDM was investigated.

Materials and methods

Ethics statements

The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. It was approved by the local ethics committee and all subjects provided written informed consent.

Subjects and tissues collection

Twenty-four ($n = 24$) pregnant women: 12 with gestational diabetes mellitus (GDM) and 12 with normal glucose tolerance (NGT) scheduled for elective cesarean section were recruited in the outpatient clinics of Policlinico Umberto I, "Sapienza" University Hospital of Rome. Diagnosis of GDM was defined in accordance with current recommendations [22]. Specifically, an oral glucose tolerance test with 75 g glucose was performed at 24–28 weeks of gestation and glycemia was measured at baseline, 60 and 120 min after load. The glycemia thresholds which established the presence of GDM were 92 mg/dl at 0′, 180 mg/dl at 60′ and 153 mg/dl at 120′. All subjects were enrolled at third trimester of pregnancy. Women with non-Caucasian ethnicity; pre-pregnancy BMI ≥ 35 kg/m^2; pre-pregnancy impaired fasting glucose (100–125 mg/dl); multiple or induced pregnancy; infectious, inflammatory and autoimmune diseases; polycystic ovary syndrome; psychiatric diseases; alcohol and drug abuse and steroid therapy were excluded. Clinical and biochemical parameters of the subjects are presented in Table 1. Six patients with GDM were treated with diet only, and the other six with insulin. Anthropometric/vital (weight, BMI, blood pressure and heart rate) and laboratory parameters were obtained (Table 1). Information about therapy for GDM (diet or insulin) and other therapies was also acquired (antihypertensive, other drugs). Fetal ultrasound parameters at third trimester and delivery data were collected (Table 2).

Maternal visceral omental adipose tissue (VAT), fetal membranes (FMs; amnion and corion) and a maternal venous blood sample were obtained from GDM and NGT women who delivered healthy, singleton infants at term (> 37 weeks gestation). FMs and VAT were collected immediately after the cesarean section (CS), delivered to laboratory within 15 min and processed immediately. Blood samples without anticoagulant were centrifuged and serum samples were aliquoted and stored at − 80 °C until assay.

Table 1 Clinical and biochemical parameters of subjects

	NGT ($n=12$)	GDM ($n=12$)	p
Age (years)	33.0 ± 6.2	35.3 ± 4.0	0.30
Gestational week (n)	37.0 ± 1.1	36.4 ± 1.2	0.23
Pre-pregnancy BMI (kg/m^2)	24.5 ± 4.2	27.3 ± 3.4	0.09
Weight increase (kg)	11.2 ± 4.4	7.6 ± 7.2	0.16
III trimester BMI (kg/m^2)	28.0 ± 4.0	30.1 ± 2.5	0.12
Nulliparity (n)	3	0	0.22
Gestational hypertension (n)	0	3	0.22
Systolic blood pressure (mmHg)	110.0 ± 11.0	110.4 ± 10.4	0.93
Diastolic blood pressure (mmHg)	69.6 ± 5.4	71.3 ± 10.0	0.61
Insulin therapy (n)	–	6	–
Total cholesterol (mg/dl)	300.1 ± 71.1	254.3 ± 68.1	0.17
HDL cholesterol (mg/dl)	75.1 ± 12.1	64.2 ± 12.2	0.07
LDL cholesterol (mg/dl)	182.6 ± 81.1	152.4 ± 56.0	0.43
Triglycerides (mg/dl)	252.1 ± 116.8	275.1 ± 117.4.3	0.68
Non-HDL cholesterol (mg/dl)	225.0 ± 79.5	190.0 ± 64.7	0.31
HbA1c (%)	5.0 ± 0.1	5.1 ± 0.6	0.86
eGFR MDRD (ml/min/1.73 m^2)	130.0 ± 16.3	122.6 ± 21.7	0.39
eGFR CKD EPI (ml/min/1.73 m^2)	125.2 ± 5.2	121.8 ± 9.3	0.29
TSH (μUI/ml)	2.6 ± 1.4	1.7 ± 1.7	0.17
C-Reactive Protein (μg/l)	4642 ± 2306	4875 ± 3399	0.88
Ferritin (μg/l)	18.2 ± 11.1	26.2 ± 28.6	0.42
Fibrinogen (g/l)	4.6 ± 0.8	4.9 ± 0.7	0.34
Leukocytes (10^9/l)	8.8 ± 1.7	8.9 ± 2.6	0.72

LDL low-density lipoprotein, *HbA1c* glycated hemoglobin, *eGFR* estimated glomerular filtration rate, *CKD EPI* chronic kidney disease epidemiology collaboration, *TSH* thyroid-stimulating hormone, *BMI* body mass index, *GDM* gestational diabetes mellitus, *HDL* high-density lipoprotein

