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

Moulinath BANERJEE  
Simon G ANDERSON  
Rayaz A MALIK  
Clare E AUSTIN<sup>‡</sup>  
J KennedyCRUICKSHANK<sup>‡\*\*</sup>

<sup>‡</sup>Joint senior authors

Authors' Affiliation:

Cardiovascular Sciences Research Group, University of Manchester, UK

Address:

Core Technology Facility  
46 Grafton Street  
University of Manchester  
Manchester, UK  
M13 9NT  
Tel: 0161-275 1191  
Fax: 0161-2751183

Correspondence to:

e-mail: [kennedy.cruickshank@manchester.ac.uk](mailto:kennedy.cruickshank@manchester.ac.uk)

**Short title:** Glycaemia during pregnancy and later arterial function

**Key words:** Endothelial function, gestational diabetes, adiposity, small arteries, women

**ABSTRACT**

Gestational diabetes (GDM) is associated with later adverse cardiovascular risk. This study examined the relationship between glycaemia during pregnancy **and small artery function and structures some 2 years post-partum.**

Women were originally enrolled in the HAPO study from which they were classified by their glycaemic distribution during pregnancy as controls (in the lower half of the distribution), upper quartile (UQ -in the upper quartile of the glycaemic distribution) or having had overt GDM. Sub-cutaneous arteries from 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 to carbachol was impaired in arteries from both GDM (43.3%, n=8, p=0.01) and UQ (51.7%, n=13, p=0.04) women despite generally 'normal' current glycaemia (controls (72.7%, n=8). Inhibition of nitric oxide synthase ((NOS) significantly reduced maximum endothelium-dependent dilatation 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 & UQ women when compared with the control group. Multiple regression analysis suggested that BMI 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.

## INTRODUCTION:

Type 2 diabetes (T2DM) is characterised by a 3-4 fold excess of cardiovascular mortality and is associated with abnormalities in both the macro and microcirculation[1]. **Mortality also increases with progressive glucose intolerance**[2-4]. Small artery structure and function is 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 impaired glucose tolerance(IGT)[12, 13] suggesting that vascular abnormalities may exist in subjects at risk of developing T2DM.

Hyperglycaemia in pregnancy or gestational diabetes (GDM), that is hyperglycaemia with onset or first recognition during pregnancy[14], is common and evident in around 2% of pregnancies[15]. Women with GDM have >9 fold risk of developing overt T2DM when compared to women who had normal pregnancy glycaemia, with fasting blood glucose during pregnancy having the most predictive value, together with increased BMI[16-18]. GDM is also associated with an increased risk of developing cardiovascular disease in later life[4]. Recent evidence from The Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study 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] while impaired nitric oxide (NO) 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 post-partum when elevated glycaemia is no longer evident. It is also unclear whether women with mild glucose intolerance during pregnancy exhibit microvascular disorders post-partum. This study was designed to test the impact of glycaemia during pregnancy on resistance artery structure and function post-partum.

## METHODS:

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

The enrolled women were invited for 2-yearly follow-up visits where detailed anthropometry, blood pressure (BP) - using the validated semi-automatic Omron 705 CP), fasting lipid profile, fasting blood glucose (or if possible standard 75 gram (OGTT) were undertaken. We recruited a sub-group of 29 women who gave informed consent to undergo gluteal fat biopsy at approximately 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 2.5-4.0cm of the top of the natal cleft and 5cm from the midline on either side of the gluteal region. An elliptical incision was made and a block of subcutaneous fat tissue (2 x 0.5 x 1cm) with overlying skin was removed and the site sutured following haemostasis. The biopsy was immediately placed in ice-cold physiological saline solution (PSS) ( $10^{-3}$ M) NaCl 119, KCl 4.7,  $MgSO_4 \cdot 7H_2O$  1.2,  $NaHCO_3$  25,  $KH_2PO_4$  1.17,  $K_2EDTA$  0.03, glucose 5.5,  $CaCl_2 \cdot 2H_2O$  2.5, pH 7.4), and immediately transferred to the laboratory. Within 1 hour small arteries, diameter 180-400  $\mu$ m, were dissected, mounted on a wire myograph between 40  $\mu$ m diameter wires (Dual Wire Myograph System - 410, Danish Myo Technology A/S), bathed in PSS at 37°C, and gassed with 5%  $CO_2$ /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 was carried out by MB in the laboratory of CA 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. MB 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 mins equilibration, all vessels were 'normalised' – a procedure to find the lumen diameter with optimised stretch equivalent to 13.3 kPa *in vivo*, and then set at 90%. This normalised diameter represents maximal active force production of the vessel[22]. Vessels were then re-equilibrated for 30 minutes then assessed for viability by sequential addition of high potassium ( $6 \times 10^{-3}$  M) physiological salt solution (KPSS) (iso-osmotically substituted for Na) either alone or in the presence of  $10^{-5}$  M Noradrenaline. Vessels were washed with PSS at least 3 times following addition of each agent. Once vessels were relaxed to baseline tension, they were left for 5 minutes before addition of any subsequent stimuli.

