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Title: Delivery of a growth restricted offspring and the association with early onset impaired maternal endothelial function.

Short title: Maternal postnatal FMD

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STRUCTURED ABSTRACT

Background: Women who have had small for gestational age offspring have an increased risk of cardiovascular disease in later life. Endothelial dysfunction is a subclinical sign of early cardiovascular disease. It is unknown whether women who have recently had a pregnancy complicated by fetal growth restriction, in the absence of other maternal and fetal disease, have subclinical endothelial dysfunction.

Objective: To assess maternal endothelial function six months after a pregnancy complicated by fetal growth restriction.

Methods: This was a case-control study, conducted in a tertiary referral hospital in London over a 15-month period. Flow-mediated dilatation (FMD) of the brachial artery was measured in women, six months (+/- 2.5 months) after the birth of their child. Forty-four women were studied; 15 had a growth restricted offspring (customised birth centile 1.9 ± 2.3; mean ± SD) and 29 women had delivered an appropriately grown baby (customized birth centile 47.5 ± 26.3). Results were analysed using an unpaired t-test and the means of the primary continuous variable (FMD % change).

Results: Women who had a growth restricted offspring had lower postpartum FMD (6.79% ± 0.95%) compared with mothers who had an appropriately grown offspring, FMD 10.26% ± 2.44%; 95% CI -5.37 to -1.57 (p=0.0007;). There were no differences in postnatal measures of maternal blood pressure, abdominal circumference, weight, glucose, insulin and lipid profiles between the two groups.

Conclusions: Within six months of childbirth, women who have had a pregnancy affected by fetal growth restriction due to likely placental failure, but in the absence of pre-eclampsia, have evidence of subclinical endothelial dysfunction. These women may benefit from lifestyle measures focused on the primary prevention of cardiovascular disease. Further research, with larger populations, is needed to discover whether this postpartum maternal endothelial dysfunction is a pregnancy-induced phenomenon, or related to pre-existing maternal phenotype and indeed whether it persists long term.

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INTRODUCTION

Endothelial dependent flow mediated dilation (FMD) is a non-invasive way to assess vascular function.[1] By measuring the change in brachial artery diameter in response to increased blood flow, endothelial function can be assessed contemporaneously.[2] Women, who have experienced pre-eclampsia, are at increased risk of cardiovascular disease.[3] Subclinical endothelial dysfunction is evident within 6 months of a woman having a pregnancy affected by pre-eclampsia, demonstrated through lower flow mediated dilation.[4]

Women who have had a baby in the lowest birth-weight quintile for gestational age, as well as those who delivered a small-for-gestational age (SGA) infant (independent of other recognised risk factors) have an increased lifetime risk of ischaemic heart disease (IHD).[5] This risk is dose dependent, varying according to severity of SGA and previous number of SGA pregnancies.[6] It is less clear whether early impaired endothelial dysfunction is present in women who have delivered a growth restricted infant.

As pregnancy progresses, high resistance maternal uterine arteries are transformed into low resistance, high capacitance vessels to facilitate the provision of oxygen and nutrients, for placental development and optimal fetal growth. Dysfunction within the placenta is thought to a central component of both pre-eclampsia and fetal growth restriction.[7] It is possible that aberrant remodelling of the utero-placental circulation resulting in a growth restricted fetus might be influenced by underlying maternal vascular phenotype or conversely, compromised placental development may itself influence future maternal vascular health. In this study we sought to

contemporaneously investigate women who had a pregnancy complicated by fetal growth restriction, independent of other maternal and fetal disease, hypothesising that they would have impaired endothelial function evident shortly after an affected pregnancy, similar to that seen in pre-eclampsia.

Given that subclinical endothelial dysfunction is a prelude to the later development of cardiovascular disease[8,9] and brachial artery FMD is inversely correlated with future cardiovascular events,[10] then identification, using FMD, of a cohort of women, with post-partum sub-clinical endothelial dysfunction **would provide a novel way of identifying women at future risk of cardiovascular disease. This finding then has the potential to prevent or ameliorate disease in these women through personalised lifestyle adjustments and monitoring.**

METHODS

This was a case control study undertaken at the Clinical Research Facility, University College London Hospital (UCLH). Ethical approval for this study was granted by the Joint UCLH/UCL (alpha) Ethical Committee (09/H0715/28).

Couples were recruited antenatally, over a 15month period. Recruitment took place in the antenatal period to give time for couples to consider the study and to consent to collection of fetal samples after delivery. Eligible participants were approached in a range of hospital settings, as has been described in our previous publication.[11]

Fetal growth restriction (FGR) was identified by ultrasound and was defined as an antenatal customised birth weight less than the 10th centile.[12] All cases of FGR were confirmed by postpartum measurements. Pregnancies affected by fetal anomaly

were excluded. We also excluded women who smoked, who also developed pre-eclampsia or who had gestational hypertension (>140/90mmHg) to best elicit placentally derived growth restriction, independent of the effect of pre-eclampsia.

