Markers of endothelial cell dysfunction are increased in human omental adipose tissue from women with pre-existing maternal obesity and gestational diabetes

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Word count of text: 3770

Word count of abstract: 253

Number of references: 79

Number of tables: 1

Number of figures: 7

The author declares no potential conflicts of interest.

ABSTRACT

Objective: To determine the effect of maternal obesity and gestational diabetes mellitus (GDM) on the expression and release of genes involved in endothelial cell dysfunction in human placenta and omental adipose tissue.

Materials/Methods: Human placental and omental adipose tissue were obtained from non-obese and obese normal glucose tolerant (NGT) women and women with GDM at the time of Caesarean section. Quantitative RT-PCR was performed to determine the level of expression. Tissue explants were performed to determine the release of proteins of interest.

Results: There was no effect of pre-existing maternal obesity or GDM on placental gene expression or secretion of members of the VEGF family members (PLGF and VEGF-A expression and secretion; sFlt-1 release; VEGFR1 and VEGFR2 mRNA expression); FGFR1 mRNA expression, FGF2 mRNA expression and secretion; endoglin mRNA expression and secretion (sEng); and the adhesion molecules ICAM-1 and VCAM-1. On the other hand, in omental adipose tissue, pre-existing maternal obesity and GDM were associated with increased gene expression of PLGF, endoglin and ICAM-1 and increased secretion of PLGF, sFlt-1, FGF2, sEng and sICAM-1. There was, however, no effect of maternal pre-existing obesity and GDM on VEGF-A, VEGFR1, VEGFR2, FGFR1 and VCAM-1 expression or secretion.

Conclusions: This study demonstrated the presence of abnormal expression and secretion of angiogenic proteins and adhesion molecules in omental adipose tissue, but not placenta, from pregnant women with GDM and pre-existing maternal obesity. Increased angiogenic and adhesion molecules released from adipose tissue may affect angiogenesis, inflammation and or lipid and glucose metabolism in both mum and her offspring.

Keywords: adipose tissue, obesity, gestational diabetes, endothelial cell dysfunction

List of Abbreviations: GDM, gestational diabetes mellitus; NGT, normal glucose tolerant; qRT-PCR, quantitative RT-PCR; sICAM, soluble intracellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; Flt-1, fms-like tyrosine kinase 1; sFlt-1, soluble fms-like tyrosine kinase 1; sEng, soluble endoglin; PLGF, placental growth factor; FGF2, basic fibroblast growth factor; FGFR1, fibroblast growth factor receptor 1.

INTRODUCTION

The prevalence of gestational diabetes mellitus (GDM) and maternal obesity during pregnancy is increasing; affecting up to 20% of all pregnancies [2-5]. These metabolic disturbances are associated with increased risk of adverse pregnancy and infant outcomes [6-8]. Additionally, there is an increased the risk of developing obesity, type 2 diabetes and cardiovascular disease later in life for both mother and child [6-8]. The economic costs associated with GDM and maternal obesity during pregnancy are substantial and extend well into adulthood [6, 9]. There is now increasing evidence that the obese and diabetic environment may induce a number of changes in both placenta and maternal adipose tissue which may play an important role in the growth and development of the fetus [10-12].

Endothelial cell dysfunction is a feature of obese and GDM pregnancies. For example, maternal obesity is associated with impaired endothelial function [13], and non-branching angiogenesis is evident in the placentas from obese women [14]. Women with GDM develop endothelial dysfunction during pregnancy [15], and despite returning to normal glucose tolerance, endothelial dysfunction is still evident in women 1 year post GDM pregnancy [16]. Indeed, women with GDM have increased circulating levels of the adhesion molecules soluble intracellular adhesion molecule (sICAM)-1 and soluble vascular cell adhesion molecule (sVCAM)-1 levels [1]. The angiogenic markers vascular endothelial growth factor (VEGF), fms-like tyrosine kinase 1 (Flt-1), endoglin, placental growth factor (PLGF), and basic fibroblast growth factor (FGF2), and the adhesion molecules ICAM-1 and VCAM-1 play an important role in the development of endothelial dysfunction [17-22]. In preeclampsia, a systemic syndrome of pregnancy characterised by widespread maternal endothelial dysfunction [23], placental trophoblast cells produce significantly more sEng, sFlt-1, and PLGF compared with those from normal placenta [24, 25]. There is,

however, a paucity of data on the effect of GDM and maternal obesity on the expression and secretion of these markers in placenta and adipose tissue.

