Previous gestational diabetes impairs long-term endothelial function in a mouse model of complicated pregnancy

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Stanley JL, Sankaralingam S, Baker PN, Davidge ST. Previous gestational diabetes impairs long-term endothelial function in a mouse model of complicated pregnancy. Am J Physiol Regul Integr Comp Physiol 299: R862–R870, 2010. First published June 16, 2010; doi:10.1152/ajpregu.00200.2010.—Women who develop gestational diabetes mellitus (GDM) display endothelial dysfunction up to 1 yr after pregnancy, despite a return to normoglycemia. It is unknown whether this dysfunction was preexisting or whether GDM pregnancy leads to long-term endothelial dysfunction. A mouse model that spontaneously develops GDM (Leprdb/db) was used to determine whether the endothelial dysfunction that develops during GDM is evident in later life. Heterozygous and wild-type (WT) controls were allowed to litter once, then age to 9–10 mo, and were compared with virgin controls. Vascular function of small mesenteric arteries was assessed using wire myography. Concentration response curves to the thromboxane A2 mimetic U46619 and the endothelium-dependent vasodilator methacholine were constructed. Superoxide production and peroxynitrite formation was also measured. Mice with previous GDM displayed blood glucose concentrations similar to previously pregnant WT mice (8.0 ± 0.1 vs. 7.1 ± 0.3 mmol/l, P > 0.05). Arteries from mice with previous GDM displayed increased sensitivity to U46619 (EC50 5.2 ± 0.7 vs. 45.2 ± 1.0 mmol/l, P < 0.01) and impaired endothelium-dependent relaxation compared with WT controls (29 ± 8 vs. 58 ± 16 percent relaxation, P < 0.05). This was associated with increased superoxide production (93.3 ± 2.3 vs. 64.6 ± 1.6 mean fluorescence intensity, P < 0.001) and increased peroxynitrite formation (173.5 ± 11.0 vs. 57.4 ± 16.2 mean fluorescence intensity, P < 0.01) compared with virgin controls. In summary, endothelial dysfunction was evident in mice with previous GDM compared with previously healthy pregnant mice or virgin controls. These data suggest that GDM affects endothelial function and may contribute to an increased risk of cardiovascular disease.

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observed (11, 23); these differences parallel the signs observed in women with GDM, and the db/db model was thus used in the studies described herein. By using this model, we will be able to assess the long-term effects of previous GDM on endothelial function in small systemic arteries. Mice with a previously healthy pregnancy (Lepr+/−) and virgin control mice will also be assessed to confirm the impact of previous GDM on endothelial function i.e., the impact of a pregnancy complicated by GDM may be separated from that of a healthy pregnancy and the genetic differences. We hypothesize that small systemic arteries from mice with previous GDM will demonstrate endothelial dysfunction compared with mice that had a healthy pregnancy or virgin controls.

MATERIALS AND METHODS

All protocols were approved by the University of Alberta Health Sciences Animal Policy and Welfare committee in accordance with the Canadian Council on Animal Care and conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Animal Model of GDM

Twelve-week-old virgin female mice, heterozygous for a leptin receptor mutation (Leprdb/db) or WT littermate controls (Lepr+/-), were used (24 mice total, acquired from the Jackson Laboratory). WT and He females (n = 6) were housed overnight with control males; the presence of a vaginal plug the following morning was deemed day 0.5 of gestation. Mice were weighed at day 18.5 of gestation, and a blood sample was collected via venopuncture to measure blood glucose concentration (to confirm development of GDM). Mice were allowed to litter and nurse their pups, which were weaned at 3 wk of age. The dams were then group housed according to genotype. At 9–10 mo of age (6–7 mo after the index pregnancy) mesenteric artery function was assessed. Arteries obtained from age-matched virgin control mice (both He and WT, n = 6) were also investigated.

Blood Glucose Concentration

Fasting blood glucose concentration was measured at day 18.5 of gestation, then again at 9 mo of age. Mice were fasted for 3 h before testing. A blood sample was collected following tail venopuncture, and blood glucose concentration was measured using a glucometer (Accu-Check Advantage; Roche Diagnostics, Mannheim, Germany).

