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The key to halting progression of CKD might be in the produce market, not in the pharmacy

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***In vitro*, experimental, and clinical work suggests that metabolic acidosis, either directly or indirectly, can promote the progression of chronic kidney disease (CKD). Goraya *et al.* demonstrate that both oral alkali supplementation and a diet rich in fruits and vegetables are equally effective at decreasing urinary excretion of markers of renal injury in patients with stage 2 CKD. Although this study is promising, the short duration and use of only urinary markers as a surrogate outcome weaken the conclusions.**

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In this issue of *Kidney International*, Goraya *et al.*[1] report that reduction in acid load to the body by increased intake of alkali in the form of either a diet rich in fruits and vegetables or oral sodium bicarbonate reduces urinary markers of kidney damage in patients with stage 2 chronic kidney disease (CKD).[1] The article is a natural extension of the authors' previous works to establish a relationship between metabolic acidosis and progression of CKD.[2] In this Commentary we will address three issues: (1) Is alkali therapy effective in slowing loss of kidney function in CKD? (2) What are the potential mechanisms? (3) How does the article by Goraya *et al.*[1] clarify the first two questions?

A number of studies in animals have shown beneficial effects of alkali supplementation in slowing the progression of kidney disease,[3] although these findings have not been universal. Two studies in particular, both from the same authors, showed the opposite finding, namely, that ammonium chloride was beneficial. However, in the latter study, the protective effect of ammonium chloride was observed with a high-phosphorus diet that resulted in very high serum phosphorus.[4]

Clinically, the association between metabolic acidosis and CKD progression has been suggested by observational studies and by several small and nonrandomized studies in which alkali supplementation slowed the loss of kidney function in CKD patients.[3] More recently, however, two small randomized clinical trials have demonstrated a beneficial effect of oral alkali supplementation in the progression of kidney disease. The first one was a randomized trial of oral sodium bicarbonate supplementation vs. standard care performed in 134 adults with stage 4 CKD (glomerular filtration rate 15–30 ml/min per 1.73 m^2) and serum bicarbonate concentrations between 16 and 20 mmol/l.[5] At the end of two years of follow-up, 22 patients in the control group and four in the bicarbonate group progressed to dialysis (6.5 vs. 33%, respectively). Among patients who did not require initiation of dialysis, those in the bicarbonate group were significantly less likely to experience rapid progression defined by a loss of glomerular filtration rate of more than 3 ml/min per 1.73 m^2 per year (9 vs. 45%).[5] Although the end points were clinically meaningful and their differences were statistically significant, this study lacked a placebo control group, and it was not blinded. The second study was a prospective, randomized, placebo-controlled, blinded intervention of daily oral $NaHCO_3$ vs. NaCl or placebo in subjects with macroalbuminuric hypertensive nephropathy and stage 2 CKD.[2] After 5 years, the rate of estimated glomerular filtration rate (eGFR) decline was slower and eGFR was higher in patients given $NaHCO_3$ than in those given placebo or NaCl.[2] The main limitation of the study was the relatively small number of patients ($n = 40$ per group). The above data support the incorporation of oral alkali supplementation as part of the routine clinical care of patients with CKD with a low serum bicarbonate concentration or in the low range of normal. Despite the fact that patients in the study by Mahajan *et al.*[2] had baseline serum bicarbonate of 26 mmol/l, the benefits of oral alkali supplements in patients with 'normal' serum bicarbonate remain to be clearly demonstrated in a larger number of subjects. If alkali therapy is indeed effective, what is the mediating mechanism or mechanisms? There are several hypotheses. One possible mechanism is that the acidic pH itself increases production of reactive oxygen species, which in turn will cause renal damage through oxidative injury. Another mechanism is acidosis-induced upregulation of the endothelin gene, leading to vasoconstriction and fibrosis.[2] Another theory is that acidic pH increases ammonia production in the proximal tubule, and high ammonia causes injury by activating the alternative pathway of complement.[6] There is a substantial amount of evidence that ammonia can activate C3. C3 normally forms an intramolecular thioester bond by reaction of the SH group of cysteine-988 with the amide nitrogen of glutamine-991, and the creation of this thioester bond apparently confers stability. The reaction occurs with the release of ammonia and formation of a thioester bond and a ring structure consisting of four amino acids (cysteine, glycine, glutamic acid, and glutamine).[7] In an environment with high ammonia concentration,

