Differential effects of maternal nutrient restriction through pregnancy on kidney development and later blood pressure control in the resulting offspring

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Abstract

The mechanisms whereby maternal nutritional manipulation through pregnancy result in altered blood pressure in the offspring may include changes in fetal and newborn and adult renal prostaglandin (PG) synthesis, metabolism and receptor expression. As the postnatal effects of nutrient restriction on the renal PG synthesis and receptor system during nephrogenesis in conjunction with nephron numbers and blood pressure have not been evaluated in the rat, the present study examined the effect of reducing maternal food intake by 50% of ad libitum through pregnancy on young male rats. Six control fed mothers and 8 nutrient restricted pregnant rats with single litter mates were used at each sampling time point, most of which occurred during nephrogenesis. Offspring of nutrient restricted dams were lighter from birth to 3 days. This was accompanied by reduced PGE₂, with smaller kidneys up to 14 days. Nutrient restriction also decreased mRNA expression of the PG synthesis (PGHS-2) enzyme, had little effect on the PG receptors, and increased mRNA expression of the degradation enzyme (PGDH) during nephrogenesis and the glucocorticoid receptor in the adult kidney. These mRNA changes were normally accompanied by similar changes in protein. Nephron number was also reduced from 7 days up to adulthood when blood pressure (measured by telemetry) did not increase as much as in control offspring during the dark, active period. In conclusion, maternal nutrient restriction suppressed renal PG concentrations in the offspring, and this was associated with suppressed kidney growth and development, and decreased blood pressure.
Introduction

Maternal nutrient restriction during pregnancy has been previously shown to affect the renal development of the offspring. In the sheep, nutrient restriction targeted to the period of early kidney development subsequently increases organ size as well as affecting kidney shape and promotes glucocorticoid receptor (GCR) mRNA abundance (32). Global nutrient restriction (9) and protein restriction in particular can both reduce the total number of nephrons formed in developing rat kidneys but this does not necessarily result in raised blood pressure in the offspring (11). The mechanisms by which maternal nutritional manipulation acts to compromise fetal development and ultimately adult health remain uncertain. It has been proposed this is dependent in part on increased maternal corticosterone concentrations acting directly on the fetus (16). This proposal is supported by the finding that maternal administration of dexamethasone, which crosses the placenta to the fetus during pregnancy, can cause similar cardiovascular outcomes as observed with maternal food deprivation (40); indeed dexamethasone administration also results in reduced maternal food consumption, suggesting a behavioural effect upon appetite (37, 40). Recently it has been shown that a transient increase in maternal corticosterone on days 14 and 15 of pregnancy in rats can impair development of the intra-renal renin-angiotensin system (RAS) thereby leading to reduced nephron number and raised blood pressure in the adult offspring (25). The overall dexamethasone effect, however, is dose-dependent. At low doses it reduces maternal food intake and weight gain, but there is no effect on blood pressure in their adult male offspring (37).
Nephron development in the kidney is dependent upon the intra-renal RAS (38, 39) and on the synthesis of intra-renal prostaglandins (PGs). Indeed, studies examining the effects of knocking out or inhibiting PG synthesis indicate they are crucial for normal metanephric development (5, 14). Renal prostaglandin endoperoxide H synthase-2 (PGHS-2, COX-2) may also be responsible for renin mRNA expression (34). Interestingly, corticosterone is able to decrease the expression of PGHS-2 in the rat kidney cortex (42) but effects on the degradation enzyme PG dehydrogenase (PGDH) or blood pressure are not known.

The intriguing possibility therefore exists that maternal nutrient restriction decreases intra-renal PG synthesis, metabolism and receptor levels, perhaps via elevated fetal or newborn plasma corticosterone levels. Should renal PG synthesis or action be suppressed during nephrogenesis, this could ultimately lead to decreased nephron numbers and consequent raised blood pressure in the young adult offspring. Surprisingly, the effects of nutrient restriction during pregnancy on the PG synthesis-receptor system in the kidney during development and into adulthood have not been evaluated in rodents. Our hypothesis was that maternal nutrient restriction through pregnancy would result in postnatal alterations in the renal PG synthesis and receptor system that may then persist into adulthood. These changes would be accompanied by increased plasma corticosterone, increased renal glucocorticoid receptors, and decreased nephron numbers that would be accompanied by a resetting of blood pressure profiles and responsiveness during early adulthood. In this first study, our objective was to examine closely the changes in the PG synthesis-receptor system and compare these to changes in the other parameters studied.
Materials and Methods

