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PROGRAMMING OF GROWTH, INSULIN RESISTANCE AND VASCULAR DYSFUNCTION IN OFFSPRING OF LATE GESTATION DIABETIC RATS

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Abstract

The offspring of diabetic mothers (ODM) have an increased risk of developing metabolic and cardiovascular dysfunction. However, few studies have focused on susceptibility to disease in offspring of mothers developing diabetes during pregnancy. We developed an animal model of late-gestation diabetic pregnancy and characterized metabolic and vascular function in the offspring. Diabetes was induced by streptozotocin (50 mg/kg, i.p.) in pregnant rats on gestational day 13 and partially controlled by twice-daily injections of insulin. At 2 months of age, ODM had slightly better glucose tolerance than controls ($p < 0.05$), however, by 6 months of age this trend reversed. Hyperinsulinemic-euglycemic clamp revealed insulin resistance in male ODM ($p < 0.05$). In 6-8 mo old female ODM, aortas showed significantly enhanced contractility to potassium chloride (KCl), endothelin-1 (ET-1) and noradrenaline (NA). No differences in responses to endothelin-1 and noradrenaline were apparent with co-administration of N^G -nitro-L-arginine (L-NNA). Relaxation to acetylcholine but not nitroprusside was significantly impaired in female ODM. In contrast, males displayed no between group differences in response to vasoconstrictors while relaxation to nitroprusside and acetylcholine was greater in ODM compared to control animals. Thus, development of diabetes during pregnancy programs gender specific insulin resistance and vascular dysfunction in adult offspring.

Keywords

glucose; endothelium; vascular smooth muscle; cardiovascular disease

INTRODUCTION

There is increasing evidence that adverse factors in the perinatal environment predispose an individual to disease later in life. This concept of “developmental programming of adult onset diseases” has primarily focused on maternal undernutrition and/or poor fetal growth and the later development of adult-onset diseases. [1-3]. However, other maternal conditions, including diabetes, produce an adverse environment for the developing fetus resulting in increased risk of obesity, hypertension, insulin resistance and dyslipidemia in the offspring [4-6]. There is a paucity of understanding of the mechanisms that underlie the adverse long-term metabolic and cardiovascular programming that occurs after exposure to maternal diabetes.

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Previous studies in animals have demonstrated that maternal diabetes promotes alterations in metabolic function and vascular reactivity in offspring [7-15]. However, these studies have primarily used animals made diabetic either prior to pregnancy [8,13,14], or early in pregnancy [7,10]. The effect of the development of diabetes during the last third of pregnancy, as commonly occurs with gestational diabetes, on long term metabolic and cardiovascular function is not known. We hypothesized that offspring of maternal rats made diabetic during the last third of gestation would demonstrate altered growth, glucose metabolism and vascular reactivity. To address this hypothesis, we examined glucose tolerance, insulin sensitivity and aortic reactivity to vasodilatory and vasorelaxing agents in 6-8 mo old offspring of rats made diabetic during the last week of pregnancy.

METHODS

Animals

All procedures were performed within the regulations of the Animal Welfare Act and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved by the Institutional Animal Care and Use Committee of the University of Iowa. Pregnant Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were injected with either streptozotocin (STZ, 50 mg/kg, 10mM citrate buffer, pH 3.5; IP) or an equivalent volume of citrate buffer on gestational day 13. STZ injected rats had blood glucose measured twice daily via tail-nicking using the LifeScan OneTouch Ultra Blood Glucose Monitoring System (LifeScan Inc., Milpitas, CA). Hyperglycemia was partially controlled and ketosis avoided by subcutaneous injection of regular insulin (Humulin, Eli Lilly and Co., Indianapolis, IN) in the morning and insulin-glargine (Lantus; Sanofi-Aventis, Bridgewater, NJ) each evening to maintain blood glucose between 100-400 mg/dl. Control rats were injected with saline twice daily. Dams were allowed to delivery spontaneously. All pups were cross-fostered to non-diabetic post-partum dams. Litters were culled to 10 pups, selecting to preserve the largest pups. Pups were weaned to standard rat chow on day of life 21.

