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Fructokinase activity mediates dehydration-induced renal injury

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The epidemic of chronic kidney disease in Nicaragua (Mesoamerican nephropathy) has been linked with recurrent dehydration. Here we tested whether recurrent dehydration may cause renal injury by activation of the polyol pathway, resulting in the generation of endogenous fructose in the kidney that might subsequently induce renal injury via metabolism by fructokinase. Wild-type and fructokinase-deficient mice were subjected to recurrent heat-induced dehydration. One group of each genotype was provided water throughout the day and the other group was hydrated at night, after the dehydration. Both groups received the same total hydration in 24 h. Wild-type mice that received delayed hydration developed renal injury, with elevated serum creatinine, increased urinary NGAL, proximal tubular injury, and renal inflammation and fibrosis. This was associated with activation of the polyol pathway, with increased renal cortical sorbitol and fructose levels. Fructokinase-knockout mice with delayed hydration were protected from renal injury. Thus, recurrent dehydration can induce renal injury via a fructokinase-dependent mechanism, likely from the generation of endogenous fructose via the polyol pathway. Access to sufficient water during the dehydration period can protect mice from developing renal injury. These studies provide a potential mechanism for Mesoamerican nephropathy.

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An epidemic of chronic kidney disease is occurring in the hot coastal communities of Central America, especially among men working in sugarcane fields.[1–3] The cause of the disease, which has been called 'Mesoamerican nephropathy,'[1–3] has remained a mystery, as it is not associated with the usual causes of chronic kidney disease such as diabetes or hypertension. Studies to date have not identified heavy metals, agrochemicals, or other toxins as likely candidates for causing the epidemic. However, a common predisposing feature appears to be exposure to heat and the development of recurrent dehydration.[1–3]

Dehydration is commonly associated with 'prerenal' dysfunction with retention of urea, but classically this is thought to reflect hemodynamic changes and not to be associated with true renal injury. If dehydration is severe, subjects can develop acute kidney injury from heat stroke, rhabdomyolysis, and hypotension, but such individuals would likely have substantial symptoms and not present with the insidious onset of chronic kidney disease as typically occurs. However, a favorite hypothesis is that recurrent dehydration may lead to low-grade renal injury that over time results in chronic kidney disease. Dehydration from sweat and heat is known to activate the release of vasopressin. In turn, vasopressin has recently been recognized to have a potential role as a mediator of chronic kidney disease.[4–6] However, although a role for vasopressin in increasing the rate of progression in chronic kidney disease has been shown in animals,[4,7] its role in acute renal injury associated with dehydration remains largely unexplored.

Dehydration can also result in an increase in serum osmolarity and in the activation of aldose reductase.[8] Activation of aldose reductase is important in the renal medulla, as here it generates sorbitol that can help protect tubular cells from the high osmolarity in the extracellular environment at this site, thus facilitating urinary concentration. However, activation of aldose reductase in the proximal tubule may not be beneficial, as the conversion of glucose to sorbitol may

result in its further degradation by sorbitol dehydrogenase to fructose. Although fructose itself may not be toxic, when it is metabolized by fructokinase, it results in transient adenosine triphosphate depletion with the generation of uric acid, oxidants, and inflammatory mediators.[9–11] The proximal tubule is one of the major sites where fructokinase is expressed.[12] This raises the hypothesis that recurrent dehydration may result in repeated stimulation of aldose reductase with the generation of fructose in the proximal tubule, leading to tubular injury and inflammation. To test this hypothesis, we designed the following study.

RESULTS

Effect of dehydration on daily weight

The dehydration protocol resulted in the mice sweating and losing weight acutely. Figure 1a shows the change in mean weight during the 7-h dehydration period. Mice that were not provided water during the day (water at night (WAN)) lost a mean of 15% body weight over the 7-h dehydration period (mean 15.4% for wild-type (WT) mice and 14.6% for the fructokinase (ketohexokinase) A and C knockout (KHK-KO) mice, $P =$ nonsignificant). In contrast, mice who had access to water during the day (water all time (WAT)) showed significantly less weight loss during the day (7.8% and 8.1%, respectively, for the WT and KHK-KO mice, $P =$ nonsignificant). This degree of weight loss was observed both at the beginning and at the end of the study and there was no evidence of adaptation over time (data not shown). Not surprisingly, the mice that had water withheld during the day drank more water at night (Figure 1b), and the mice that had water all the time during the day drank more water compared with the control group in 7 h (Figure 1c), such that total water intake over 24 h was identical in the WAT and WAN groups (Figure 1d).