Thus, the aim of this study was to determine the effect of maternal obesity and GDM on the (i) gene expression of PLGF, FGF2 and FGF receptor 1 (FGFR1), VEGF-A and its receptors VEGFR1 (also known as Flt-1), VEGFR2, endoglin, ICAM-1 and VCAM-1 and (ii) release of PLGF, FGF2, soluble Flt-1 (sFlt-1), VEGF, soluble endoglin (sEng), sICAM-1 and sVCAM-1 from human placenta and omental adipose tissue.

MATERIALS AND METHODS

Tissue Collection and Preparation

Approval for this study was obtained from the Mercy Hospital for Women's Research and Ethics Committee and written informed consent was obtained from all participating subjects. Human placenta and omental adipose tissue were obtained from a total of 60 pregnant women (28 NGT and 32 GDM). Tissues were obtained within fifteen minutes of delivery.

Women were invited to provide samples on the day of admission for surgery. Sample collection occurred between November 2011 and July 2013. All tissues were obtained at the time of term Caesarean section in the absence of labour. Indications for Caesarean section included repeat Caesarean section or breech presentation. Women with any adverse underlying medical condition (i.e. including asthma, preeclampsia and pregestational diabetes) were excluded. Samples were collected from non-obese (BMI between 18-29 kg/m²) and obese (BMI $\geq$ 30 kg/m²) subjects. The women were classified as non-obese or obese based on their pre-pregnancy BMI. Women with GDM were diagnosed according to the criteria of the Australasian Diabetes in Pregnancy Society (ADIPS) by either a fasting venous plasma glucose level of $\geq$ 5.5 mmol/l glucose, and/or $\geq$ 8.0 mmol/l glucose 2 h after a 75 g oral glucose load at approximately 26-28 weeks gestation. Nineteen women with GDM were prescribed insulin in the third trimester of pregnancy according to hospital guidelines for insulin therapy in GDM. All pregnant women were screened for GDM, and women participating in the NGT group had a negative screen. The relevant clinical details of the subjects are detailed in Table 1.

Tissue explants

Human placenta and omental adipose tissue were obtained from 28 NGT and 32 GDM (13 diet- and 19 insulin-treated) pregnant women. Placental and omental adipose tissue explants were performed

as previously described [26, 27]. Briefly, adipose tissue was washed thoroughly in ice-cold PBS to remove any blood, and then cut into 2 mm² pieces. Placental lobules (cotyledons) were obtained from various locations of the placenta; the basal plate and chorionic surface were removed from the cotyledon, and villous tissue was obtained. A piece of placenta and adipose tissue (~100 mg) was snap frozen in liquid nitrogen and stored at -80°C until required for RNA extraction. The remaining tissue was placed in DMEM (supplemented with penicillin G and streptomycin) at 37°C in a humidified atmosphere of 5% CO₂ and 8% O₂ for placenta, and 5% CO₂ and 21% O₂ for adipose tissue for 1 h. Tissues were blotted dry on sterile filter paper and transferred to 24-well tissue culture plates (100 mg wet weight/well). The explants were incubated in 1 ml DMEM (containing 100 U/ml penicillin and 100 µg/ml streptomycin) at 37°C in a humidified atmosphere of 5% CO₂ and 8% O₂ for placenta, and 5% CO₂ and 21% O₂ for adipose tissue. After 20 h incubation, tissue and media were collected and stored separately at minus 80°C. Media was analysed for FGF2, VEGF-A, sFlt-1, sEng, PLGF, soluble ICAM-1 (sICAM-1) and soluble VCAM-1 (sVCAM-1) levels by ELISA as detailed below. Tissues were collected and assayed for total protein using BCA protein assay (Pierce, Rockford, USA), as previously described [28-31].

