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Chorionic plate arterial function is altered in maternal obesity

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ABSTRACT

Objectives: To characterise Chorionic Plate Artery (CPA) function in maternal obesity, and investigate whether leptin exposure reproduces the obese CPA phenotype in normal-BMI women.

Study design: CPA responses to the thromboxane-A_2 mimetic U46619 (pre/post leptin incubation), to the nitric oxide donor sodium nitroprusside (SNP) and the occurrence of tone oscillations (pre/post leptin incubation) were assessed in 46 term placentas from women of normal (18.5–24.9) or obese (>30) Body Mass Index (BMI).

Outcome measures: Area Under the dose response Curve (AUC), maximum response (V_{max}), sensitivity (EC_{50}) to U46619 (pre/post leptin) and SNP; average vessel tone, oscillation amplitude and frequency (pre/post leptin).

Results: U46619 vasoconstriction was similar between BMI categories ($p > 0.05$), however vasodilatation to SNP was reduced in obesity (AUC $p = 0.02$, V_{max} $p = 0.04$) compared to normal-BMI women. Leptin incubation altered responses to U46619 in both normal-BMI (EC_{50} at 100 ng/ml leptin; $p < 0.05$) and obese women (AUC at 50 ng/ml; $p < 0.05$) but vasomotion was unaffected ($p > 0.05$).

Conclusions: Maternal obesity is associated with altered placental vascular function which may adversely affect placental oxygen and nutrient transport, placing the fetus at risk. Leptin incubation altered CPA vascular function but did not reproduce the obese phenotype.

1. Introduction

The obesity epidemic is one of the most significant challenges to 21st century health. In 2008 59% of the obese population worldwide was female [1]. Recent figures show that in North-West England nearly 20% of women registering for antenatal care are obese [2,3] as defined by Body Mass Index (BMI $\geq$ 30 kg/m^2; weight (kg)/height (m)^2) [4]. Maternal obesity increases the risk of a range of complications including fetal growth restriction (FGR) [5], fetal overgrowth [6], and related complications including stillbirth, birth injury and intervention in labour [7]. Indeed infants stillborn to obese mothers are commonly of low-normal birth weight [5], suggesting that failure to achieve individual growth potential despite remaining within an arbitrary population "normal" weight range may have contributed to their demise. Both FGR and large for gestational age (LGA) are major causes of morbidity and mortality with long-term health consequences [8].

Fetal growth is dependent on a number of factors, particularly oxygen and nutrient delivery via maternal and fetoplacental blood supplies. Susceptibility to FGR or LGA birth could arise from reduced or elevated uteroplacental blood flow respectively. Chorionic plate arteries (CPAs) have the features of resistance arteries in other circulations [9] and thus have the potential to regulate tone resistance in the placental circulation. Placental vascular dysfunction in FGR pregnancies is observed *in vivo* by umbilical artery Doppler measurements [10], and *in vitro* when using wire myography to measure vascular responses to agonists [11] and examine tone oscillations (rhythmic vasoconstriction and vasodilation) which are thought to acutely modulate local blood flow in the placenta and determine end organ perfusion [12]. However, vascular function has not been extensively assessed in maternal obesity or fetal overgrowth, with findings of umbilical artery Doppler studies often being compounded by co-occurrence of gestational diabetes [13,14]. There have been no previous studies of placental chorionic artery function in relation to maternal obesity.

Increasing BMI correlates with increased adipose tissue mass [15]. Adipose tissue represents a highly active endocrine organ which secretes prothrombotic and proinflammatory substances e.g. leptin, endothelin-1, tumour necrosis factor (TNF)-α, plasminogen

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activator inhibitor (PAI)-1, interleukins (IL)-6 and IL-8, and vasoprotective factors, such as adiponectin [16]. In obesity, the increased adipose tissue mass results in derangements in a number of these circulating hormones and factors, often referred to as adipokines, which may alter homeostatic regulation of uteroplacental vascular tone and hence transplacental oxygen and nutrient delivery. This altered hormonal environment has been proposed as a potential link between obesity and the increased risk of disorders of vascular endothelial origin [17]. In particular a key adipokine, leptin is known to have vasoactive properties [18]. Altered umbilical cord blood leptin concentrations are observed in pregnancies associated with maternal obesity [19] and in pregnancies resulting in birth of FGR or LGA infants [20].

Aberrant fetal growth in maternal obesity might arise as a consequence of dysregulated nutrient delivery to the fetus via altered placental blood flow. This study tested the hypotheses that (a) maternal obesity is associated with altered function of small CPAs and (b) leptin exposure replicates the obese CPA phenotype.

2. Materials and methods

2.1. Ethical information

North West (Haydock Park) Research Ethics Committee (Ref: 08/H1010/55) approved the study. Written informed consent was obtained from all women.