*$p<0.05$. Data are expressed as mean value ± standard deviation or frequency

Table 2 Fetal ultrasound and neonatal parameters

	NGT ($n=12$)	GDM ($n=12$)	p
Fetal ultrasound parameters			
Gestational week (n)	33.9 ± 2.0	34.3 ± 2.2	0.63
Abdominal circumference (mm)	298.1 ± 25.0	307.8 ± 27.1	0.42
Head circumference (mm)	308.6 ± 18.2	293.7 ± 48.9	0.46
HC/AC ratio	1.05 ± 0.06	0.97 ± 0.18	0.30
Bi-parietal diameter (mm)	85.8 ± 4.5	86.0 ± 4.6	0.93
Femur length (mm)	64.9 ± 4.7	67.8 ± 4.2	0.14
Humerus length (mm)	58.0 ± 4.1	60.8 ± 5.3	0.20
Estimated fetal weight (g)	2308 ± 509	2535 ± 473	0.27
Neonatal parameters			
Weight (g)	3292 ± 356	3182 ± 420	0.50
Head circumference (cm)	34.8 ± 1.2	34.5 ± 1.3	0.58
Length (cm)	49.1 ± 1.4	49.0 ± 1.6	0.82
Gestational week at delivery (n)	38.8 ± 0.8	38.5 ± 1.0	0.39
Apgar 5′	9.3 ± 0.6	9.6 ± 0.5	0.17
Male/female (n/n)	6/6	7/5	1.00
Fetal complications	1	2	1.00
Neonatal jaundice (n)	1	1	
ARDS (n)	0	1	

HC head circumference, *AC* abdominal circumference, *ARDS* acute respiratory distress syndrome

*$p<0.05$. Data are expressed as mean ± standard deviation or frequency

Omental adipose tissue explants and culture

Pieces of maternal VAT were obtained from women with GDM ($n=12$) and with NGT ($n=12$). The tissue biopsies were blunt dissected to remove visible connective tissue and vessels, then washed in 0.9% NaCl, and cut into ~ 200 mg tissue samples and incubated at 37 °C in 2 ml of low glucose, serum-free DMEM containing 1% BSA for 18 h. After incubation both tissue and medium were collected and stored separately at − 80 °C for further analysis.

Fetal membrane explants and culture

After delivery of placenta, samples of macroscopically recognized fetal membranes (FMs; amnion and corion) were collected immediately with a surgical blade quite distant from both the free edge and the placental plate.

Tissue samples were washed several times with saline buffer solution to remove blood and clots, then put in Petri multi-plates containing RPMI 1640 culture medium (Gibco, Grand Island N.Y.) 2 ml, penicillin (100 U/ml) and streptomycin (100 mg/ml) and incubated for 24 h in atmosphere of 95% air and 5%CO_2. At the end of incubation time, the culture media and tissue samples were collected and stored at − 80 °C. Representative samples of FMs were examined histologically and consisted of amnion, chorion and in a minor part of decidual cells.

Western blotting

Tissue lysates and Western blotting were prepared as previously described [23].

Immunoblotting analysis was carried out using specific antibodies for HMGB1 (R & D Systems, Minneapolis, MN, USA), RAGE (clone DD/A11 Millipore), VIP (M-19, SC7841, Santa Cruz Biotechnology, Santa Cruz, CA), VPAC1(H-130; SC-30019 Santa Cruz),VPAC2 (H-50, SC-30020, Santa Cruz). Blots were treated with appropriate secondary antibodies conjugated with horseradish

peroxidase followed by ECL detection (Amersham Bio-Sciences, Buckinghamshire, UK). Equal loading of proteins was verified by immunoblotting with goat anti-cyclophilin antibody. Densitometric analysis was performed using a molecular imager FX (Bio-Rad, Hercules, CA, USA).

Cytokines detection

HMGB1 and VIP concentration were analyzed in serum and tissue culture supernatants using specific ELISA kits according to the manufacturer's instructions as follows: HMGB1 (Aviva Systems Biology, San Diego CA) and VIP (BioSite, Taby, Sweden). The limits of detection were 0.156 ng/ml for HMGB1 and 7.81 pg/ml for VIP, respectively.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD) or as median (Interquartile range, IR). Categorical variables are reported as percentage. Continuous variables were tested for normality with Kolmogorov–Smirnov test. Differences between the two groups were evaluated with independent sample *t* test for normally distributed continuous variables and with Mann–Whitney *U* test for not normally distributed continuous variables. Categorical variables between groups were compared using Chi-square. A p value < 0.05 was considered statistically significant. Statistical analysis was performed with IBM SPSS Statistics software version 23 (Chicago, IL, USA).

Results

Characteristics of study subjects

Clinical and biochemical parameters of GDM and NGT women are reported in Table 1. Fetal ultrasound parameters at third trimester and neonatal parameters are reported in Table 2. Mothers in both groups were comparable in maternal age, pre-pregnancy BMI, third trimester BMI, weight gain in pregnancy, gestational age and fasting glucose at third trimester. Fetal and neonatal parameters did not differ between the two groups. The frequency of male sex of the offspring was not significantly different between the two groups.

