

Enhancement of Endothelial Function by Pregnancy

Inadequate response in women with type 1 diabetes

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OBJECTIVE — Pregnant women with type 1 diabetes have a substantially increased risk of vascular complications. Our aim was to study vascular function and metabolic and inflammatory risk factors during the antenatal and postpartum periods in women with type 1 diabetes compared with healthy control subjects.

RESEARCH DESIGN AND METHODS — A total of 15 women with diabetes and 30 control subjects were recruited in the third trimester of pregnancy. Of these women, 9 case subjects and 16 control subjects were reinvestigated in the postnatal period. Blood samples were collected and microvascular skin perfusion was assessed in vivo using laser Doppler imaging and iontophoretic administration of endothelial-dependent (acetylcholine [ACH]) and endothelial-independent (sodium nitroprusside [SNP]) vasodilators.

RESULTS — Microvascular responses in both control subjects (ACH, $P = 0.018$; SNP, $P = 0.01$) and diabetic women (ACH, $P = 0.029$; SNP, $P = 0.105$) were better during pregnancy than in the postnatal period, although responses in women with diabetes were significantly inferior to those in control subjects during both periods (all $P < 0.001$, two-way ANOVA). This dysfunction existed despite similar lipoprotein profiles. The difference in vascular responsivity between case and control subjects was significantly attenuated by adjustment for differences in HbA_{1c} but not C-reactive protein concentrations in the two groups.

CONCLUSIONS — Pregnancy enhances microvascular function, but in women with diabetes, such improvements are insufficient to attain responses seen in healthy nonpregnant women. This suggests a persistent vascular defect in young women with type 1 diabetes that may contribute to adverse pregnancy outcome. Our data suggest a role for the chronic effects of hyperglycemia in the impaired vascular responsiveness in such women.

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In the mother with type 1 diabetes, pregnancy is associated with a significant risk of poor maternal outcome. The frequency of maternal vascular disease, such as preeclampsia, is 12–20% (1,2), and this risk increases in relation to

severity of diabetes, particularly preexisting microalbuminemia (1). Currently, mechanisms for these associations are poorly understood.

A plethora of data exists concerning mechanisms of vascular disease in non-

pregnant type 1 diabetes (3). One hypothesis involves altered microvascular reactivity, particularly impairment of the nitric oxide system. However, studies of endothelial function in type 1 diabetes have produced conflicting results (4). In pregnant women with type 1 diabetes, impaired endothelial function has been demonstrated using ex vivo techniques (5). However, currently, prospective data examining in vivo vascular function, both during and after pregnancy, are absent.

The aim of this investigation was to examine microvascular function using a well-tolerated, in vivo technique during the antenatal and postpartum periods in women with type 1 diabetes and in healthy control subjects. We also examined whether conventional (lipids) or novel (inflammatory parameters) vascular risk factors were perturbed in such patients. Our hypotheses were that 1) microvascular function would be impaired in nonpregnant diabetic women as compared with healthy control subjects, 2) despite pregnancy-induced improvements in both groups, the impairment in vascular function in women with diabetes would persist; and 3) vascular risk factors, other than chronic glycemia, may contribute to such dysfunction.

RESEARCH DESIGN AND METHODS

The study was divided into two portions comparing diabetic women and healthy control subjects during the antenatal and postnatal periods. All work was performed according to the Declaration of Helsinki, approval was granted by the institutional ethics committee, and all patients gave written informed consent.

I. A total of 15 women with type 1 diabetes (no microscopic proteinuria, retinopathy, or ketonuria) and 30 normal pregnant control subjects were recruited consecutively from antenatal clinics in the third trimester of pregnancy. Women participating in the study were healthy and normotensive with no significant medical history, such as peripheral vascu-

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Abbreviations: ACH, acetylcholine; AUC, area under the perfusion time curve; CRP, C-reactive protein; SNP, sodium nitroprusside; VCAM, vascular cellular adhesion molecule.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

lar abnormalities or dermatological diseases, and no relevant complications of pregnancy.

