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Streptozotocin diabetes in the pregnant rat induces cardiovascular dysfunction in adult offspring

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Summary Severe diabetes in pregnant rats produces persistent metabolic consequences in adult offspring. This study investigated whether diabetes in pregnant rats could also lead to cardiovascular abnormalities in the adult offspring. Blood pressure, heart rate and in vitro vascular reactivity of small arteries were evaluated in female adult offspring of control rats and of rats rendered diabetic with streptozotocin. Rise in blood pressures were similar in both groups of offspring but heart rate was lower in the diabetic offspring ($p < 0.05$). The rise in blood pressure associated with infusion of a nitric oxide synthase inhibitor was similar in both groups, but the associated decrease in heart rate was more pronounced in diabetic offspring ($p < 0.01$). Small mesenteric arteries from this group showed enhanced sensitivity to noradrenaline ($p < 0.05$) and abnormal endothelium-dependent relaxation to acetylcholine ($p < 0.01$) and bradykinin ($p < 0.05$). Reduction in acetylcholine induced relaxation, reflected reduced synthesis of nitric oxide or a cyclooxygenase product and was not attributable to an endothelium-derived hyperpolarizing factor. Sensitivity to exogenous nitric oxide was normal. A subgroup of pups born to diabetic dams were suckled by control maternal dams and a subgroup of those born to controls by diabetic dams. Suckling was an important determinant of impaired growth; offspring of diabetic rats suckled by their own mother and those of control rats by diabetic dams showed impaired growth rates whereas growth of offspring of diabetic rats suckled by control dams paralleled those of control rats suckled by their own mother. [Diabetologia (1999) 42: 81–89]

Keywords Insulin dependent diabetes, rat offspring, vascular function, endothelium, streptozotocin.

The hypothesized association between disturbance of the in utero environment and disease in later life is based largely on retrospective epidemiological studies which have focused upon the long-term consequences of in utero malnutrition [1]. Some population-based studies also point to prolonged effects of maternal diabetes on the fetus, including an increase in the frequency of gestational diabetes and of insulin resistance in adult offspring [2, 3]. Prospective investigation of potential associations between a diabetic intrauterine milieu and disease in later life has been facilitated by animal models of chemically induced diabetes. These provide a means to evaluate responses to the intrauterine environment per se without the influence of hereditary traits [4–9]. Hyperglycaemia during rat gestation causes perturbations of glucose homeostasis in the offspring [4–9]; adult offspring of rats rendered diabetic with streptozotocin exhibit high plasma insulin concentrations during a glucose tolerance test [5], increased renal clearance of insulin [10] and are strongly resistant to the action of insulin as shown by the eugly-

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Abbreviations: ACh, Acetylcholine; BK, bradykinin; INDO, indomethacin; NA, noradrenaline; L-NAME, Nω-nitro L-arginine methyl ester; O-CR, offspring a of control rats; O-DR, offspring of diabetic rats; O-CR:DL, offspring of a control pregnant rat lactated by a diabetic maternal rat; O-DR:CL, offspring of diabetic pregnant rat lactated by a control maternal rat; ODQ, oxidazole quinoxalin; SNP, sodium nitroprusside; EDHF, endothelium-derived hyperpolarizing factor.

caemic hyperinsulinaemic clamp [11]. The decreased sensitivity to insulin is observed in the liver as well as the extrahepatic tissues [11] and peripheral glucose uptake is specifically reduced in skeletal muscles [6]. Insulin sensitivity in any tissue is dependent not only on the ability of insulin to stimulate cellular glucose uptake but is also influenced by the arteriovenous glucose gradient and, potentially, blood flow [12, 13]. It is therefore also of interest to determine whether diabetes in pregnancy could evoke an increase in peripheral vascular tone in the offspring and, in so doing, contribute to insulin resistance. Alternatively, it could be proposed that the observed insulin resistance in the adult offspring of diabetic rats, and the associated dyslipidaemia gives rise to vascular malfunction and overt cardiovascular disease. In this study, an investigation designed to evaluate whether maternal diabetes can induce in utero 'imprinting' of cardiovascular dysfunction, we have determined whether severe maternal diabetes in pregnant rats influenced the blood pressure and heart rate of the adult offspring. Using the technique of small vessel myography we have also determined whether diabetes in the maternal rat leads to constrictor or dilator dysfunction in isolated small arteries (mesenteric circulation) of the adult offspring. Additionally, by fostering the offspring with non-diabetic dams we have evaluated the contribution of the suckling period (and therefore of maternal milk) to the growth retardation observed in the offspring.

Materials and methods

The entire protocol was reviewed and approved by the local ethics committee for animal procedures (K.U. Leuven, Belgium).

