Maternal stress and development of atherosclerosis in the adult apolipoprotein E-deficient mouse offspring

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Andersson IJ, Jiang Y, Davidge ST. Maternal stress and development of atherosclerosis in the adult apolipoprotein E-deficient mouse offspring. Am J Physiol Regul Integr Comp Physiol 296: R663–R671, 2009. First published January 7, 2009; doi:10.1152/ajpregu.90768.2008.—Stress is a risk factor for cardiovascular disease, such as atherosclerosis. Stress during pregnancy (maternal stress) may have long-term consequences for the health of the offspring. However, it is not known whether maternal stress affects the offspring’s predisposition to develop atherosclerosis. Atherosclerosis is often related to vascular endothelial dysfunction. We hypothesized that maternal stress affects vascular endothelial function and accelerates development of atherosclerosis in offspring of apolipoprotein E-deficient mice, a model commonly used for atherosclerosis research. Stress was induced by restraining dams in small cylinders for five consecutive days (2 h/day) beginning on gestational day 8 ± 0.5. Vascular function and development of atherosclerosis in the aorta were determined in male and female offspring at 11–15 wk of age (with early lesions) and at 22–26 wk of age (with established lesions). Endothelium-dependent vasorelaxation was determined using methacholine (0.0001–10 μmol/l) in the absence or presence of the nitric oxide synthase inhibitor Nω-nitro-l-arginine methyl ester hydrochloride (l-NAME; 100 μmol/l). Male offspring (11–15 wk old) from stressed dams were less dependent on nitric oxide for relaxation compared with controls (l-NAME inhibition: 38 ± 10 vs. 69 ± 6%, P < 0.05). Atherosclerotic lesion area was larger in male and female 25- to 26-wk-old offspring from stressed dams compared with corresponding controls [median (interquartile range): males: 6.8 (5.4–7.7) vs. 5.1 (4.4–5.5), P < 0.05, females: 10.0 (8.9–10.9) vs. 7.0 (4.7–8.7), P < 0.05]. In conclusion, maternal stress renders the apolipoprotein E-deficient offspring more susceptible to develop atherosclerosis.

fetal origins of adult disease; endothelial function; aorta

It is well established that stress is a risk factor for cardiovascular disease during adult life. In particular, a number of studies have shown that long-term and/or repeat activation of stress systems may lead to a deterioration of endothelial function and eventually atherosclerosis (for review, see Ref. 23). Endothelial dysfunction is considered an early event in the development of atherosclerosis (24). However, sensitivity to stress differs among individuals, and neither stress nor established risk factors such as dyslipidemia, hypertension, and smoking can explain all cases of cardiovascular disease or the differing susceptibility among populations. It has been proposed that the development of cardiovascular diseases in adults can be influenced by the in utero environment (3). The “Barker hypothesis” suggests that fetal adaptations to a suboptimal in utero environment results in permanent structural and functional differences in key homeostatic systems, predisposing the individual to later metabolic and cardiovascular diseases (1).

There is now a growing body of evidence that emotional/psychological stress during pregnancy (maternal stress) may have long-lasting effects on physical development, behavior, neurochemistry, and regulation of the hypothalamic-pituitary-adrenal (HPA) axis in both human and animal offspring (reviewed in Refs. 27 and 29). Stress generally results in heightened activity of the sympathetic-adrenal medullary branch of the autonomic nervous system and of the HPA axis. The effects of maternal stress on the fetus are proposed to be mediated via increased activity of these systems, especially of the HPA axis (12). When the HPA axis is activated, glucocorticoids (the major one being cortisol in humans and corticosterone in rodents) are released from the adrenal gland. Excess exposure of the fetus to glucocorticoids is linked to hypertension (9, 21) and changes in HPA axis activity (4) in the adult offspring. These alterations are potential risk factors for development of atherosclerosis. Yet, to our knowledge, no studies have addressed the question as to whether changes in the in utero environment caused by maternal stress influence the susceptibility of the offspring to develop atherosclerosis.