II. The above groups were invited to return at least 4 months after delivery. Of the original group, 9 of the diabetic subjects and 16 of the control participants attended for postpartum assessment. Exactly the same protocol was followed for the postpartum and antenatal assessments.

Clinical and laboratory measurements

Control subjects attended for participation in the study after an overnight fast and underwent testing between 9:00 and 11:00 A.M.

Women with type 1 diabetes were seen before the midday meal and were instructed to eat a light breakfast and take their morning insulin before 7:00 A.M. Examination was performed after noon, before the midday insulin administration. This approach minimized the risk of hypoglycemic episodes in outpatients in whom glycemic control is tight. All women were examined after at least a 5-h fast. Maximum detrimental effects on endothelial function in response to a fat load are observed 2 h after ingestion, with effects lasting up to a maximum of 4 h (6).

Blood pressure was recorded by the same operator using a standard mercury sphygmomanometer and an appropriately sized cuff, and diastolic pressure was recorded as Korotkoff phase V. Blood was collected for lipid profile (reagent kits 704121 and 704113; Boehringer, East Sussex, U.K.), HbA_{1c} (high-performance liquid chromatography, HA8121 analyzer; Menarini Diagnostics, Berkshire, U.K.), soluble vascular cellular adhesion molecule (VCAM) quantification (Parameter Human sVCAM-1 Immunoassay; R&D Systems, Oxon, U.K.), and C-reactive protein (CRP) (double antibody sandwich enzyme-linked immunoassay with rabbit anti-human CRP and peroxidase-conjugated rabbit anti-human CRP, DK-2600; Dako, Glostrup, Denmark). Standard curves for CRP measurement were linear up to 5 mg/l and logarithmic thereafter. The interassay and intra-assay coefficients of variation were <7% across the range of measured results. In the diabetic women, random capillary glucose was recorded before testing (One II One glucometer and reagent strips; Lifescan, Buckinghamshire, U.K.).

Perfusion measurements

No caffeine-containing drinks or over-the-counter medications were consumed by participants before testing. During a 10-min period of acclimatization in a temperature-controlled room, the women lay in a semirecumbent position with the flexor aspect of the forearm exposed on an armrest. Noninvasive measurement of skin perfusion was performed by laser Doppler imaging (LDI) using an instrument equipped with a red laser (wavelength 633 nm, power 1 mW, beam diameter 1 mm; Moor Instruments, U.K.), as previously described in detail (7). A total of 20 repetitive scans were taken; the first was a control (precurrent administration), which was followed by an incremental iontophoretic current protocol (14 scans) and five additional scans with no current administration. An assessment of the overall response to drugs was obtained by taking the area under the perfusion time curve (AUC).

Iontophoresis

This technique is based on the principle that a charged molecule migrates across the skin under the influence of an applied electrical field. Iontophoresis of acetylcholine (ACH) examines endothelial-dependent function whereas delivery of sodium nitroprusside (SNP), a nitric oxide (NO) donor, is used as an endothelium-independent control. A total of 2.5 ml of 1% ACH (Sigma, St. Louis, MO) was introduced to the anodal chamber while 2.5 ml of 1% SNP (Sigma) was placed in the cathodal chamber. The vehicle for these drugs was 0.5% NaCl. The experiment was also performed with vehicle alone as a control experiment (results not shown). The mean (\pm SD) between-day coefficient of variation for the ACH response was $6.4 \pm 3.3\%$, whereas the within-day, between-site coefficient of variation measured in both forearms was $8.9 \pm 5.3\%$.

Statistical analyses

Dose-response curves were expressed as means \pm SE, and comparisons were by two-way ANOVA. Other data are expressed as medians with the interquartile range as the measure of variability. For these data, the Mann-Whitney *U* test for comparisons between groups and Wilcoxon's test for comparison within groups were used as appropriate. Linear relationships between variables were examined

using the Pearson correlation coefficient after normalizing skewed variables by log transformation. Regression analysis was used to test for independent associations between variables.