Pregnant women were recruited as controls, at the same time point and from the same clinical area, as cases, if they were thought to be having a normally grown baby with an estimated fetal weight between 10th and 95th customised centile as assessed by an antenatal ultrasound and which was confirmed by postnatal assessment. Only non-smoking women who did not develop pre-eclampsia or gestational hypertension were included as controls or cases, in order to try and minimize confounding influences of extrinsic maternal factors.

Each parent also completed a questionnaire enquiring about past medical, family and treatment history. Own birth weight was remembered personally, or from a parent. Maternal antenatal notes were reviewed to provide pertinent antenatal data.

Women were invited back for postpartum study, by letter, email or telephone between four months and one year after the birth of their child, after breastfeeding had ceased, on a day when not menstruating. The study room was temperature controlled at 24°C. Participants were asked to fast overnight for at least 10h before study and all postnatal studies were performed in the morning. Weight, height and abdominal circumference were recorded. After resting, two measures of supine blood pressure (BP) were taken 15 minutes apart. The same single trained operator measured blood pressure by auscultatory sphygmomanometry, using an aneroid sphygmomanometer, in sitting position. Where appropriate, cuff sizes were adjusted

to arm circumference. The mean value of two separate measurements 15 minutes apart was used for analysis.

Fasting venous insulin, glucose and lipid levels were measured. Insulin resistance (IR) was calculated using the HOMA model.[13] Blood was spun within 1hr of venepuncture and plasma and serum were frozen at -80ºC. All blood samples were processed in the same laboratory.

Endothelial function was assessed using brachial artery flow-mediated dilatation (FMD). **A single operator, trained and validated within an established group using FMD[14], performed the scans, using established protocols as previously reported for conducting flow-mediated dilatation.[14]** Briefly, the right arm was rested in an arm holder and the forearm was raised slightly using a foam pad. An 8.5cm wide pneumatic cuff was then placed around the upper forearm, 2cm distal to the medial epicondyle (figure 1).

Cuff pressure inflation was controlled by an automatic cuff inflator (Hokanson Cuff Inflator, PMS Instruments Ltd, Maidenhead, UK). Using an ultrasound probe in the longitudinal plane, a segment of brachial artery was identified and studied before and after a five-minute compressive arterial occlusion.

An Aloka SSD 5000 ultrasound machine was used with a 13MHz linear array transducer probe (Aloka Holding Europe, AG Switzerland). The image was acquired in B-mode and the probe fixed so that a 5-10cm segment of the brachial artery proximal to the antecubital fossa was in view. Longitudinal end diastolic images, in conjunction with ECG tracing were acquired every three seconds during the eleven minute recording (one minute baseline, five minutes with cuff inflation at 300mmHg

and five minutes with cuff deflated). Blood flow velocity was continuously monitored during the scan by switching to a B/D (Doppler) mode for the test duration.

Images were acquired and analysed using automated software (Brachial Tools Medical Imaging Applications, Iowa, USA). With the initial segment recorded a smaller region of maximum clarity was selected for analysis. To provide internal reproducibility two different segments within each scan were analysed and compared for similarity. Stringent inclusion criteria were applied. Analysis exclusion criteria included; poor image quality, if recording was deemed to be unrepresentative of the true vessel diameter or if the image did not return to near baseline after reperfusion. Each image-recording was validated by a second operator blinded to the subject's group. FMD was calculated as a percentage change from baseline brachial artery diameter (mm) to maximum dilation (mm) after reperfusion, using semi automated continuous capture software. Scans were excluded if the FMD results from two different segments or second operator validation differed by >1.5%.

Statistical Analysis

Cases and controls were assessed for comparability with simple descriptive statistics. Baseline antenatal characteristics of cases and controls are summarised with means and standard deviations (SD). Postnatal measurements are summarised with means and SD. For continuous variables, 95% confidence limits are given and percentages for categorical variables, e.g. sex. Statistical analysis was carried out using STATA 10 (StataCorp LP, Texas USA) and GraphPad Prism version 5. Visual assessment of normality curves for variables was undertaken to assess for normality. Analysis was done using the unpaired t-test and means were used for the

primary continuous variable (FMD % change). Two tailed P-values <0.05 were considered statistically significant.

