Effects of Maternal Iron Restriction in the Rat on Blood Pressure, Glucose Tolerance, and Serum Lipids in the 3-Month-Old Offspring

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Epidemiologic studies have demonstrated associations between low birth weight and increased rates of adult diseases such as hypertension and diabetes. Maternal iron restriction in the rat has been reported to both reduce birth weight and to elevate blood pressure at 40 days of age. The aim of the present study was to extend these findings to investigate the effects of maternal iron restriction on glucose tolerance and serum lipids, 2 important components of the metabolic syndrome, in adult offspring. Blood pressure, glucose tolerance, and serum lipids were measured in the 3-month-old offspring of iron-restricted dams. Rats were placed on control or iron-restricted diets 1 week before mating. At term, dams on the iron-restricted diet were anemic with decreased haemoglobin, red blood cell (RBC) count, hematocrit, and mean RBC volume compared with controls. Neonates from iron-restricted litters were more severely anemic than the dams. At birth, body weight was lower in the offspring of iron-restricted dams than in controls and was still decreased at 3 months of age. At this same age, systolic blood pressure was significantly elevated in the offspring of iron-restricted dams. Glucose tolerance was improved in the maternal iron-restricted group. Fasting serum insulin levels were not different between the control and maternal iron-restricted groups. Fasting serum triglyceride was decreased in the offspring of iron-restricted dams compared with controls. Fasting serum cholesterol and free fatty acid concentrations were similar in both groups. These results suggest that maternal iron restriction has long-term effects on physiology and metabolism in the offspring. Some of these findings are comparable to those reported for the maternal protein-restriction model. It is thus speculated that the long-term effects of maternal dietary restriction may result from common fetal metabolic responses to this restriction.

EPIDEMIOLOGIC STUDIES suggest that there is an inverse relationship between birth weight and adult blood pressure.[1] A similar relationship has also been demonstrated between birth weight and glucose tolerance[2] and serum lipids.[3] Blood pressure, glucose tolerance, and dyslipidemia frequently occur together in humans and may be linked by a common metabolic disorder such as insulin resistance.[4]

A number of animal models have been established in an attempt to understand the mechanistic basis of this relationship. Maternal caloric restriction,[5] protein restriction,[6,7] maternal dexamethasone treatment,[8] umbilical artery ligation,[9] and maternal iron restriction[10] have all been shown to cause fetal growth retardation and also to elevate blood pressure in the offspring. It is not clear whether the increased blood pressure observed in these models is the consequence of a common pathway resulting from the fetal growth retardation or whether there are specific mechanisms associated with each particular insult. Maternal low-protein diets have also been reported to affect the offspring's serum cholesterol, triglycerides, and glucose tolerance.[11-13] Maternal dexamethasone treatment has also been reported to decrease glucose tolerance in the offspring.[14] These studies raise the question as to whether the long-term consequences are specific to the cause of fetal growth retardation or whether a common pathway mediates them.

Iron deficiency and anemia are common during pregnancy, particularly in the developing world.[15] Maternal anemia is associated with low birth weight and increased rates of perinatal mortality and morbidity.[15-18] In the rat, maternal iron restriction causes fetal growth retardation[10,13] and has been shown to cause elevated blood pressure in the 40-day-old offspring.[10]

The aim of this study was to investigate whether maternal iron deficiency had effects on blood pressure, glucose tolerance, and serum lipids in the 3-month-old offspring.

MATERIALS AND METHODS

Animals

All animal procedures were performed, under license, in accordance with the Scientific Procedures Act (1986). Virgin Wistar females were housed individually and maintained at 22°C. They were fed ad libitum either control (K4447.01) or low-iron diets (K4447.00; Hope Farms, Waerden, Holland) and provided with deionized water. The control diet was made by adding 150 mg/kg iron (as iron subcarbonate) to the low-iron diet, which contained 3 mg/kg of iron, so that in all other respects the 2 experimental diets were identical. The diets contained 17.5% crude protein, 5.2% crude fat, and 66.2% sugar and starch, with a gross energy content of 4.03 MJ/kg.

Prior to the study rats were fed LAD 1 diet (Special Diet Services, Witham, Essex, UK). One week before mating, dams were placed onto either the control diet or the low-iron diet. After 7 days on the diet dams were mated; those that did not mate within 4 days were excluded from the study. Dams were maintained on the control or low-iron diet throughout gestation. From the day of birth until weaning all dams were fed the control diet. Dams were weighed daily and their food intake recorded throughout the experiment.