Enzyme immunoassays

The release of sFlt-1, sEng, PLGF and sVCAM-1 was performed by sandwich ELISA from R&D Systems according to the manufacturer's instructions. The release of VEGF, FGF2 and sICAM-1 was performed by sandwich ELISA from Peprotech according to the manufacturer's instructions. All data were corrected for total protein and expressed as either pg or ng per mg protein. The calculated interassay and intraassay coefficients of variation (CV) were all less than 10%.

RNA extraction and qRT-PCR

Total RNA was extracted from approximately 100 mg of tissue using Tri Reagent according to manufacturer's instructions (Sigma-Aldrich, Saint Louis Missouri). RNA concentrations were

quantified using a spectrophotometer (Smart Spec, Bio-Rad). RNA quality and integrity was determined via the A_{260}/A_{280} ratio and agarose gels electrophoresis. One μg of RNA was converted to cDNA using the Tetro cDNA Synthesis Kit according to the manufacturer's instructions (Bioline, Alexandria, NSW, Australia). The cDNA was diluted ten-fold and 2 μl of this was used to perform RT-PCR using SensiMix SYBR No-ROX Kit (Bioline, Alexandria, NSW, Australia) with 100 nM primer. All primers were purchased from Qiagen (Germantown, Maryland, USA). The RT-PCR was performed using CFX384 from Bio-Rad Laboratories (Hercules, California, USA). The specificity of the product was assessed from melting curve analysis. RNA without reverse transcriptase during cDNA synthesis as well as PCR reactions using water instead of template showed no amplification. Following baseline correction, the fluorescence threshold level was set during the geometric (exponential) phase of PCR amplification to generate the threshold cycle (C_T) value for each amplification curve. Average gene C_T values were normalised to the average actin RNA C_T values of the same cDNA sample. Variations in gene expression were calculated by the comparative C_T method. Fold differences are expressed relative to NGT non-obese patients.

Statistical Analysis

Statistics was performed on the normalised data unless otherwise specified. All statistical analyses were undertaken using GraphPad Prism (GraphPad Software, La Jolla, CA). For two sample comparisons, an unpaired Student's t-test was used to assess statistical significance between normally distributed data; otherwise, the nonparametric Mann-Whitney U (unpaired) or the Wilcoxon (matched pairs) tests were used. Statistical significance was ascribed to P value <0.05 . Data were expressed as mean $\pm$ standard error of the mean (SEM).

RESULTS

Participants

Demographic data of the participants involved in this investigation are summarised in Table 1. The NGT and GDM women were stratified according to their pre-pregnancy BMI as either non-obese or obese. There were no significant differences in maternal age, gestational age at delivery and fetal birthweight between any of the groups. As expected maternal BMI (both pre-pregnancy and at delivery) was significantly higher in the non-obese patients compared to the obese patients. One-hour and two-hour plasma glucose concentrations at OGTT were significantly greater in women with non-obese GDM compared with non-obese NGT women. In obese women, fasting, one-hour and two-hour plasma glucose concentrations at OGTT were significantly greater in women with GDM compared with NGT women.

Effect of pre-existing maternal obesity and GDM on the expression and secretion of angiogenic markers

The effect of pre-existing maternal obesity and GDM on the gene expression of PLGF, FGF2 and FGF receptor 1 (FGFR1), VEGF-A and its receptors VEGFR1 and VEGFR2, and endoglin from human placenta and pregnant omental adipose tissue is presented in Figures 1-5. Additionally, the effect of pre-existing maternal obesity and GDM on the release of PLGF, FGF2, sFlt-1, VEGF and sEng are presented in Figures 1-5.

There was no effect of pre-existing maternal obesity and GDM on PLGF mRNA expression or secretion from placenta (Figures 1A,B). In omental adipose tissue, there was no difference in the expression or secretion of PLGF between non-obese NGT and obese NGT pregnant women (Figures 1C,D). On the other hand, PLGF expression and secretion was significantly higher in obese GDM women compared to non-obese GDM women. In the obese women, adipose tissue

expression was also higher in women with GDM compared to NGT women (Figures 1C,D).

