Increased myogenic tone in 7-month-old adult male but not female offspring from rat dams exposed to hypoxia during pregnancy

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Hemmings, D. G., S. J. Williams, and S. T. Davidge. Increased myogenic tone in 7-month-old adult male but not female offspring from rat dams exposed to hypoxia during pregnancy. Am J Physiol Heart Circ Physiol 289: H674–H682, 2005. First published April 15, 2005; doi:10.1152/ajpheart.00191.2005.—Intrauterine growth restriction (IUGR) increases the risk of cardiovascular disease later in life. Vascular dysfunction occurs in adult offspring from animal models of IUGR including maternal undernutrition, but the influence of reduced fetal oxygen supply on adult vascular function is unclear. Myogenic responses, essential for vascular tone regulation, have not been evaluated in these offspring. We hypothesized that 7-mo-old offspring from hypoxic (12% O2; H) or nutrient-restricted (40% of control; NR) rat dams would show greater myogenic responses than their 4-mo-old littermates or control (C) offspring through impaired modulation by vasodilators. Growth restriction occurred in male H (P < 0.01), male NR (P < 0.01), and female NR (P < 0.02), but not female H, offspring. Myogenic responses in mesenteric arteries from males but not females were increased at 7 mo in H (P < 0.01) and NR (P < 0.05) vs. C offspring. There was less modulation of myogenic responses after inhibition of nitric oxide synthase (P < 0.05), prostaglandin H synthase (P < 0.005), or both enzymes (P < 0.001) in arteries from 7-mo male H vs. C offspring. Thus reduced vasodilator modulation may explain elevated myogenic responses in 7-mo male H offspring. In contrast, there was increased modulation of myogenic responses in arteries from 7-mo female H vs. C or NR offspring after inhibition of both enzymes (P < 0.05). Thus increased vasodilator modulation may maintain myogenic responses in female H offspring at control levels. In summary, vascular responses in adult offspring from adverse intrauterine environments are impaired in a gender-specific, age-dependent, and maternal insult-dependent manner, with males more profoundly affected.

epidepidemiologic studies suggest that intrauterine growth-restricted infants are at increased risk of developing hypertension and other cardiovascular diseases in adult life (1). Fetal adaptations to an adverse intrauterine environment may include altered cellular differentiation and tissue growth to ensure short-term survival but may also lead to impaired cardiovascular and endocrine function later in adult life (1). Intrauterine growth restriction, in many cases, is thought to occur as a result of placental dysfunction leading to impaired oxygen and/or nutrient transfer to the fetus. Experimentally, undernutrition during pregnancy (2, 6, 10, 18, 28, 32, 40) or fetal exposure to elevated glucocorticoid levels (35, 36, 38) results in vascular dysfunction in adult offspring. However, the long-term effects of prenatal hypoxia are unclear.

Exposure to chronic hypoxia in ovo results in reduced nitric oxide modulation of vascular responses in arteries from both embryonic and adult chickens (42, 43). In mammals, the vascular effects of prenatal hypoxia have been examined primarily in the fetus (33, 49) or the neonate (54). One recent study, however, demonstrated decreased vasoconstriction in the pulmonary vasculature of adult rat offspring after perinatal exposure to reduced oxygen, but these results are from a specialized vascular bed and the length of exposure to hypoxia included a 1-wk postnatal period (24). Thus the specific effects of chronic hypoxia in utero on peripheral vascular function in mammalian adult offspring are currently unknown.

The myogenic response, defined as vasoconstriction or relaxation in response to increased or decreased intraluminal pressure, respectively, plays a critical role in development of vascular tone and regulation of peripheral vascular resistance (4). This response to pressure is vascular bed dependent (30) and occurs through stretch-activated mechanisms in the vascular smooth muscle but is also modulated by endothelium-derived factors. A role for nitric oxide and/or prostaglandins in modulation of myogenic responses has been shown in a number of vascular beds (8, 15, 30, 47) including the mesentery (13, 30, 41, 44). Evaluation of this response and the factors that modify it are of crucial importance for understanding mechanisms of vascular dysfunction, as has been shown in disease states such as hypertension (9, 20).

