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Authors: Hayward, Christina E., Cowley, Elizabeth J., Mills, Tracey A., Sibley, Colin P., and Wareing, Mark

Source: Biology of Reproduction, 90(3)

Published By: Society for the Study of Reproduction

URL: https://doi.org/10.1095/biolreprod.113.112623

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BIOLOGY OF REPRODUCTION (2014) **90**(3):65, 1–9
Published online before print 29 January 2014.
DOI 10.1095/biolreprod.113.112623

Maternal Obesity Impairs Specific Regulatory Pathways in Human Myometrial Arteries[1]

Christina E. Hayward,[2,3,4] Elizabeth J. Cowley,[3,4] Tracey A. Mills,[3,4,5] Colin P. Sibley,[3,4] and Mark Wareing[3,4]

[3]*Maternal and Fetal Health Research Centre, Institute of Human Development, Faculty of Medical and Human Sciences, The University of Manchester, Manchester, United Kingdom*
[4]*St. Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, United Kingdom*
[5]*School of Nursing, Midwifery and Social Work, The University of Manchester, Manchester, United Kingdom*

ABSTRACT

Obese women (body mass index ≥30 kg/m^2) are at greater risk than normal weight women of pregnancy complications associated with maternal and infant morbidity, particularly the development of cardiovascular disease and metabolic disorders in later life; why this occurs is unknown. Nonpregnant, obese individuals exhibit systemic vascular endothelial dysfunction. We tested the hypothesis that obese pregnant women have altered myometrial arterial function compared to pregnant women of normal (18–24 kg/m^2) and overweight (25–29 kg/m^2) body mass index. Responses to vasoconstrictors, U46619 (thromboxane mimetic) and arginine vasopressin, and vasodilators, bradykinin and the nitric oxide donor sodium nitroprusside, were assessed by wire myography in myometrial arteries from normal weight (n = 18), overweight (n = 18), and obese (n = 20) women with uncomplicated pregnancies. Thromboxane-prostanoid receptor expression was assessed using immunostaining in myometrial arteries of normal weight and obese women. Vasoconstriction and vasodilatation were impaired in myometrial arteries from obese women with otherwise uncomplicated pregnancies. Disparate agonist responses suggest that vascular function in obese women is not globally dysregulated but may be specific to thromboxane and nitric oxide pathways. Because obesity rates are escalating, it is important to identify the mechanisms underlying impaired vascular function and establish why some obese women compensate for vascular dysfunction and some do not. Future studies are needed to determine whether central adiposity results in an altered endocrine milieu that may promote vascular dysfunction by altering the function of perivascular adipose tissue.

body mass index, myometrium, nitric oxide, preeclampsia, pregnancy, thromboxane, vascular

INTRODUCTION

Obesity, defined by the World Health Organization as a body mass index (BMI) ≥30 kg/m^2, is one of the most significant risks to 21st-century global health, challenging traditional concerns of undernutrition and infectious disease as a cause of ill health in the general population [1]. Between 1980 and 2008, worldwide obesity rates doubled for men (to 10%) and women (to 14%) [2]. Similar trends were observed in the number of obese pregnant women registering for antenatal care [3–5]; the United Kingdom national average has doubled to 16% since 1992 with even higher rates in Northern England where nearly one in five pregnant women is clinically obese [6, 7]. In pregnancy, maternal obesity is an independent risk factor for serious maternal and fetal complications, including hypertension, preeclampsia (PE), aberrant fetal growth (both fetal overgrowth and fetal growth restriction), stillbirth, congenital abnormalities such as spina bifida and cardiac defects, gestational diabetes, and intervention in labor (e.g., Cesarean section) [6–9].

Healthy pregnancies depend on the maternal cardiovascular system, and uterine vasculature undergoing a series of complex physiological adaptations (e.g., <40% increase in cardiac output [10], remodeling of uterine spiral arteries [11], and altered vascular resistance [12]) to ensure optimal placental vascular bed perfusion. In obese pregnant women, cardiac output and blood pressure (though still in normal range) exceeds that in nonpregnant obese subjects and that normally observed in pregnancy [13, 14].

Systemic vascular tone is normally modulated by the activity and interaction of the endothelium and adjacent smooth muscle. However, nonpregnant, obese individuals exhibit systemic vascular endothelial dysfunction [15]. Similar dysfunction has been observed in women with the maternal syndrome PE [16, 17]. Preeclampsia, which affects 3%–5% of pregnancies, is characterized by hypertension (blood pressure ≥140/90 mmHg) and proteinuria (≥300 mg/24 h). It is a leading cause of maternal and perinatal morbidity and mortality [18] and carries a particular additive risk of future cardiovascular and metabolic disorders (e.g., cardiovascular disease and diabetes) for both mother and baby [19–21]. The origins of PE are unclear but the current pathogenic model suggests a two-step process [22]: 1) uteroplacental hypoperfusion and subsequent placental oxidative stress promotes 2) release of factor(s) into the maternal circulation that trigger widespread maternal inflammation and systemic endothelial dysfunction. Epidemiological evidence demonstrates that maternal obesity (BMI >30 kg/m^2) triples the risk of developing PE [23]. Thus, obese women may be

[1]Funded by the British Heart Foundation, and infrastructure support was provided by the National Institute for Health Research (NIHR) Manchester Biomedical Research Centre and Tommy's the Baby Charity. Presented, in part, at the 58th Annual Meeting of the Society for Gynecologic Investigation, 16–19 March 2011, Miami Beach, FL.
[2]Correspondence: Christina E. Hayward, Maternal and Fetal Health Research Centre, The University of Manchester, 5th floor (Research), St. Mary's Hospital, Oxford Road, Manchester, M13 9WL, United Kingdom. E-mail: Christina.Hayward@manchester.ac.uk

Received: 24 July 2013.
First decision: 15 August 2013.
Accepted: 22 January 2014.
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eISSN: 1529-7268 http://www.biolreprod.org
ISSN: 0006-3363

