Altered cardiac leptin signalling in offspring born small for gestational age

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Introduction: Cardiovascular disease is the leading cause of death worldwide and affects one in six Australians. In humans, uteroplacental insufficiency resulting in intrauterine growth restriction has been associated with development of cardiovascular disease, coronary heart disease and increased blood pressure (Barker *et al.*, 1993). Our group have previously identified that being born small for gestational age due to uteroplacental insufficiency is associated with a reduction in cardiomyocyte number in male Wistar Kyoto (WKY) rats (Black *et al.*, 2012) and this is accompanied with an increase in blood pressure and left ventricular hypertrophy at 5 months of age (Wlodek *et al.*, 2007). The protein hormone leptin provides an important target due to the potential effects it has in the heart on cell growth, differentiation and proliferation. In addition, rats undergo a leptin surge during the first two weeks of development (Ahima *et al.*, 1998) which is important in postantal organ development (Attig *et al.*, 2011). Given these findings, investigation into the potential changes in cardiac signalling is needed in the growth restricted offspring. The aim of this research was to characterise plasma leptin concentrations in growth restricted offspring and determine the levels of gene expression for leptin signalling targets in the heart .

Methods: Female WKY rats were mated and at day 18 of pregnancy (E18: term is E22), females were randomly allocated to undergo sham surgery or bilateral uterine vessel ligation to induce uteroplacental insufficiency, animals were anaesthetised with a tail vein injection of mixed solution containing ketamine (50 mg/kg) and ilium Xylazil-20 (10 mg/kg). Post mortem of offspring were conducted at embryonic day (E) 20, postnatal day (PN) 1, PN7 and PN35. Offspring plasma and hearts were collected. Plasma leptin concentration was quantified using a Leptin ELISA and gene abundance of JAK2, STAT3, SOCS3 and PI3K was characterised by Real Time PCR.

Results: Growth restriction was associated with a significantly decreased plasma leptin concentration in females at E20 and PN1, and males at PN1 and PN7 ($P < 0.05$). A plasma leptin peak occurred in both sexes at PN7. Gene expression in the heart was significantly altered in leptin signalling targets in restricted animals. Specifically, there was a significant difference between the gene profiles between the males and females ($P < 0.05$). In males, JAK2 and STAT3 were reduced in the restricted. In females, there was a significant difference between control and restricted offspring for STAT3.

Conclusion: We have demonstrated that a reduction in plasma leptin concentrations in growth restricted offspring is associated with altered gene expression of leptin signalling targets in the heart during developmental stages, and these differences are sex specific. These findings may provide biochemical pathways for the observed alterations in cardiac physiology in growth restricted offspring and a potential role for the plasma leptin surge on cardiac development.

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