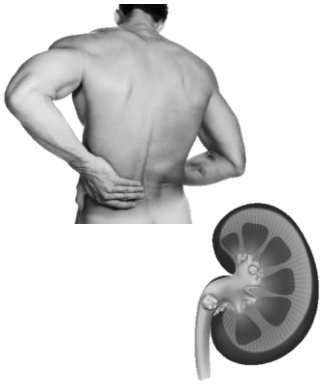
Medicine Grand Rounds
25th September 2015
University of Texas Southwestern Medical Center

A "Garden Variety" Case of Kidney Stones

Viewed by a Clinical Physiologist

Orson W, Moe, M.D.



Orson Moe has disclosed that he has served on Advisory Boards or performed Consultations for Abbvie, Ardelyx, Allena, Genzyme-Sanofi, and Takeda in the last 5 years. There will not be discussions of off-label use of any products.

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Research and Clinical Interests: Epithelial membrane transport and metabolism. Nephrolithiasis. Mineral complications of CKD. Acid-base and electrolyte disorders.

Purpose & Overview: Using a very common cause of kidney stones as a starting point, an analysis of clinical physiology and pathophysiology is presented that espouse the view that physician are human biologists and the pathophysiologic approach to disease in fact renders the practice more interesting and also improves patient care.

Education Objectives:

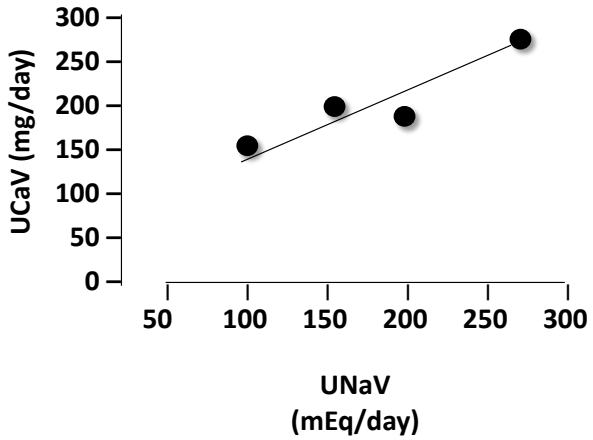
1. Nephrolithiasis is not a diagnosis *per se* but a presentation of a diverse number of underlying conditions.
2. Understand how a calcium phosphate-based skeleton can have profound effects on many aspects of human physiology.
3. Understand the underlying biology for the dependence of urine calcium excretion on dietary sodium intake and the concept of physiologic hypercalciuria.
4. Understand the dual role of citrate in urine as a base and as the major chelator of calcium and conditions when there is conflict between these roles which forms the basis of dietary protein induced hypocitraturia.
5. Extreme ranges of physiology can sometimes lead to undesirable phenotypes called “disease”.

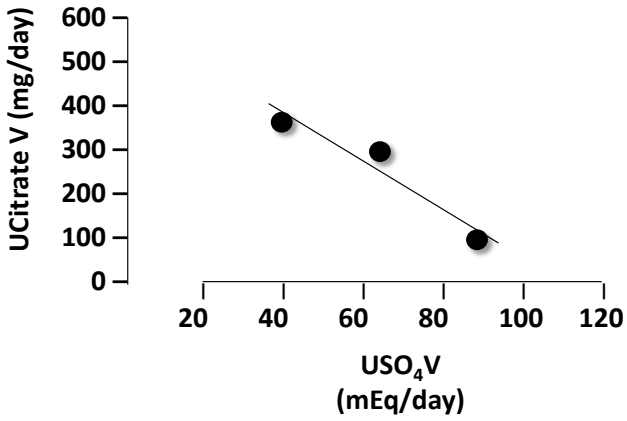
Patient

37 year old Caucasian veteran (retired captain)
Left-sided renal colic with spontaneous passage
More stones visible on plain X-Ray
Healthy otherwise
Served in the gulf war. Retired captain of the US Army
Physical exam- healthy young male
Serum chemistry- all normal

24 hr urine stone risk on random diet

Parameter			Desired value to reduce relative stone risk
Calcium	278 mg	6.95 mmoles	<250 mg/day (6.3 mmoles/day)
Oxalate	32 mg	0.36 mmoles	<45 mg/day (0.51 mmoles/day)
Uric Acid	689 mg	4.10 mmoles	<700 mg/day (4 mmoles)
Citrate	125 mg	0.66 mmoles	>320 mg/day (1.7 mmoles/day)
pH	5.6		5.5 - 7.0
TV	1.98 L		> 2 L/day
Na	267 mEq		< 200 mEq/day
K	30 mEq		> 50 mEq/day
SO_4	86 mEq		< 30 mmol/day (<60 mEq/day)
P	1220 mg	39 mmoles	< 1100 mg/day (11.6 mmoles/day)
Mg	65 mg	2.7 mmoles	> 60 mg/day (2.5 mmoles/day)
Cl	251 mEq		<200 mEq/day
NH_4	79 mEq		< 45 mEq /day
Creatinine	2050 mg	18.1 mmoles	





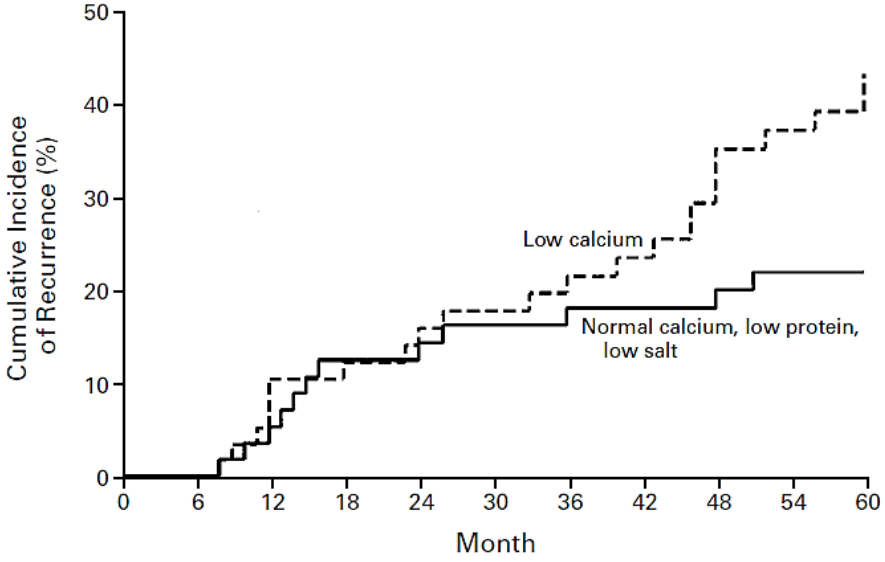
The two major urinary stone risk factors- hypercalciuria and hypocitraturia varied as the patient made dietary changes in sodium and acid (protein) intake.