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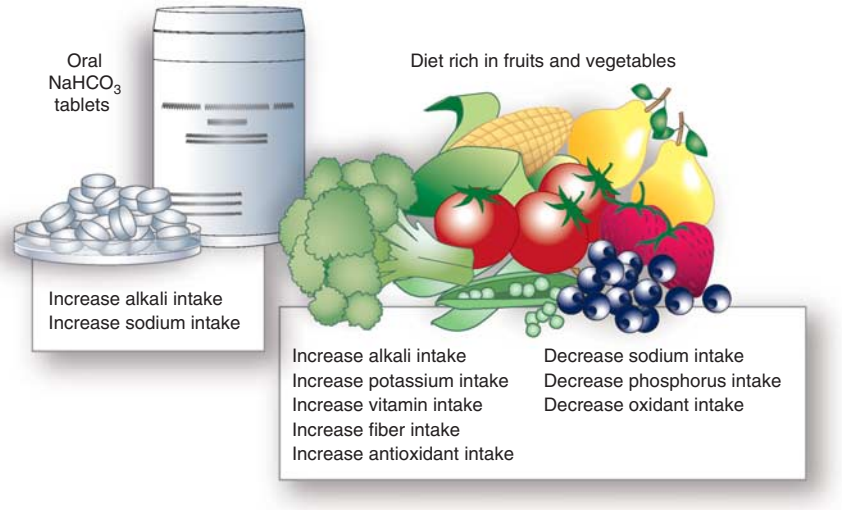

Figure 1 | Alkali supplementation in CKD: $NaHCO_3$ or a diet rich in fruits and vegetables?

the thioester bond is attacked by ammonia, which is quite nucleophilic. As the bond is broken, ammonia is inserted into glutamine, forming an amide bond.[7] The resulting C3 without a thioester bond undergoes conformational changes and acquires the enzyme activity of C3b, which can convert C3 to C3a and C3b; this is the start of the activation of the alternative complement pathway. If an increased load of ammonia per kidney mass is responsible for activation of complement and therefore progression of kidney injury, one would have expected that other conditions characterized by high urinary ammonia would also accelerate renal disease. In fact, associations of CKD and chronic hypokalemia (as with surreptitious diuretic use, eating disorders, laxative abuse, or primary aldosteronism) have been observed for many years, and hypokalemic nephropathy is an entity clearly described, although not widely recognized in clinical nephrology.

The current study by Goraya *et al.*[1] tests the hypothesis that, as daily acid load is primarily dependent on the type of food ingested, increasing the daily intake of fruits and vegetables might have effects similar to those of alkali therapy in preserving renal function. Indeed, the study showed similar effects of oral $NaHCO_3$ and a diet rich in fruits and vegetables (calculated to reduce acid load by 50%) in patients with stage 2 CKD, although no effects were noted in stage 1 CKD patients. A major problem with this study, however, is the use of indirect markers (urinary excretion of albumin, *N*-acetyl-β-D-glucosaminidase, and transforming growth factor-β) and not changes in glomerular filtration rate as primary outcome, and therefore the conclusions should be accepted with caution. As we do not know the actual mechanisms of how alkali supplementation slows down renal injury, the effect of the intervention on these markers may turn out to be irrelevant in terms of the real outcome: preservation of renal function. For example, there is no supportive evidence that *N*-acetyl-β-D-glucosaminidase has any direct adverse effect on the kidney. Interestingly, however, ficolin can be activated by binding to *N*-acetyl-β-D-glucosamine, which is usually found on the cell membrane of microorganisms but can be found on human tissues, especially when they are damaged. It is tempting to speculate that increased *N*-acetyl-β-D-glucosaminidase represents availability of *N*-acetyl-β-D-glucosamine on renal tissue, which can activate the lectin-mediated complement system. Ficolin, like mannose-binding lectin, can activate the complement system through its action on C4 and C2, once it binds a suitable ligand such as *N*-acetyl-β-D-glucosamine.[8] Moreover, a diet rich in fruits and vegetables could potentially slow down renal disease progression by several effects independent of decreasing acid load, for example, relative increase of potassium over sodium intake, decreased phosphorus load, and increased intake of fibers, antioxidants, vitamins, and chemicals such as sulforaphane (Figure 1). The latter compound is known to activate nuclear factor (erythroid-derived 2)-like 2 (Nrf2), and other compounds that activate Nrf2, such as bardoxolone, have been shown to improve renal function.[9] Goraya *et al.*[1] recognize that a significant effect of the fruit-and-vegetable-rich diet is decreasing blood pressure, a factor known to slow loss of kidney function. Regardless of mechanisms, a fruit-and-vegetable-rich diet has so many potential beneficial effects that it could be easily accepted for implementation. This study is also important because it illustrates a very simple and safe way to treat metabolic acidosis, an intervention to slow CKD progression that has not received enough attention.