Gender- or age-related differences in myogenic responses have not been well studied in the mesenteric vascular bed, which contributes to peripheral vascular resistance (3, 12). However, studies conducted in other vascular beds (11, 21, 26) indicate that myogenic responses tend to be lower in females than males, possibly as a result of increased nitric oxide modulation (11, 21, 26, 39). In addition, although myogenic responses were decreased in arteries from aged compared with young animals (12, 37), those with a predisposition to hypertension have elevated myogenic responses with increasing age (9, 12), which may be related to decreased nitric oxide modulation (27). The few studies that have evaluated gender differences in the vascular function of adult offspring from perturbed pregnancies show that dysfunction occurs in both genders but affects each gender differently (35, 38) and may be more severe in male offspring (6, 40). Age-related decreases in endothelium-dependent relaxation (2) and increased sensitivity
to vasoconstrictors (40) have also been observed in adult offspring from nutrient-restricted dams. Whether these gender- or age-related differences in adult offspring are reflective of the intrinsic ability of these vessels to respond to pressure (i.e., myogenic responses) is unknown.

The objectives of this study were to assess the myogenic response and evaluate the role of nitric oxide and prostaglan-
dins in modulating that response in mesenteric arteries from two age groups of female and male adult offspring of rat dams subjected to hypoxia in the last week of pregnancy. To examine the effect of prenatal hypoxia on an aging profile, we chose the ages of 4 and 7 mo so that the rats were still within the active reproductive stage and thus without the additional complications caused by gonadal insufficiencies. Because the dams undergoing hypoxia treatment showed significantly reduced food intake (54), as has been previously observed (5, 51), a group with comparable nutrient restriction was also included. We hypothesized that mesenteric arteries from the older male offspring of either treatment group would demonstrate greater myogenic responses than their younger male littersmates, age-matched female littersmates, or offspring from control dams, as a result of reduced modulation by vasodilators.

MATERIALS AND METHODS

Animals. The University of Alberta Animal Welfare Committee approved this study, and all procedures followed the guidelines of the Canadian Council on Animal Care. Female Sprague-Dawley rats (Charles River) were mated at 3 mo of age. A vaginal smear obtained the following morning was examined for the presence of sperm, which signified day 0 of pregnancy (term = 22 days). From day 0 to day 15 of pregnancy, all rats were fed standard lab rat chow ad libitum. On day 15, rats were randomly assigned to control, maternal hypoxia, or maternal nutrient restriction protocols as previously described (54). Rats in the control group (n = 11) were housed in room air and fed ad libitum throughout pregnancy. All pregnant rats were assessed for food intake and weight gain on a daily basis.

Maternal hypoxia. On day 15 of pregnancy, rats (n = 8) were put into a Plexiglas chamber (volume 140 liters) that could hold a typical adult rat. The pregnant rats (n = 8) random-
ized to a maternal nutrient restriction protocol on day 15 of pregnancy were restricted to the lowest food intake before delivery on day 22 of pregnancy; however, six of the eight rats delivered on day 21 and were removed from the chamber at this time. After delivery, all rat dams were returned to a normal diet. These animal models were previously described in detail, including maternal food intake and weight gain throughout pregnancy (54).

Female and male offspring. All pups were weighed within 3–12 h after birth, and the litter size was reduced to eight pups by random selection of an equal sex ratio to ensure consistent feeding. Two or three randomly selected culled pups per litter (at least 1 female and 1 male) were weighed, and gender was confirmed by dissection after decapitation (reported in Table 1). Vascular myogenic response studies (see below) were performed in one or two pups per litter at 16–18 wk (4 mo) and 26–28 wk (7 mo) of age. These two ages were chosen to represent an aging profile in rats still within their reproductive capacity so that interpretation of the data would not be complicated by gonadal insufficiencies. Adult body weights were assessed at time of death.

Vessel preparation and arteriograph mounting. The rats attained surgical plane anesthesia after an intraperitoneal injection of 42.25 mg/kg body wt pentobarbital sodium (Somnotol, MTC Pharmaceuticals) and were subsequently exsanguinated. A section of mesentery 5–10 cm distal to the pylorus was excised and placed in ice-cold HEPES-physiological saline solution [HEPES-PSS (pH 7.4), in mmol/l: 142 NaCl, 4.7 KCl, 1.17 MgSO4, 1.18 KH2PO4, 1.56 CaCl2, 5–10 cm distal to the pylorus was excised and placed in ice-cold HEPES-physiological saline solution [HEPES-PSS (pH 7.4), in mmol/l: 142 NaCl, 4.7 KCl, 1.17 MgSO4, 1.18 KH2PO4, 1.56 CaCl2, 10 HEPES, 5.5 glucose], where two third-order arteries were dissected free of adipose and connective tissue. Arteries in each treatment group were mounted in the two-chamber arteriograph. The myo-

diameters as previously described (14, 17).

