Acute leptin exposure alters the expression of fibrotic mediators in renal proximal tubule cells *in vitro*

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Introduction: Obesity rates and associated co-morbidities including end stage renal disease have increased significantly worldwide in the past decade. Adipocytes produce the hormone leptin, with plasma leptin concentrations reflecting adipose stores. Leptin is filtered from the blood by the kidneys, and is reabsorbed by proximal tubule cells (PTCs) by the scavenger receptor megalin, with negligible levels of leptin present in the urine even in obese individuals. Typically obese individuals have altered tubular function which results in microalbuminuria and proteinuria, increasing the risk of chronic kidney disease (CKD); with elevated leptin levels correlating with albuminuria. Several studies have now demonstrated that elevated leptin increases the expression of the fibrotic cytokine transforming growth factor β (TGF-β) and collagen type IV in the glomerulus (Wolf *et al.*, 1999). However, the effect elevated leptin has on these fibrotic mediators in the renal proximal tubule has not been investigated. The aim of this study is to investigate the effect elevated leptin has on the expression of TGF-β1 and collagen type IV in renal PTCs. As recent research has demonstrated that AMP-activated protein kinase α (AMPK-α) is an activator of TGF-β-activated kinase 1 (Kim *et al.*, 2012) we will also investigate the effect leptin has on AMPK phosphorylation.

Method: We used the established Opossum kidney (OK) PTCs cell line model. OK cells were treated with 0.05, 0.10, 0.25 and 0.50 μg/mL leptin for 2 hours and mRNA and protein was extracted. 'Real Time' PCR was used to determine any changes in TGF-β1 mRNA expression. Western blot analysis of signalling mediators was performed using equal quantities (50 μg) of protein (Briffa *et al.*, 2014). Samples were probed for TGF-β1, collagen type IV, AMPK-α and AMPK-β as well as their phosphorylated forms, with analysis of the blots determined using densitometry analysis. To determine the effect leptin has on TGF-β1 secretion from PTCs we also performed an ELISA assay following the manufacturers' protocol, and the data was standardized to total protein from each sample.

Results: Densitometry analysis identified a significant increase in phosphorylated AMPK-α at 0.05, 0.25 and 0.50 μg/mL leptin, and a significant decrease in phosphorylated AMPK-β at 0.50 μg/mL leptin. 'Real Time' PCR analysis identified a significant increase in TGF-β1 mRNA expression at 0.50 μg/mL, with Western blot analysis identifying a significant reduction in mature TGF-β1 protein expression at this concentration. TGF-β1 ELISA analysis identified that leptin treatment significantly increases TGF-β1 secretion from OK cells across all leptin treatments (0.05 - 0.50 μg/mL).

Conclusion: Therefore, we have identified that leptin increases PTC expression of phosphorylated AMPK-α and AMPK-β showing that leptin upregulates the AMPK pathway. This work also identified that leptin significantly increases the secretion of TGF-β1 in PTCs and upregulated TGF-β1 mRNA. These data are the first to show that leptin upregulates fibrotic mediators in PTCs and suggest a potential role for AMPK in mediating these fibrotic mediators.

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