Maternal low-protein diet during mouse pre-implantation development induces vascular dysfunction and altered renin–angiotensin-system homeostasis in the offspring

Adam J. Watkins1*, Emma S. Lucas1, Christopher Torrens2, Jane K. Cleal2, Lauren Green1, Clive Osmond3, Judith J. Eckert1,2, William P. Gray4, Mark A. Hanson2 and Tom P. Fleming1

1School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, UK
2Institute of Developmental Sciences, Developmental Origins of Health and Disease (DOHaD) Division, School of Medicine, University of Southampton, Southampton General Hospital, Southampton SO16 6YD, UK
3MRC Epidemiology Resource Centre, Southampton General Hospital, Southampton SO16 6YD, UK
4Division of Clinical Neurosciences, School of Medicine, University of Southampton, Southampton General Hospital, Southampton SO16 6YD, UK

(Received 15 July 2009 – Revised 8 December 2009 – Accepted 10 December 2009 – First published online 4 February 2010)

Environmental perturbations during early mammalian development can affect aspects of offspring growth and cardiovascular health. We have demonstrated previously that maternal gestational dietary protein restriction in mice significantly elevated adult offspring systolic blood pressure. Therefore, the present study investigates the key mechanisms of blood pressure regulation in these mice. Following mating, female MF-1 mice were assigned to either a normal-protein diet (NPD; 18 % casein) or an isocaloric low-protein diet throughout gestation (LPD; 9 % casein), or fed the LPD exclusively during the pre-implantation period (3·5 d) before returning to the NPD for the remainder of gestation (Emb-LPD).

All offspring received standard chow. At 22 weeks, isolated mesenteric arteries from LPD and Emb-LPD males displayed significantly attenuated vasodilatation in response to isoprenaline (P=0·04 and P=0·025, respectively), when compared with NPD arteries. At 28 weeks, stereological analysis of glomerular number in female left kidneys revealed no significant difference between the groups. Real-time RT-PCR analysis of type 1a angiotensin II receptor, Na+/K+ ATPase transporter subunits and glucocorticoid receptor expression in male and female left kidneys revealed no significant differences between the groups. LPD females displayed elevated serum angiotensin-converting enzyme (ACE) activity (P=0·044), whilst Emb-LPD males had elevated lung ACE activity (P=0·001), when compared with NPD offspring. These data demonstrate that elevated offspring systolic blood pressure following maternal gestational protein undernutrition is associated with impaired arterial vasodilatation in male offspring, elevated serum and lung ACE activity in female and male offspring, respectively, but kidney glomerular number in females and kidney gene expression in male and female offspring appear unaffected.

CVD: Maternal diet: Pre-implantation embryo development

The development of adult CVD has been associated typically with classical risk factors such as poor diet, a sedentary lifestyle, smoking and genetic predisposition. However, the ‘Developmental Origins of Health and Disease’ (DOHaD) hypothesis identifies the uterine environment and associated maternal nutritional status as critical components in the origin of postnatal cardiovascular and metabolic disease(1–3). The DOHaD concept proposes that maternal nutritional status influences embryonic and fetal growth and physiology so that the setting of homeostatic mechanisms matches the nutrient availability in postnatal life. However, mismatch between pre- and postnatal nutrient environments is associated with adult onset disease(1–3).

Rodent and domestic animal studies have demonstrated that maternal gestational undernutrition can alter cardiovascular homeostasis in the offspring(4–8). The precise mechanisms underlying dietary programmed hypertension are likely to be multifactorial and complex, originating from more than one individual pathway. Vascular dysfunction in adult programmeed animals in response to prolonged gestational undernutrition is characterised by an enhanced constrictive and impaired endothelial-dependent dilatation phenotype. In the sheep, maternal nutrient restriction encompassing either preconception or early gestation enhances vasconstriction and attenuates vasodilatation in offspring coronary, thoracic and renal arteries(9–11). However, a more prolonged maternal gestational

Abbreviations: ACE, angiotensin-converting enzyme; Emb-LPD, low-protein diet for the first 3·5 d before switching to normal diet for the remainder of gestation; LPD, low-protein diet; NPD, normal-protein diet; pEC50, – log effective concentration equal to 50% of the maximal response; RAS, renin–angiotensin system; SBP, systolic blood pressure.