RESULTS

Recruitment occurred over a 15-month period. Women were invited back by telephone, email or letter. Eight could not be contacted due to out of date details or no response. **Of the 94 women contacted, 61 (65%) were studied in the postnatal period. Of those women not studied; 8 were pregnant at the time of recall; 10 women declined to come back having moved area or having been out of area initially and 15 women declined due to work or family commitments.**

In total, 24 women who had pregnancies affected by fetal growth restriction (FGR; cases) and 37 women who had an appropriately growth baby (AGA; controls) were able to attend postnatal appointments. Of FGR cases, 4 women also experienced pre-eclampsia so were excluded from final analysis. After quality checks of FMD recordings (as described in the methods section), **13 studied were excluded; 8 controls and 5 cases. Four studies (2 cases and 2 controls) were excluded because 2nd operator value varied >1.5%; 5 further studies (3 controls, 2 cases) were rejected as the baseline did not return by the end of the recording; in 2 control studies the recording not completed and the recording was rejected due to image quality in 2 studies (1 case and 1 control).** Thus, 29 studies of postnatal FMD scans on mothers with AGA offspring were eligible for analysis compared with 15 FMD scans on mothers of FGR offspring.

Baseline characteristics of these 44 offspring confirmed that case and control offspring subjects met the study criteria. In cases, mean customised birth weight centile was 1.9th (± 2.3) with a mean birth weight of 2254g (± 547g) and gestation of 268 days (± 19.4 days). The mean customised birth centile of controls was 47.5th (± 26.3) centile with a mean birth weight of 3566g (± 350g) and gestation of 285 days (±9.6 days).

Maternal pre-pregnancy age, weight (kg), height (cm) and parity revealed no differences between cases and controls (table 1). There was no difference between the groups in BP at 10-12 weeks gestation, no women in either group developed sustained hypertension >140/90mmHg before childbirth or peripartum. Maternal BP at time of FMD study remained similar (table 2).

Antenatally and peripartum, and no women reported pre-pregnancy hypertension or significant medical conditions.

Postnatal study was carried out at 6.9 ± 2.5 months after the birth of their baby (mothers of AGA offspring) and at 6.8-± 2.5 months post-partum (mothers of FGR offspring) (p=0.92 95% CI -1.537 to 1.690). Postpartum, there was no difference between case and control women in relation to maternal weight, waist circumference or blood pressure (table 2). More mothers of FGR pregnancies had a caesarean section 63.9% compared with 27% mothers of AGA offspring.

Women whose pregnancy had been complicated by FGR had lower FMD (6.79±0.95%) compared with women who had an appropriately grown baby (FMD 10.26% ± 2.44%; 95% CI -5.37 to -1.57 (p=0.0007).

Postpartum fasting glucose and insulin levels were similar between control and case mothers, which reflected no difference in insulin resistance, as defined by the HOMA-IR index. There were also no differences in lipid profile, renal function, 25 (OH) D, B12, or folate levels between cases and controls.

DISCUSSION

Principal Findings of the Study

In healthy individuals, flow mediated dilatation has been reported to range from 8-15%.[2] The data generated by this study supports the concept that impaired endothelial function is present postnatally in women who have had pregnancies affected by growth restriction. **Fetal growth restriction, like pre-eclampsia, might be acting as a maternal 'stressor' triggering a pre existing underlying maternal vascular phenotype.**

To avoid the influence of known risk factors for endothelial dysfunction, women who had chronic diseases, pre-eclampsia or who smoked and those mothers who were still breast-feeding were excluded from analysis. **Given this, then it is also possible that compromised placental development may itself be influencing future maternal vascular health.**

Comparison with findings of previous studies

Maternal endothelial dysfunction is known to be impaired during and after a pregnancy complicated by pre-eclampsia.[15, 16] We found very similar FMD measurements in mothers who had pregnancies affected by FGR as were found following pregnancies affected by pre-eclampsia (FMD of pre-eclampsia cases

7.3±2.4%).[16] Large-scale retrospective population studies have shown that women who have had an SGA baby have an increased risk of IHD. [5,6,17] It was been reported that within 4 years, a woman who experiences a pregnancy affected by growth restriction, exhibits worse endothelial function and metabolic profiles than a control population.[18, 19] However, in one study, maternal blood pressures were higher during pregnancies affected by SGA and the postpartum study period compared with women who had AGA offspring. In our study, maternal blood pressures were similar at booking and during labour between FGR and AGA pregnancies.

