Chronic high fat feeding increases megalin expression and causes albuminuria and proteinuria in rats

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Introduction: Obesity rates and associated co-morbidities including end stage renal disease have increased significantly worldwide in the past decade. Typically obese individuals have altered tubular function which results in albuminuria and proteinuria, increasing the risk of chronic kidney disease (CKD). Importantly, elevated concentrations of the adipokine leptin correlate with the degree of albuminuria. The main cause of decreased protein handling by the kidneys in CKD is unclear. It may either be caused by an increase in glomerular permeability, altered protein handling by cells of the proximal tubule, or a combination of the two. The scavenger receptor megalin and its associated transmembrane proteins are essential for normal protein handling by the kidneys; whereby any alterations in megalin expression is likely to alter protein handling. Several studies have demonstrated that obesity is associated with fibrotic changes to the kidney, specifically the glomerulus. Only one study to date has shown a reduction in megalin expression in obese Zucker rats (Habibi *et al.*, 2011), which is likely due to a complication of diabetes and not obesity (Tojo *et al.*, 2001). Therefore, the aim of this study was to investigate the effect a chronic high fat diet has on renal morphology, albumin and protein handling, fibrotic mediator expression, and macromolecular complex expression in the kidney.

Method: All experiments were approved by the Howard Florey Animal Ethics Committee. Seventeen male Sprague-Dawley rats (six weeks old) were randomly assigned to receive either a control diet (5% fat) or high fat diet (21% fat) for 10 weeks. Twenty-four hour urine samples were collected from the animals using metabolic cages in week 10 to evaluate urinary protein, albumin, sodium and creatinine. Following this, the animals were deeply anaesthetised with sodium pentobarbitone (100 mg/kg) and killed by cardiac puncture and the plasma was collected to evaluate plasma creatinine. Renal tissues were removed and either snapped frozen in liquid nitrogen for mRNA and protein expression or frozen in optimal cutting solution for histological analysis.

Results: High fat feeding for 10 weeks did not alter body weight gain compared to standard chow; however the animals had an increase in body fat composition which was consistent with an increase in circulating leptin concentrations. Urinary analysis identified that high fat feeding for 10 weeks increased protein and albumin secretion, reduced sodium excretion and reduced glomerular filtration rate (determined by creatinine clearance), indicating altered renal function in these animals. Histological analysis identified no changes in glomerular cross sectional area but an increase in tubule cross sectional area. Further, there was no change in TGF-β1, collagen IV and fibronectin in the kidney of obese animals. Western blotting analysis identified an increase in megalin expression, and decreased expression of the sodium hydrogen exchanger 3 (NHE3) and sodium potassium pump in the kidneys of obese rats. Akt stimulates NHE3 expression (Lee-Kwon *et al.*, 2001), and not surprisingly, obesity decreased total and phosphorylated Akt proteins in the kidney.

Conclusion: Therefore, we have identified that chronic high fat feeding alters albumin and protein handling in the kidneys prior to structural changes to the glomerulus, which indicates the early stages of CKD are likely due to altered tubule function.

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