2.2. Participants and placental collection

Participants were identified upon admission to hospital for delivery at 37–42 weeks gestation ($N = 46$). Women with diabetes and hypertension (pre-existing or gestational) or other maternal or fetal conditions, including antenatally diagnosed SGA or LGA pregnancies, were excluded. First trimester maternal BMI was used to categorise participants as normal-BMI (18.5–24.9 kg/m^2) or obese ($\geq$30 kg/m^2). An individualised birth weight ratio (IBR) was calculated for each infant using Gestation-Related Optimal Weight software (Customised Weight Centile Calculator v5.12/6.2 2009; www.gestation.net) taking into account maternal variables, gestation and gender; use of IBR has been shown to improve identification of pregnancies with aberrant fetal growth [21]. Infants were categorised as small for gestational age (SGA; IBR <10th centile), appropriate for gestational age (AGA; IBR 10th–90th centile), or LGA (IBR >90th centile). Whilst antenatally diagnosed cases of SGA or LGA were excluded from the study (as antenatal/intrapartum management of these pregnancies may have been affected by this diagnosis, including elective delivery at a relatively earlier gestation and the potential administration of corticosteroids) those postnatally diagnosed SGA or LGA pregnancies were not excluded.

2.3. Wire myography

Placentas were collected within 30 min of delivery. Chorionic plate biopsies were placed into physiologic salt solution (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, 0.034 mM EDTA; pH 7.4). Small CPA branches (100–500 μm) were dissected, cut into 2 mm lengths and mounted on two 40 μm steel wires in a Danish Myotechnology M610 wire myograph (Danish Myotech, Aarhus, Denmark) filled with PSS gassed with 5%CO_2/5%O_2/N_2 (Normal-BMI $N = 17$ placentas, $n = 77$ vessels; obese $N = 13$ placentas, $n = 65$ vessels). Arteries were normalised to an internal diameter of 0.9 of $L_{5.1kPa}$ equivalent to an intraluminal pressure of 25 mm Hg, as described previously [22] (Myodaq software version 2.02; Danish Myotech Aarhus). Following equilibration (20–30 min), the bath solution was changed to 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, 0.034 mM EDTA; pH 7.4) to assess vessel viability. After repeated washing to baseline tone, constriction was assessed using incremental doses of the thromboxane-A_2 mimetic U46619 (10^{-10}–$10^{-5.7}$ M; response recorded at plateau or after five minutes if plateau not reached). Arteries were again washed and the experiment proceeded using one of the following protocols (Fig. 1).

2.4. Protocol 1: Endothelium-independent relaxation

CPAs from normal-BMI ($N = 8$ placentas, $n = 28$ vessels) and obese ($N = 10$ placentas, $n = 38$ vessels) women were pre-constricted with an EC_{80} (concentration required to achieve 80% of maximal constriction for each vessel) concentration of U46619 for 20 min, then exposed to incremental doses of the nitric oxide donor, sodium nitroprusside (SNP; 10^{-10}–10^{-4} M; intervals as above).

2.5. Protocol 2: Effect of leptin on vessel constriction

Placentas utilized for protocol 2 were all collected from women who had delivered AGA babies. CPAs from normal-BMI ($N = 12$ placentas, $n = 12$ vessels for control and each individual leptin concentration) and obese ($N = 7$ placentas, $n = 7$ vessels for control and each individual leptin concentration) women were incubated for 1 h with vehicle diluent without leptin or 20, 50 or 100 ng/ml leptin (reconstituted in 0.5 ml 15 mM HCl and 0.3 ml 7.5 mM NaOH) to represent an internal time control, normal circulating levels of leptin, obese circulating levels of leptin and supra-physiological levels of leptin respectively in maternal serum and cord blood [23–25]. Following incubation, the U46619 dose response curve was repeated.

2.6. Protocol 3: Effect of leptin on endothelial function

Tone oscillations (rhythmic vasoconstriction and vasodilation) which are a feature of CPA function *in vitro* and are altered in FGR [12], are thought to be modulated by vascular endothelium and have the potential to acutely modulate local blood flow. These were assessed in placentas from normal-BMI ($N = 11$ placentas, $n = 14$ vessels for control and each individual leptin concentration) delivering AGA infants using a method adapted from Sweeney and colleagues [12] (Fig. 2). CPAs were exposed to a sub-maximal concentration (30 nM) of U46619 for 30 min to induce oscillations, followed by 15 min incubation with the endothelium-dependent vasodilator bradykinin (final concentration 1 μM; BK). The arteries were then washed with PSS and incubated for 1 h with vehicle diluent or 20, 50 or 100 ng/ml leptin. Following incubation, exposure to U46619 and BK was repeated.

2.7. General chemicals

Chemicals and pharmacological agents were purchased from Sigma–Aldrich (Poole, Dorset, UK) or BDH (Poole, Dorset, UK).