HMGB1-RAGE expression in fetal membranes and adipose tissue

The Western blotting analyses for HMGB1 and RAGE expression in FMs are shown in Fig. 1a, b. The expression of HMGB1 was higher in fetal membranes in GDM patients than in NGT women (GDM 1.10 ± 0.38 vs NGT 0.58 ± 0.36, $p = 0.02$); no difference was observed in the expression of RAGE (GDM 0.93 ± 0.40 vs NGT 0.82 ± 0.50, $p = 0.62$) between the two groups. In VAT, HMGB1 protein expression was comparable (Fig. 1c) in both groups (GDM 1.74 ± 1.37 vs NGT 1.90 ± 0.94, $p = 0.76$), whereas the expression of its receptor RAGE (Fig. 1d) was significantly higher in GDM (GDM 2.10 ± 1.32 vs NGT 1.15 ± 0.59, $p = 0.03$) compared to NGT controls.

VPACs and VIP expression in fetal membranes and adipose tissue

VPAC1, VPAC-2 and VIP expression in FMs are shown in Fig. 2a–c. Even though there was no significant difference in the expression of VPAC1 (GDM 1.19 ± 0.56 vs NGT 0.76 ± 0.54, $p = 0.17$), VPAC2 (GDM 1.10 ± 0.55 vs NGT 1.06 ± 0.79, $p = 0.97$) and VIP (GDM 1.18 ± 0.60 vs NGT 0.89 ± 0.29, $p = 0.28$) between GDM and NGT women, VPAC-1 and VIP levels tended to be higher in GDM compared to NGT women.

As showed in Fig. 2e, VPAC2 protein expression was significantly higher in VAT obtained from women with GDM (GDM 1.28 ± 0.63 vs NGT 0.83 ± 0.29, $p = 0.03$) compared to NGT subjects. There was no effect of GDM on VPAC1 (GDM 1.87 ± 1.05 vs NGT 1.63 ± 1.47, $p = 0.70$, Fig. 2d) expression. VIP protein expression was not detectable in VAT of both GDM and NGT women.

Circulating levels of HMGB1 and VIP

HMGB1 serum levels were not different in GDM compared to NGT women [GDM 0.7 (0.63–3.82) vs NGT 0.7 (0.42–1.03) ng/ml; $p = 0.33$; Table 3]; and also VIP levels did not differ between the two groups [NGT 154 (101–183) vs GDM 107 (81–122) pg/ml, $p = 0.15$; Table 3].

HMGB1and VIP "in vitro" release by fetal membranes and adipose tissue

High variability of HMGB1 levels was observed in fetal membranes culture supernatant in NGT women [NGT 1.12 (0.11–3.6) ng/ml], whereas HMGB1 was not detectable in culture supernatants in GDM fetal membranes (Table 3).

HMGB1 release was observed in VAT culture supernatant in both NGT and GDM women [NGT 0.23 (0.11–1.4) vs GDM: 0.15 (0.12–0.31) ng/ml $p = 0.28$]. No significant differences were observed between the two groups,

VIP protein expression was not detectable in adipose tissue samples by Western blot analysis, but VIP secretion was determined in VAT culture supernatant. It tended to be lower in women with GDM than in control subjects [NGT 35 (24–72.5) vs GDM 19 (11.25–28) pg/ml, $p = 0.05$]. VIP levels in supernatant of fetal membranes cultures did not

