The effect of prenatal diet and glucocorticoids on growth and systolic blood pressure in the rat

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Hypertension is a major cardiovascular risk factor for stroke and CHD in Westernized societies (Thom et al. 1992). Whilst many factors contribute towards elevated blood pressure, such as low physical activity, obesity, saturated fat, salt and alcohol intake (Ward, 1993), they only explain a proportion of cardiovascular risk. Since adult systolic blood pressure (SBP) tracks growth from infancy, i.e. SBP increases in proportion to the increase in blood pressure in the rat. The relation between birth weight and SBP in childhood. The relationship between growth leading to an asymmetric or a proportionately-small baby at term also influence future cardiovascular risk (Barker, 1994).

Cross-fostering and sibling studies have clearly shown that the maternal environment, rather than genetic factors, largely accounts for variations in birth weight (Walton & Hammond, 1938; M"uller & Gluckman, 1996). Intrauterine growth is primarily substrate driven (Karleberg et al. 1994) and, thus, the supply of substrate may influence the intrauterine growth process. Indeed, low birth weight in man is associated with a reduced maternal intake of protein coupled with a high intake of carbohydrate (Godfrey et al. 1996 and indices of poor maternal nutritional status, such as reduced skinfold thickness (Godfrey et al. 1994) and reduced maternal Fe stores (Godfrey et al. 1991).

SBP in childhood and adult life is inversely related to the same indices of poor maternal nutritional status, i.e. maternal anaemia (Law et al. 1991) and reduced maternal skinfold thickness (Godfrey et al. 1994). A low intake of protein coupled with a high intake of carbohydrate during gestation is associated with an elevated SBP in the offspring when measured 40 years later (Campbell et al. 1996). The propensity towards hypertension and thus towards CHD, therefore, is partially determined in utero by nutritional factors. The underlying physiological processes relating maternal nutrition, intrauterine growth patterns and adult hypertension are as yet unknown.

Research using animal models has provided some insight into the mechanism of intrauterine programming of adult hypertension. Whilst hypertension can be experimentally produced in animal models through reno-vascular manipulations such as Goldblatts or aortic coarctation (Wilkinson, 1994), or steroid-induced using deoxycorticosterone acetate or dexamethasone (Kenyon & Morton, 1994), these methodologies do not account for an early origin of adult hypertension. Steroid-induced hypertension associated with reduced birth weight can be reproduced, however, by either dexamethasone (Benediktsson et al. 1993; Levitt et al. 1996) or carbenoxolone (Lindsay et al. 1996) injections during pregnancy in the rat.

Maternal glucocorticoids (GC) are metabolized by the placental enzyme 11ß-hydroxysteroid dehydrogenase type 2 (EC 1.1.1.146; 11-HSD2; Seckl, 1997). Placental 11-HSD2 is inhibited by carbenoxolone and has weak activity towards dexamethasone. Thus, increased fetal exposure to excess maternal GC reduces birth weight and renders the resultant offspring hypertensive. Importantly, hypertension associated with carbenoxolone injections requires a product of the maternal adrenal gland, since carbenoxolone injections to adrenalectomized dams have no effect on birth weight or SBP of the resultant offspring (Lindsay et al. 1996). The activity of placental 11-HSD2 is positively correlated with birth weight in rats (Benediktsson et al. 1993) and man (Stewart et al. 1995), and activity at term predicts birth weight (Benediktsson et al. 1995). Thus, Edwards et al. (1993) contend that placental 11-HSD2 has an important role in determining birth weight and, through maternal GC influence, may mediate the fetal origins of hypertension in the rat.
adult cardiovascular disease. Nutritional factors are clearly important, however, in determining future cardiovascular risk (Barker & Osmond, 1986; Barker et al. 1993).

Nutritional programming of adult hypertension has been demonstrated in the rat. Reductions in either food intake (Woodall et al. 1996) or maternal Fe stores (Crowe et al. 1995) throughout pregnancy render the resultant adult offspring hypertensive. Similarly, a maternal low-protein isoenergetic (MLP) diet both reduces birth weight and elevates the SBP of the resultant offspring (Langley & Jackson, 1994; Isherwood-Peel et al. 1997). The greater SBP exhibited by the offspring of MLP dams is of early onset and apparently lifelong (for review, see Langley-Evans et al. 1997).

Pharmacological blockade of GC synthesis throughout the first 2 weeks of rat pregnancy prevents the development of MLP-induced hypertension (Langley-Evans, 1997a). In addition, MLP-induced hypertension is associated with a reduction in both the activity (Langley-Evans et al. 1996c; DS Gardner, unpublished results) and expression (CB Whorwood and SC Langley-Evans, unpublished results) of placental 11-HSD2 from mid to late gestation. Thus, exposure to excess maternal GC during gestation in the rat may represent a common factor between MLP-induced hypertension and steroid-induced hypertension (Seckl, 1997b). Indeed, injections of carbenoxolone to protein-replete rat dams reduces the birth weight and elevates the SBP of the resultant offspring as effectively as maternal protein restriction (Langley-Evans, 1997b).