Glucose Tolerance and Insulin Sensitivity

Glucose tolerance was assessed in all offspring at 2 and 6 months of age after 3-5 hour fast by measuring blood glucose at baseline and 15, 30, 60 and 120 min after intraperitoneal injection of 2 g/kg dextrose (20% solution). Testing was typically performed in the early afternoon (12:00 – 14:00). Serum insulin was measured at baseline and 30 min after the injection using a rat insulin ELISA kit (Crystal Chem Inc, Downer's Grove, IL). An intravenous glucose tolerance test was performed in a subset of the 5 mo old offspring after overnight fast by measuring central venous blood glucose and serum insulin at baseline and 1, 2, 3, 5, 10, 15, 25, 40 and 60 min after 0.5g/kg dextrose infused via the left carotid artery. In a separate subset of offspring, insulin sensitivity was measured in overnight fasted offspring by euglycemic-hyperinsulinemic clamp, infusing regular insulin at 20 mU/kg/min via a right internal jugular catheter and adjusting the dextrose infusion to maintain blood glucose at a target of ~90 mg/dl. Euglycemic-hyperinsulinemic clamps and ivGTTs were performed as terminal procedures under pentobarbital anesthesia, utilizing acute catheterization of the left carotid artery and right jugular vein.

Vascular reactivity vessel preparation and arteriography mounting

Offspring used for vascular reactivity studies (n = 14 female, 14 male) were products of diabetic (n = 5) or control (n = 5) female rats. At 6-8 mo of age, offspring were euthanized and descending thoracic aorta segments harvested, cleansed of adherent connective tissue and sectioned into 2-mm-long rings. Two ring segments were denuded of endothelium by inserting forceps tips into the lumen and gently rolling the vessel ring. Aortic rings were mounted in individual 18-ml isolated organ chambers (Radnoti Glass Technology Inc., Monrovia, CA)

and connected to an isometric force transducer. Contractile responses were recorded with a MacLab 8E (ADInstruments, Colorado Springs, CO) and stored on a Power Macintosh 8600 computer. Passive stretch was set at 90% of the tension required to obtain peak responses to potassium chloride (KCl) (2.5 g, determined in preliminary studies) and the rings were allowed to equilibrate in bicarbonate-buffered physiological salt solution (PSS) at 37°C for 60 min before the start of experimentation. The composition of the PSS was as follows (in mM): 130 NaCl, 4.7 KCl, 1.18 KH₂PO₄, 1.17 MgSO₄·7 H₂O, 14.9 NaHCO₃, 1.6 CaCl₂·H₂O, 5.5 dextrose and 0.03 CaNa₂-EDTA (pH 7.30). PSS was aerated with a mixture of 95% O₂-5% CO₂.

Experimental protocols for vascular function

Separate baths were used to assess the cumulative concentration responses to angiotensin II (ANG II, 10⁻¹¹ to 10⁻⁷ M), endothelin-1 (ET-1, 10⁻¹¹ to 10⁻⁷ M), noradrenaline (NA, 10⁻¹¹ to 10⁻⁷ M) and serotonin (5-HT, 10⁻¹¹ to 10⁻⁷ M). Arteries were re-equilibrated with washes of PSS before measurement of vasoconstrictor responsiveness in the presence of N^G-nitro-L-arginine (L-NNA, 10⁻⁵ M). Separate baths were used to assess cumulative concentration-vasorelaxant responses to sodium nitroprusside (SNP, 10⁻⁹ to 10⁻⁵ mmol/l) or acetylcholine (ACh, 10⁻¹⁰ to 10⁻⁷ mmol/l) after precontraction with NA (10⁻⁵ mmol/l). All PSS reagents and vasoactive compounds were acquired from Sigma with the exception of ET-1 (Alexis, San Diego, CA).

