Small artery function 2 years postpartum in women with altered glycaemic distributions in their preceding pregnancy


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ABSTRACT

GDM (gestational diabetes mellitus) is associated with later adverse cardiovascular risk. The present study examined the relationship between glycaemia during pregnancy and small artery function and structures approx. 2 years postpartum. Women were originally enrolled in the HAPO (Hyperglycaemia and Adverse Pregnancy Outcome) study from which they were classified by their glycaemic distribution during pregnancy as controls (in the lower half of the distribution), UQ (upper quartile; in the UQ of the glycaemic distribution) or having had overt GDM. Subcutaneous arteries from a gluteal fat biopsy taken at follow-up 2 years later were examined using wire myography. Small artery structure, stiffness and vasoconstrictor responses were similar across groups. Maximal endothelium-dependent dilation in response to carbachol was impaired in arteries from both GDM (43.3%, n = 8 and P = 0.01) and UQ (51.7%, n = 13 and P = 0.04) women despite generally ‘normal’ current glycaemia (controls, 72.7% and n = 8). Inhibition of NOS (nitric oxide synthase) significantly reduced maximum endothelium-dependent dilation in controls but had no effect on arteries from UQ and GDM women, suggesting impaired NOS activity in these groups. Endothelium-independent dilation was unaffected in arteries from previous GDM and UQ women when compared with the control group. Multiple regression analysis suggested that BMI (body mass index) at biopsy was the most potent factor independently associated with small artery function, with no effect of current glycaemia. Overweight women with either GDM or marginally raised glycaemia during pregnancy (our UQ group) had normal vascular structure and stiffness, but clearly detectable progressively impaired endothelium-dependent function at 2 years follow-up. These results suggest that vascular pathology, which may still be reversible, is detectable very early in women at risk of decline into Type 2 diabetes mellitus.

INTRODUCTION

T2DM (Type 2 diabetes mellitus) is characterized by a 3–4-fold excess of cardiovascular mortality and is associated with abnormalities in both the macro- and micro-circulation [1]. Mortality also increases with progressive glucose intolerance [2–4]. Small artery structure and function are abnormal in patients with...
T2DM with both impaired endothelium-dependent dilation and hypertrophic remodelling being evident in small arteries from both normotensive and hypertensive T2DM patients [5–8]. These changes are early indicators of target organ damage and are strong predictors of cardiovascular mortality [9–11]. Impaired endothelium-dependent dilation has been observed in healthy normoglycaemic offspring with a parental history of T2DM and in subjects displaying IGT (impaired glucose tolerance) [12,13], suggesting that vascular abnormalities may exist in subjects at risk of developing T2DM.

Hyperglycaemia in pregnancy or GDM (gestational diabetes mellitus), which is hyperglycaemia with onset or first recognition during pregnancy [14], is common and evident in approx. 2 % of pregnancies [15]. Women with GDM have >9-fold risk of developing overt T2DM when compared with women who had normal pregnancy glycaemia, with fasting blood glucose during pregnancy having the most predictive value, together with increased BMI (body mass index) [16–18]. GDM is also associated with an increased risk of developing CVD (cardiovascular disease) in later life [4]. Evidence from the HAPO (Hyperglycaemia and Adverse Pregnancy Outcome) study has suggested that glycaemic levels below what is currently considered GDM are also associated with adverse pregnancy outcomes, sparking debate about the diagnostic criteria for GDM [19]. Small arteries from women with GDM exhibit impaired endothelial function at term [20], and impaired NOS (nitric oxide synthase) activity has been demonstrated in stem villous vessels collected from the placenta of women with GDM at term [21]. It is unclear whether alterations in maternal endothelial function exist postpartum when elevated glycaemia is no longer evident. It is also unclear whether women with mild glucose intolerance during pregnancy exhibit microvascular disorders postpartum. The present study was designed to test the impact of glycaemia during pregnancy on resistance artery structure and function postpartum.