Table 1. Male and female offspring body weight at birth and 4 and 7 mo of age

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Sex</th>
<th>Birth Weight (g)</th>
<th>4 mo</th>
<th>7 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male control</td>
<td></td>
<td>0.67±0.0165</td>
<td>0.620±0.21</td>
<td>0.688±0.2445</td>
</tr>
<tr>
<td>Male hypoxia</td>
<td></td>
<td>0.52±0.0302</td>
<td>0.543±0.1526</td>
<td>0.598±0.3835</td>
</tr>
<tr>
<td>Male nutrient restricted</td>
<td></td>
<td>0.45±0.2144</td>
<td>0.613±0.1476</td>
<td>0.642±0.2080</td>
</tr>
<tr>
<td>Female control</td>
<td></td>
<td>0.61±0.1781</td>
<td>0.327±0.1195</td>
<td>0.432±0.2666</td>
</tr>
<tr>
<td>Female hypoxia</td>
<td></td>
<td>0.62±0.1941</td>
<td>0.342±0.2666</td>
<td>0.360±0.3115</td>
</tr>
<tr>
<td>Female nutrient restricted</td>
<td></td>
<td>0.56±0.1781</td>
<td>0.327±0.1106</td>
<td>0.427±0.3188</td>
</tr>
</tbody>
</table>

Values are means ± SE body weights (g) for no. of offspring indicated in parentheses. Significant differences within each age group for each gender were assessed with I-way ANOVAs and the Fisher least significant difference post hoc test, with differences denoted by different superscripted letters (a, b) as follows: male neonate offspring (P < 0.01), male 4-mo offspring (P < 0.01), and female neonate offspring (P < 0.02).

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myogenic response in the absence of drugs was then examined in each vessel. Intraluminal pressure was reduced to 20 mmHg, and the initial lumen diameter was measured. Changes in lumen diameter were then measured after stepwise increases in pressure (20–130 mmHg), allowing 4 min between steps. After completion of the myogenic response curves, the pressure was returned to 60 mmHg, the bath medium was changed, and the vessel equilibrated for 20 min. The myogenic response curves were then repeated in the presence of either the prostaglandin H synthase (PGHS) inhibitor meclofenamate (Meclo; 1.0 μmol/l), or the nitric oxide synthase (NOS) inhibitor N^ω-nitro-L-arginine methyl ester (L-NAME; 100 μmol/l). Each drug was incubated for 20 min before the pressure was reduced to 20 mmHg and the pressure steps outlined above were repeated. After completion of the curves, the pressure was returned to 60 mmHg, the bath medium was changed, and the vessel equilibrated for 20 min. The myogenic response was then assessed in the presence of both Meclo (1.0 μmol/l) and L-NAME (100 μmol/l) as described above. To assess the passive response curve of each vessel, the pressure was returned to 60 mmHg, the vessels were extensively washed in EGTA-Ca^2+-free PSS (in mmol/l: 142 NaCl, 4.7 KCl, 1.17 MgSO_4, 1.18 KH_2PO_4, 10 HEPES, 2 EGTA) and equilibrated for 10 min in the presence of papaverine (Sigma; 0.1 mmol/l), and the pressure steps were repeated as outlined above.

Calculations. Percent myogenic tone at each pressure step was calculated with the following formula: percent myogenic tone = (D_1 - D_2)/D_1 × 100, where D_1 is the arterial lumen diameter in EGTA-Ca^2+-free PSS and papaverine and D_2 is the arterial lumen diameter in HEPES-PSS containing Ca^2+. For each animal, percent myogenic tone from both mounted vessels in the absence of drugs was averaged, with the error not greater than 2 SD, and this value was then used in calculating the means for each experimental group. The percent myogenic response due to the influence of a drug(s) was calculated by the following formula: difference in percent myogenic tone = MR_1 - MR_2, where MR_1 is the percent myogenic tone (as calculated above) in the presence of a drug and MR_2 is the percent myogenic tone (as calculated above) taken at the same pressure in the absence of any drugs. The percent myogenic tone calculated for a single vessel from each animal was used in calculating the means for each experimental group.