Stone analysis: Calcium oxalate

Patient

Diagnosis:	Calcium oxalate nephrolithiasis
Pathogenesis:	Hypercalciuria Hypocitraturia
Etiologies:	Salt-induced hypercalciuria Protein-induced hypercalciuria Acid-induced hypocitraturia
Treatment:	Dietary modification

A diet low in protein and salt has indeed been proven in a randomized controlled trial to reduce stone recurrence (Borghi 2002).



No. at Risk											
Low calcium	60	59	51	49	46	44	42	39	33	31	28
Normal calcium, low protein, low salt	60	57	53	47	46	45	44	43	41	40	40

Borghi et al. NEJM 2002

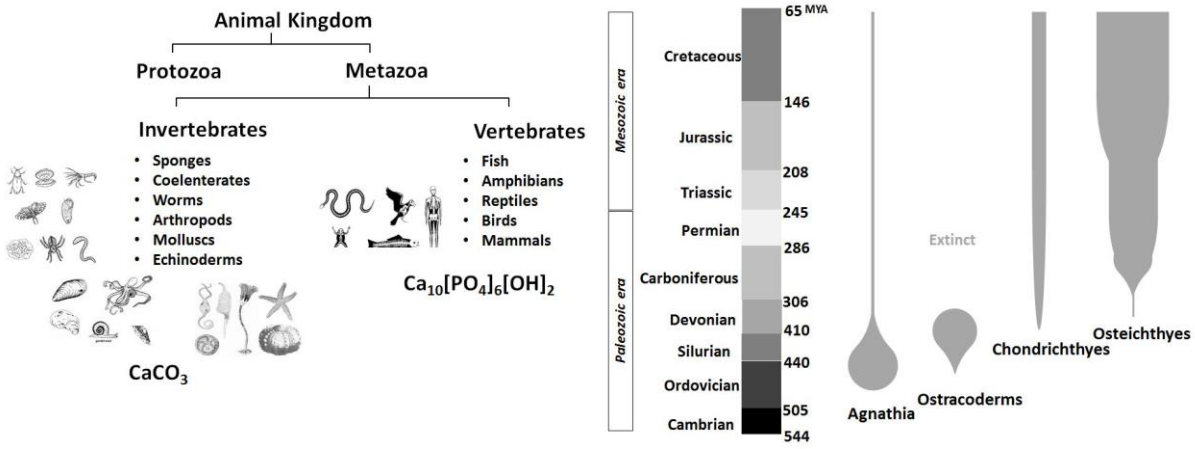
Salt and protein restriction should reverse the hypercalciuria and hypocitraturia and dramatically reduce stone risk. No further investigation and therapy is required. In our stone clinic's experience, this is indeed the case in most of these patients.

On the surface, the right laboratory tests were ordered, and "evidence-based" therapy is prescribed. This appears to be all a physician need to practice. In this presentation, an alternative view is presented where the practitioner assumes the role of a clinical physiologist.

CALCIUM

Apatite Endoskeleton

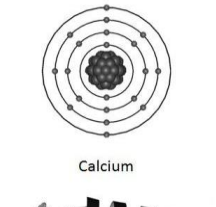
Calcium ions (Ca^{2+}) impact every aspect of cellular and organ function. Calcium is a constituent of the skeleton, which has evolved over more than 500 million years. Some invertebrates have primarily exoskeletons (outside the body) while vertebrates have endoskeletons (inside the body). There is a complete switch from $CaCO_3$ to $Ca_{10}[PO_4]_6[OH]_2$ (apatite) when vertebrates evolved. The oldest vertebrate skeletons are the jawless fish (agnathia). The ostracoderms (armored jawless fishes of the Paleozoic Era, 500 to 250 million years ago) are extinct with only fossil records. DNA data on the ostracoderms would have been valuable. The cartilaginous and the true bony fish populate the present day ocean.



Another important landmark for the skeleton was the water-to-land migration in the Devonian period (about 420 to 360 million years ago). Terrestrial existence imparted significant demands on physiology, many of which impacted on the skeleton and renal function. A few examples:

- Air breathing
- Thermoregulation
- Loss of buoyancy – weight-bearing skeleton
- Fast and famine physiology – energy storage
- Sodium and water conservation
- Crystallization of urine

Another salient and unique feature of the calcium cation is its ability to interact with biologic molecules such as proteins. An incomplete but rather representative list of intracellular and extracellular calcium binding proteins is shown. There is more than one type of calcium-binding motifs and many of these proteins have been around since the evolution of single cell life forms (Williams 1976, Yanez 2012).