Additionally, when the non-obese and obese women were combined in the two cohorts, PLGF gene expression was significantly higher GDM women compared to NGT women (Figure 1C).

For both placenta and omental adipose tissue, there was no effect of pre-existing maternal obesity and GDM on VEGF-A mRNA expression (Figures 2A,B). In omental adipose tissue, there was also no effect of pre-existing maternal obesity and GDM on VEGF-A secretion (Figure 2C). In this study, the concentration of VEGF-A release by placenta was below the sensitivity of the assay (data not shown).

For both placenta and omental adipose tissue, there was no effect of pre-existing maternal obesity and GDM on VEGFR1 and VEGFR2 mRNA expression (Figures 3A,B,D,E). For placenta, there was no effect of pre-existing maternal obesity and GDM on sFlt-1 secretion (Figure 3C). On the other hand, omental sFlt-1 secretion was significantly higher in non-obese GDM women compared to non-obese NGT women (Figure 3F). The release of sFlt-1 was also higher in obese GDM women compared to obese NGT; however, this just failed to reach significance. When the non-obese and obese women were combined in the two cohorts, sFlt-1 release was again significantly higher in the women with GDM when compared to the NGT women (Figure 3F).

There was no effect of pre-existing maternal obesity and GDM on placental FGFR1 and FGF2 mRNA expression (Figures 4A,B), placental FGF2 secretion (Figure 4C), and omental FGFR1 mRNA expression (Figure 4D). FGF2 gene expression was higher in omental adipose tissue from obese GDM women compared to obese NGT women; however, this just failed to reach significance (Figure 4E). The release of FGF2 was significantly higher in omental adipose tissue obtained from women with GDM (Figure 4F).

The effect of pre-existing maternal obesity and GDM on the mRNA expression and the secretion of endoglin from human placenta and omental adipose tissue is presented in Figures 5. There was no effect of pre-existing maternal obesity and GDM on endoglin mRNA expression or secretion from placenta (Figures 5A,B). In the NGT women, the release of sEng was higher in omental adipose tissue from obese women compared to non-obese women; however, this just failed to reach significance (Figure 5D). In the non-obese women only, the release of sEng was also significantly higher in GDM compared to NGT omental adipose tissue (Figure 5D). When the non-obese and obese women were combined in the two cohorts, both endoglin gene expression (Figure 5C) and sEng release (Figure 5D) from omental adipose tissue were significantly higher in the women with GDM when compared to the NGT women.

Effect of pre-existing maternal obesity and GDM on the expression and secretion of adhesion molecules

In placenta, ICAM-1 mRNA expression and sICAM-1 release was not different between non-obese and obese women, and between GDM and NGT women (Figures 6A,B). On the other hand, in the NGT cohort, the release of sICAM-1 was significantly higher in omental adipose tissue from obese women compared to non-obese women (Figure 6D). Additionally, in the non-obese women only, the release of sICAM-1 was also significantly higher in omental adipose tissue from GDM women compared to NGT women (Figure 6D). Furthermore, when the non-obese and obese women were combined from the two cohorts, sICAM-1 release was significantly higher in the women with GDM when compared to the NGT women (Figure 6D).

There was no effect of pre-existing maternal obesity and GDM on VCAM-1 mRNA expression or sVCAM-1 secretion from human placenta (Figures 7A,B) and omental adipose tissue (Figures 7C,D).

DISCUSSION

In this study, obesity and GDM-associated changes in the expression and secretion of angiogenic proteins and adhesion molecules in human placenta and adipose tissue was assessed. In placenta, pre-existing maternal obesity or GDM had no effect on the VEGF and FGF family members, endoglin, and the adhesion molecules ICAM-1 and VCAM-1. On the other hand, in omental adipose tissue, pre-existing maternal obesity and GDM was associated with increased gene expression of PLGF, endoglin and ICAM-1 and increased secretion of PLGF, sFlt-1, FGF2, sEng and sICAM-1.

