Growth restriction before or after birth reduces nephron number and increases blood pressure in male rats

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Impaired growth *in utero* predicts a low nephron number and high blood pressure later in life as does slowed or accelerated growth after a normal birth weight. We measured the effects of early postnatal growth restriction, with or without prenatal growth restriction, on blood pressure and nephron number in male rat offspring. Bilateral uterine artery and vein ligation were performed to induce uteroplacental insufficiency (Restricted) on day 18 of pregnancy. Postnatal growth restriction was induced in a subset of sham operated control animals by reducing the number of pups at birth to that of the Restricted group (Reduced Litter). Compared to Controls, Restricted pups were born smaller while Reduced Litter pups weighed less by postnatal day 3 and both groups remained lighter throughout lactation. By 10 weeks of age all animals were of similar weight but the Reduced Litter rats had elevated blood pressure. At 22 weeks, Restricted but not Reduced Litter offspring were smaller and the blood pressure was increased in both groups. Restricted and Reduced Litter groups had fewer glomeruli and greater left ventricular mass than Controls. These results suggest that restriction of both perinatal and early postnatal growth increase blood pressure in male offspring. This study also demonstrates that the early postnatal period is a critical time for nephron endowment in the rat.

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Poor intrauterine growth results in small for gestational age babies and low birth weight predicts an increased risk of developing many adult onset diseases, including hypertension.[1] It is thought that the fetus, upon exposure to a suboptimal intrauterine environment, makes adaptations, which increase short-term survival. These adaptations impact on normal growth and functional development increasing predisposition to later disease.[2] Growth in the early postnatal period has also been implicated to independently affect later health and result in disease development.[3,4] Accelerated growth in early infancy has been shown to be associated with the later development of coronary artery disease[5] and type II diabetes,[6] although other studies suggest that slowed postnatal growth, especially during the first year, can lead to increased risk of coronary heart disease[7] and insulin resistance.[8] Together, these studies suggest that factors that affect prenatal and/or postnatal growth, including nutrition, are important in determining later cardiovascular and metabolic health.

In animal studies, maternal dietary manipulations (low protein or calorie restriction during pregnancy) restrict fetal growth and produce offspring that have a reduced nephron number[9] and develop hypertension as adults.[10,11] Of particular concern is that a congenital nephron deficit has been implicated as a mechanism through which a prenatal perturbation may result in later hypertension.[12] However, previous studies have not assessed the effects of an impaired postnatal lactational environment, a period when nephrogenesis continues in the rodent. Of interest is that a pregnancy-induced lactation deficit may persist even when the mother is returned to a normal diet at birth. Although it is evident that offspring undergo variable postnatal growth following maternal nutritional modulation, the role of this in the development of later hypertension has not been studied extensively.

In Western society, much of the fetal growth restriction that occurs reflects placental insufficiency and impaired uteroplacental blood flow. Uteroplacental insufficiency and growth restriction, either by bilateral uterine artery and vein ligation[13,14] or aortic clip,[15–18] have been experimentally

induced in several species, including the rat. We have utilized the bilateral uterine vessel ligation model to examine fetal growth and development.[14,18,19] Although this model has previously been thought to affect intrauterine growth only, we have demonstrated that uteroplacental insufficiency impairs mammary development and the lactational environment and thus reduces postnatal nutrition and growth of the offspring.[19] Experimentally induced uteroplacental insufficiency also reduces the number of pups at birth.[14,18] To control for this reduction in litter size on postnatal growth, we have incorporated a second control group in our studies in which the number of pups from a control litter is reduced on day 1 to match the number of offspring in the growth-restricted (Restricted) group. We previously demonstrated that this results in postnatal growth restriction in pups born of normal weight.[19] In some studies, a 60–70% reduction in litter size soon after birth increases milk delivery to individual offspring, resulting in increased early postnatal growth and the development of adult obesity.[20–22] In comparison, we have previously used a more modest reduction in the number of pups (from approximately 10 to 5) to match that resulting from uterine artery ligation and have found that this leads to impaired nutrition postnatally.[20–22] This may be due to the reduced number of pups decreasing the suckling stimulus to the dam, which in turn decreases milk production and pup growth.[19,23] Thus, we have used this litter size reduction to induce postnatal growth restriction alone.