RESULTS

I. Antenatal diabetic versus control

Demographic characteristics are shown in Table 1. Although all women were tested in the third trimester, gestation differed by three weeks between the two groups. Despite excellent glycemic control, the birth centile was significantly greater in the diabetic births: 85 (64–98) vs. 50 (25–75) ($P = 0.0067$).

Endothelial-dependent vasodilatation.

There was a significant difference in dose-dependent perfusion responses to ACH between diabetic and control groups ($P < 0.001$). Median response to ACH (as defined by corrected AUC) was significantly lower in the diabetic group: 8,176 (3,618–11,434) vs. 13,235 (9,083–16,809) PUM Ω /min ($P = 0.0017$). This difference remained after adjusting for differences in gestation between the two groups ($T = -2.39$, $P = 0.022$).

Endothelial-independent vasodilatation.

Dose-dependent perfusion responses to SNP were also lower in the pregnant diabetic women as compared with healthy control subjects ($P = < 0.001$). Median vascular response to SNP (AUC) was also significantly less in the diabetic women: 6,339 (2,712–7,568) vs. 9,130 (7,462–11,489) PUM Ω /min ($P = 0.0007$). This difference remained after adjusting for gestation ($T = -2.83$, $P = 0.007$).

Plasma analyses. Results of plasma measurements are shown in Table 2. The concentration of HbA_{1c} was greater in the women with diabetes than in the control subjects. The median capillary blood glucose in women with diabetes at the time of testing was 6.15 mmol/l (interquartile range 5.38–9.73). There was no correlation between AUC for ACH response in relation to capillary blood glucose ($r = -0.069$, $P = 0.815$), although a nonsignificant inverse relationship was observed between HbA_{1c} and ACH response ($r = -0.384$, $P = 0.16$). Adjustment for HbA_{1c} resulted in a loss of significance in ACH response between case and control subjects ($T = -0.64$, $P = 0.523$). There were no differences in plasma lipoproteins in the antenatal period between the

Table 1—Demographic characteristics of study subjects

	Antenatal diabetic women	Antenatal control subjects	P value
<i>n</i>	15	30	
Age (years)	29 (25–31)	29 (25–32)	0.99
Gestation (weeks)	32 (29–35)	35 (34–37)	0.001
BMI (kg/m ²)	26.5 (23.4–28)	26.7 (21.8–30.2)	0.8
Systolic blood pressure (mmHg)	120 (110–130)	120 (110–130)	0.4
Diastolic blood pressure (mmHg)	75 (67–80)	75 (69.5–80)	0.8
First-trimester systolic blood pressure (mmHg)	114 (100–120)	119 (112–126)	0.13
First-trimester diastolic blood pressure (mmHg)	67 (60–72)	71 (62–77)	0.25
Primigravid (<i>n</i>)	9	21	0.8*
Smokers (<i>n</i>)	2	5	0.959*

Data are median (interquartile range). Statistical analysis was performed using the Mann-Whitney *U* test. * χ^2 test. All diabetic women were treated with insulin and had no microalbuminemia.

groups. Soluble VCAM-1 concentration was similar in both groups. Compared with controls, CRP concentrations were significantly higher in diabetic women in the antenatal period, although this observation did not persist after adjustment for gestation ($T = 1.43$, $P = 0.162$). CRP did correlate with AUC for ACH response in the control group ($r = -0.37$, $P = 0.043$) but did not in the women with diabetes ($r = 0.041$, $P = 0.885$). Adjustment for CRP did not attenuate the case-control difference in ACH response ($T = -2.98$, $P = 0.005$).

IIa. Postnatal results: diabetic versus control

Postnatal comparison of control subjects and diabetic women showed no significant difference in BMI (22.7 vs. 25.6 kg/m², $P = 0.28$), blood pressure (systolic 115 vs. 112 mmHg, $P = 0.6$; diastolic 76 vs. 76 mmHg, $P = 0.98$), or weeks elapsed since delivery (31.5 vs. 28, $P = 0.23$). The proportions of subjects breast-

feeding (3 [18.8%] vs. 3 [30%], $\chi^2 P = 0.412$) or taking oral contraceptives (4 [25%] and 2 [22.2%], $\chi^2 P = 0.88$) were also not significantly different.