Apolipoprotein E-deficient mice (apoe−/−) are often used as an animal model in atherosclerosis research because they develop atherosclerotic lesions similar in type and distribution to those lesions seen in humans (20). Atherosclerotic fatty streaks normally start to develop around 10 wk of age when apoe−/− mice are fed normal chow. More advanced, fibrous plaques can start to develop around 20 wk of age (20). In humans, development of atherosclerosis is preceded by endothelial dysfunction, caused by an imbalance in endothelium-derived vasoactive factors that play an important regulatory role in vascular homeostasis. Nitric oxide (NO) is a potent vasodilator produced by endothelial cells. In addition, it can inhibit monocyte adhesion, platelet aggregation, and vascular smooth muscle cell proliferation. These functions have an inhibitory effect on the atherosclerotic process and thus NO bioavailability may play a role in the development of atherosclerosis. Treatment with the NO synthase inhibitor Nω-nitro-l-arginine methyl ester hydrochloride (l-NAME) for 8 wk inhibited NO-mediated vascular responses and increased atherosclerotic lesion area in aortas of apoe−/− mice (13). Thus the importance of NO in the development of atherosclerosis has been demonstrated in apoe−/− mice (13), whereas it is not

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clear whether endothelial dysfunction is an early marker of atherosclerosis or rather a marker of established atherosclerosis in this mouse model (6, 32).

In this study, we hypothesized that maternal stress will have long-term effects on vascular NO-dependent endothelial function and on the development of atherosclerosis in offspring of apoe-/- mice. Because fetal exposure to glucocorticoids may have differential effects in males and females (21), we further hypothesized that male and female mice would be differently affected by maternal stress. To this end, we studied endothelial function and quantified atherosclerosis in the aorta of male and female apoe-/- offspring of dams exposed to stress during pregnancy.

MATERIALS AND METHODS

Maternal Stress

Male and female apoe-/- mice were purchased at 7 wk of age (Jackson Laboratory, Bar Harbor, ME) and mated at 8–12 wk of age.

Day 0.5 of gestation was defined as the morning of the day a vaginal plug was found after overnight mating. Pregnant nulliparous females were randomly assigned to a control (n = 11) or stress (n = 10) group.

The dams in the stress group were exposed to restraint stress for 2 h/day starting at gestational day 8 ± 0.5. This was repeated for five consecutive days. The time frame was chosen to avoid stress during implantation and during the last week of pregnancy to ensure that timing of delivery was not affected. The restraint container consisted of a 50-ml perforated plastic centrifugation tube. Thus the animal could breathe freely, but movements were restricted. The stress always began between 10:00 A.M. and 1:00 P.M. Tail vein blood samples were taken from each mouse on a random day during the stress period to determine whether stress affected the levels of plasma corticosterone. One sample was taken just before the stress period and the other immediately after. Blood samples were also taken from the control dams at matching days and times, but except from that the controls were left undisturbed. All mice had free access to water and standard chow.

All procedures in this study were approved by the University of Alberta Animal Care and Use Committee: Health Sciences and are in accordance with federal, state, and local laws and regulations. They are also in compliance with the Institute for Laboratory Animal Research Guide for Care and Use of Laboratory Animals, Washington, DC: National Academy Press, 1996.

Offspring

Pup weights were recorded within 12 h of birth, and pups were weaned at 19–21 days of age. For clarity, the offspring born to control mothers are referred to as “control” and offspring of mothers exposed to stress during pregnancy as “stress.” Individual pups were considered experimental units, since the outcome measures could differ between the offspring within a litter.

Male and female offspring were divided into the following two broad age groups: 11–15 and 22–26 wk old. These ages were chosen with the anticipated progression of atherosclerotic lesions in mind. Mice in the younger group were expected to have developed stage I lesions (based on the American Heart Association-defined stages of human atherosclerosis (25)). The 22- to 26-wk-old mice were expected to have developed stage II lesions (31). For simplicity, the age groups will be referred to as “12w” and “25w,” which are the age medians for the corresponding groups the day they were killed and used for experiment. The numbers and age distribution for each group are shown in Table 1. All offspring had free access to water and standard chow.

At the time points mentioned above, offspring were killed by an overdose of isoflurane (Baxter). The abdominal aorta was excised and kept in ice-cold HEPES-buffered PSS containing (in mmol/l): 142.0 NaCl, 10.0 HEPES, 6.6 CaCl2, 5.5 D-glucose, 4.7 KCl, 2.4 MgSO4, and 1.2 KH2PO4. Kidneys, spleen, adrenal glands, and thyroid gland were removed, cleaned of adherent tissue, and weighed. Thereafter, the thoracic aorta was perfused with saline to clear the vascular lumen before being fixated with 4% paraformaldehyde at −100 mmHg for 7 min. The thoracic aorta was cleaned of adherent tissue and kept in 4% paraformaldehyde until the atherosclerotic lesion area was quantified.