Wire Myography

Mice were euthanized at 9–10 mo. The mesentery and the surrounding tissue were removed and placed in ice-cold physiological salt solution (PSS) (in mmol/l: 10 HEPES, 1.56 CaCl2, 142 NaCl, 4.7 KCl, 1.17 MgSO4, 1.18 KH2PO4, 5.5 glucose; pH 7.4). Vascular function was assessed in second-order mesenteric arteries; these are demonstrated that denuded murine second-order mesenteric arteries do not relax in response to MCh, thus confirming the endothelial-dependency of this response. Vessels were again washed with PSS and then incubated for 20 min with an NOS inhibitor (N-nitro-L-arginine methyl ester; L-NAME, 100 μmol/l; Sigma), a cyclooxygenase (COX) inhibitor (meclofenamate, 1 μmol/l; Sigma), or left in PSS to serve as a time control. Concentration-response curves to MCh were then repeated. Vessels were again washed with PSS, constricted with U46619, and a concentration-response curve to the endothelium-independent vasodilator sodium nitroprusside (SNP; 0.1–10 μmol/l) was constructed. Finally, 7 ml of 120 mmol/l potassium solution (KPSS; in mmol/l: 10 HEPES, 24 NaCl, 124 KCl, 2.4 MgSO4, 4.9 CaCl2, 1.18 KH2PO4, 5.5 glucose; pH 7.4) was added to each vessel segment and the constriction allowed to plateau.

Superoxide Production and Peroxynitrite Formation

Superoxide production was assessed using dihydroethidium according to a previously developed protocol (19). Peroxynitrite formation was measured using dihydrorhodamine. Twenty-micrometer second-order mesenteric artery sections were prepared and stored at −80°C. Before use, sections were equilibrated for 10 min at 37°C using HBSS (GIBCO). Sections were then incubated with 10 μmol/l dihydroethidium (Molecular Probes, Mississauga, ON, Canada) or 25 μM dihydrorhodamine (Sigma) in HBSS for 20 or 25 min, respectively, at 37°C. Sections were then washed with HBSS ×3, and coverslips applied. Slides were visualized immediately, and images were taken using a fluorescence microscope (Olympus IX 81) using the CY3 filter. Images were analyzed using Adobe Photoshop to determine mean fluorescence intensity/pixel in each vessel. Two arterial sections per animal were used, and the mean value was determined; arteries from three animals per group were studied.

Statistical Analysis

The distribution of all data was tested for normality using the Kolmogorov-Smirnov test prior to statistical analysis. All normally distributed data are expressed as means ± SE and were compared using a two-way ANOVA followed by Bonferroni post hoc test. Sigmoidal curve fitting was performed on wire myography concentration-response curve data using Graphpad Prism software; these curves were then used to determine EC50 and EC80 values.

RESULTS

Body Weight and Fasting Blood Glucose Concentration

The effect of previous GDM on both body weight and fasting blood glucose concentration was assessed. At 9 mo of age, He mice that were previously pregnant were heavier than all other mice, but their fasting blood glucose concentration was not significantly different (Fig. 1). The increase in body weight following GDM reflects the larger increase in weight gain observed during pregnancy. He mice gained significantly more weight during pregnancy than their WT controls (19.3 vs. 13.2 g; P < 0.01). Pregnant He mice also displayed a significant increase in blood glucose concentration compared with pregnant WT mice (7.9 ± 0.5 vs. 5.1 ± 0.1 mmol/l; P < 0.01).
Effect of Previous Gestational Diabetes on Mesenteric Artery Function

**Agonist-induced vasoconstriction.** Previous GDM had a significantly affected endothelium-dependent vasodilation, and a significant interaction between genotype and previous pregnancy was observed \((P < 0.01)\). MCh-induced maximum relaxation of arteries from mice with previous GDM was significantly reduced compared with those from previously pregnant WT mice \((29 \pm 8\% \text{ vs. } 58 \pm 16\% \text{ relaxation}; \ P < 0.05, \text{Fig. 3A})\). The maximum relaxation response of arteries from virgin mice, or those with a previously healthy pregnancy, was in line with that previously observed by our laboratory \((5)\). Similarly, sensitivity of arteries from mice with previous GDM to MCh was significantly decreased compared with that of arteries from previously pregnant WT mice \((P < 0.01, \text{Fig. 3B})\). In comparison, there was no effect of genotype in virgin animals. Maximal relaxation of arteries from virgin WT and He mice \((54 \pm 5 \text{ and } 57 \pm 12\% \text{ relaxation}, \text{respectively}; \text{Fig. 3A})\) was comparable to that seen in arteries from previously pregnant WT mice. There was also no difference in sensitivity between vessels from never-pregnant WT and He mice \((\text{Fig. 3B})\).