Cardiovascular function in the offspring of streptozotocin-diabetic rats. Adult offspring (O-CR, $n = 9$) of three control pregnant Wistar rats (Leuven/pfd, K.U. Leuven Breeding Centre, Leuven, Belgium) and of three diabetic pregnant Wistar rats (O-DR, $n = 9$) were studied. Diabetes mellitus in the maternal rat was induced experimentally by a single i.v. injection of streptozotocin (35 mg/kg body weight; Upjohn, Puurs, Belgium) on day 1 of pregnancy (the day of the copulation plug). On day 20, a tail vein blood sample was removed for evaluation of plasma glucose concentrations. Only offspring of rats with eight or more fetuses were included in the study. All rats had free access to a standard rat laboratory chow (Trouw, Gent, Belgium) and tap water. After weaning only the female offspring of control and diabetic rats were kept and raised till adulthood. This allowed comparison with previous similar studies from our laboratory [4–7, 11] of metabolic disorders, particularly insulin resistance in female offspring of diabetic rats. The offspring were weighed on postnatal days 21, 28, 35, 49, 70 and 91. Pups were not weighed at birth to prevent maternal rejection.

Measurement of blood pressure and heart rate. At 91 days of age the offspring were transported to the Janssen Research Foundation (Beerse, Belgium). After 1 week of acclimatisation (at 98 days of age) the measurement of blood pressure and heart rate was carried out as described previously [14]. Briefly, the animals were anaesthetised with ether and a femoral artery and vein dissected free of surrounding tissue and cannulated. The animals were restrained in Bollman cages and local anaesthesia induced by applying 2% lidocaine (Astra, Huizingen, Belgium) to the wound. Heart rate and blood pressure measurements were recorded from the output of a pressure transducer (Janssen Scientific Instruments Division, Beerse, Belgium) inserted into the femoral artery catheter. When the animals were fully awake (approximately 60 min after ether withdrawal), systolic and diastolic arterial blood pressure and heart rate were recorded for an equilibration period of 60 min (MacLab, AD Instruments, PTY Ltd., Castle Hill, New South Wales, Australia), during which time systolic and diastolic blood pressures and heart rate reached a stable plateau. Recording was then carried out for a further 60 min experimental period over which the mean values were calculated for the three variables using a customized computer program (Janssen Scientific Instruments Division, Beerse, Belgium). In order to evaluate the contribution of nitric oxide to tonic lowering of blood pressure, 1.25 mg/kg of the nitric oxide synthase inhibitor Nω-nitro L-arginine methyl ester (L-NAME; Sigma, Bornem, Belgium) was then injected into the venous catheter and systolic and diastolic arterial blood pressure and heart rate recorded for a further 120 min. The mean systolic and diastolic blood pressure and heart rate were calculated over the 120 min period.

Vascular function in isolated arteries. Further offspring (O-CR, total $n = 21$) of 11 normal rats and a group of offspring (O-DR, total $n = 20$) of 10 diabetic pregnant rats were investigated in two studies of vascular function (sub-groups 1 and 2). No more than two offspring of any mother were studied. On day 20 of pregnancy a tail blood sample was obtained for measurement of plasma glucose. All pups were nursed by their mothers in standard laboratory conditions. After weaning, the female offspring of rats with eight or more pups were kept on ad libitum food intake and in vitro vascular function determined between 100 and 120 days of age. In sub-group 1, at 80 days of age, a tail blood sample was obtained after an overnight fast for determination of concentrations of plasma triglycerides (Triglycerides GPO-PAP; Boehringer, Mannheim, Germany), total cholesterol (Cholesterol CHOL-PAP; Boehringer, Mannheim, Germany) and glucose (glucose analyser 2300STAT; Yellow Spring Instruments, Yellow Springs, Ohio, USA). Plasma insulin was assessed by radioimmunoassay using rat insulin as a standard [7].

Mounting of vessels. Small mesenteric arteries were mounted on a small vessel wire myograph as described previously [15]. Arteries were bathed in PSS (constituents in mmol/l: NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.16, EDTA 0.026 and glucose 6.0), pH 7.4 at 37 °C and gassed with 5% CO_2 in O_2. The passive tension-internal circumference characteristics of the arteries were determined by stretching to achieve an internal circumference equivalent to 90% of that which would be attained when relaxed in situ under a transmural pressure of 100 mmHg.

Assessment of vascular function. To confirm viability of the arteries four contractions (4 min duration) were done to 5×10^{-6} mol/l noradrenaline (NA; Winthrop, Guilford, UK), KPSS (125 mmol/l KCl in PSS) or a combination of both. Arteries failing to produce active tension equivalent to 100 mmHg were rejected. A cumulative concentration response to NA (10^{-9} to 10^{-5} mol/l) was constructed and arteries then preconstricted with NA at a concentration of 5×10^{-6} mol/l NA (to give a submaximal constrictor response).

Sub-group 1. Vasodilator responses to acetylcholine (ACh) (10^{-9} to 10^{-5} mol/l; Sigma, Poole, Dorset, UK) were determined with additions at 2 min intervals, and after further preconstrictions to NA, responses to bradykinin (BK) (10^{-9} to 10^{-5} mol/l; Sigma, Poole, Dorset, UK) and sodium nitroprusside (SNP) (10^{-9} to 10^{-5} mol/l; Sigma, Poole, Dorset, UK) were evaluated.

