Received: 22 February 2024 | Revised: 8 April 2024 | Accepted: 22 April 2024

DOI: 10.1111/apha.14155

ACTA PHYSIOLOGICA

REVIEW ARTICLE

# Effects of thiazides and new findings on kidney stones and dysglycemic side effects

**Matteo Bargagli**[1,2,3] | **Manuel A. Anderegg**[1,2,3] | **Daniel G. Fuster**[1,2,3]

[1]Department of Nephrology and Hypertension, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

[2]Swiss National Centre of Competence in Research (NCCR) Kidney.CH, University of Zürich, Zürich, Switzerland

[3]Department for Biomedical Research (DBMR), University of Bern, Bern, Switzerland

**Correspondence**
Daniel G. Fuster, Department of Nephrology and Hypertension, Inselspital, Bern University Hospital, University of Bern, Freiburgstrasse 15, Bern 3010, Switzerland.
Email: daniel.fuster@unibe.ch

**Funding information**
Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung; Swiss National Centre of Competence in Research Kidney Control of Homeostasis; Inselspital, Universitätsspital Bern; Novartis Foundation for Medical-Biological Research

**Abstract**

Thiazide and thiazide-like diuretics (thiazides) belong to the most frequently prescribed drugs worldwide. By virtue of their natriuretic and vasodilating properties, thiazides effectively lower blood pressure and prevent adverse cardiovascular outcomes. In addition, through their unique characteristic of reducing urine calcium, thiazides are also widely employed for the prevention of kidney stone recurrence and reduction of bone fracture risk. Since their introduction into clinical medicine in the early 1960s, thiazides have been recognized for their association with metabolic side effects, particularly impaired glucose tolerance, and new-onset diabetes mellitus. Numerous hypotheses have been advanced to explain thiazide-induced glucose intolerance, yet underlying mechanisms remain poorly defined. Regrettably, the lack of understanding and unpredictability of these side effects has prompted numerous physicians to refrain from prescribing these effective, inexpensive, and widely accessible drugs. In this review, we outline the pharmacology and mechanism of action of thiazides, highlight recent advances in the understanding of thiazide-induced glucose intolerance, and provide an up-to-date discussion on the role of thiazides in kidney stone prevention.

**KEYWORDS**

diabetes mellitus, glucose, intolerance, kidney stone, nephrolithiasis, thiazide

## 1 | INTRODUCTION

Before the advent of modern pharmacology, variably effective treatments for volume overload, historically referred to as "dropsy," were largely based on herbal remedies such as purgatives (e.g., castor oil, tamarind)[1] dandelion, foxglove, strophanthus seeds, and juniper berries, as well as dietary restrictions and physical remedies like sweating and bleeding.[2–5] By the 18th century, mercurial diuretics, used for treatment of syphilis and already mentioned by the Swiss physician and alchemist Paracelsus for treatment of "dropsy" in 1520,[5] emerged as a significant advancement in the treatment of volume overload, despite their narrow therapeutic window and severe and sometimes idiosyncratic side effects.[6] The discovery of the diuretic effect of sulfonamides via proximal tubular carbonic anhydrase (CA) inhibition in 1937[7–9] motivated chemists to synthesize new compounds to inhibit CA, leading to the detection of acetazolamide (AZM) in 1950 (Figure 1).[10,11] CA inhibitors, however, proved insufficiently effective due

Matteo Bargagli and Manuel A. Anderegg contributed equally to this study.