* Corresponding author: Dr Adam Watkins, fax +44 2380 594459, email ajw7@soton.ac.uk
nutrition restriction associates with a higher offspring blood pressure and altered baroreflex responses to noradrenaline infusion\(^{(12)}\). In the rat, male offspring of dams either globally undernourished (30 % less food than controls), or fed a low-protein diet (LPD), throughout pregnancy display significantly attenuated vascular responsiveness to both endothelial-independent and dependent vasodilators\(^{(4–8,13)}\). However, the magnitude of this vascular impairment was unaffected by a subsequent postnatal high-fat dietary challenge\(^{(13)}\), highlighting the detrimental effect of poor maternal nutrition during pregnancy on vascular function.

Along with vascular function, homeostatic regulation of the renin–angiotensin system (RAS) also appears susceptible to programming in response to dietary and environmental challenges during gestation. The production of angiotensin II, a potent vasoconstrictor, from angiotensin I is mediated by angiotensin-converting enzyme (ACE)\(^{(14)}\). The binding of angiotensin II to its receptor\(^{(15)}\) results in vasoconstriction, renal tubular Na reabsorption and aldosterone and vasopressin secretion\(^{(16)}\). Angiotensin II has also been implicated in endothelial dysfunction through its ability to increase oxidative stress\(^{(17)}\). As a result, altered RAS regulation has been associated with the development of the hypertensive state\(^{(15)}\) and with several cardiovascular and renal pathologies\(^{(18,19)}\). The role of RAS in the aetiology of programmed adult hypertension following maternal nutrient restriction has been demonstrated via the normalisation of blood pressure in hypertensive adult offspring from LPD-fed rat dams after administration of the ACE inhibitor, captopril\(^{(20)}\), or through the blockade of the angiotensin I receptor (AT\(_1\)-R) with losartan\(^{(21)}\). Offspring from rats fed LPD during gestation display significantly altered mRNA and protein levels for the angiotensin I and II receptors (AT\(_1\)-R and AT\(_2\)-R) in kidney tissue\(^{(22,23)}\). Maternal LPD has also been shown to alter expression levels of the glucocorticoid receptor and the Na/K-adenosine triphosphatase subunits in the kidney\(^{(24)}\). These findings have led to the suggestion that maternal undernutrition could alter renal Na retention, contributing to the increased systolic blood pressure (SBP) observed in these animals.

The pre-implantation period of development has been shown to be particularly sensitive to changes in its immediate environment, whether \textit{in vivo} or \textit{in vitro}, resulting in a myriad of changes in offspring phenotype\(^{(25–29)}\). Altered cardiovascular and metabolic phenotype, including raised SBP and diastolic blood pressure and altered glucose homeostasis, have been identified in \textit{human in vitro} fertilisation children compared with age-matched controls\(^{(30)}\). In the sheep, culture of pre-implantation embryos in the presence of serum resulted in significantly altered rates of blastocyst development, and elevated growth of placental and fetal tissues\(^{(31)}\). Impaired blastocyst and fetal development have also been reported following the culture of mouse pre-implantation embryos under high ammonia\(^{(32)}\) or reduced oxygen\(^{(33)}\) environments. Studies from our laboratory have demonstrated that maternal LPD diet targeted exclusively to the pre-implantation period in rodents significantly alters blastocyst cell numbers and gene expression patterns in fetal liver tissue\(^{(26,29,34,35)}\), whilst elevating offspring postnatal growth, behavioural patterns and mean lifelong SBP\(^{(29)}\).

Whilst the development of offspring hypertension following maternal gestational undernutrition is well established, the mechanisms and pathways involved are still poorly defined. In the present study we evaluate for the first time key mechanisms of adult blood pressure regulation, in order to gain a better understanding of how maternal undernutrition during discrete windows of gestation can programme disease in the adult offspring.