Maternal blood was collected from the tail for hematologic measurements on day 21 of gestation. Full blood counts were performed in the Department of Haematology at Addenbrooke's Hospital, Cambridge, UK.

On postnatal day 2, three pups were culled from each of a subset of

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litters to collect blood for hematologic determinations. Pups were weighed on days 3, 7, 14, and 21. To standardize conditions, litters were culled to 8 on postnatal day 3. At 21 days of age, 2 male and 2 female offspring from each litter were weaned onto our standard LAD 1 diet. Eight control litters and 6 iron-restricted litters were bred successfully. In 1 iron-restricted litter, no males survived to weaning and 4 females were kept instead. The offspring were weighed weekly.

Blood Pressure Measurement

Blood pressures were recorded between 100 and 106 days of age using the indirect tail-cuff method. Rats were trained in the perspex restraining tubes for 10 to 15 minutes on 3 occasions in the 2 weeks before blood pressure was recorded. Rats were placed in perspex restraining tubes and acclimatized in a warmed chamber (27 to 29°C). Five systolic blood pressure recordings were made from each rat. Blood pressure was read from the traces by a blinded investigator. The highest and lowest blood pressure readings were excluded and the remaining 3 were averaged. To assess the repeatability of the results, 1 female and 1 male rat had blood pressures recorded on 4 separate occasions. The intra-assay coefficient of variation for the male rat was 5.8% and for the female rat 5.0%.

Intraperitoneal Glucose Tolerance Tests

Intraperitoneal glucose tolerance tests (IPGTTs) were performed on conscious rats between 106 and 118 days of age after an overnight fast (18 hours). Fasting glucose was measured from tail blood using a HemoCue Glucose Analyser (HemoCue, Sheffield, UK) and then 1 mL/100 g of body weight of 10% (wt/vol) glucose in 0.9% NaCl was administered by intraperitoneal injection. Glucose levels were then measured at 15, 30, 60, 120, and 180 minutes after the injection.

Serum Measurements

Blood was collected from the offspring between 114 and 124 days of age following an overnight fast. Blood from the tail was collected onto ice. Once clotted, the blood was centrifuged and the serum removed and stored at –20°C.

Serum insulin was measured using a rat insulin radioimmunoassay (RIA) kit (Linco Research, St Charles, MI). All samples were assayed in duplicate.

Serum lipids were measured enzymatically using a Monarch autoanalyzer (Instrumentation Laboratories, Lexington, MA). Fasting serum free fatty acids were measured using the Free Fatty Acids, Halfmicro test (Boehringer Mannheim, Mannheim, Germany, Cat #1383 175). Fasting serum triglyceride was measured using GPO trinder reagents (Sigma Diagnostics, St Louis, MO, Cat #337-A). Fasting serum cholesterol was measured using Infinity cholesterol reagent (Sigma Diagnostics, Cat #401-25P).

Statistical Analysis

For maternal and neonatal data, differences between the control and iron-restricted groups were analyzed using the unpaired *t* test. Data collected from the offspring after weaning at day 21 were analyzed using 2-way analysis of variance (ANOVA) to investigate effects of maternal diet and the sex of the offspring. To determine whether differences in body weight were influencing the results, analyses were also performed with body weight included as a covariate. Body weight data were analyzed by repeated-measures ANOVA. Analysis of the IPGTT curve was initially performed by repeated-measures ANOVA to demonstrate that there were differences overall, and individual time points were then analyzed by 2-way ANOVA. Data are presented as the mean ± SD except for corrected means (estimated marginal means), which are presented in the text as the mean ± SE. The insulin data had a skewed distribution and were analyzed using log-transformed data. The data for insulin are therefore presented as geometric means (95% confidence interval). Where data were abnormally distributed or there was unequal variance between the groups, analysis was performed using ranked data presented as the median (interquartile range). Significance was assumed at a P value of .05.

