Gender-Linked Hypertension in Offspring of Lard-Fed Pregnant Rats

Imran Y. Khan, Paul. D. Taylor, Vasia Dekou, Paul T. Seed, Lorin Lakasing, Delyth Graham, Anna F. Dominiczak, Mark A. Hanson, Lucilla Poston

Abstract—Epidemiological studies suggest an association between maternal nutrition and offspring cardiovascular disease. We previously demonstrated endothelial dysfunction and abnormal aortic fatty acid composition in adult female offspring of rats fed animal lard during pregnancy. We have now further investigated this model. Female Sprague-Dawley rats were fed a control breeding diet (5.3% fat) or a diet rich in lard (25.7% fat) 10 days before and throughout pregnancy and lactation. Male and female offspring were implanted with radiotelemeters for recording of blood pressure, heart rate, and activity at 80, 180, and 360 days of age. Reactivity to acetylcholine and to nitric oxide were assessed in isolated small mesenteric arteries from 80- and 180-day-old littermates. Systolic blood pressure (awake phase) was raised in female offspring (180 days: offspring of control, 130.7±1.6 mm Hg, n=5, versus offspring of lard-fed, 138.1±2.9, n=5, P=0.029; 360 days: offspring of control, 129.7±3.7 mm Hg, n=6, versus offspring of lard-fed, 142.1±3.2, n=6, P=0.005). Diastolic blood pressure was also raised at 180 days (offspring of control, 87.6±1.0 mm Hg, n=5, versus offspring of lard-fed, 94.7±2.6, n=5, P=0.011). Blood pressure was not raised in male offspring. Endothelium-dependent relaxation to acetylcholine was blunted in male and female offspring of lard-fed dams (80 and 180 days). Feeding a diet rich in lard to pregnant rats leads to gender-related cardiovascular dysfunction in normally fed offspring. (Hypertension. 2003;41:168-175.)

Key Words: pregnancy ■ rats ■ diet ■ blood pressure ■ endothelium

Epidemiological studies have reported associations between low birth weight and adult cardiovascular disease, giving rise to the “fetal origins of adulthood disease” hypothesis. The majority of investigations have focused on associations of smallness at birth or of disproportionate growth with later disease, but it is now widely appreciated that these outcomes, which are easily measurable indexes of fetal undernutrition, may not be the most appropriate markers of fetal nutritional status. Fetal nutrition may be compromised not only by overt maternal undernutrition and/or placental insufficiency leading to growth restriction but also through maternal nutritional imbalance, with less obvious or measurable alterations in parameters of neonatal outcome.

The most common maternal dietary imbalance in Western populations is an excessive intake of dietary fat. Although the cardiovascular hazards of a raised intake of dietary fat in adults are well recognized, it is not known whether a maternal diet containing a disproportionately high fat content in pregnancy may predispose the offspring to cardiovascular disease, although indirect evidence suggests that this may be so. Autopsies of the children of hypercholesterolemic women have shown aortic streaks, with the size of the lesion being related to maternal risk factors for atherosclerosis. Maternal fat intake during late pregnancy has also been found to correlate with a reduction in placental size and birth weight.

Fetal “programming” may be investigated in a more controlled manner by using laboratory animals that age rapidly and have similar genetic backgrounds. Previously, we have demonstrated abnormalities in plasma lipids, vascular fatty acids, and evidence for reduced endothelium-dependent relaxation in adult offspring of Sprague-Dawley rats fed a lard-rich diet during pregnancy. This diet, which was designed to mimic the fat intake of a typical high-fat Western diet, also caused a reduction in the content of the cardioprotective fatty acids docosahexaenoic acid (DHA) and arachidonic acid in the aortas of the adult offspring. Others have reported an elevation in blood pressure in 7-week-old offspring of rat dams fed coconut oil during pregnancy and fatty lesions in neonatal and adult offspring of rabbits whose dams were fed a diet rich in cholesterol.
pressure in conscious, unrestrained animals. Offspring were studied at 80, 180, and 360 days of age. Vascular endothelial function was also assessed at 80 and 180 days in isolated small mesenteric arteries in littermates by using the technique of small-vessel myography. The measurement of plasma lipids was also undertaken.