Morphometric analysis

Distal segments of thoracic aorta were incubated for 10 min in PSS containing 10⁻⁵ M sodium nitroprusside, fixed in Pen-Fix (Richard Allen Scientific, Kalamazoo, MI) and paraffin embedded. Rings were cut in cross section, mounted on glass slides and stained. Sections were imaged using a digital camera and Spot software (Diagnostic Instruments, Sterling Heights, MI). Aortic media thickness, external vessel diameter, luminal diameter, and media thickness were measured at six points and averaged from two sections per vessel.

Statistical Analysis

Comparisons between groups were performed by Student's unpaired, two-tailed *t*-test or ANOVA, factoring for treatment group, drug concentration and presence of endothelium. If ANOVA identified significant differences (*P* < 0.05), pairwise comparisons were made using the Tukey test, with *P* < 0.05 considered significant. Variances were compared using the variance ratio test (F-test). Genders were analyzed separately. Data are reported as means ± SE.

RESULTS

Dams injected with STZ had peak blood glucose levels on gestational days 15 and 16 and remained hyperglycemic throughout the remainder of gestation (Figure 1). The number of pups per litter were similar in the STZ group (12.6 ± 1.1) and the control (CON) group (13.4 ± 0.5) (*p*=0.54). Likewise, there were no group differences in the gestational day of delivery. A total of 87 male (offspring of diabetic mothers (ODM), *n*=45; CON, *n*=42) and 38 female (ODM, *n*=18; CON, *n*=20) offspring from 7 diabetic and 7 control dams were used for the study. Differences in male and female numbers likely resulted from culling of the smallest pups at birth without attention to gender.

Birth weight and growth

Perinatal weights of CON offspring exhibited a bell shaped distribution (Figure 2). In contrast, ODM exhibited an increased number of high and low birthweight pups. There was no difference in mean birthweights between male ODM and CON pups, however, the variance in birthweight

among male ODM pups was 3.7 fold higher than among the CON group ($p < 0.0001$). A similar pattern was also in females. Male ODM weight gain was initially similar to CON offspring, began to lag behind at approximately two months of age, and remained slightly less at 140 d (absolute weights at 140 d: 630 ± 7 vs. 611 ± 9 gm, CON and ODM, respectively, $p < 0.05$). No differences in growth were detected among female offspring. For male ODM, birth weight was a positive predictor of weight at young ages, but transitioned to a negative predictor of weight persisting through at least 140 days ($r = -0.36$, $p < 0.05$, $n = 44$). This negative correlation was not observed in the CON offspring, however a similar trend was apparent among the female ODM. Animal weights at the time of metabolic and vascular testing, outlined below, were not significantly different between groups at any time point.

Glucose Tolerance and Insulin Sensitivity

Glucose tolerance was measured at 2 and 5 months of age. Glucose values were lower in 2 mo old male ODM at 30 min compared to (CON 225 ± 8 vs. 248 ± 7 mg/dl, $p < 0.05$), no other differences between groups were identified at any time point (Figure 3). Plasma insulin values at 0 and 30 minutes during the GTT were not different between experimental groups (data not shown).

Intravenous glucose tolerance tests (performed only in male offspring at 6 mo of age) demonstrated no difference in glucose levels between ODM and CON offspring (Figure 4). Euglycemic hyperinsulinemic clamp demonstrated significant insulin resistance among male ODM, as they required 30% less glucose at steady state (120-140 minutes of insulin infusion) than the CON offspring to maintain euglycemia (17.0 ± 0.8 mg/kg/min vs. 24.3 ± 1.5 mg/kg/min for ODM and CON, respectively, $p < 0.05$).

Vascular function in female offspring of diabetic mothers

Aorta segments from female ODM exhibited similar maximal responses to KCl (90 mM) compared to CON, although responses at lesser concentrations were slightly but significantly increased ($P < 0.05$) (Figure 5A). Vasoconstrictive responses to 5-HT, NA, and ET-1 were significantly enhanced in aorta from ODM relative to controls (Figures 5B-D); responses to ANG II were not different (Figure 5E).