Angiogenic growth factors and their receptors, including FGFs and the VEGF family, are crucial for normal placental development and pregnancy [32-34]. Preeclampsia is a systemic syndrome of pregnancy characterised by widespread maternal endothelial dysfunction [23]. Trophoblast cells from human preeclamptic placenta produce significantly more sEng, sFlt-1, and PLGF compared with those from normal placenta [24]. In this study, there was no effect of pre-existing maternal obesity on placental villous expression and or secretion of VEGF family members and endoglin. Previous studies in GDM placenta have yielded contradictory results. Placental mRNA levels of VEGFR1 and VEGFR2 were significantly higher in women with insulin-treated GDM compared to NGT women [35], whereas placental protein expression of VEGF-A and VEGFR-2 were lower in women with GDM [36]. On the other hand, in keeping with the findings of this study, there was no effect of pre-existing type I diabetes on the gene expression of VEGF, VEGFR1 and VEGFR2 [37]. There are no other published studies in the effect of obesity on the expression or release of angiogenic factors from human placenta; however high fat fed dams at gestational day 18.5 have increased levels of VEGF-A expression in placental vascular endothelial cell [38].

FGFR1 and FGF2 are important mediators of fetal growth. FGFR1-deficient mice display severe growth restriction in utero [39] and the levels of FGF2 in maternal serum correlate positively with fetal size both in the second trimester and at term [40, 41]. In this study, there was no effect of maternal obesity or GDM on placental FGF2 expression or secretion or FGFR1 expression. In contrast, other studies report that FGF2 mRNA levels are significantly higher in the GDM placenta than non-GDM placenta, while FGFR2 showed no difference [40, 42]. Additionally, the serum concentrations of FGF2 are higher in GDM women when compared to NGT women [40, 41]. It is of note, that FGF2 and FGFR1 mRNA expression was not different in normal and type I diabetes placenta [37].

Atherosclerosis is predominantly mediated by cellular adhesion molecules, which are expressed on the vascular endothelium and on circulating leukocytes in response to several inflammatory stimuli. Soluble forms of ICAM-1 and VCAM-1 are found to enter the systemic circulation, as a result of proteolytic cleavage at the cell surface, releasing the extracellular domain. The levels of sICAM-1 and sVCAM-1 are elevated in a broad array of disease states, including insulin resistance, obesity and type 2 diabetes [43-45]. Additionally, sICAM-1 and sVCAM-1 levels have been shown to correlate to various cardiovascular risk factors [46, 47]. In this study, there was no effect of maternal obesity or GDM on placental ICAM-1 and VCAM-1 expression or secretion. Similarly, ICAM-1 expression in the umbilical artery, vein or the placenta of women GDM was not different when compared to NGT women [48]. Of note, placental expression of adhesion molecules is also not altered with preeclampsia [49-51].

Unlike many other organs and tissues, adipose tissue can expand many-fold, and in obese individuals, it can comprise more than 40% of total body composition. The expansion of the vasculature occurs through angiogenesis, and inappropriate angiogenesis may underlie adipose tissue dysfunction in obesity, which in turn increases type 2 diabetes risk [17, 52]. Numerous pro

and anti-angiogenic factors secreted by adipocytes are likely to control adipose tissue angiogenesis; recent proteomic studies have revealed that up to 50% of the adipose tissue secretome is comprised of proteins that have been implicated in angiogenesis [53].

In this study, PLGF and endoglin mRNA expression and the release of sFlt-1 and sEng were significantly higher in adipose tissue obtained from women with GDM when compared to NGT women. Additionally, sEng release was also higher from obese adipose tissue when compared to non-obese adipose tissue in NGT women. There was, however, no effect of pre-existing maternal obesity or GDM on VEGF-A expression and secretion, and VEGFR1 and VEGFR2 mRNA expression. To my knowledge, there have been no other studies on the VEGF family and endoglin in adipose tissue from pregnant women. However, and in keeping with the findings of this study, PLGF is up-regulated in the gonadal adipose tissue of ob/ob compared with WT mice, but is unchanged in nutritionally induced obesity [54]; and subcutaneous adipose tissue of VEGF-A levels and VEGFR1-3 mRNA levels were not markedly modulated by obesity in mice [54]. However, both VEGF-A mRNA and protein levels were found to be higher in 14-wk-old, compared with 6-wk-old, db/db mice and in obese KK-A^Y mice, compared with WT mice [55].