**MATERIALS AND METHODS**

**Subjects**

The MMHVH (Manchester Mothers’ Heart and Vascular Health) study prospectively followed women sampled from the first 957 women screened in the Manchester site of the HAPO project. All women enrolled in HAPO underwent a 75 g OGTT (oral glucose tolerance test) at approx. 28 (range 24–32) weeks in pregnancy [19]. That glycaemic profile was used to stratify women into three groups in the present study: (i) controls with fasting plasma glucose <4.5 mmol/l and 2 h values <5.8 mmol/l; (ii) a marginally raised glycaemic group, arbitrarily formed here by the UQ (upper quartile) of that glucose distribution, fasting glucose (4.8–6.9 mmol/l) and/or 2 h glucose (6.8–7.8 mmol/l) and (iii) WHO (World Health Organization)-defined GDM (fasting glucose >7.0 mmol/l and/or 2 h >7.8 mmol/l). These criteria allowed us to examine the impact of both overt GDM and mild glycaemia during pregnancy on postpartum microvascular structure and function.

The enrolled women were invited for 2-yearly follow-up visits where detailed anthropometry, BP (blood pressure; using the validated semi-automatic Omron 705 CP), fasting lipid profile, and fasting blood glucose (or if possible a standard 75 g OGTT) were undertaken. We recruited a sub-group of 29 women who gave informed consent to undergo gluteal fat biopsy at approx. 2 years after delivery of their HAPO baby. Ethical approval was obtained from the Central Manchester Committee (03/CM/477). The study conforms to the Declaration of Helsinki (2000) of the World Medical Association.

**Isolation and mounting of resistance arteries**

A single subcutaneous gluteal fat biopsy was obtained from each participant, following infiltration with 1 % lignocaine. Each biopsy site was within 2.5–4.0 cm of the top of the natal cleft and 5 cm from the midline on either side of the gluteal region. An elliptical incision was made and a block of subcutaneous fat tissue (2 cm × 0.5 cm × 1 cm) with overlying skin was removed and the site sutured following haemostasis. The biopsy was immediately placed in ice-cold PSS (physiological saline solution; 119 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄·7H₂O, 25 mM NaHCO₃, 1.17 mM KH₂PO₄, 0.03 mM K₂EDTA, 5.5 mM glucose and 2.5 mM CaCl₂·2H₂O, pH 7.4), and immediately transferred to the laboratory. Within 1 h small arteries, diameter 180–400 μm, were dissected, mounted on a wire myograph between 40 μm diameter wires (Dual Wire Myograph System 410; Danish Myo Technology), bathed in PSS at 37 °C, and gassed with 5%CO₂/95% air [22]. All vessels were dissected out and mounted under a dissection microscope. Great care was taken to ensure that the wires did not touch or damage the endothelium during the mounting process. All dissection and mounting were carried out by M.B. in the laboratory of C.E.A., who has long experience in the technique of wire myography and the dissection of human small arteries [7]. One vessel from each subject was included and the full experimental protocol was carried out on each vessel. M.B. is an experienced myographer and we are confident that the functional integrity of arteries was not compromised during the dissection and mounting process.

Following 30 min of equilibration, all vessels were ‘normalized’, a procedure to find the lumen diameter with optimized stretch equivalent to 13.3 kPa in vitro, and then set at 90 %. This normalized diameter represents the maximal active force production of the vessel [22].
Vessels were then re-equilibrated for 30 min and then assessed for viability by sequential addition of high KPSS (potassium (6 mM) PSS; iso-osmotically substituted for sodium) either alone or in the presence of $10^{-5}$ M noradrenaline. Vessels were washed with PSS at least three times following the addition of each agent. Once vessels were relaxed to baseline tension, they were left for 5 min before addition of any subsequent stimuli.

**Assessment of resistance arterial contractility**

**Vasoconstrictor responses**

To assess vasoconstrictor responses of subcutaneous resistance arteries, cumulative concentration–response curves (final bath concentrations of $10^{-4}$, $3\times10^{-5}$, $10^{-5}$, $3\times10^{-6}$, $10^{-6}$, $3\times10^{-6}$ and $10^{-5}$ M) to the adrenergic agonist noradrenaline were constructed for each artery. Arteries were left undisturbed for 5 min after addition of each dose to ensure attainment of stable contraction.