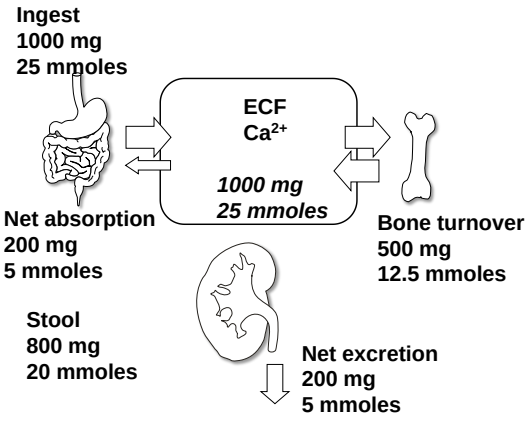


Calcium binding proteins

Yanez et al. *Adv Exp Med Bio* 2012

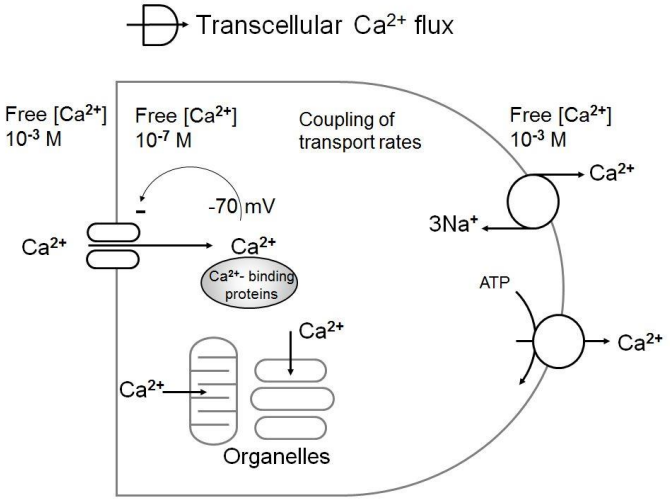
Intracellular	Extracellular
EF hand	EF hand
Calmodulin family	Osteonectin family
S100 family	EGF-like domains
Parvalbumin family	Factor VII, IX, X
Calcineurin	Proteins C and S
Neuronal Ca sensor	Fibrillin
Non-EF hand	Notch/delta receptors
Calreticulin	LDL Receptor
Calnexin	γ-COOH gltutamic acid rich
Grp94	Factor II, VII, IX, X
BiP/Grp78	Protein C and Z
Protein disulfide isomerase	Osteocalcin
Calsquestrin	Matrix GLA
Annexins	Periostin
C2 domain proteins	Cadherin
Protein kinase C	Classical cadherin
Synaptotagmin	Protocadherin
Phospholipase C	Atypical cadherin
Phospholipase A	C type lectin domain
	Selectins
	Mannose receptor family
	ICAM3
	Collectins
	GPCRs
	Ca sensing receptor
	Metabotropic glutamate receptors
	GABA receptors

Internal calcium balance is between the extracellular fluid volume and two compartments- intracellular space and the large bony skeleton. External calcium balance is maintained mainly by the intestine and the kidney. Internal calcium balance is between the extracellular fluid volume and two tissue compartments – intracellular and the large bony skeleton. These three pools of calcium in the body (bone, extracellular and intracellular) have vastly different concentrations and total amount.



	Three pools of Ca^{2+}		
	Bone	ECF	ICF
$[Ca^{2+}]$	-	10^{-3}	10^{-7} M
Ca^{2+} content	1.3×10^6 ~ 30,000	10^3 ~25	<0.1 mg <1 mmole

Moving calcium within different body compartments can be challenging; the task is fulfilled by many calcium transporting epithelia. One has to move calcium between two ECF compartments with $[Ca^{2+}]$ in mM concentrations while traversing the intracellular compartment with $[Ca^{2+}]$ at 100 nM. Several mechanisms are in place to forestall a “calcium catastrophe”. 1. The rise in intracellular $[Ca^{2+}]$ per se inhibits apical calcium entry. 2. Intracellular $[Ca^{2+}]$ is buffered by calcium binding proteins in high concentrations. 3. Calcium can be transiently sent into organelles such as mitochondria and endoplasmic reticulum. 4. There is tight cross-talk and coupling of apical calcium entry and basolateral calcium exit. With this type of cell, colossal amounts of calcium can be moved across the cell without affecting cell health (Hoenderop 2005).

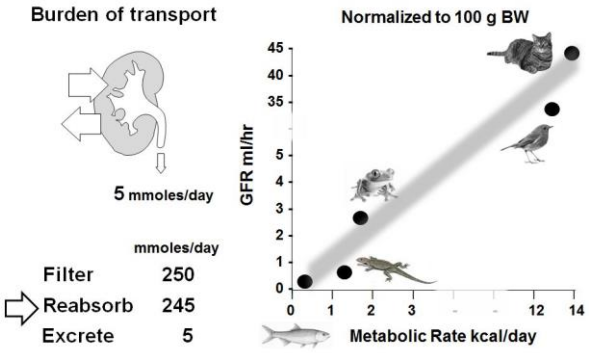


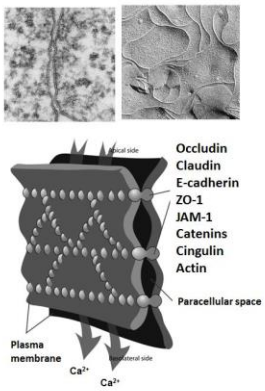
Transcellular Ca^{2+} flux in epithelia

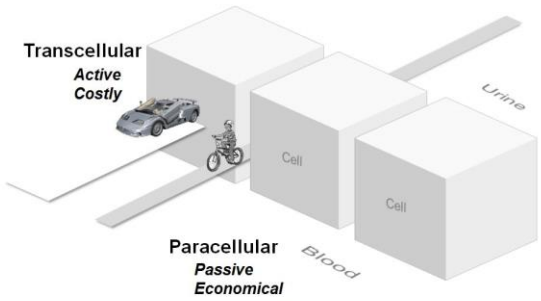
	nmoles/cm²/hr
Mammalian distal convoluted tubule	**200**
Crustacean carapace	**5,000**
Avian uterus	**20,000**

Non-mammalian species actually have much higher transcellular calcium transport than mammals. In crustaceans immediately after moulting, the exoskeleton has to be re-calcified in a very short time (Neufeld 1993). In birds, the eggs in the oviduct have to be covered in calcium carbonate (Panheleux 1999). In either of these examples, the requirement for calcium movement is much greater than the mammalian kidney. Thus transcellular transport of calcium is much better developed in these non-mammalian species. It is true that mammals do not moult or lay eggs, however, there are other demands in mammals.