Sub-group 2. To evaluate the contribution of prostanoids and nitric oxide to ACh induced relaxation, NA and ACh responses were again carried out and ACh responses then repeated in a group of arteries after incubation (20 min) with and in the continued presence of indomethacin [(INDO) 10^{-5} mol/l; Sigma, Poole, Dorset, UK]. A further ACh concentration response was then done after incubation (20 min) with INDO, L-NAME (10^{-4} mol/l) and the soluble guanylate cyclase inhibitor, oxidazole quinoxalin, ODQ (10^{-6} mol/l; Alexis Corporation, Nottingham, UK). To determine the contribution of an endothelium-derived hyperpolarizing factor (EDHF) in the persistent relaxation observed in the presence of these inhibitors, ACh responses were then evaluated in arteries preconstricted to a similar degree of tone with NA ($2–4 \times 10^{-6}$ mol/l) and 25 mmol/l KCl (equimolar substitution for NaCl in PSS). In most experiments, two arteries from each rat were investigated simultaneously and the results expressed as the mean of the data pair.

Effect of a diabetic perinatal milieu on the growth of the offspring. The O-DR showed growth retardation throughout the period studied. This could have been acquired in utero or during the lactation period. In order to evaluate the contribution of the suckling period to growth retardation in the offspring, newborn pups of three diabetic dams were cross-fostered with three control dams. Nine offspring of diabetic pregnant rats suckled with control dams (O-DR:CL), and nine of control pregnant rats suckled with diabetic dams (O-CR:DL); they were weighed at intervals up to 91 days of age and compared with O-DR and O-CR.

Statistical analysis

Heart rate and blood pressure measurement. Data for blood pressure, heart rates and body weights are given as means ± SEM. Statistical analyses for heart rate and blood pressure were carried out using paired *t*-test, unpaired *t*-test or Mann-Witney U-test as appropriate to assess differences within or between the groups. Two-tailed probabilities p less than 0.05 were considered significant. Growth curves were analysed by analysis of variance (ANOVA) for multiple comparison ("Statistica"; Statsoft, Oklahoma, USA).

Vascular function in isolated arteries. All values are given as the means ± SEM. Tension was calculated as mN/mm artery length. Concentration responses to NA were expressed as absolute tension and, to account for possible variation in smooth muscle mass, as a percentage of the contractile response to a depolarizing potassium buffer (124 mmol/l KCl). Relaxation to ACh, BK and SNP was expressed as a percentage of the initial precontraction to NA. The pEC_{50} was calculated for each concentration responses curve, when appropriate, and maximum responses were compared by unpaired Students' two tailed *t* test. When calculation of the pEC_{50} was not appropriate, responses to vasodilators were compared only by the maximum responses to the agonist. Unpaired Students two tailed *t*-test or the Mann-Whitney U-test was used for parametric and non-parametric data respectively ("Instat"; GraphPad Software Inc., San Diego, Calif., USA). Significance was assumed if p was less than 0.05.

Table 1. Body weight and plasma glucose concentrations in control and diabetic maternal rats on day 20 of gestation (studies 1 and 2)

	n	Control 17	Diabetic 16
Body weight (g)		296 ± 3	275 ± 4[a]
Glucose (mmol/l)		4.73 ± 0.16	26.68 ± 0.89[a]

Values are means ± SEM. [a] $p < 0.001$ vs control

Table 2. Heart rate, systolic and diastolic blood pressure in basal conditions and in response to i. v. L-NAME infusion in adult female offspring of control and diabetic pregnant rats

	O-CR	O-DR
Basal		
Heart rate (beats/min)	451 ± 8	408 ± 13[a]
SBP (mm Hg)	176 ± 4	176 ± 4
DBP (mm Hg)	127 ± 3	130 ± 6
L-NAME		
Heart rate (beats /min)	408 ± 11[b]	356 ± 11[b,c]
SBP (mm Hg)	190 ± 3[b]	193 ± 2[b]
DBP (mm Hg)	148 ± 4[b]	150 ± 3[b]

Values are given as means ± SEM of nine experiments. O-CR, female offspring of control pregnant rats suckled by their own mother; O-DR, female offspring of diabetic pregnant rats suckled by their own mother; [a] $p < 0.05$ vs O-CR basal, [b] $p < 0.001$ vs basal values for same group, [c] $p < 0.01$ vs O-CR post L-NAME. SBP, systolic blood pressure; DBP, diastolic blood pressure

Results

Maternal weights and plasma glucose concentrations for all animals in the study are given in Table 1. Two litters of diabetic dams had fewer than eight pups and were therefore not included in the study. Litter sizes of the diabetic and control dams of those included in the study were similar (10.14 ± 0.14 pups per litter in controls and 10.25 ± 1.1 pups per litter in the diabetic group). Mortality rates were comparable in both the controls and diabetic animals (1.4 % in control and 2.8 % in the diabetic group).