2.8. Statistics

Data were analysed using GraphPad Prism 5 for Windows (GraphPad Software, San Diego, CA) according to maternal obesity status; subgroup analyses were performed for AGA pregnancies and for fetal gender. *N* represents the number of placentas studied; *n* represents the number of vessels.

Vessel tone was expressed as active effective pressure (kPa): tension (mN/mm) × [diameter (μm)/2000]. Area Under the dose response Curve (AUC; arbitrary units; calculated as the area between the dose response curve and the line of $y = 0$), maximum response (V_{max}; in kPa for U46619 and percentage change from baseline pre-constriction for SNP irrespective of the agonist dose at which that response occurred) and sensitivity (EC_{50}; in nM for concentration of agonist required to achieve 50% maximal response) were calculated for each artery and averaged per placenta.

Fluctuations in vascular tone were observed in CPAs when contraction was stimulated with a sub-maximal dose (30 nM) of U46619 and subsequently after addition of BK. These tone fluctuations were defined as oscillations when the amplitude (change in tone from top to bottom) exceeded 10% of the maximum (peak) constriction to 30 nM U46619. The number of oscillations in the final 15 min of the U46619 incubation was counted and expressed as a frequency (min^{-1}). Maximum response (V_{max}) to BK, oscillation amplitude (percentage of peak U46619 constriction) and oscillation frequency were compared pre/post-leptin incubation for each artery.

Categorical data are presented as frequencies and tested by χ^2 analysis, continuous data are presented as medians (range in parentheses) and analysed by Mann-Whitney U tests; subgroup analysis of AGA infants was also performed to exclude the possibility of confounding effects of infant growth phenotypes. Paired data (pre/post-leptin incubation) were compared using Wilcoxon matched-pairs signed rank test (for AUC, V_{max} and EC_{50} to U46619) or Friedman test (for oscillation and BK data). Statistical significance was set at $p < 0.05$.

Based on a previous study examining CPA vasoconstriction to U46619 in differing oxygen concentrations [26], we calculated that 10 patients/group would be required to detect a similar magnitude of difference in maximal contraction (mean difference 4.0 kPa, standard deviation 2.8 kPa) with power of 90% and significance level of 5%.

3. Results

3.1. Demographic and outcome data

Clinical data are presented in Table 1. Other than for BMI, maternal characteristics were comparable between groups. Infants of obese women were more frequently male, had higher birth weights and IBRs, and were more likely to be LGA compared to infants of normal-BMI women. Subgroup analysis by fetal gender revealed no significant difference in birth weight, IBR or rates of macrosomia and LGA between male and female offspring ($p > 0.05$)