In the course of evolution from lower to higher vertebrates, the progressively higher metabolic rates (e.g. as mandated by homeothermy), required higher glomerular filtration rates (GFR). The rising GFR in a filtration-reabsorption nephron requires higher and higher reabsorption of all solutes including calcium. The burden of transport is therefore not the excreted calcium but rather the reabsorbed calcium. When the demand for calcium transport rose during mammalian evolution, the solution was not to "reverse evolve" back to the epithelia of crustaceans or birds. New mechanisms of transport developed. When one cannot send adequate amount of calcium through the cell (transcellular transport), one sends the calcium around the cell (paracellular transport).

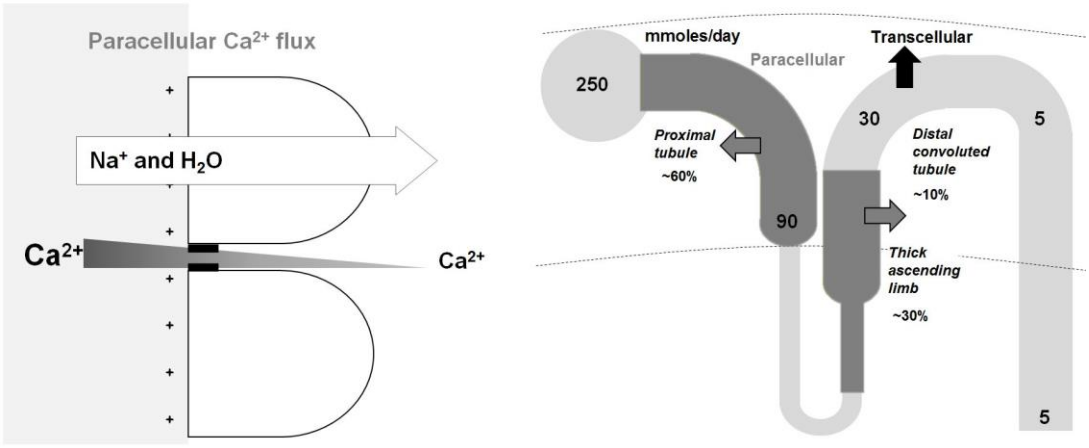




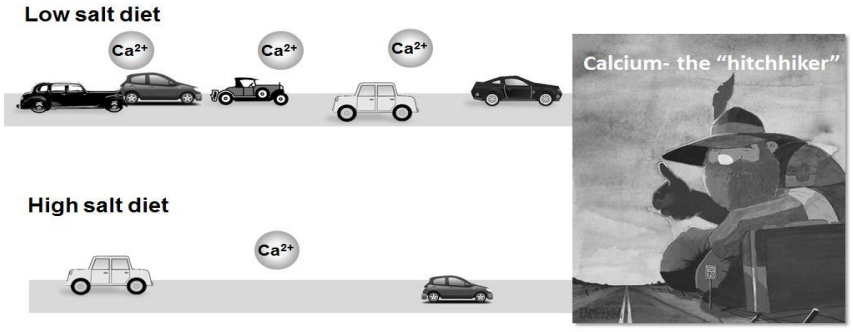


While transcellular transport "costs" the cell energy and proteins dedicated to moving calcium and protecting the cell from calcium overload, paracellular transport uses different pathways. Mainly, it uses passive driving forces and it involves the participation of paracellular proteins, especially occludins and claudins. These proteins form pores that confer permeability and selectivity to certain ions and solutes allowing them to traverse the space around the cell.

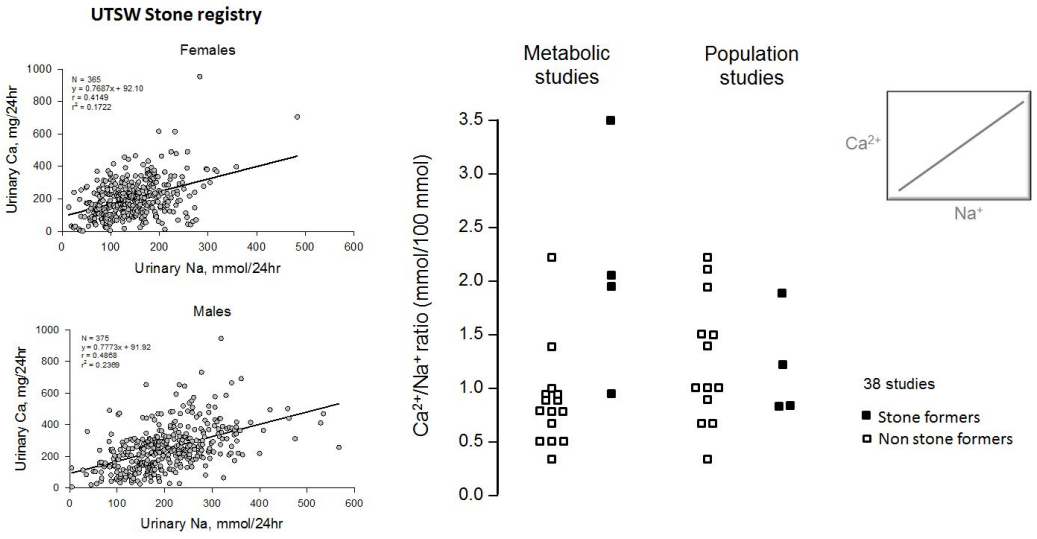
There are two elements that permit paracellular transport- permeability (conferred by the paracellular proteins) and a driving force that pushes them through. In general, diffusive transport is driven by a chemical gradient, electrical gradient, or both. In the case of paracellular calcium transport in the kidney, a chemical driving force predominates in the proximal tubule and an electrical driving force predominates in the thick ascending limb. In concert, the proximal tubule and the thick ascending limb are responsible for majority of calcium reabsorption and all of the paracellular transport leaving transcellular transport to be conducted by the distal convoluted tubule.