Systolic and diastolic blood pressure and heart rate. Under basal conditions, systolic and diastolic blood pressure was normal in O-DR. The heart rate was lower in O-DR than O-CR ($p < 0.05$). Treatment with L-NAME decreased heart rate and increased systolic and diastolic blood pressure in both groups ($p < 0.001$). The mean systolic and diastolic blood pressure after L-NAME were similar in O-CR and O-DR. After L-NAME the difference in heart rate between O-DR and O-CR was more pronounced than the difference recorded in the basal period ($p < 0.01$), (Table 2, Fig. 1).

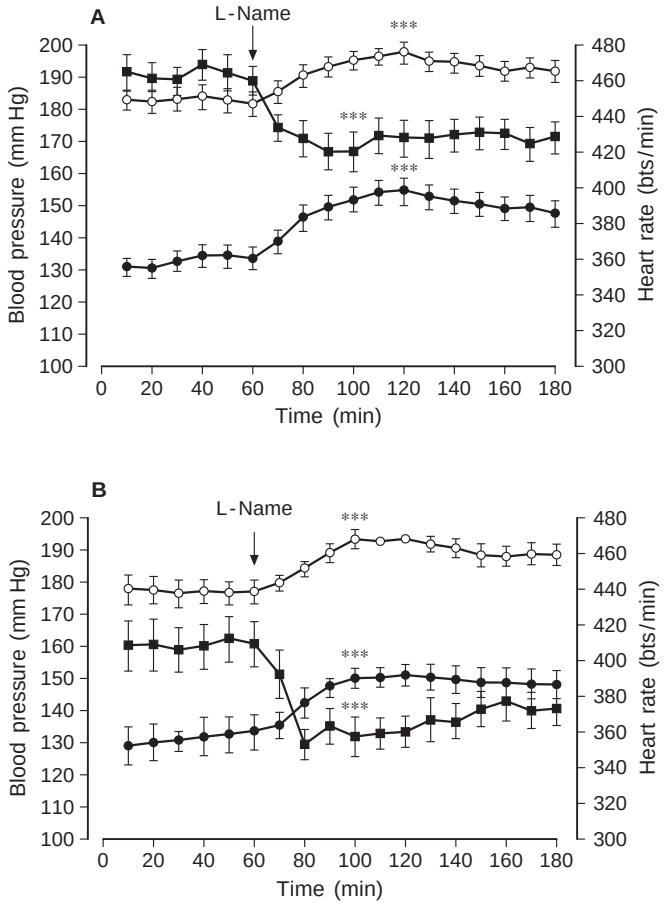

Fig. 1A,B. Systolic, diastolic blood pressure and heart rate in offspring of control and diabetic rats. ↓ indicates i. v. treatment with 1.25 mg/kg L-NAME; ○: systolic blood pressure; ●: diastolic blood pressure; ■: heart rate. **A** O-CR (female offspring of control pregnant rats) **B** O-DR (female offspring of diabetic pregnant rats) Values are given as means ± SEM of nine rats ***$p < 0.001$ maximum response vs basal value

Vascular function in adult diabetic offspring. The 80-day-old O-DR had lower body weights than O-CR and in the 11 offspring studied (sub-group 1) fasting triglyceride, cholesterol, glucose and insulin concentrations were higher than in controls (Table 3).

At 100–120 days of age, when vascular function was assessed, O-DR were lighter in weight than O-CR (179 ± 3 g, $n = 20$ vs 204 ± 4 g, $n = 22$, $p < 0.001$). Mean vessel internal diameter of the arteries from O-CR and O-DR were 299 ± 6 μm ($n = 37$) and 284 ± 8 μm ($n = 37$) respectively (p NS). The sensitivity, but not maximum constrictor response, to NA was different in O-DR compared with O-CR when expressed as absolute tension or as a percentage of K^+ induced tension ($p < 0.05$) (Table 4, Fig. 2). Maximum responses to the 125 mmol/l KCl buffer were reduced in O-DR compared with O-CR (3.77 ± 0.2 mN/mm vs 4.42 ± 0.21; $p < 0.05$). Preconstrictor tension to NA prior to relaxation responses to ACh (in the absence or presence of any inhibitor), BK or SNP were no different between the two groups. Sensitivity and maximum relaxation to ACh were impaired in arteries from O-DR (Table 4, Fig. 3 A). Cycloogygenase inhibition (with INDO) resulted in a small but significant rightward shift in the ACh response in O-CR only (Table 4, Fig. 3 B). Nevertheless O-DR responses remained significantly reduced at an ACh concentration of 10^{-7} mol/l. The residual relaxation in the presence of INDO was substantially reduced in both groups by the addition of an NO synthase (NOS) inhibitor (L-NAME) and oxidazole quinoxalin (ODQ). Some residual relaxation was still evident but was similar in both groups (Table 4, Fig. 3 c). The subsequent addition of 25 mmol/l KCl (to prevent relaxation to an EDHF) in the continued presence of INDO, L-NAME and ODQ resulted in complete inhibition of relaxation to ACh in both groups (Table 4, Fig. 3 D). Further evidence for endothelial dysfunction in arteries from O-DR was indicated by reduced maximum relaxation to BK (Table 4, Fig. 4). Relaxation to the nitric oxide donor SNP was similar in O-DR and O-CR (Table 4, Fig. 5).

