# Maternal nutrient restriction alters renal development and blood pressure regulation of the offspring

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Studies have shown that the risk of hypertension in adulthood can be affected by the *in utero* environment. It is established that hypertension is linked to compromised kidney function and that factors affecting organogenesis can increase the risk of later disease. Prostaglandins (PG) and growth factors are known to play an important role in regulating kidney function and renal organogenesis. The extent, however, to which global energy restriction (where all nutrients are reduced) of the mother can programme later blood pressure control or renal PG and growth factor status is unknown. A study is described that aimed to examine the long-term effects of maternal nutrient restriction (NR) and elucidate their relationship with compromised kidney development. First, it was necessary to establish animal models. A sheep model of 50% NR during specific stages of gestation was used to investigate fetal renal development, whilst a rat model of 50% NR throughout pregnancy was used to investigate postnatal kidney development and adult functioning. Molecular analysis has shown that expression of the growth hormone–insulin-like growth factor (GH–IGF) axis is affected by NR in the fetal sheep kidneys, and that changes are dependent on the timing of NR and whether the fetus is a singleton or a twin. Analysis of the kidneys from the rat model has shown nutritional differences in the expression of PG receptors and the enzymes responsible for PG synthesis and degradation that persist into adulthood. In conclusion, NR does affect the GH–IGF and PG axes, and these changes may be important in the nutritional programming of renal functioning and adult blood pressure control.

**Maternal nutrient restriction: Blood pressure: Kidney development: Prostaglandin axis: Growth hormone–insulin-like growth factor axis**

## Fetal programming

CVD is a major cause of mortality and morbidity in the Western world and hypertension is one of the main risk factors. Whilst adult lifestyle and diet undoubtedly play major roles in determining who is affected, there is an increasing body of evidence to suggest that the *in utero* environment is also involved. The incidence of CVD is not uniform across the UK, with mortality being highest in the North-west and lowest in the South-east (Knox, 1973; Fulton *et al*. 1978; Pocock *et al*. 1980). It has been found that individuals born in a high-risk area still have an increased risk even if they move to an area with lower risk (Osmond *et al*. 1990). It would seem, therefore, that the risk of CVD is determined, at least in part, by the place of birth and consequently moves with the individual.

Many retrospective studies have been carried out on medical records to examine the relationship between birth weight and both CVD in general (Barker, 2002) and hypertension specifically (Barker *et al*. 1990). Hypertension is linked to impaired development of the kidney, in particular a reduction in nephron number (Mackenzie & Brenner, 1995). There is some evidence from human observational studies that the uterine environment can affect the growth of the kidneys. This evidence includes a study by Konje *et al*. (1996), which has found that slowly-growing fetuses have disproportionately small kidneys. Similarly, intra-uterine growth restriction decreases nephron number in

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**Table 1.** The effects of maternal nutrient restriction on blood pressure of the offspring investigated using rat and sheep models

| Species | Restriction | Measurement technique | Result | Age at measurement (weeks) | Gender | Reference |
|---|---|---|---|---|---|---|
| Sheep | Energy | Catheter | ↓ | 26 | M and F | Gopalakrishnan *et al.* (2005) |
| Sheep | Energy | Catheter | ↑ | 156 | M | Gopalakrishnan *et al.* (2004) |
| Rat | Protein | Tail cuff | ↑ | 6 | M | Gardner *et al.* (1997) |
| Rat | Protein | Tail cuff | ↑ | 4 | M and F | McMullen *et al.* (2004) |
| Rat | Protein | Telemetry | ↑ | 13 | M | Tonkiss *et al.* (1998) |
| Rat | Protein | Telemetry | = | 52 | M and F | Jensen *et al.* (2005) |
| Rat | Protein | Telemetry | = | 21 | F | Woods *et al.* (2005) |
| Rat | Energy | Catheter | ↑ | 28 | M and F | Ozaki *et al.* (2001) |
| Rat | Energy | Tail cuff | ↑ | 6 | M and F | Franco *et al.* (2002) |

M, male; F, female; ↑, increased; ↓, decreased; = , no difference in blood pressure compared with control.

infants (Hinchliffe *et al.* 1992). It is not possible, however, to determine from these studies the extent to which nutrition is responsible for the outcomes described.