There was no effect of maternal pre-existing obesity on adipose tissue FGFR1 and FGF2 mRNA expression and secretion of FGF2. This data is consistent with studies showing FGF2 gene expression is not altered in adipose tissue in mouse models of obesity [54]. In contrast to these findings, FGF2 mRNA levels are increased in preadipocytes from massively obese humans compared with lean ones [56] where it has been shown to enhance adipocyte differentiation *in vivo* [57]. Although FGF2 was unaltered with obesity, its expression and secretion was higher in adipose tissue from women with GDM. In keeping with these findings, FGF2 is higher in the kidneys of diabetic patients [58] and high glucose has been shown to increase FGF2 mRNA, protein synthesis and secretion compared with normal glucose [58].

There was no effect of pre-existing maternal obesity and GDM on adipose tissue VCAM-1 mRNA levels or sVCAM-1 secretion. On the other hand, ICAM-1 mRNA expression and sICAM-1 secretion was significantly higher in GDM adipose tissue compared to NGT adipose tissue. Furthermore, in the NGT cohort, sICAM-1 release was higher in adipose tissue from obese women compared to non-obese women. There are no other published studies on adhesion molecules in pregnant adipose tissue; however, these findings are in agreement with studies in non-pregnant adipose tissue from both humans and animals. For example, obesity is associated with increased ICAM-1 and VCAM-1 mRNA and protein expression in visceral adipose tissue from non-pregnant women [59]. In mice, a high fat-diet was associated with increased ICAM-1 mRNA expression and sICAM-1 levels significantly correlated with body weight and abdominal fat mass [60].

The factors responsible for the increased expression and secretion of angiogenic proteins and adhesion molecules observed in this study from obese and GDM adipose tissue are not known. However, it is hypothesised that cytokines, growth factors and adhesion molecules are released in response to tissue hypoxia [17]. Indeed, adipose tissue hypoxia has consistently been reported in various animal models of obesity, including genetically obese ob/ob and db/db mice, obese Zucker rats, and high-fat diet (HFD)-fed mice [61-63]. It is thus possible that obesity and diabetes during pregnancy may also induce a hypoxic environment in adipose tissue.

Adipokines may also play a crucial role in the modulation of adipose angiogenesis [64]. For example, leptin upregulates the expression of members of the VEGF family [65-67], expression of adhesion molecules [68, 69] and induces angiogenesis [70]. Of note, my previous studies have shown that there is expression of leptin in visceral adipose tissue from women with pre-existing maternal obesity and GDM [27]. Thus, it is possible, that in both obese and GDM pregnancies,

increased expression of adipokines in adipose tissue may contribute to increased expression and secretion angiogenic and adhesion molecules which associated with these conditions.

In addition to their well-established role in angiogenesis, VEGF and FGF2 family members, and endoglin may act as inducers of inflammation. For example, my previous studies have shown that PLGF increases inflammation in human placenta [26]. Additionally, in non-gestational tissues, FGF2 potentiates inflammatory mediator-induced leukocyte recruitment [71] and endoglin induces inflammation [72, 73]. It is thus possible that increased secretion of angiogenic molecules from obese and GDM adipose tissue may be responsible for the increase in placental and adipose tissue inflammation observed in these conditions [27, 74-78]. They may also act as physiological mediators of lipid and glucose metabolism; heterozygous endoglin deficiency in mice decreases high fat diet-induced hepatic triglyceride content and insulin levels [79]. Therefore, it is feasible that increased adipose tissue endoglin secretion may lead to higher circulating lipid levels leading to excess substrate supply that drives maternal–fetal energy transfer and increased neonatal adiposity. Indeed, infants of women with pre-existing maternal obesity and GDM often display dyslipidemia and have increased fat deposition even when they are average weight for gestational age [7].