**Methods**

**Animal Husbandry and Experimental Diets**

Female Sprague-Dawley (100 to 120 days) rats were fed, for 10 days before mating and throughout pregnancy and lactation, either a control breeding diet of normal laboratory chow (5.3% fat [corn oil], 21.2% protein, 57.4% carbohydrate, 4.6% fiber; Rat and Mouse Diet No. 3 [RM3], Special Diet Services) or the RM3 diet supplemented with animal lard and with added vitamins, minerals, and protein to compensate for reduction in these components incurred by addition of lard on a weight-to-weight basis (final analysis: 25.7% fat, 19.5% protein, 41.3% carbohydrate, 3.5% fiber; estimated fats: palmitic acid 4.50%, stearic acid 1.99%, palmitoleic acid 0.12%, oleic acid 6.86%, linoleic acid 2.58%, α-linolenic acid 0.25%, arachidonic acid 0.19%, Special Diet Services). Composition was confirmed by independent analysis (Eclipse Scientific Group). Maternal food intake was measured daily. At the 1-week postpartum examination, all litters were reduced to 8 pups (except 1 of the control and 2 lard-fed litters in which 7 pups were delivered), and, where possible, to equal numbers of male and female pups. After weaning, all offspring were fed ad libitum the standard maintenance diet (RM1). Food intake and animal weights were recorded from 1 week of age (to avoid maternal rejection of the pups) until animals were fully grown.

**Radiotelemetry Monitoring of Offspring Blood Pressure, Heart Rate, and Activity**

Blood pressure (systolic, diastolic, and mean pressure), heart rate, and activity were assessed by radiotelemetry (Dataquest IV, Data Sciences International). Randomly selected littermates (1 male, 1 female, from 6 litters) at 80 days ±7 days, 180 days ±7 days, and 360 days ±14 days of age were administered buprenorphine (0.1 mg/kg SC) before surgery and anesthetized with isoflurane. A flexible catheter was secured in the abdominal aorta, and the telemetry transmitter was sutured to the abdominal wall. On recovery, rats were housed in individual cages, and each cage was placed over a receiver panel with output to a PC. After a 1-week recovery period, heart rate, systolic, diastolic, mean arterial blood pressures, and activity were recorded for 10 seconds every 5 minutes for 1 week. Twelve-hour day and night mean values were computed. At the end of this week, animals were fasted overnight and then killed by CO2 inhalation. Blood samples for total cholesterol (UNIMATE CHOL, Roche Diagnostic Systems), triglycerides (UNIMATE TRIG, Roche Diagnostic Systems), high density lipoprotein (Alpha Laboratories), glucose (HK/G6P-DH enzymatic UV test, Roche Diagnostic Systems), and insulin (DGR Instruments) were obtained by cardiac puncture, and plasma was stored at −70°C.

**Offspring Mesenteric Artery Function**

Mesenteric vascular function was assessed in 80- and 180-day-old littermates of animals used for telemetric recording. Rats were killed by CO2 inhalation. The liver, lungs, heart, kidney, spleen, and brain were weighed after swabbing. One male and one female offspring from each litter were studied. Third-order branches of the mesenteric arcade were dissected and mounted in physiological salt solution (PSS) on a Mulvany-Halpern Small Vessel Myograph (J.P. Trading, DK-8000). Concentration responses to norepinephrine (NE; 10−5 to 10−7 mol/L) and nitric oxide (NO; 10−5 to 10−7 mol/L) were determined in arteries submaximally constricted with NE (80% of maximal concentration), to acetylcholine (ACh; 10−6 to 10−5 mol/L) and the receptor panel with output to a PC. After a 1-week recovery period, heart rate, systolic, diastolic, mean arterial blood pressures, and activity were recorded for 10 seconds every 5 minutes for 1 week. Twelve-hour day and night mean values were computed. At the end of this week, animals were fasted overnight and then killed by CO2 inhalation. Blood samples for total cholesterol (UNIMATE CHOL, Roche Diagnostic Systems), triglycerides (UNIMATE TRIG, Roche Diagnostic Systems), high density lipoprotein (Alpha Laboratories), glucose (HK/G6P-DH enzymatic UV test, Roche Diagnostic Systems), and insulin (DGR Instruments) were obtained by cardiac puncture, and plasma was stored at −70°C.