Statistics. Data are expressed as means ± SE. One-way ANOVAs with the Fisher least significant difference (LSD) post hoc test were used to compare body weights within each age group for each gender. Entire curves were compared with two-way ANOVAs and the Fisher LSD post hoc test. Statistical significance was accepted at P < 0.05.

RESULTS

Characteristics of offspring. The birth weights of male H (P < 0.01) and NR (P < 0.01) offspring were significantly reduced compared with male C offspring (Table 1). Reduced body weight persisted to 4 mo of age in male H (P < 0.01) but not male NR offspring. Significantly reduced body weights of female NR but not H offspring were observed (Table 1; P < 0.02). However, by either 4 or 7 mo of age there were no differences in body weight of female offspring among treatment groups (Table 1).

Myogenic responses among treatment groups. There were no significant differences in the passive curves between genders or ages or among treatment groups (data not shown). When myogenic responses in mesenteric arteries from male offspring were compared among treatment groups at each age, only 7-mo-old male H offspring showed significant elevation compared with aged-matched male C (Fig. 1B; P < 0.01) or NR (Fig. 1B; P < 0.05) offspring. Myogenic responses were also significantly increased at 7 mo compared with 4 mo of age in male H (P < 0.01) and NR (P < 0.05) but not C offspring.

In contrast, arteries from female NR offspring showed significantly reduced myogenic responses compared with age-matched female C or H offspring (Fig. 2A; P < 0.05). These differences did not persist in the 7-mo-old females (Fig. 2B). Myogenic responses in female offspring did not differ between 4 and 7 mo of age in any treatment group.

Myogenic responses between genders. Myogenic responses were also compared between male and female offspring from each treatment group at each age. Increased myogenic responses at pressures >60 mmHg were found in arteries from 4-mo-old female C compared with male C offspring (P < 0.05). By 7 mo of age, there were no longer any gender-specific differences in this treatment group. Although there were no gender differences at 4 mo of age in H offspring, by 7 mo of age, arteries from male H offspring showed signifi-

Fig. 1. Male offspring: % myogenic responses in mesenteric arteries from 4-mo-old (A) and 7-mo-old (B) male offspring. Lumen diameter changes were assessed from arteries from offspring of control (C), hypoxic (H), and nutrient-restricted (NR) dams in response to increasing intraluminal pressure and depicted as % of the initial diameter at 20 mmHg. Significant differences among groups were determined with a 2-way ANOVA and the Fisher least significant difference (LSD) post hoc test: *H compared with C offspring (P < 0.01); #H compared with NR offspring (P < 0.05).
significantly enhanced myogenic tone compared with aged-matched female H offspring at pressures between 60 and 100 mmHg (P < 0.05). There were no significant differences between genders in NR offspring at either age.

Modulation of myogenic tone by inhibition of NOS. Modulation of active myogenic responses by pretreatment with a drug was calculated at any one pressure as the difference in percent myogenic tone (% myogenic tone) in the presence and absence of drug(s). Entire % myogenic tone curves were statistically compared among treatment groups or between ages for each gender and were found to be significantly different over a range of pressures. However, for simplicity of presentation, these data were summarized by presenting Δpercent myogenic tone at 90 mmHg in male offspring and 100 mmHg in female offspring because these pressures corresponded to the largest differences observed in either the active myogenic tone in the absence of drug(s) among treatment groups (male offspring) or the active myogenic tone compared with myogenic tone in the presence of a drug or drugs (female offspring).

Interestingly, the pattern of nitric oxide modulation of myogenic tone in the mesenteric vasculature differed between ages and genders and among treatment groups. In male offspring, there were no significant treatment effects at 4 mo of age. However, Δpercent myogenic tone was significantly reduced in the presence of l-NAME in arteries from 7-mo-old male H offspring compared with C or NR offspring (Fig. 3A; P < 0.05). There were no age-related differences in Δpercent myogenic tone in the presence of l-NAME within any offspring group (Fig. 3A).