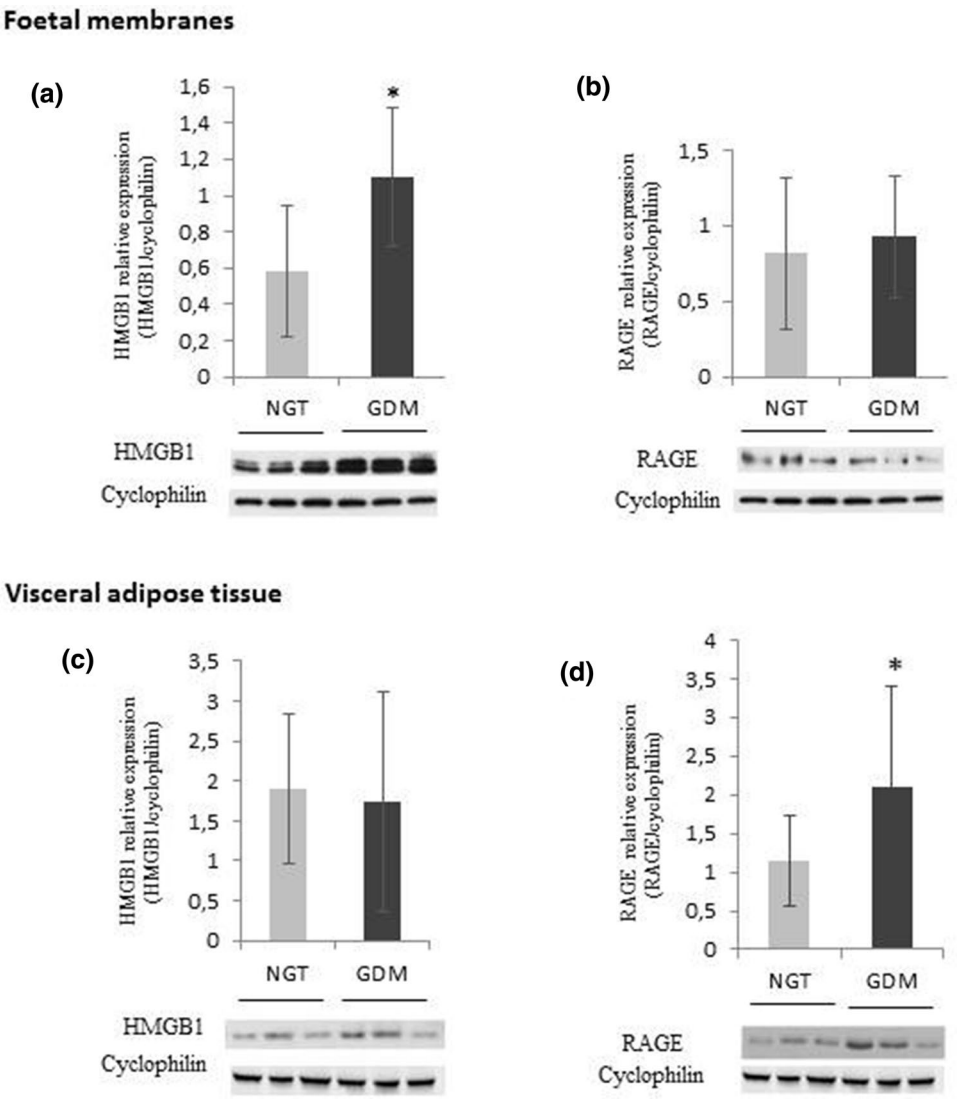

Fig. 1 Comparison of high mobility group box 1 protein (HMGB1) and receptor for advanced glycation end products (RAGE) protein expression in fetal membranes (**a, b**) and visceral adipose tissue (**c, d**) from normal glucose tolerance (NGT) and gestational diabetes mellitus (GDM) women. *$p < 0.05$. Data are expressed as mean value ± standard deviation. Representative blots are shown

differ between GDM and controls [NGT 39 (20.5–69.5) vs GDM 22 (17.25–41.25) pg/ml, $p = 0.43$].

Discussion

In this study GDM condition was found to be associated with an increased protein expression of HMGB1 in fetal membranes, and a high VPAC2 (VIP receptor) and RAGE (HMGB1 receptor) expression in omental adipose tissue. In addition, in vitro cultures from GDM women showed that FMs did not secrete detectable levels of HMGB1, and that VAT released lower levels of VIP, compared with the NGT.

Despite the small number of analyzed subjects, these results highlight the importance of these pro-, anti-inflammatory mediators and pathways in regulating the function of both tissues in pregnancy and support the concept that both fetal membranes and omental adipose tissue take part to the immune metabolic modifications in GDM.

Literature data indicate that placenta and adipose tissue have a regulatory role in the modulation of an increasing number of not well-known molecules necessary for the maintenance of pregnancy to the delivery [7, 9]. Of interest, in the last few years emerging evidence has shown that not only fetal membranes provide a barrier between the feto-placental and maternal compartments but they might have also a basic role in fetal growth [8]. FMs are able to produce a large spectrum of molecules at different stages of gestation and appear to be involved in the signature of inflammation during pregnancy both in physiological and pathological settings [24] The higher expression of the pro-inflammatory protein HMGB1 observed in fetal membranes of GDM women compared to NGT is in line with the previous results obtained in fetal membranes in human spontaneous preterm birth (PTB) [13], and in damaged fetal membrane