Interestingly, use of sodium bicarbonate infusion prior to use of intravenous contrast media is now widely practiced, but it is not known whether some or all the above mechanisms are responsible for its effectiveness, if it is indeed effective.

Finally, in view of the above discussion, it is tempting to speculate that the age-related decline in renal function is also due to the ammonia toxicity that results partly from the greater acid load of the modern diet in comparison with the Paleolithic diet. Sebastian *et al.*[10] have argued that so-called normal values for serum bicarbonate vary with the dietary intake of alkali. It is likely that the renal production of ammonia on the Paleolithic diet was substantially lower than that on the modern diet. Perhaps, the so-called age-related decline in renal function is a result of damage induced by ammonia overproduction.

DISCLOSURE
The authors declared no competing interests.

REFERENCES

1. Goraya N, Simoni J, Jo C *et al.* Dietary acid reduction with fruits and vegetables or bicarbonate attenuates kidney injury in patients with a moderately reduced glomerular filtration rate due to hypertensive nephropathy. *Kidney Int* 2012; **81**: 86–93.
2. Mahajan A, Simoni J, Sheather SJ *et al.* Daily oral sodium bicarbonate preserves glomerular filtration rate by slowing its decline in early hypertensive nephropathy. *Kidney Int* 2010; **78**: 303–309.
3. Kraut JA. Effect of metabolic acidosis on progression of chronic kidney disease. *Am J Physiol Renal Physiol* 2011; **300**: F828–F829.

4. Jara A, Felsenfeld AJ, Bover J *et al.* Chronic metabolic acidosis in azotemic rats on a high-phosphate diet halts the progression of renal disease. *Kidney Int* 2000; **58**: 1023–1032.
5. De Brito-Ashurst I, Varagunam M, Raftery MJ *et al.* Bicarbonate supplementation slows progression of CKD and improves nutritional status. *J Am Soc Nephrol* 2009; **20**: 2075–2084.
6. Nath KA, Hostetter MK, Hostetter TH. Increased ammoniagenesis as a determinant of progressive renal injury. *Am J Kidney Dis* 1991; **17**: 654–657.
7. Pangburn MK. Spontaneous reformation of the intramolecular thioester in complement protein C3 and low temperature capture of a conformational intermediate capable of reformation. *J Biol Chem* 1992; **267**: 8584–8590.
8. Matsushita M. Ficolins: complement-activating lectins involved in innate immunity. *J Innate Immun* 2009; **2**: 24–32.
9. Pergola PE, Raskin P, Toto RD *et al.* Bardoxolone methyl and kidney function in CKD with type 2 diabetes. *N Engl J Med* 2011; **365**: 327–336.
10. Sebastian A, Frassetto LA, Sellmeyer DE *et al.* Estimation of the net acid load of the diet of ancestral preagricultural Homo sapiens and their hominid ancestors. *Am J Clin Nutr* 2002; **76**: 1308–1316.

