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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 ($p > 0.05$)