The effect of intrauterine growth restriction on Ca^{2+}-activated force in β-escin skinned mesenteric arteries of 6-month old male Wistar-Kyoto rats

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An analysis of epidemiological cohort studies by Barker and colleagues (1990) found intrauterine growth restricted (IUGR) infants had a significant risk of developing vascular dysfunction and hypertension in adulthood. Vascular smooth muscle retains remarkable plasticity with the ability to shift from a contractile to proliferative state and vice versa depending upon environmental cues such as high blood pressure. Extensive evidence of an altered extracellular vascular responsiveness to certain vasoactive chemicals has been recognized in several IUGR rat models (Williams *et al.,* 2005; Anderson *et al.,* 2006; Tare *et al.,* 2012), with the specific shift in agonist reactivity depending upon numerous factors, including age and sex. However, it is unclear whether these observed changes in vascular sensitivity are driven by changes in receptor number, a change in the phenotypic state, or by changes in downstream events that lead to force development, specifically, the Ca^{2+}-regulated activation of the contractile apparatus. The aim of this study is to therefore examine the effects of IUGR on Ca^{2+}-activated force production, and potential changes in contractile/proliferative protein and receptor expression in adult (6-month old) male Wistar-Kyoto (WKY) rat mesenteric arteries.

Pregnant WKY rats were randomly assigned to a sham (n = 8) or bilateral uterine vessel ligation surgery (BUVL; n = 8) as described previously (Wlodek *et al*., 2005). At 6 months of age, male rats were euthanised by an overdose of isoflurane (4% v/v) in accordance with NHMRC guidelines and approval from the Animal Ethics Committee of La Trobe University. Individual mesenteric arterial segments were collected and prepared for Western blot analysis. While single segments of intact mesenteric artery were dissected and mounted on a single wire myograph system (320A, ADInstruments) and placed in physiologic saline solution (PSS; containing in mM: 10 HEPES, 150 NaCl, 3 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$ and 5.5 glucose; pH 7.3) while constantly exposed to 100% O_2. Segments were normalized to achieve optimised internal circumference for development of tension described previously (Mulvany and Halpern, 1977). Extracellular force responses to both KCl (150mM) and phenylephrine (10^{-8} to 10^{-4}M) were measured. The same artery segment was then skinned with β-escin (50μM in Ca^{2+}-free EGTA solution; containing in mM: 90 HEPES, 50 EGTA, 9 total Mg^{2+}, 1 NaN_3 8 ATP, 10 creatine phosphate; pH 7.1) using a modified procedure described previously (Satoh *et al*., 1994). Skinned artery segments were then exposed to a series of highly buffered Ca^{2+}-EGTA solutions containing increasing levels of free [Ca^{2+}] (between 0.1μM and 20μM) (equivalent in composition to the Ca^{2+}-free EGTA solution above but with added Ca^{2+}).

Maximum responsiveness to KCl was significantly decreased in BUVL rats (P<0.05). Phenylephrine induced significantly reduced force responses in BUVL compared to sham rats (P<0.05) consistent with previous observations by Tare *et al*. (2012). In β-escin permeabilized mesenteric arteries the Ca^{2+}-sensitivity was unchanged between experimental groups. However, the maximum Ca^{2+}-activated force response was found to be significantly decreased in BUVL adult male rats (P<0.05). Western blot analysis of phenotypic differentiation protein markers found no significant differences between experimental groups. While relative amounts of important receptor proteins were significantly reduced in BUVL male rats (P<0.05). These results suggest that the vascular dysfunction of reduced extracellular responsiveness in male adult rats (6 months old) is driven by down-regulation of key receptors and reduced maximum Ca^{2+}-activated force production, while a possible phenotypic switch to a proliferative state is unlikely to have occurred or contributed to the vascular dysfunction.

Anderson C, Lopez F, Zimmer A, Benoit N. (2006) *Biol of Reprod* **74**(3), 538-544.
Barker D, Bull A, Osmond C, Simmonds S. (1990) *Brit Med J* **301**(6746), 259-262.
Mulvany M and Halpern W. (1977) *Circ Res* **41**(1), 19-26.
Satoh S, Kreutz R, Wilm C, Ganten D, Pfitzer G. (1994) *J Clin Invest* **94**(4), 1397-1403.
Tare M, Parkington H, Bubb K, Wlodek M. (2012) *Hypertension* **60**, 378-386.
Williams S, Campbell M, McMillen C and Davidge S. (2005) *Am J Physiol Regul Integr Comp Physiol* **288**(2), 360-367.
Wlodek M, Westcott K, O'Dowd R, Serruto A, Wassef L, Moritz K, Moseley J. (2005) *Am J Physiol Regul Integr Comp Physiol* **288**, 1620-1627.