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TABLE 1. Demographic, biophysical, and obstetric data for the participants.[a]

Category	Normal weight (n = 18)	Overweight (n = 18)	Obese (n = 20)	*P* value[b]
Age (yr)	32.0 (22.0–39.0)	32.5 (26.0–42.0)	31.0 (24.0–47.0)	ns
Ethnicity: Caucasian no. (%)	16 (88.9%)	17 (94.4%)	16 (80.0%)	ns
Parity	1 (0–8)	1 (0–3)	1 (0–7)	ns
Smoker no. (%)	1 (5.6%)	2 (11.1%)*	4 (20.0%)***,+	*$P < 0.05$ (N vs. Ov) ***$P < 0.0001$ (N vs. Ob) +$P < 0.05$ (Ov vs. Ob)
BMI at booking (kg/m^2)	22.8 (19.0–24.9)	28.1 (25.3–29.9)**	32.9 (30.0–42.3)***,+++	**$P < 0.01$ (N vs. Ov) ***$P < 0.0001$ (N vs. Ob) +++$P < 0.0001$ (Ov vs. Ob)
Gestation (wk^{+days})	39^{+2} (38^{+6}–41^{+2})	39^{+1} (37^{+3}–40^{+2})	39^{+0} (37^{+1}–40^{+3})	ns
Birthweight (g)	3400 (2900–3710)	3380 (2650–3990)	3520 (3170–4100)	ns
IBR	50 (16–89)	43 (11–84)	59 (17–89)	ns
Male infant no. (%)	10 (55.6%)	10 (55.6%)	15 (75.0%)*,+	*$P < 0.05$ (N vs. Ob) +$P < 0.05$ (Ov vs. Ob)

[a] Median (range) unless stated otherwise.
[b] N, normal weight; Ov, overweight; Ob, obese; ns, nonsignificant.

susceptible to developing PE because they have preexisting subclinical vascular endothelial dysfunction.

Maintenance of efficient end organ perfusion is promoted by tone oscillations (rhythmic constriction and dilation) in small peripheral blood vessels [24, 25]. Oscillation initiation and propagation requires cross talk between the endothelium and vascular smooth muscle leading to alterations in intracellular calcium (Ca^{2+}) levels in the latter [26]. Abnormalities in endothelial cell (EC) function result in abnormal blood vessel oscillatory activity and subsequent reduced perfusion of a range of vascular beds; this may account for the increased vascular resistance observed in PE [27] and prevalence of peripheral vascular disease in diabetic patients [28, 29]. Endothelial dysfunction is associated with obesity but whether tone oscillations are altered in obese pregnancy is unknown. This study aimed to determine whether there is a relationship between BMI and myometrial artery (MA) function in pregnancy and tested the hypothesis that obese pregnant women would have altered myometrial vascular function compared to normal weight pregnant women.

MATERIALS AND METHODS

Ethical Information

This study was approved by the NRES North West Haydock Ethics Committee (08/H1010/55), and written informed consent was obtained from all participants prior to delivery. This investigation conformed to the principles outlined in the Declaration of Helsinki.

Participants and Tissue Collection

Myometrial biopsies were collected from women (N = 56, where N = number of tissue samples and n = number of vessels) with uncomplicated singleton pregnancies undergoing elective Cesarean sections at term (37–42 wk gestation). Women with preexisting medical disorders (e.g., diabetes) or pregnancy complications (e.g., PE, gestational diabetes, or small or large for gestational age infants) were excluded. Maternal BMI was recorded prior to 12 wk gestation, and women were categorized as normal weight (BMI 18.5–24.9 kg/m^2), overweight (BMI 25–29.9 kg/m^2), or obese (BMI $\geq$30 kg/m^2). An individualized birthweight ratio (IBR) was calculated for each infant using the Gestation-Related Optimal Weight software (Customized Weight Centile Calculator version 5.12/6.2 2009 downloaded from www.gestation.net); only biopsies from mothers who delivered appropriate for gestational age infants (IBR 11–89) were included. Maternal demographics as biophysical and obstetric data are presented in Table 1.

General Chemicals

Chemicals were purchased from Sigma-Aldrich (Poole, Dorset, U.K.) unless stated otherwise.

Wire Myography

Myometrial biopsies, taken from the upper lip of the uterine incision, were transferred to ice cold tissue collection buffer [30] (154 mM NaCl, 5.4 mM KCl, 1.2 mM $MgSO_4$, 1.6 mM $CaCl_2$, 10 mM MOPS, and 5.5 mM glucose, pH 7.4), and arteries ($\leq$500 μm diameter) were carefully dissected away from the adjacent connective tissue. Arterial sections (2 mm) were mounted on a Danish Myotechnology M610 wire myograph (Danish Myotech, Aarhus, Denmark), normalized to an internal diameter of 0.9 of $L_{13.3\ kPa}$ (luminal pressure ~45 mmHg) and left to equilibrate (37°C; gassed with air containing 5% CO_2) in 6 ml physiological salt solution [31] (PSS: 119 mM NaCl, 25 mM $NaHCO_3$, 4.69 mM KCl, 2.4 mM $MgSO_4$, 1.6 mM $CaCl_2$, 1.18 mM KH_2PO_4, 6.05 mM glucose, and 0.034 mM ethylenediaminetetraacetic acid, pH 7.4) as previously described [32]. Vessel viability was assessed using a high potassium solution [31] (KPSS: 11 mM NaCl, 25 mM $NaHCO_3$, 120 mM KCl, 2.4 mM $MgSO_4$, 1.6 mM $CaCl_2$, 1.18 mM KH_2PO_4, 6.05 mM glucose, and 0.034 mM ethylenediaminetetraacetic acid, pH 7.4). Protocol 1 was used to assess vascular function in MAs from all three BMI groups. During the period of this study, more overweight women than normal weight women were delivered by elective Cesarean section, thus myometrial biopsies were more frequently available from this BMI group. It was also apparent from protocol 1 that MAs from overweight women have similar functional characteristics to those from normal weight women. For these reasons, only MAs from overweight women were used in protocol 2 to determine whether indomethacin altered vascular function.