**Endothelium-dependent responses**

Endothelial function was assessed in the same tissues by constructing cumulative concentration–response curves to the endothelium-dependent dilator carbachol ($10^{-9}$–$10^{-5}$ M) following pre-constriction with $10^{-5}$ M noradrenaline. Arteries were again left undisturbed for 5 min at each concentration to ensure stable relaxation. To assess the contribution of NO to responses, vasodilator responses to carbachol were then re-examined following incubation (30 min) with $5\times10^{-5}$ M N-nitro-L-arginine (L-NNA; nitro-L-arginine), a well-characterized inhibitor of eNOS (endothelial NOS), widely used to investigate the effects of NO on resistance arterial contractility [7].

**Endothelium-independent responses**

Following assessment of endothelium-dependent responses, arteries were washed at least three times with PSS and tension allowed to return to resting levels. Arteries were then left undisturbed for a further 5 min before being pre-constricted with $10^{-5}$ M noradrenaline and concentration–response curves constructed to the endothelium-independent dilator SNP (sodium nitroprusside) ($10^{-9}$–$10^{-3}$ M).

Contractile responses were recorded on a chart recorder and changes in force (mN/mm) computed using calibration coefficients of the transducers and arterial segment length. Relaxation responses are expressed as a percentage of stable contractile response to noradrenaline ($10^{-5}$ M). EC$_{50}$ (effective concentration for 50% response) values were calculated for individual curves using Prism v3.0.

**Passive properties of resistance arteries**

To determine the passive properties of arteries, mounted arteries were placed on the stage of a Leitz Biomed microscope where they were viewed with a $\times 40$ water-immersion objective. Measurements of wall thickness (mean of right- and left-hand walls) and lumen diameter of arteries mounted under minimal stretch (to prevent thinning of walls due to the force of the wires which is evident at higher stretches) were made via a calibrated graticule as has been described previously [23]. Measurements were taken at three points along each vessel and mean values were determined. Mean wall/lumen ratios were calculated for each vessel.

Lumen diameter, media cross-sectional area and resultant tensions were calculated in response to sequential stretches (carried out during normalization). Stress was then calculated from the equation:

$$S = T \times 10^{-3}/M$$

where $S$ is the stress (in kPa), $M$ is the media thickness (in $\mu$m) (assuming constant media cross-sectional area) and $T$ is the tension (in M/m).

Strain ($Z$) was calculated from the equation

$$Z = (C - C_{\text{min}})/C_{\text{min}},$$

where $C_{\text{min}}$ is the circumference at which morphological measurements were made and $C$ is the circumference at a given stretch.

Stress–strains relationships were calculated for each vessel from the equation

$$\log_{10} S = xZ + y$$

where $x$ and $y$ are the slope and intercept respectively.

The Incremental Young’s modulus was calculated as the slope of the stress–strain relationship at 13.3 kPa and was used as a measure of small arterial wall stiffness. This methodology has been described previously [23].