RESULTS

Maternal Body Weights

On the first day of pregnancy, there were no significant differences in maternal weight between the groups (Table 1). From day 14 of gestation until term, the iron-restricted dams were lighter than the control dams ($P < .05$, data not shown). At term, on day 22 of gestation, the iron-restricted dams were 14% lighter than the controls ($P < .001$, Table 1). Despite switching to the control diet at birth, the previously iron-restricted dams remained significantly lighter than control dams during lactation. On day 21 maternal weights were 347 ± 28 g in the control group and 318 ± 13 g in the iron-restricted group ($P < .05$).

In the week prior to mating there were no differences in maternal food intake between the control and low-iron diets. During gestation, food intake relative to body weight was lower in the iron-restricted dams ($P < .001$, Table 1). Maternal food intake relative to body weight was not significantly different during lactation (Table 1).

Hematology

On day 21 of gestation, maternal hemoglobin, hematocrit, and red blood cell (RBC) count were lower in the iron-restricted dams compared with the control dams ($P < .001$, Table 2). Maternal mean cell volume was not significantly different between the groups, while RBC distribution width was significantly greater in the iron-restricted dams ($P < .001$, Table 2).

On neonatal day 2, hemoglobin ($P < .001$, Table 3), RBC count ($P < .003$), hematocrit ($P < .005$), mean cell volume ($P < .002$), and RBC distribution width ($P < .05$) were all decreased in the offspring of iron-restricted dams.

Growth Characteristics of the Offspring

The offspring of iron-restricted dams had lower body weights on day 3 (6.2 ± 0.8 g *v* 4.8 ± 0.3 g, $P < .003$), day 7 (12.8 ± 2.4 g *v* 8.3 ± 1.8 g, $P < .002$), day 14 (29.6 ± 4.1 g *v* 22.1 ± 5.3 g, $P < .01$), and day 21 (48.6 ± 5.2 g *v* 39.9 ±

Table 1. Material Weight and Food Intake in Control and Iron-Restricted Dams

	Control (n = 8)	Iron-Restricted (n = 6)
Maternal weight on day 1 of gestation (g)	231 ± 10	239 ± 11
Maternal weight on day 22 of gestation (g)	437 ± 26	385 ± 12*
Maternal food intake, gestation (mg/day/g body wt)	87 ± 4	70 ± 3*
Maternal food intake, lactation (mg/day/g body wt)	116 ± 14	133 ± 15

NOTE. Data are means ± SD.
*$P < .001$.

Table 2. Maternal Hematology on Day 21 of Gestation in Control and Iron-Restricted Dams

	Control (n = 8)	Iron-Restricted (n = 6)
Hemoglobin (g/dL)	13.11 ± 1.07	10.00 ± 0.67*
Hematocrit (%)	39 ± 3	28 ± 4*
RBC count (× 10^9/L)	6.59 ± 0.56	5.19 ± 0.21*
Mean RBC volume (μm^3)	59.14 ± 2.66	55.48 ± 3.75
RBC distribution width	13.5 (12.5-14.5)	15.2 (15.0-16.8)*

NOTE. Data are means ± SD or medians (interquartile range).
*$P < .001$.

5.2 g, $P < .01$). After weaning, the offspring of iron-restricted dams were significantly smaller than the controls at each weekly measurement up to 14 weeks of age when experimentation began ($P < .001$, Fig 1). From 5 weeks of age, females were smaller than males in both groups ($P < .001$, Fig 1). Growth velocities (g/wk) were lower in the offspring of iron-restricted dams during the periods of maximal growth velocity ($P < .05$). In the males, growth velocity was lower in the iron-restricted offspring on weeks 5, 6, 7, and 8 when growth velocity was highest ($P < .05$). In females, where there was a more limited period of maximal growth velocity, growth velocity was lower at 5 weeks of age in the offspring of iron-restricted dams ($P < .05$).

Blood Pressure

Maternal diet had a significant effect on blood pressure ($P < .001$) and there was also an interaction between maternal diet and the sex of the offspring ($P < .05$, Table 4). This indicates that blood pressure was higher in the offspring of iron-restricted dams, but that the effect of maternal diet on blood pressure was strongest in the male offspring.

Glucose Tolerance

There were significant overall differences in the IPGTT results between the offspring of control and iron-restricted as determined by repeated-measures ANOVA ($P < .001$). There were no significant differences in fasting glucose between the groups (Fig 2). During the IPGTT, blood glucose was lower in the offspring of iron-restricted dams at 30 minutes ($P < .001$), 60 minutes ($P < .001$), and 120 minutes ($P < .05$, Fig 2), but was not significantly different between the sexes. Total area under the curve was decreased in the offspring of iron-restricted dams compared with controls ($P < .005$, Table 4). Area under the glucose tolerance curve was not significantly different between the sexes. Fasting serum insulin levels were not different between the control offspring and the offspring of iron-restricted dams (Table 4). Serum insulin levels were higher in males than in females ($P < .001$, Table 4).