As expected, removal of the endothelium increased the constrictive responses to 5-HT, NA and ET-1 in both groups (except CON ET-1) (Figures 5B-D). However, the differences in the responses to 5-HT and NA between ODM and CON were lost, suggesting endothelium buffers vasoconstriction to a greater extent in CON compared to ODM. In the presence of the L-NNA, CON but not ODM aorta displayed an increased vasoconstrictive response to ET-1 and NA (Figure 5F). (Figure 5G). As a result, the vasoconstrictive response of ODM and CON aorta to NA were similar in the presence of L-NNA, whereas the response to ET-1 remained greater in ODM.

Relaxation was investigated by examining responses to the endothelium independent vasodilator SNP and the endothelium dependent vasodilator Ach. In endothelium intact aorta, relaxation to SNP was similar in both groups (Figure 6A). However, responses to acetylcholine were markedly different between groups, relaxation being significantly greater in aortas from CON offspring compared to ODM (Figure 6B). In endothelial-denuded aortas, responses to SNP were decreased in both groups; however, the attenuation was significantly greater in ODM than controls.

Vascular Dimensions—Sections of distal thoracic aorta were used to examine external vessel diameter, luminal diameter, and media thickness. No significant differences between female ODM and CON offspring were identified for any of these parameters (Figure 7).

Male offspring of diabetic mothers

No significant differences between male groups in the contractile responses to KCL, 5-HT, ANG II, or ET-1 were seen (Figures 8A-D). The response to NA was significantly greater in intact aorta from ODM compared to CON, similar to that seen in the females (Figure 8E). In endothelium denuded vessels, contractile responses to 5-HT, ET-1 and NA were similar in the two groups (Figures 8B, D, E) while responses to ANG II were significantly enhanced in ODM compared to controls (Figure 8C). The presence of L-NNA resulted in increased contractile responses to NA in CON but had no effect of ET-1 in either group (Figures 8F, G).

Vasorelaxation responses to SNP and Ach were also significantly different between groups. Specifically, intact aorta from male ODM displayed greater relaxation to SNP and Ach compared to control animals ($p < 0.05$, Figures 9A, B). This finding is in sharp contrast to that displayed in female ODM, where decreased sensitivity to Ach and SNP were observed in ODM relative to controls. Removal of endothelium abolished the between group differences in response to SNP.

Male ODM displayed significantly decreased aorta wall diameter and wall area compared to controls ($p < 0.05$, Figure 7). No differences were detected in aorta lumen area between groups.

DISCUSSION

Exposure to diabetes *in utero* is a significant risk factor for development of components of metabolic syndrome, including glucose intolerance, insulin resistance and hypertension [4, 16]. The majority of previous studies evaluating the effects diabetes during pregnancy have studied offspring of mothers made diabetic before, or relatively early in gestation. We have developed a rat model of diabetes with maternal hyperglycemia limited to the last third of pregnancy, at a time beyond the embryonic development period (<http://embryology.med.unsw.edu.au/OtherEmb/Rat.htm>). Our model results in a spectrum of birthweights, with ODM maintaining relatively normal glucose tolerance, but males developing insulin resistance. Female, but not male ODM also displayed altered vascular reactivity, suggesting gender-specific cardiovascular dysfunction can be induced in offspring of mothers displaying a “gestational-onset” form of diabetes.

Our model of late gestation maternal hyperglycemia produced both microsome and macrosomic pups. The heavier pups tended to come from mothers with mild hyperglycemia, whereas more severe hyperglycemia predicted underweight pups ($r = -0.54$ $p = 0.0001$). Postnatal weight gain among male ODM was slightly reduced, consistent with a prior study using STZ to induce more severe gestational diabetes [12]. Pre-gestational maternal diabetes induced by STZ has produced both large [17,18] or small [9,11,19,20] offspring. In these studies, adult size correlated with birthweight [9,17-19,21]. In contrast in our study, birth weight inversely correlated with adult size.