**Statistical analysis**

Statistical analyses were performed using SPSS version 16.0. The results are expressed as arithmetic means [95 % CI (confidence interval)] or medians (interquartile range). Skewed data were log-transformed and are presented as geometric means (95 % CI). Tests for differences in medians used the Mann–Whitney rank sum test. Tests for differences in proportions used $\chi^2$ tests. ANOVAs were used to test for statistically significant differences between the groups. Although aware of the limitations of multiple regression models with relatively small numbers studied, this approach is useful, and indeed, may be the only way to explore the independent influence of the risk factors over the vascular parameters tested. $P < 0.05$ were considered significant.
Table 1  Characteristics of women in the three different glycaemic groups at follow-up
Values are expressed as means (95 % CI), unless otherwise stated. The glycaemic profile during the previous pregnancy was used to stratify women. *P < 0.05 compared with control. SBP, systolic BP; DBP, diastolic BP; MAP, mean arterial pressure; HbA1c, glycated haemoglobin.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n = 8)</th>
<th>UQ (n = 13)</th>
<th>GDM (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.7 (33.6–37.8)</td>
<td>35.1 (32.8–37.3)</td>
<td>39.3 (36.3–42.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.9 (21.0–22.8)</td>
<td>27.7 (25.1–30.3)*</td>
<td>32.1 (30.9–33.3)*</td>
</tr>
<tr>
<td>Smoking habits (n) (current/ex-/non-smokers)</td>
<td>4/0/4</td>
<td>1/6/6</td>
<td>0/0/8</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>108.2 (103.3–113.2)</td>
<td>118.3 (114.3–122.4)*</td>
<td>114.5 (109.6–119.5)*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>66.1 (61.7–70.4)</td>
<td>74.5 (71.2–77.7)*</td>
<td>73.1 (70.3–76.0)*</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.7 (4.6–4.8)</td>
<td>4.8 (4.5–5.0)*</td>
<td>5.5 (4.9–6.2)*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.8 (4.6–4.9)</td>
<td>5.0 (4.9–5.0)*</td>
<td>5.1 (4.8–5.4)*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.3 (3.9–4.8)</td>
<td>4.4 (4.0–4.8)</td>
<td>4.5 (3.6–5.3)</td>
</tr>
<tr>
<td>TAG (mmol/l)†</td>
<td>0.7 (0.5–0.9)</td>
<td>1.0 (0.9–1.2)*</td>
<td>1.1 (0.8–1.3)*</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.5 (1.0–1.9)</td>
<td>1.5 (1.4–1.7)</td>
<td>1.2 (1.1–1.3)*</td>
</tr>
<tr>
<td>Family history of T2DM (%)</td>
<td>12.5</td>
<td>8.3</td>
<td>37.5*</td>
</tr>
</tbody>
</table>

†Results are expressed as geometric means (95 % CI).

RESULTS

At total of 29 women consented to and underwent biopsies for inclusion in the present study. At follow-up (mean, 22.4 months after index pregnancy), two (25 %) of the GDM group had overt T2DM and one (12.5 %) had IGT, whereas two participants (15.4 %) from the UQ group had IGT. One woman in each group had hypertension. None of these women had been prescribed pharmacological treatment for hypertension or diabetes. Women in the UQ and GDM groups were of similar age and had similar serum cholesterol concentrations, but had significantly higher adiposity, BP and serum TAG (triacylglycerol) concentrations than controls (Table 1). There were no differences in the anthropometric and clinical parameters of this sub-sample of women (mean age, 35.4 years; BMI, 27.3 kg/m²) compared with the larger parent cohort (MMHVH Study, originally recruited for HAPO), except for mean diastolic BP (68.1 ± 8.3 compared with 71.9 ± 8.4 mmHg; P = 0.03).

Impact of glycaemia during pregnancy

Small artery function

Constrictor responses

Contractile responses of arteries from women in the different glycaemic groups were similar with maximal contraction to noradrenaline being 4.0 (2.6–5.5), 3.7 (2.6–4.9) and 4.3 (3.0–5.6) mN/mm [values are means (95 % CI)] for women in the control, UQ and GDM groups respectively (Figure 1). Sensitivity, as shown by EC50 values, was unaffected by glycaemic status during pregnancy [control, 4.90×10⁻⁷ (2.20×10⁻⁷–1.1×10⁻⁶) M; UQ, 2.27×10⁻⁷ (4.10×10⁻⁸–0.30×10⁻⁶) M; and GDM, 5.93×10⁻⁷ (2.10×10⁻⁷–1.7×10⁻⁶) M]. Contractile responses due to depolarization by high potassium were also similar between the different groups [control, 3.3 (2.3–4.3) mN/mm; UQ, 2.8 (2.1–3.5) mN/mm; and GDM, 2.6 (2.1–3.2) mN/mm].