Animals, diet and tissue sampling

All experimental animal work was approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee and followed the Canadian Council on Animal Care guidelines. Fourteen female Long-Evans rats were received at the animal housing facility at 10 weeks of age and allowed to acclimatise for 1 week. Animals were then mated and pregnancy was confirmed by the presence of sperm in a vaginal smear examined microscopically the following morning. This was considered day 0 of pregnancy and dams were randomly separated into control (n = 6) or nutrient restricted (NR; n = 8) groups. Food intake in the control group was measured daily, and 50% of this amount was given to the nutrient restricted group for the duration of pregnancy. Control dams thus consumed 20 ± 1 g per day at the start of pregnancy and 25 ± 2 g near to term, whilst the nutrient restricted group consumed half this amount. The Laboratory Rodent Diet (LabDiet 5001, Richmond, IN, www.labdiet.com) comprised 23.4% protein, 10% total fat, 49.9% carbohydrates, and 6.9% ash plus water. All dams were weighed daily. Following birth and for the remainder of the experiment, both groups had *ad libitum* access to food which was recorded on a daily basis. Each pup was weighed daily for the first three weeks after birth and then weekly after weaning during which time food intake was measured daily. At weaning all dams were euthanased by sodium pentobarbital overdose (100 mg/kg injected into the peritoneum) to enable the weights of hearts, livers, lungs and kidneys to be assessed.

Shortly after birth litter sizes were reduced to ten pups per dam and the remaining animals were all euthanased by decapitation. These pups were selected depending on their size with
the smallest and largest pups being selected as well as up to three medium-sized animals, depending on litter size, with only one medium sized offspring being used in any kidney analyses. Then at postnatal day seven, a further two medium sized pups from each litter were euthanased by sodium pentobarbital overdose. Only male pups from each litter were sampled at postnatal days 14, 21 and 28 as this is coincident with maturation of the hypothalamic-pituitary axis and to avoid any potential for a confounding influence of gender. Then following blood pressure assessments, as described below, each adult male was euthanased at 12 weeks of age. For all animals, the brain, heart, liver, lung and kidney were removed, weighed and snap frozen in liquid nitrogen then stored at -80 °C.

Heparinized blood samples were also taken from selected pups at every sampling point. In the neonatal pups killed by decapitation this was achieved by collecting trunk blood (approximately 1 ml) of the medium-sized pups. At all other time points blood was taken by cardiac puncture once the animals were anaesthetized by sodium pentobarbital. Blood samples were immediately centrifuged at 3000 rpm for 10 minutes and the plasma removed and stored at -20 °C. In order to obtain sufficient plasma for later analyses, plasma from the three pups at day 0 in each litter were combined.

**Radiotelemetry monitoring of offspring blood pressure and heart rate**

Twenty four hour measurements of systolic, diastolic and mean blood pressure together with heart rate were made by radiotelemetry (PA-C40 Data Sciences International, St Paul, Minnesota, USA). This was undertaken in one male animal from each litter that was moved to the surgical facility between 9-10 weeks of age. They were then allowed at least seven
days to acclimatise before undergoing surgery to enable implantation of a catheter into the aorta and the transmitter attached to the abdominal wall as described by Khan et al. (12). Recordings were then made at least one week after surgery by which time feed intake of each animal was the same as prior to surgery. Each animal was placed in a cage adjacent to one of the receiving devices and following 12 hours acclimatization, continuous recordings were taken over a period of 24 hours using WINDAQ Acquisition software (Dataq Instruments, Akron, Ohio, USA). In addition, to measure the animal’s response to an acute stress exposure, their cages were connected to an air pipe. This pipe was then switched on for a period of ten seconds producing a loud noise and a blast of air into the cage. This test was always carried out between 13:00 and 14:00 hours whilst the animals were resting.

**Messenger RNA detection**

Total RNA was extracted from ~0.1 g of kidney using TRIzol™ (Invitrogen, Ontario, Canada). Reverse transcription was then performed by adding 2 µg of RNA to an initial mixture (1 µl random primers, 2 µl dNTP mix, 19 µl water) and heating at 65°C for 5 minutes then chilling on ice. A second mixture was then added (8 µl 5x RT buffer, 4 µl 0.1M DTT, 2 µl RNase Out) and either 2 µl of Superscript II Reverse Transcriptase enzyme or 2 µl water and incubated accordingly.

Oligonucleotide primers specific for each gene were optimized for annealing temperature (Table 1). Correct product formation was confirmed by determining size by agarose electrophoresis. PCR reactions of 25 µl were prepared containing 12.5 µl 2x SYBR Green, 0.25 µl Amp Erase, 0.5 µl each of 10mM forward and reverse primers and 10.25 µl water.
Briefly, the protocol used for RT-PCR was: initial denaturation step (95°C for 10 min) followed by 40 cycles of a denaturation step (95°C for 20 seconds) and an amplification and quantification step (annealing temperature for 1 min). This was followed by a melt curve step which involved the incremental (0.5°C/12 seconds) heating from 55 to 95°C to establish that every product melted at the same temperature, thus confirming that they were all the same. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a housekeeping gene, was tested as a standard comparator and found to be unaffected by the animal’s age and dietary history (e.g. Day 0 - C 16.9 ± 0.2; NR 16.5 ± 0.4; Adult - 16.1 ± 0.4; NR 16.9 ± 0.7 arbitrary units). In addition, single samples were run on each plate to assess inter-assay variation and all samples run in triplicate. Both the inter and intra-assay variation were < 10 %. Threshold cycle results were normalised to GAPDH expression for each sample and expressed as a % of the reference sample run on all plates.