Protocol 1: Effect of Maternal BMI on Vasoconstriction and Vasodilatation in MAs

Arteries were exposed to incremental doses of the thromboxane A_2 (TXA_2) mimetic U46619 (10^{-10}–$10^{-5.7}$ M; 6 × 2min intervals; Merck Chemicals, Nottingham, U.K.) or arginine vasopressin (AVP) (10^{-10}–10^{-8} M; 5 × 2 min intervals). Following washing, arteries were precontracted for 15min with an EC_{80} concentration of U46619 or AVP (an effective concentration to induce 80% of the maximum contraction observed in the previous dose-response curve) and then exposed to the endothelial-dependent vasodilator bradykinin (BK) (10^{-10}–10^{-5} M; 6 × 2 min intervals) or nitric oxide (NO) donor sodium nitroprusside (SNP) (10^{-11}–10^{-6} M; 6 × 2 min intervals). Following PSS washout, KPSS was applied to confirm vessel viability.

Protocol 2: Effect of Indomethacin on MA Function

Tone oscillations were observed in a high proportion of MAs when exposed to EC_{80} concentrations of either agonist (predominantly AVP) and incremental doses of BK and SNP, but what mediated these oscillations was unclear. A previous study observed similar oscillatory activity in MAs and ruled out NO as a potential modulator [27]; another study indicated prostaglandins might contribute to vascular oscillations [33]. Thus, the current study used indomethacin, an inhibitor of prostaglandin biosynthesis [34], to investigate whether prostaglandins regulated these oscillations.

Paired arteries were treated with indomethacin (10^{-5} M) or an equivalent concentration of dimethyl sulfoxide (DMSO), the drug diluent for the control. After 30 min incubation, incremental doses of AVP (10^{-10}–10^{-8} M; 5 × 2 min intervals) were added. Arteries were washed to baseline with PSS containing indomethacin or DMSO (as appropriate). Arteries were precontracted with an

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EC_{80} concentration of AVP for 15 min and then exposed to BK (10^{-10}–10^{-5} M; 6 × 2 min intervals) or SNP (10^{-11}–10^{-6} M; 6 × 2 min intervals). Following PSS washout, KPSS was used to confirm vessel viability.

Immunohistochemistry

Myometrial tissue from normal weight (N = 6) and obese (N = 6) pregnant women was fixed in 10% neutral buffered formalin at 4°C overnight and wax embedded. Immunohistochemistry was performed as previously described [35] on serial tissue sections (5 μm) using the following primary antibodies: polyclonal rabbit anti-human thromboxane-prostanoid (TBXA2R) receptor (3 μg/ml; Cayman Chemicals, Cambridge Bioscience Ltd., Cambridge, U.K.); monoclonal mouse anti-human smooth muscle α-actin (15.25 μg/ml); and endothelium markers CD31 (2 μg/ml; Dako U.K. Ltd., Ely, U.K.) and von Willebrand factor (VWF) (4 μg/ml; Dako U.K. Ltd.). For negative controls, the primary antibody was substituted with an equivalent concentration of nonimmunized immunoglobulin G (containing all the immunoglobulin G isotypes) derived from animal serum corresponding to the animal of primary antibody origin. Negative controls were included in every staining run.

Immunohistochemical Analyses

Images of stained tissue were obtained using an Olympus BX41 microscope with a QI Cam Fast 1394 camera and Image Pro Plus software (Media Cybernetics U.K., Marlow, U.K.). Smooth muscle α-actin and endothelial markers CD31 and VWF were used to confirm the presence of arteries in the myometrial tissue. A scoring system was established to allow semiquantitative analysis of TBXA2R staining intensity in the endothelium and smooth muscle of all the arteries present in the sample; n = 17 ± 1 (mean ± SEM). There was no difference in the number of arteries scored per sample in the normal weight compared to the obese cohort. Staining scores were 0 = no staining, 1 = faint/patchy, 2 = moderate, 3 = strong, 4 = very strong. Four independent scorers were given the same example photos for each score and blinded to sample identity. Median scores for endothelium and smooth muscle were calculated for each myometrium.

Statistics

Data were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA). Demographic data (median and range) were analyzed by chi square and Kruskal-Wallis tests (Dunn posttest used when appropriate). Vessel tone (mN/mm) was converted into active effective pressure (kPa) by normalizing for vessel diameter. Dose-response curves (mean ± SEM) were analyzed by two-way ANOVA (Bonferroni posttest used when appropriate). Area under the curve (AUC, in arbitrary units), maximum response (contraction or relaxation; V_{max}, in kPa or percentage), and sensitivity (EC_{50}, in nM) for each artery and agonist are presented as median and interquartile range and analyzed using Mann Whitney, Kruskal-Wallis, or Wilcoxon matched-pairs signed rank tests as appropriate. Median TBXA2R staining scores were analyzed using a Kruskal-Wallis test.

Changes in vascular tone were defined as oscillations if the amplitude (peak to trough) was >10% of the maximum AVP-induced contraction. Oscillation amplitude and frequency (number per min) were recorded for the last 10 min of the precontraction in each artery. $P < 0.05$ was considered significant.

RESULTS

Effect of Maternal BMI on MA Vasoconstriction

Artery diameters were comparable between normal weight (N = 13; n = 25; mean ± SEM, 317 ± 15 μm), overweight (N = 12; n = 47; 333 ± 20 μm), and obese (N = 20; n = 33; 332 ± 15 μm) cohorts. Maximum contraction to KPSS was not affected by maternal BMI (8.1 kPa [6.9–12.0 kPa], 9.9 kPa [7.2–11.8 kPa], and 10.2 kPa [6.1–12.4 kPa]).