**Chemicals**

Chemicals used were NE (Abbott Laboratories Ltd), ACh, and U46619 (Sigma Chemical Co), and NO (BDH). All chemicals for PSS were from BDH.

**Statistical Analysis**

**Power Calculations**

On the basis of previously published studies from other laboratories for telemetric recording of blood pressure, at least 6 animals were required to achieve 90% power to detect a 10–mm Hg difference, with a probability of P < 0.05. In two groups (offspring of control females and offspring of lard-fed females at 180 days of age), data from only 5 animals is given because 1 animal died in surgery. For vascular studies, an estimated 12 animals were required to achieve...
Blood Pressure, Heart Rate, and Activity

All values are given as mean ± SEM. To evaluate changes at each time point, multiple regression with generalized estimating equations (GEE) was used to estimate differences between maternal diet groups and to test for interactions between groups and gender of offspring. Standard errors were corrected for multiple observations on each animal by use of the Huber sandwich estimator.\textsuperscript{15} Statistical significance was assumed at a value of $P<0.05$.

Vascular Function and Plasma Analyses

All values are given as mean ± SEM. The negative log of the concentration (mol/L) of a drug required to produce 50% of the maximum response ($pEC_{50}$) was calculated after data were fitted to a sigmoidal curve (GraphPad Software Inc). Statistical comparisons were made by Student’s $t$ test. Statistical significance was assumed at a value of $P<0.05$.

Results

Successful pregnancies in animals fed the different dietary regimes occurred in 88% of control rats and 100% of lard-fed rats. Data are presented for the offspring of a total of 12 and 14 litters fed the control and lard-rich diets, respectively.

Maternal Food Intake

In both control and lard-fed dams, food intake did not alter with gestation over the first 15 days of pregnancy but fell sharply toward term in both groups (20 to 21 days). Dams on the lard diet consumed less food daily than those on the standard rat chow (average daily intake day 0 to 20, 25.3 ± 0.66 g/d for control versus 19.82 ± 0.60 g/d for lard fed, $P<0.001$), such that a caloric intake similar to that of the control rats was consumed (average daily gross energy intake; control rats, 379.0 ± 9.9 kJ versus 388.3 ± 11.8 kJ for lard-fed rats), as previously reported in similar studies.\textsuperscript{16} As a result, the lard-fed dams consumed 5.09 g fat per day compared with 1.34 g in the control rats, a 380% increase.

Offspring Food Intake and Body Weight

Male offspring consumed more food than female offspring in both groups, but among male and female offspring, food intake was not different between groups from weaning until adulthood. Body weight was no different at 7 days in offspring (male and female offspring combined) from litters of the two groups (offspring of control rats, 11.8 ± 1.25, $n=12$, versus offspring of lard-fed rats, 11.3 ± 1.25, $n=12$), and, after weaning, weight was only different between the groups in the female offspring at 360 days (offspring of control, 399.1 ± 17.82 g, $n=8$, versus offspring of lard-fed rats, 458.27 ± 14.65 g, $n=11$, $P=0.020$) (Figure 1). Body and organ (liver, lungs, heart, kidney, spleen, and brain) weights were not different between

### TABLE 1. Cardiovascular Parameters and Activity in Offspring of Control (C) and Lard Fed (LF) Dams at 80, 180, and 360 Days

<table>
<thead>
<tr>
<th>Parameter</th>
<th>80 Days</th>
<th>180 Days</th>
<th>360 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C Male</td>
<td>LF Male</td>
<td>C Female</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Night</td>
<td>105.9±4.0</td>
<td>109.4±4.6</td>
<td>107.1±2.7</td>
</tr>
<tr>
<td>Day</td>
<td>103.2±3.8</td>
<td>105.5±4.2</td>
<td>103.0±2.2</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Night</td>
<td>424.0±7.5</td>
<td>430.6±7.9</td>
<td>375.2±9.1</td>
</tr>
<tr>
<td>Day</td>
<td>444.7±9.1</td>
<td>449.4±7.5</td>
<td>398.2±9.6</td>
</tr>
<tr>
<td>Activity</td>
<td>4.3±0.4</td>
<td>4.4±0.3</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>Day</td>
<td>1.4±0.1</td>
<td>1.6±0.1</td>
<td>2.2±0.1</td>
</tr>
</tbody>
</table>