Table 3. Neonatal Day 2 Hematology in Offspring of Control and Iron-Restricted Dams

	Control (n = 4)	Iron-Restricted (n = 3)
Hemoglobin (g/dL)	12.48 ± 0.62	5.53 ± 0.76†
RBC count (× 10^9/L)	2.48 ± 0.22	1.75 ± 0.14†
Hematocrit (%)	37 ± 1	17 ± 3†
Mean cell volume (μm^3)	151.05 ± 14.78	95.60 ± 8.79†
RBC distribution width	33.05 ± 3.38	27.67 ± 0.9*

NOTE. Data are means ± SD. (n) refers to the number of litters.
*$P < .05$.
†$P < .005$.

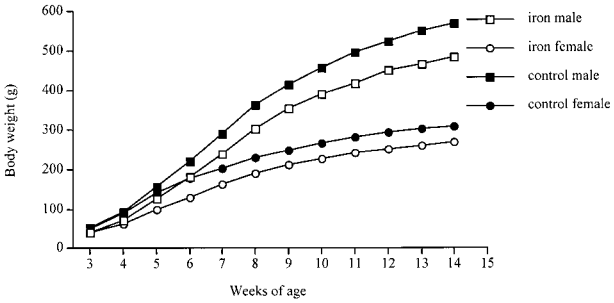

Fig 1. Body weight in control and maternal iron-restricted rats from weaning until 14 weeks of age. The offspring of iron-restricted dams were significantly smaller than the controls every week up to 14 weeks of age when experimentation began ($P < .001$). From 5 weeks of age, females were smaller than males in both groups ($P < .001$).

Serum Lipids

Serum triglycerides were lower in the offspring of iron-restricted dams than in controls ($P < .001$,Table 5) and were lower in females than in males ($P < .001$, Table 5). Serum cholesterol was not significantly different between the control and maternal iron-restricted groups, but was higher in females than in males ($P < .001$, Table 5). There were no significant differences in serum free fatty acids between the 2 maternal diet groups or between the sexes (Table 5).

Influence of Body Weight

To determine whether differences in body weight between the groups could be influencing these findings, the statistical analyses were repeated including body weight as a covariate. This did not affect the outcome for blood pressure or serum free fatty acids. However, differences in glucose tolerance, serum insulin, serum triglycerides, and serum cholesterol levels did appear to be associated with differences in body weight. When body weight was included as a covariate, there was no longer a significant difference in area under the glucose tolerance curve between the control and maternal iron-restricted groups ($P = .3$). However, the area under the curve was less in males than in females ($P < .05$; corrected means, 1,046 ± 55 mmol · min *v* 1,285 ± 52 mmol · min). It should be noted that for the IPGTTs the adjustment for body weight was investigating how an animal of a particular weight responded to a specific dose of glucose (1 mL of 10% glucose/100 g of body weight). We are not correcting for the amount of glucose given, as each animal received the same dose. When body weight was included as a covariate there was no longer a significant effect of sex on serum insulin levels. However, there was a significant effect of maternal diet ($P < .02$) and an interaction between maternal diet and sex, indicating that insulin was lower in the male

Table 4. Blood Pressure, Glucose Tolerance, and Fasting Serum Insulin From 3-Month-Old Offspring of Control or Maternal Iron-Restricted Dams

	Control Male (n = 16)	Control Female (n = 16)	Iron-Restricted Male (n = 10)	Iron-Restricted Female (n = 14)
Blood pressure (mm Hg)*†	141 ± 16	149 ± 14	170 ± 20	157 ± 14
Area under glucose curve (mmol · min)*	1,246 ± 133	1,213 ± 171	1,148 ± 128	1,059 ± 80
Serum insulin pmol/L‡	161 (123-214)	69 (52-91)	199 (138-287)	64 (45-83)

NOTE. Data are means ± SD or geometric mean (95% confidence interval).

*Denotes an effect of maternal diet, $P < .001$.