Concentration-dependent vasoconstriction to U46619 was significantly shifted to the right in the obese subgroup, demonstrating that a higher concentration of U46619 was required to induce a similar level of contraction in MAs from obese compared to normal weight pregnant women ($P < 0.05$; two-way ANOVA; Fig. 1A). Myometrial arteries from overweight women had a similar response to increasing concentrations of U46619 to that in MAs from normal weight women, but this was not significantly different from the obese subgroup. AUC (median 18.8 [interquartile range: 14.8–30.5], 17.4 [12.8–23.3], and 15.3 [12.7–25.3]), maximal contraction (11.1 kPa [9.4–15.4 kPa], 12.5 kPa [8.3–16.4 kPa], and 9.6 kPa [8.5–16.0 kPa]), and sensitivity (41.9 nM [10.5–59.3 nM], 54.2 nM [38.0–119.2 nM], and 61.0 nM [20.7–109.6 nM]) to U46619 were comparable between normal weight, overweight, and obese groups.

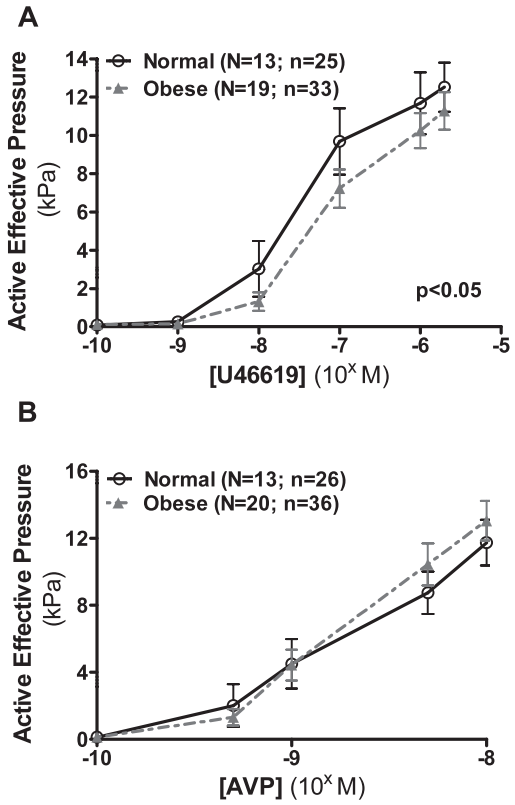

FIG. 1. Vasoconstriction of MAs from normal weight and obese pregnant women in response to U46619 (**A**) and AVP (**B**). Data are mean ± SEM. $P < 0.05$; two-way ANOVA.

AVP-induced vasoconstriction was not different between BMI cohorts (Fig. 1B). AUC (7.6 [4.8–11.3], 10.6 [7.1–17.2], and 9.8 [4.8–14.3]), maximal contraction (11.4 kPa [8.1–14.6 kPa], 12.0 kPa [9.9–19.6 kPa], and 13.5 kPa [8.3–15.7 kPa]), and sensitivity (2.6 nM [0.8–5.0 nM], 1.1 nM [0.6–4.4 nM], and 2.5 nM [1.0–5.6 nM]) to AVP were comparable between the normal weight, overweight, and obese groups.

Effect of Maternal BMI on Endothelial-Dependent Vasodilatation

There was no difference in U46619 or AVP precontraction between BMI groups (data not shown). Bradykinin exposure induced marked relaxation in precontracted (U46619 and AVP) MAs from normal weight (residual contraction in U46619-precontracted arteries; N = 13, n = 15: 6.6% [4.1%–18.8%]; in AVP-precontracted arteries; N = 13, n = 14: 6.8% [2.3%–12.7%]), overweight (U46619 arteries; N = 11, n = 15: 6.4% [3.6%–12.6%]; AVP arteries; N = 9, n = 13: 4.5% [0.2%–8.1%]), and obese women (U46619 arteries; N = 18, n = 20: 14.0% [6.6%–31.4%]; AVP arteries; N = 20, n = 21: 6.9% [2.3%–12.0%]). Bradykinin-induced relaxation in U46619-precontracted MAs (Fig. 2A) was not affected by maternal BMI. However, there was an upward shift in BK-induced relaxation in AVP-precontracted arteries from obese compared

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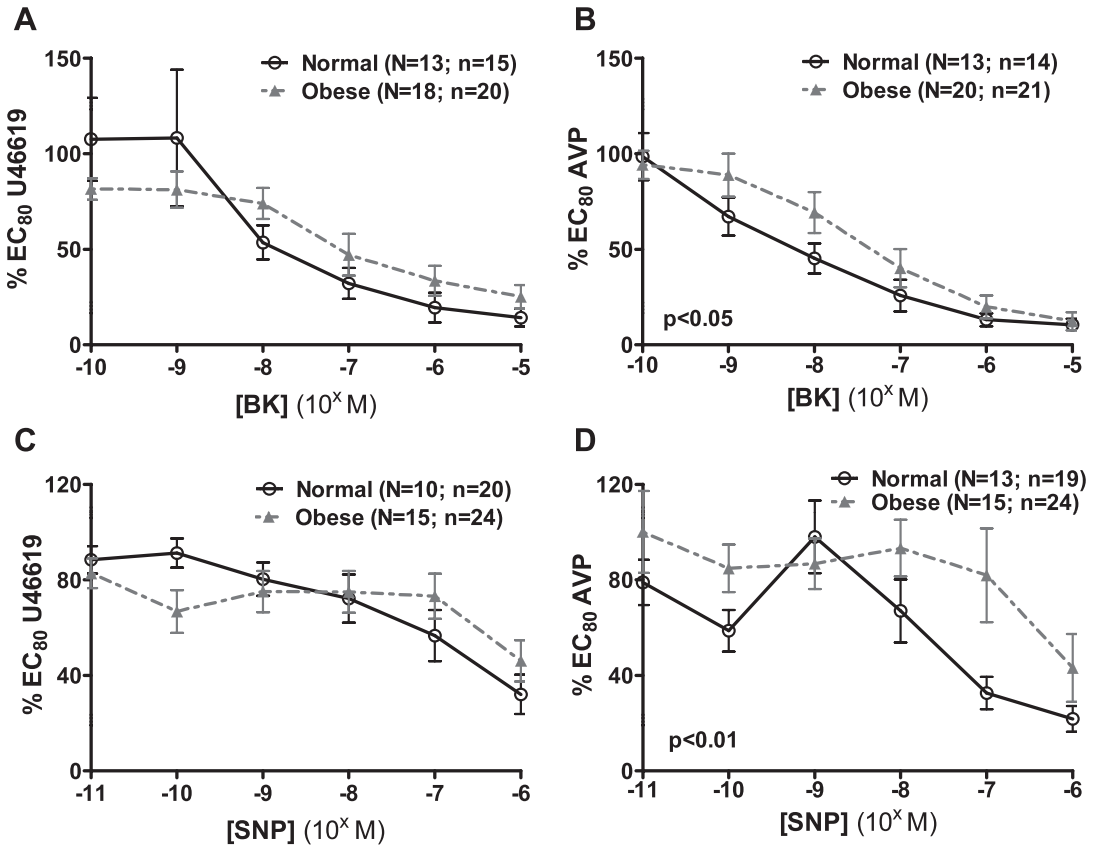