Table 3. Body weight, fasting plasma triglyceride and cholesterol concentrations in 80-day-old offspring of control rats (O-CR) and diabetic rats (O-DR)

	O-CR	O-DR
body weight (g)	179 ± 4 ($n = 11$)	143 ± 2[a] ($n = 11$)
triglycerides (mmol/l)	0.61 ± 0.04 ($n = 11$)	0.85 ± 0.08[b] ($n = 11$)
cholesterol (mmol/l)	1.73 ± 0.08 ($n = 11$)	2.10 ± 0.04[c] ($n = 11$)
glucose (mmol/l)	4.88 ± 0.08 ($n = 8$)	5.84 ± 0.27[c] ($n = 6$)
insulin (mmol/l)	0.04 ± 0.01 ($n = 8$)	0.10 ± 0.02[b] ($n = 6$)

Values are means ± SEM. [a] $p < 0.001$, [b] $p < 0.05$, [c] $p < 0.01$ vs control

Effect of a diabetic perinatal milieu on the growth of the offspring. At weaning, body weight was 50 % lower in O-DR than in O-CR (23 ± 4 g in O-DR, $n = 9$, vs 46 ± 2 g in O-CR, $n = 9$; $p < 0.001$) and 66 % lower in O-CR:DL than in O-CR (15 ± 1 g, $n = 9$; $p < 0.001$ vs O-CR). Growth rates of all offspring suckled by a diabetic mother were lower than those of control offspring suckled by their own mothers. Thus, although the growth of both O-DR and O-CR:DL after weaning was parallel to the growth in O-CR, the body weight at each point of measurement was lower than in O-CR ($p < 0.001$). Offspring of O-DR:CL recovered well during the suckling period (40 ± 1 g in O-DR:CL vs 46 ± 2 g in O-CR; $p < 0.01$) and body weight was similar to O-CR by 28 days of age (68 ± 2 g vs 73 ± 2 g in O-CR; p NS). The growth curve of O-DR:CL rats was very similar to that of O-CR

Table 4. Responses to constrictor and dilator agonists in rat small mesenteric arteries from offspring of control (O-CR) and diabetic rats (O-DR)

	O-CR	O-DR
NA	(n = 21)	(n = 20)
pEC_{50} (μmol/l)	5.71 ± 0.20	5.88 ± 0.27[a]
Max constriction (% K^+)	102.61 ± 2.26	109.97 ± 3.66
ACh	(n = 21)	(n = 20)
pEC_{50} (μmol/l)	7.14 ± 0.05	6.76 ± 0.09[b]
Max relaxation	91.92 ± 2.02	82.23 ± 3.13[a]
ACh (INDO)	(n = 10)	(n = 9)
pEC_{50} (μmol/l)	6.89 ± 0.08	6.55 ± 0.14
Max relaxation	89.52 ± 3.63	91.75 ± 2.82
ACh (INDO, L-NAME, ODQ)	(n = 10)	(n = 9)
Max relaxation	70.12 ± 6.39	58.47 ± 8.71
ACh (INDO, L-NAME, ODQ) in 25 mmol/l KCl	(n = 10)	(n = 9)
Max relaxation	3.64 ± 2.21	2.71 ± 1.23
BK	(n = 11)	(n = 11)
Max relaxation	40.97 ± 2.80	30.93 ± 3.15[a]
SNP	(n = 10)	(n = 11)
pEC_{50} (μmol/l)	7.50 ± 0.13	7.36 ± 0.12
Max relaxation	70.43 ± 4.43	68.9 ± 3.62

Values are given as means ± SEM. [a] $p < 0.05$, [b] $p < 0.01$ vs control. Max = maximum

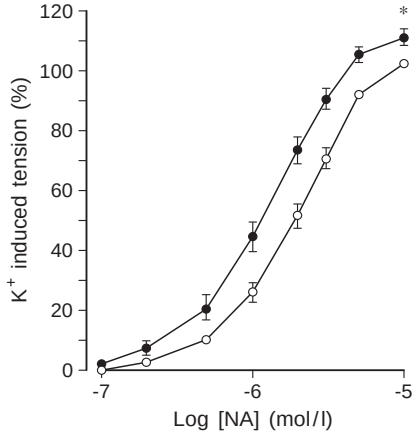

Fig. 2. Tension development to noradrenaline (NA) in mesenteric small arteries from offspring of control pregnant rats (○; O-CR: n = 21) and offspring of diabetic pregnant rats (●; O-DR, n = 20). Tension is expressed as a percentage of constriction to potassium (K^+, 124 mmol/l). Values are given as means ± SEM. When absent, error bars lie within symbols. *pEC_{50} $p < 0.05$ maximum response O-DR vs O-CR

(195 ± 3 g in O-DR:CL vs 200 ± 3 g in O-CR at 91 days of age; p NS), (Fig. 6).