†Denotes an interaction between maternal diet and sex, $P < .05$.

‡Denotes an effect of the offsprings sex, $P < .001$.

offspring of the control dams than in the other groups ($P < .05$). The corrected geometric means (95% confidence interval) for serum insulin were as follows: control male, 60.5 (34.6 to 105.6) pmol/L; control female, 124.1 (84.2 to 183.1) pmol/L; iron-restricted male, 120.6 (79.9 to 182.2) pmol/L; and iron-restricted female, 138.7 (84.2 to 228.4) pmol/L. When body weight was included as a covariate in the analysis of serum triglyceride levels there was no longer an effect of sex. However, the effect of maternal diet on serum triglycerides remained, indicating that they were lower in the offspring of iron-restricted dams ($P < .01$; corrected means, 1.67 ± 0.13 mmol/L *v* 1.09 ± 0.14 mmol/L). When body weight was included as a covariate in the analysis of serum cholesterol levels there was no longer an effect of sex. However, there was an effect of maternal diet on cholesterol level, indicating that it was lower in the offspring of iron-restricted dams ($P < .05$; corrected means, 2.95 ± 0.12 mmol/L *v* 2.25 ± 0.13 mmol/L).

DISCUSSION

Epidemiologic studies have shown strong relationships between early growth restriction and the subsequent development of features of the metabolic syndrome (glucose intolerance, hypertension, and hyperlipidemia). The present study was designed to investigate whether there are long-term effects of maternal iron restriction on blood pressure, glucose tolerance, and serum lipid concentrations. This study demonstrates that maternal iron restriction during gestation affects blood pressure, glucose tolerance, and serum triglyceride in the adult offspring. This provides further evidence for programming of adult physiology and metabolism by alterations in maternal nutrition.

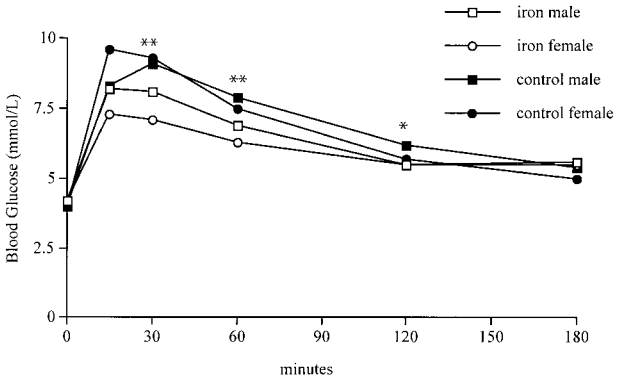

Fig 2. Glucose tolerance curves for the 3-month-old offspring of control and iron-restricted dams. There was no significant difference between the sexes so indicators of significance refer to control *v* iron-restricted offspring: **P* < .05, ***P* < .001.

As previously reported, iron deficiency caused growth restriction.[10] It is not immediately clear how iron restriction causes growth retardation; however, reduced maternal food intake could be a contributing factor. There were no differences in maternal food intake in the week before mating, suggesting that the diets were equally palatable. Decreased food intake during gestation in the iron-restricted litters is therefore likely to be an effect of iron restriction rather than differential palatability of the diets.

Elevated blood pressure has previously been reported in the 40-day-old offspring of iron-restricted dams.[10] In the present study, elevated blood pressure was found in adult rats at 3 months of age. This study differs from that of Crowe et al[10] in that the dams in the current study became anemic during pregnancy rather than before pregnancy, blood pressure was measured in adult rats rather than adolescent rats, and a difference in the magnitude of the effect between the sexes was apparent. Together these studies provide strong evidence for elevated blood pressure in the offspring of iron-restricted dams.

There is a relationship between growth in childhood and adolescence and rises in blood pressure.[19,20] Early rises in blood pressure have been found to occur at the same time as growth spurts, suggesting that the rise in blood pressure may be growth-related.[20] It is therefore interesting that in this study there were significant differences in growth velocity during the growth spurts following weaning.