FIG. 2. Endothelial-dependent and -independent vasodilatation in MAs isolated from normal weight and obese pregnant women. Vasodilatation to bradykinin (BK) in MAs preconstricted to U46619 (**A**) and AVP (**B**); and vasodilatation to sodium nitroprusside (SNP) in arteries preconstricted to U46619 (**C**) and AVP (**D**). Data are mean ± SEM. $P < 0.05$; two-way ANOVA.

to normal weight subgroups ($P < 0.05$; Fig. 2B); AUC was higher in MAs from obese (307.1 [202.1–344.0]) compared to normal weight (218.0 [133.4–250.3]) women ($P < 0.05$). Maximum relaxation (as above) and sensitivity (EC_{50}; normal weight 10.5 nM [1.0–37.1 nM] vs. obese 22.9 nM [7.4–150.4 nM]) to BK were unaffected by maternal BMI. Bradykinin-induced vasodilatation in U46619 and AVP-precontracted MAs from overweight women resembled that measured in MAs from the normal weight cohort, but there was no difference in relaxation compared to MAs from the obese cohort (data not shown).

Effect of Maternal BMI on Endothelial-Independent Vasodilatation

Precontraction (U46619 or AVP) was unaffected by maternal BMI (data not shown). There was no difference in maximum relaxation, AUC, and sensitivity to SNP in U46619-precontracted arteries between normal weight (N = 10; n = 20) and obese (N = 15, n = 24) cohorts (Fig. 2C). In contrast, SNP-induced vasodilatation was shifted upward in AVP-precontracted arteries in obese (N = 15, n = 24) compared to normal weight women (N = 13, n = 19; $P < 0.01$; Fig. 2D). AUC was greater in arteries from obese (419.8 [313.9–475.8]) compared to normal weight (277.7 [243.1–392.7]) women ($P < 0.05$); however, maximum relaxation (residual contraction; normal weight 11.3% [7.7%–19.9%], overweight 11.4% [8.6%–20.3%], and obese 15.3% [6.7%–48.7%]), and sensitivity (8.0 nM [2.4–86.1 nM], 26.2 nM [4.9–54.7 nM], and 39.7 nM [4.6–140.6 nM]) to SNP in AVP arteries were unaffected by maternal BMI. SNP-induced relaxation in both U46619- and AVP-precontracted MAs from overweight women (N = 11; n = 19) was similar to that in MAs from normal weight women, but there was no difference compared to MAs from obese women (data not shown).

Effect of Indomethacin on MA Function

Although marked relaxation was induced in MAs, the characteristic sigmoid curve relationship to increasing concentrations of SNP was disrupted by oscillations in the vascular tone. Tone oscillations were observed in 65% of MAs in response to precontraction (both U46619 and AVP) and concentration-dependent relaxation curves (both BK and SNP). There was no difference in the incidence of MA oscillations between BMI groups. Approximately 66% of oscillations occurred in AVP-precontracted arteries; therefore, further experiments to examine oscillations were performed using AVP according to protocol 2. In Figure 3, there are examples of original traces illustrating the absence (Fig. 3A) and presence (Fig. 3B) of tone oscillations and the effect of DMSO (Fig. 3C) and indomethacin (Fig. 3D) in MAs.

Indomethacin did not affect MA responses to AVP (Fig. 3E); AUC (9.0 [4.0–11.1] vs. 7.5 [7.1–17.2]), maximum contraction (11.9 kPa [9.3–17.0 kPa] vs. 13.8 kPa [9.7–15.5 kPa]), and sensitivity (2.5 nM [2.4-3.2 nM] vs. 2.4 nM [2.1–13.5 nM]) to AVP were comparable in MAs exposed to DMSO and indomethacin, respectively. There was no difference in the response to AVP in arteries used for protocol 2 and arteries from overweight women used in protocol 1.

Maximum response to AVP precontraction was similar between MAs exposed to DMSO (13.4 kPa [8.6–17.2 kPa])