**Methods**

**Animal treatments**

All experiments were conducted using protocols approved by the UK Home Office and local ethics committee. Female MF-1 mice were bred in-house (University of Southampton Biomedical Research Facility) and maintained on a 07.00 to 19.00 hours light cycle with controlled temperature and standard chow and \textit{water ad libitum}. All offspring and archived tissues investigated in the present study were taken from the same cohort of animals as described previously\(^{(29)}\). Briefly, virgin MF-1 females (aged 7–8.5 weeks) were mated naturally with MF-1 studs (Harlan Link Ltd, Bicester, Oxon, UK) and randomly assigned to either (a) a normal protein diet (NPD; 18 % casein) throughout gestation, (b) an isocaloric low-protein diet (LPD; 9 % casein) throughout gestation, or (c) the LPD for the first 3.5 d before switching to the NPD for the remainder of gestation (Emb-LPD). The morning after mating, females were individually caged and fed such that the experimenter was blinded to the diet each female received, and subsequently did not know what dietary treatment any offspring pertained to. Nineteen litters of each treatment group were generated. Mothers from the day of birth, and offspring (after weaning at 3 weeks), were fed standard chow. Litter size was adjusted to six at birth (three per sex) and the sexes caged separately from weaning. Previously, we demonstrated that these dietary regimens were sufficient to induce mean lifelong hypertension in both male (elevated by 3.2 mmHg) and female (elevated by 3.0 mmHg) offspring as assessed by tail-cuff plethysmography\(^{(29)}\), and that female Emb-LPD offspring displayed increased body weight from birth for up to 28 weeks (increased by 11 % at birth, and 8.3 % at 28 weeks, respectively, over controls\(^{(29)}\). Analysis of the mean lifelong SBP (measurements taken at 9, 15 and 21 weeks of age) of the cohort of males and females used within all experiments within the present study (fifty-five, fifty-seven and sixty-one per respective, over controls\(^{(29)}\). Therefore, the mice in the cohort analysed in the present study were considered as being representative of the entire population\(^{(29)}\) from which they were selected.

**Mesenteric artery vasoreactivity**

Vascular function in male offspring was assessed at 22 weeks in isolated small mesenteric artery segments following cervical dislocation as described previously\(^{(7,8,13,36)}\). Male offspring alone were analysed in order to minimise potential variation caused from the differing levels of circulating oestrogen in female offspring during the oestrous cycle, and the
effect this has on vascular responsiveness (37). Briefly, small mesenteric artery segments (luminal diameter about 100–300 μm) were isolated and mounted on a wire myograph (Danish Myo Technology A/S, Aarhus, Denmark). Following normalisation, cumulative concentration response curves were measured for the α₁-adrenergic agonist phenylephrine (10⁻⁹ to 10⁻⁴ mol/l). After pre-constriction with a submaximal dose of phenylephrine (80% of maximal excitatory concentration; EC₈₀), cumulative concentration response curves to the endothelial-dependent and -independent vasodilators acetylcholine (10⁻⁹ to 10⁻⁵ mol/l) and isoprenaline (10⁻¹⁰ to 10⁻⁵ mol/l), respectively, and the NO donor, sodium nitroprusside (10⁻¹¹ to 10⁻⁵ mol/l), were conducted in that order in the same mesenteric arteries from seven or eight individual males, all from different litters. All drugs were purchased from Sigma (Poole, Dorset, UK).