Exposure to MLP diet during gestation leads to patterns of disproportionate fetal growth that appear to favour maintenance of brain growth (Langley-Evans et al. 1996a). Thus, whilst growth of the fetal brain over late gestation was maintained in proportion to body weight, the growth of the liver, lungs and trunk was retarded (Fig. 1).

In association with deficits in organ growth, the offspring from MLP dams exhibit long-term programming of the hypothalamic–pituitary–adrenal axis. Specific elevations in the activities of central and peripheral GC-inducible enzymes, despite similar circulating corticosterone concentrations, indicate a hypersensitivity to GC action in adult life (Langley-Evans et al. 1996b). Disproportionate fetal growth patterns and hypersensitivity to GC action may represent a manifestation of a common phenomenon. Thus, the fetuses from MLP dams enter the third week of rat pregnancy prevents the development of hypertension. If maternal GC concentrations are reduced during gestation, either by pharmacological (Langley-Evans, 1997a) or surgical adrenalectomy (Fig. 2), then MLP-induced hypertension is prevented. Furthermore, replacement of corticosterone to either pharmacologically (Langley-Evans, 1997a)- or surgically (DS Gardner, unpublished results)-adrenalectomized rat dams restores the hypertensive state of MLP offspring. Exposure to maternal GC, therefore, appears essential for programming of hypertension in MLP rats.

![Fig. 1. The percentage increase in mass and length between day 20 and full term in rat fetuses exposed to either a control (■) or low-protein (MLP; □) maternal diet. Values are expressed as a percentage of the fetal mass or length as determined at day 20 gestation. A total of twenty rat dams were fed on either 180 g casein/kg (control; n 10) or 90 g casein/kg (MLP; n 10) for 2 weeks before mating and throughout gestation. At day 20 gestation, five dams from each dietary group were killed and fetal body and organ masses recorded. The remaining pregnancies proceeded to term. Offspring were killed within 12 h of the dam giving birth and organ weights determined. (Redrawn from Langley-Evans et al. 1996a.)](https://www.cambridge.org/core/terms)
suggest programming at the central level (Langley-Evans et al. 1996b). Altered central metabolism of glutamate, indicated by increased glutamine synthetase (EC 6.3.1.2) activity in MLP (Langley-Evans et al. 1996b), may influence blood pressure control, since glutamate is excitatory in the nucleus tractus solitarius (the cardiovascular control centre in the medulla; Talman et al. 1984). Interestingly, transplantation of hypothalamic tissue grafts from day 16 spontaneously-hypertensive rat embryos to normotensive adult Wistar-Kyoto rats significantly elevates the SBP of the Wistar-Kyoto rats (Elam et al. 1991), indicating that hypothalamic factors underlie hypertension in spontaneously-hypertensive rats. Later menarche in women is associated with reduced birth weight (Cooper et al. 1996), and growth hormone secretion in adulthood is related to growth in infancy (C Fall, P Hindmarsh, E Dennison, S Kellingray, D Barker and C Cooper, unpublished results). Both are suggestive of centrally-oriented programming in early life.

In the periphery, redistribution of blood flow causing shifts in haemodynamic loads are known permanently to alter the structural properties of vascular smooth muscle (Berry 1978). In fetal vascular tissues, elevated GC receptor densities may facilitate increased SBP and the redistribution of blood flow in MLP, since GC have many hypertensionogenic actions in the vasculature (Walker & Williams, 1992; Whitworth et al. 1995). In postnatal life an increased sensitivity to GC action in MLP may predispose towards elevated SBP. Adrenalectomy of 6-week-old male rats from MLP dams significantly reduced SBP to yield pressures similar to controls (Gardner et al. 1997). No effect of adrenalectomy on SBP of control rats were observed. Furthermore, the hypotensive effect of adrenalectomy in MLP rats was prevented by corticosterone replacement (Table 1). Thus, maintenance of MLP-induced hypertension in adult life is dependent on an intact adrenal gland and, in particular, corticosterone.

However, maintenance of hypertension in MLP rats does not appear to be entirely through GC receptors, since blockade of GC receptors using RU486, an antiGC, has no

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**Fig. 2.** Systolic blood pressures (SBP) of 6-week-old offspring from maternally adrenalectomized (MADX) rats fed on either a control or low-protein (MLP) diet during pregnancy. Twelve rats were bilaterally adrenalectomized 2 months before mating. On conception rats received either a control (180 g casein/kg; n 6) or MLP (90 g casein/kg; n 6) diet throughout pregnancy. At birth the diet was substituted for standard chow and litters culled to eight pups (four male and four female). Offspring were weaned onto chow at 4 weeks of age. SBP was determined at 6 weeks of age on male (□) and female (■) pups using the indirect tail-cuff method and compared with age-matched offspring from adrenal-intact control dams. Values are means with their standard errors represented by vertical bars. Two-way ANOVA indicated a significant effect of diet (F = 11.99, P = 0.002) and sex (F = 16.21, P = 0.001) on the SBP of the offspring. a, b, c, Values with unlike superscript letters were significantly different (post hoc analyses by Student's t-test; P < 0.01).