Endothelium-dependent dilation

Endothelium-dependent dilation to carbachol was significantly reduced in arteries from women in both the UQ and GDM groups compared with controls, with a highly significant test for trend across all groups (P = 0.02; Figure 2A). The sensitivity to carbachol (measured as EC50 values) was similar across the groups [control, 2.69×10⁻⁷ (8.8×10⁻⁸–8.2×10⁻⁶) M;...
Glycaemia during pregnancy and later arterial function

Figure 2  Small arterial response to carbachol (A) and carbachol following incubation with l-NNA (B)
(A) Carbachol-induced vasodilator response of isolated subcutaneous arteries isolated at follow-up (mean, 22.4 months after index pregnancy) from patients stratified by glycaemic status during pregnancy by the HAPO study into control (n = 8), UQ (n = 13) and GDM (n = 8). Arteries were pre-constricted with noradrenaline. Values are means ± S.E.M. Differences between means used ANOVA.

P < 0.05. (B) Carbachol-induced vasodilator response of isolated subcutaneous arteries isolated at follow-up (mean 22.4 months after index pregnancy) from patients stratified by glycaemic status during pregnancy by the HAPO study into control (n = 8), UQ (n = 13) and GDM (n = 8) in the presence of the NOS inhibitor L-NNA (5 × 10⁻⁵ M). Arteries were pre-constricted with noradrenaline. Values are means ± S.E.M. 1.00E-09 etc., 1 × 10⁻⁹ etc., UQ, 1.74 × 10⁻⁹ (1.8 × 10⁻⁸–1.7 × 10⁻⁶) M; and GDM, 3.50 × 10⁻⁸ (4.9 × 10⁻⁹–2.5 × 10⁻⁶) M. Inhibition of NOS with l-NNA significantly reduced dilatory responses to carbachol in arteries from control women, but had no significant effect on the responses of arteries from women in the UQ and GDM groups. In the presence of l-NNA, responses to carbachol were similar across the groups (Figures 2B and 3).

Endothelium-independent vasodilation
All arteries exhibited concentration-dependent dilations to the endothelium-independent dilator SNP. Maximal dilation was similar in arteries from women in all of the three sub-groups (Figure 4). Sensitivity to SNP was similar across the groups [control, 7.32 × 10⁻⁸ (2.68 × 10⁻⁹–2.0 × 10⁻⁷) M; UQ, 5.34 × 10⁻⁸ (2.45 × 10⁻⁹–1.16 × 10⁻⁶) M; and GDM, 1.87 × 10⁻⁸ (1.66 × 10⁻⁹–2.11 × 10⁻⁷) M].

Small artery morphology and arterial stiffness
Small artery morphology estimated by wall thickness, lumen diameter, media/lumen ratios and stiffness
Table 2  Morphological characteristics of small arteries at the time of biopsy in each group according to glycaemic status during pregnancy as defined by the HAPO study

Results are means (95 % CI).

<table>
<thead>
<tr>
<th>Morphological features</th>
<th>Control (n = 8)</th>
<th>UQ (n = 13)</th>
<th>GDM (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen diameter (μm)</td>
<td>313.1 (283.5–337.6)</td>
<td>280.5 (259.6–303.1)</td>
<td>319.2 (266.2–382.7)</td>
</tr>
<tr>
<td>Wall thickness (μm)</td>
<td>14.4 (10.7–19.5)</td>
<td>13.8 (11.5–16.5)</td>
<td>11.5 (8.3–16.0)</td>
</tr>
<tr>
<td>Wall/lumen ratio</td>
<td>46.7 (33.6–64.8)</td>
<td>49.1 (40.7–59.3)</td>
<td>31.1 (19.6–49.3)</td>
</tr>
<tr>
<td>Incremental elastic modulus at 13.3 kPa (kPa)</td>
<td>42.5 (29.4–61.5)</td>
<td>42.4 (31.0–58.0)</td>
<td>46.2 (27.0–79.0)</td>
</tr>
</tbody>
</table>

Table 3  Multivariate regression analyses of the relationship between vascular parameters [incremental Young’s modulus (model 1), maximum noradrenaline contraction (model 2), maximum carbachol dilation (model 3) and SNP dilation (model 4)] and cardiovascular risk factors

Results are β-coefficient (per 1 unit change) and 95 % CIs. If fasting glucose was first added to the model instead of total cholesterol, this made no contribution to any of the vessel parameters (lowest P value, P = 0.1).