### Assessment of resistance arterial contractility

#### a) Vasoconstrictor responses

To assess vasoconstrictor responses of subcutaneous resistance arteries, cumulative concentration responses curves (final bath concentrations of  $10^{-9}$  M,  $3 \times 10^{-9}$  M,  $10^{-8}$  M,  $3 \times 10^{-8}$  M,  $10^{-7}$  M,  $3 \times 10^{-7}$  M,  $10^{-6}$  M,  $3 \times 10^{-6}$  M and  $10^{-5}$  M) to the adrenergic agonist

Noradrenaline were constructed for each artery. Arteries were left undisturbed for 5 minutes after addition of each dose to ensure attainment of stable contraction.

*b) 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 minutes at each concentration to ensure stable relaxation. To assess the contribution of nitric oxide (NO) to responses, vasodilator responses to carbachol were then re-examined following incubation (30 minutes) with  $5 \times 10^{-5}$  M LNNA (N( $\omega$ )-Nitro-L-Arginine, a well characterised inhibitor of endothelial NOS, widely used to investigate the effects of NO on resistance arterial contractility[7].

*c) Endothelium independent responses*

**Following assessment of endothelium dependent responses, arteries were washed at least 3 times with PSS and tension allowed to return to resting levels.** Arteries were then left undisturbed for a further 5 minutes before being pre-constricted with  $10^{-5}$  M Noradrenaline and concentration-response curves constructed to the endothelium-independent dilator Sodium nitroprusside (SNP) ( $10^{-9}$ – $10^{-5}$ 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 were expressed as percentage of stable contractile response to Noradrenaline ( $10^{-5}$  M).  $EC_{50}$ s (effective concentration for 50% response) 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 x40 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 previously described[23]. Measurements were taken at three points along each vessel and mean values 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 normalisation). Stress was then calculated from:

$$S = T10^{-3}/M$$

Where S = stress (kPa), M = media thickness ( $\mu$ m) (assuming constant media cross sectional area) and T = tension ( $Nm^{-1}$ ).

Strain (z) was calculated from:

$$Z = (C - Cmin)/Cmin$$

Where Cmin = circumference at which morphological measurements were made and C= circumference at a given stretch.

Stress-strains relationships were calculated for each vessel from:

$$\text{Log}_{10}S = xZ + y$$

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 previously described[23].

### Statistical analysis

Statistical analyses were performed using SPSS version 16.0. **The data are expressed as arithmetic means and 95% confidence intervals (CI) or median (IQR). Skewed data were log transformed and presented as geometric means (95%CI). Tests for differences in medians used the Mann Whitney rank sumtest. Tests for differences in proportions used Chi squared tests. ANOVA were used to test for statistically significant differences between the groups. While aware of the limitations of multiple regression models with relatively small numbers studied, this approach is useful, indeed may be the only way, to explore the independent influence of the risk factors over the vascular parameters tested. P-values < 0.05 were considered significant.**