The use of animal nutritional intervention studies has enabled more controlled investigations of maternal diet and kidney development. Reduced kidney size and impaired nephron development have been found in rats following both protein-specific (Langley-Evans *et al.* 1999) and global energy (Lucas *et al.* 1997) restriction (where all nutrients are reduced). Another study carried out in sheep has found that global energy restriction during early to mid gestation actually increases organ size at birth and also affects kidney shape (Whorwood *et al.* 2001), and these offspring subsequently have lower nephron numbers (Gopalakrishnan *et al.* 2005). These differences in outcome are likely to be a result of species-specific differences in the timing of kidney development.

Ovine kidney development is similar to that of man in that the total number of nephrons is determined before birth. In sheep nephrogenesis is complete at approximately gestational day 130 (Moritz *et al.* 2003) of a 147 d pregnancy, whilst in man it is complete at approximately week 32 of a 38-week pregnancy (Potter, 1972). Renal development is timed differently in the rat, which has only approximately 20 % of its total nephron complement formed before birth. Nephrogenesis then continues after birth, finishing at approximately postnatal day 8 (Moritz *et al.* 2003). Thus, these characteristics make the sheep a good animal model for investigating the effects of nutrient restriction during different periods of gestation, whilst the rat is a useful model for studying animals at different time points of kidney development, since it is possible to use littermates from the same pregnancy.

There is also evidence from animal models that nutrient restriction can alter kidney function in adult offspring. In the majority of studies kidney function has not been measured directly but effects have been seen on the animal's blood pressure, suggesting that regulatory mechanisms are disrupted. Table 1 summarises the findings of some previous studies. In sheep global energy restriction has been shown to be associated with reduced blood pressure at 6 months of age (Gopalakrishnan *et al.* 2005) but increased blood pressure at 3 years of age (Gopalakrishnan *et al.* 2004), suggesting that age at measurement is an important factor when determining the effects of a nutritional insult. Several studies have shown that a maternal low-protein diet in the rat leads to an increase in blood pressure of approximately 23 mmHg in the offspring when measured by the tail-cuff technique (Langley & Jackson, 1994; Gardner *et al.* 1997; McMullen *et al.* 2004). When blood pressure is measured by telemetry, however, there is either a smaller increase in pressure of approximately 4·5 mmHg (Tonkiss *et al.* 1998) or no significant difference is found (Jensen *et al.* 2005), suggesting that the method of measurement is important. This effect may be gender specific because no significant difference in blood pressure has been found for female animals (Woods *et al.* 2005). Finally, global energy restriction has also been found to increase blood pressure in rats (Ozaki *et al.* 2001; Franco *et al.* 2002), although this finding has yet to be confirmed by telemetry. In addition, Franco *et al.* (2002) have found that both glomerular filtration rate and renal plasma flow are reduced in animals that are nutrient restricted, in combination with an acceleration of glomerularsclerosis.

The mechanisms and mediators responsible for translating energy or protein restriction during pregnancy into altered blood pressure in adult offspring are undefined. The overall purpose of the studies to be described was, therefore, to examine the effects of undernutrition on two potential mediators of nephron development, the growth hormone (GH)–insulin-like growth factor (IGF) axis and prostaglandins (PG) in the developing kidney.