Therefore paracellular calcium transport in the kidney can be compared to “hitchhiking”. When there is plenty of paracellular sodium transport (e.g. under conditions of low salt intake), there will be plenty of calcium reabsorption by the kidney. When there is reduced paracellular sodium transport (e.g. under conditions of high salt intake), paracellular calcium transport is reduced and hypercalciuria can ensue.



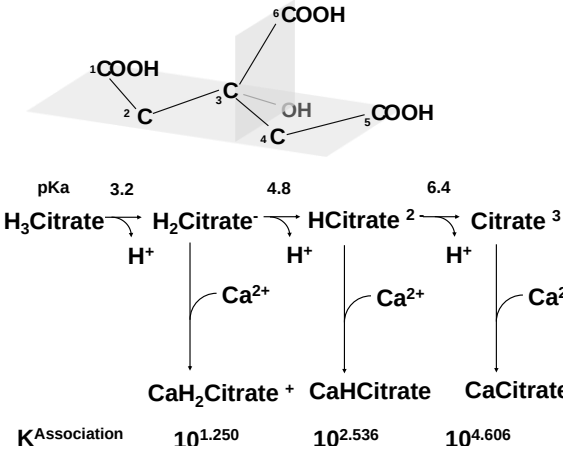
Hypercalciuria is a normal physiologic consequence of high salt intake and does not represent a disease state. In humans (both non stone formers and stone formers), there is a linear relationship between urinary calcium and sodium excretion



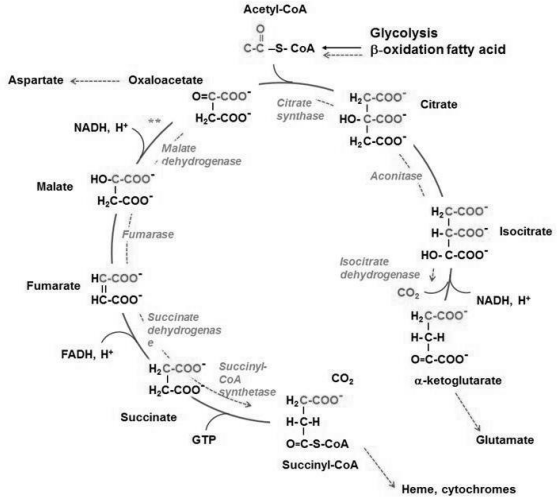
CONCLUSION: High dietary sodium intake begets hypercalciuria

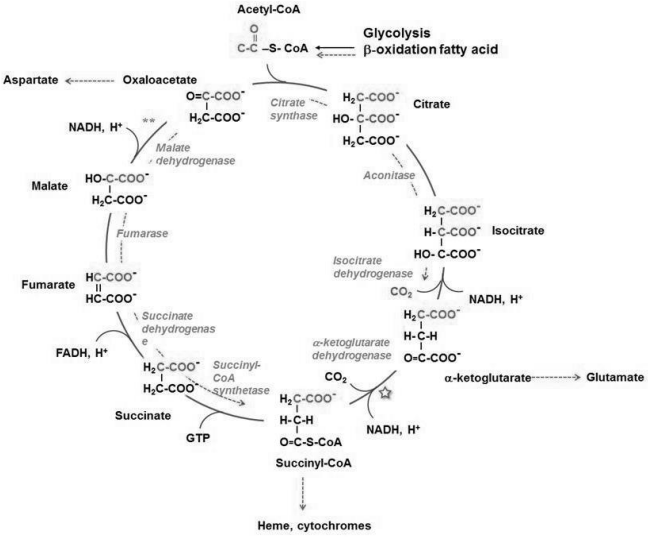
Citrate

Citrate is a tricarboxylic six carbon anion that exists as a polytopic acid in equilibrium with H^+'s to form its conjugate citric acid. The pKa values that best approximates the urinary environment are listed in the Figure. In the plasma with an ambient pH of 7.4, citrate exists mostly as citrate^{3-} ~ 100 μM. In the urine, citrate concentration is much higher in mM and is distributed between divalent and trivalent citrate. Citrate chelates a variety of cations with different stabilities.



The classic functions of citrate are: supply of reducing equivalents, source of acetyl groups, and allosteric regulator of enzymes. In the urine, citrate's ability to chelate calcium with high affinity and the high solubility of the complex is the most fundamental reasons for citrate's presence in urine in vertebrates. The association constants (KA's) of 3 different species of citrate with calcium are shown in the Figure.





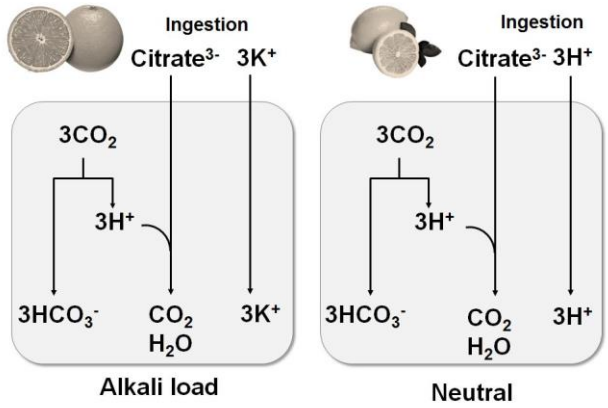
The regulation of urinary citrate excretion was described years before Hans Krebs published the components of the famous cycle. The current model of the evolution of the TCA cycle favors a union of biosynthesis and bioenergetics. The original TCA cycle was more of TCA horseshow for synthesis of glutamate, heme, and cytochromes. The introduction of α-keto-glutarate dehydrogenase transformed the horseshow into a circle and from purely synthetic into a bioenergetic network. Some has cited the TCA cycle as an evolutionary conundrum that defies explanation by natural selection. How could natural selection construct such a complicated structure when the intermediate stages do not confer survival or reproductive fitness? In the TCA cycle, the intermediary stages

are each useful for different purposes, with the totality in its final completed form being an example of opportunistic usage of existing pathways.