This is the first study performed in adipose tissue from pregnant women that has identified changes in angiogenic factors and adhesion molecules induced by maternal obesity and GDM. This study, however, has a number of limitations which may impact on the interpretation of the results. Firstly, this study assessed whole placenta and adipose tissue; it is possible that analysing the different cells types (e.g trophoblasts, adipocytes, immunocytes and vascular cells) may yield different results. Secondly, all samples were collected at the time of term Caesarean section. Thus, whether the observed effects in gene expression and secretion are due to metabolic changes during pregnancy or present only during the time tissue sampling occurred is not known. Although not possible in this study as the samples were not available, it would be of interest to measure the circulating levels of

the angiogenic markers and adhesion molecules in maternal plasma/serum. Lastly, these results do not take into account the effect of gestational weight gain or fat mass; it would be of interest to determine what effect GWG has on the expression of angiogenic proteins and adhesion molecules in omental adipose tissue. Future studies are required to determine the effect of hypoxia and adipokines on the expression and release of angiogenic proteins and adhesion molecules in omental adipose tissue. Furthermore, future studies should also determine the role of these angiogenic proteins and adhesion molecules on inflammation, and lipid and glucose metabolism in both adipose tissue and placenta.

In conclusion, this study demonstrated the presence of abnormal expression and secretion of angiogenic proteins and adhesion molecules in omental adipose tissue, but not placenta, from pregnant women with GDM and pre-existing maternal obesity. Increased angiogenic and adhesion molecules released from adipose tissue may affect angiogenesis, inflammation and or lipid and glucose metabolism in both mum and her offspring.

DECLARATION OF INTEREST, FUNDING, AND ACKNOWLEDGEMENTS**Declaration of interest**

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

Associate Professor Martha Lappas was a recipient of a Career Development Fellowship from National Health and Medical Research Council (NHMRC; grant no. 1047025). The work described in this manuscript was funded by the Medical Research Foundation for Women and Babies and Diabetes Australia Research Trust (DART).

Acknowledgements

Dr. Ratana Lim and Gillian Barker (Obstetrics, Nutrition and Endocrinology Group, Department of Obstetrics and Gynaecology, University of Melbourne) are thanked for their excellent technical assistance. The clinical research midwives Genevieve Christophers, Debra Jinks, Rachel Murdoch and Gabrielle Fleming, are gratefully acknowledged for assistance with sample collection. The Obstetrics and Midwifery staff of the Mercy Hospital for Women is also thanked for their co-operation.

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Table 1. Characteristics of the study group.

	NGT Non-obese (n=12)	NGT Obese (n=16)	GDM Non-obese (n=17)	GDM Obese (n=15)
Maternal age (years)	31.7 ± 0.6	30.9 ± 1.2	30.5 ± 1.0	32.2 ± 1.0
Pre-pregnancy BMI (kg/m ²)	24.3 ± 0.9**	38.0 ± 2.0*	24.1 ± 0.7	37.0 ± 1.1 [#]
Maternal BMI at delivery (kg/m ²)	29.3 ± 1.2**	39.7 ± 1.8*	28.5 ± 0.7	38.8 ± 0.9 [#]
Gestational age at birth (weeks)	38.6 ± 0.2	38.6 ± 0.3	38.7 ± 0.2	38.4 ± 0.2
Fetal birth weight (g)	3315 ± 105	3395 ± 85	3360 ± 66	3575 ± 61
Fetal Gender	5 Female 7 Male	10 Female 6 Male	7 Female 10 Male	8 Female 7 Male
OGTT at ~28 weeks gestation				
...Fasting plasma OGTT (mmol/l)	4.5 ± 0.1	4.5 ± 0.1	4.9 ± 0.2	5.4 ± 0.3**
...1 hour plasma OGTT (mmol/l)	6.4 ± 0.4 [#]	6.9 ± 0.4	9.2 ± 0.4	9.5 ± 0.4**
...2 hour plasma OGTT (mmol/l)	5.6 ± 0.4 [#]	5.5 ± 0.2	8.7 ± 0.3	8.0 ± 0.4**

Values represent mean ± SEM

OGTT, oral glucose tolerance test

* $P < 0.05$ vs. NGT non-obese; ** $P < 0.05$ vs. NGT obese; [#] $P < 0.05$ vs. GDM non-obese (one-way ANOVA)

Figure 1. Effect of pre-existing obesity and GDM on PLGF mRNA expression and release

(A,C) PLGF mRNA expression and (B,D) PLGF release from human (A,B) placenta and (C,D) omental adipose tissue obtained from NGT non-obese (n=12 patients), NGT obese (n=16 patients), GDM non-obese (n=17 patients), GDM obese (n=15 patients) pregnant women. Each bar represents the mean $\pm$ SEM. * P <0.05.

