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A rat model of chronic kidney disease-mineral bone disorder

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Chronic Kidney Disease-Mineral Bone Disorder (CKD-MBD) is a newly defined syndrome encompassing patients with chronic kidney disease that have a triad of biochemical alterations in calcium, phosphorus and parathyroid hormone, vascular calcification, and bone abnormalities. Here we describe a novel Cy/+ rat model of slowly progressive kidney disease spontaneously developing the three components of CKD-MBD when fed a normal phosphorus diet. Since the renal disorder progressed 'naturally' we studied the effect of dietary manipulation during the course of the disease. Animals with early, but established, chronic kidney disease were fed a casein-based or a grain-based protein diet both of which had equivalent total phosphorus contents. The two different sources of dietary protein had profound effects on the progression of CKD-MBD, likely due to differences in intestinal bioavailability of phosphorus. Although both dietary treatments resulted in the same serum phosphorous levels, the casein-fed animals had increased urinary phosphorus excretion and elevated serum FGF23 compared to the grain-fed rats. This model should help identify early changes in the course of chronic kidney disease that may lead to CKD-MBD.

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Vascular calcification is common in patients with chronic kidney disease (CKD), appearing in 30–65% of patients with stages 3–5 CKD and 50–80% of patients with stage 5D CKD, and is associated with increased morbidity and mortality.[1–3] In the coronary arteries, this calcification is typically intimal, within atherosclerotic plaques or as circumferential intimal lesions, whereas in the aorta, calcification occurs in both the intimal and medial layers of the vessel wall (atherosclerosis and Mönkeberg's medial calcific sclerosis). The pathogenesis of vascular calcification is complex, but *in vitro* studies support the concept of an initial transformation of vascular smooth muscle cells to chondrocyte/osteoblast-like cells in response to hyperphosphatemia, uremia, inflammation, and elevated glucose levels.[4] These transformed cells then lay down a matrix of collagen and non-collagenous proteins and produce matrix vesicles that serve as the initial nidus for calcification, similar to the process of normal bone mineralization. This process is accelerated in the clinical setting of CKD, possibly because of hyperphosphatemia and hyperparathyroidism, the use of high dose calcium salts as phosphate binders that increase the overall calcium load, abnormal bone remodeling, and relative deficiencies of circulating and locally produced inhibitors of calcification. This interrelationship of vascular calcification with abnormal serum biochemistries and abnormal bone remodeling is the basis for the recently named syndrome, chronic kidney disease-mineral bone disorder (CKD-MBD).[5] The complexity of this interrelationship makes studies in humans difficult, as one cannot easily control one variable without impacting another. Thus, there is a clear need for an appropriate animal model in which to understand the progressive pathophysiology of CKD-MBD, as well as to test interventions.

Several animal models of vascular calcification exist, including the adenine nephrotoxic model[6,7] and the 5/6th nephrectomy models in rats.[8,9] In both models, animals develop severe hyperphosphatemia and hyperparathyroidism due to an acute kidney injury, which is followed by CKD. These models have provided useful information on the pathophysiology of vascular calcification. However, they represent advanced CKD, due to the severity of acute injury,

with creatinine clearances that approximate late 4 or 5 stage human CKD. In both disease states, diet is an important factor. The 5/6th nephrectomy rat model is generally fed a high-phosphorus diet to induce hyperphosphatemia.[8,9] The adenine model has a more rapid onset and severe course of kidney disease as well as hyperparathyroidism and arterial calcification than the 5/6th nephrectomy model. Curiously, the adenine model develops more consistent arterial calcification on a low-protein diet.[7] The type of calcium deposition may also differ in these models.[10] In mice, spontaneous calcification in the setting of surgically induced acute kidney injury followed by CKD, is not found unless there is a concomitant genetic abnormality that is proatherogenic, such as ablation of the low-density lipoprotein receptor or the *ApoE* genes.[11,12] Although useful, none of these rodent models provide the opportunity to study a slowly progressive CKD, nor earlier stages of CKD-MBD. This is important because studies demonstrate that patients in earlier stages of CKD also have coronary artery calcification[1,13,14] suggesting that the process begins before beginning dialysis. Therefore, there is a clear need for additional animal models to provide the opportunity to study slowly progressive CKD to better understand the triad of CKD-MBD: (1) abnormal serum biochemistries, (2) abnormal bone remodeling, and (3) vascular calcification. In this report, we describe a novel model of progressive CKD-MBD, the Cy/+ rat, and demonstrate its usefulness for evaluating early pathogenesis and the impact of different dietary regimens on the course of CKD-MBD.