In the simplest paradigm, one can summarize that citrate has two role in the urine (Moe 2006)

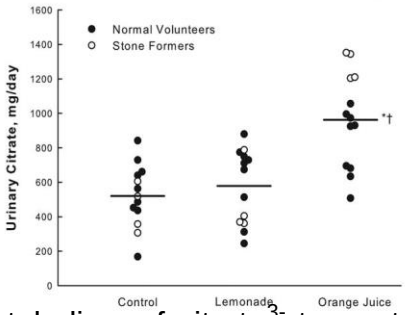
1. Major urinary base and
2. Chelator of calcium.

As a urinary base



Parameter	Control	Lemonade	Orange Juice	ANOVA *P*
Total volume (L/d)	2.55	2.61	2.51	0.51
Ca^{2+} (mg/d)	159	154	146	0.32
Cr (mg/d)	1589	1565	1585	0.81
Na^+ (mEq/d)	70	58	66	0.28
K^+ (mEq/d)	38	39	85	<0.0001
Ammonium (mEq/d)	37	36	23	<0.0001
NAE (mEq/d)	55	49	17	<0.0001

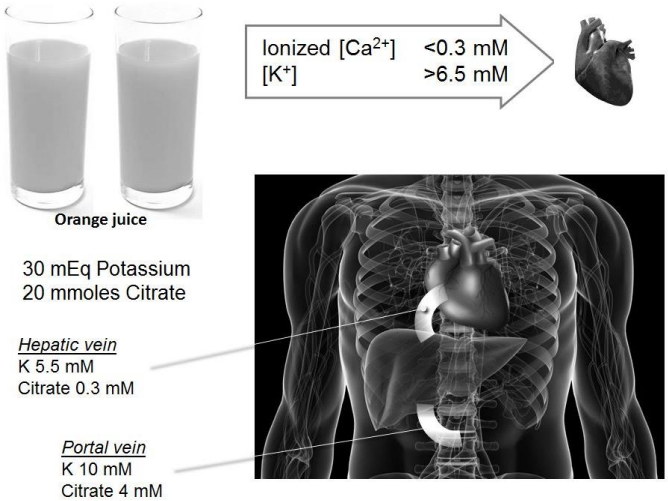
Odvina et al. *JASN* 2007

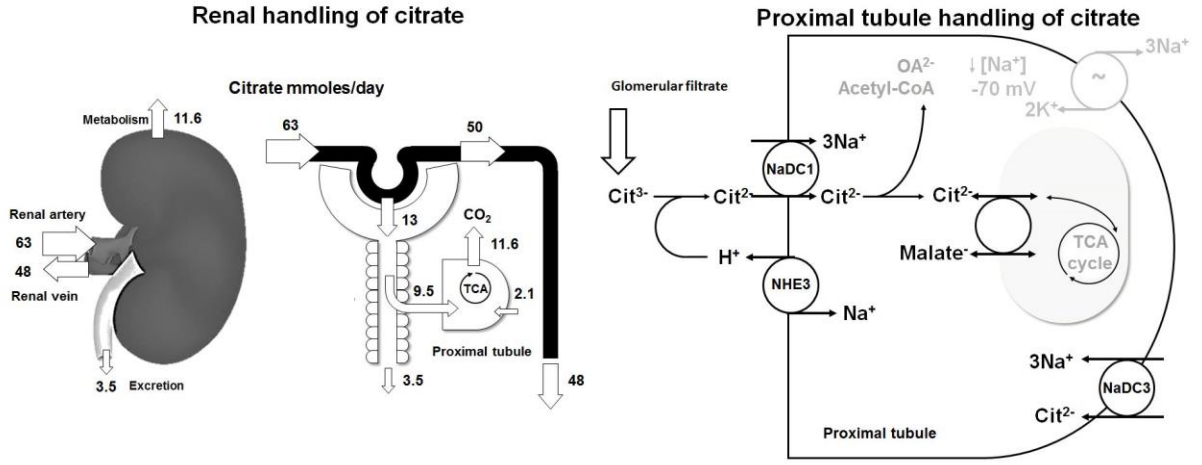


Citrate is not directly metabolized to bicarbonate but metabolism of citrate^{3-} to neutral CO_2 and water results in consumption of one H^+ which was derived from metabolic CO_2, thus leaving behind one HCO_3^-; hence the equivalence of citrate and HCO_3^- (Simpson 1983)

One good illustrative example is that ingestion of lemonade (H_3Citrate) which is neutral in acid-base terms, does not alter urinary acid excretion. In contrast, the ingestion of orange juice, which is largely K_3Citrate, leads to decreased renal net acid excretion and increased citraturia.

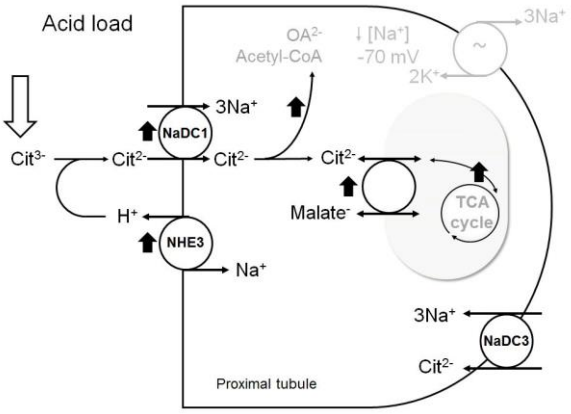
The rapid and complete metabolism of citrate is critical because the bolus of potassium and citrate from a large ingestion of orange juice can conceivably lead to life-threatening combination of hypocalcemia and hyperkalemia. Therefore, the hepatic metabolism of citrate and the temporary housing of potassium are critical in maintain life when our foraging ancestors binged on a large bolus of fruits with high K_3Citrate.