Discussion

In agreement with earlier reports, this study of the streptozotocin-diabetic rat has shown clearly that exposure to maternal diabetes during the fetal and perinatal period has profound consequences in the offspring. The novelty of the present investigation lies in the observation that cardiovascular function of the offspring is compromised.

There was no suggestion in this study that diabetes in rat pregnancy 'programmes' the fetus to develop hypertension, but there was evidence of pronounced bradycardia in the adult offspring. Theoretically, this could arise from resetting of the baroreceptor response, or, through neuropathy [16] acquired in utero and permanently altered sympathetic discharge at the synoatrial node. Growth restriction per se was unlikely to be involved as we have not found bradycardia in the adult offspring of semistarved rats [17] with comparable growth retardation at term [7].

The offspring of the diabetic animals also showed abnormalities of vascular function in vitro. Previously, we have shown reduced relaxation to endothelium dependent dilators and enhanced constriction to NA in small mesenteric arteries from adult diabetic rats when compared with non-diabetic controls [18]. These defects were also present, to a lesser degree, in the arteries isolated from O-DR, proving that exposure to maternal diabetes in utero or in the perinatal period or both induces vascular dysfunction in the adult offspring. The enhanced sensitivity, but similar maximum response, to noradrenaline is indicative of abnormal receptor mediated tension development. Again, neuoropathy could have a role, with reduced noradrenaline release evoking up-regulation of the α/β adrenoceptors. The lower potassium induced tension development observed in O-DR could reflect reduced smooth muscle mass or an abnormality of electromechanical coupling in response to potassium induced depolarization.

The reduction in relaxation to ACh and BK is indicative of impaired synthesis of endothelium-derived vasodilators. The normal sensitivity to sodium nitroprusside suggests that the defect does not arise from reduced sensitivity of the smooth muscle to nitric oxide (NO). In the rat mesenteric circulation, ACh and BK evoke relaxation through release of NO, prostacyclin (PGI_2) [19] and the putative endothelium derived hyperpolarizing factor [20]. In this study, the defect in sensitivity and maximum relaxation to ACh between O-CR and O-DR was not observed in the presence of indomethacin, suggesting reduced PGI_2 synthesis in O-DR. Differences in relaxation to individual concentrations of ACh remained, however, and might suggest insufficient power to detect small differences in ACh sensitivity. With NO synthase (NOS) and guanylate synthase inhibition there was no difference in residual relaxation to ACh and we conclude that PGI_2/NO induced relaxation is responsible for the defect in O-DR endothelium-dependent relaxation, and not an EDHF. The observation that the residual relaxation with cycloogygenase and NOS blockade was totally inhibited in both groups