Analysis of Plasma Corticosterone

Plasma corticosterone concentration was measured in pregnant females to confirm the stress effect. In addition, to determine if maternal stress caused changes in baseline corticosterone in the offspring, plasma corticosterone was measured at 5–8 wk of age. Tail vein blood was collected in heparin tubes within a maximum of 2 min after taking the mouse out of its cage, since corticosterone levels can change rapidly in response to handling. The plasma was stored at −80°C until corticosterone was analyzed using a correlate-EIA kit (Assay Designs, Ann Arbor, MI) according to the manufacturer’s protocol.

Analysis of Serum Cholesterol and Triglycerides

Serum cholesterol and triglycerides were analyzed in 24- to 26-wk-old male and female offspring. Blood samples were collected from vena cava after the mice were killed with isoflurane, and the thoracic cavity was opened. The blood samples were allowed to clot on ice and then centrifuged at 5,000 g, 4°C for 5 min. The serum was kept in −80°C until prepared for analysis of plasma cholesterol and triglycerides by gas chromatography as described previously (18).

Vascular Endothelial Function

Two segments (2 mm each) of the abdominal aorta were dissected and mounted in a Multimyograph 610M (Danish Myo Technology, Aarhus, Denmark) and warmed to 37°C for 20 min before they were gradually stretched to 0.6 g/mm over 20 min. The chosen tension was based on previous work (10). The vessels were activated with phenylephrine (PE, 10 μmol/l) two times, with a 15-min wash in between. Methacholine (MCh, 10 μmol/l) was added at the end of the second PE-induced contraction to determine the integrity of the endothelium. A PE dose-response curve (0.01–100 μmol/l in eight steps) was performed followed by careful washing and a 30-min incubation period with the NO synthase inhibitor l-NAME (100 μmol/l). One vessel was left untreated as a control. The vessels were precontracted with PE, and the endothelium-dependent relaxation response was tested by a cumulative dose-response curve to MCh (0.0001–10 μmol/l in nine steps). Finally a dose-response curve to sodium nitroprusside (SNP, 0.0001–10 μmol/l in eight steps) was performed to validate endothelium-independent NO-mediated smooth muscle relaxation. All chemicals were obtained from Sigma (St Louis, MO).

Quantification of Aortic Plaque Area

Thoracic aorta plaque area was quantified as previously described (11). Briefly, the fixated thoracic aorta was cut open longitudinally,
Student's using the Mann-Whitney post hoc test when possible. Differences in plaque area were tested for groups were analyzed by two-way ANOVA followed by Bonferroni. Statistically significant differences between means of more than two 

The average number of pups per litter of control dams (litter was captured, and the aorta and lesions were outlined using AxioVison LE Rel.4.5 (Carl Zeiss, Toronto, Canada), followed by calculation of the percent lesion area.

Statistics

Data are expressed as means ± SE or median (interquartile range). Statistically significant differences between means of more than two groups were analyzed by two-way ANOVA followed by Bonferroni post hoc test when possible. Differences in plaque area were tested for using the Mann-Whitney U-test followed by multiple-comparison Bonferroni correction. All other variables were analyzed by unpaired Student's t-test. P < 0.05 was considered statistically significant.

RESULTS

Litter Size and Maternal Corticosterone

The length of gestation was 19 ± 0.5 days for all females. The average number of pups per litter of control dams (litter n = 11) was 7.8 ± 0.6 pups (78 ± 7% of the pups survived until weaning) while stressed dams (litter n = 10) gave birth to 7.3 ± 0.3 pups (of which 87 ± 4% survived until weaning). The sex distribution was not different between the groups; the proportion of males was 56 ± 8% among litters from control dams and 43 ± 4% among litters from stressed dams. The average male control pup weight (1.19 ± 0.02 g) was not different from male stress pup weight (1.20 ± 0.02 g). Likewise, the control female pup weight (1.14 ± 0.03 g) was similar to that of female stress pups (1.16 ± 0.03 g).