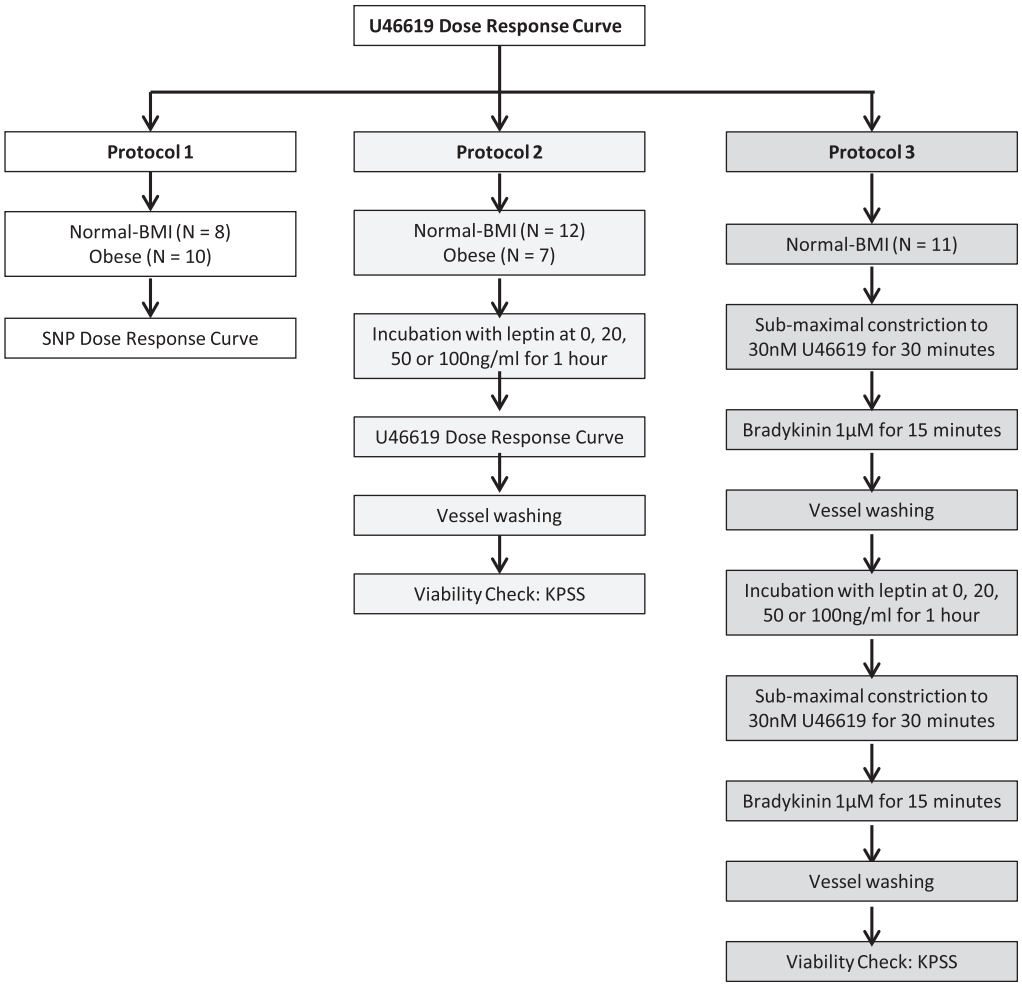

Fig. 1. Study protocol.

or between male offspring of normal-BMI or obese mothers, however female offspring of obese mothers were statistically larger in terms of both birth weight ($p = 0.002$) and IBR ($p = 0.005$).

3.2. CPA constriction is unaffected by maternal BMI

U46619-induced vasoconstriction in the obese cohort did not differ to that from normal-BMI women (Fig. 3a–c), although maternal obesity was associated with a trend towards increased V_{max} which failed to reach significance ($p = 0.08$).

To ensure there were no confounding effects of infant birth weight on the relationship between maternal BMI and placental vascular function, comparisons were made between AGA births in the three BMI cohorts; AUC, V_{max} and EC_{50} values to U46619 were comparable ($p > 0.05$) between groups and between male and female offspring.

3.3. Protocol 1: Raised maternal BMI impairs placental CPA vasodilatation

CPA vasodilatation to SNP in the obese cohort was significantly reduced compared to normal-BMI women (Fig. 4a–b). The dose response curve to SNP was significantly non-sigmoidal in 50% of all obese participants (in keeping with studies in FGR placentas) and therefore an SNP EC_{50} value could not be calculated for these placentas. Examination of only the vasodilatory phase of SNP dose response curves also failed to fit a sigmoidal curve. Consequently sensitivity to SNP has not been compared between cohorts. In analysing only AGA pregnancies SNP V_{max} remained significantly different between normal-BMI and obese cohorts [53.9% (36.5–62.5) vs. 27.7% (17.5–40.1) respectively, $p = 0.03$]. No statistically significant difference was observed between fetal genders in terms of AUC or V_{max} ($p > 0.05$) and SNP dose response curves remained non-sigmoidal.

3.4. Protocol 2: Leptin exposure impairs CPA vasoconstriction

Leptin exposure data on CPA constriction are summarised in Table 2. U46619-induced constriction was unaffected by experimental duration ($p > 0.05$, data not shown). In normal-BMI women sensitivity to U46619 significantly decreased (higher EC_{50}) post-incubation with 100 ng/ml leptin ($p < 0.01$) but other parameters were unaffected by leptin incubation. In the obese cohort, U46619 AUC was significantly reduced post-incubation with 50 ng/ml leptin ($p < 0.05$) but not at higher leptin concentrations.

3.5. Protocol 3: No effect of leptin on tone oscillations in CPAs

Tone oscillations were observed in 61% of CPAs prior to leptin incubation. Peak constriction and average tone to U46619 (Fig. 2)