**Kidney glomerular counting**

Due to the fact that male offspring alone had been analysed for the study of mesenteric artery responsiveness (for reasons given above), only female kidneys were analysed in order to maintain a balanced number of male and female offspring for the final analysis of organ allometry at 28 weeks (organ allometry data previously detailed by Watkins et al. (29)). This also kept the present study in line with our previous study(36), making it possible to compare results. Stereological analysis of kidney glomerular number was conducted as previously described(36). Briefly, left kidneys from female offspring were perfusion-fixed with neutral buffered formalin (BDH Laboratory Supplies, Poole, Dorset, UK). Kidney volume was determined by suspended immersion into neutral buffered formalin placed on a balance, with the increase in weight recorded being equal to the volume of the kidney. Three separate measurements of volume per kidney were taken and an average calculated. Fixed kidneys were then processed into glycol methacrylate (JB-4 Embedding Kit; Polysciences Europe GmbH, Eppelheim, Germany) and exhaustively sectioned at 2 μm. Every 100th and 110th section was analysed using the Stereo Investigator software (Microbrightfield, Williston, VT, USA). An unbiased counting frame (500 μm²) was placed randomly over the 100th section; glomeruli present within the 110th but absent from the 100th section were counted and an estimate of kidney glomerular number was obtained using the fractionator principle(38).

**RNA extraction and quantitative RT-PCR for gene expression analysis**

Whole left kidneys from eight male and eight female offspring per treatment group at 28 weeks, archived from our previous study(29), were powdered and the RNA extracted using the RNeasy® Lipid Tissue Mini Kit (QIAGEN, Crawley, West Sussex, UK) according to the manufacturer’s instructions. On-column DNase I digestion was performed to remove contaminating genomic DNA. RNA was reverse transcribed to cDNA using the ImProm®II kit (Promega, Southampton, Hants, UK), according to the manufacturer’s instructions. Intron-spanning primers used in the present study are detailed in Table 1. For real-time PCR, a mastermix was prepared consisting of 10μl 2X Precision Mastermix containing

---

**Table 1. Real-time PCR primer details for gene expression studies**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Accession no.</th>
<th>Genesymbol</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Amplicon length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II receptor, type 1a</td>
<td>NM_177323</td>
<td>Agtr1a</td>
<td>ACTCACAGCAACCCTCCAAG</td>
<td>CTCAGACACTGTTCAAAATGCAC</td>
<td>62</td>
</tr>
<tr>
<td>Monocarboxylic acid transporter, Na⁺/K⁺</td>
<td>NM_144900.1</td>
<td>Atp1a1</td>
<td>CTTGGAGAACGTGTGCTAGGT</td>
<td>TTCCGGGAAACTGTTCGTCA</td>
<td>60</td>
</tr>
<tr>
<td>Succinate dehydrogenase complex, subunit A,</td>
<td>NM_023281</td>
<td>Sdha</td>
<td>TGTTCAGTTCCACCCCACA</td>
<td>TCTCCACGACACCCTTCTGT</td>
<td>66</td>
</tr>
<tr>
<td>Phosphoglycerate kinase 1</td>
<td>NM_008828</td>
<td>Pgk1</td>
<td>TACCTGCTGGCTGGATGG</td>
<td>CACAGCCTCGGCATATTTCT</td>
<td>65</td>
</tr>
<tr>
<td>Nuclear receptor subfamily 3, group C,</td>
<td>NM_008173.3</td>
<td>Nr3c1</td>
<td>TGACGTGTGGAAGCTGTAAAGT</td>
<td>CATTTCTTCCAGCACAAAGGT</td>
<td>78</td>
</tr>
<tr>
<td>Phosphoglycerate kinase 1</td>
<td>NM_007502.4</td>
<td>Pgk1</td>
<td>CTGCACTCTTCACATTCACA</td>
<td>CACAGAATATGGCTTTAGGA</td>
<td>206</td>
</tr>
</tbody>
</table>