### Growth hormone–insulin-like growth factor axis

The main components of the GH–IGF axis are summarised in Fig. 1(a). GH stimulates production of IGF-I via the GH receptor and is known to be the major stimulus for IGF-I production in the adult kidney, although its role in the fetus is less clear (Hammerman, 1989). IGF-I and -II can both bind to the IGF-I receptor and stimulate growth. IGF-II can also bind to the IGF-II receptor, which acts as a scavenger, removing excess IGF-II from the system (Hammerman, 1989).
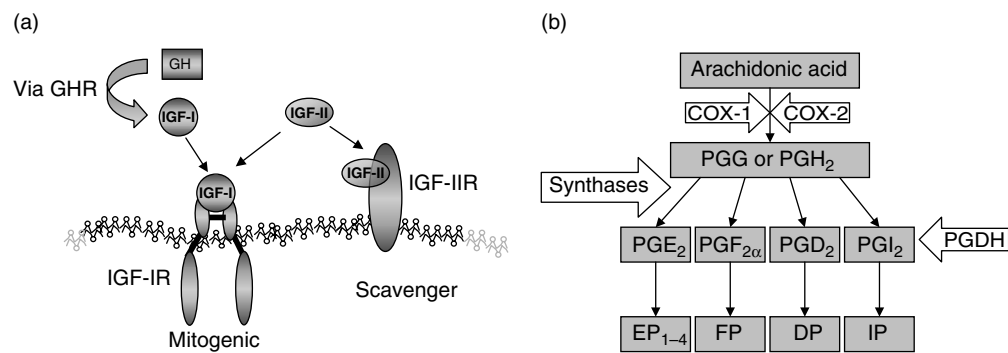
**Fig. 1.** (a) The growth hormone (GH)–insulin-like growth factor (IGF) axis. R, receptor. (Adapted from Hyatt et al. 2004.) (b) The prostaglandin (PG) axis. COX, cyclooxygenase; EP, FP, DP, IP, E-, F-, D-, I-prostanoid receptors respectively; PGDH, PG dehydrogenase.

### Rationale for the investigation

The effects of maternal nutrient restriction (NR) on the GH–IGF axis have been investigated because it is known to play an important role in kidney development. Both the GH receptor and IGF-I are expressed at very high levels in the developing kidney, suggesting that they are important (Ymer & Herington, 1992). IGF-I is first detectable at approximately gestational day 11 in the rat, rising 8·6-fold during the subsequent 48 h (Hammerman, 1989). This timing coincides with that of metanephric differentiation, indicating that IGF-I may play a primary role. IGF-I is also produced by metanephroi cultures and blocking its expression with either antibodies or oligonucleotides prevents further growth or development (Wada et al. 1993). A similar effect is seen with the addition of antibodies or oligonucleotides specific for IGF-II (Hammerman & Miller, 1993). In addition, animals with the IGF-I gene knocked out have severely-impaired renal development (Rogers et al. 1999).

As well as a role in kidney development there is evidence to suggest that the GH–IGF axis can also affect kidney function. Infusion of GH into normal adult human subjects causes an increase in both glomerular filtration rate and renal plasma flow. This effect is only found once GH levels have returned to normal, but whilst plasma IGF-I is still elevated, suggesting this effect is indirect (Parving et al. 1978).

The fetal and adult GH–IGF axis is nutritionally sensitive. For example, NR affects IGF-I and -II expression in both the liver and skeletal muscle (Brameld et al. 2000), whilst kidneys from growth-restricted sheep fetuses have greater sensitivity to GH infusion (Bauer et al. 2003) than normally-growing controls (Bauer et al. 2000). Importantly, abnormalities within the GH–IGF axis have been found in many of the adult diseases associated with low birth weight (Holt, 2002).