In humans, *in utero* exposure to diabetes is associated with long term risk of developing type 2 diabetes [5] and/or the insulin resistance syndrome [4]. Likewise, the male offspring in our study developed insulin resistance by 6 months of age despite initially improved glucose tolerance at 2 months of age. Similar results have been observed by other investigators in the offspring of STZ-induced diabetic mothers [12]. In contrast, offspring of mothers with hyperglycemia induced by glucose infusion experience impaired glucose tolerance due to progressive loss of glucose-stimulated insulin secretion [22]. The reasons for this discrepancy are unclear, though the infusion model provides excess glucose-based calories, potentially reducing normal dietary intake of other nutrients by the mothers.

The STZ model of maternal diabetes has been used by other investigators to examine cardiovascular consequences in the offspring. Holemans et al. [10] reported that sensitivity of mesenteric arteries to noradrenaline was enhanced in female offspring of mothers made diabetic on day one of pregnancy with STZ while sensitivity and maximum relaxation to Ach but not SNP were reduced. Our findings in female ODM of impaired relaxation to Ach, but not SNP, are consistent with those of Holemans and suggest exposure to maternal hyperglycemia even late in gestation results in offspring with endothelial dysfunction. Because relaxation to SNP was similar in the two groups, it is less likely there are intrinsic differences in vascular smooth muscle sensitivity to nitric oxide. The presence of endothelial dysfunction is further suggested by the findings that between group differences in vasoconstrictive responses were attenuated by removal of endothelium and that L-NNA enhanced vasoconstrictive responses to ET-1 and NA in CON offspring by not ODM.

The gender specific differences in vascular responsiveness in ODM, with females being primarily affected, are distinct from findings of other investigators. Rocha et al. found that the *in vivo* response of mesenteric microvessels to Ach and bradykinin, but not SNP, were significantly attenuated in male offspring of dams made diabetic prior to mating [14]. Male offspring from dams treated with STZ on the 7th day of pregnancy also exhibited increased vascular responsiveness to phenylephrine but not SNP [7]. In contrast, we found that intact aorta from male ODM and CON offspring displayed similar contractile responses to KCl, ANG II, 5-HT, or ET-1. In addition, aorta from male ODM displayed significantly greater relaxation to Ach and SNP compared to control animals, suggesting enhancement of smooth muscle mediated vasodilation. Studies in other models of adverse fetal environments also demonstrate sex differences in the pathophysiological responses, with female offspring having a lower incidence of hypertension and vascular dysfunction [23]. A role for sex hormones interacting with other regulatory mechanism, including the renin-angiotensin system, has been suggested. Reasons for the discrepant findings in effected gender between our study and others are unclear, but may be related to underlying differences in the models and timing of the *in utero* insult.

Vessel morphometry, specifically aorta diameter, luminal diameter, and media thickness were not different between female groups. Similarly, Holemans et al. found no differences in vessel internal diameter [8]. However, in male offspring, aorta wall diameter and wall area were significantly decreased in ODM compared to controls. This finding suggests the diabetic environment *in utero* resulted in alterations in vascular smooth muscle development in males. The effects of in utero hyperglycemia on vascular smooth muscle cell proliferation, have to our knowledge, not been explored.

Blood pressure was not measured in the animals used in this study. Thus, it is possible that the alterations in vascular reactivity are secondary changes due to hypertension rather than primary effects of an altered in-utero environment. Holemans et al. found that female offspring of diabetic mothers had normal blood pressures [12] while male offspring of dams made diabetic prior to mating or on the 7th day of pregnancy were hypertensive by 2-3 mo of age [10]. Rocha et al. also found male offspring of dams made diabetic prior to mating were hypertensive by 3 mo of age relative to controls [11].