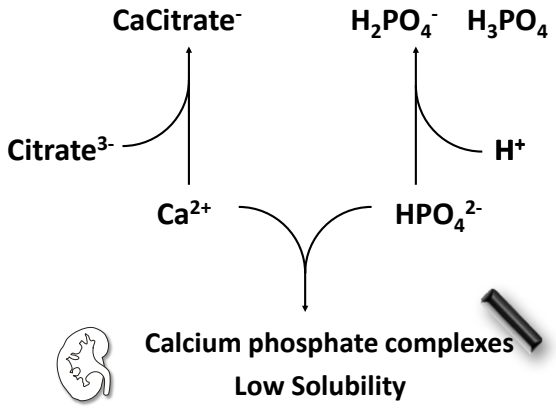


The renal handling of citrate is shown in the Figure. The renal A-V difference in citrate is due to both renal metabolism and urinary excretion (Neith 1966). The proximal tubule is the site where all the handling occurs. The proximal tubule normally puts very little citrate into the blood. In fact, it usually takes up citrate from the glomerular filtrate and the blood and metabolizes it to CO_2. Hence, when one considers proximal tubule citrate handling, it is a combination of transport and metabolism (Hamm 1990).

When the kidney senses the imposition of an acid load, the proximal tubule responds by limiting the excretion of citrate in urine which is adaptive as urinary citrate excretion is tantamount to base loss. This is a sophisticated and well-coordinated physiologic reaction involving the up-regulation of luminal uptake, mitochondria uptake, cytoplasmic and mitochondrial metabolism of citrate. Much of this work was done in UTSW by Preisig and Alpern, and colleagues.

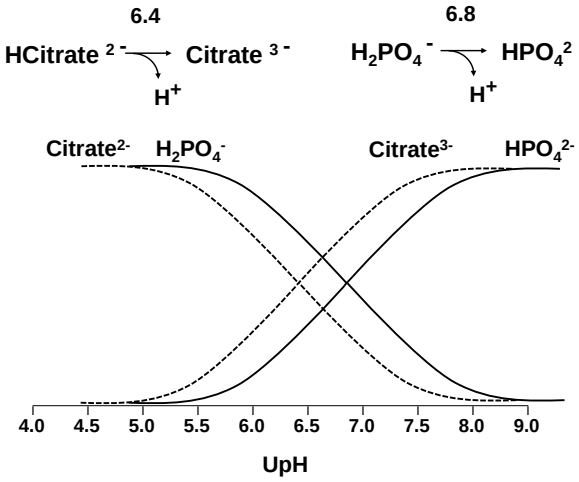


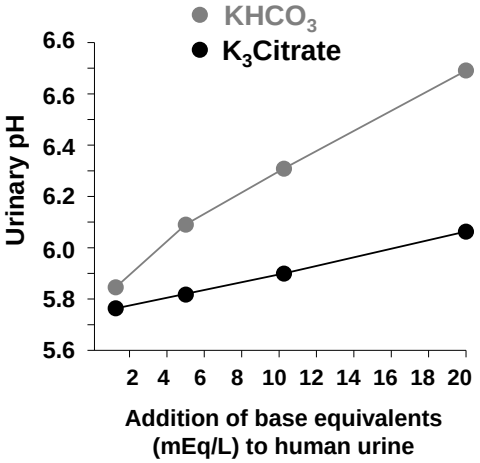
As a calcium chelator



The second function of citrate in the urine is to chelate calcium in a soluble complex so calcium does not react with phosphate and oxalate to precipitate in the urine. With an apatite skeleton that is metabolically active, there is a constant turnover that mandates a minimal amount of calcium and phosphate to be present in the urine. This actually conflicts with the water conservation required to live on land.

The kidney deals with this with a “hijack” strategy. The high urinary citrate (mM) will chelate the calcium, and the low urinary pH will convert bivalent phosphate to monovalent phosphate which is much less likely to form insoluble calcium phosphate complex. If one compares the pKa of citrate (6.4 divalent vs. trivalent) and phosphate (6.8 monovalent vs. divalent), one envision that the citrate titration curve just trails behind the phosphate one. When urine pH rises which enhances the risk of calcium phosphate precipitation with divalent phosphate, divalent citrate also gets titrated to trivalent citrate which will chelate the calcium so the divalent phosphate will lack a partner to bind.





Increasing base intake

Acid

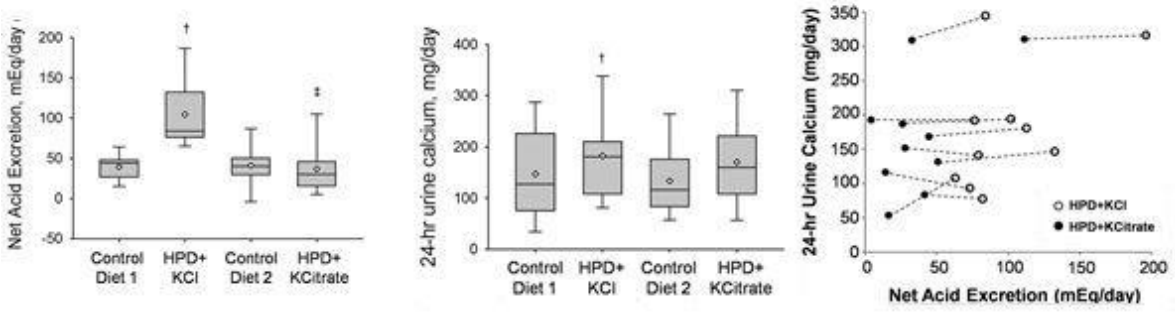
	Day 1	*2*	*3*	*4*	*5*	*6*
pH	5.8	5.7	5.9	6.0	6.1	6.2
NH_4	34	41	30	28	25	22
Titratable acid	17	22	19	16	14	12
SO_4	50	63	50	49	52	51
K	56	59	60	71	79	85
HCO_3	1.3	1.0	1.5	2.0	2.5	2.9
Citrate	8	2	6	9	12	15
Net acid excretion	42	60	41	33	25	17
NAE/SO_4	0.8	1.0	0.8	0.7	0.5	0.3

Therefore when one desires to add base into the urine, the most appropriate species is not bicarbonate, but rather citrate. For the same amount of base added to urine, the excretion of citrate will raise the urine pH much less than bicarbonate. This is seen in real life when alkali intake is varied over a small range. The increased urinary base excretion is primarily citrate and not bicarbonate. Bicarbonaturia ensues when the subject is given a very large base load such as sodium bicarbonate assault by an investigator.