∗P < 0.05, ∗∗P < 0.01 and ∗∗∗P < 0.001. MAP, mean arterial pressure; NA, noradrenaline.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explanatory variable</td>
<td>Incremental Young’s modulus</td>
<td>Maximum NA contraction</td>
<td>Maximum carbachol dilation (%)</td>
<td>Maximum SNP dilation (%)</td>
</tr>
<tr>
<td>Age (months)</td>
<td>1.93 (–1.15 to 5.01)</td>
<td>0.16 (–0.13 to 0.16)</td>
<td>–0.51 (–1.89 to 0.86)</td>
<td>2.34 (0.16 to 4.51)</td>
</tr>
<tr>
<td>BMI</td>
<td>4.67 (1.05 to 8.29)*</td>
<td>0.15 (0.01 to 0.30)*</td>
<td>–2.72 (–4.17 to –1.28)**</td>
<td>–2.60 (–4.69 to –0.31)*</td>
</tr>
<tr>
<td>MAP</td>
<td>–1.74 (–4.14 to 0.67)</td>
<td>–0.12 (–0.22 to –0.02)*</td>
<td>–0.95 (–1.06 to 0.87)</td>
<td>0.73 (–0.80 to 2.26)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>–2.39 (19.22 to 14.44)</td>
<td>0.10 (–0.62 to 0.83)</td>
<td>–14.4 (–21.3 to 17.50)**</td>
<td>–4.51 (–15.49 to 6.47)</td>
</tr>
</tbody>
</table>

(Young’s modulus) were similar across all three subgroups and were not influenced by glycaemic status during pregnancy (Table 2).

**Impact of serum cholesterol but not glycaemia at the time of biopsy on arterial structure and function**

Multiple regression analysis was performed using each vessel’s characteristic in turn as the dependent variable, adjusted for age, BMI and mean arterial pressure. If current fasting plasma glucose at the time of biopsy, was added next, it had no influence on any of the parameters. Then if serum total cholesterol levels, also at the time of biopsy, was substituted, its result on endothelial function was clearly additional (Table 3). In all the models, the impact of adiposity, measured as BMI, was clear; it directly influenced arterial stiffness (model $R^2 = 0.21$) and contractile response to noradrenaline ($R^2 = 0.25$), but was inversely related to both maximum endothelium-dependent ($R^2 = 0.64$) and endothelium-independent ($R^2 = 0.30$) dilation.

**DISCUSSION**

Pregnancy complicated by GDM is associated with an adverse cardiovascular risk in later life [4]. The results of the present study show that women who had GDM and those who exhibited marginally raised glycaemia during pregnancy (our UQ group) had normal vascular structure and stiffness, but clearly detectable impaired endothelium-dependent function at 2 years of follow-up. Thus even mild hyperglycaemia during pregnancy has long-term effects on endothelial dysfunction of resistance arteries. The endothelial impairment was not associated with current glycaemia, but was associated with BMI.

Endothelial dysfunction has previously been demonstrated in systemic and placental resistance arteries of women with GDM at delivery [20,21] and in a mouse model of GDM 6–7 months after delivery [24]. Flow-mediated dilation of the brachial artery has also been demonstrated 3–6 months postpartum in both obese and non-obese women who have suffered from GDM [25]. In the present study, we show that 2 years after delivery, endothelial dysfunction was also evident in resistance arteries from women with GDM during pregnancy. This effect was probably due to reduced NO bioavailability, as has been demonstrated previously in placental vessels from women with GDM at delivery [21]. The underlying mechanisms for this are unknown, but it is interesting to note that reduced postpartum endothelium-dependent dilation previously reported in a mouse model of GDM was associated with increases in superoxide production [24]. Further studies are required to determine whether
this contributes to the dysfunction observed in the present study. It should be noted, however, that there are numerous ways whereby NO bioavailability may be modulated. Current diagnostic criteria for GDM are a matter of on-going debate, as they are not based on maternal and perinatal outcome. Results from the HAPO study show that both maternal and fetal outcomes are continuously related to hyperglycaemia during pregnancy, and that glycaemia below that currently defined as GDM may have an an impact on outcome [19].