Several additional limitations of the present study exist. Because we used STZ to induce maternal diabetes, it is possible that the agent crossed the placenta and had direct effects on the fetus. However, we believe this to be unlikely since insulin levels were similar in young offspring regardless of exposure to STZ. Schroeder et al. also found that administration of STZ to pregnant dams at 12-13 had no significant effects on fetal (day 20-21) insulin values [15]. Our model is also one of maternal insulinopenia and hyperglycemia and not gestational diabetes, a condition of insulin resistance. Additionally, the vascular reactivity studies were performed in aorta, and the findings may not be reflective of endothelial and vascular smooth

muscle function in resistance vessels. Finally, culling of the smallest newborns resulted in a bias of studying the larger offspring.

The majority of other studies using the STZ model to investigate cardiovascular or metabolic function in offspring of diabetic mothers have not cross-fostered offspring after birth. Because milk volume or content may be altered in diabetic rats, programming effects in these studies may be related to postnatal nutrition. Since all offspring were cross fostered in the present study, the metabolic and cardiovascular changes we identified in ODM are likely related to the abnormal intrauterine environment rather than postnatal nutrition. Diabetes increases the levels of many macronutrients in the maternal circulation, including glucose, triglyceride, non-esterified fatty acids, and ketone-bodies [24,25]. Most metabolic fuels are readily transferred from the maternal circulation across the placenta either by passive diffusion, facilitated diffusion or by facilitated transport [26]. Thus, the growing fetus is exposed to an oversupply of most circulating fuels during diabetic pregnancy. It is not known which macronutrients may be deleterious to the offspring with regards to long term health.

Our results suggest that exposure to a hyperglycemic milieu during the last third of gestation results in sex specific metabolic and cardiovascular abnormalities in the offspring. The consequences of being born to a diabetic mother present tremendous health implications. With improved understanding of the cellular and molecular responses of the metabolic pathways and the cardiovascular system to the intrauterine diabetic milieu, it may be possible that early pharmacologic interventions can be designed to prevent or attenuate the development of disease later in life.

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Abbreviations

Ach	Acetylcholine
ANG II	Angiotensin II
CON	Control
ET-1	Endothelin 1
KCl	Potassium chloride
L-NNA	<i>N</i> ^G -nitro-L-arginine
NA	Noradrenaline
ODM	Offspring of diabetic mothers
SNP	Sodium nitroprusside
STZ	Streptozotocin
5-HT	Serotonin

Figure 1. Daily blood glucose values in pregnant dams with diabetes induced by injection of STZ (■) on day 13 of gestation and sham controls (●). Glucose values represent average daily values. Values are means $\pm$ SE. ($p < 0.05$ between groups for all data points.) The mean values in the control groups ranged from 97.8 to 99.4 mg/dl with SE range of 2.73 - 4.08 mg/dl.

Figure 2. Weight distribution of 1 day old male (A) and female (B) rat pups of diabetic (STZ) (—) or control mothers (■). The distribution is shown as a normalized frequency plot. Tick marks representing weights of each pup are shown below the x-axis. N, number of animals in each group.

Figure 3.

Blood glucose levels during intraperitoneal glucose tolerance tests in female offspring of a diabetic (ODM, (■), n = 17 at 2 and 5 months) and control (CON (●), n = 19 at 2 and 5 months) and in male offspring of diabetic (n = 44 at 2 months, n = 42 at 5 months) and control (n = 39 at 2 months, n = 37 at 5 months) mothers. A, females at 2 months; B, males at 2 months; C, females at 5 months; D, males at 5 months. Values are means $\pm$ SE. * p < 0.05 compared to control at same time point. Total area under the curve, as determined by the trapezoidal rule, was not significantly different between CON and ODM offspring for either gender at any age.

Figure 4.

Blood glucose (left panel) and plasma insulin (right panel) levels during intravenous glucose tolerance tests in 6 mo old male offspring of diabetic (ODM (■), n = 6) or control (CON, n = 6 (●)) rats. Values are means $\pm$ SE. No differences in any time point or in total area under the curve, as determined by the trapezoidal rule, were detected.

Figure 5.

