Effects of IUGR on contractile protein expression and Ca^{2+}-activated force in β-escin permeabilised mesenteric arteries of adult (6-month old) and aged (1-year old) WKY rats

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Epidemiological studies by Barker and colleagues (1990) found intrauterine growth restricted (IUGR) infants had a significant risk of developing vascular dysfunction and hypertension in adulthood. Evidence suggests that IUGR alters extracellular vascular responsiveness, with the shift in reactivity depending upon numerous factors, including both age and sex (Williams *et al*., 2005; Anderson *et al*., 2006; Tare *et al*., 2012). However, the underlying mechanisms responsible for this shift in reactivity remains to be determined. These changes may be due to downstream events leading to force development, specifically, the Ca^{2+}-regulated activation of the contractile apparatus, shifts in the expression of important contractile proteins or a change in the phenotypic state. Therefore, we aimed to examine the effects of growth restriction on Ca^{2+}-activated force production, and contractile/proliferative protein and receptor expression in adult (6-month old) and aged (1-year old) male and female Wistar-Kyoto (WKY) rat resistance mesenteric arteries.

Pregnant WKY rats were randomly assigned to a sham (Control) or a bilateral uterine vessel ligation surgery (Restricted) as described previously (Wlodek *et al.*, 2005). Post mortem of restricted and control offspring were performed at 6 months and 1 year. Mesenteric arterial segments were collected for Western blot analysis. Single segments of intact mesenteric artery were mounted on a single wire myography system and placed in physiologic saline solution while constantly exposed to 100% O_2. Segments were normalized to achieve optimised internal circumference for tension development. Extracellular force responses to a high $[K^+]$ solution (150 mM) and phenylephrine (PE; 10^{-8} to 10^{-4} M) were measured. The same artery segment was then permeabilized with β-escin (50 μM), using a modified procedure described previously (Satoh *et al*., 1994). Chemically permeabilized segments were then exposed to a series of highly buffered Ca^{2+}-EGTA solutions containing increasing levels of free $[Ca^{2+}]$.

The peak contractile response to a K^+-induced depolarization and PE-stimulation were significantly decreased in 6-month old restricted males. At 1-year of age, restricted males had a significant increase in contractile responsiveness; thus, highlighting the age-dependent effect of IUGR. This change in responsiveness was at least partly due to an identical directional shift in maximum Ca^{2+}-activated force, which was not accompanied by a change in relative Ca^{2+}-sensitivity. Analysis of the relative protein expression discovered that the mesenteric artery from 6-month old male restricted rats underwent a phenotypic switch to a more 'non-contractile' state, through a decreased expression of important receptor and contractile proteins. However, 1-year old restricted males had a significant increase in expression of these key contractile proteins, which likely contributed to the reported increase in Ca^{2+}-activated force and extracellular responsiveness. These same physiological and biochemical changes were not observed in restricted female offspring; highlighting a sex-specific disparity.

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