### RESULTS

29 women consented to, and underwent biopsies for inclusion in the present study. At follow up (mean 22.4 months after index pregnancy), 2 (25%) of the GDM group had overt type 2 diabetes, and one (12.5%) had IGT, while 2 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, had similar serum cholesterol concentrations, but had significantly higher adiposity, BP and serum triglyceride 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/m<sup>2</sup>) compared to the larger parent cohort (Manchester Mothers' Heart and Vascular Health Study, originally recruited for HAPO), except for mean diastolic BP (68.1±8.3 vs. 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 (mean (95% CI) 4.0(2.6-5.5), 3.7(2.6-4.9) and 4.3(3.0-5.6) mN/mm for women in control, UQ and GDM groups

respectively [Fig. 1]. Sensitivity, as evidenced by  $EC_{50}$  values, was unaffected by glycaemic status during pregnancy [Control:  $4.90 \times 10^{-7}$  ( $2.20 \times 10^{-7}$ - $1.1 \times 10^{-6}$ M), UQ:  $2.27 \times 10^{-7}$  ( $4.10 \times 10^{-8}$ - $0.30 \times 10^{-6}$ M) and GDM:  $5.93 \times 10^{-7}$  ( $2.10 \times 10^{-7}$ - $1.7 \times 10^{-6}$ M)]. Contractile responses due to depolarization by high potassium were also similar between the different groups [Control: 3.3 (2.3-4.3), UQ 2.8(2.1-3.5) 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 UQ and GDM groups compared with controls, with a highly significant test for trend across all groups ( $p = 0.02$ ; Fig. 2A). The sensitivity to carbachol (measured as  $EC_{50}$ ) was similar across groups (Controls:  $2.69 \times 10^{-7}$  ( $8.8 \times 10^{-9}$  to  $8.2 \times 10^{-6}$  M), UQ:  $1.74 \times 10^{-7}$  ( $1.8 \times 10^{-8}$  to  $1.7 \times 10^{-6}$  M) and GDM:  $3.50 \times 10^{-8}$  ( $4.9 \times 10^{-10}$  to  $2.5 \times 10^{-6}$  M)). Inhibition of NOS with LNA significantly reduced dilatory responses to carbachol in arteries from control women but had no significant effect on responses of arteries from women in the UQ and 'GDM' groups. **In the presence of LNA responses to carbachol were similar across the groups [Fig. 2B & 3].**

#### *Endothelium-independent vasodilatation:*

All arteries exhibited concentration-dependent dilations to the endothelium-independent dilator SNP. Maximal dilation was similar in arteries from women of all three sub-groups [Fig 4]. Sensitivity to SNP was similar across groups [Controls:  $7.32 \times 10^{-8}$  ( $2.68 \times 10^{-9}$  to  $2.0 \times 10^{-6}$ M), UQ:  $5.34 \times 10^{-8}$  ( $2.45 \times 10^{-9}$  to  $1.16 \times 10^{-6}$ M) and GDM:  $1.87 \times 10^{-8}$  ( $1.66 \times 10^{-9}$  to  $2.11 \times 10^{-7}$ M)].

#### *Small artery morphology and arterial stiffness:*

Small artery morphology estimated by wall thickness, lumen diameter, media: lumen ratio and stiffness (Young's modulus) were similar across all three subgroups and was not influenced by glycaemic status during pregnancy (Table 2).

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

Multiple regression analysis was performed using each vessel characteristic in turn as the dependent variable, adjusted for age, BMI, 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 Nor-adrenaline ( $R^2 = 0.25$ ) 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 this study show that women who had GDM and those which 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 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 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 post-partum in both obese and non-obese women who have suffered from GDM[25]. Here we show that 2 years after delivery, endothelial dysfunction was also evident in resistance arteries from women with GDM during pregnancy. This effect was likely due to reduced NO bioavailability, as has previously been demonstrated 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 post-partum 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 HAPO show that both maternal and fetal outcomes are continuously related to hyperglycaemia during pregnancy, and that glycaemia below that currently defined as GDM may impact on outcome[19].