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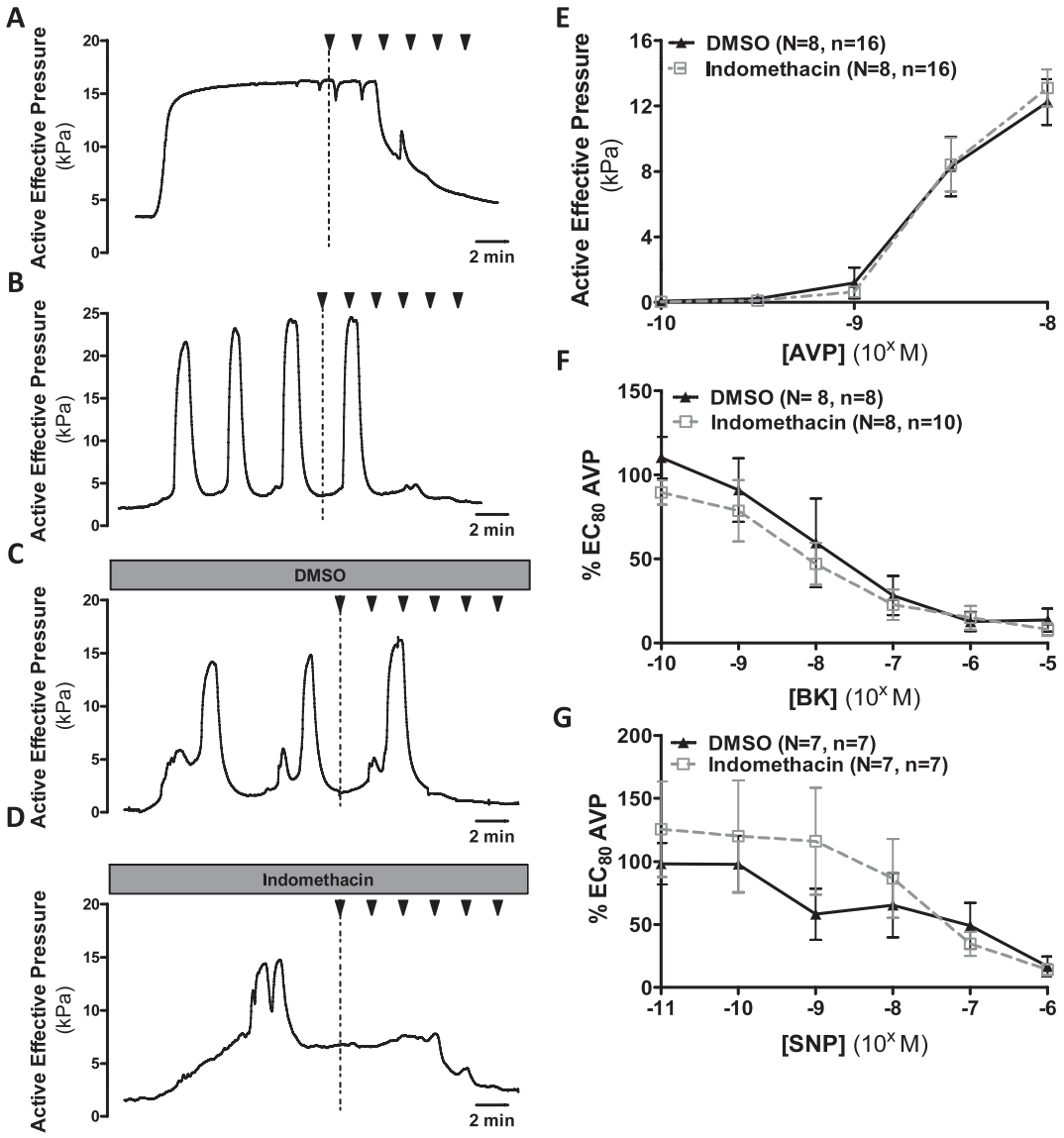

FIG. 3. Tone oscillations in MAs. **A**, **B**) Original traces of arteries contracted to arginine vasopressin (AVP) for 15 min before incremental doses (from dotted line) of either bradykinin (BK) or sodium nitroprusside (SNP) were added at 2 min intervals (arrows). Changes in vascular tone were defined as oscillations if the amplitude (peak to trough) was >10% of the maximum AVP-induced contraction. Oscillation amplitude and frequency (number per min) were recorded for the last 10 min of the precontraction in each artery. Example traces presented are of an artery, which was exposed to BK but did not oscillate (**A**), and AVP-induced tone oscillations in an artery exposed to SNP (**B**). **C**, **D**) AVP-induced tone oscillations developed in two sections of the same artery, one exposed to DMSO (drug diluent control; **C**) and the other to indomethacin (10^{-5} M; **D**) prior to a BK concentration response curve. Amplitude, but not frequency, of these oscillations was reduced in arteries exposed to indomethacin. The effect of indomethacin was also assessed on arterial responses to AVP (**E**), BK (**F**), and SNP (**G**). Data are mean ± SEM.

and indomethacin (16.8 kPa [10.9–19.1 kPa]). There was no difference in BK-induced (Fig. 3F) or SNP-induced (Fig. 3G) vasodilatation between arteries exposed to DMSO and indomethacin. AUC, maximum response and sensitivity to AVP, BK, and SNP were similar in the DMSO and indomethacin groups compared to the overweight cohort.

The oscillation amplitude during AVP precontraction was reduced in MAs exposed to indomethacin (1.2 kPa [1.2–3.9 kPa]) compared to those exposed to DMSO (7.0 kPa [2.6–11.5 kPa]; $P < 0.05$), but the frequency of oscillations was unaffected (0.15 [0.10–0.76] vs. 0.15 [0.10–0.34]).

TBXA2R Protein Expression

Positive staining for TBXA2R (Fig. 4A) was observed in MA smooth muscle cells (SMCs) and ECs in both normal weight and obese samples. Endothelial cells and SMCs were identified using serial tissue sections immunostained with endothelial markers CD31 (Fig. 4B) and VWF (picture not shown) and smooth muscle α-actin (Fig. 4C). There was a trend for higher TBXA2R expression in ECs than SMCs ($P = 0.06$), but there was no difference in staining intensity between normal weight and obese subgroups (Fig. 4E).