Baseline corticosterone concentration did not differ between the control and stressed dams (data not shown). As expected, corticosterone concentration was significantly higher after stress than in controls at the corresponding time of day (334 ± 30 vs. 60 ± 6 ng/mL, P < 0.001).

Offspring Body and Organ Weights

Body weight on the experimental day did not differ between stress and control offspring (Tables 2 and 3). The weight of various organs did not differ between 12w stress and control mice, in either males or females (Table 2). However, the weight of kidneys, adrenal glands, spleen, and thyroid gland were lower in male 25w stress mice than in the corresponding controls, whereas there was no difference between female 25w control and stress mice (Table 3).

Offspring Corticosterone

Plasma corticosterone concentrations did not differ between 5- to 8-wk-old male stress and control offspring (31.6 ± 4, n = 16, vs. 43.0 ± 9.8, n = 10, ng/mL). In females, the levels were also similar between stress and control offspring (41.4 ± 5.8, n = 19 vs. 44.3 ± 7.1, n = 12, ng/mL).

Offspring Serum Cholesterol and Triglycerides

Serum cholesterol did not differ between male stress and control offspring (404.2 ± 22.5, n = 5 vs. 387.4 ± 33.2, n = 5, mg/dl) nor did serum cholesterol differ between female stress and control offspring (379.0 ± 26.4, n = 5 vs. 321.6 ± 4.9, n = 5, mg/dl). Furthermore, triglycerides did not differ between male stress and control offspring (131.6 ± 16.3, n = 5 vs. 100.2 ± 11.2, n = 5, mg/dl). Likewise, serum triglycerides did not differ between female stress and control offspring (47.4 ± 6.4, n = 5 vs. 38.4 ± 5.8, n = 5, mg/dl).

Vascular Function

Contractile responses. Aortas from male 12w stress mice had a lower maximal PE-induced contraction compared with

Table 2. Body and organ weights of 12w male and female offspring of control and stressed dams

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<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Stress</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>26.0±0.9</td>
<td>25.4±0.6</td>
</tr>
<tr>
<td>Kidney, (g·g body wt⁻¹)·10²</td>
<td>1.31±0.064</td>
<td>1.22±0.061</td>
</tr>
<tr>
<td>Thyroid gland, (g·g body wt⁻¹)·10³</td>
<td>5.78±0.92</td>
<td>6.60±0.29</td>
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<tr>
<td>Adrenal gland, (g·g body wt⁻¹)·10⁴</td>
<td>1.71±0.061</td>
<td>1.61±0.11</td>
</tr>
<tr>
<td>Spleen, (g·g body wt⁻¹)·10³</td>
<td>3.24±0.20</td>
<td>3.33±0.14</td>
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<td></td>
<td>21.2±0.35</td>
<td>19.9±0.96</td>
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<td></td>
<td>1.21±0.028</td>
<td>1.21±0.13</td>
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<td></td>
<td>4.77±0.54</td>
<td>5.26±0.37</td>
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<td>2.12±0.24</td>
<td>2.75±0.20</td>
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<td>4.82±0.34</td>
<td>4.65±0.24</td>
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Values are means ± SE; n = 4–8 offspring in each group. Organ weight is normalized to body wt. P values reflect control vs. stress within each sex.

Table 3. Body and organ weights of 25w male and female offspring of control and stressed dams

<table>
<thead>
<tr>
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<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Stress</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>27.5±0.6</td>
<td>28.1±0.8</td>
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<tr>
<td>Kidney, (g·g body wt⁻¹)·10²</td>
<td>1.34±0.025</td>
<td>1.16±0.027</td>
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<tr>
<td>Thyroid gland, (g·g body wt⁻¹)·10³</td>
<td>7.41±0.11</td>
<td>6.46±0.17</td>
</tr>
<tr>
<td>Adrenal gland, (g·g body wt⁻¹)·10⁴</td>
<td>2.15±0.20</td>
<td>1.40±0.059</td>
</tr>
<tr>
<td>Spleen, (g·g body wt⁻¹)·10³</td>
<td>3.69±0.12</td>
<td>3.05±0.22</td>
</tr>
<tr>
<td></td>
<td>22.8±1.2</td>
<td>21.9±1.4</td>
</tr>
<tr>
<td></td>
<td>1.23±0.028</td>
<td>1.19±0.036</td>
</tr>
<tr>
<td></td>
<td>4.78±0.51</td>
<td>4.07±0.12</td>
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<tr>
<td></td>
<td>2.37±0.14</td>
<td>2.08±0.28</td>
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<tr>
<td></td>
<td>4.57±0.46</td>
<td>4.69±0.22</td>
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Values are means ± SE; n = 4–14 offspring in each group. Organ weight is normalized to body wt. P values reflect control vs. stress within each sex.