### Animal model utilised

In order to investigate the effects of NR on the GH–IGF axis a sheep model was established, the design of which is illustrated in Fig. 2(a). Briefly, sheep were randomly allocated to one of five groups on the day of mating (nine to sixteen per group). The control group (group 1) was fed 100% of their metabolisable energy requirements throughout the experiment. Groups 2–4 were restricted to eating 50% of their metabolisable energy requirements from gestational days 0–30, 31–65 and 66–110 respectively. Group 5 was nutrient restricted throughout the experiment. At 110 d of gestation all animals were killed and the fetal kidneys sampled. In all five groups there were both singleton and twin pregnancies. RT–PCR was used to measure the mRNA abundance of the components of the GH–IGF axis.
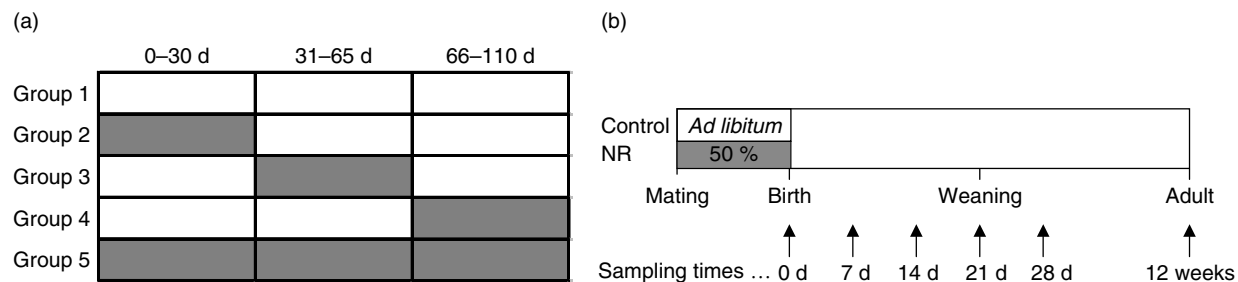


**Fig. 2.** (a) Sheep model design for investigating the effects of maternal nutrient restriction (NR) during different periods of gestation. (□), 100% metabolisable energy requirements; (▨), 50% metabolisable energy requirements. (b) Rat model design for investigating the effects of NR at different time points of kidney development. From conception to birth the controls (□) were allowed ad libitum access to food, while the NR group (▨) was fed 50% of the amount eaten by the control group. After birth and for the duration of the experiment all animals were allowed ad libitum access to food.

## Nutritional effects on fetal kidney development

It was found that kidney or body weights of the fetuses are not affected by NR (Brennan *et al.* 2005). This finding differs from that of Whorwood *et al.* (2001), who have reported an enlargement of kidney size. This disparity may be because the latter study sampled the kidneys of newborn lambs and their increased size may have been related to an acceleration of kidney growth after 110 d of gestation. The only effect of NR on GH–IGF expression in singleton animals is an increase in the expression of the IGF-I receptor in animals from group 4 (Brennan *et al.* 2005). Interestingly, this increase is not seen in animals that were nutrient restricted throughout the experiment, suggesting that it is the switch from a well-fed diet to a restricted diet that is the trigger; a similar finding has been reported in relation to the nutritional programming of fetal adipose tissue and liver development (Brameld *et al.* 2000; Bispham *et al.* 2003).

Examination of the expression of the GH–IGF axis in twin animals has shown that expression of all the genes is lower in twins compared with singleton animals, but only in animals that have been previously nutrient restricted (Brennan *et al.* 2005). This finding suggests that there is a combined effect of being a twin and being nutrient restricted. Similar results have been found in studies of the hypothalamic–pituitary–adrenal axis of twin sheep fetuses (Edwards & McMillen, 2002). There is also some evidence that growth-restricted twin sheep have a reduction in nephron number compared with singletons (Mitchell *et al.* 2003). Given that the GH–IGF axis is one of the key regulators of the nephron complement it is quite possible that changes in expression of the axis are involved. A possible explanation for why twin fetuses may respond differently from the effects of NR is that they experience a different maternal endocrine environment from that of singleton fetuses (Budge *et al.* 2003) and maturation of the fetal hypothalamic–pituitary–adrenal axis is delayed (Gardner *et al.* 2004*a*).

## Summary

This model of NR has no effect on kidney size at the time of sampling (110 d of gestation). This finding does not preclude the possibility that kidney growth might be affected later in gestation, which indeed appears to be the case (Whorwood *et al.* 2001). NR also appears to have a time-dependent stimulatory effect on the IGF-I receptor in the kidneys of singleton fetuses, an effect that is not seen in twin animals. As a consequence twins may be protected from some of the adverse effects of exposure to a reduction in maternal food intake, which may explain why blood pressure control can be similar between singleton and twin offspring in later life despite *in utero* competition (Gardner *et al.* 2004*b*; Gopalakrishnan *et al.* 2005).