---

A. J. Watkins et al. British Journal of Nutrition

Downloaded from https://www.cambridge.org/core. IP address: 54.70.40.11, on 31 Oct 2019 at 23:01:52, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
SYBRgreen (PrimerDesign, Southampton, Hants, UK), 1·2 μl primer mix (containing 5 μM each forward and reverse primers) and 7·8 μl water per reaction. Mastermix was aliquotted to ninety-six-well PCR plates (Axygen, Union City, CA, USA) in 19 μl volumes and 5 ng cDNA was added to sample wells, or water to control (no template) wells, giving a final reaction volume of 20 μl. All samples were run in triplicate. An inter-plate calibrator was included on all runs to control for inter-assay variation. Amplification and detection were performed using a DNA Engine thermal cycler and Chromo4 Real-Time Detector and data were acquired using Opticon Monitor version 3·1 software (Bio-Rad, Hemel Hempstead, Herts, UK). A post-amplification melting curve confirmed the presence of specific products for each primer set. Data were normalised to the expression of succinyl dehydrogenase α (Sdhα) and phosphoglycerate kinase 1 (Pgtk1) using geNorm software (Ghent University, Ghent, Belgium)(39), with which we determined these to be the two most stable reference genes in adult mouse kidney (data not shown).

Angiotensin-converting enzyme activity

Serum and lung ACE activity was analysed as previously described(28,40). Briefly, samples were incubated in the presence of hippuryl-l-histidine-l-leucine acetate salt (Sigma, Poole, Dorset, UK) solution in buffer at 37°C followed by the addition of cyanuric chloride (Sigma) in 1,4 dioxane (Sigma). Each sample was analysed in duplicate, with four readings per duplicate analysed at 380 nm. Samples containing only serum or lung lysate and buffer were used as negative controls. Sample readings were analysed against a standard curve ranging from 50 μM to 1 mM, prepared in buffer and treated exactly as the samples. Serum and lung ACE activity was expressed as nmol hippurate formed per ml serum per min or per mg protein per min, respectively.

Statistical analysis

For analysis of vascular responsiveness data, a four-parameter logistic equation using non-linear regression was used to obtain the − log effective concentration equal to 50 % of the maximal response (pEC50) and maximum response for each of the cumulative concentration response curves analysed. Differences in maximal response and pEC50 were analysed using two-way ANOVA (Prism 3·0, GraphPad Software Inc., San Diego, CA, USA) with Bonferroni post hoc correction as described previously (7,13,36). Kidney glomerular number and gene transcript analysis and ACE data were analysed using a multilevel random effects regression model (SPSS version 15; SPSS, Inc., Chicago, IL, USA) taking into account the hierarchical nature of the data with between-mother and within-mother variation and different parameters measured from individual animals (28,29,41). Therefore, statistical differences identified between treatment groups were independent of maternal origin of litter, gestational litter size and body weight, unless otherwise stated. All data are presented as mean values with their standard errors of the mean.

Results

Mesenteric artery vasoreactivity

Isolated male arteries from all treatment groups displayed a concentration-dependent vasoconstriction to the α1-adrenergic agonist phenylephrine, which was not different between treatments (Fig. 1(a)). Following pre-constriction with phenylephrine, no difference in responsiveness to the endothelium-dependent vasodilator acetylcholine was observed between treatment groups (Fig. 1(b)). However, Emb-LPD males displayed an attenuated response to the β-adrenoceptor agonist isoprenaline when compared with NPD arteries (% maximal response 62·22 (SEM 4·59) v. 76·11 (SEM 3·58), respectively; P = 0·025) whilst the response in LPD arteries was shifted significantly to the right (significantly different pEC50) when compared with NPD arteries (pEC50 6·99 (SEM 0·07) and 7·56 (SEM 0·13), respectively; P = 0·04; Fig. 1(c)), both of which were independent of litter size and body weight effects. No differences in response to sodium nitroprusside were observed between the treatment groups.

Kidney glomerular counts

Stereological analysis of female left kidneys at 28 weeks revealed no difference in mean glomerular number between the treatment groups, or in mean kidney weight or mean kidney volume (Fig. 2).

Kidney gene expression analysis

Real-time analysis of type 1a angiotensin II receptor (Agtr1A), the α1, β1, 2 and 3 subunits of the Na+/K+ ATPase transporter (Atp1a1, Atp1b1, Atp1b2, Atp1b3) and glucocorticoid receptor (Nrr3c1) mRNA expression in male and female left kidneys revealed no significant differences between any of the treatment groups (Table 2).