The results of this study show for the first time that post-partum 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 response to carbachol but there was a relation 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 type-1[27] and type-2 [28] diabetes, 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 post-partum structure of resistance arteries has not previously been studied. Here, we show that glycaemic status during pregnancy did not influence either wallstructure or distensibility post-partum. The reason for this is unclear but proliferative[32] and anti-proliferative[33] effects of hyperglycaemia on vascular smooth

muscle cells may contribute. **However, more likely in our view, 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 (endothelial-dependent) function and prognosis longer-term for affected patients [10]. Earlier data 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 here 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 T2 diabetes is a topic of ongoing work.

**Implications:** We found that post-natal endothelial dysfunction occurs in small arteries of overweight women with both prior GDM and with glycaemic levels below that used to define GDM previously, the latter in the absence of current diabetes. Results from the HAPO study [19] suggest that hyperglycaemia during pregnancy, at levels previously considered below those defining GDM, are associated with adverse foetal 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 study is the relatively small number of participants, as expected with the invasive nature of subcutaneous biopsies as well as 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 Type 2 diabetes, before their current glycaemia becomes elevated.

#### **Author Contributions**

M.B., C.E.A., S.G.A., R.A.M. and J.K.C. participated in the study concept and design, acquisition of data, interpretation of data and critical revision of the manuscript. All authors provided input to the manuscript, and none of the authors had any conflict of financial or personal interest. S.G.A. is currently an NIHR Academic Clinical Fellow in Cardiology.

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### Figure legends

**Figure 1.** Contractile responses to Noradrenaline of isolated subcutaneous small arteries isolated at follow up (mean 22.4 months after index pregnancy) from patients stratified by glycaemic status during pregnancy by HAPO into control (n=8), UQ (upper quartile; n=13) and GDM (gestational diabetes; n=8). Differences between means used ANOVA. \*P<0.05

### Figure 2.

**A.** Carbachol induced vasodilator response (Mean +/- SEM) of isolated subcutaneous arteries isolated at follow up (mean 22.4 months after index pregnancy) from patients stratified by glycaemic status during pregnancy by HAPO into control (n=8), UQ (upper quartile; n=13) and GDM (gestational diabetes; n=8). Differences between means used ANOVA. \*P<0.05. Arteries were pre-constricted with Noradrenaline.

**B.** Carbachol induced vasodilator response (Mean +/- SEM) of isolated subcutaneous arteries isolated at follow up (mean 22.4 months after index pregnancy) from patients stratified by glycaemic status during pregnancy by HAPO into control (n=8), UQ (upper quartile) (n=13) and GDM (gestational diabetes)(n=8) in the presence of the nitric oxide synthase inhibitor LNNA (N( $\omega$ )-Nitro-L-Arginine:  $5 \times 10^{-5}$  M/l). Arteries were pre-constricted with Noradrenaline.

**Figure 3.** Maximum carbachol induced vasodilator response (Median & upper and lower quartile in box & whisker plot) of isolated subcutaneous arteries from the different groups of women in the presence and absence of LNNA. L-NNA significantly reduced maximum dilation in arteries from control women but had no effect on those isolated from women in UQ and GDM groups.

**Figure 4.** Sodium nitroprusside (SNP) induced vasodilator response (Mean +/- SEM) of isolated subcutaneous arteries isolated at follow up (mean 22.4 months after index pregnancy) from patients stratified by glycaemic status during pregnancy by HAPO into control (n=8), UQ (upper quartile) (n=13) and GDM (gestational diabetes)(n=8). Arteries were pre-constricted with Noradrenaline.

**Table 1.** Characteristics of women in the three different glycaemic groups at follow up. The glycaemic profile during the previous pregnancy was used to stratify women (see Methods).