Figure 2. Effect of pre-existing obesity and GDM on VEGF-A mRNA expression and release

(A,B) VEGF-A mRNA expression and (C) VEGF release from (A) human placenta and (B,C) omental adipose tissue obtained from NGT non-obese (n=12 patients), NGT obese (n=16 patients), GDM non-obese (n=17 patients), GDM obese (n=15 patients) pregnant women. Each bar represents the mean $\pm$ SEM. * P <0.05.

Figure 3. Effect of pre-existing obesity and GDM on VEGFR1 and VEGFR2 mRNA expression and sFlt-1 release

(A,D) VEGFR1, (B,E) VEGFR2 mRNA expression and (C,F) sFlt-1 release from human (A-C) placenta and (D-F) omental adipose tissue obtained from NGT non-obese (n=12 patients), NGT obese (n=16 patients), GDM non-obese (n=17 patients), GDM obese (n=15 patients) pregnant women. Each bar represents the mean $\pm$ SEM. * P <0.05.

Figure 4. Effect of pre-existing obesity and GDM on FGFR1 and FGF2 mRNA expression and FGF2 release

(A,D) FGFR1 mRNA expression, (B,E) FGF2 mRNA expression and (C,F) FGF2 release from human (A-C) placenta and (D-F) omental adipose tissue obtained from NGT non-obese (n=12 patients), NGT obese (n=16 patients), GDM non-obese (n=17 patients), GDM obese (n=15 patients) pregnant women. Each bar represents the mean $\pm$ SEM. * P <0.05.

Figure 5. Effect of pre-existing obesity and GDM on endoglin mRNA expression and release

(A,C) Endoglin mRNA expression and (B,D) sEng release from h human (A,B) placenta and (C,D) omental adipose tissue obtained from NGT non-obese (n=12 patients), NGT obese (n=16 patients), GDM non-obese (n=17 patients), GDM obese (n=15 patients) pregnant women. Each bar represents the mean $\pm$ SEM. * P <0.05.

Figure 6. Effect of pre-existing obesity and GDM on ICAM-1 mRNA expression and release

(A,C) ICAM-1 mRNA expression and (B,D) sICAM-1 release from human (A,B) placenta and (C,D) omental adipose tissue obtained from NGT non-obese (n=12 patients), NGT obese (n=16 patients), GDM non-obese (n=17 patients), GDM obese (n=15 patients) pregnant women. Each bar represents the mean $\pm$ SEM. * P <0.05.

Figure 7. Effect of pre-existing obesity and GDM on VCAM-1 mRNA expression and release

(A,C) VCAM-1 mRNA expression and (B,D) sVCAM-1 release from human (A,B) placenta and (C,D) omental adipose tissue obtained from NGT non-obese (n=12 patients), NGT obese (n=16 patients), GDM non-obese (n=17 patients), GDM obese (n=15 patients) pregnant women. Each bar represents the mean $\pm$ SEM. * P <0.05.

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Title:

Markers of endothelial cell dysfunction are increased in human mental adipose tissue from women with pre-existing maternal obesity and gestational diabetes

Date:

2014-06-01

Citation:

Lappas, M. (2014). Markers of endothelial cell dysfunction are increased in human mental adipose tissue from women with pre-existing maternal obesity and gestational diabetes. METABOLISM-CLINICAL AND EXPERIMENTAL, 63 (6), pp.860-873. <https://doi.org/10.1016/j.metabol.2014.03.007>.

Publication Status:

Accepted manuscript

Persistent Link:

<http://hdl.handle.net/11343/41907>