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"Proximal Tubular Cell-Specific Heterozygous Ablation of Carnitine Acetyltransferase Causes Cellular and Respiratory Remodeling as a Component of Tubular Disease"

Abstract

Alterations in mitochondrial fatty acid metabolism are a component of type 2 diabetes in tissues that are affected in diabetic complications. However, it is not well known how mitochondrial substrate overload affects the development of a major diabetic complication, kidney disease. This lab has previously shown that modeling mitochondrial overload in proximal tubular cells by creating a cell-specific homozygous knockout of the enzyme Carnitine Acetyltransferase (CrAT) is associated with energy deficit and impaired respiration before the onset of pathology. These results suggest that mitochondrial dysfunction may be causative to chronic kidney disease. The respiratory and ultrastructural effects of proximal tubular cell-specific heterozygous ablation of CrAT (PT-CrAT KO^{HET}) were studied, which seem to have similarly profound effects as evidenced by recent respiratory studies. This highlights the importance of even mild inhibition of CrAT observed in states of lipid stress in the progression of renal disease. Our analysis of transmission electron microscopy images of heterozygous CrAT KO kidneys in mice (aged 12-16 months) reveals more numerous lipid droplets, extensive deterioration of mitochondrial architecture, increased levels of mitophagy, and the presence of multilamellar bodies compared to control mice. Ongoing respiratory studies involve providing isolated proximal tubule CrAT heterozygous knockout segments from mice of ages 6, 9, or 12 months with pyruvate to investigate how the segments respirate differently with less CrAT using a XF24 Analyzer (Agilent Seahorse). Since it has been proposed that excess acetyl-coA can inhibit pyruvate dehydrogenase (PDH), it was hypothesized that these tubule segments would catabolize pyruvate less efficiently than control PT segments. Contrary to this hypothesis, our data indicated that PT-CrAT KO^{HET} mitochondria exhibit greater basal oxygen consumption rate and decreased reserve capacity when respiring on pyruvate compared to control PT segments. One possible explanation is that PT-CrAT KO^{HET} mitochondria may exhibit greater basal proton leak, indicating a possible greater amount of uncoupled respiration and cellular reactive oxygen species compared to control PT segments. Other possible explanations include that acetyl CoA levels in the heterozygous knockout, at least at the ages examined herein, are perhaps not high enough to block PDH, or that proximal tubular cells switch and prefer carbohydrate-based substrates in these culture conditions. PT-CrAT KO^{HET} mitochondria may also not be equipped to handle oxidative stress as well as control mitochondria.