	Control (n=8)			UQ (n=13)			GDM (n=8)		
	Mean	95% CI		Mean	95% CI		Mean	95% CI	
Age (years)	<b>35.7</b>	33.6	37.8	<b>35.1</b>	32.8	37.3	<b>39.3</b>	36.3	42.2
BMI (kg/m <sup>2</sup> )	<b>21.9</b>	21.0	22.8	<b>27.7*</b>	25.1	30.3	<b>32.1*</b>	30.9	33.3
Smoking habits (n for current / ex-/ non-smokers)	<b>4/0/4</b>			<b>1/6/6</b>			<b>0/0/8</b>		
SBP (mm Hg)	<b>108.2</b>	103.3	113.2	<b>118.3*</b>	114.3	122.4	<b>114.5*</b>	109.6	119.5
DBP (mm Hg)	<b>66.1</b>	61.7	70.4	<b>74.5*</b>	71.2	77.7	<b>73.1*</b>	70.3	76.0
Fasting glucose (mmol/l)	<b>4.7</b>	4.6	4.8	<b>4.8</b>	4.5	5.0	<b>5.5*</b>	4.9	6.2
HbA1c (%)	<b>4.8</b>	4.6	4.9	<b>5.0</b>	4.9	5.0	<b>5.1*</b>	4.8	5.4
Total cholesterol (mmol/l)	<b>4.3</b>	3.9	4.8	<b>4.4</b>	4.0	4.8	<b>4.5</b>	3.6	5.3
Triglycerides (mmol/l) <sup>†</sup>	<b>0.7</b>	0.5	0.9	<b>1.0*</b>	0.9	1.2	<b>1.1*</b>	0.8	1.3
HDL-cholesterol (mmol/l)	<b>1.5</b>	1.0	1.9	<b>1.5</b>	1.4	1.7	<b>1.2*</b>	1.1	1.3
Family history of type 2 DM (%)	<b>12.5</b>	-	-	<b>8.3</b>	-	-	<b>37.5*</b>	-	-

<sup>†</sup>Data expressed as geometric mean. BMI- Body mass index, SBP- Systolic blood pressure, DBP- Diastolic blood pressure and **MAP - Mean arterial pressure**. P values for differences between mean (\*p<0.05).

**Table 2.** Morphological characteristics of small arteries at the time of biopsy in each group according to glycaemic status during pregnancy as defined by HAPO.

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

Data are means and 95%CI.

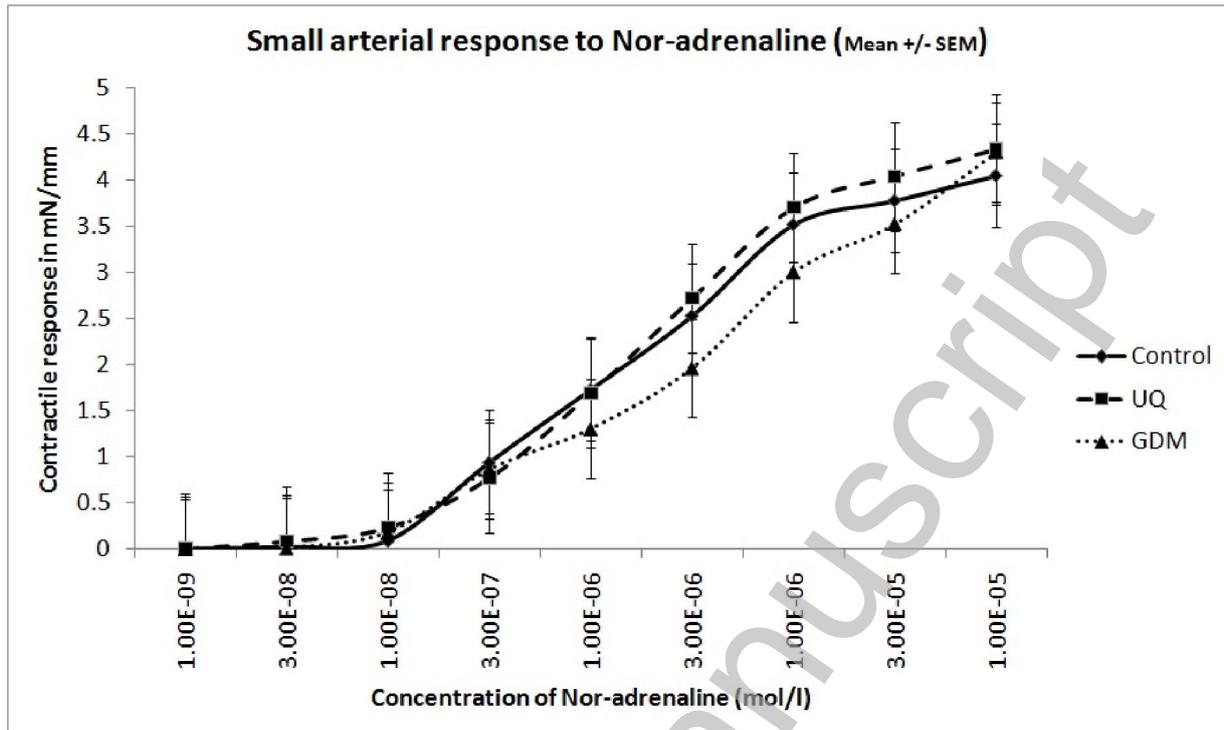
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Table 3. Multivariate regression analyses of relation between vascular parameters: Incremental Young's modulus (model 1), Maximum Noradrenalin (NA) contraction (model 2), Maximum carbachol (model 3), and SNP dilatation (model 4) and cardiovascular risk factors<sup>‡</sup>