DISCUSSION

Obese women with otherwise uncomplicated pregnancies had impaired MA function linked to specific vascular regulatory pathways. Contraction to the TXA_2 mimetic U46619 was reduced in MAs from obese compared to normal weight pregnant women; however, AVP-induced contraction was unaffected by maternal BMI. U46619 and AVP were both

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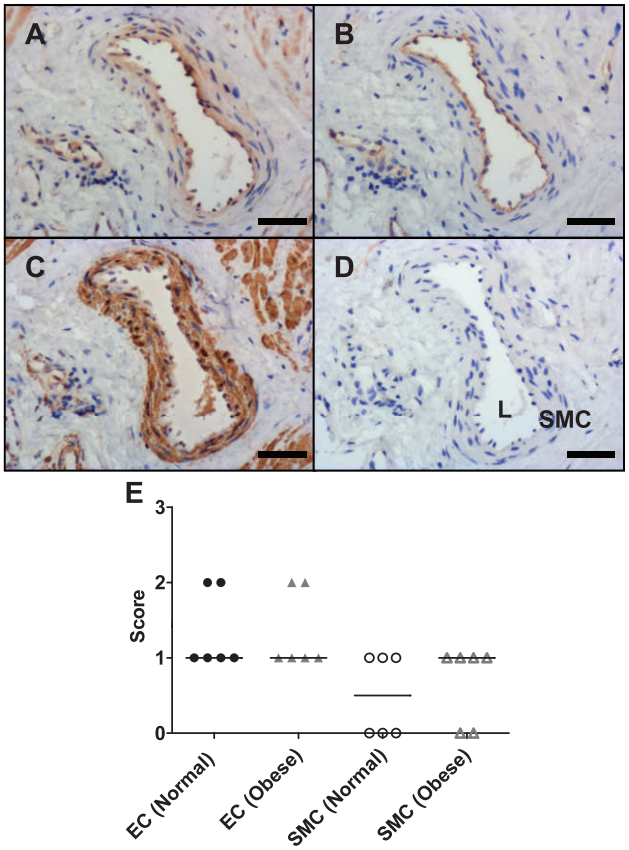

FIG. 4. Thromboxane-prostanoid (TBXA2R) receptor immunostaining in MAs from normal weight and obese women. Representative photomicrographs of serial immunostaining: TBXA2R (**A**), CD31 (**B**), smooth muscle α-actin (**C**), and negative control (**D**). Positive 3,3′-diaminobenzidine staining (brown) and hematoxylin counterstain (blue). Bars = 50 μm. L, lumen. **E**) Semiquantitative analysis of TBXA2R immunostaining in the endothelium (EC) and smooth muscle (SMC) of MAs from normal weight and obese pregnant women. Horizontal lines = median.

used to assess vasoconstriction in this study because they induce vascular function via distinct receptor-mediated processes that directly and indirectly affect intracellular Ca^{2+} levels [36–38]. Disparate responses to U46619 and AVP indicate that MA function is not globally impaired in obese women, but disruptions may be specific to the TXA_2 pathway. TXA_2-induced contraction results from numerous steps mediated by ECs and SMCs [37, 39, 40], any one of which might be disrupted in obesity.

Aberrant TXA_2 production is associated with obesity; in the nonpregnant state, serum TXB_2 (a stable metabolite of TXA_2) levels are higher in obese, and lower in morbidly obese, women than their normal weight counterparts [41]. Abnormal artery function in obese pregnancies may thus arise from aberrant levels of and/or altered responses to TXA_2. The location and distribution of TBXA2R was assessed in myometrial tissue from normal weight and obese pregnant women because receptor expression or density could be a limiting factor in TXA_2-induced contraction. However, TBXA2R expression on ECs and SMCs was not affected by maternal BMI, indicating that other stages and/or metabolites of the TXA_2 pathway are altered in obese pregnancies. Unfortunately, it is unknown if TXA_2 levels change in human obese pregnancies.

The origin of vascular dysfunction in obesity might arise from increased visceral adipose tissue mass. Adipose tissue produces numerous signaling factors (often referred to as adipokines) [42, 43] that mediate a range of physiological processes, including vascular function. In obesity, there are increased systemic circulating concentrations of prothrombotic, proinflammatory and vasoactive factors, for example, leptin, tumor necrosis factor, interleukin 6 (IL6) and IL8, and decreased levels of vasoprotective adipokines, such as adiponectin [43, 44]. Women with PE have a similarly altered endocrine profile [45, 46]. Aberrant vasoactive adipokines may stimulate the systemic, utero- and fetoplacental vascular dysfunction evident in obese mothers. In support of this, hyperleptinemia, as observed in systemic and umbilical circulations in obesity, potentially promotes endothelial dysfunction via an imbalance in NO bioavailability and increasing oxidative stress [47]. Also, exposure to adipokines (e.g., leptin or IL6), produced from cultured human adipocytes, alters human umbilical venous EC function by up-regulating monocyte adhesion (a mechanism associated with vascular disease) [48]. Increased central adiposity has been linked to an impaired ability to process fatty acids, which results in excessive fatty acids in the maternal circulation and oxidative stress [49, 50]. These lead to oxidized lipids and lipotoxicity, which have been hypothesized to promote vascular dysfunction and impair placental development in obese mothers [51, 52]. An imbalanced hormonal milieu and/or altered lipid metabolism may therefore link maternal obesity with circulatory disorders during pregnancy and in later life by promoting chronic inflammation and vascular dysfunction.

Our findings cannot be directly extrapolated to PE as preeclamptic women were not included in the current study. However, it is interesting to note that there are similarities in the vascular function observed in obesity and PE. Reduced MA vasodilatation in obese compared to normal weight pregnant women was consistent with that demonstrated in previous studies of obesity [53] and PE [16]. However, impaired relaxation was only identified in MAs from obese women precontracted to AVP and not U46619. Similar results have been observed in some studies of MAs from PE pregnancies [16, 54] but not in all; BK-induced vasodilation was attenuated in U46619-precontracted vessels from PE pregnancies [55]. No published studies of obesity or PE have assessed vasodilatation in both AVP- and U46619-precontracted arteries dissected from the same biopsy, thus, no direct comparisons can be made with the current study. Differential vasodilatation mechanisms indicate that specific vasoregulatory pathways may be altered by maternal obesity, but this requires further study.

