The effects of intra-uterine growth restriction on metabolic organ growth, cardiomyocyte Ca^{2+}-handling properties, and contractile function in juvenile rats

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Intra-uterine growth restriction (IUGR) describes the inadequate supply of oxygen and/or nutrients during fetal development, and is defined as a birth weight below the 10th percentile (Barker & Osmond, 1986). Correlative evidence suggests that these small for gestational age offspring have an increased risk of developing cardiovascular disease (CVD) in adulthood. Calcium ions (Ca^{2+}) are an integral messenger for several of the steps that lead from initial stimulation, to contraction of cardiac muscle. Any changes in Ca^{2+} storage and release from the cardiac cells sarcoplasmic reticulum (SR), or sensitivity of the cardiac cells contractile apparatus may identify why IUGR increases the risk of CVD. This study aimed to explore the possible effects of IUGR on both the growth patterns of the heart and other primary organs (namely, liver, pancreas and kidney) and on cardiac function; both calcium-handling by the SR and force development by the contractile apparatus.

All procedures were approved by the La Trobe University animal ethics committee. Long Evans Hooded rats (7-10 wk old) born to both control and nutrient restricted (60% restricted diet for final trimester of gestation) rat dams were euthanised *via* isoflurane inhalation (4% volume: volume) and used to evaluate the effects of IUGR on the above mentioned parameters. SR Ca^{2+} content and contractile apparatus sensitivity was determined in chemically-skinned cardiomyocyte bundles (150-300 μm diameter) attached to a sensitive force transducer, and force responses were recorded on a Powerlab system (ADinstruments). When examining the SR Ca^{2+} content, intact bundles were treated with saponin (50 $\mu g \cdot ml^{-1}$ in a K-HDTA solution; containing mM: 90 HEPES, 8.6 total Mg^{2+}, 0.125 EGTA, 50 HDTA, 1 NaN_3, 8 ATP, 10 creatine phosphate; pH 7.1) to permabilize the surface membrane. Bundles were then washed in a K-HDTA solution (no saponin and containing 125 μM EGTA) and then the SR was fully emptied of Ca^{2+} using an equivalent K-HDTA solution containing 0.5 mM EGTA and 30 mM caffeine. The SR of permeablized bundles was then reloaded with Ca^{2+} for periods between 5-40 min in an equivalent K-HDTA solution (containing extra Ca^{2+} - pCa 6.0). Between each loading time the SR was depleted of Ca^{2+} and the area under the curve of the caffeine force response re-determined. This area has been shown to be a good indicator of the total SR Ca^{2+} content (Fryer & Stephenson, 1996). To examine the contractile apparatus specifically, the same bundles were then further skinned in a Ca^{2+}-free EGTA solution (containing mM: 90 HEPES, 9 total Mg^{2+}, 50 EGTA, 1 NaN_3, 8 ATP, 10 creatine phosphate; Triton X-100 (2%v/v) at pH 7.1) to permeablize all membranes. The Ca^{2+}-sensitivity of the contractile apparatus was determined by examining force responses to a series of Ca^{2+}-EGTA solutions progressively increasing free [Ca^{2+}] (between 0.1 μM and 20 μM) (equivalent in composition to the Ca^{2+}-free EGTA solution above but with added Ca^{2+}). Sub-maximal force responses were normalized to the maximum Ca^{2+}-activated force and fitted with a modified Hill equation and the pCa50 (force at 50% maximum force) and the Hill coefficient determined. The maximum force per cross sectional area was also determined.

While male heart weights were decreased and kidney weights increased (proportional to body weight) in IUGR rats (Control: n=16, IUGR: n=10; $P<0.05$), female organ weights displayed no significant change (C: n=14, IUGR: n=9; $p>0.05$). Conversely, males displayed no change in SR Ca^{2+}-contents at any loading time ($P>0.05$) between groups, while IUGR females showed significantly greater Ca^{2+}-contents across most loading times compared with controls ($P<0.05$). While maximum Ca^{2+}-activated force was unchanged in both males and females, there was a tendency for larger force output in IUGR males compared with controls ($P=0.053$). In juvenile IUGR males, the possible increased force per cross sectional area suggests these hearts are displaying early stage hypertrophy and that older rats may display more significant increases. In juvenile IUGR females, the increase in SR Ca^{2+} content suggests a different response to IUGR that may contribute to altered Ca^{2+} handling in the adult. These findings show novel, different responses to IUGR on cardiac muscle development between males and females which may have important implications in the development of CVD later in life.

Barker DJ, Osmond C. (1986) Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* **1:** 1077-1081.

Fryer MW, Stephenson DG. (1996) Total and sarcoplasmic reticulum calcium contents of skinned fibres from rat skeletal muscle. *Journal of Physiology* **493:** 357-370.