## Prostaglandins

The kidneys are major sites of both PG synthesis and catabolism (Gleason, 1987); the major components of the PG axis are illustrated in Fig. 1(b). The first step in the pathway is the conversion of arachidonic acid to $PGH_2$. This reaction is catalysed by the cyclooxygenase (COX) enzyme, of which there are two isoforms, 1 and 2. The subsequent step forms the different types of PG by the actions of several specific synthase enzymes (Campean *et al.* 2003). The main PG in the kidney is $PGE_2$ (Gleason, 1987), which has four different receptors (E-prostanoid $(EP)_{1-4}$) that elicit different responses when activated (Audoly *et al.* 1999). All PG are degraded by the same enzyme, PG dehydrogenase, which catalyses the oxidation of the 15-hydroxyl group making them inactive (Nasjletti *et al.* 1984; Johnson *et al.* 2004).

## Rationale for the investigation

PG have an established role in regulating kidney function through modulations of vascular tone, water and ion absorption and also renin release (Breyer & Breyer, 2000; Jensen *et al.* 2001). Inhibiting PG synthesis affects fetal renal function, altering renal blood flow and increasing urine osmolarity (Matson *et al.* 1981). Recent studies suggest that PG has a direct role in regulating kidney development. Because, COX-2 has a very specific pattern of expression during organ development (Vio *et al.* 1999; Ogawa *et al.* 2001; Madsen *et al.* 2004), disruption of which, either by the use of COX inhibitors (Komhoff *et al.* 2000) or by gene knock-out (Dinchuk *et al.* 1995), reduces the total number of nephrons formed and those formed can be abnormal.

In contrast to the GH–IGF axis the effects of NR on PG have not been well investigated. One study (Williams *et al.* 2002) has shown that a model of placental restriction throughout pregnancy in sheep increases the expression of both COX-2 and $EP_2$ mRNA in the offspring's kidney. It has, however, been suggested that these effects are a result of fetal hypoxia rather than nutrient deficiency. Feeding a low-protein diet to rats through pregnancy decreases PG dehydrogenase activity and increases $PGE_2$ excretion in the offspring (Sherman *et al.* 1999). The effects of a maternal global energy restriction on PG have yet to be investigated.

## Animal model utilised

A rat model was used to study the PG axis because: (1) rats are litter bearing, which means it is possible to determine effects on PG expression at a range of time points in pups from the same pregnancy; (2) rats have a shorter lifespan, which means that the outcome in relation to the adult kidney can be established over a relatively short experimental time period.

The study design is summarised in Fig. 2(b). From conception to birth the controls were allowed *ad libitum* access to food, while the NR group was fed 50% of the amount eaten by the control group. After birth and for the duration of the experiment all animals were allowed *ad libitum* access to food. Animals from each litter were killed shortly after birth, then further animals were killed at postnatal days 7, 14, 21, 28 and at 12 weeks of age.
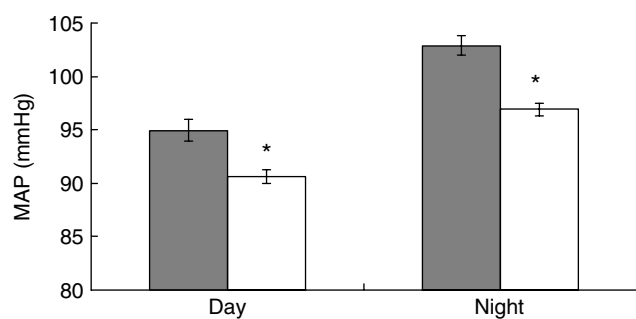
**Fig. 3.** The effect of maternal nutrient restriction (NR) on mean arterial pressure (MAP) in adult male rats. From conception to birth the controls (■) were allowed *ad libitum* access to food, while the NR group (□) was fed 50% of the amount eaten by the control group. After birth and for the duration of the experiment all animals were allowed *ad libitum* access to food (for details of the model design, see Fig. 2(b)). Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those for the controls: *$P<0.05$.