In female offspring, Δpercent myogenic tone in the presence of l-NAME was reduced in arteries from 4-mo-old H offspring Fig. 2. Female offspring: % myogenic responses in mesenteric arteries from 4-mo-old (A) and 7-mo-old (B) female offspring. Lumen diameter changes were assessed as described in Fig. 1 in arteries from C, H, and NR offspring. Significant differences among groups were determined with a 2-way ANOVA and the Fisher LSD post hoc test: *NR compared with C offspring (P < 0.05); **NR compared with H offspring (P < 0.05).

Fig. 3. Effect of nitric oxide synthase (NOS) inhibition on the myogenic response. The difference (Δ) in % myogenic tone was calculated in mesenteric arteries with and without Nω-nitro-l-arginine methyl ester (l-NAME) treatment (100 μmol/l) at 90 or 100 mmHg from 4- and 7-mo-old male (A; n = 4–7) and female (B; n = 4–7) offspring, respectively. Significant differences were determined with a 2-way ANOVA and the Fisher LSD post hoc test on the entire pressure curve. *Significant differences among treatment groups at each age: male H offspring (Hoff) vs. male C offspring (Coff) or male NR offspring (NRoff) at 7 mo (P < 0.05, A); female H offspring vs. female C offspring or female NR offspring at 4 mo (P < 0.05, B).
compared with C or NR offspring (Fig. 3B; \( P < 0.05 \)). However, by 7 mo of age the ∆percent myogenic tone in the presence of l-NAME had recovered to levels similar to those found in C offspring, which resulted in a significant increase in 7-mo-old H offspring compared with 4-mo-old H offspring (Fig. 3B; \( P < 0.01 \)).

Modulation of myogenic tone by inhibition of PGHS. Although the response in arteries from male H offspring at 4 mo of age was similar to that in age-matched control animals, by 7 mo of age ∆percent myogenic tone was significantly reduced after Meclo treatment compared with age-matched controls (Fig. 4A; \( P < 0.005 \)). In contrast, at 4 mo of age, arteries from male NR offspring showed reduced ∆percent myogenic tone after inhibition of PGHS by Meclo treatment compared with age-matched male C or H offspring (Fig. 4A; \( P < 0.01 \)). By 7 mo of age, arteries from these offspring showed ∆percent myogenic tone in the presence of Meclo comparable to that in age-matched C offspring. This resulted in a significant increase at 7 mo compared with 4 mo of age in male NR offspring (Fig. 4A; \( P < 0.04 \)).

The pattern of changes in myogenic tone of mesenteric arteries from the female offspring to pretreatment with Meclo differs from that of the male offspring. There was significantly reduced ∆percent myogenic tone in arteries from 4-mo-old H offspring compared with C offspring (Fig. 4B; \( P < 0.01 \)). By 7 mo of age, ∆percent myogenic tone in the presence of Meclo was restored to levels seen in C offspring, resulting in a significant difference between 4- and 7-mo-old female H offspring (Fig. 4B; \( P < 0.04 \)). There were no differences in ∆percent myogenic tone after Meclo treatment in arteries from female NR offspring compared with C offspring at either age (Fig. 4B).

Modulation of myogenic tone by inhibition of both NOS and PGHS. The pattern of differences in ∆percent myogenic tone in arteries from male offspring in the presence of both inhibitors was similar to that found in the presence of each individual inhibitor. The difference in percent myogenic tone in the presence of both inhibitors was significantly reduced in arteries from 7-mo-old male H offspring compared with those from both their 4-mo-old littermates (Fig. 5A; \( P < 0.03 \)) and age-matched C offspring (Fig. 5A; \( P < 0.001 \)). In contrast, ∆percent myogenic tone was significantly increased in the presence of both inhibitors in 7-mo-old male NR offspring (Fig. 5A; \( P < 0.05 \)). This pattern resembled that of controls, although there were no significant differences between ages in arteries from male C offspring.

In female offspring, in the presence of both inhibitors, there were no significant differences in ∆percent myogenic tone found at 4 mo of age in female H compared with C offspring (Fig. 5B). In contrast, ∆percent myogenic tone was significantly increased in arteries from female H offspring at 7 mo compared with 4 mo of age (Fig. 5B; \( P < 0.01 \)) and also compared with age-matched female C and NR offspring (Fig. 5B; \( P < 0.05 \)). There were also no differences at either age in arteries from NR compared with C offspring.