*P<0.05 offspring of control vs offspring of lard fed; †P<0.01 offspring of control vs offspring of lard fed.
groups at 80 days or 180 days of age when vascular function was measured (data not shown).

Radiotelemetry Monitoring of Offspring Blood Pressure, Heart Rate, and Activity

Male Rats
There were no significant differences between the male offspring of control or lard-fed dams in diastolic, systolic, and mean arterial blood pressure (MAP), activity, or heart rate at 80, 180, or 360 days (Figure 2 and Table 1).

Female Rats
Systolic blood pressure was not raised in 80-day-old offspring of lard-fed dams when compared with control rats. However, systolic blood pressure was significantly raised at 180 days of age (awake phase: offspring of control rats, 130.7±1.6 mm Hg, n=5, versus offspring of lard-fed rats, 138.1±2.9, n=5; P=0.029) and 360 days (offspring of control rats, 129.7±3.7 mm Hg, n=6, versus offspring of lard-fed rats, 142.1±3.2, n=6; P=0.005) (Figure 3). Diastolic blood pressure was not different in the two groups at 80 days of age. Diastolic blood pressure was raised in the 180-day-old female offspring of lard-fed dams (offspring of control rats, 87.6±1.0 mm Hg, n=5, versus offspring of lard-fed rats, 94.7±2.6, n=5; P=0.011), and, similarly, at 360 days (offspring of control rats, 87.9±2.3 mm Hg, n=6, versus offspring of lard-fed rats, 94.3±2.7, n=6; P=0.011) (Figure 3). Mean arterial pressure was also raised at 180 days (P=0.008) and 360 days (P=0.007) in female offspring of lard-fed dams. Activity was reduced in the female offspring of lard-fed dams during the waking phase at 180 days (P<0.001). Heart rate was unaffected at any of the time points studied.

Offspring Mesenteric Artery Function
Endothelium-Dependent Relaxation

Male rats.
Sensitivity to ACh (as assessed by EC50) was not different between the two groups at 80 or 180 days. Maximum endothelium-dependent relaxation to ACh in small mesenteric arteries was reduced in the male offspring of lard-fed dams at 80 days (P=0.003). Maximal relaxation was also reduced in the 180-day male offspring of lard-fed animals (P=0.001) (Figure 4 and Table 2).
The novelty of this study lies in the observation that supplementation of the maternal diet with animal lard during rat pregnancy leads to an increase in blood pressure in the offspring and that this was confined to the female rats. In contrast, both male and female offspring demonstrated abnormalities of endothelium-dependent dilation. There was thus a clear dissociation between endothelial dysfunction and elevation of blood pressure.

The method of radiotelemetry has marked advantages over the tail-cuff method for the measurement of blood pressure in rats because it enables recording of systolic and diastolic blood pressures in conscious unrestrained and unanesthetized animals. The majority of reports in which offspring blood pressure has been implicated in rat models of fetal programming have used the tail-cuff plethysmography method of measurement, which records only systolic blood pressure. This also involves restraint of the animal and could artifactually imply substantive elevation in basal blood pressure while failing to observe a selective increase in pressor responses. Radiotelemetry also provides a contrast between day (sleep) and night time (awake) values, together with heart rate and an estimate of activity. The observation in the current study that the increase in systolic and diastolic blood pressures were confined to the female offspring is unexpected and interesting. The female offspring alone also showed reduced activity at 180 days during the awake phase (dark period), but this was not related to a reduction in heart rate, which

**Female rats.**
Sensitivity to ACh was not different between the groups. Maximum endothelium-dependent relaxation to ACh was reduced in female offspring at 80 days (P=0.023). This defect persisted to 180 days of age (P=0.012) (Figure 4 and Table 2).