Serum and lung angiotensin-converting enzyme activity

Analysis of serum ACE activity showed no differences between male treatment groups; however, LPD females had an elevated activity compared with NPD females (135·45 (SEM 9·48) v. 113·17 (SEM 9·48) nmol hippurate formed/ml serum per min, respectively; Fig. 3(a); P = 0·04). Analysis of lung ACE activity showed that Emb-LPD males had an elevated activity when compared with NPD and LPD males (3·98 (SEM 0·48) v. 1·69 (SEM 0·16) and 2·33 (SEM 0·29) nmol hippurate formed/mg protein per min, respectively; Fig. 3(b); P = 0·005). Whilst Emb-LPD females had an elevated activity when compared with LPD females (3·31 (SEM 0·55) v. 2·29 (SEM 0·41) nmol hippurate formed/mg protein per min, respectively; Fig. 3(b); P = 0·05), there was no difference between Emb-LPD females and LPD and control females. All differences were independent of litter size effects; however, body weight at 28 weeks of age had a negative influence on female lung ACE activity (− 0·104 nmol hippurate formed/mg protein per min per g increase in body weight; P = 0·018).
Discussion

The earliest stages of development have been highlighted as critically sensitive periods during which perturbations, either in vivo or in vitro, can have long-term effects on offspring health and physiology (25–29,31,33,36). Previously, we have demonstrated that a maternal LPD treatment exclusively during the pre-implantation period (the first 3·5 d of gestation; Emb-LPD) or throughout gestation (LPD) significantly elevated offspring SBP (29). Therefore, the present study investigated the mechanisms underlying the observed elevated SBP in a cohort of these same offspring, focusing on vascular function, the RAS and changes in nephron number and kidney gene expression patterns. We observed impaired vasodilation in male LPD and Emb-LPD offspring, elevated patterns

![Graph showing vasoreactivity of isolated mesenteric arteries from male offspring at 22 weeks of age from dams fed a low-protein diet throughout gestation (LPD; ○) or exclusively during the pre-implantation period (Emb-LPD; □) compared with arteries of offspring of normal-protein diet (NPD)-fed dams (△). Cumulative additions of (a) phenylephrine (PE) and, after pre-constriction with PE, of the vasodilators (b) acetylcholine (ACh), (c) isoprenaline (ISO) and (d) sodium nitroprusside (SNP). Values are means of seven to eight males, each from different litters representing each treatment group, with standard errors represented by vertical bars. * Response was significantly different from that of the NPD arteries (P<0.05).](image)

**Fig. 1.** Vasoreactivity of isolated mesenteric arteries from male offspring at 22 weeks of age from dams fed a low-protein diet throughout gestation (LPD; ○) or exclusively during the pre-implantation period (Emb-LPD; □) compared with arteries of offspring of normal-protein diet (NPD)-fed dams (△). Cumulative additions of (a) phenylephrine (PE) and, after pre-constriction with PE, of the vasodilators (b) acetylcholine (ACh), (c) isoprenaline (ISO) and (d) sodium nitroprusside (SNP). Values are means of seven to eight males, each from different litters representing each treatment group, with standard errors represented by vertical bars. * Response was significantly different from that of the NPD arteries (P<0.05).

![Bar graph showing effect of maternal low-protein diet either throughout gestation (LPD) or exclusively during the pre-implantation period (Emb-LPD) compared with maternal normal-protein diet (NPD) on kidney glomerular number (a), kidney weight (b) and kidney volume (c) in female offspring at 28 weeks. Values are means for six kidneys per treatment, each from different litters representing each treatment group, with standard errors represented by vertical bars.](image)