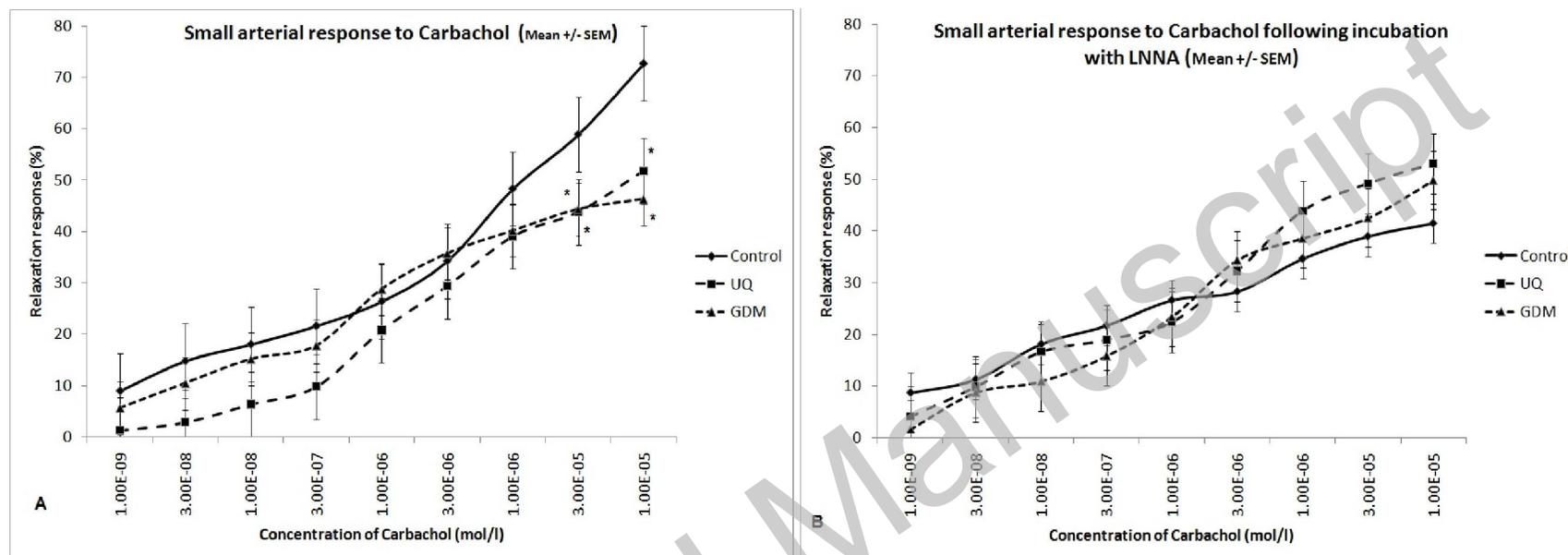
Explanatory variables	Model 1		Model 2		Model 3		Model 4	
	Dependent Variables							
	Incremental Young's Modulus		Max. NA contraction		Max. Carbachol Dilatation (%)		Max. SNP Dilatation (%)	
	$\beta$	95% CI	$\beta$	95% CI	$\beta$	95% CI	$\beta$	95% CI
Age (months)	1.93	-1.15 to 5.01	0.16	-0.13 to 0.16	-0.51	-1.89 to 0.86	<b>2.34</b>	<b>0.16 to 4.51*</b>
BMI	<b>4.67</b>	<b>1.05 to 8.29*</b>	<b>0.15</b>	<b>0.01 to 0.30*</b>	<b>-2.72</b>	<b>-4.17 to -1.28***</b>	<b>-2.60</b>	<b>-4.69 to -0.31*</b>
MAP	-1.74	-4.14 to 0.67	<b>-0.12</b>	<b>-0.22 to -0.02*</b>	-0.95	-1.06 to 0.87	0.73	-0.80 to 2.26
Total Cholesterol	-2.39	19.22 to 14.44	0.10	-0.62 to 0.83	<b>-14.4</b>	<b>-21.3 to 17.50***</b>	-4.51	-15.49 to 6.47