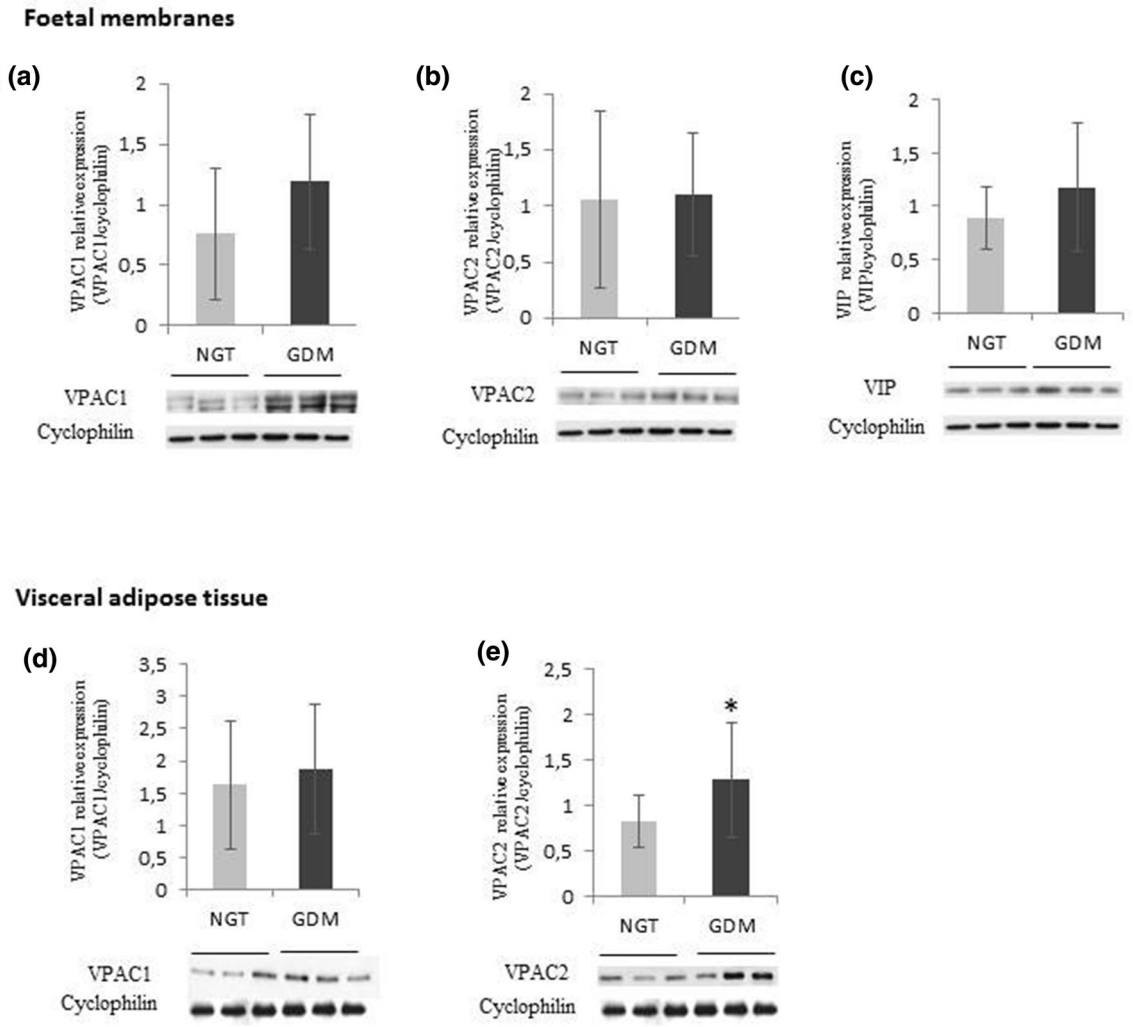

Fig. 2 Comparison of vasoactive intestinal peptide (VIP) and its receptors (VPAC-1 and VPAC-2) protein expression in tissues from normal glucose tolerance (NGT) and gestational diabetes mellitus (GDM) women. VPAC1 (**a**), VPAC2 (**b**) and VIP (**c**) in fetal membranes; and VPAC1 (**d**) and VPAC2 (**e**) in visceral adipose tissue. $^{*}p < 0.05$. Data are expressed as mean value ± standard deviation. Representative blots are shown

from women with intra-amniotic inflammation [25]. This evidence supports a significant role of this cytokine in maintaining the inflammatory milieu in pregnancy complications. In addition, in this study FMs in GDM patients did not secrete detectable levels of HMGB1 in vitro, in contrast to NGT women. It is known that this cytokine exerts multiple extracellular and intracellular activities by activating several signaling pathways [12, 13, 26]. Recent data indicate that the release of HMGB1 from FMs to other tissues is part of the physiological inflammatory signaling required to promote parturition [27, 28]. Specifically, it appears that HMGB1 mediates physiological senescence of fetal membranes to promote labor at term through the activation of p38MAPK [13]. Since we have obtained FMs at cesarean section (not in labor), we speculate that the absence of HMGB1 secretion in fetal membranes of GDM women might alter the physiological response in promoting labor.

Different from Giacobbe et al. study [11], we did not observe higher circulating levels of HMGB1 in GDM women.

Then, we investigated the expression of RAGE, one of the receptors through which HMGB1 exerts its inflammatory activity [12]. However, no differences were observed in fetal membranes between the two groups.