The effect of the endothelium-independent vasodilator SNP was also assessed. There was no effect of previous GDM on this response \((\text{Fig. 4})\).

Fig. 1. Body weight was significantly increased in mice with previous gestational diabetes mellitus (GDM) but fasting blood glucose was unchanged. A: there was a significant increase in body weight in mice with previous GDM compared with their virgin counterparts, as well as with both groups of wild-type \((\text{Lepr}^{+/+}; \text{WT})\) mice. B: there was no difference in fasting blood glucose between virgin WT and heterozygous for a leptin receptor mutation \((\text{Lepr}^{db/db}; \text{He})\) mice. This did not change following pregnancy. Means \(\pm SE, \text{n} = 6; \text{two-way ANOVA, } **P < 0.01\).

**Effect of Previous Gestational Diabetes on Mesenteric Artery Function**

In comparison, there was no effect of genotype on maximum constriction in response to U46619 in mesenteric arteries obtained from virgin WT and He mice \((136 \pm 12 \text{ and } 151 \pm 18\% \text{ maximum response to KPSS; Fig. 2A})\). Furthermore, there was also no difference in sensitivity between arteries from virgin WT and He mice \((\text{Fig. 2B})\).

**Agonist-induced vasodilation.** Previous GDM also significantly affected endothelium-dependent vasodilation, and a significant interaction between genotype and previous pregnancy was observed \((P < 0.01)\). MCh-induced maximum relaxation of arteries from mice with previous GDM was significantly reduced compared with those from previously pregnant WT mice \((29 \pm 8\% \text{ vs. } 58 \pm 16\% \text{ relaxation}; \ P < 0.05, \text{Fig. 3A})\). The maximum relaxation response of arteries from virgin mice, or those with a previously healthy pregnancy, was in line with that previously observed by our laboratory \((5)\). Similarly, sensitivity of arteries from mice with previous GDM to MCh was significantly decreased compared with that of arteries from previously pregnant WT mice \((P < 0.01, \text{Fig. 3B})\). In comparison, there was no effect of genotype in virgin animals. Maximal relaxation of arteries from virgin WT and He mice \((54 \pm 5 \text{ and } 57 \pm 12\% \text{ relaxation}, \text{respectively}; \text{Fig. 3A})\) was comparable to that seen in arteries from previously pregnant WT mice. There was also no difference in sensitivity between vessels from never-pregnant WT and He mice \((\text{Fig. 3B})\).

The effect of the endothelium-independent vasodilator SNP was also assessed. There was no effect of previous GDM on this response \((\text{Fig. 4})\).
relaxation compared with that seen with MCh alone (44 ± 10 vs. 51 ± 6% relaxation; Fig. 6A). When arteries from mice with previous GDM were investigated, however, l-NAME significantly reduced maximal relaxation compared with that seen in response to MCh alone (7 ± 4% vs. 29 ± 8% relaxation; Fig. 6D). Similar effects were seen when vessels were incubated with l-NAME and meclofenamate in combination. There was no effect on relaxation in arteries from previously pregnant WT animals (38 ± 3% relaxation; Fig. 6C). The maximal response seen in arteries from mice with previous GDM was significantly impaired (10 ± 5% relaxation; Fig. 6F). Again there was no effect of meclofenamate on arteries from either previously pregnant WT mice or mice with previous GDM (50 ± 3% and 27 ± 10% relaxation; Fig. 6, B and E).

