SNPs of metabolism, not stones

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DACARBOXYLATES ARE THE SUBSTRATES for oxidative phosphorylation, aka the Krebs cycle or citric acid cycle. Some of these substrates are synthesized within cells. However, epithelia with high metabolic needs (e.g., the small intestine and kidney) bring these substrates directly into cells. The plasma membrane of cells separates the outside world from the inside due to the hydrophobic core of this lipid bilayer. This latter property means that ions and solutes (for metabolism or otherwise) must be carried by a transporter protein through the environment. These transporters use either the energy of ATP hydrolysis or of the electrochemical gradient to accomplish this task. The Na\(^+\)-coupled dicarboxylate cotransporter (NaDC1, SLC13A2) is localized at the apical membrane of these epithelia (15, 19) and is responsible for renal and gut dicarboxylate as well as citrate (tricarboxylate) uptake. As expected, knocking out NaDC1 in mice causes elevated urinary citrate and other metabolites (7).

In the average 70-kg human, cellular metabolism generates \(\sim 70\) mmol of acid (\(\text{H}^+\)). Urinary buffers such as citrate are also known as titratable acids because they can take on this \(\text{H}^+\) from metabolism. Ironically, this means that \(\text{H}^+\) acceptors are considered “bases.” The renal proximal tubule absorbs 70–90% of urinary citrate, which enters the nephron from the filtered blood. Proximal tubule metabolism of citrate results in intracellular \(\text{HCO}_3^-\), which either buffers intracellular \(\text{pH}\) or is absorbed into the blood via the electrogenic \(\text{Na}^+\)-\(\text{HCO}_3^-\) cotransporter (NBCe1-A) (4, 8, 18), leading to transepithelial \(\text{NaHCO}_3\) absorption. Acidosis stimulates \(\text{HCO}_3^-\) absorption but is also known to increase the amount of NaDC1 mRNA and protein (2) as well as NaDC1 activity (1, 6). Since citrate metabolism leads to \(\text{HCO}_3^-\) production and increases blood \(\text{HCO}_3^-\) concentration, acidosis decreases urinary citrate (increased NaDC1 activity \(\rightarrow\) a decrease in citrate in the ultrafiltrate). Alkalosis reduces transepithelial \(\text{HCO}_3^-\) absorption by reducing basolateral \(\text{HCO}_3^-\) exit (via NBCe1-A). Alkalosis causes a \(\text{pH}\) increase of the proximal ultrafiltrate, and this elevated luminal \(\text{pH}\) decrease citrate absorption and enhances urinary citrate excretion (3, 9, 11, 20). Alkalosis (in opossum kidney cells) does seem to decrease NaDC1 cotransport of citrate but not succinate (1). This observation seems consistent: a decreasing \(\text{H}-\text{citrate}\) concentration (preferred dicarboxylate) and increasing citrate\(^3^-\) concentration (tricarboxylate) as \(\text{pH}\) increases beyond 6.4 (\(\text{pK}_a\) 6.4; \(\text{H}-\text{citrate}\) \(\rightarrow\) \(\text{H}^+\) + citrate\(^3^-\)). This \(\text{pH}\) shift, thus results in less of the transported substrate (H-citrate\(^2^-\)). That is, the proximal tubule responds by limiting citrate absorption, resulting in higher urinary citrate (3, 7).

Low urinary citrate (hypocitraturia) is associated with kidney stones (nephrolithiasis) (12) as is acidosis (3, 17). In addition, as above, acidosis increases apical NaDC1 protein (2) as well as activity (citrate transport) (1). In fact, one of the most common treatments for physiologically dissolving uric acid kidney stones is to alkalize the urine, usually with K-citrate or possibly lemon juice (high citrate). Medically, calcium stones are also often treated by giving K-citrate. While from an acid-base prospective this treatment seems a bit counterintuitive, there is an additional role of citrate per se.

Citrate can complex ionized \(\text{Ca}^{2+}\). This means that there is a dual role for citrate: \(\text{H}^+\) buffer and \(\text{Ca}^{2+}\) chelator. For Ca-oxalate stones, the chelation role is critical. If free-ionized \(\text{Ca}^{2+}\) is complexed, the \(\text{Ca}^{2+}\) cannot complex with oxalate (only slightly soluble and therefore stone initiating). Accordingly, high urine citrate should decrease the ability of Ca-oxalate stones to initiate (protective against stones). Similarly, low urine citrate (hypocitraturia) would result in more ionized \(\text{Ca}^{2+}\) and increase the likelihood that a stone could form.

With the cloning of NaDC1 (14), it was hypothesized that this cotransporter might be associated with controlling urinary citrate, and thus urinary \(\text{pH}\), and subsequently the ability to form urinary stones. In fact, one allele (I550V) has been implicated as causative in recurrent stone formers (13).

Pajor and Sun (16) have examined the protein and physiological impact of allelic variation (single nucleotide polymorphisms; SNPs) in NaDC1 (SLC13A2). After examining the published and database (http://www.ncbi.nlm.nih.gov/projects/SNP) SNPs, they have determined the biophysical impact of the coding SNPs (cSNPs) on NaDC1 function (see Table 1).

While these data are of themselves interesting for the function of NaDC1, there is a larger implication. Due to decreased protein and function, all of these NaDC1-SNPs are expected to decrease intestinal and renal citrate absorption, thereby increasing urinary citrate. Importantly, since nephrolithiasis is associated with decreased urinary citrate, these allelic variations would not be causative of nephrolithiasis and perhaps even preventative.

Is the case closed for NaDC1 being causative of nephrolithiasis? Probably not. As we have learned with many rare disorders, the rare allelic variations (cSNPs) are typically not represented in these population databases. What Pajor and Sun’s work (16) does indicate is that NaDC1 mutations are unlikely to be a common cause of hypocitraturia and nephrolithiasis. These results do illustrate that for NaDC1 to result in hypocitraturia would require either increases in NaDC1 protein expression or hyperactivity of the transporter. Thus far, such increases in NaDC1 protein or activity have not been reported.

However, as with oxalate transport (5, 10), decreased NaDC1 activity under certain circumstances could result in low urinary citrate. For example, decreased intestinal absorption of citrate (through decreased gut NaDC1 activity) would tend to
decrease blood citrate concentration. This reduced gut citrate absorption in turn could lead to overall reduced urinary citrate, i.e., hypocitraturia, which would be intestinal not renal in origin. On the other hand for citrate (as an intermediate of metabolism), other metabolic defects would likely have to exist simultaneously. As indicated, citrate can also be produced through metabolism, other metabolic defects would likely have to exist simultaneously. As indicated, citrate can also be produced through metabolism, other metabolic defects would likely have to exist simultaneously. As indicated, citrate can also be produced through metabolism, other metabolic defects would likely have to exist simultaneously. As indicated, citrate can also be produced through metabolism, other metabolic defects would likely have to exist simultaneously. As indicated, citrate can also be produced through metabolism, other metabolic defects would likely have to exist simultaneously.

As with many diseases and pathophysologies, single gene mutations (initially thought to explain many human diseases) seem to be the exception rather than the rule. Our cells perform complicated functions, with most solutes having multiple roles as evidenced with citrate. Moreover, these cellular units form tissues with further physiological complexity. This complexity is particularly true for the “simple” epithelium of the nephron. The work of Pajor and Sun (16) gives a strong indication that single and common allelic variations of NaDC1 (SLC13A2) are unlikely to directly cause hypocitraturia and nephrolithiasis. Now the more difficult task rests on the physiology community to unravel these complex disorders.

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REFERENCES