Female aortic responsiveness to KCl (A), serotonin (5-HT) (B), noradrenaline (NA) (C), endothelin-1 (ET-1) (D), and angiotensin II (ANG II) (E) in the presence or absence (rubbed) of vascular endothelium and to noradrenaline (NA) (F) and endothelin-1 (ET-1) (G) in the presence or absence of L-NNA ($n = 7$ for each group). For figures A-E: endothelium intact ODM (■), intact control (●), rubbed ODM (▣), rubbed control (◐). For figures F,G: ODM (■), control (●), ODM + L-NNA (▣), control + L-NNA (◐). Values are means $\pm$ SE. ** $p < 0.05$ between endothelium intact ODM and intact control at specific concentrations. * $p < 0.05$ between rubbed and intact within same treatment groups (ANOVA). ¶ $p < 0.05$ between intact ODM and intact control (for all figures), or between rubbed ODM and rubbed control (figure B-E) or between L-NNA treated ODM and L-NNA treated controls (figures F,G) (treatment group effect, ANOVA).

Figure 6.

Concentration-response curves for female aorta vasodilation produced by sodium nitroprusside (A) and acetylcholine (B) ($n = 7$ for each group). Endothelium intact ODM (■), intact control (●), rubbed ODM (□) and rubbed control (○). Values are means $\pm$ SE. * $p < 0.05$ between intact and rubbed within same treatment group (ANOVA). ¶ $p < 0.05$ between intact ODM and intact control, or between rubbed ODM and rubbed control (treatment group effect, ANOVA).

Figure 7. Abdominal aorta lumen area and wall area and diameter in female (left column) and male (right column) offspring of control (black columns) and diabetic (white columns) mothers. Values are means $\pm$ SE. * $p < 0.05$ compared to control.

Figure 8.

Male aortic responsiveness to KCl (A), serotonin (5-HT) (B), angiotensin II (AII) (C), endothelin-1 (ET-1) (D), and noradrenaline (NA) (E) in the presence or absence (rubbed) of vascular endothelium and to ET-1 (F) and NA (G) in the presence or absence of L-NNA ($n = 7$ for each group). For figures A-E: endothelium intact ODM (—■—), intact control (—●—), rubbed ODM (---□---), rubbed control (---○---). For figures F,G: ODM (—■—), control (—●—), ODM + L-NNA (---■---), control + L-NNA (---○---). Values are means $\pm$ SE. * $p < 0.05$ between rubbed and intact within same treatment groups (ANOVA). ** $p < 0.05$ between intact ODM and intact control (for all figures), or between rubbed ODM and rubbed control (figure B-E) (treatment group effect, ANOVA).

Figure 9. Concentration-response curves for male aorta vasodilation produced by sodium nitroprusside (A) and acetylcholine (B) ($n = 7$ for each group). Endothelium intact ODM (■), intact control (○), rubbed ODM (●) and rubbed control (□). Values are means $\pm$ SE. * $p < 0.05$ between intact and rubbed within same treatment group (ANOVA). ‡ $p < 0.05$ between intact ODM and intact control (treatment group effect, ANOVA).

Table

Animal weights (grams) at times of metabolic and vascular testing.

		<u>ipGTT (2 mo)</u>	<u>ipGTT (6 mo)</u>	<u>Clamp</u>	<u>ivGTT</u>	<u>VF</u>
Female	Control	243 ± 4	362 ± 8	–	–	422 ± 17
	ODM	237 ± 6	371 ± 10	–	–	444 ± 18
Male	Control	362 ± 5	660 ± 8	638 ± 18	650 ± 18	767 ± 16
	ODM	358 ± 5	642 ± 10	636 ± 21	712 ± 37	719 ± 29

ODM, offspring of diabetic mothers; ipGTT, intraperitoneal injection of dextrose for glucose tolerance; Clamp, euglycemic-hyperinsulinemic clamp; ivGTT, intravenous injection of dextrose for glucose tolerance; VF, vascular function. Clamp and ivGTT not performed in females. Data are mean ± SE.