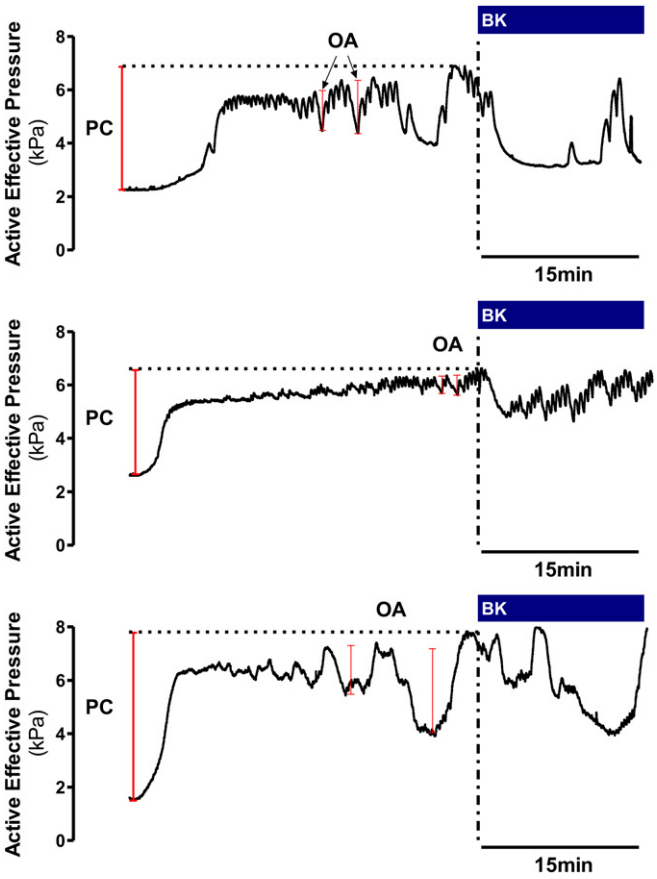

Fig. 2. Examples of original tracings illustrating U46619-induced tone oscillations with 1 μM bradykinin (BK) added at the end of the exposure period. The peak constriction (PC) and average tone over the last 15 min of the U46619 constriction were calculated for each CPA. Oscillations during the U46619 constriction were defined as a change in tone (peak to trough) of >10% compared to the PC. Mean amplitude (OA) and frequency (min^{-1}) of oscillations to U46619 were calculated for each artery. The maximum response, average tone, oscillation amplitude and frequency over 15 min were calculated for BK exposure.

were 5.4 kPa (0.2–13.9 kPa) and 3.7 kPa (0.1–12.8 kPa) respectively with amplitude and frequency of 24.6% (0.6–70.7%) and 0.05 per min (0.02–0.7 per min) respectively. BK exposure significantly reduced peak constriction by 50.5% (14.4–94.7%; V_{max}; $p < 0.0001$) and increased oscillation amplitude to 47.7% (12.5–90.9%) of peak U46619 constriction ($p < 0.05$) but had no significant effect on oscillation frequency (0.07 per min [0.03–2.0 per min]). CPA tone, oscillation amplitude and frequency or relaxation to BK were unaffected by leptin exposure (Table 3).

4. Discussion

This study demonstrates altered placental vascular reactivity in maternal obesity and is the first to examine the effect of leptin on placental vascular function. U46619-induced CPA constriction was unaffected by maternal BMI whilst SNP induced CPA relaxation was impaired in obesity. These data indicate that term placental vascular function is impaired in women who commence pregnancy with an increased BMI. It is interesting to note that these differences in vascular function between placentas of normal-BMI and obese women have been observed in pregnancies resulting predominantly in AGA offspring and that in this cohort (although underpowered to study the effect of fetal gender in detail) maternal obesity appears to promote growth of female fetuses but not of male fetuses. We believe that this may result (in part) from the fetus compensating for adverse intrauterine conditions, regulating its own growth despite the adverse maternal environment.

If altered CPA reactivity translates to a less vasodilated phenotype as suggested by the findings of this study, aberrant placental blood flow and lower birth weights would be expected. However, the impairment of CPA vasodilatation to SNP contrasts with the enhanced CPA vasodilatation in response to this endothelium-independent vasodilator observed in idiopathic FGR pregnancies [11]. We hypothesise that this may have an effect on organ perfusion and alter the homeostatic regulation of blood pressure and blood flow in the offspring, predisposing to cardiovascular disease in later life [27].

In keeping with other studies, CPAs from both BMI cohorts demonstrated the characteristic biphasic response to SNP; a vasodilatation followed by vasoconstriction with doses above 10^{-6} M (Fig. 4a) [11,26]. The mechanisms underlying this have not been explored as yet. Although impaired CPA vasodilatation may be expected to reduce nutrient transfer across the placenta by impairing placental blood flow and hence lead to reduced fetal growth, in this small cohort there was a high incidence of LGA birth amongst obese women, despite exclusion of pregnancies complicated by antenatally diagnosed aberrant fetal growth. This demonstrates that the link between CPA tone, placental blood flow and fetal growth is not simple.

Wire myography studies suggest that isolated CPAs are relatively unresponsive to endothelial-dependent agonists, such as acetylcholine and BK, following EC_{80} or V_{max} doses of U46619 [28]. However, in pressure myography studies of CPAs, flow-induced nitric oxide release induces vasodilation which suggests that shear-stress promotes nitric oxide release from the endothelium that can influence basal tone [29]. Here, we replicated the findings of Sweeney and colleagues who demonstrated that in CPAs exposed to a sub-maximal dose of U46619, vascular tone could be reduced by BK (Fig. 2; Table 3), and tone oscillations (vasomotion) could be observed [12]. Vasomotion (rhythmic oscillations in vascular tone) has been observed in many vascular beds but its function has not been fully elucidated. The general consensus is that oscillations occur via interactions between the endothelium and underlying smooth muscle to promote efficient blood flow to optimise end-

Table 1
Demographic, biophysical and obstetric data for participants. Data are median (range) unless stated otherwise. Abbreviations; IBR, individualised birth weight ratio; BMI, body mass index; SGA, small for gestational age; LGA, large for gestational age. SGA is defined as IBR < 10th centile, LGA is defined as IBR > 90th centile and macrosomia defined as birth weight >4 kg.