Superoxide Production and Peroxynitrite Formation

Similarly, previous GDM had a significant effect on the production of superoxide in mesenteric arteries, and a significant interaction between genotype and previous pregnancy was observed (P < 0.01). When superoxide production was measured in arteries taken from WT mice, there was a significant reduction in vessels from previously pregnant mice (Fig. 7C) compared with those from virgin controls (Fig. 7A, 64.6 ± 1.6 vs. 84.1 ± 2.1, mean fluorescence intensity; P < 0.01; Fig. 7E). In comparison, superoxide production in arteries taken from mice with previous GDM (Fig. 7D) was significantly increased compared with that seen in arteries from virgin He mice (Fig. 7B, 93.3 ± 2.3 vs. 59.6 ± 1.7, mean fluorescence intensity; P < 0.01, Fig. 7E). There was also a significant reduction in superoxide production in arteries from virgin He mice compared with virgin WT mice (P < 0.01, Fig. 7E).

Previous GDM had a significant effect on peroxynitrite formation, and a significant interaction between genotype and pregnancy was demonstrated (P < 0.01). Peroxynitrite formation was significantly increased in arteries from mice with previous GDM compared with their virgin controls (173.5 ± 11.0 vs. 57.4 ± 16.2, mean fluorescence intensity; P < 0.01; Fig. 8). There was also a significant increase in peroxynitrite formation in arteries from mice with previous GDM compared with arteries from previously pregnant WT mice (133.7 ± 11.3, mean fluorescence intensity; P < 0.05; Fig. 8). Similar to

Mechanisms of Endothelium-Dependent Vasodilation

To determine the effect of previous GDM on mechanisms of endothelial-dependent relaxation, the response to MCh was assessed in the presence of inhibitors of NOS (l-NAME) and COX (meclofenamate). This demonstrated a significant effect of previous GDM on the mechanisms of endothelial-dependent relaxation. When arteries from virgin WT and He mice were investigated, maximal relaxation was significantly reduced in the presence of l-NAME compared with that seen in response to MCh alone (16 ± 7 vs. 51 ± 6% relaxation and 14 ± 10 vs. 50 ± 12% relaxation, respectively, Fig. 5, A and D). A similar reduction of maximal relaxation was observed in arteries from virgin WT and He mice in the presence of l-NAME and meclofenamate (23 ± 11% and 24 ± 7% relaxation, Fig. 5, C and F). There was, however, no effect of meclofenamate alone in vessels from either virgin WT or He mice (39 ± 8 and 52 ± 11% relaxation, respectively, Fig. 5, B and E).

In comparison, when arteries from previously pregnant WT mice were used, there was no effect of l-NAME on maximal relaxation compared with that seen with MCh alone (44 ± 10 vs. 51 ± 6% relaxation; Fig. 6A). When arteries from mice with previous GDM were investigated, however, l-NAME significantly reduced maximal relaxation compared with that seen in response to MCh alone (7 ± 4% vs. 29 ± 8% relaxation; Fig. 6D). Similar effects were seen when vessels were incubated with l-NAME and meclofenamate in combination. There was no effect on relaxation in arteries from previously pregnant WT animals (38 ± 3% relaxation; Fig. 6C). The maximal response seen in arteries from mice with previous GDM was significantly impaired (10 ± 5% relaxation; Fig. 6F). Again there was no effect of meclofenamate on arteries from either previously pregnant WT mice or mice with previous GDM (50 ± 3% and 27 ± 10% relaxation; Fig. 6, B and E).

Superoxide Production and Peroxynitrite Formation

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the superoxide levels, there was a significant reduction in peroxynitrite formation in arteries from virgin He mice compared with the WT mice ($P < 0.05$, Fig. 8).

**DISCUSSION**

This study investigated the vascular function of small systemic arteries from 9-mo-old mice with previous GDM, as well as age-matched mice with a previously healthy pregnancy and virgin controls. Arteries from mice with previous GDM demonstrated an increased sensitivity to the vasoconstrictor U46619 and reduced endothelium-dependent vasodilation compared with arteries from mice with a previously healthy pregnancy or virgin controls. Within these vessels an increase in oxidative stress (increased superoxide production and increased peroxynitrite formation) was also observed. These results suggest that a pregnancy complicated by GDM has a significant impact on later-life endothelial function and may be an additional risk factor for the development of CVD.

