Elevated intra-cardiac angiotensin II modulates myocardial autophagic signalling induced by insulin resistance

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Our recent investigations have demonstrated a role for myocardial autophagy in fructose-induced insulin resistant cardiomyopathy (Mellor *et al.,* 2011). We have previously determined that cardiomyocyte autophagy is regulated by angiotensin II (AngII) receptors (Porrello *et al.*, 2009). Our aim was to investigate the interactions between dietary fructose and cardiac AngII upregulation in myocardial autophagy modulation.

Male mice with cardiac-specific overexpression of the angiotensinogen (AOGN, TG) gene or wildtype (WT) were fed a high fructose (60%, Fruc) diet or nutrient-matched control (Ctrl) diet for 12 weeks. At the completion of the 12 week dietary intervention, hearts were excised from anaesthetized mice (100mg/kg i.p. sodium pentobarbitone) and cardiac weight indices evaluated. Myocardial insulin resistance and autophagy induction was determined by protein expression analysis of signalling intermediates.

Fructose feeding was not associated with hypertension or obesity. Cardiac AngII upregulation induced cardiac hypertrophy in control-fed but not fructose-fed mice (heart weight/tibia length: Ctrl-WT 8.7±0.4mg/mm *vs* Ctrl-TG 10.3±0.4mg/mm *vs* Fruc-WT 8.3±0.3mg/mm *vs* Fruc-TG 9.0±0.3mg/mm; diet × genotype interaction $P<0.05$). Dietary fructose increased cardiac protein content of beclin-1 and p62 (involved in autophagy initiation and recruitment of materials to the autophagosome respectively), in both WT and TG. In contrast, protein content of the autophagy activity marker LC3BII:I ratio was increased in fructose-fed WT (62% increase) but not TG mouse hearts (diet × genotype interaction $P<0.05$). Correlation analyses identified a positive relationship between LC3BII and heart weight ($R^2=0.726$, $P<0.05$) in fructose-fed TG mice, in contrast to the negative correlations observed in control-fed WT & TG and fructose-fed WT mice.

These findings suggest that cardiac AngII upregulation interacts with fructose-induced autophagy at the LC3B-mediated stage of autophagosome formation and modifies cardiomyocyte trophic/survival signalling balance. This study provides novel insight into the mechanisms of cardiac autophagy upregulation in fructose-induced insulin resistance and suggests a role for AngII in this context.

Mellor KM, Bell JR, Young MJ, Ritchie RH, Delbridge LMD. (2011) Myocardial autophagy activation and suppressed growth signaling is associated with insulin resistance in fructose-fed mice. *Journal of Molecular and Cellular Cardiology* **50:** 1035-1043.

Porrello ER, D'Amore A, Curl CL, Allen AM, Harrap SB, Thomas WG & Delbridge LMD. (2009) Angiotensin II type 2 receptor antagonizes angiotensin II type 1 receptor-mediated cardiomyocyte autophagy. *Hypertension* **53:** 1032-1040.