Category	Normal ($n = 26$)	Obese ($n = 20$)	p
Age: Years	29 (16–42)	30 (20–39)	ns
Caucasian: Number (%)	20 (77)	12 (60)	ns
Parity	1 (0–3)	1 (0–4)	ns
Smoker: Number (%)	3 (12)	1 (5)	ns
BMI at booking: Kg/m^2	22.5 (18.5–24.7)	34.2 (30.4–49.6)	<0.05
Gestation: Weeks	39^{+1} (37^{+6}–41^{+4})	39^{+2} (38^{+0}–41^{+6})	ns
Laboured: Number (%)	4 (15.4)	2 (10.0)	ns
Caesarean: Number (%)	22 (84.6)	18 (90.0)	ns
Previous Caesarean section: Number (%)	14 (51.9%)	10 (50.0%)	ns
Birth weight: Grams	3215 (2500–3840)	3790 (2880.0–4850.0)	<0.05
IBR: Centile	40 (4–99)	76 (5–100)	<0.05
Male infant: Number (%)	9 (35)	13 (68)	<0.05
SGA: Number (%)	3 (12)	1 (5)	ns
LGA: Number (%)	2 (8)	5 (25)	<0.05
Macrosomia: Number (%)	0 (0)	5 (25)	<0.05

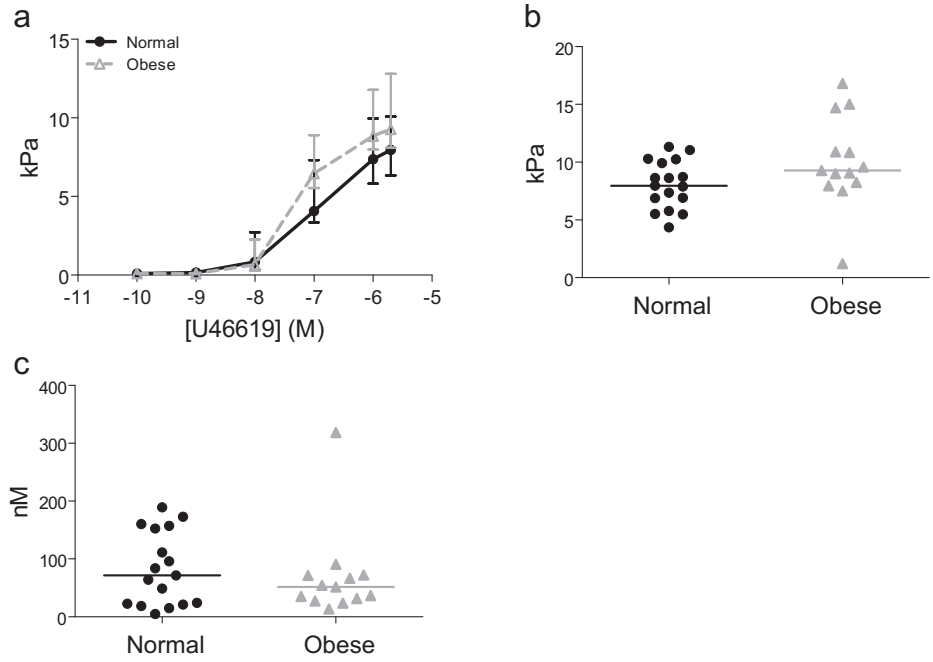

Fig. 3. Effect of maternal BMI on chorionic plate artery vasoconstriction. Vasoconstriction response of CPAs to the thromboxane-A2 mimetic U46619 was unaltered between BMI cohorts: a) Dose response curves; Area under the curve (AUC; $p > 0.05$). b) Maximal response (V_{max}; $p > 0.05$). c) Sensitivity (EC_{50}; $p > 0.05$).

organ perfusion [30]. These findings further support a role for the endothelium in the control of basal CPA tone.