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controls (Fig. 1A, P < 0.05). However, as the mice grew older, aortas from 25w stress mice contracted more in response to PE than aortas from control mice (Fig. 1B, P < 0.001). Whereas maximal contractile force in response to PE was different between the groups, the sensitivity to PE was not different between the aortas from male stress and control mice at either age (data not shown). K⁺-induced maximal smooth muscle contraction did not differ between male 12w stress and control mice (1.4 ± 0.26 vs. 1.5 ± 0.14 mN/mm). However, contrary to the higher contraction in response to PE, maximal K⁺-induced contraction was lower in male 25w stress mice compared with their controls (1.5 ± 0.10 vs. 2.9 ± 0.35 mN/mm, P < 0.01).

Aortas from female mice had a similar maximal response and sensitivity to PE irrespective of group and age (data not shown). The maximal K⁺-induced contraction was also similar between female stress mice and controls (data not shown).
**Endothelium-dependent relaxation.** In males, relaxation in response to MCh was not different between aortas from either 12w or 25w stress and control mice (Fig. 2, A and B, respectively). Similarly, in females, relaxation in response to MCh did not differ between aortas from either 12w or 25w stress and control females (Fig. 3, A and B, respectively).

**NO-dependent relaxation.** L-NAME was used to study NO contribution to total relaxation. In aortas from 12w stress males, L-NAME had less effect on relaxation compared with their corresponding controls (Fig. 2A), indicating a reduced NO contribution to total relaxation. The effect of L-NAME was similar in aortas from 25w stress and control offspring (Fig. 2B). There was a decreased NO dependency with aging in control mice that was absent in the stress offspring, as shown in Fig. 2C, which summarizes the NO contribution to total relaxation.

In females, L-NAME had a similar effect on aortic relaxation in stress and control mice at both 12w and 25w (Fig. 3, A and B, respectively). The NO contribution to total relaxation of aortas from females is summarized in Fig. 3C.

**Endothelium-independent relaxation.** A SNP dose-response curve was conducted to test endothelium-independent NO-mediated smooth muscle relaxation. There was no difference in maximal SNP-induced relaxation between aortas from male 12w stress and control mice (97 ± 13 vs. 95 ± 13%), nor was there a difference between aortas from male 25w stress and control mice (98 ± 14 vs. 100 ± 13%). Likewise, aortas from female 12w stress mice showed similar response to controls (92 ± 13 vs. 90 ± 12%). Finally, aortas from female 25w stress mice had a maximum SNP-induced relaxation of 92 ± 13% and aortas from controls relaxed 93 ± 13%. The sensitivity to SNP was similar in all groups.

**Atherosclerotic lesion area.** Atherosclerosis was quantified in the thoracic aorta from male and female 12w and 25w mice. No differences were found between stress and control groups among 12w mice. Similarly, there was no difference between male 25w stress and control mice, whereas female 25w stress mice had a larger lesion area compared with controls (Fig. 4). Furthermore, lesion area increased significantly with increasing age (the actual ages of the mice in the 25w groups ranged from 22 to 26 wk); thus, separate comparisons of a more narrow age range that included only 25- to 26-wk-old mice showed that male and female stress offspring had larger total lesion area than controls (Fig. 5).

**DISCUSSION**

The hypothesis of this study was that maternal stress during pregnancy would alter vascular endothelial function and accelerate the development of atherosclerosis in the adult apoe<sup>−/−</sup> offspring. The first main finding was that male offspring of dams exposed to stress during pregnancy had developed endothelial dysfunction at 11–15 wk of age and more atherosclerosis at 25–26 wk of age compared with control mice. The second main finding was that female offspring of dams exposed to stress during pregnancy also had developed more atherosclerosis at 25–26 wk of age.