Endothelial cells initiate vasodilatation by releasing factors, such as NO. Normally, EC activation (e.g., by shear stress or agonist) induces Ca^{2+}-calmodulin binding that stimulates NO synthase (NOS) to produce NO [56]. NOS activity is regulated by binding to caveolin. Endothelium-dependent agonists (e.g., BK) dissociate the NOS/caveolin complex and initiate NO production; endothelium-independent agonists (e.g., SNP) circumvent this pathway by directly donating NO to SMCs. NO permeation into SMCs activates a cascade of phosphorylation events resulting in reduced intracellular Ca^{2+} (via sarcoplasmic reticulum reuptake or extrusion from the cell), cellular hyperpolarization, and attenuated Ca^{2+} sensitivity, which lead to vasodilatation. Blunted endothelial-dependent (BK-induced) and NO-dependent (SNP-induced) vasodilatation in obese pregnant women suggest there may be abnormalities 1) in the ability of ECs to synthesize or release vasodilators and/or 2) in the capacity of the smooth muscle to respond (e.g., desensitization) to vasodilators and elicit downstream signaling pathways. These data are comparable to studies that have examined the potential role of perivascular

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adipose tissue (PVAT; adipocytes surrounding blood vessels) in regulating vascular function. PVAT, similar to visceral fat, releases factors that exert an anticontractile effect on the adjacent blood vessel through endothelial-dependent (NO release and Ca^{2+}-dependent potassium channel activation) and/or endothelial-independent mechanisms (e.g., hydrogen peroxide production) [57, 58]. In obesity, this anticontractile phenotype is attenuated [58], but how this is achieved is unknown. It may be that larger perivascular adipocytes observed in obesity release altered levels of cytokines, which promote localized hypoxia, oxidative stress, and inflammation, or reduce NO bioavailability, and result in impaired vasodilatation [58]. Altered PVAT function might therefore underpin reduced relaxation in MAs from obese mothers. Alternatively, MAs from obese women may be less responsive than those from normal weight women due to high amounts of PVAT mass increasing vascular stiffness [59]. Further investigations are required to confirm these suggestions.

Bradykinin is commonly used as a potent vasodilator when assessing vascular function, particularly using myography [16, 17, 27, 32]. However, previous data demonstrate that BK has direct and indirect effects on the vasculature and can induce vasodilatation, via NO and endothelium-derived relaxing factor (EDRF)-based mechanisms [60, 61], but can have more of a contractile effect on vascular SMCs mediated by prostaglandins (including TXA_2) when the endothelium is damaged [62, 63]. As endothelial dysregulation has been linked to obesity [15], these properties might explain the impaired ability of MAs from obese pregnant women to relax to BK; either the endothelial-dependent mechanisms are unable to overcome AVP-induced contraction or prostaglandins impede relaxation. Previous studies have demonstrated that BK-induced contraction can be inhibited by blocking TBXA2R [64], potentially suggesting that when U46619 is present, it occupies the receptor enabling the BK to initiate relaxation via other pathways. More detailed experiments would be required to confirm or deny the interactions that occur between U46619, AVP, and BK in MAs.

Large-amplitude, long-duration (>2min) oscillations were observed in a high proportion of MAs when exposed to precontraction concentrations of U46619, but more often to AVP. Oscillations were also occasionally triggered by the addition of BK or SNP. An initial examination of the potential mechanisms that underpin these agonist-induced oscillations was made but was not the main focus of the current study. There were no differences in oscillations between BMI cohorts, indicating that vascular pathways (e.g., TXA_2 or NO), which are disrupted by maternal obesity, do not mediate local oscillatory activity. However, it is important to study MA tone oscillations further as they are altered in pregnancies complicated by PE and fetal growth restriction [27], suggestive of increased vascular resistance and subsequent changes in blood flow. Endothelial cell and SMC interactions mediate oscillatory activity but if disrupted, for example, by the altered hormonal environment in obesity, could prime blood vessels to be more susceptible to stimuli that promote vascular dysfunction as observed in pregnancy complications, for example, PE. Tone oscillations, which acutely regulate blood flow to ensure optimal oxygen and nutrient supply to the tissue, may have a different stimuli, for example, prostaglandins [33, 34]. Here indomethacin, an irreversible inhibitor of cyclooxygenases that participate in prostaglandin biosynthesis [34], reduced MA oscillation amplitude but not incidence, indicating that cyclooxygenase/prostaglandin-dependent [34] and -independent [65] mechanisms contribute to AVP-induced oscillations. A previous study investigated whether NO contributed to tone oscillations but demonstrated that MA oscillations were unaffected by the administration of L-NNA, a NOS inhibitor, indicating that there are other mechanisms that must contribute to oscillatory activity [27].

It is important to note that despite aberrant MA function, the obese pregnant women studied here had uncomplicated pregnancies and delivered appropriate for gestational age babies. This indicates there are mechanisms that can compensate for an adverse intrauterine environment, enabling these women to overcome impaired vascular function and deliver a normal-sized baby. However, this compensation appears to be short-term because reports suggest that irrespective of birthweight, being born to an obese mother confers an increased risk of ill health in later life [66]; children of obese mothers are already clinically categorized as obese by 4 yr of age [67]. Childhood obesity is associated with chronic metabolic and cardiovascular disorders in adulthood [68]. This emphasizes the need to reduce maternal obesity and its associated pathologies to prevent adverse fetal programming and improve the health of subsequent generations. However, as obesity rates are currently escalating, it is important that future research should concentrate on determining the underlying mechanisms of impaired vascular function as well as identifying how some obese women are able to compensate for this and have a good pregnancy outcome whilst others do not. Future studies are needed to elucidate whether central adiposity results in an altered endocrine milieu that may promote vascular dysfunction potentially by altering the function of PVAT.

ACKNOWLEDGMENT

We offer our sincere thanks to our research midwives, Helen Miller, Linda Peacock, and Christine Hughes, and clinical fellows who recruited participants and collected data. Many thanks go to Dr. Lucy Higgins who scored the TBXA2R staining. Our thanks also go to the women and staff of St. Mary's Hospital, Manchester, U.K., for participating in the study.

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