Data are  $\beta$  coefficient (per 1 unit change) and 95% confidence intervals. P values for independent variables in each model(\*P<0.05; \*\*P<0.01, \*\*\*P<0.001). Independent variables: Age, BMI - Body mass index, MAP - Mean Arterial pressure. <sup>‡</sup>If fasting glucose was first added to the model *instead of* total cholesterol, this made no contribution to any of the vessel parameters above (lowest p value, p=0.1).



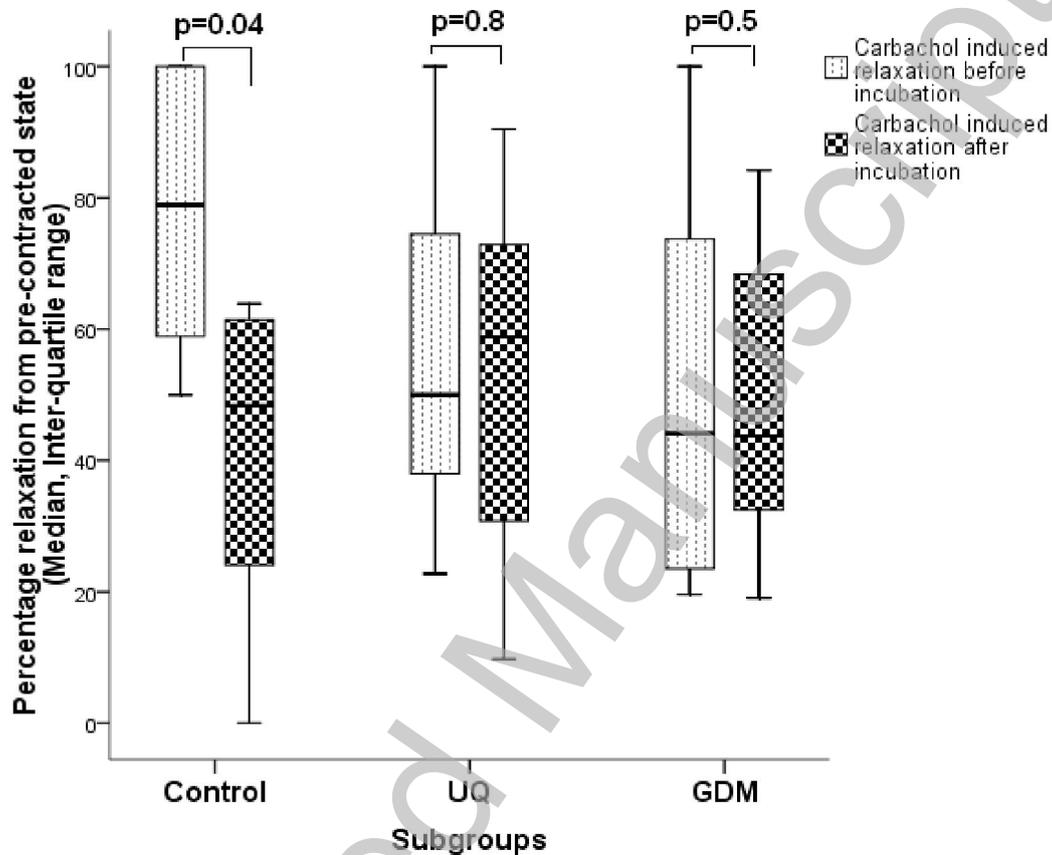