Evidence from literature suggests that HMGB1–RAGE axis contributes to the pathogenesis of the chronic vascular complications of diabetes [29]. Our data are in line with a previous study, which did not identify any differences in RAGE protein expression in fetal membranes between GDM and NGT women [30]. It suggests that the higher expression

Table 3 HMGB1 and VIP circulating levels and release from tissues in vitro

	NGT	GDM	*p*
Serum			
HMGB1(ng/ml)	0.7 (0.42–1.03)	0.7 (0.63–3.83)	0.33
VIP (pg/ml)	154 (101–183)	107 (81–122)	0.15
In vitro release			
HMGB1(ng/ml)			
FM	1.12 (0.11–3.6)	ND	
VAT	0.23 (0.11–1.4)	0.15 (0.12–0.31)	0.28
VIP (pg/ml)			
FM	39 (20.5–69.5)	22 (17.25–41.25)	0.43
VAT	35 (24–72.5)	19 (11.25–28)	0.05

HMGB1 High Mobility Group Box 1, *VIP* vasoactive intestinal peptide, *FM* fetal membranes, *VAT* visceral adipose tissue

*$p < 0.05$. Results are expressed as median (Interquartile range). ND: not detected

of HMGB1 might be sufficient to stimulate the inflammatory response, by activating the transcriptor factor nuclear factor-kB (NF-k B), which in turn stimulates the production of inflammatory cytokines [31].

Because of the function of FMs in fetal development and protection throughout pregnancy, we can speculate that inflamed FMs could have a considerable impact on the offspring outcome as well.

It has been observed that increased expression of HMGB1 in adipose tissue might play a role in maintaining the chronic inflammatory state in obesity and insulin resistance [14]. Indeed, in human SW872 preadipocyte cell line, the interaction between HMGB1 and RAGE induced the secretion of inflammatory cytokines [32].

Although we did not observe any differences in the protein expression of HMGB1 in VAT between the two groups, the increased expression of RAGE in omental adipose tissue in GDM women might indicate an amplified inflammatory activity mediated by this receptor.

This finding is in accordance with the results of a previous, in vitro, study, which showed that the overexpression of RAGE, via NFkB activation, induced the transcription of inflammatory cytokines and was involved in adipocyte hypertrophy and in reducing insulin sensitivity [15]. It is possible to hypothesize that other ligands activate RAGE in GDM adipose tissue, since several exogenous and endogenous ligands, including advanced glycation end products (AGEs), advanced oxidation protein products, calcium-binding S100 proteins, lipopolysaccharide (LPS), recognize RAGE and appear to be involved in inflammation and diabetes progression [33].

Among the wide array of molecules involved in fetal membranes and adipose tissue alterations in GDM, we evaluated if VIP–VPACs pathway could have a role in GDM pathophysiology, because of its involvement in the immune response [20], in the development of placental tissues [34] and in promotion insulin secretion [18]. We did not observe any differences in the expression of VIP, VPAC1 and VPAC2 in FMs from GDM and NGT pregnant women.

Considering that VPAC1 and VPAC2 receptors are expressed in normal human female genital tract (endometrium, myometrium, ovary and Fallopian tube) [35], and recent data indicated that VIP/VPACs pathway has a role in regulating pregnancy by controlling inflammatory response [16], we can hypothesize that, in this study, this signaling pathway is not involved in FMs modifications in GDM.

In this study, VAT in GDM showed increased expression of VPAC2 and released lower levels of VIP "in vitro". Physiological pregnancy is characterized by a fine balance between pro- and anti-inflammatory cytokines [36]. Giving the anti-inflammatory activity of VIP/VPAC2 pathway [20], it is possible to speculate that this pathway exerts this activity in autocrine manner via VPAC2 overexpression in VAT, by inhibiting and promoting the production of inflammatory and anti-inflammatory cytokines, respectively.

Moreover, VIP and VPACs receptors can activate several signaling pathways which can modulate adipose tissue metabolism and regulate appetite and body composition [37]. Remarkable changes in maternal and fetal lipid metabolism take place during pregnancy, and adipose tissue plays a significant role in GDM development [38]. An in vitro study has shown that VIP induced lipolysis through VPAC2 activation, in primary rat adipocytes [39]. The authors assume that lipolytic action of VIP via VPAC2 by increasing free fatty acid levels potentiates insulin secretion, contributing to maintain energy homeostasis both in conditions of energy deprivation and after food intake. Thus, it is conceivable to hypothesize that the increased expression of VPAC2 observed in VAT of GDM patients can be an attempt to manage hyperglycemia. Moreover, we can not exclude that other molecules, including the pituitary adenylate cyclase activating peptide (PACAP), a new member of VIP family, can activate the receptor VPAC2 and exert antidiabetic effects [40]. There are no literature data regarding the role of VIP–VIPACs pathways in the third trimester of pregnancy.