**Protein detection**

Protein was extracted from ~0.1 g of kidney and Western blotting was then used to determine the abundance of each protein using the antibodies detailed in Table 2. Densitometric analysis was performed using the Flour-S Max software (Biorad, California, USA) and data expressed as a % of a single reference sample that was run on all gels, except for PGHS-2 and GCR proteins where commercially available standards were run on each gel and used for quantification (Cayman Chemicals, Ontario, Canada (No. 360120)) and Santa Cruz, California, USA (No. 121-420). Where standards were not available, antibody specificity was established by comparing band patterns produced to those produced when the antibody was incubated with a specific blocking peptide.
**Measurement of glomeruli number**

The number of glomeruli was determined using the mild acid-hydrolysis method (31) which enables the number of individual glomeruli to be counted (1). For the kidneys sampled at postnatal days 0 and 7, the entire kidney was incubated in 1 ml of 1 mol/l hydrochloric acid for 30 minutes at 37°C whilst for the older animals 1 g of tissue (obtained from the same region in all animals) was used. The acid was then removed and the tissue blotted dry and then placed into 1 ml 50 mM PBS pH 7.4 and homogenised, after which three 20 µl aliquots of the sample were placed on slides and the number of glomeruli counted under a 10 x objective lens. All counting was undertaken by the same individual who was blinded to both the age and nutritional group of the sample. Mean results were averaged then used to calculate the number of glomeruli for each animal.

**Prostaglandin E2 Assay**

Prostaglandins were extracted from adult kidney samples by homogenizing 0.1 g tissue in 500 µl of ethanol to which 4 ml of 50 mM citrate buffer (pH 3.5) was then added in order to reduce the ethanol concentration to < 15%. The samples were then centrifuged at 1250 x g for 10 min and then purified using C-18 solid phase extraction (SPE) Sep-Pak cartridges. The protein content of the pellet was then determined using the Micro BCA Protein Assay Kit and following homogenization the PGE2 content determined using EIA Assay (Cayman Chemicals, Ontario, Canada, No. 514010). The intra-assay coefficient of variation was 9%.
Statistical Analyses

All data were analysed by two way analysis of variance (SigmaStat, Systat, Point Richmond, California, USA) with diet and age as the two variables. In addition for the tissue data at 0 days of age the additional effect of birth weight was assessed. When a significant F value was obtained this was followed by a Holm-Sidak post-hoc test to differentiate treatment effects. Prostaglandin E$_2$ assay data were analysed using the Student’s t-test. A P value < 0.05 has accepted as significant.

For the cardiovascular measurements hourly means from each animal were combined to obtain a 24 hour mean of the effects of nutrient restriction on blood pressure and heart rate. The effect of time of day and diet were then determined. These data were analysed to compare measurements from the light (06:00 - 18:00 h) to dark periods (18:00 - 06:00 h) using a Mann-Whitney U test because of unequal variances. To assess the effect of maternal diet on the circadian variability of blood pressure, the Z-score was calculated for each animal during the light and dark periods using the following equation:

\[
Z\text{-score} = \frac{\text{period average} - \text{overall average}}{\text{standard deviation for the whole period}}
\]
Results

Effects of nutrient restriction on maternal body weight, offspring birth weight and later growth

Nutrient restricted dams immediately lost weight and remained lighter than controls throughout pregnancy (Figure 1a). Controls gained in weight through pregnancy whereas nutrient restricted dams lost weight until ~ 15-16 days gestation. Although all dams gained weight through lactation nutrient restricted dams remained lighter but the weights of all major body organs were similar between groups (data not shown). There was no difference in litter size between groups (C 14.9 ± 0.6 (n=6); NR 14.8 ± 0.3 (n=8) pups/dam).

Pups born to the nutrient restricted dams were significantly lighter than controls and remained so until 3 days of age (Figure 1b). By 21 days of age the male nutrient restricted rats tended to weigh more than control rats a pattern that continued to the end of the study when they remained heavier than controls (C 348 ± 8; NR 396 ± 10 g, (P=0.004)). The nutrient restricted males ate significantly more than control males from 6 weeks of age until the end of the study (e.g. week 10 - C 190 ± 13; NR 230 ± 8 g/week (P=0.04)).