Endothelial-dependent and -independent vasodilatation. Dose-dependent perfusion responses to ACH and SNP were less in the women with diabetes than in the control group, both $P < 0.001$ (two-way ANOVA) (Fig. 1A and B).

IIb. Antenatal versus postnatal in diabetic and control groups

Endothelial-dependent vasodilatation. There was a greater response to ACH in pregnancy compared with postpartum, as represented by dose-dependent perfusion responses in both control ($P = 0.018$) and diabetic ($P = 0.029$) groups (Fig. 1A).

Endothelial-independent vasodilatation. In healthy control subjects, a greater dose-dependent perfusion response to SNP was observed in pregnancy as compared with postpartum ($P = 0.01$). In the diabetic women, this effect was also

observed but did not attain significance ($P = 0.105$) (Fig. 1B).

Plasma analyses (Table 3). In both groups, lipid and lipoproteins were significantly elevated in pregnancy compared with postpartum. CRP was elevated during pregnancy in patients with diabetes ($P < 0.10$). HbA_{1c} levels were similar in the control group during and after pregnancy, but a marked increase was noted in women with diabetes postpartum, despite similar capillary blood glucose concentrations at both time points. Postpartum VCAM-1 concentrations increased in women with diabetes and decreased in the control subjects, although these changes did not attain significance. However, VCAM-1 levels were higher in the diabetic women than in the control subjects in the postpartum period ($P < 0.01$).

CONCLUSIONS — We have demonstrated, using a noninvasive, in vivo technique, the presence of microvascular

Table 2—Results of lipid profiles, HbA_{1c}, CRP, and soluble VCAM in pregnant diabetic women versus control subjects

	Antenatal diabetic women	Antenatal control subjects	P value	Adjusted P value*
<i>n</i>	15	30		
Cholesterol (mmol/l)	6.05 (5–6.95)	6.47 (5.7–7.2)	0.25	0.44
Triglyceride (mmol/l)	2.45 (2–2.95)	2.37 (2.05–3)	0.66	0.59
VLDL cholesterol (mmol/l)	0.55 (0.25–0.8)	0.6 (0.34–0.8)	0.64	0.84
LDL cholesterol (mmol/l)	3.75 (2.5–4.4)	4.05 (3.5–5.07)	0.15	0.24
HDL cholesterol (mmol/l)	1.8 (1.65–2.0)	1.55 (1.4–1.8)	0.06	0.32
HbA _{1c} (%)	5.8 (5.5–6.1)	4.55 (4.3–4.7)	<0.001	<0.001
CRP (mg/ml)	3.81 (2.47–8.15)	2.74 (1.53–4.4)	0.03	0.162
VCAM (ng/ml)	323 (254–370)	330 (249–390)	0.82	0.37

Data are medians (interquartile ranges). Statistical analysis was performed using the Mann-Whitney *U* test. *P value after adjustment for gestation.