**DISCUSSION**

The primary aim of this study was to assess myogenic responses in mesenteric arteries at 4 and 7 mo of age in male and female offspring from dams exposed to either hypoxia or nutrient restriction during pregnancy compared with control offspring. The major findings of this study are first that these maternal treatments impair fetal growth and alter vascular responses in adult offspring in a gender-specific and age-dependent manner that is also dependent on the nature of the maternal insult. Second, vascular function appears to be more profoundly affected in male than female offspring, particularly as they age.

Intrauterine growth restriction resulting from an adverse intrauterine environment may have long-term consequences for adult cardiovascular health (1). In this study, both maternal nutrient restriction and maternal exposure to hypoxia led to reduced birth weights of male offspring along with increased myogenic responses in H offspring compared with either NR or C offspring at 7 mo of age and age-dependent increased...
The significant elevation of myogenic responses in male but not female H offspring compared with gender-matched C offspring along with the reduced (male) and elevated (female) nitric oxide modulation of those responses emphasizes the responses in both treatment groups that were not observed in control animals. Thus the adaptations of male fetuses to an adverse intrauterine environment may have led to vascular dysfunction. In female offspring, only maternal nutrient restriction led to reduced birth weights, with little accompanying vascular changes in the adults. Interestingly, even though maternal exposure to hypoxia resulted in normal birth weights in female offspring and no overt changes in myogenic responses, there were significant alterations in modulation of these responses by nitric oxide and prostaglandins. This suggests that the underlying regulation of vascular function in adult offspring from complicated pregnancies is altered even in the absence of intrauterine growth restriction. These results are comparable to others that have also shown vascular dysfunction in normally grown offspring from protein-restricted rat dams (2, 50).

The nutrient-restricted group was included in this study because dams treated with hypoxia substantially reduced their food intake (54), consistent with previous reports (5, 51). It is evident in the current study that hypoxia treatment during the latter third of pregnancy induced vascular alterations in both male and female offspring that were independent of the effects of nutrient restriction alone. In males, there was a greater increase in myogenic responses in H than NR offspring at 7 months of age and at 7 mo only male H offspring showed significantly increased responses compared with control animals. Moreover, modulation of these responses by nitric oxide and prostaglandins in each treatment group was qualitatively different. In females, the only detectable effect on vascular function in adult NR offspring was a reduction in myogenic responses at 4 mo of age compared with control animals. There were no changes in nitric oxide or prostaglandins but no changes in overall myogenic responses compared with C or NR offspring. These results indicate that the altered vascular responses in adult offspring after exposure to prenatal hypoxia are independent of reduced nutrient supply.

It is interesting that myogenic responses in arteries from female H offspring at 4 mo of age were maintained at control levels even though nitric oxide and prostaglandin modulation of these responses were reduced compared with age-matched controls. These results suggest that compensation by other vasodilators such as endothelium-derived hyperpolarizing factor (EDHF) (44). Female H offspring, on the other hand, showed significant changes in modulation of myogenic responses by nitric oxide and prostaglandins but no changes in overall myogenic responses compared with C or NR offspring. These results indicate that the altered vascular responses in adult offspring after exposure to prenatal hypoxia are independent of reduced nutrient supply.

Few studies have examined gender differences of myogenic responses in the mesenteric vasculature (12, 53), although it is clear that gender differences exist in a number of other vascular beds (11, 21, 26, 39). Elevated myogenic responses in males compared with females has been demonstrated in rat gracilis muscle (21) and mouse cerebral (11), rat coronary (26), and mouse mesenteric (53) arteries. The presence of estrogen in females may reduce myogenic responses in an endothelial nitric oxide synthase-dependent manner (11, 19, 45). In contrast, we found that mesenteric arteries from 4-mo-old female C offspring exhibited significantly greater myogenic responses than those from age-matched male C offspring, with the differences resolved by 7 mo of age. This difference could not be explained by differential nitric oxide or prostaglandin modulation of the response between genders.