The apoe<sup>−/−</sup> mice are a common animal model in atherosclerosis research, since they develop atherosclerotic lesion...
Lesion development occurs spontaneously in $\text{apoE}^{-/-}$ mice, but, despite this, high-fat diet is commonly used in atherosclerosis research since high-fat content accelerates lesion development. However, we fed mice normal chow, as to not interfere with the spontaneous lesion development. Our studies were designed to determine whether maternal stress has an impact on spontaneous atherosclerosis lesion development in the offspring. We used restraint to induce a physiological stress response in pregnant mice. Restraint has been used in many studies on maternal stress, for example, to study how stress affects behavior and physical development of the offspring later in life (16, 29). However, it has not been used in studies of atherosclerosis development. Our study demonstrated that maternal stress leads to larger atherosclerotic lesion area in both male and female offspring.

RestRAINT of mice results in an immediate adrenocortical release of corticosterone into the circulation. Repeated stress may cause habituation and decreased release of corticosterone. However, in our study, restraint of pregnant dams for 2 h/day did not cause any decrease of corticosterone release over time. In fact, the maternal corticosterone levels after restraint were roughly 200% higher than the levels in controls, even on the 5th day of restraint. The lack of habituation is in agreement with earlier studies showing that repeated stress does not lead to habituation in mice (17, 26).

One mechanism by which glucocorticoids are thought to cause fetal programming is by inhibiting fetal growth. Indeed, elevated maternal corticosterone levels because of psychological stress at midgestation have been found to be related to lower fetal weight (7). It is clear from both human and animal studies that reduced size at birth is a predictor for later cardiovascular and metabolic disease (2). However, birth weight can also be regarded a crude outcome measure for an adverse in utero environment, and some of the many factors that may affect the in utero environment may be responsible for fetal programming without affecting birth weight. For example, short gestational exposure to cortisol leads to hypertension in adult sheep without affecting birth weight (8). Similarly, we did not detect any birth weight reduction resulting from maternal stress in our study.

High blood pressure is a well-known risk factor for atherosclerosis in humans. For $\text{apoE}^{-/-}$ mice, however, there is a
substantial amount of data suggesting that hypertension is a result rather than a cause of atherosclerotic lesion development (30, 32). As an example, mice treated with ANG II infusion did develop both high blood pressure and more atherosclerosis, but a similar increase of blood pressure induced by norepinephrine infusion only minimally affected lesion development (30). In terms of programming of blood pressure, it has been shown that administration with the synthetic corticosteroid dexamethasone to rats during part of their pregnancy resulted in hypo-

Fig. 5. Quantification of atherosclerotic lesion area. Graphs of atherosclerotic lesion area (expressed as %total aorta area) in thoracic aorta from 25- to 26-wk-old offspring. The control offspring were born to control dams, and the stress offspring were born to dams exposed to repeated restraint stress during pregnancy. A: lesion area was larger in male stress offspring (n = 6) than in control offspring (n = 10). B: lesion area was larger in female stress offspring (n = 8) than in control offspring (n = 13). *P < 0.05, Mann-Whitney U-test with multiple-comparison Bonferroni correction to avoid the effect of multiple comparisons, since the data are derived from Fig. 4. The line and bars represent median and interquartile range, respectively.

smooth muscle-mediated response to NO was not causative of the altered relaxation. There are many potential reasons for the finding that L-NAME was less effective in stress offspring that we can only speculate upon. For example, there could be a shift in the balance between endothelial vasodilators, with endothelium-derived hyperpolarizing factors or prostaglandins playing a greater role in the male offspring of stressed mothers than in controls. This could be a compensatory mechanism for lower NO modulation of vascular tone in this model. Interestingly, the observed shift between vasoactive factors was not seen in female offspring of stressed mothers.

In aortas from male control offspring, aging from 12w to 25w reduced NO contribution to total relaxation, as observed by the reduced effect of L-NAME on vasorelaxation in the 25w controls. The 12w stress offspring had reduced NO contribution to total relaxation compared with 12w control offspring, but no effect of aging was observed in the stress offspring. It is intriguing to speculate that the similarities between the male 12w stress offspring’s and the 25w control offspring’s vascular phenotypes are the result of “accelerated ageing” of the male stress offspring’s vasculature, which was not evident in females.