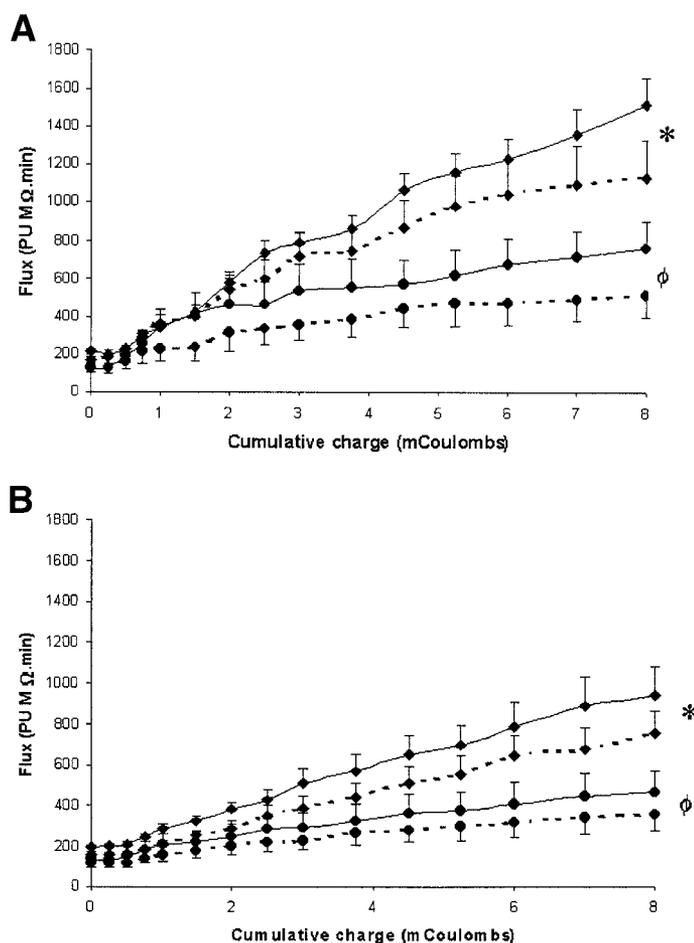


Figure 1—A: Endothelial-dependent (ACH) vasodilatation (control subjects and diabetic women): antenatal versus postnatal. Dose-dependent perfusion response to ACH in control women: antenatal (n = 16; ◆) versus postnatal (n = 16; dashed line ◆), and diabetic women: antenatal (n = 9; ●) versus postnatal (n = 9, dashed line ●). Data are mean ± SE. *Control antenatal versus postnatal, P = 0.018; ϕ diabetic antenatal versus postnatal, P = 0.029, two-way ANOVA. B: Endothelial-independent SNP vasodilatation (control subjects and diabetic women): antenatal versus postnatal. Dose-dependent perfusion response to SNP in control women: antenatal (n = 16; ◆) versus postnatal (n = 16; dashed line ◆), and diabetic women: antenatal (n = 9; ●) versus postnatal (n = 9, dashed line ●). Data are mean ± SE. *Control antenatal versus postnatal, P = 0.01; ϕ diabetic antenatal versus postnatal, P = 0.105, two-way ANOVA.

dysfunction in forearm skin resistance vessels of pregnant women with type 1 diabetes and no clinical evidence of microvascular complications. We demonstrate reduced vasodilator responses to both endothelial-dependent and -independent stimuli in contrast to previously reported work in diabetic pregnancy (5). We have also provided data concerning microvascular function in the nonpregnant state. Our results indicate that pregnancy enhances vascular function in both healthy women and those with diabetes. However, in women with diabetes, this improvement, which is likely to be mediated by pregnancy hormones, is insufficient even to attain vascular responsive-

ness seen in healthy nonpregnant women. This finding suggests either a persistent vascular defect in young women with type 1 diabetes or the presence of toxic circulating factors. Our data, which considered circulating lipids, CRP, and HbA_{1c}, suggest that even a modest level of chronic hyperglycemia is detrimental to vascular function.

Previous investigations of microvascular function in nonpregnant women with type 1 diabetes have proposed that endothelial dysfunction contributes to an increased incidence of cardiovascular complications (8). However, we have described reduced responses to both endothelial-dependent and -independent

vasodilators, thus excluding any presumption concerning the function or integrity of the endothelial cell. These results concur with similar findings in nonpregnant subjects with type 1 diabetes and in healthy relatives of individuals with diabetes (9–11). There may be impaired production of endothelial-derived NO; however, our results suggest alterations further downstream in the microvascular tree at the level of the vascular smooth muscle. This may indicate a problem with inactivation of endothelial-derived NO or inadequate receptor binding and function at the level of the vascular smooth muscle cell. In many studies of diabetic vascular function, prolonged exposure to hyperglycemia is proposed as the main protagonist in the mediation of complications. Hyperglycemia is believed to induce multiple changes in intracellular metabolism, such as activation of the polyol pathway. Also, hyperglycemia results in nonenzymatic glycation of circulating cells and proteins, resulting in production of advanced glycation end products (AGEs) (4,12). These molecules quench NO and, in combination with free radicals such as superoxide anions, which directly inactivate NO, are responsible for reducing the bioavailability of NO (8). In our subjects, the difference between ACH responses in women with diabetes and control subjects was eliminated after adjustment for HbA_{1c}. Our observation of a combined reduction in responses to endothelial-dependent and -independent vasodilatation may also reflect microvascular sclerosis, a more physical loss of vasodilator reserve (13). Some groups have suggested that, in early stages of diabetes, increased microvascular flow and consequent increased capillary pressure results in upregulated tissue perfusion, which may result in further shear stress applied to the vessel wall and consequent extravascular matrix protein production as an injury response. The resultant effect may result in sclerosis and impairment in vasodilatory reserve and autoregulation (13)