Effect of NR on offspring organ development

Absolute and relative kidney weights were significantly reduced in nutrient restricted offspring at birth, 7 and 14 days of age (e.g. 14 days - C 0.37 ± 0.01; NR 0.30 ± 0.01 g (P<0.05); C 1.16 ± 0.02; NR 0.95 ± 0.02 % body weight, (P<0.05)) but were not accompanied by any difference in the weights of other organs with the exception of the liver that was only reduced at birth (C 282 ± 23; NR 194 ± 12 mg, (P=0.003)) but not later in
development. Furthermore, this decrease in nutrient restricted kidney weight was found in small, medium and large offspring (e.g. smallest pup per litter: C 59 ± 4; NR 42 ± 1 mg, P<0.001). The reduced rate of kidney growth for the first two weeks after birth was accompanied by a pronounced reduction in nephron number per kidney that persisted into adulthood (Figure 2).

**Radiotelemetry monitoring of offspring blood pressure and heart rate**

Systolic, diastolic and mean arterial blood pressures in young male adult offspring were higher at night than during the day, and reduced in nutrient restricted offspring, largely as a result of the nocturnal rise being blunted (Figure 3). There was no significant difference in Z-score for blood pressure between groups (C 0.6 ± 0.05, NR 0.6 ± 0.04) indicating no effect of maternal diet on circadian variation. Although heart rate was higher at night than during the day, this was not different between nutritional groups (Figure 3). Measurement of blood pressure response to acute stress between 13:00 and 14:00 hours showed no significant difference between nutritional groups in either maximal pressure (C 127 ± 5; NR 119 ± 6 mmHg) or magnitude of response (C 38 ± 2; NR 33 ± 2 mmHg). Unfortunately, due to the acute nature of this challenge, consistent values for heart rate were not measurable.

**PGHS and PGDH Expression in the kidney and its PGE₂ content**

PGHS-2 mRNA (Figure 4a) and protein (data not shown) increased after birth in the control group, peaked at postnatal day 14 and then decreased to adulthood. In nutrient restricted animals the peak in mRNA abundance was earlier, occurring on day 7, then levels significantly decreased (P<0.05) on postnatal day 14 and thereafter to adulthood. Further,
these levels in NR offspring were considerably lower (P<0.05) than in control offspring on
day 14. Both mRNA and protein expression in NR offspring were slightly, but significantly
(P<0.05), higher than in control offspring in adult animals (Protein: C 10 ± 2; NR 18 ± 1 %
of reference; (P<0.05)). When cortical and medullary protein expression were analysed
separately in the adult kidney, it was found that they were both significantly increased by
maternal nutrient restriction. In contrast, there were no significant differences in PGHS-1
protein expression with age or between nutritional groups (data not shown). Whilst from
birth and postnatal day 7 the mRNA expression of PGHS-1 significantly increased 3-fold and
then maintained at a steady state.

Expression of PGDH mRNA (Figure 4b) and protein (data not shown) increased after birth
and then decreased to adult levels. Nutrient restricted animals had raised mRNA abundance
at birth and then showed an accelerated decrease in both mRNA and protein expression after
21 days of age so that it was significantly lower at postnatal day 28. Protein abundance then
remained unchanged in offspring born to nutrient restricted mothers so that as adults this was
significantly raised (C 70 ± 30; NR 144 ± 28 % of reference, (P<0.05)). The tissue content of
PGE₂ in the adult, nutrient restricted animals, was significantly lower compared to controls
(C 4630 ± 380; NR 3120 ± 95 pg PGE₂/mg protein (P<0.05)).

**EP Expression**

Expression of EP₁ mRNA (Figure 5a) and protein (data not shown) increased after birth and
then decreased to significantly lower adult values with no differences between control and
nutrient restricted animals at any time. Messenger RNA (Figure 5b) and protein (data not
shown) abundance for EP2 also increased after birth to then decrease by day 14. This response was delayed in nutrient restricted animals in which EP2 expression was significantly higher at 14 days of age. Then as adults nutrient restricted animals showed decreased protein expression (C 190 ± 50; NR 35 ± 14 % of reference (P<0.05)). Expression of EP3 mRNA (Figure 5c) did not rise immediately after birth but showed a sudden increase between postnatal days 21 and 28, followed by a decrease to adult levels in control but not nutrient restricted offspring. EP3 protein abundance (Figure 5d) showed a more gradual increase in expression, reaching the adult level by day 28. Nutrient restricted animals had a delayed increase in protein expression with blunted increase in mRNA expression, and as adults exhibited significantly raised mRNA and protein abundance. Expression of EP4 mRNA (Figure 5e) and protein (Figure 5f) increased after birth then decreased to adult levels. Nutrient restricted animals showed a greater increase in mRNA expression that was significantly higher at day 14 and was followed by a greater decrease in expression so that the adults had significantly lower abundance of mRNA but not protein.