The significant elevation of myogenic responses in male but not female H offspring compared with gender-matched C offspring along with the reduced (male) and elevated (female) nitric oxide modulation of those responses emphasizes the
gender-specific differences after exposure to an adverse intra-uterine environment. Although it is not surprising that each gender was affected differently by a particular in utero insult simply based on hormonal differences (19, 39), it is particularly interesting that the vascular effects for each gender differed depending on the insult. These gender-specific changes could be directly due to differences in sensitivity and adaptation to the specific insult while still in utero, or females may have greater vascular compensatory mechanisms after birth. These gender-specific differences are in contrast with a study of impaired endothelium-dependent relaxation in a maternal lard-fed model, which found no differences between genders (25). However, results from the present study are consistent with other models of maternal undernutrition showing either greater adverse vascular effects in male compared with female offspring (6, 40) or gender-specific impairment of adult cardiovascular and metabolic function (35, 38). These differing effects in male and female offspring may involve gender-specific differences in vasodilatory modulation of vascular responses and compensatory mechanisms when one or more of the vasodilation pathways are absent or deficient (44, 56).

In this study, male but not female offspring from both treatment groups showed an age-dependent increase in myogenic responses that was not seen in controls. In contrast, in normal aging, either myogenic responses were reduced (37) or the pressure at which a myogenic response occurred was increased (22). Interestingly, our results are similar to those found in young spontaneously hypertensive rats, in which myogenic responses were increased in skeletal (20) and mesenteric (23) arteries. Myogenic responses were also increased in rats with chronic heart failure (13) and in murine models of Type 2 diabetes (29) and oxidative stress (53). Our results in male offspring are therefore consistent with those found in other animal models of vascular dysfunction. In addition, we recently demonstrated (16, 52) elevated myogenic responses in uterine arteries from pregnant and nonpregnant female offspring of nutrient-restricted dams. In other studies, the aging vasculature in adult offspring from nutrient-restricted dams had decreased endothelium-dependent relaxation (2) and increased sensitivity to vasoconstrictors (40).

The reduced modulation of myogenic responses in 7-mo-old male H compared with C offspring by nitric oxide, prostaglandins, or a combination of the two suggests a mechanism for the enhanced myogenic responses observed in this group compared with controls. Reduced nitric oxide modulation of vascular responses has also been found in fetal sheep exposed to chronic hypoxia at high altitude (33) and in embryonic and adult chickens after chronic exposure to hypoxia in ovo (42, 43). Moreover, we recently demonstrated (55) decreased nitric oxide modulation of endothelium-dependent relaxation in 7-mo-old male offspring from dams exposed to hypoxia during pregnancy. Similar effects have also been observed in various nutrient restriction (2, 6, 31) and glucocorticoid overexposure (36) models. Interestingly, offspring from nutrient-restricted dams with impaired endothelium-dependent vasodilation showed increased levels of superoxide anion, suggesting a mechanism for the reduced bioavailable nitric oxide observed in these models (7).

Our data demonstrating decreased myogenic responses in the presence of a PGHS inhibitor in male H compared with C offspring at 7 mo of age suggest that, in addition to reduced nitric oxide modulation, there is a role for prostaglandins in the increased myogenic tone observed in this treatment group. The results are consistent with either an increase in PGHS-mediated vasoconstrictors or a decrease in PGHS-mediated vasodilators. A role for PGHS vasodilators in modulation of myogenic responses in arteries from young normal rats has been shown (30). However, PGHS-mediated constrictors have been shown to modulate myogenic responses of arteries from normal rats (47) and have been implicated in the increased myogenic responses observed both in an insulin-resistant mouse model (29) and in spontaneously hypertensive rats (20). This suggests that adult male offspring from adverse pregnancies may also experience an enhanced PGHS-dependent release of vasoconstrictors resulting in increased myogenic responses. It is interesting that the pattern of nitric oxide and prostaglandin involvement in the vascular response of 7-mo-old male offspring observed in this study has also been observed in 12- to 14-mo-old aged rats (17, 34, 46), suggesting that fetal adaptation to an adverse in utero environment may lead to premature aging of the vasculature in male offspring and increased cardiovascular complications.

Understanding how fetal adaptations to an adverse intrauterine environment permanently affect vascular function, particularly before the onset of detectable cardiovascular complications, is essential for development of early intervention and treatment strategies. Our results suggest that although vascular changes are evident in both genders by 7 mo of age, only males show overt functional changes that could contribute to increased peripheral vascular resistance and cardiovascular disease. The other important finding of this study is that the vascular changes observed in adult offspring from complicated pregnancies differ in a gender-specific way depending on the specific nature of adverse maternal treatment. These results not only suggest the importance of monitoring vascular function parameters early in adult life, particularly in males from suspected adverse pregnancies, but also the importance of investigating the potential cause of the original pregnancy complication.

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REFERENCES