**Endothelium-Independent Relaxation**

**Male and female rats.** Neither sensitivity to exogenous NO nor maximum relaxation was different between the groups at 80 or 180 days of age.

**Plasma Analyses**

**Male Rats**
None of the biochemical parameters tested were significantly different among the groups at 80 days or 180 days (see Table 3). The male offspring at 360 days had reduced HDL (P=0.008) and reduced total cholesterol (P=0.024).

**Female Rats**
Biochemical parameters were similarly unaffected at 80 and 180 days. At 360 days, however, the female offspring had raised fasting glucose (P=0.004), raised triglycerides (P=0.019), and reduced HDL (P=0.006).

**Discussion**

The table below shows the vascular function in offspring of control (C) and lard fed (LF) dams at 80 and 180 days.

<table>
<thead>
<tr>
<th>Vascular Parameters</th>
<th>C Male</th>
<th>LF Male</th>
<th>C Female</th>
<th>LF Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 days, n</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Lumen diameter, μm</td>
<td>285.00±11.73</td>
<td>257.24±14.19</td>
<td>234.65±12.73</td>
<td>231.89±15.61</td>
</tr>
<tr>
<td>Maximal contraction</td>
<td>3.50±0.24</td>
<td>3.96±0.35</td>
<td>2.53±0.28</td>
<td>2.54±0.24</td>
</tr>
<tr>
<td>Noradrenaline, nM/mm</td>
<td>84.58±3.21</td>
<td>67.60±3.70</td>
<td>80.74±5.23</td>
<td>63.10±5.00*</td>
</tr>
<tr>
<td>Maximal relaxation, %</td>
<td>91.94±1.04</td>
<td>89.63±0.79</td>
<td>85.86±2.56</td>
<td>89.17±1.74</td>
</tr>
<tr>
<td>pEC50</td>
<td>-5.70±0.04</td>
<td>-5.67±0.06</td>
<td>-5.74±0.04</td>
<td>-5.66±0.04</td>
</tr>
<tr>
<td>NA</td>
<td>-7.31±0.04</td>
<td>-7.44±0.06</td>
<td>-7.08±0.07</td>
<td>-7.19±0.08</td>
</tr>
<tr>
<td>ACh</td>
<td>-6.73±0.08</td>
<td>-6.62±0.05</td>
<td>-7.08±0.08</td>
<td>-6.83±0.06</td>
</tr>
<tr>
<td>180 days, n</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Lumen diameter, μm</td>
<td>281.72±15.07</td>
<td>280.45±20.48</td>
<td>256.01±7.34</td>
<td>249.88±15.08</td>
</tr>
<tr>
<td>Maximal contraction</td>
<td>3.91±0.30</td>
<td>4.48±0.42</td>
<td>3.40±0.44</td>
<td>2.51±0.32</td>
</tr>
<tr>
<td>Noradrenaline, nM/mm</td>
<td>74.93±4.33</td>
<td>48.00±5.01*</td>
<td>71.22±6.40</td>
<td>45.58±6.64*</td>
</tr>
<tr>
<td>Maximal relaxation, %</td>
<td>89.25±3.56</td>
<td>85.84±2.98</td>
<td>87.18±2.70</td>
<td>87.12±2.68</td>
</tr>
<tr>
<td>pEC50</td>
<td>-5.53±0.06</td>
<td>-5.57±0.05</td>
<td>-5.68±0.07</td>
<td>-5.72±0.08</td>
</tr>
<tr>
<td>NA</td>
<td>-7.32±0.12</td>
<td>-7.40±0.10</td>
<td>-7.10±0.16</td>
<td>-6.94±0.14</td>
</tr>
<tr>
<td>ACh</td>
<td>-6.53±0.07</td>
<td>-6.56±0.07</td>
<td>-6.93±0.27</td>
<td>-6.52±0.08</td>
</tr>
</tbody>
</table>

*P<0.05 offspring of control vs offspring of lard fed; †P<0.01 offspring of control vs offspring of lard fed; ‡P<0.001 offspring of control vs offspring of lard fed.
may infer an increase in baseline sympathetic activity and so contribute to elevation of the blood pressure.