In the event of an acid load, there is a conflict between acid-base balance and urinary calcium chelation. Quite surprisingly, the tubules devote priority to acid-base balance and put the urine at risk for calcium precipitation.

CONCLUSION: High acid (protein) intake begets hypocitraturia

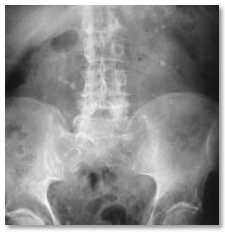
In addition to hypocitraturia, there is an independent effect of protein loading on urinary calcium excretion. The acid content of protein demands buffering by bone which releases calcium and increases calciuria. In addition to the acid content, there are other constituents of protein (other than acid) that can cause hypercalciuria. The best illustration of this phenomenon was shown by Maalouf and colleagues. When the acid content of protein was neutralized by potassium citrate, it did not abolish the hypercalciuria. These non-acid generators of hypercalciuria are yet to be determined (Maalouf 2011).



Kidney Stones

Now, one can revisited our patient who developed common calcium oxalate stones due to dietary salt and protein excess. One approach is to use a diagnostic algorithm to identify diagnoses and treatment options that are billable.

AN



How about the captain?

- **No significant intrinsic disease**
- **Extremes of physiology**

	ICD-10
Renal calculus	**N20.0**
Hypercalciuria	**E83.52**
Hypocitraturia	**-**
Inappropriate diet	**V69.1**

Rx: Dietary counselling & modification

Follow-up:

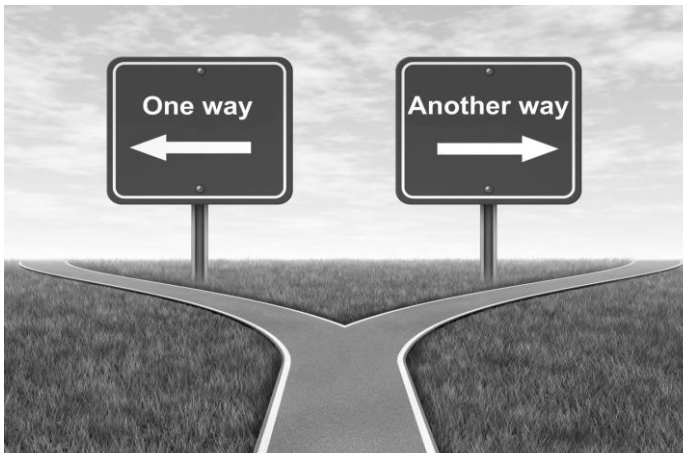
1. **24 hr urine**
2. **KUB**
3. **Clinical events**

Level 4 New Patient Office Visit (99204)

45 minutes face-to-face with the patient
Medicare allowable: $166
2.43 work RVUs.

Documentation:

1) Comprehensive History
2) Comprehensive Exam



An alternative approach is to view this as an example of the biology of harboring an apatite skeleton.

- Turnover of the calcium phosphate skeleton mandates a minimal amount of calcium and phosphate in the urine, which is in direct conflict with the low urine volume required for terrestrial existence
- To prevent calcium phosphate precipitation in the urine, to tactics are employed to keep calcium from forming in soluble complexes with bivalent phosphate.
- In the presence of low urine pH, bivalent phosphate is titrated to monovalent phosphate, which is much less likely to bind calcium.
- Citrate has dual roles in the urine- it is utilized as the main urinary base and at the same time, sequesters calcium in a soluble form.
- The ingestion of a high protein (acidogenic) diet leads to physiologic hypocitraturia.
- The high glomerular filtration rate demands a large amount of filtered calcium to be reabsorbed; which exceed the capacity of the transcellular transport.
- A paracellular pathway for calcium reabsorption solves the problem but places calcium reabsorption at the mercy of sodium reabsorption.
- A low salt diet, which prevailed when terrestrial vertebrate evolved, leads to high sodium reabsorption which promotes paracellular calcium reabsorption. However, the modern day high salt diet limits the paracellular calcium reabsorption which leads to renal calcium leak.
- A high salt and high protein diet leads to sodium-induced physiologic hypercalciuria and a high protein (acid) diet leads to physiologic hypocitraturia.
- The combination of two extreme physiologic states promotes calcium stones.

Citations

Borghi L, Schianchi T, Meschi T, Guerra A, Allegri F, Maggiore U, Novarini A. Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. *N Engl J Med*. 346:77-84. 2002

Hamm LL. Renal handling of citrate. *Kidney Int.* 38:728-735,1990

Hoenderop JG, Nilius B, Bindels RJ. Calcium absorption across epithelia. *Physiol Rev.* 85:373-422. 2005

Maalouf NM, Moe OW, Adams-Huet B, Sakhaee K. Hypercalciuria associated with high dietary protein intake is not due to acid load. *J Clin Endocrinol Metab.* 96:3733-3740, 2011.

Moe OW, Preisig PA. Dual role of citrate in mammalian urine. *Curr Opin Nephrol Hypertens.* 15:419-424, 2006.

Neufeld DS, Cameron JN. Transepithelial movement of calcium in crustaceans *J Exp Biol.* 184, 1–16, 1993.

Nieth, H., and Schollmeyer. P. Substrate-utilization of the human kidney. *Nature London* 209: 1244-1245, 1966.

Panheleux M, Kaèlin O, Gautron J, Nys Y. Features of eggshell formation in guinea fowl: kinetics of shell deposition, uterine protein secretion and uterine histology. *British Poultry Science* 40: 632–643, 1999.

Simpson DP. Citrate excretion: a window on renal metabolism. *Am J Physiol.* 244:F223-234. 1983

Yanez M, Gil-Longo J, Campos-Toimil M. Calcium binding proteins *Adv Exp Med Bio* 740:461-82.2012.

Williams RJP. Calcium chemistry and its relations to biological function. *Symp Soc Exp Biol* 30:1-17, 1976.