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**Table 1.** The effect of postnatal adrenalectomy on the systolic blood pressure of animals exposed to either a maternal control or low-protein (MLP) diet* (From Gardner et al. 1997)

<table>
<thead>
<tr>
<th>Dietary group†‡</th>
<th>Control</th>
<th>MLP</th>
<th>Control</th>
<th>MLP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>7 d</td>
<td>14 d</td>
<td>Initial</td>
</tr>
<tr>
<td>Treatment group</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
</tr>
<tr>
<td>SV</td>
<td>147 4</td>
<td>149 5</td>
<td>137 7</td>
<td>161 1</td>
</tr>
<tr>
<td>AV</td>
<td>142 5</td>
<td>149 2</td>
<td>137 10</td>
<td>162 1</td>
</tr>
<tr>
<td>SC</td>
<td>146 8</td>
<td>166†</td>
<td>146 7</td>
<td>175 5</td>
</tr>
<tr>
<td>AC</td>
<td>143 7</td>
<td>174†</td>
<td>168†</td>
<td>165 6</td>
</tr>
</tbody>
</table>

* The effect of diet was significant (ANOVA; F = 29.85, P < 0.0001).
† The effect of corticosterone replacement was significant (ANOVA; F = 50.67, P < 0.0001).
‡ The interaction between diet and corticosterone replacement was significant (ANOVA; F = 3.47, P < 0.06).
‡‡ Mean value was significantly different from the initial value for MLP-AV rats (Bonferroni/Dunn test; P < 0.05).
* A total of twenty-four male offspring from control (180 g casein/kg) or MLP (90 g casein/kg, n 20) dams were either bilaterally adrenalectomized (A) or sham-operated (S) under pentobarbital anaesthesia at 47 ± 1 d of age. Corticosterone (C) replacement (20 mg/kg in 0.1 ml arachis oil) or vehicle replacement (V; 0.1 ml arachis oil) began the following day (subcutaneously twice daily for 14 d). The systolic blood pressure of rats was determined by the indirect tail-cuff method before surgery (initial) and subsequently at days 7 and 14 following either adrenalectomy or sham operation (day 0).
The dose of angiotensin peripherally programmed factors and a synergistic interplay between pressor response was established to be significantly lower in MLP rats than controls (MLP 37 n; controls, 44 ng angiotensin II, n = 5; P = 0.01).

In adult life, MLP-induced hypertension is, therefore, a consequence of a steeper rise in SBP due to centrally-programmed factors and a synergistic interplay between peripheral hypothalamic–pituitary–adrenal activity, the renin–angiotensin system and the kidney. With regard to the kidney, the MLP diet consistently impairs renal growth as a whole (Langley-Evans et al. 1996d) and more specifically, the formation of nephrons (SJM Welham, unpublished results). A reduced nephron complement at birth is associated with an increased susceptibility to hypertension (Mackenzie & Brenner, 1995). In the periphery, the combination of altered blood-flow profiles in utero, increased vascular sensitivity to pressors and increased pressor action, which perhaps are mediated by GC, may comprise the initial stimulus leading to primary hypertension. Postnatally, these exaggerated responses persist, facilitating structural cardiovascular adaptations that predispose to higher blood pressure and secondary hypertension (Folkow, 1978). Structural adaptation within baroreceptor sites may reset the ‘barostat’ function of baroreceptors at a higher level, further contributing to the hypersensitive state (Sleight et al. 1975).

In conclusion, increased maternal glucocorticoid exposure, therefore, may primarily facilitate fetal peripheral adaptation to an MLP diet. A consequence of the increased GC exposure is programming or imprinting of the immature fetal hypothalamus. Fetal physiology is thus programmed in utero towards an increased propensity for SBP to rise. Postnatally, summation of the central and peripheral programmed alterations result in a greater rate of increase of SBP in early life, leading towards hypertension in later life. If the mechanisms, e.g. angiotensin II, facilitating secondary structural adaptation in postnatal life are prevented, then hypertension does not develop (Sherman et al. 1997). Furthermore, the early rise in SBP is exacerbated by conditions of nutritional excess. The effects of early exposure to a low-protein diet on SBP followed by exposure to a cafeteria diet in later life are cumulative (Petry et al. 1997).

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References


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