As far as we are aware, this is the first investigation to have undertaken a comprehensive longitudinal study with the use of radiotelemetry for the measurement of blood pressure in any animal model of fetal programming. A previous study by Tonkiss et al, in which blood pressure was measured at 96 days of age over one 24-hour time period, had suggested a small (4 mm Hg) but significant rise in the awake-phase diastolic blood pressure in male offspring of dams fed a diet with severely reduced protein content (6% versus 25% in control rats). Female offspring were not studied. In that study, pressor responses to ammonia odor also showed an abnormal profile in the offspring of the undernourished rats, with a considerable increase over that shown by control rats in the initial pressor response to stress, emphasizing the importance of recording in conscious unrestrained animals.

Insight into potential mechanisms of hypertension in the female offspring may be gained from observations that fat feeding in nonpregnant rats leads to activation of the hypothalamic-pituitary-adrenal (HPA) axis. Furthermore, activation of the HPA axis in pregnant rats has been reported to lead to permanent and female specific alteration in the offspring HPA axis. It is also highly relevant that stress-induced ACTH secretion has been shown to be reduced in 35-day-old offspring of dams fed a 20% fat diet in the last week of pregnancy and during weaning, although male and female rats were considered together in the analyses. In view of the reported increase in blood pressure in offspring of sheep and rats administered synthetic glucocorticoids in pregnancy, these observations may imply that the lard-fed model is another example of fetal cardiovascular programming through maternal glucocorticoid excess. Retrospective analysis of plasma samples from the dams killed by asphyxia and cervical dislocation at day 20 indicated an elevation of plasma corticosterone (control rats, 541 ± 96 ng/mL, n = 11; versus lard-fed rats, 1134 ± 171, n = 11; P = 0.007). Studies are now underway to evaluate pressor responses to stress and biochemical markers of the HPA axis and of sympathetic activity in our model.

The recent observation that dietary-induced deficiency of the cardioprotective n-3 polyunsaturated fatty acid, DHA, in rats during the perinatal period is associated with the later development of hypertension may also be relevant to the development of hypertension in this study, as we have previously described a significant reduction in the female offspring aorta DHA content in this model. Cardiovascular dysfunction could also arise from an imbalance between the fat, carbohydrate, and protein composition of the diet in the control and lard-fed dams, which would also occur in pregnant women whose diet was similarly rich in dietary fat. However, the modest reduction in protein composition (19.5% in lard diet versus 21.2% in control diet) in the lard-fed dams compared with control dams is unlikely to have played a role, as 21-week-old offspring of dams fed as little as 12% protein diet are normotensive.

The endothelial dysfunction observed in the offspring of the lard-fed dams is consistent with our previous reports in young and adult female offspring, although we previously