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Protecting podocytes: how good do we need to be?

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Progression of many glomerular diseases has been firmly tied to a loss of podocytes, followed by a deterioration of glomerular architectural stability eventuating in segmental, and ultimately global, sclerosis. Recent studies have begun to clarify the nature of the autonomous (disease-independent) aspects of this process, as well as to explore mechanistically the 'unreasonable effectiveness' of angiotensin blockade in slowing glomerular disease progression. Quantitative monitoring of podocyte loss (e.g., to assess therapy) remains a challenge.

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The first indications of the importance of podocyte loss in the progression of kidney diseases came from animal models[1] and from cross-sectional studies of human disease.[2] The concept of podocyte 'insufficiency,' first clearly enunciated by Rennke and co-workers,[1] was further developed by Kriz and co-workers[3,4] into the current canonical model for the development of glomerular sclerosis: a loss of podocytes leads to 'bare areas' of glomerular basement membrane, which in turn lead to formation of synechiae to Bowman's capsule and then to segmental and finally global glomerular sclerosis.

More recently, ingenious animal models have been developed in the mouse[5] and the rat[6] that allow a much more precise titration of the degree of podocyte loss than was possible with previous models using podocyte toxins such as puromycin or adriamycin. This has allowed those aspects of glomerular disease progression due solely to reduced podocyte number to be studied in isolation. In some sense, this represents the intraglomerular analog of the subtotal nephrectomy model introduced decades ago to study the effects of reduced nephron number on renal disease progression. Also analogous to the subtotal nephrectomy model is the existence of an apparent threshold level of initial podocyte loss necessary for the subsequent development of an autonomous phase of podocyte loss.

Fukuda and colleagues[7] (this issue) now present a meticulous study of the natural history of the autonomous progression of podocyte loss and consequent glomerular sclerosis, and their modulation through angiotensin blockade. The study is based largely on the authors' technique of targeting podocytes for killing by diphtheria toxin (DT) in a transgenic rat model in which the human DT receptor (hDTR) is expressed under the control of the (human) podocin promoter.[6] The rodent analog of the hDTR does not bind DT, so wild-type rats are naturally resistant to its cytotoxic effects. Since, under the control of the podocin promoter, the hDTR is expressed only in podocytes of the transgenic rats, it is only these cells that take up and are killed by administered DT. Different levels of initial podocyte loss can be achieved by varying doses of DT. Other rat models (subtotal nephrectomy, puromycin) support the generality of Fukuda and colleagues'[7] findings in this model.

The authors estimate glomerular podocyte number by two complementary methods: the thick-and-thin-section method to determine the number of WT-1-positive nuclei (podocytes) per unit glomerular volume, and the fraction of glomerular tuft staining for GLEPP1, a podocyte-expressed protein. In addition, the authors use a surrogate indicator for urinary podocyte loss—mRNA for the podocyte proteins podocin and nephrin in the urinary sediment. These more novel indicators of progression are correlated with the incidence of glomerular sclerosis and quantitative proteinuria.

Fukuda and colleagues[7] show that a process of autonomous progression—based on ongoing podocyte loss—follows the initial loss of a threshold fraction of podocytes (greater than about 30%) induced by DT. Ongoing loss of podocytes 'destabilizes' the glomerulus, leading to glomerular sclerosis. Progression can be prevented by combined angiotensin blockade (with enalapril and losartan). Interestingly, the effect of angiotensin blockade on proteinuria is quite rapid (presumably reflecting improved podocyte function), whereas beneficial effects on urinary podocyte excretion (as reflected in urine podocin mRNA) are delayed by 2 weeks. If angiotensin blockade is stopped, the several indices of autonomous progression recommence.

It has long been known that angiotensin blockade is 'unreasonably effective' in preventing progression of many renal diseases. So what does this study add to our understanding of how angiotensin blockade prevents progression? The most significant finding is that prevention of autonomous

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