Hemodynamic differences due to perinatal anemia could affect vascularization and cause the elevated blood pressure in these animals. Crowe et al[10] reported that blood pressure was decreased in the day 20 offspring of iron-restricted dams, at a time when hemoglobin values were still only 44% of the control value. This could be explained by decreased blood viscosity and decreased peripheral resistance. On day 40, hemoglobin values had risen to 91% of the control value and blood pressure was higher in the offspring of iron-restricted dams. Interestingly, the spontaneously hypertensive rat is also anemic during fetal life,[21] and nutritional anemia prevents the onset of hypertension in this strain.[22]

Glucose tolerance was better in the offspring of iron-restricted dams compared with the controls. Fasting serum insulin was not different between the 2 groups. However, there could be differences between the groups in the secretion of insulin in

Table 5. Fasting Serum Lipids in 3-Month-Old Offspring of Control or Maternal Iron-Restricted Dams

	Control Male (n = 16)	Control Female (n = 16)	Iron-Restricted Male (n = 10)	Iron-Restricted Female (n = 14)
Free fatty acids (μmol/L)	934 ± 193.1	953 ± 221.3	1,038 ± 201	1,025 ± 265
Triglyceride (mmol/L)*†	2.06 (1.71-2.64)	1.32 (0.88-1.72)	1.29 (0.69-1.78)	0.70 (0.53-1.23)
Cholesterol (mmol/L)†	2.48 ± 0.78	3.22 ± 0.52	2.28 ± 0.49	2.96 ± 0.39

NOTE. Data are means ± SD or median (interquartile range).
*Denotes an effect of maternal diet, $P < .001$.
†Denotes an effect of the offsprings sex, $P < .001$.

response to the glucose load, which may explain the differences between the groups. Improved glucose tolerance has also been reported in 6- and 9-week-old offspring of dams fed low-protein diets.[12,23] Although the offspring of protein-restricted dams have better glucose tolerance at 3 months of age, their glucose tolerance decreases more rapidly than controls, and at 15 months of age they have impaired glucose tolerance.[24]

The lower levels of serum triglycerides in the offspring of iron-restricted dams may indicate that the metabolism of these animals has been altered by iron restriction in early life or could relate to persistent differences in body composition between the groups. Iron deficiency is reported to affect lipid metabolism in the rat; however, the reports are contradictory, with some finding increased levels of serum triglyceride and others decreased levels.[25-28] However, there are no reports on the effect of maternal iron restriction on lipid metabolism in the offspring. The effects of maternal iron restriction on lipid metabolism could be due to long-term changes in tissue iron or to alterations in the metabolic set points. Maternal iron restriction has been shown to decrease brain iron content in the adult offspring.[29] This raises the possibility that fetal iron availability may have a long-term effect on tissue iron levels in other tissues such as the liver, which may be affecting lipid metabolism.

Interestingly, adjusting for body weight, by including body weight as a covariate in the analysis, affected the results for glucose tolerance, serum insulin, serum triglycerides, and serum cholesterol. This raises the possibility that the differences observed for these factors may be related to the differences in body weight between the groups. It is unlikely that body weight itself is causing these effects, but it may be a surrogate indicator for other factors with a more direct bearing on the factor being measured. For example, adiposity and muscle mass could affect both body weight and glucose tolerance.

There are a number of similarities in the long-term effects on the offspring of the iron-restricted and maternal low-protein models. Both models induce early growth retardation. In the low-protein model, blood pressure has been reported to be elevated,[6,7] glucose tolerance to be improved at 6 and 9 weeks of age,[12,23] and serum triglyceride to be decreased.[11] As these 2 different maternal nutritional deficiencies appear to affect these offspring in a similar manner, there may be common factors initiating these effects. As the 2 models involve different nutritional insults, it is possible that any common long-term effects are due to a common mechanism of response to nutritional deprivation rather than specific to the nutritional insult. If this is the case, then many different nutritional deficiencies may have similar effects on the offspring.

The difference in the magnitude of the effect on blood pressure between the sexes in the iron-restricted offspring is an example of a gender-specific effect of maternal environment on the adult offspring. A recent investigation of the long-term effects of uterine artery ligation on the offspring reported that the effects on glucose tolerance, insulin secretion, and catecholamines were only observed in female offspring.[30] Gender differences in the response of the offspring to maternal malnutrition have also been observed in organ growth in the maternal low-protein model.[31] These findings demonstrate the importance of investigating the effects on both male and female offspring.

This study provides further evidence that maternal nutritional status can have long-term effects on the offspring. The common features of this model and the maternal low-protein model suggest that similar underlying mechanisms may be involved in initiating these processes.

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