**GCR Expression**

Expression of GCR mRNA (Figure 6a) increased by day 7, peaking at postnatal day 14 and then decreased to neonatal levels in the adults. The increase in GCR protein (Figure 6b) occurred later and did not decrease until after postnatal day 28. Nutrient restricted animals had significantly lower GCR protein levels at birth which then increased to become significantly higher than controls at postnatal day 7 only. They also showed a transiently delayed decrease in mRNA whereas protein abundance was again significantly raised in adulthood.
Discussion

The major finding of the present study is that maternal nutrient restriction leads to maternal weight loss and fetal growth restriction, decreased newborn PG synthetic capacity, and decreased blood pressure in young adult males. These changes are accompanied by increased newborn plasma corticosterone (2) that is followed by decreased renal weight and nephron numbers from 7 days of age in conjunction with lower PGE₂ concentrations in adult kidneys. These offspring did not then achieve the same elevation of blood pressure relative to the control offspring during the nocturnal (i.e. active period) hours. The elevated plasma concentrations of corticosterone are expected, and the sensitivity of the growing kidney to maternal nutrient restriction is in accord with a number of other studies but this does not necessarily equate with raised blood pressure (11). We achieved our principal goal of associating changes in NR offspring renal PG synthesis capacity and concentrations with nutrition restriction together with a reduction in blood pressure. Such data suggests the possibility that renal PGs mediate, in part, the effect of NR on blood pressure.

A global reduction in maternal food intake is expected to result in an immediate weight loss in the dam (15, 30). It is therefore not until day 15 of gestation that nutrient restricted dams start to increase their body weight in conjunction with the rapid rise in feto-placental mass and coincident with the time at which maternal food intake in the control dams increased significantly above non-pregnant intake. Our study is the first to report that nutrient restricted dams remain significantly lighter than controls throughout lactation despite their food intake being similar. The apparent failure to restore maternal body weight is in contrast to the catch-up growth in the offspring whose weight is the same as controls by three days after birth. It is
interesting to note that as with all other studies of this type, litter size is unaffected (30).

Taken together these findings suggest the intriguing possibility that rats have evolved to adapt to adverse nutritional conditions by maintaining the maximum number of pups at the expense of both pup and dam size, with any deficit in fetal growth being overcome during lactation (21) which is the time at which maturation of the hypothalamic-pituitary axis in the offspring occurs. This could explain the critical importance of this stage of development with regard to amplifying later adult outcomes (26).

The reduction in pup birth weight seen in the present study is less marked than other studies which have used a more severe nutritional challenge, i.e. fed a 70% rather than 50% reduction of ad libitum nutrition (17, 20, 36). Previous studies using a 70% nutrition reduction have demonstrated that both hyperphagia and sedentary behaviour patterns can be programmed by maternal nutrient restriction (17, 30). We also observed an increase in food intake of nutrient restricted offspring and greater weight as adults. Our 50% nutrient restriction diet caused a small but statistically significant reduction in kidney size (both absolute and relative to body weight) at birth, and this observation is consistent with the findings of previous studies of the effect of both low protein (11) and global nutrient restriction in rats (17, 30). Furthermore we observed that primarily the kidney was affected for the first two weeks of life, as most other organs were normal during this period. The liver was smaller only at birth, but caught up to control weights by day 7. This supports the hypothesis that the kidneys are particularly susceptible to the effects of maternal nutrient restriction and is an adaptation that occurs irrespective of birth weight (32).
In association with their smaller size in the newborn period, the kidneys of the nutrient restricted animals had significantly fewer nephrons from 7 days after birth to adulthood, but not at birth, which was coincident with the time at which plasma corticosterone remained at the relatively high post partum concentrations in nutrient restricted offspring, rather than falling as in controls (2). Interestingly, a similar magnitude of maternal nutrient restriction but commencing on day 13 of gestation in Wistar rats led to elevated fetal plasma corticosterone levels on day 21 of gestation and lower placental levels of 11β-hydroxysteroid dehydrogenase, type 2 mRNA (16). However, by 2 hours after birth, plasma corticosterone was lower in nutrient restricted offspring compared to controls, although no further determinations were made in older animals.

In the present study plasma corticosterone concentrations were the same in control and nutrient restricted offspring at birth (2), indicating they were not obviously higher in nutrient restricted fetuses compared with controls during late gestation. At day 14, higher plasma corticosterone in postnatal nutrient restricted offspring was observed (2). It is established that nephrogenesis in the fetal rat begins at 12 days gestation and is largely complete by postnatal day 14 (35), so elevated plasma corticosterone would only inhibit this process if this occurred prior to this date. In other species such as the sheep, that have comparable numbers (and between animal variation) of glomeruli to the human (27) and are born after a long gestation and nephron number is set in utero, maternal dexamethasone administration around the time of development of the mesonephros leads to impaired kidney function in later life (6). This can lead to an increase in blood pressure in castrated offspring (6). Interestingly when maternal nutrient restriction is targeted over a similar period of development although
nephron number is reduced, blood pressure is not raised when the offspring remain intact (10) and the kidney is actually protected from the adverse structural and molecular responses to later obesity (33).