### Table 3. Plasma Analyses in Offspring of Control (C) and Lard Fed (LF) Dams at 80, 180, and 360 Days

<table>
<thead>
<tr>
<th>Fasting Plasma Concentration</th>
<th>C Male</th>
<th>LF Male</th>
<th>C Female</th>
<th>LF Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 days, n</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>8.05 ± 0.77</td>
<td>8.16 ± 1.56</td>
<td>8.70 ± 3.04</td>
<td>5.60 ± 0.72</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>1.18 ± 0.07</td>
<td>1.46 ± 0.20</td>
<td>1.60 ± 0.09</td>
<td>1.52 ± 0.20</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.06 ± 0.14</td>
<td>1.08 ± 0.22</td>
<td>0.80 ± 0.19</td>
<td>0.72 ± 0.13</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>0.68 ± 0.14</td>
<td>0.90 ± 0.17</td>
<td>1.2 ± 0.00</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>180 days, n</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>13.68 ± 1.59</td>
<td>15.27 ± 1.38</td>
<td>10.69 ± 1.70</td>
<td>15.98 ± 3.73</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>2.96 ± 0.15</td>
<td>2.38 ± 0.27</td>
<td>3.07 ± 0.19</td>
<td>2.87 ± 0.20</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.42 ± 0.07</td>
<td>1.35 ± 0.24</td>
<td>0.96 ± 0.14</td>
<td>1.23 ± 0.09</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.47 ± 0.12</td>
<td>1.22 ± 0.16</td>
<td>1.73 ± 0.18</td>
<td>1.53 ± 0.15</td>
</tr>
<tr>
<td>Insulin, mmol/L</td>
<td>0.92 ± 0.36</td>
<td>0.55 ± 0.13</td>
<td>0.44 ± 0.18</td>
<td>0.71 ± 0.17</td>
</tr>
<tr>
<td>360 days, n</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>19.38 ± 2.89</td>
<td>20.27 ± 4.60</td>
<td>12.73 ± 2.19</td>
<td>22.53 ± 1.36</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>2.84 ± 0.19</td>
<td>2.16 ± 0.17</td>
<td>3.52 ± 0.24</td>
<td>3.60 ± 0.44</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.39 ± 0.14</td>
<td>1.28 ± 0.12</td>
<td>1.65 ± 0.15</td>
<td>2.51 ± 0.27</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.66 ± 0.12</td>
<td>1.16 ± 0.09</td>
<td>2.20 ± 0.13</td>
<td>1.60 ± 0.11</td>
</tr>
<tr>
<td>Insulin, mmol/L</td>
<td>0.98 ± 0.15</td>
<td>1.64 ± 0.60</td>
<td>1.96 ± 1.17</td>
<td>4.02 ± 1.46</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM.

*P < 0.05 offspring of control vs offspring of lard fed; †P < 0.01 offspring of control vs offspring of lard fed.
studied the femoral circulation.\textsuperscript{7,8} In our previous study, a similar defect of a blunted response to acetylcholine was observed in the femoral arteries of animals of a similar age.\textsuperscript{7} In this study, we chose mesenteric arteries in preference because the maximal relaxation to acetylcholine is more pronounced in comparison to that of femoral vessels. In the previous studies, the diet was not supplemented with micronutrients (as in this investigation), and a role for micronutrient deficiency could not be ruled out. The current data strongly suggest that endothelial dysfunction (and elevation of female blood pressure) is a direct result of the addition of the fat to the diet. Because there was no substantive defect in the response to NO, the defect appears to reside predominantly at the level of the endothelial cell. This may implicate deficiency in NO, prostacyclin, or endothelium-derived hyperpolarizing factor. A similar degree of endothelial dysfunction has been observed in the offspring of rat dams fed a low protein diet,\textsuperscript{30} which are also reported to be hypertensive.\textsuperscript{18,31} The HPA axis has also been implicated in the low protein model.\textsuperscript{32} A commonality of mechanisms may be implied between the low protein and lard models. Insulin resistance may also be a common link and is associated with endothelial dysfunction in humans\textsuperscript{33} and has been reported in offspring of rats fed a low protein diet\textsuperscript{34} or of dams administered dexamethasone.\textsuperscript{35} We have previously reported a lipid profile indicative of insulin resistance in the maternal lard-fed,\textsuperscript{36} and in the current study, the female offspring demonstrated raised fasting glucose and triglyceride levels at 360 days. Accordingly, insulin resistance is now being more rigorously assessed by the euglycemic hyperinsulinemic clamp.

The clear dissociation between blood pressure and endothelial dysfunction as observed in the male offspring has been substantiated previously in hypertensive rats,\textsuperscript{26} subordinating many reports that these two parameters are not invariably linked. In the female offspring, mechanisms other than failure of endothelium-dependent relaxation may contribute to the hypertension observed.

**Perspectives**

We have shown that the male and female offspring of dams fed a diet rich in lard demonstrate severely abnormal endothelial function, which in the female rats is associated with hypertension. This occurred despite these animals being fed a normal low fat diet from weaning and is likely to reflect an adverse influence of the maternal diet during the fetal or neonatal period. The balance of nutrients in the maternal lard diet was not dissimilar in composition from that eaten by many women in developed countries. If applicable to human pregnancy, fetal programming of adulthood endothelial dysfunction and of hypertension by raised maternal fat consumption may have major implications for the cause of cardiovascular disease in humans.

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**References**


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