It is possible that altered circulating adipokine concentrations in obesity may contribute to altered placental vascular reactivity. Hyperleptinemia, as observed in obese humans and in animal models of maternal obesity, is thought to promote an imbalance in nitric oxide bioavailability and increased oxidative stress in the systemic circulation [31]. Leptin receptors are present in the placenta and on vascular endothelial cells [31,32] indicating that the placenta has the potential to detect/respond to differing leptin concentrations and this may offer a mechanism by which adipokines are potential dysregulators of fetoplacental blood flow. We

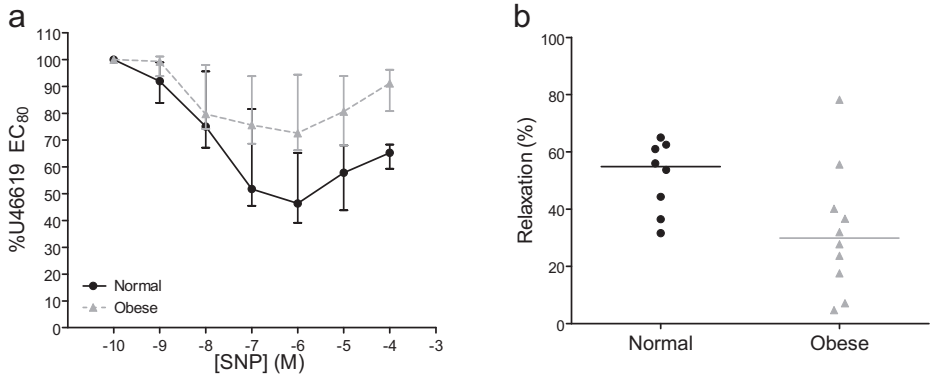

Fig. 4. Effect of maternal obesity on chorionic plate artery vasodilation. Vasodilation response of CPAs to the nitric oxide donor sodium nitroprusside is impaired by maternal obesity: a) Dose response curve (AUC; $p = 0.02$). b) Maximal response (V_{max}; $p = 0.04$).

Table 2
Leptin exposure impairs chorionic plate artery vasoconstriction. Demonstrating the effects of vessel incubation with differing concentrations of leptin on constriction to U46619. Abbreviations; TC, time control. Data are median and (range). $^{*}P < 0.05$, $^{**}P < 0.01$ pre/post-incubation.

BMI Category	Leptin concentration ng/ml	AUC arbitrary units		V_{max} kPa		EC_{50} nM	
		Pre	Post	Pre	Post	Pre	Post
Normal	0 [TC]	13.0 (6.8–28.1)	13.5 (3.2–23.0)	9.0 (5.5–15.1)	8.5 (6.1–16.8)	105.7 (9.0–258.2)	85.9 (5.3–679.2)
	50	8.4 (3.7–35.5)	9.5 (4.3–28.8)	7.3 (2.8–22.5)	7.7 (3.4–23.5)	82.5 (25.4–347.5)	113.8 (1.8–382.8)
	100	13.4 (7.7–33.4)	9.2 (6.9–39.3)	8.2 (6.0–21.3)	7.8 (6.0–24.8)	79.4 (11.1–137.4)	224.4** (43.3–466.7)
Obese	0 [TC]	13.2 (7.9–38.3)	16.0 (5.7–31.0)	10.1 (5.6–22.2)	10.1 (6.1–22.8)	61.1 (14.4–135.2)	56.6 (42.0–358.1)
	50	20.0 (11.0–35.6)	13.4* (9.9–21.4)	14.0 (9.2–27.1)	10.9 (5.1–13.8)	89.1 (27.5–161.1)	59.0 (20.9–769.1)
	100	14.8 (9.7–28.7)	18.2 (9.9–28.8)	10.3 (7.7–18.0)	9.8 (8.9–17.7)	60.1 (37.8–349.0)	66.4 (12.1–343.6)

Table 3
Chorionic plate artery vasomotion in normal-BMI women is unaltered by leptin exposure. No parameters studied were altered by treatment with leptin ($p > 0.05$). Abbreviations; TC, time control. Data are median (range).

Leptin concentration ng/ml	Peak constriction kPa	Average tone kPa	Amplitude % peak U46619 constriction	Frequency min^{-1}	BK V_{max} % peak U46619 constriction
0 (TC)	4.5 (1.9–10.9)	3.0 (1.4–9.6)	29.2 (11.2–133.3)	0.1 (0.03–0.17)	63.2 (21.8–93.8)
50	5.7 (1.5–13.0)	3.4 (1.0–8.9)	25.3 (11.0–40.3)	0.1 (0.03–0.73)	55.7 (19.3–193.3)
100	5.5 (1.4–16.2)	3.6 (0.7–10.1)	41.7 (22.2–97.0)	0.07 (0.03–0.13)	40.4 (16.4–87.9)

noted that *in vitro* short-term leptin exposure altered CPA vasoconstriction in response to a thromboxane-A_2 mimetic but had no effect on endothelial-dependent CPA relaxation induced by BK or on tone oscillations in CPAs. Our findings therefore contradict previous studies [18,33] that suggest a direct or acute role of leptin in modulation of CPA endothelial function, but agree with other studies [34] which indicate leptin affects endothelial-independent mechanisms.