In the present study we only measured blood pressure at one age i.e. between 10-11 weeks of age. It is thus possible that the lower values we find in NR offspring from between 00.00-12.00 h could increase with age. A majority of studies in the rat, however, show little change in blood pressure after this age under a range of genetic and nutritional programming models (13, 28). One notable exception is found in female offspring born to mothers fed a high fat diet (i.e. 4 times greater fat content compared to controls) through pregnancy and lactation in which there is no difference in blood pressure at 10 weeks of age, but it is higher in these offspring at 6 and 12 months of age (12). Interestingly this adaptation is accompanied by an increased body mass with age despite no difference in food intake and has not been shown to date to have any effect on the kidney.

In order to further establish whether blood pressure responsiveness may have been reset in NR offspring we subjected each male to a stress challenge and found no difference between groups. This is of interest as it contrasts with the effect of intra-uterine growth retardation induced by uterine artery ligation 4 days before term (22), and may be indicative that blood pressure remains lower in the longer term in NR offspring. One alternative explanation is that they are developing a gradual rise in blood volume thereby promoting peripheral vasodilatation. Ultimately this may lead to higher blood pressure. An adaptation of this type is partly in accord with the enhanced rise in blood volume seen in pregnant sheep following
maternal nutrient restriction between early-to-mid gestation followed by normal dietary intake up to term (4). Clearly additional studies are required to test this hypothesis with regard to the impact of the kidney on blood volume regulation (7) and how they may potentially be reset in utero.

In spite of the smaller birth size, elevated corticosterone levels, and appreciably lower number of nephrons, these nutrient restricted young adult males displayed a lower increase in blood pressure at night than controls as measured by radiotelemetry. Importantly there was no time point over the 24 h recording period in which blood pressure was raised and is thus in accord with most recent findings in the offspring that are maintained on a low protein diet through their life cycle that similarly have fewer nephrons (11). Indeed, the magnitude of reduction in nephron number observed in this earlier study as determined using morphometric methods is very similar to our results in offspring born to mothers subjected to a global reduction in food intake through pregnancy. Our additional analyses of the kidney with regard to PGs, EP and GCR receptors provide some novel insights into the potential mechanisms by which maternal nutrient restriction through pregnancy can impact on its development from birth up to adulthood.

The observation that maternal nutrient restriction changes the expression of PGHS-2 is novel. In this regard the reduction in mRNA expression at day 14 was not matched by protein and could thus be indicative of a slower turnover of protein following maternal nutrient restriction. Any adaptation of this type is transient as by 28 days both mRNA and protein were similarly reduced in nutrient restricted offspring. By adulthood, however, both these
indices of renal PGHS-2 were raised in the renal cortex and medulla. Similar adaptations were found for PGDH at 28 days and in the adult that was accompanied by a lower PGE$_2$ content in the adult kidney. Taken together these findings suggest enhanced degradation of PGs in the kidney as seen in offspring born to dams fed a low protein diet (24).

One potential mechanism by which PGHS-2 abundance was raised in the adult offspring born to nutrient restricted dams is through elevated plasma corticosterone which persisted from 14 days of postnatal life through to adulthood. Corticosterone inhibits cortical PGHS-2 (18, 42) but promotes medullary PGHS-2 expression (43) although it is only thought to be significantly expressed in the medulla after weaning (41). An adaptation of this type may therefore have been mediated by the increased GCR in nutrient restricted adults that may have similarly impacted upon PGDH expression (8, 19). Interestingly it was protein and not mRNA abundance for the GCR that was specifically up-regulated in the adult nutrient restricted offspring which may relate to a slower rate of loss of this receptor with aging in these animals. In order to further examine the role of corticosterone on kidney development following maternal nutrient restriction through pregnancy, the impact of GCR antagonists could now be explored.

Nutrient restriction had differential effects on EP expression in the developing and adult kidney, with EP$_1$ being unaffected suggesting it is not involved in the nutritional programming of renal development. Interestingly, the ontogenic decrease in EP$_2$ expression is delayed with nutrient restriction and could reflect a delay in nephron maturation or altered blood flow to the vasa recta (3). With respect to the reduced EP$_2$ protein abundance in
adulthood following maternal nutrient restriction, this may be reflective of differences in PGE$_2$ action between the two groups or decreased nephron number as demonstrated in earlier studies (9). In contrast, EP$_3$ abundance was raised in these offspring and since activation of this receptor concentrates urine, nutrient restricted offspring could potentially retain more water than their control counterparts. An adaptation of this type has previously been shown to lead to hypertension (17) but is clearly not the case in our study and may relate to the differential effects of raised GCR. The extent to which the accelerated rise in EP$_4$ mRNA abundance and subsequent decline contributed to any functional changes in the kidney of nutrient restricted offspring is unclear as there were no accompanying effects on protein content. One possible outcome is reduced renin production (23) which is in accord with adaptations found in offspring born to dams fed a low protein diet through pregnancy (29) that also have fewer nephrons (11).