This is a preliminary study and its main limits are the number of the included subjects and the lack of some parameters to test insulin resistance and beta-cell function (i.e., serum insulin, HOMA index and C-peptide).

The results of this study support the concept that both fetal membranes and omental adipose tissue take part to the immune metabolic modifications in GDM, and highlight the importance of these molecules in regulating the function of both tissues in pregnancy.

Conclusions

In conclusion, this pilot study provides relevant data that suggest a complex cross talk between FMs and VAT throughout pregnancy, despite the limitation due to the small number of women involved.

In regard to GDM status, there is an attempt to balance the inflammatory milieu due to higher HMGB1 levels in FMs and higher RAGE expression in VAT, with the activation of the anti-inflammatory pathway represented by the higher expression of VPAC2 in VAT. The role of FMs and VAT in maintaining immune homeostasis likely occurs by fine tuning regulation that involves HMGB1/RAGE and VIP/VAPCs pathways. Further studies are necessary to clarify the mechanisms underlying these findings and the role of these mediators on fetal and maternal outcomes in GDM.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Pintaudi B, Fresa R, Dalfra M et al (2018) The risk stratification of adverse neonatal outcomes in women with gestational diabetes (STRONG) study. Acta Diabetol 55(12):1261–1273
2. Leybovitz-Haleluya N, Wainstock T, Landau D, Sheiner E (2018) Maternal gestational diabetes mellitus and the risk of subsequent pediatric cardiovascular diseases of the offspring: a population-based cohort study with up to 18 years of follow up. Acta Diabetol 55:1037–1042
3. American Diabetes A (2018) 13. Management of diabetes in pregnancy: standards of medical care in diabetes-2018. Diabetes Care 41(Suppl 1):S137–S143
4. Bao W, Yeung E, Tobias DK et al (2015) Long-term risk of type 2 diabetes mellitus in relation to BMI and weight change among women with a history of gestational diabetes mellitus: a prospective cohort study. Diabetologia 58(6):1212–1219
5. McKenzie-Sampson S, Paradis G, Healy-Profitos J, St-Pierre F, Auger N (2018) Gestational diabetes and risk of cardiovascular disease up to 25 years after pregnancy: a retrospective cohort study. Acta Diabetol 55(4):315–322
6. Howell KR, Powell TL (2017) Effects of maternal obesity on placental function and fetal development. Reproduction 153(3):R97–R108
7. Jayabalan N, Nair S, Nuzhat Z et al (2017) Cross talk between adipose tissue and placenta in obese and gestational diabetes mellitus pregnancies via exosomes. Front Endocrinol 8:239
8. Menon R, Richardson LS (2017) Preterm prelabor rupture of the membranes: a disease of the fetal membranes. Semin Perinatol 41(7):409–419
9. Pantham P, Aye IL, Powell TL (2015) Inflammation in maternal obesity and gestational diabetes mellitus. Placenta 36(7):709–715
10. Skrha J Jr, Kalousova M, Svarcova J et al (2012) Relationship of soluble RAGE and RAGE ligands HMGB1 and EN-RAGE to endothelial dysfunction in type 1 and type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 120(5):277–281
11. Giacobbe A, Granese R, Grasso R et al (2016) Association between maternal serum high mobility group box 1 levels and pregnancy complicated by gestational diabetes mellitus. Nutrition, metabolism, and cardiovascular diseases. NMCD 26(5):414–418
12. Kang R, Chen R, Zhang Q et al (2014) HMGB1 in health and disease. Mol Asp Med 40:1–116
13. Bredeson S, Papaconstantinou J, Deford JH et al (2014) HMGB1 promotes a p38MAPK associated non-infectious inflammatory response pathway in human fetal membranes. PLoS One 9(12):e113799
14. Zhang J, Zhang L, Zhang S et al (2017) HMGB1, an innate alarmin, plays a critical role in chronic inflammation of adipose tissue in obesity. Mol Cell Endocrinol 454:103–111
15. Monden M, Koyama H, Otsuka Y et al (2013) Receptor for advanced glycation end products regulates adipocyte hypertrophy and insulin sensitivity in mice: involvement of Toll-like receptor 2. Diabetes 62(2):478–489
16. Ramhorst R, Calo G, Paparini D et al (2018) Control of the inflammatory response during pregnancy: potential role of VIP as a regulatory peptide. Ann N Y Acad Sci 1437:15–21
17. Ibrahim H, Barrow P, Foster N (2011) Transcriptional modulation by VIP: a rational target against inflammatory disease. Clin Epigenetics 2(2):213–222
18. Giordanetto F, Revell JD, Knerr L et al (2013) Stapled vasoactive intestinal peptide (VIP) derivatives improve VPAC2 agonism and glucose-dependent insulin secretion. ACS Med Chem Lett 4(12):1163–1168
19. Forghani P, Petersen CT, Waller EK (2017) Activation of VIP signaling enhances immunosuppressive effect of MDSCs on CMV-induced adaptive immunity. Oncotarget 8(47):81873–81879
20. Delgado M, Ganea D (2013) Vasoactive intestinal peptide: a neuropeptide with pleiotropic immune functions. Amino Acids 45(1):25–39
21. Sekar R, Wang L, Chow BK (2017) Central control of feeding behavior by the secretin, PACAP, and glucagon family of peptides. Front Endocrinol 8:18
22. Associazione Medici Diabetologi (AMD) (2018) Società Italiana di Diabetologia (SID) Standard Italani per la cura del diabete mellito. http://www.standarditaliani.it. Accessed 16 Jan 2019
23. Scazzocchio B, Vari R, Filesi C et al (2015) Protocatechuic acid activates key components of insulin signaling pathway mimicking insulin activity. Mol Nutr Food Res 59(8):1472–1481
24. Menon R, Richardson LS, Lappas M (2018) Fetal membrane architecture, aging and inflammation in pregnancy and parturition. Placenta. https://doi.org/10.1016/j.placenta.2018.11.003
25. Baumbusch MA, Buhimschi CS, Oliver EA et al (2016) High Mobility Group-Box 1 (HMGB1) levels are increased in amniotic fluid of women with intra-amniotic inflammation-determined preterm birth, and the source may be the damaged fetal membranes. Cytokine 81:82–87