RESULTS

Main study assessment of CKD

To assess the magnitude of uremia, we compared blood urea nitrogen (BUN), creatinine, weight, and hematocrit in the 38-week Cy/+ CKD animals each treated for 18 weeks with the casein protein-based diet with either 0.7% (normal) or 0.2% phosphorus (low), comparing these CKD animals to normal littermates. The 38-week animals with CKD had elevated BUN at 20 weeks, which persisted (Figure 1a). The animals in the CKD groups also had elevated serum creatinine values (normal, 0.62 ± 0.01 mg/100 ml; CKD/0.7% Pi, 3.05 ± 0.27 mg/100 ml; CKD/0.2% Pi, 1.93 ± 0.25 mg/100 ml; $P < 0.01$). The low-phosphorus diet attenuated the progressive CKD as assessed by BUN (Figure 1a). In the 38-week end-point groups, the CKD animals in both treatment groups weighed less than normal littermate

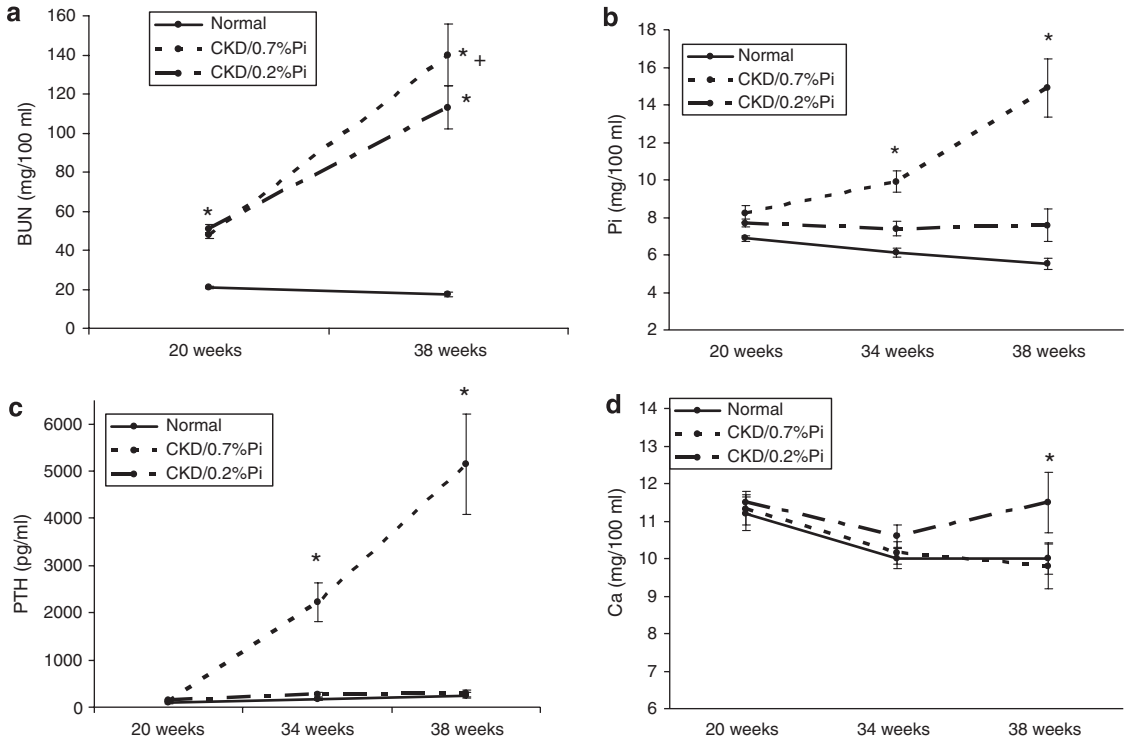

Figure 1 | Biochemical changes over time. Cy/+ rats were placed on the study diet at 20 weeks of age. The 38-week-old animals had an intermediate blood draw at 34 weeks for phosphorus, parathyroid hormone, and calcium for a total of three time points, whereas BUN was measured at 20 and 38 weeks. The graphs show changes in plasma levels over time for BUN (**a**), phosphorus (**b**), intact parathyroid hormone (**c**), and calcium (**d**). The data demonstrate that CKD animals fed a 0.7% phosphorus diet had progression of CKD as assessed by BUN, hyperphosphatemia, and hyperparathyroidism compared to the normal littermates. Feeding a low-phosphorus diet (0.2%) ameliorated these changes. As detailed in the text, these changes over time were significant ($P < 0.001$), even when adjusted for baseline values and final BUN. At 38 weeks, the calcium levels were greater in the CKD/0.2% Pi animals than in the CKD/0.7% Pi fed animals ($P = 0.04$). $n = 15$–17 per group. *$P < 0.05$ compared to normal animals, $^{+}P < 0.05$ 0.2% phosphorus-treated CKD animals versus 0.7% phosphorus-treated CKD animals.