The effect of the dehydration procedure on overall changes in morning basal weight was also assessed. At the end of 5 weeks, both WT and KHK-KO mice exposed to dehydration tended to maintain but not increase their weight (Figure 1e). In contrast, control WT and control KHK mice tended to increase their weight over the 5-week period.

Dehydration activates the aldose reductase pathway in the renal cortex of WT mice

WT and KHK-KO mice that were dehydrated without water (WAN) showed an increase in serum osmolarity at the end of the 7 h dehydration period (Figure 2a). Urinary osmolarity was increased in the mice exposed to heat in samples collected at both week 1 and week 4 (Figure 2b and c). Hyperosmolarity is expected to increase aldose reductase levels, but no differences were observed in aldose reductase protein levels by western blot of the renal cortex at the time of killing, although there was a tendency for higher aldose reductase levels in the WT WAN group (Figure 2d). Nevertheless, both renal cortical sorbitol (Figure 2e) and renal cortical fructose levels (Figure 2f) were increased in WT mice dehydrated without water during the day (WAN group), consistent with an increase in aldose reductase activity. Sorbitol levels were not increased in the KHK-KO mice independent of groups. In contrast, renal fructose levels were elevated in all three groups of KHK-KO mice, consistent with earlier studies showing that fructose levels are high in mice in which fructokinase is absent.[13]

Effect of dehydration on blood pressure, renal function, and renal histology

As shown in Figure 2 the WT mice that were dehydrated during the day without water (the WAN group) constituted the group that showed evidence for an increase in aldose reductase activity, as noted by an increase in renal sorbitol and fructose levels. This was also the only group that showed evidence of renal injury, as noted by an increase in urinary neutrophil gelatinase-associated lipocalin (Figure 3a), serum creatinine (Figure 3b), and evidence of proximal tubular injury by renal histology (Figure 4), and with a loss of proximal tubular brush border based on staining for angiotensin-converting enzyme using computer image analysis (Figure 5). Injury was present in both proximal tubules in the renal cortex and in the outer stripe. The injury was not due to rhabdomyolysis, as serum creatine phosphokinase levels at the time of killing were normal in all mice regardless of group (creatine phosphokinase levels <10 ng/ml). Consistent with the known effects of fructose to stimulate the chemokine monocyte chemotactic protein-1 (MCP-1) in proximal tubular cells,[10] we also found both an increase in renal cortical MCP-1 levels (Figure 6a) and an increase in infiltrating macrophages in the WAN group compared with the other groups of WT mice (Figure 6b) as we can see in the F4/80 stain (Figure 7). An increase in interstitial fibrosis (collagen III staining) was also observed in the WT WAN group (Figures 8 and 9).

Importantly, KHK-KO mice were protected from renal injury. Despite the KHK-KO WAN group having the same degree of weight loss and increase in serum osmolarity, the KHK-KO mice did not show an increase in serum creatinine, tubular injury, inflammation, or fibrosis by histology, or increased expression of the chemokine MCP-1 (Figures 6–9).

Blood pressure was also measured in both WT and KHK-KO mice at the end of a dehydration period at week 4 (Figure 10). Blood pressure was increased in the WT WAN mice compared with all other groups. KHK-KO mice that were dehydrated without water (WAN) also showed a higher blood pressure, but it was significantly less than that of the WAN WT mice (Figure 10).

Uric acid levels

Previous studies have found that uric acid generated during fructose metabolism may be partially responsible for the stimulation of MCP-1.[10] Consistent with this hypothesis, an increase in kidney cortical uric acid was observed in the WT WAN mice (Figure 11a). However, renal cortical uric acid levels were also high in the KHK-KO WAN mice despite no stimulation of MCP-1 (Figure 11a). We also noted