## Nutritional effects

Kidney weight in the NR group was found to be significantly decreased at birth and at postnatal days 7 and 14, whereas total body weight is only reduced at birth (KA Brennan, DM Olson and ME Symonds, unpublished results). Surprisingly, NR was found to have no effect on any other major organ size, confirming that the kidneys are particularly sensitive to NR.

Using the adult males to measure the effect of NR on offspring blood pressure it was found that mean arterial pressure in the NR group is significantly lower than that of the controls ($P<0.05$; Fig. 3). These findings differ in some aspects from those reported previously following global nutrient or protein restriction (see Table 1), with the most likely explanation being the method used to measure blood pressure, i.e. telemetry *v.* tail cuff.

The present model of NR affects the expression of the COX-2 enzyme, as summarised in Fig. 4. The expression pattern for controls follows that previously reported (Vio *et al.* 1999; Ogawa *et al.* 2001; Madsen *et al.* 2004) but is altered in NR offspring for which expression is significantly ($P<0.05$) reduced at postnatal days 14 and 28 compared with the controls. Given the importance of the COX-2 expression pattern for renal development, these changes could be highly disruptive to later kidney function. In contrast, COX-2 expression is significantly ($P<0.05$) raised in the NR adults compared with the controls. COX expression and its relationship with blood pressure have been widely investigated and meta-analyses of these results have found that COX inhibitors elevate blood pressure (Pope *et al.* 1993; Johnson *et al.* 1994). This finding has not been supported by more recent studies (Catella-Lawson *et al.* 1999; Dilger *et al.* 2002; Gertz *et al.* 2002), and in the study of Pope *et al.* (1993) raised blood pressure was found only in those animals that were hypertensive before the administration of the inhibitors. The increase in COX-2 expression in the animals from the present rat model, coupled with their reduced blood pressure, would support a role for COX in blood pressure
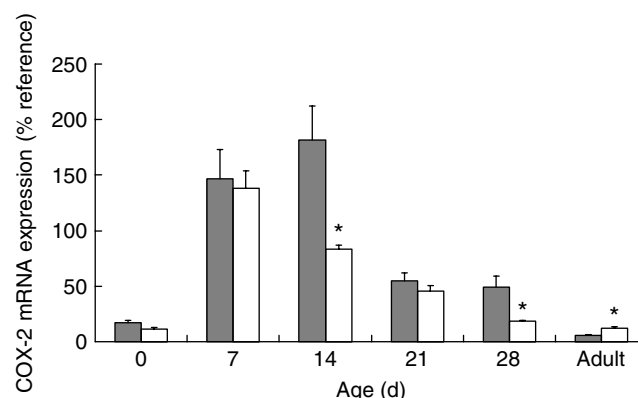


**Fig. 4.** The effect of maternal nutrient restriction (NR) on rat kidney cyclooxygenase (COX)-2 mRNA expression. From conception to birth the controls (■) were allowed *ad libitum* access to food, while the NR group (□) was fed 50% of the amount eaten by the control group. After birth and for the duration of the experiment all animals were allowed *ad libitum* access to food (for details of the model design, see Fig. 2(b)). Data were calculated as a percentage of a single reference sample. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those for the controls: *$P<0.05$.

regulation. In addition, the $PGE_2$ content of adult kidneys was found to be reduced in NR offspring (KA Brennan, DM Olson and ME Symonds, unpublished results), suggesting that either the increase in COX expression does not increase PG synthesis or that the rate of degradation is also enhanced. This finding is surprising because previous studies have found that $PGE_1$ administration leads to an increase in blood pressure (Kailasam *et al.* 1994; Ishibe *et al.* 1998).