**Fig. 2.** Effect of maternal low-protein diet either throughout gestation (LPD) or exclusively during the pre-implantation period (Emb-LPD) compared with maternal normal-protein diet (NPD) on kidney glomerular number (a), kidney weight (b) and kidney volume (c) in female offspring at 28 weeks. Values are means for six kidneys per treatment, each from different litters representing each treatment group, with standard errors represented by vertical bars.
The present data, however, identify inhibiting phosphodiesterase breakdown of cAMP(46). Endothelial NO release can modulate isoprenaline responses (45). One suggestion is that basal mouse mesenteric arteries this is dependent on a functioning endothelial NO synthase (46). From the present study the role of NO is unclear, as responses were not performed in the presence of an NO synthase inhibitor such as N-nitro-L-arginine-methyl ester. Furthermore, reductions in the expression of guanylate cyclase or intracellular cGMP may be contributing to the blunted vasodilatation therefore possible that alterations in guanylate cyclase or intracellular cGMP can modulate isoprenaline responses (47–49). It is therefore possible that alterations in guanylate cyclase or cGMP may be contributing to the blunted vasodilatation responses observed in the male offspring within the present study; however, this would require further investigation to confirm. Similarly, whether differences in vasodilatation exist for female offspring from LPD and Emb-LPD mothers also remains to be determined. However, vascular responsiveness at different stages of the oestrous cycle would have to be quantified independently.

The second major alteration in postnatal phenotype that we observed was that female LPD offspring displayed significantly elevated serum ACE activities (28,40). It is therefore possible that alterations in guanylate cyclase or cGMP may be contributing to the blunted vasodilatation responses observed in the male offspring within the present study; however, this would require further investigation to confirm. Similarly, whether differences in vasodilatation exist for female offspring from LPD and Emb-LPD mothers also remains to be determined. However, vascular responsiveness at different stages of the oestrous cycle would have to be determined so that both dietary and hormonal effects could be quantified independently.

Maternal undernutrition and vascular disease

Table 2. Real-time RT-PCR relative expression values from male and female kidney tissue*

*Mean values with their standard errors

<table>
<thead>
<tr>
<th>Gene of interest†</th>
<th>NPD Mean ± SEM</th>
<th>LPD Mean ± SEM</th>
<th>Emb-LPD Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agtr1a †</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1·5 ± 0·12</td>
<td>1·65 ± 0·11</td>
<td>1·48 ± 0·12</td>
</tr>
<tr>
<td>Females</td>
<td>1·28 ± 0·13</td>
<td>1·58 ± 0·13</td>
<td>1·28 ± 0·12</td>
</tr>
<tr>
<td>Nrc3c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1·5 ± 0·11</td>
<td>1·55 ± 0·12</td>
<td>1·71 ± 0·06</td>
</tr>
<tr>
<td>Females</td>
<td>1·49 ± 0·13</td>
<td>1·34 ± 0·09</td>
<td>1·54 ± 0·13</td>
</tr>
<tr>
<td>Atp1a1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1·15 ± 0·11</td>
<td>1·23 ± 0·11</td>
<td>1·2 ± 0·06</td>
</tr>
<tr>
<td>Females</td>
<td>1·37 ± 0·14</td>
<td>1·31 ± 0·13</td>
<td>1·25 ± 0·11</td>
</tr>
<tr>
<td>Atp1b1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1·09 ± 0·09</td>
<td>1·22 ± 0·06</td>
<td>1·24 ± 0·07</td>
</tr>
<tr>
<td>Females</td>
<td>1·23 ± 0·13</td>
<td>1·22 ± 0·13</td>
<td>1·22 ± 0·09</td>
</tr>
<tr>
<td>Atp1b2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1·15 ± 0·16</td>
<td>1·29 ± 0·11</td>
<td>1·24 ± 0·09</td>
</tr>
<tr>
<td>Females</td>
<td>1·33 ± 0·11</td>
<td>1·53 ± 0·15</td>
<td>1·26 ± 0·07</td>
</tr>
<tr>
<td>Atp1b3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1·37 ± 0·12</td>
<td>1·37 ± 0·1</td>
<td>1·37 ± 0·07</td>
</tr>
<tr>
<td>Females</td>
<td>1·30 ± 0·14</td>
<td>1·28 ± 0·12</td>
<td>1·17 ± 0·06</td>
</tr>
</tbody>
</table>

NPD, normal-protein diet; LPD, isocaloric low-protein diet; Emb-LPD, low-protein diet for the first 3·5 d before switching to normal diet for the remainder of gestation.