The importance of endogenous NO has been demonstrated in apoe<sup>-/-</sup> mice, where chronic inhibition of NO accelerates lesion formation (13). Because NO is proposed to be anti-atherosclerotic, it is possible that the 12w male stress mice were lacking NO at an early age, which contributed to accelerated lesion formation. We therefore studied atherosclerotic lesion area in the thoracic aorta, initially using 12w mice. However, atherosclerotic lesion area did not differ between either male or female stress and control mice. The lesion areas were ~1% of the total luminal areas, with lesions concentrated to the aortic arch and very few lesions on the thoracic part, as has been reported previously (11).

We then determined lesion area in 22- to 26-wk-old stress and control offspring. At first, we did not detect any difference in the extent of atherosclerosis between male stress and control mice at this age. However, the dispersion in lesion area was large, and the age difference between mice within this group was up to 5 wk. It is therefore likely that the variation in age caused some of the dispersion seen in plaque area within the groups. Because apoe<sup>-/-</sup> mice do have a progressive development of atherosclerosis (20), we separately analyzed the data from only the oldest mice, 25–26 wk old. This revealed that male stress offspring had distinctively larger lesion area of thoracic aorta than control mice. In addition to having impaired endothelial function and more atherosclerosis, the weight of kidneys, adrenal glands, spleen, and thyroid gland from male stress offspring was reduced compared with organs from controls. Because body weights did not differ between stress and control offspring, organ size seemed to be specifically targeted. Taken together, maternal stress had consequences for the male offspring’s vascular endothelial function as well as for development of atherosclerosis.

In females, maternal stress had no significant effect on endothelial function at any age. In regard to lesion area, there was no difference between the younger stress and control mice. However, stress female of the broad older age range (22–26 wk) displayed larger lesion area compared with control females. Stress females also had a larger lesion area when the more narrow age ranges (25–26 wk) were compared. Overall, females appeared to develop more lesions than males, irrespec-
tive of maternal condition. Most of the earlier studies have not compared males and females, making it difficult to compare our findings with existing literature. Comparisons are further complicated by inconsistencies in the methods used for quantification (i.e., aortic root, whole aorta, or thoracic aorta) and in the way data are reported (in mm² or as percent). However, one study that looked at 12 wk-old male and female apoe⁻/⁻ mice found that the lesion area did not differ between the sexes (15).

The apoe⁻/⁻ mice are hypercholesterolemic because of their lack of the glycoprotein apolipoprotein E, which serves as a ligand for receptor-mediated clearance of serum lipids. Thus hypercholesterolemia predisposes these mice, as well as humans, to development of atherosclerosis. Because prenatal overexposure to glucocorticoids has been shown to alter lipid metabolism (5), it is possible that the offspring’s serum lipid levels (5) are affected by maternal stress. However, we did not find any differences in cholesterol or triglyceride levels between control and stress offspring. Thus it is not likely that altered lipid metabolism played a major role for the increase in atherosclerotic lesion area in this study.

The effects of stress during pregnancy can be studied in humans, but the results are often difficult to evaluate, since it is uncertain whether the outcome is attributable to the stress itself or confounding factors (14). Furthermore, the measures of stress, whether they are subjective or objective, have some drawbacks. One drawback of subjective measures is the risk of underreporting while objective methods measure the stressful events themselves rather that the perceived stress. Perceived stress cannot be estimated in animal models, but the advantages are that the timing and amount of stress can be more precisely applied, and it is possible to control for confounding social and environmental factors.

**Perspectives and Significance**

This study demonstrates that vascular physiology can be affected in the offspring even by mild emotional maternal stress that does not alter birth weight of the offspring. Larger atherosclerotic lesion area in both male and female apoe⁻/⁻ offspring suggests that both sexes ultimately are vulnerable to maternal stress. However, this study also suggests that the mechanisms by which maternal stress affects the vasculature might differ between male and female offspring. Effects on endothelial function were only obvious in males. Future studies could address the specific mechanisms behind the increased development of atherothrombosis following maternal stress. They could also pay special attention to the observed differences in vascular function between the sexes. In conclusion, maternal stress may be of importance for the future cardiovascular health of the offspring by influencing the development of atherosclerosis.

**ACKNOWLEDGMENTS**

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