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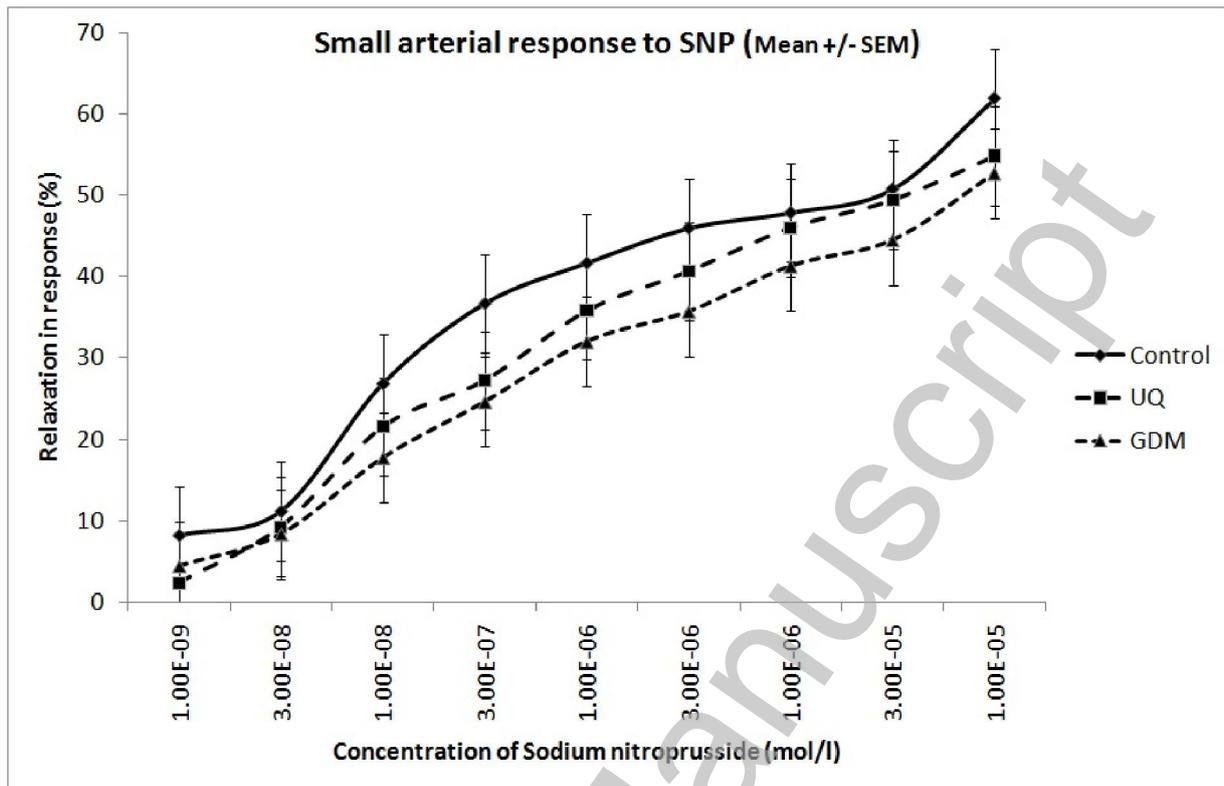


**Figure 2. A.** Carbachol induced vasodilator response (Mean +/- SEM) of isolated subcutaneous arteries isolated at follow up (mean 22.4 months after index pregnancy) from patients stratified by glycaemic status during pregnancy by HAPO into control (n=8), UQ (upper quartile) (n=13) and GDM (gestational diabetes)(n=8). Arteries were pre-constricted with Noradrenaline. Differences between means used ANOVA. \*P<0.05.

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