Maternal nutrient restriction alters Ca^{2+} handling properties and contractile function of isolated left ventricle bundles in male but not female rats

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Intrauterine growth restriction (IUGR), defined as a birth weight below the 10th centile, may be caused by maternal undernutrition, with evidence that IUGR offspring have an increased risk of cardiovascular disease (Rich-Edwards *et al.*, 1997). Ca^{2+} is an integral messenger for several steps associated with excitation-contraction coupling. Any changes in Ca^{2+} storage and release from the sarcoplasmic reticulum (SR), or sensitivity of the contractile apparatus to Ca^{2+} may underlie the mechanism linking IUGR to an increased risk of CVD. This study aimed to explore the effects of maternal nutrient restriction on cardiac function, including Ca^{2+} handling by the SR and force development by the contractile apparatus, in conjunction with quantitative biochemical analysis of key Ca^{2+} handling and contractile proteins.

Experiments were approved by the Animal Ethics Committee La Trobe University and comply with the NHMRC guidelines. 10–12 week old rats born to Control (C) and nutrient restricted (NR; 60% restricted diet for final trimester of gestation) dams were anaesthetized *via* isoflurane inhalation (4% v/v) for collection of the heart. Left ventricular myocytes bundles (150-300μm diameter) were attached to a force transducer, and force responses were recorded on a Powerlab system (ADinstruments). SR Ca^{2+} handling: Bundles were skinned with saponin (50 μg/ml in a solution; containing mM: 136 K^+; 26 Na^+, 90 HEPES, 8.6 total Mg^{2+}, 0.125 EGTA, 50 $HDTA^{2-}$, 8 ATP, 10 creatine phosphate; pH 7.1) for 30 mins to permabilize the surface membrane. Bundles were then washed in a K-HDTA solution (125μM EGTA) and the SR was then 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+}). The rate of rise of each elicited force response after a given load was measured and normalized to maximum Ca^{2+}-activated force.

Contractile apparatus experiments: The same bundles were then further skinned in a Ca^{2+}-free EGTA solution (containing mM: 136 K^+, 26 Na^+, 90 HEPES, 9 total Mg^{2+}, 50 EGTA, 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 with progressively increasing free [Ca^{2+}](between 0.1μM and 20μM; 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. The pCa50, Hill coefficient and the maximum force per cross sectional area for each bundle was determined. Additional samples of ventricle were used for western blot analysis, to determine the relative content of proteins associated with Ca^{2+} handling *via* the SR, and force development by the contractile machinery. Only male NR offspring displayed increased maximum Ca^{2+}-activated force (C: 7.9 mN/mm2 ± 0.59 *vs* NR: 10.57 ± 0.25; $P<0.05$), and decreased protein content of troponin I (cTnI) compared to C males ($P<0.05$). Furthermore, male NR offspring showed a reduction in rate of rise of the caffeine-induced force response ($F(1,6) = 13.74$, $P<0.05$) and a decrease in the protein content of ryanodine receptor (RYR2; $P<0.05$) . These findings illustrate a sex-specific effect of maternal NR on cardiac development, and also highlight a possible mechanism for the development of hypertension and hypertrophy in male NR offspring.

Rich-Edwards JW, Stampfer MJ, Manson JE, Rosner B, Hankinson SE, Colditz GA, Willet WC, Hennekens CH. (1997). Birth weight and risk of cardiovascular disease in a cohort of women followed up since 1976. *BMJ* **315**, 396–400.