The results of the present study show for the first time that postpartum endothelial function is also influenced by mild glycaemia during pregnancy supporting this notion. Regression analysis suggested that glycaemic status at follow-up was not an independent influence on endothelial responses to carbachol, but there was a relationship with BMI and cholesterol, known CVD risk factors [25,26]. The reduced dilatory responses observed to carbachol were not attributable to a reduced sensitivity of smooth muscle to NO, as responses to SNP were unaffected by glycaemic status during pregnancy.

Alterations in small vessel structure and distensibility have been found previously in patients with established T2DM and are also believed to contribute to increased cardiovascular risk. Increased arterial media thickness has been reported in established T1DM (Type 1 diabetes mellitus) [27] and T2DM [28], as in those at high risk of diabetes [29,30]. These remain unaltered in T1DM of short duration (3–4 years) [31]. The effect of glycaemia during pregnancy on the postpartum structure of resistance arteries has not been studied previously. In the present study, we show that glycaemic status during pregnancy did not influence either wall structure or distensibility postpartum. The reason for this is unclear, but proliferative [32] and anti-proliferative [33] effects of hyperglycaemia on VSMCs (vascular smooth muscle cells) may contribute. However, more likely in our view is that, glycaemia may come secondary to vasocrine signals from fat in determining vessel function.

Recently, it has become clear that perivascular fat plays a key role in maintaining the integrity of vascular function in related small (arterial resistance) vessels. Apart from our earlier work [7], an impressive body of evidence shows the impact of vessel wall re-modelling on both their (endothelium-dependent) function and prognosis longer-term for affected patients [10]. Earlier findings showed the impact of large vessel stiffening across the glucose tolerance spectrum [2]. Since a previous hypothesis [34], with effects on capillary bed recruitment in relation to body fat distribution [35], work in this laboratory has implicated quality of fat and its infiltration with inflammatory macrophages in determining small vessel function [36,37]. Circulating inflammatory markers were related in a population study to arginine availability, affecting endothelial function [38], found compromised in the present study in our sample of ‘UQ’ potentially pre-diabetic women. How total body fat and its distribution affect blood vessel function and whether this is integral to what becomes T2DM is a topic of on-going work.

**Implications**

In the present study, we have found that postnatal endothelial dysfunction occurs in small arteries of overweight women with both prior GDM and with glycaemic levels below that used previously to define GDM, the latter in the absence of current diabetes. Results from the HAPO study [19] suggest that hyperglycaemia during pregnancy, at levels considered previously to be below those defining GDM, are associated with adverse fetal outcome. Whether plasma glucose is the primary cause of such pathology or other causal pathways such as those related to adipose tissue may initiate vascular impairment (as almost all gestational glucose intolerance occurs in overweight women) remains unclear. A limitation of our present study is the relatively small number of participants, as expected with the invasive nature of subcutaneous biopsies as well as the young age of the participants. These results suggest that early vascular pathology, which may still be reversible, is detectable very early in women at risk of decline into T2DM, before their current glycaemia becomes elevated.

**AUTHOR CONTRIBUTION**

Moulnath Banerjee, Simon Anderson, Rayaz Malik, Clare Austin and Kennedy Cruickshank participated in the study concept and design, acquisition of the data, interpretation of the data and critical revision of the paper. All authors provided input to the paper, and none of the authors had any conflict of financial or personal interest.

**ACKNOWLEDGEMENT**

The Wellcome Trust Clinical Research Facility Manchester provided measurement and laboratory support.

**FUNDING**

This work was supported by a British Heart Foundation Clinical Training Fellowship (to M. B.). S.A. is currently an National Institute of Health Research Academic Clinical Fellow in Cardiology

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Received 21 January 2011/27 June 2011; accepted 11 July 2011
Published as Immediate Publication 11 July 2011, doi:10.1042/CS20110033