**Perspectives and Significance**

A global reduction in maternal food intake through pregnancy does alter growth of the fetus and resulting offspring with kidney development being specifically compromised. Prostaglandin synthesis, metabolism and possibly receptors are associated with renal developmental changes which may lead to other downstream physiological changes, such as with blood pressure. This nutrition restriction does not, however, contribute to raised blood pressure and indicates the adaptive capacity of the fetus and newborn to resist later disease is greater than previously appreciated. Future experiments should confirm the mechanisms where by nutrition restriction alters renal PGs and their consequent actions on renal development and other physiological outcomes.
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References


Table 1. Primer sequences and annealing temperatures used for the detection of mRNA expression for prostaglandin endoperoxide H synthase-2 (PGHS-2), prostaglandin dehydrogenase (PGDH), prostaglandin receptors - EP1-4, and the glucocorticoid receptor (GCR).

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>Product Size (base pairs)</th>
<th>Primer Sequence</th>
<th>Annealing Temp (°C)</th>
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</thead>
<tbody>
<tr>
<td>PGHS-1</td>
<td>117</td>
<td>For: 5’-GGA ATT CAA CCA CCT CTA TGA CTC-3’&lt;br&gt;Rev: 5’-GAC ACC GTA GTC CAC CAG CAT-3’</td>
<td>61</td>
</tr>
<tr>
<td>PGHS-2</td>
<td>130</td>
<td>For: 5’-CCT TGA ACA CGG ACT TGC TCA C-3’&lt;br&gt;Rev: 5’-TCT CTC TGC TCT GGT CAA TGG A-3’</td>
<td>62</td>
</tr>
<tr>
<td>PGDH</td>
<td>283</td>
<td>For: 5’-ATG CAC GTG AAC GGC AAA GTG-3’&lt;br&gt;Rev: 5’-TTC ACT CCT GCC TTG TTG ACC-3’</td>
<td>62</td>
</tr>
<tr>
<td>EP1</td>
<td>98</td>
<td>For: 5’-AAC TGC TTC GCC TCC TAC-3’&lt;br&gt;Rev: 5’-AAC TAC GCA GTG AAC TGG-3’</td>
<td>60</td>
</tr>
<tr>
<td>EP2</td>
<td>301</td>
<td>For: 5’-TTC GGA GCA AAA GAA GCC-3’&lt;br&gt;Rev: 5’-GAG CGC ATT AGT CTC AGG-3’</td>
<td>62</td>
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<tr>
<td>EP3</td>
<td>310</td>
<td>For: 5’-GCT GTC TGT GCT CGC CTT-3’&lt;br&gt;Rev: 5’-CCA TAA GCT GGA TAG-3’</td>
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</tr>
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<td>EP4</td>
<td>240</td>
<td>For: 5’-GGA AGA CTG TGC TCA GTA-3’&lt;br&gt;Rev: 5’-GAA GCA AAT TCT TGC CTC-3’</td>
<td>59</td>
</tr>
<tr>
<td>GCR</td>
<td>210</td>
<td>For: 5’- AGG GAT TCA GCA AGC CAC -3’&lt;br&gt;Rev: 5’- CGC CCA CCT AAC ATG TTG -3’</td>
<td>60</td>
</tr>
<tr>
<td>GAPDH</td>
<td>110</td>
<td>For: 5’-GGC AAG TTC AAT GGC ACA GT-3’&lt;br&gt;Rev: 5’-TGG TGA AGA CGC CAG TAG ACT C-3’</td>
<td>60</td>
</tr>
</tbody>
</table>
Table 2. Summary of antibody suppliers and dilutions used for the detection of protein abundance for prostaglandin endoperoxide H synthase-2 (PGHS-2), prostaglandin dehydrogenase (PGDH), prostaglandin receptors - EP₁-₄, and the glucocorticoid receptor (GCR).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Company</th>
<th>Catalogue Number</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGHS-1</td>
<td>Santa Cruz</td>
<td>H-62</td>
<td>1:100</td>
</tr>
<tr>
<td>PGHS-2</td>
<td>Cayman</td>
<td>160126</td>
<td>1:1000</td>
</tr>
<tr>
<td>PGDH</td>
<td>Cayman</td>
<td>160615</td>
<td>1:250</td>
</tr>
<tr>
<td>EP₁</td>
<td>Cayman</td>
<td>101740</td>
<td>1:250</td>
</tr>
<tr>
<td>EP₂</td>
<td>Cayman</td>
<td>101750</td>
<td>1:250</td>
</tr>
<tr>
<td>EP₃</td>
<td>Cayman</td>
<td>101760</td>
<td>1:250</td>
</tr>
<tr>
<td>EP₄</td>
<td>Cayman</td>
<td>101770</td>
<td>1:500</td>
</tr>
<tr>
<td>GCR</td>
<td>Santa Cruz</td>
<td>H-300</td>
<td>1:200</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1. Effect of maternal nutrient restriction through pregnancy on maternal weight during pregnancy and pup growth over the first 21 days of lactation. a) Pregnant rats were either fed ad libitum (control, closed symbols (n=6)) or 50% of this amount (nutrient restricted (NR), open symbols (n=8)). Data are means ± standard errors. Significant difference between control and nutrient restricted indicated by a. Time points when nutrient restricted maternal weight is significantly higher than at start indicated by b. Time points when nutrient restricted maternal weight is significantly lower than at start indicated by c.