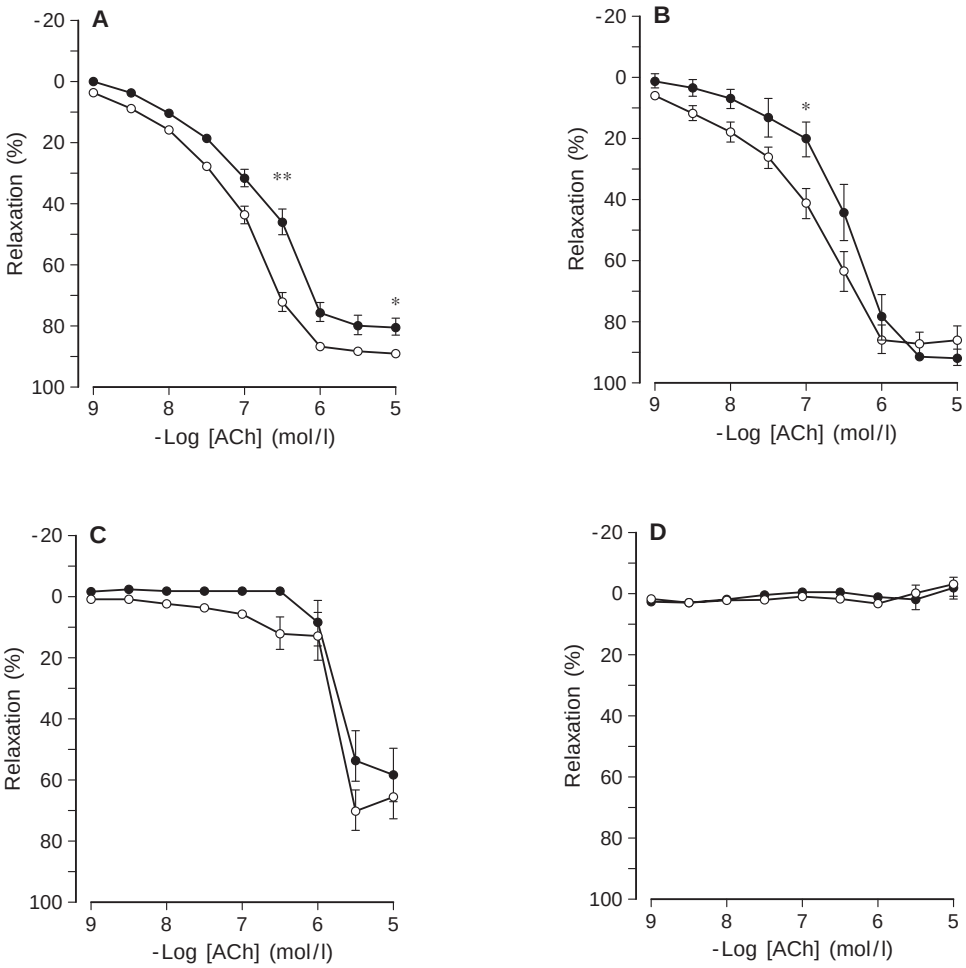

Fig. 3 A–D. **A** Concentration-dependent relaxation to acetylcholine (ACh) in mesenteric small arteries preconstricted with noradrenaline from offspring of control pregnant rats (○; O-CR, $n = 21$) and offspring of diabetic pregnant rats (●; O-DR, $n = 20$). Tension is given as percentage relaxation of initial response to noradrenaline. **pEC_{50} $p < 0.01$ O-DR vs O-CR, *$p < 0.05$ maximum response O-DR vs O-CR. **B** Concentration-dependent relaxation to acetylcholine (ACh) in mesenteric small arteries preconstricted with noradrenaline after 20 min incubation with and in the continued presence of INDO. Arteries from offspring of control pregnant rats (○; O-CR, $n = 10$) and offspring of diabetic pregnant rats (●; O-DR, $n = 9$). Tension is given as percentage relaxation of initial response to noradrenaline. *$p < 0.05$ for 10^{-7} mol/l ACh O-DR vs O-CR. **C** Concentration-dependent relaxation to acetylcholine (ACh) in mesenteric small arteries preconstricted with noradrenaline after 20 min incubation with and in the continued presence of INDO, L-NAME and ODQ. Arteries from offspring of control pregnant rats (○; O-CR, $n = 10$) and offspring of diabetic pregnant rats (●; O-DR, $n = 9$). Tension is given as percentage relaxation of initial response to noradrenaline. **D** Concentration-dependent relaxation to acetylcholine (ACh) in mesenteric small arteries preconstricted with noradrenaline and 25 mmol/l KCl after 20 min incubation with and in the continued presence of INDO, L-NAME and ODQ. Arteries from offspring of control pregnant rats (○; O-CR, $n = 10$) and offspring of diabetic pregnant rats (●; O-DR, $n = 9$). Tension is given as percentage relaxation of initial response to noradrenaline. Values are given as means ± SEM. When absent, error bars lie within the symbols

by depolarization to KCl also confirms that the EDHF component of relaxation was no different in O-CR and O-DR.

The increase in blood pressure induced by the NOS inhibitor, L-NAME confirms that NO contributes to tonic lowering of the blood pressure. The similar rise in blood pressure in O-CR and O-DR might indicate that the defect observed in the isolated arteries is not confined to a reduction in NO mediated relaxation as suggested above. Extrapolation of the in vivo data, providing information on basal NO release, however, to that of the isolated arteries relating to agonist induced synthesis may not be appropriate as different signal transduction pathways contribute to each mode of NO production.

The importance of abnormal endothelium-dependent dilatation, as observed in O-DR, lies in the possible pathological sequelae. Reduced stimulation of NO may contribute to coagulation, to leucocyte migration and atherosclerosis, as well as to increased peripheral resistance [19]. Endothelial dysfunction, similar to that we report here in O-DR, is not only observed in adult diabetic subjects [21] and animals [18] but in other conditions with high cardiovascular risk, particularly hypercholesterolaemia [22, 23]. It is possible, therefore, that the intrauterine diabetic mi-

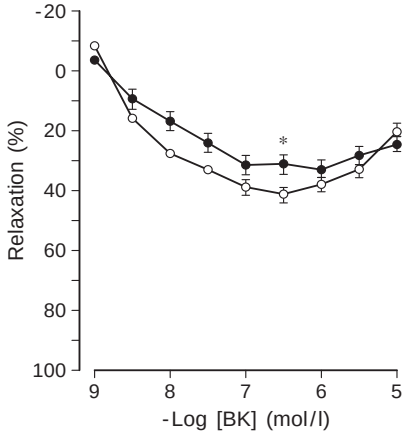

Fig. 4. Concentration-dependent relaxation to bradykinin (BK) in mesenteric small arteries preconstricted with noradrenaline from offspring of control pregnant rats (◯; O-CR, $n = 11$) and offspring of diabetic pregnant rats (●; O-DR, $n = 11$). Tension is given as percentage relaxation of initial response to noradrenaline. *$p < 0.05$ maximum response O-DR vs O-CR. Values are given as means ± SEM. When absent, error bars lie within the symbols.

