Hyperinsulinaemia and elevated levels of phosphorylated Akt/PKB in the neonatal Hypertrophic Heart Rat (HHR) precede the onset of cardiac hypertrophy

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There is experimental and epidemiological evidence supporting the 'foetal origins of adult disease' hypothesis. It is well established that low birth weight is associated with increased cardiovascular risk and diabetes in adulthood. However, relatively little is known about the molecular and cellular mechanisms controlling neonatal programming of adult cardiovascular disease. Cardiac hypertrophy is a major cardiovascular risk factor and is often seen in association with diabetes. The developmental origins of this condition have not been extensively studied. The Hypertrophic Heart Rat (HHR) is a normotensive model of cardiac and cardiomyocyte hypertrophy (Harrap *et al.*, 2002). The aim of this study was to determine whether the growth-regulating protein kinase PKB/Akt is implicated in the neonatal 'programming' of the HHR adult cardiac phenotype.

HHR and control strain Normal Heart Rat (NHR) neonates were killed by decapitation at post-natal day 2. Male NHR and HHR were also culled at 4, 6, 8 and 12 weeks of age (n = 8-11 per group) by overdose of halothane. Hearts were immediately excised post-mortem and cardiac mass was normalized to body mass to give a cardiac weight index (CWI, mg/g). Trunk blood was collected from fasted (30 min for neonates and overnight for 12 week old) NHR and HHR for analysis of blood glucose (glucometer, Accu-Check®) and plasma insulin (radioimmunoassay, Linco Research) levels. At post-natal day 2, HHR hearts were significantly smaller than NHR (4.33 ± 0.19 *vs.* 5.01 ± 0.08 mg/g, p = 0.024). HHR hearts were similar to NHR by 4 weeks (5.10 ± 0.15 *vs.* 5.16 ± 0.11 mg/g) and were 11% larger than NHR by 8 weeks of age. This was followed by a rapid period of growth, and by 12 weeks of age HHR CWIs were 27% larger than NHR (p = 0.003). Blood glucose and plasma insulin levels were both significantly higher in HHR neonates (glucose: 3.51 ± 0.16 *vs.* 5.00 ± 0.12 mM; insulin: 0.68 ± 0.06 *vs.* 2.07 ± 0.27 ng/ml, $p < 0.001$). At 12 weeks of age, HHR were still hyperglycaemic (5.97 ± 0.35 *vs.* 8.08 ± 0.43 mM, $p < 0.01$), but plasma insulin levels were not different (2.69 ± 0.91 *vs.* 2.34 ± 0.62 ng/ml).

Phosphorylated (Ser473) and total Akt levels were determined in NHR and HHR neonatal and 12 week old ventricular lysates by western blot. The ratio of phosphorylated to total Akt was 2.3-fold higher in the HHR heart at post-natal day 2 (0.99 ± 0.07 *vs.* 2.31 ± 0.53, p = 0.029), but was not significantly different at 12 weeks of age (1.00 ± 0.05 *vs.* 1.14 ± 0.09, p = 0.18). As Akt suppresses cardiomyocyte apoptosis, we sought to quantify the incidence of apoptosis in the HHR neonatal heart. Cryosections from the mid-ventricle of post-natal day 2 rats were stained with TUNEL to label apoptotic nuclei. Sections were counter-stained with DAPI to label total nuclei. The number of TUNEL positive cells in the myocardium was normalized to the total area of DAPI stained nuclei to obtain an apoptotic index. The apoptotic index was suppressed almost 3-fold in the neonatal HHR heart (0.002 ± 0.0005% *vs.* 0.0007 ± 0.0002%, p = 0.02).

These findings demonstrate that cardiac growth restriction and hyperinsulinaemia in the neonate precede the onset of cardiac hypertrophy in the HHR. The hyperactivation of Akt signalling and suppression of myocardial apoptosis in the neonatal HHR heart may implicate these molecular and cellular mechanisms in the neonatal 'programming' of adult cardiovascular disease.

Harrap SB, Danes VR, Ellis JA, Griffiths CD, Jones EF & Delbridge LM. (2002) *Physiological Genomics*, **9:** 43-8.