Women in our study were asymptomatic without marked metabolic dysfunction, yet had sub-clinical endothelial dysfunction; a recognised prelude to metabolic syndrome and cardiovascular disease, soon after a growth restricted pregnancy. A meta-analysis of 14 prospective studies has revealed that the risk of a cardiovascular event is reduced by 13% for every 1% increase in FMD.[20]

Limitations of the study

We included well phenotyped cases of fetal growth restriction, but the postnatal study was carried out six months after initial recruitment, with stringent exclusion criteria. **A limitation of this study was the loss (~40%) to follow up, of antenatal participants recruited. However, when analysed, drop out rates and reasons for non-attendance were similar in both groups, likely limiting any bias effect.**

We found that women who had FGR, had a 3.47% lower FMD than mothers of AGA offspring. Although this value could represent a meaningful difference in

cardiovascular risk between the groups, it is possible that the association between FMD and CV disease is not as strong in asymptomatic populations.[21] **Due to stringent exclusion criteria, a number of FMD results were rejected. The number rejected due to 2nd operator disagreement was only ~6%, which is consistent with previously published data[22] but the overall number of studied rejected due to additional criteria is higher. Given the number of exclusions caution in interpreting results is appropriate. However, a sub-analysis of rejected images and pre-eclamptic subjects did not alter the overall finding of lower FMD in case women.** FMD values can vary according to a number of environmental factors such as; time of day, food consumption and phase of the menstrual cycle.[1] Whilst we tried to account for these factors they may be confounding the result. A bigger longitudinal cohort, replicating these initial results, would strengthen the association identified **and is planned as part of a follow on study.**

Implications for Practice

The pathophysiological pathway by which poor placental function with FGR, in the absence of maternal gestational hypertension/pre-eclampsia, is associated with maternal endothelial dysfunction is yet to be elucidated. A prospective study of pregnancy outcomes, with preconception and postnatal measures of maternal FMD in the same women, would elucidate the role of pregnancy-induced endothelial dysfunction. **Reproducibility of the technique, as evidenced with this study, along with its high operator dependency, are issues that need to be taken into account for future study designs or if implementation in a clinical arena is**

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being considered.

Longitudinal follow up could also elicit whether decreased FMD persists in these individuals as there is some evidence to suggest FMD changes can resolve after 10 years.[23, 24]

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TABLES

Antenatal characteristics	Mothers with FGR		n= 15		Mothers with AGA		n=29	
	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Age (years)	33.2	3.3	20	40	32.6	3.7	20	39
Weight (kg)	60.0	7.8	48	80	64.1	10.7	44	84
Height (cm)	164.5	5.3	152	175	166.0	6.1	156	185
Booking BMI (kg/m^2)	22.1	2.3	20	27	23.1	3.2	20	30
Systolic BP (mmHg)	110.8	8.3	93	121	110.4	10.9	93	136
Diastolic BP (mmHg)	65.6	9.2	51	84	65.7	9.9	46	85
Nulliparous Multiparous	11 73.3% 4 (26.7%)				22 (75.9%) 7 (24.1%)			
Neonatal Birth weight	2254g (± 547g)				3566g (± 350g)			

Table 1. Maternal endothelial function in growth restriction

Maternal Antenatal Baseline characteristics at 10-12 weeks gestation and offspring birth weights (g)

	Mothers with FGR Mean (SD)	Mothers with AGA Mean (SD)	p-value	95% CI
Number (n)	15	29		
Weight (kg)	61.4 (8.9)	64.0 (8.8)	0.50	(-10.2 to 5.05)
Waist Circumference (cm)	81.9 (6.7)	82.9 (6.5)	0.76	(-7.63 to 5.66)
Systolic BP (mmHg)	105.6 (13)	109.1 (7.9)	0.15	(-8.45 to 1.31)
Diastolic BP (mmHg)	64.0 (7.68)	63.9 (7.68)	0.98	(-3.68 to 3.80)
FMD (%)	6.79 (0.95)	10.26 (2.44)	**0.0007**	(-5.37 to -1.57)

Table 2. Maternal endothelial function in growth restriction

Maternal measurements 6.8 (± 2.5) months postpartum

TABLE LEGENDS

Table 1. Baseline characteristics of mothers with FGR offspring (<10th customised birth weight centile: n=15) compared with mothers who had AGA offspring (10th - 95th customised birth weight centiles; n=29). Assessment at 10-12 weeks gestation including; age (years), weight (kg) at first booking visit, height, body mass index (BMI) kg/m^2, recorded blood pressure in antenatal clinic (mmHg) (sitting and by same operator) and parity of the women.

Table 2. Physical measurements of the 29 women with AGA offspring originally recruited and 15 women who had FGR offspring studied at 6.8 ± 2.5 months. Measurements included weight (kg), height (cm), abdominal circumference (cm) and blood pressure (mmHg), and revealed no significant differences. Flow mediated dilatation (FMD), as expressed as % change from baseline, was significantly different between cases and controls.

FIGURE LEGENDS

Figure 1: Flow mediated dilatation equipment. Image shows configuration of the equipment necessary to record the diameter of the brachial artery.