Effects on the expression of the EP receptors were also found, as summarised in Table 2. With the exception of $EP_1$ expression patterns of all these receptors are affected by NR. Interestingly, there are no differences in expression at days 0 and 7, immediately following the insult and during the time when the kidneys were growth retarded, but the differences persist into adulthood. The $EP_2$ receptor acts to stimulate water and salt re-absorption in the kidney, so reduced expression would lower re-absorption, thereby decreasing blood pressure. Indeed, $EP_2$ receptor knock-out mice have reduced blood pressure (Tilley *et al.* 1999).

**Table 2.** Kidney E-prostanoid (EP) receptor expression in rat pups whose mothers had been nutrient restricted during pregnancy†

| Postnatal day … | 0 | 7 | 14 | 21 | 28 | Adult |
|---|---|---|---|---|---|---|
| $EP_1$ | | | | | | |
| $EP_2$ | | | ↑* | | | ↓* |
| $EP_3$ | | | | | ↓* | ↑* |
| $EP_4$ | | | ↑* | | ↓* | ↓* |

↑, Increase; ↓, decrease.
Receptor expression was significantly different from that of the controls: *$P<0.05$.
†From conception to birth the controls were allowed *ad libitum* access to food, while the NR group was fed 50% of the amount eaten by the control group. After birth and for the duration of the experiment all animals were allowed *ad libitum* access to food (for details of the model design, see Fig. 2(b)).

Conversely, activation of the $EP_3$ receptor decreases salt and water re-absorption so increased expression of this receptor would have the same outcome. Finally, reduced expression of the $EP_4$ receptor could reduce renin levels (Schweda *et al.* 2004) and therefore reduce angiotensin II and blood pressure (Lemmer *et al.* 2000).

## Summary

The present model of NR clearly affects kidney growth, an effect that is not seen in the other organs examined. There are also changes in the PG axis of these animals that persist into adulthood. Whilst the decrease in $PGE_2$ content might suggest an increase in systemic blood pressure, the increased COX-2 expression and changes in EP expression could explain the reduction in blood pressure seen in this model.

## Possible mechanism by which maternal nutrient restriction programmes kidney development

Having established that NR can affect both the GH–IGF axis and PG in the kidney it was important to determine how these changes were mediated. Glucocorticoids are a candidate mechanism because other studies have previously found that they are affected by NR. A low-protein diet in rats has been found to increase renal expression of the glucocorticoid receptor (Bertram *et al.* 2001) and a similar result has been found following global NR in sheep (Whorwood *et al.* 2001). Cortisol treatment during pregnancy has also been linked with increased blood pressure in the adult offspring (Dodic *et al.* 2002). Recent studies of rats with raised blood pressure following dexamethasone treatment have found a similar rise in blood pressure in animals pair fed to the dexamethasone group (Woods & Weeks, 2005). This finding suggests that programming by glucocorticoids and maternal nutrition may share a common mechanism. To this extent NR during late gestation in rats causes the fetus to be overexposed to maternal glucocorticoids (Lesage *et al.* 2001) and glucocorticoids have been shown to be essential in maintaining hypertension programmed by a maternal low-protein diet (Gardner *et al.* 1997).

The GH–IGF axis can be regulated by glucocorticoids through changes in gene transcription and receptor abundance (Hochberg, 2002), as can PG expression (Goppelt-Struebe, 1997). Adrenalectomy increases both COX-2 expression and activity in the renal cortex, an effect that is reversed with replacement corticosterone (Zhang *et al.* 1999). In rats the postnatal decrease then subsequent increase in circulating corticosterone is responsible for the developmental expression pattern of renal COX-2 (Vio *et al.* 1999; Madsen *et al.* 2004). Infusion of cortisol into fetal sheep during nephrogenesis, however, has no effect on the expression of COX-2, $EP_2$ or $EP_4$ in the kidney (Williams *et al.* 2004).