It has been consistently demonstrated that women with GDM do develop endothelial dysfunction during pregnancy, in both systemic (14) and uterine arteries (4). There has, however, been little investigation of whether the GDM pregnancy causes the endothelial dysfunction observed, or whether this endothelial dysfunction was evident prior to pregnancy. A study of women with previous GDM, 3–6 mo after the complicated pregnancy, observed impaired flow-mediated dilation in the brachial artery despite a return to normal glucose tolerance (3). Similar changes were observed by Pleiner et al (17); changes in forearm blood flow in response to vasoactive compounds were investigated in women with previous GDM, but again, normal glucose tolerance, up to 1 yr after the complicated pregnancy. Impaired endothelium-dependent vasodilation was observed that correlated with increased levels of an endogenous NO inhibitor (asymmetric dimethylarginine), indicating the impaired response was associated reduced NO bioavailability.

In the study described here, arteries from mice with previous GDM displayed an increased sensitivity to the vasoconstrictor U46619. An increased pressor response is characteristic of endothelial dysfunction; the increase in sensitivity to U46619 observed suggests the development of vascular dysfunction in these arteries. There was also a significant effect of previous GDM on endothelial-dependent vasodilation; both maximal relaxation and sensitivity to MCh was significantly decreased in arteries from mice with previous GDM compared with all other groups. We observed that endothelium-dependent vasodilation of arteries from the other groups (a previously healthy pregnancy and the virgin controls) was comparable. Thus, it is the impact of a GDM pregnancy, as opposed to previous...
pregnancy or the genotype of the mice that results in endothelial dysfunction. Endothelium-independent vasodilation was also assessed in all arteries using SNP; this response did not differ across the groups. This demonstrates that previous GDM does not affect the ability of vascular smooth muscle to relax in response to donated NO and that the reduced vasodilation observed in arteries from mice with previous GDM is due to impaired endothelial function.

To try and understand possible mechanisms by which previous GDM mediates endothelial dysfunction, inhibitors of NOS and COX were used. In virgin animals, prior incubation with L-NAME significantly inhibited the response to MCh, suggesting that endothelium-dependent relaxation in these arteries was largely dependent on NO, regardless of genotype. An interesting observation was made when investigating mechanisms of endothelium-dependent relaxation in arteries from previously pregnant WT animals. When arteries were incubated with L-NAME and meclofenamate, alone or in combination, there was little effect on vasodilation. This suggests that the response to MCh in these arteries was largely mediated by the non-NO/non-COX component and that there may be a lasting impact of pregnancy on mediators of endothelium-dependent vasodilation. These results mirror those seen in arteries from women; a study of myometrial arteries demonstrated that while those from nonpregnant women were primarily dependent on NO, those obtained from women with a healthy pregnancy displayed both NO and EDHF components (13). It is suggested that both pathways may be enhanced during pregnancy to ensure maximal relaxant capacity in the event that one is unable to function. The data presented here suggests that the enhancement of the non-NO/non-COX pathway may be maintained long-term.