† For gene names, see Table 1.
The programming of elevated serum and tissue ACE activity has also been demonstrated following maternal cortisol infusion and co-occurring with adult elevated SBP in response to pre-implantation embryo culture conditions and maternal diet. As in our previous studies, we observed sex- and tissue-specific responses in ACE activity across treatment groups. In vivo, serum ACE is derived from the leakage of endothelial-bound somatic ACE from the lungs, vasculature and kidneys. As we only assessed the activity of ACE in the serum and lungs, we are not able to comment on the activity of other somatic sources of ACE, their relative contribution to the overall activity in the serum or in possible differences between the sexes.

The third finding was that female left kidney glomerular number was unaltered in response to maternal dietary protein levels. Significant reductions in both kidney size and glomerular number have been reported in response to maternal LPD in the rat, mouse and with decreased birth weight in humans. However, several studies have demonstrated programmed elevated blood pressure in the absence of significant changes in glomerular number. Whilst reductions in glomerular number may reflect compromised kidney development during gestation in females, in response to restricted maternal dietary protein intake (LPD), maternal cortisol infusion, and co-occurring with adult tissue ACE activity has also been demonstrated following exposure of dams to a 6% casein diet from day 12 of pregnancy. This discrepancy reinforces the hypothesis that the specific mechanistic origins of the programmed hypertension phenotype are driven by the timing and severity of the nutritional challenge.

It is of interest to compare the relative difference in postnatal phenotype observed in the present study with those occurring in response to maternal LPD during oocyte maturation. During the present study, we observed endothelial-independent impairments in vasodilatation, elevated ACE activity profiles, but no changes in glomerular number. However, in response to the LPD during oocyte maturation, we observed impaired endothelial-dependent and -independent vasodilatation, no changes in ACE activity and an elevation in glomerular number. As previously discussed in detail, these changes may reflect the different times during the reproductive cycle during which the LPD was administered (gamete maturation vs. post-fertilisation development). Alternatively, they may reflect the contribution of the paternal genome, absent during oocyte maturation. A third distinction is the connection between early postnatal phenotype and adult physiology and cardiovascular health present in this and previous associated studies which appear absent in the offspring derived from the LPD administered during oocyte maturation. Whilst both dietary protocols result in hypertensive offspring, the mechanisms underlying this state appear both similar (vascular responsiveness) and distinct (ACE activity, body weight and kidney morphology).

In conclusion, we have demonstrated that maternal LPD given either exclusively during pre-implantation embryo development, or throughout gestation, results in an impaired arterial vasodilatory capacity in male offspring, and altered activity of serum and lung ACE activity in female and male offspring, respectively. However, kidney glomerular number in females, and expression of key RAS component transcripts and blood volume regulators in both male and female offspring were unaffected. These findings demonstrate that maternal undernutrition encompassing the earliest stages of development has an impact upon interacting vascular, signalling and enzymic pathways which associate with the development of an elevated offspring SBP phenotype. These changes in offspring homeostatic regulation may have occurred in response to adaptations made during embryonic and/or fetal development to maximise postnatal development in an anticipated nutritional environment, but which then predispose the offspring to adult disease. Collectively, the present results offer novel insights into the mechanisms behind the association between poor pre-implantation maternal diet and adult cardiovascular dysfunction.

Acknowledgements

The present study was supported by the Biotechnology and Biological Sciences Research Council (BBSRC; grant no. BB/F007450), the National Institutes of Health, USA as part of the National Institute of Child Health and Human Development (NICHD) National Cooperative Program on Female Health and Egg Quality under cooperative agreement (grant no. U01 HD04435) and the Gerald Kerkut Charitable Trust. M. A. H. is supported by the British Heart Foundation. We thank the Biomedical Facility, University of Southampton, for their technical support. We also acknowledge the help of Stephanie Marfy-Smith with the preparation of the kidney tissue for gene expression analyses.

analysed the data; A. J. W., E. S. L., C. T., M. A. H. and T. P. F. wrote the manuscript.

There are no conflicts of interest associated with the present study.

References