Glucocorticoids were found to be altered in both models of NR described earlier. Twin fetuses from the sheep model were found to have significantly ($P<0.05$) lower expression of mRNA for the glucocorticoid receptor
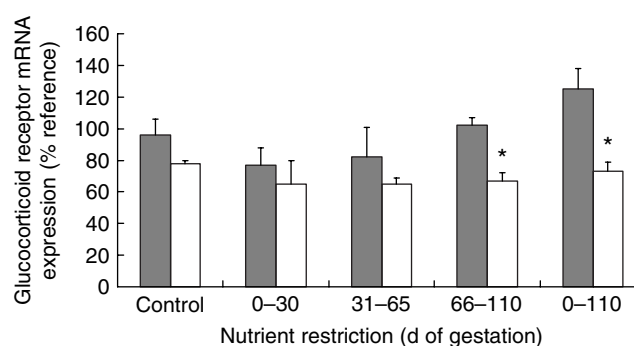
**Fig. 5.** The effect of maternal nutrient restriction (50% metabolisable energy requirements) and no. of fetuses (singletons (■) and twins (□)) on sheep kidney glucocorticoid receptor mRNA expression (for details of the model design, see Fig. 2(a)). Data were calculated as a percentage of a single reference sample. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those for the single fetus: *$P<0.05$.

following NR from 66 to 110 d of gestation and from 0 to 110 d of gestation (Fig. 5). This finding might explain the differences in GH–IGF expression seen in these two groups, but it does not explain the variation in the other groups, or why the IGF-IR receptor is affected in singletons and is altered only in the groups that were subjected to NR on days 0–65 d of gestation and not in the group nutrient restricted from 0 to 110 d of gestation. It seems unlikely, therefore, that glucocorticoids are the only mechanism involved. In the rat model glucocorticoid receptor expression was found to be unaffected by NR (KA Brennan, DM Olson and ME Symonds, unpublished results), but offspring were found to exhibit raised plasma corticosterone at postnatal days 14 and 28 and in adulthood (Fig. 6). This increased corticosterone could explain the reduced expression of COX-2 at days 14 and 28 because corticosterone inhibits cortical COX-2 (Zhang *et al.* 1999).
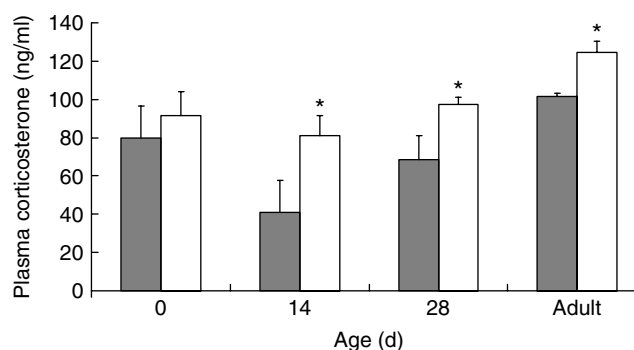
**Fig. 6.** The effect of maternal nutrient restriction (NR) on rat plasma corticosterone. From conception to birth the controls (■) were allowed *ad libitum* access to food, while the NR group (□) was fed 50% of the amount eaten by the control group. After birth and for the duration of the experiment all animals were allowed *ad libitum* access to food (for details of the model design, see Fig. 2(b)). Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those for the controls: *$P<0.05$.

The raised corticosterone in adult animals could also explain the increase in COX-2 in these animals because corticosterone can stimulate medullary COX-2 (Zhang *et al.* 2002). Studies have found that medullary COX-2 is very low in weanling rats but is greatly increased in adults (Yao *et al.* 2005). The time points measured in the present model suggest that this difference in the location of COX-2 expression occurs after day 28 but before 12 weeks of age.

Whilst glucocorticoids can regulate both the GH–IGF axis and PG in the kidney and these changes might be involved in the altered development and function following NR, it is possible for glucocorticoids to affect kidney function directly, for example, by regulating levels of ion pumps in nephrons (Devarajan & Benz, 2000). Glucocorticoid treatment has also been found to affect other systems, including the renin–angiotensin system (Dodic *et al.* 2002), that are also likely to be involved.

In conclusion, glucocorticoids are affected by NR in both the sheep and rat models. These changes could explain some of the differences seen in the GH–IGF and PG axes thereby providing a potential mechanism for their programming.

## Acknowledgements

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