To date, there has been little investigation into the effect of early postnatal growth restriction as an independent factor contributing to adult disease development, including hypertension. One study in which additional pups were cross-fostered onto a dam at birth to increase litter size and thereby to decrease nutritional supply received by individual pups showed that offspring slow their growth after birth. Only males were examined in that study, and it was found that offspring had a reduced number of enlarged glomeruli in their kidneys.[24] However, the limitation of that study was that blood pressure was not measured. Therefore, the first aim of this study was to compare the effects of perinatal growth restriction (where offspring are exposed to restraint before and after birth) with postnatal growth restriction (due to reduced litter size) alone on blood pressure in male offspring. Furthermore, as nephrogenesis continues after birth in the rat and thus may be influenced by postnatal nutrition and growth, we also aimed to determine the effects of perinatal and postnatal growth on nephron endowment in offspring. Similar to other studies,[14,25] we chose to study male offspring to address these issues, as it is reported that males tend to be more susceptible to the development of hypertension[25] and that the mechanisms controlling blood pressure may be sex specific.[26]

To further elucidate the mechanisms behind the effect of growth restriction on blood pressure, we examined components of the renin–angiotensin system, which play a critical role in renal development and the maintenance of blood pressure and fluid homeostasis. We and others have shown that alterations in the renal and cardiac renin–angiotensin system occur following growth restriction and that these changes correlate with increased blood pressure and altered renal development in offspring.[14,27–29] Specifically, the expression of the angiotensin II type 1 (AT_1) and 2 (AT_2) receptors was examined in renal and cardiac tissue at 6 months of age. Gene expression studies were also performed in the adult to examine markers of tissue remodeling (including collagen, metalloproteinases (MMPs), and the tissue inhibitors of metalloproteinases (TIMPs)), as we expected fibrosis-related disease to be developing in these offspring. We hypothesized that offspring from both restricted and reduced litter groups would have a nephron deficit with altered renal and cardiac gene expression associated with increased blood pressure.

RESULTS

Body weights and growth profiles

Litter size on day 1 along with the body weights of male offspring at days 3, 6, and 35, at week 10, and at postmortem (6 months) are shown in Table 1. On days 3 and 6 after birth, reduced litter male pups weighed less than controls, whereas restricted pups were lighter than both control and reduced litter pups ($P<0.05$). Restricted male rats underwent a degree of accelerated growth between days 6 and 35, reaching weights comparable to those of the reduced litter group at day 35. However, both reduced litter and restricted groups were still lighter than control pups ($P<0.05$). The reduced litter male offspring underwent a period of accelerated growth after weaning so that by 10 weeks of age, their weights were comparable to control animals. Reduced litter offspring were of similar weight to controls at 6 months, whereas restricted offspring remained lighter ($P<0.05$).

Blood pressure

Perinatal or postnatal growth restriction did not alter systolic blood pressure at 5 weeks of age, although animals in the reduced litter group tended to have higher blood pressure (by ~5–8 mm Hg) compared to the control or restricted group (Figure 1a). At 9 weeks of age, the reduced litter offspring had increased blood pressure (by 14 mm Hg) compared to both control and restricted male offspring ($P<0.05$; Figure 1b). At 22 weeks, the restricted and reduced litter groups both had increased blood pressure (by 9 and 7 mm Hg, respectively) compared to controls ($P<0.05$; Figure 1c).

Kidney parameters

Absolute kidney weight in male offspring of the restricted and reduced litter groups was reduced ($P<0.05$; Figure 2a), but not when corrected for body weight (Figure 2b). Kidney volume (measured in five animals per group) was not significantly different between the groups but tended to be lower in the restricted and reduced litter animals (control, $0.88 \pm 0.09\ cm^3$; restricted, $0.74 \pm 0.07\ cm^3$; and reduced litter, $0.79 \pm 0.07\ cm^3$). A reduced glomerular number (by 36