b) Offspring were born to mothers that were either fed ad libitum (control, closed symbols (n=6)) or 50% of this amount (nutrient restricted (NR) open symbols (n=8)). Data are means ± standard errors. Significant differences between groups indicated by * P < 0.05.

Figure 2. Effect of maternal nutrient restriction through pregnancy on total nephron number in the resulting offspring. Offspring were born to mothers that were either fed ad libitum (control, closed bars (n=6)) or 50% of this amount (nutrient restricted (NR), open bars (n=8)). Data are means ± standard errors. Significant differences between groups indicated by * P < 0.05.

Figure 3. Effect of maternal nutrient restriction through pregnancy on 24 h a) systolic and diastolic, b) mean blood pressure and c) heart rate profiles in the resulting male offspring as measured between 11-12 weeks of age. Offspring were born to mothers that were either fed ad libitum (control, closed symbols (n=6)) or 50% of this amount (nutrient restricted (NR),
open symbols (n=8)). Data are means ± standard errors. Significant differences between groups indicated by * P < 0.05.

Figure 4. Effect of maternal nutrient restriction on expression of a) prostaglandin endoperoxide H synthase-2 (PGHS-2) and (b) prostaglandin dehydrogenase (PGDH) mRNA. Pregnant rats were either fed *ad libitum* (control, closed bars (n=6)) or 50% of this amount (nutrient restricted (NR), open bars (n=8)). Bar graphs illustrate means with their standard errors. Significant differences between control and NR groups: * P < 0.05 and significant differences between ages in the same nutritional group of animals indicated by different superscripts: P < 0.05 (C: bold lowercase letters; NR: capital letters).

Figure 5. Effect of maternal nutrient restriction on expression of the prostaglandin receptors (a) EP₁ mRNA (b), EP₂ mRNA (c), EP₃ mRNA (d), EP₃ protein (e), EP₄ mRNA and (f) EP₄ protein (h). Pregnant rats were either fed *ad libitum* (control, closed bars (n=6)) or 50% of this amount (nutrient restricted (NR), open bars (n=8)). Bar graphs illustrate means with their standard errors. Significant differences between control and NR groups: * P < 0.05 and significant differences between ages in the same nutritional group of animals indicated by different superscripts: P < 0.05 (C: bold lowercase letters; NR: capital letters).

Figure 6. Effect of maternal nutrient restriction on expression of glucocorticoid receptor (GCR) mRNA (a) and GCR protein (b). Bar graphs illustrate means with their standard errors. Pregnant rats were either fed *ad libitum* (control, closed bars (n=6)) or 50% of this amount (nutrient restricted (NR), open bars (n=8)). Significant differences between control
and nutrient restricted groups: * P < 0.05 and significant differences between ages in the same nutritional group of animals indicated by different superscripts: P < 0.05 (C: bold lowercase letters; NR: capital letters).
Figure 1b

Age (days)

Weight (g)
Figure 2

Total Nephron Number per Kidney

Age (days postpartum)
Figure 3a

![Graph showing systolic and diastolic pressure changes over time.]

- NR Systolic
- Control Systolic
- NR Diastolic
- Control Diastolic
Figure 3c
Figure 4a – PGHS-2 mRNA

Age (days postpartum)

% ref

0 500 1000 1500 2000 2500

0 7 14 21 28 84

a B b C c AE ad
Figure 4b – PGDH mRNA
Figure 5a – EP$_1$ mRNA
Figure 5b – EP₂ mRNA
Figure 5c - EP₃ mRNA

The graph shows the expression levels of EP₃ mRNA at different ages postpartum. The x-axis represents the age in days postpartum (0, 7, 14, 21, 28, 84), and the y-axis represents the percentage of reference (% ref) expression levels.

Significant differences are indicated by letters and asterisks:
- "a," "A," "B," "C," and "C" denote different groups with significant differences.
- "*" indicates a significant increase.

The graph illustrates the dynamic changes in EP₃ mRNA expression across the specified time points.
Figure 5d - EP₃ protein
Figure 5e - EP₄ mRNA
Figure 5f - EPα protein
Figure 6a – GCR mRNA
Figure 6b – GCR protein

Age (days postpartum)

% std

0 7 14 21 28 84

a a b b b a

A B A B B A

*