In comparison, although endothelium-dependent relaxation of arteries obtained from mice with previous GDM was minimal, there was still a significant impairment of relaxation in the presence of L-NAME. This suggests that the upregulation of the non-NO/non-COX pathway that occurred following a healthy pregnancy either did not occur in animals with GDM or was not maintained long-term. These results also mirror those observed in women with previous GDM, up to 1 yr after the pregnancy. In these women, impaired endothelium-dependent vasodilation was also observed (17). There is, however, one important difference between these two studies; it is not known whether the women with previous GDM demonstrated endothelial dysfunction prior to the pregnancy. By using virgin control mice and those with a previously healthy pregnancy it was possible to demonstrate that it was the previous GDM pregnancy that affected endothelial function.
The finding that arteries from the previously GDM group remain primarily dependent on NO could pose further complications with aging due to enhanced vascular oxidative stress. Increased oxidative stress, specifically an increase in superoxide, can lead to an increase in peroxynitrite formation through a reaction with NO. In light of possible scavenging of NO by superoxide to form peroxynitrite, both superoxide production and peroxynitrite formation was measured in these vessels. Previous studies have demonstrated in the homozygous form of this mouse (Leprdb/db), which displays type 2 diabetes, that impaired endothelial-dependent relaxation is associated with increased superoxide production (1). Additionally, an increase in superoxide production has also been observed, following exposure to high glucose, in both mouse microvessel endothelial cells and cultured human endothelial cells (8, 10). Superoxide may significantly reduce the bioavailability of NO by scavenging it and forming peroxynitrite. As well as reducing the bioavailability of NO, peroxynitrite may cause long-term endothelial cell dysfunction via protein nitrosylation and DNA damage. Interestingly, in our study, we noted that superoxide production was significantly reduced in arteries from virgin He mice compared with those from virgin WT mice. The mechanism(s) responsible for this change are unclear. The reduced production of superoxide in arteries from virgin He mice, however, only further highlights the dramatic impact of previous GDM on superoxide production in these vessels. Although it was significantly reduced following a healthy pregnancy, there was a significant increase in production following GDM. These data illustrate that there is increased oxidative stress in arteries from mice with previous GDM, despite normal glucose tolerance. Peroxynitrite formation was also significantly increased in arteries from mice with previous GDM, possibly as a result of the increased superoxide formation observed. This is of particular importance as, although endothelium-dependent relaxation of arteries from mice with previous GDM is significantly reduced, it is primarily dependent on NO. The increase in superoxide production and increased scavenging of NO to form peroxynitrite may be one mechanism by which endothelial dysfunction is mediated in arteries from these mice. Again, peroxynitrite formation was significantly reduced in arteries following healthy pregnancy, but significantly increased in arteries from mice with previous GDM. Representative images of superoxide production in mesenteric arteries from WT virgin mice (A), He virgin mice (B), WT PP mice (C), and mice with previous GDM (D). E: superoxide production was significantly reduced in arteries from He virgin mice compared with WT virgin mice. Production was also reduced in arteries from PP WT mice compared with their virgin controls. In comparison, production was significantly increased in mice with previous GDM compared with their virgin controls. Means ± SE, n = 3; two-way ANOVA, **p < 0.01, ***p < 0.001; bar = 100 μm.
from virgin He mice compared with virgin WT mice; it is likely this is due to the reduced superoxide production observed in these vessels.

It was observed that, not surprisingly, mice with previous GDM were significantly heavier than either their virgin counterparts or previously pregnant WT mice. It has been demonstrated in other studies that He mice gain more weight during pregnancy than WT mice (11). This was also observed in this study, and the increase in postnatal body weight observed in the animals studied here may reflect this. Indeed, the weight of He mice at term was ~6 g more than that of WT mice; at 9 mo of age, He mice remained ~6 g heavier than WT mice. Pregnant He mice also demonstrated a significant increase in fasting blood glucose compared with all other groups. Despite the changes that occurred during pregnancy, mice with previous GDM demonstrated blood glucose concentrations that were indistinguishable from controls at the time of study (9 mo of age). This reflects the clinical observation that the majority of women with GDM return to euglycemia postpartum (7). It is possible that the endothelial dysfunction observed in mice with previous GDM may be attributable to the small but significant increase in body weight observed. Indeed, a small study of women with previous GDM observed that those who were overweight (body mass index > 25 kg/m²) showed reduced forearm blood flow in response to the endothelium-dependent vasodilator acetylcholine compared with those who were not overweight (17). This impaired response was associated with reduced NO bioavailability. Therefore, while it is possible that the small increase in body weight observed in this study may contribute to the endothelial dysfunction seen, it is likely that GDM previously experienced by these mice played an important role in mediating the impairment of endothelium-dependent relaxation.

One potential limitation of this study is that the leptin receptor mutation present in He mice may have effects independent of the rise in blood glucose concentration and increased weight gain. Throughout this study, however, virgin He mice have been used as an additional control, and results suggest specific effects of the pregnancy-associated phenotype.

GDM is currently one of the most commonly observed obstetrical complications. As the incidence of diabetes contin-
ues to rise, it is likely that cases of GDM will also increase. The results presented here highlight the long-lasting effects that GDM may have on vascular function, even in the absence of overt diabetes. Thus our data indicate that a history of GDM, in the absence of other risk factors, should be observed as a risk factor for the development of CVD.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