Inflammation is increasingly proposed as a key mediator of vascular disease (14). A recent study of nonpregnant patients with type 1 diabetes reported elevated CRP concentrations in association with elevated VCAM-1 levels, implicating a potential role for inflammatory mediators in the accelerated vascular disease of this group (15). CRP was correlated with

Table 3—Results of lipid profiles, HbA_{1c}, soluble VCAM, and CRP: antenatal versus postnatal (control subjects and diabetic women)

	Control			Type 1 diabetes			P*
	Antenatal	Postnatal	P value	Antenatal	Postnatal	P	
n	16	16		9	9	—	—
Lipoproteins							
Cholesterol (mmol/l)	6.6 (5.8–7.5)	4.8 (3.8–5)	<0.001	6.05 (5.2–6.5)	4.4 (3.9–5.0)	0.009	0.46
Triglyceride (mmol/l)	2.22 (1.9–3)	0.85 (0.7–1.1)	<0.001	2.3 (1.9–3.3)	0.85 (0.7–1.6)	0.013	0.75
VLDL cholesterol (mmol/l)	0.6 (0.3–0.9)	0.22 (0.1–0.4)	0.001	0.55 (0.3–0.7)	0.25 (0.1–0.5)	0.08	0.55
LDL cholesterol (mmol/l)	4.32 (3.5–5.2)	2.95 (2–3.5)	0.001	3.75 (2.7–4.1)	2.5 (2.1–2.8)	0.009	0.25
HDL cholesterol (mmol/l)	1.52 (1.2–1.9)	1.32 (1.1–1.7)	0.01	1.8 (1.7–2.0)	1.45 (1.2–1.7)	0.009	0.73
Inflammation							
CRP (mg/ml)	2.13 (0.9–3.9)	1.15 (0.3–3.3)	0.48	3.44 (2.3–7.6)	2.95 (0.9–6.7)	0.076	0.174
VCAM-1 (ng/ml)	369 (321–424)	344 (285–393)	0.103	351 (299–391)	419 (385–476)	0.124	0.004
Glucose control							
HbA _{1c} (%)	4.6 (4.4–4.8)	4.7 (4.4–4.9)	1.00	5.8 (5.3–5.9)	7.6 (5.9–7.7)	0.033	<0.001
Capillary blood glucose (mmol/l)	—	—	—	6.15 (5.4–9.7)	5.35 (3.6–8.1)	0.32	—

Data are medians (interquartile ranges). Statistical analysis was performed using the Mann-Whitney *U* test. *Control vs. diabetic at postnatal visit.

ACH responses in the control subjects and has been linked to endothelial function in other diseases. However, although higher in case subjects, the difference in CRP was not significant after adjustment for gestation; in addition, CRP did not attenuate the significant case-control difference in ACH responses. Clearly, larger studies are needed to address CRP changes in pregestational diabetes.

In conclusion, using an *in vivo* method of microvascular assessment, we have demonstrated that pregnancy enhances vascular function in both healthy women and those with type 1 diabetes. However, in women with diabetes, this improvement is insufficient even to attain responses seen in healthy nonpregnant women. Our data suggest a key role for the chronic effects of hyperglycemia in the impaired vascular responsiveness in such women. These important observations provide an insight into mechanisms underlying pregnancy-associated and long-term vascular risk in women with type 1 diabetes.

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