26. Banerjee S, de Freitas A, Friggeri A, Zmijewski JW, Liu G, Abraham E (2011) Intracellular HMGB1 negatively regulates efferocytosis. J Immunol 187(9):4686–4694
27. Menon R, Bonney EA, Condon J, Mesiano S, Taylor RN (2016) Novel concepts on pregnancy clocks and alarms: redundancy and synergy in human parturition. Hum Reprod Update 22(5):535–560
28. Grabowska K, Stapinska-Syniec A, Saletra A, Jarmuzek P, Bomba-Opon D (2017) Labour in women with gestational diabetes mellitus. Ginekologia polska 88(2):81–86
29. Manigrasso MB, Juranek J, Ramasamy R, Schmidt AM (2014) Unlocking the biology of RAGE in diabetic microvascular complications. Trends Endocrinol Metab 25(1):15–22
30. Alexander KL, Mejia CA, Jordan C et al (2016) Differential receptor for advanced glycation end products expression in preeclamptic, intrauterine growth restricted, and gestational diabetic placentas. Am J Reprod Immunol 75(2):172–180
31. Wang K, Yang ZQ, Yu HF, Wang YS, Guo B, Yue ZP (2018) High Mobility Group Box 1 regulates uterine decidualization through bone morphogenetic protein 2 and plays a role in Kruppel-Like factor 5-induced stromal differentiation. Cell Physiol Biochem 48(6):2399–2408
32. Nativel B, Marimoutou M, Thon-Hon VG et al (2013) Soluble HMGB1 is a novel adipokine stimulating IL-6 secretion through RAGE receptor in SW872 preadipocyte cell line: contribution to chronic inflammation in fat tissue. PLoS One 8(9):e76039
33. Yamamoto Y, Yamamoto H. RAGE-Mediated Inflammation (2013) Type 2 diabetes, and diabetic vascular complication. Front Endocrinol 4:105
34. Marzioni D, Fiore G, Giordano A et al (2005) Placental expression of substance P and vasoactive intestinal peptide: evidence for a local effect on hormone release. J Clin Endocrinol Metab 90(4):2378–2383
35. Bajo AM, Juarranz MG, Valenzuela P, Martinez P, Prieto JC, Guijarro LG (2000) Expression of vasoactive intestinal peptide (VIP) receptors in human uterus. Peptides 21(9):1383–1388
36. Soeters PB, Grimble RF (2013) The conditional role of inflammation in pregnancy and cancer. Clin Nutr 32(3):460–465
37. Vu JP, Larauche M, Flores M et al (2015) Regulation of appetite, body composition, and metabolic hormones by vasoactive intestinal polypeptide (VIP). J Mol Neurosci 56(2):377–387
38. Herrera E, Desoye G (2016) Maternal and fetal lipid metabolism under normal and gestational diabetic conditions. Horm Mol Biol Clin Investig 26(2):109–127
39. Akesson L, Ahren B, Edgren G, Degerman E (2005) VPAC2-R mediates the lipolytic effects of pituitary adenylate cyclase-activating polypeptide/vasoactive intestinal polypeptide in primary rat adipocytes. Endocrinology 146(2):744–750
40. Zhao SJ, Wang DH, Li YW et al (2017) A novel selective VPAC2 agonist peptide-conjugated chitosan modified selenium nanoparticles with enhanced anti-type 2 diabetes synergy effects. Int J Nanomed 12:2143–2160