The differences demonstrated in CPA phenotype between obese and leptin-treated normal-BMI placentas require further mechanistic study. It is noteworthy that maximal leptin effects were observed at differing concentrations in CPAs from normal-BMI compared to obese women (100 ng/ml vs. 50 ng/ml) suggestive of altered placental artery sensitivity to leptin. We hypothesise that the reasons for the failure of leptin exposure *in vitro* to reproduce the obese CPA phenotype may be related to differences in the duration of leptin exposure *in vitro* compared with *in utero* and co-exposure to deranged levels of other adipokines. It is unlikely that leptin alone would be the sole link between obesity and increased susceptibility to poor pregnancy outcome. Nonetheless, these results add to a growing literature [18,33,35] suggesting that leptin has a role to play in regulating vascular function.

4.1. Study limitations

Study participants were identified and recruited at admission for delivery. The majority of participants were delivered by elective Caesarean section as these women were pain-free at the time of approach and able to give informed consent to research participation. The decision for Caesarean section has been subtextually influenced by maternal or clinician estimated fetal size and may account for the high rate of SGA and LGA births observed in this study compared with the general population. Mode of delivery has previously been studied using a similar experimental protocol in a cohort of 145 pregnancies and was shown to have no influence on chorionic plate vascular reactivity [36]. Therefore we believe our findings truly reflect the effect of maternal obesity on CPA physiology rather than consequences of mode of delivery.

As a non-interventional study in which participants were enrolled at admission for delivery, and obesity itself is not in itself an indication for routine fetal growth and umbilical artery Doppler surveillance of the pregnancy in the United Kingdom; data on *in vivo* placental vascular function is lacking for the majority of study participants. As such we are unable to link our *ex vivo* vascular studies to *in utero* umbilical artery Doppler resistance parameters however the relationship between wire myography and Doppler parameters is debated [11,37] and is itself in need of further examination.

4.2. Future perspectives

The preponderance of male offspring amongst the obese women of this cohort is interesting for a number of reasons. In animal studies maternal nutrition at the time of conception is observed to influence gender specific survival of fetuses, with a relative excess of male offspring born at times of nutritional plenty [38]. In humans however this phenomenon is debated [39,40]. In addition gender differences in placental function have not been fully investigated and this study is underpowered to fully answer this question. Whilst there is a preponderance of male fetuses in the obese cohort, our subanalysis of fetal gender suggests that the predominant effect of maternal obesity on fetal growth is observed in female offspring whilst birth weights and IBRs of the male offspring were not different between normal-BMI and obese groups. It remains therefore a possibility that the observed differences in placental arterial function between obese and normal-BMI women's offspring may be confounded by the observed gender imbalance, with blunting of placental arterial vasodilation in order to limit fetal overgrowth that may be more marked amongst male fetuses. There is certainly a growing evidence base suggesting that male fetuses may be more susceptible to certain pregnancy complications, with a higher rate of intrauterine growth and therefore higher *in utero* nutrient demands which may fail to be met by a dysfunctional placenta [41]. Future studies are required both to establish whether fetal gender influences placental arterial function in health and in disease, and also to establish whether sex selection occurs in humans.

Whilst first trimester maternal BMI has been shown to accurately reflect maternal adiposity [15], BMI $\geq$ 30 kg/m^2 may be viewed as a relatively arbitrary cut off to define those at risk of obesity-related health problems. Indeed in some populations, notably South East Asian communities, the risk of obesity-related adverse health outcome accelerates at much lower BMI levels than for Caucasian women [42]. We recommend that in order to better understand the relationship of obesity to pregnancy outcome, longitudinal cohort studies should be conducted to examine maternal adiposity and nutrition not only using first trimester BMI but also with pre-pregnancy weight, BMI, waist-hip ratio and nutritional status and the change in adiposity across gestation.

This study of the effect of obesity and adipokines on placental vascular function is not exhaustive and many questions relating to the effect of obesity on the placenta remain unanswered, particularly regarding the mechanisms underlying such changes. Future studies examining the impact of factors such as periconceptual and gestational caloric intake, changes in maternal adiposity throughout pregnancy and relating maternal/fetal circulating adipokine concentrations to pregnancy outcome and placental vascular function are recommended.

5. Conclusion

Placental vascular function is altered in women who are obese at the start of pregnancy, despite exclusion of maternal and fetal comorbidity. Although leptin incubation of CPAs from women of normal-BMI altered vascular reactivity, the specific vascular profile observed in maternal obesity was not reproduced. The potential role for other adipokines such as adiponectin, in the obese CPA functional phenotype requires further study. The causes of obesity-related placental vascular dysfunction are potential targets for modulation in order to break the vicious cycle of maternal obesity and its burden upon healthcare resources and society.

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