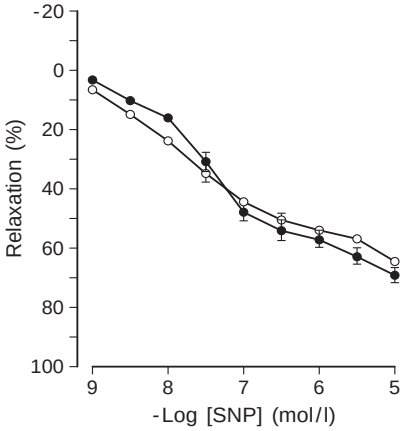

Fig. 5. Concentration-dependent relaxation to sodium nitroprusside (SNP) in mesenteric small arteries preconstricted with noradrenaline from offspring of control pregnant rats (◯; O-CR, $n = 11$) and offspring of diabetic pregnant rats (●; O-DR, $n = 11$). Tension is given as percentage relaxation of initial response to noradrenaline. Values are given as means ± SEM. When absent, error bars lie within the symbols

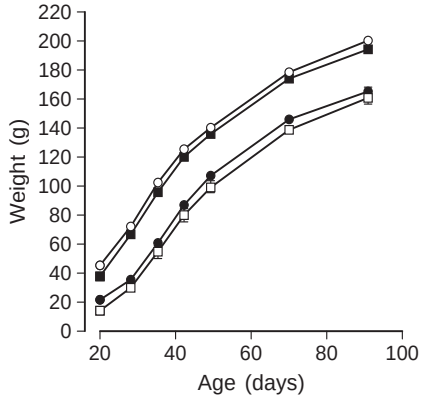

Fig. 6. Postnatal growth curves in female offspring of: control pregnant rats suckled by their own mother (◯, O-CR); of diabetic pregnant rats suckled by their own mother (●; O-DR); of diabetic pregnant rats suckled by a control rat (■; O-DR:CL) and of control pregnant rats suckled by a diabetic rat (□; O-CR:DL). Data given as means ± SEM of nine rats per group. When absent, error bars lie within the symbols

lieu has conferred upon the O-DR a predisposition to severe cardiovascular disorders in later life.

In a recent study of 16 day old O-DR, endothelium-dependent relaxation of the femoral arteries was normal [24]. Although in a different vascular bed to this study, this indicates that vascular malfunction in O-DR develops as the animal matures. Previous studies from one of our laboratories [11] and others [25, 26] have shown overt insulin resistance in adulthood O-DR. In this study, the O-DR sub-group in which plasma biochemical analyses were made also showed a plasma lipid profile characteristic of insulin resistance together with raised plasma insulin. Insulin resistance, perhaps as a result of raised plasma cholesterol [22] and triglycerides [27] is increasingly implicated in endothelial dysfunction [21]. Other candidates for endothelial impairment include transient hyperglycaemia [7], oxidative stress [28, 29] or the synthesis of advanced glycosylation end products (AGE) [30] in utero.

There is suggestion that endothelial dysfunction, as observed in O-DR, could directly lead to insulin resistance as a reduction in insulin evoked endothelium-dependent vasodilatation would reduce skeletal muscle blood flow and attenuate glucose delivery and uptake [12, 13]. Recent evidence has, however, shown a clear dissociation between insulin stimulation of blood flow and glucose uptake in normal subjects [31]. We consider, as suggested above, that it is more likely that the abnormality in O-DR is a consequence rather than a cause of insulin resistance.

It could be argued that the O-DR vascular defect observed is simply the consequence of growth retardation. Several population studies in man have linked low birth weight to adulthood cardiovascular disease [32, 33] and some reports [34, 35], although not a recent study from our laboratory [17], have documented raised blood pressure in growth restricted offspring of dietary-deprived rats. Growth retardation is unlikely, however, to have played an important part as we have shown that the growth restricted offspring of semistarved rats have only a very minor alteration in vascular function [17]. Future studies in offspring of dams with partially controlled diabetes, in which offspring are not growth retarded [25] will prove definitively whether growth retardation per se plays any part at all in the defects observed.

In the cross- fostering experiment we observed that the propensity for lower adult body weight of the offspring born to diabetic mothers is 'programmed for' or 'imprinted' upon the animal during the suckling period. The lower body weight in adult O-DR is therefore likely to arise from decreased milk volume and deficient nutrient intake during lactation [36] and contrasts with our recent study of growth retarded offspring from dietary deprived (semistarved) rats [37] in which pregnancy was more important than the lactation period in determining adult body weight.

In conclusion, this study suggests that perinatal development in a diabetic milieu in the rat does not result in hypertension in early adulthood but leads to subtle changes in the cardiovascular system which may predispose to overt cardiovascular disease. This observation, if applicable to the offspring of diabetic women, could have considerable healthcare implications.

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