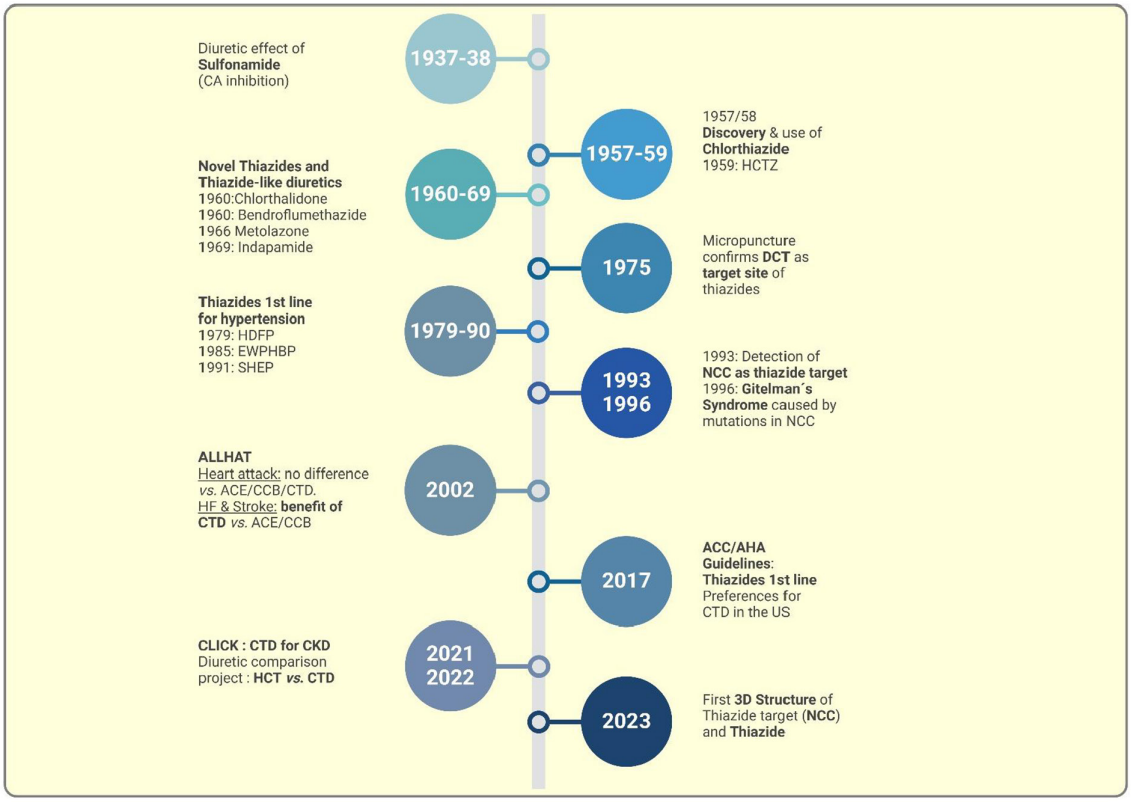



**FIGURE 1** Timeline presenting important milestones in thiazide development, mechanism of action, and implementation in clinical practice. The discovery of the diuretic effect of sulfonamides in 1937 proved catalytic for the synthesis of new diuretics, initially carbonic anhydrase (CA) inhibitors and then, rather serendipitously, thiazide diuretics (e.g., chlorothiazide) in the late 1950s. In the 1960s, other thiazide analogs such hydrochlorothiazide (HCT) and thiazide-like diuretics followed. From the 1970s onwards, thiazides were first-line therapy for hypertension, after a series of important studies, 1979 the Hypertension Detection and Follow-Up Program (HDFP), 1985 the European Working Party on High Blood Pressure in the Elderly trial (EWPHBP), 1991 the Systolic Hypertension in the Elderly Program (SHEP). After novel mechanistic results on thiazide action in the distal convoluted tubule (DCT) in 1990, the Antihypertensive and Lipid-Lowering to Prevent Heart Attack Trial (ALLHAT) study showed noninferiority or superiority of Chlorthalidone (CTD) vs ACE-inhibitors and calcium channel blockers (CCB), leading to reaffirmation of the important role of thiazides in recent guidelines. In recent years, Chlorthalidone has proven effective also in patients with chronic kidney disease (CKD), and a comparison of CTD vs HCT showed no benefit of CTD. Only last year, the 3D structure of the major thiazide target (NCC) with a thiazide bound was elucidated. Created with BioRender.com.

to their physiological limitations.[12,13] The search for novel CA inhibitors led to the rather serendipitous discovery of thiazide diuretics with the launch of chlorothiazide in 1958,[14,15] drugs seemingly targeting for the first time distal tubules and leading to increased chloride excretion.[16] Modifications of the basic benzothiadiazine core led to the development of hydrochlorothiazide (HCT) and subsequently to more distantly related thiazides-like diuretics such as chlorthalidone, metolazone, and indapamide (Figures 1 and 2).[17]

The development of thiazides was the start of a novel era in diuretic therapy and the treatment of arterial hypertension. Since the 1960s, thiazides have been investigated in numerous randomized clinical trials (RCTs) for the treatment of arterial hypertension. In these RCTs, thiazides were repeatedly shown to improve clinical outcomes of hypertensive patients and thiazides are still today recommended as first-line agents for the treatment of arterial hypertension.[18–20] In 1959, Lamberg and Kuhlbäck reported the observation that thiazides reduce urine calcium.[21] Soon thereafter, Lichtwitz and colleagues proposed that this peculiar property might be exploited to prevent recurrence of calcium-containing kidney stones.[22] Several small RCTs thereafter indeed suggested that thiazides are effective in the recurrence prevention of calcareous nephrolithiasis.[23] Based on these trials, thiazides rapidly became a cornerstone of medical stone prevention. Yet, the evidence for their effectiveness in kidney stone prevention has recently been challenged due to methodological deficiencies of the RCTs conducted thus far for this indication.[23–25] Due to their indications targeting very common human disorders and their universal availability and low cost, thiazides belong to the most widely prescribed drugs worldwide.[26]

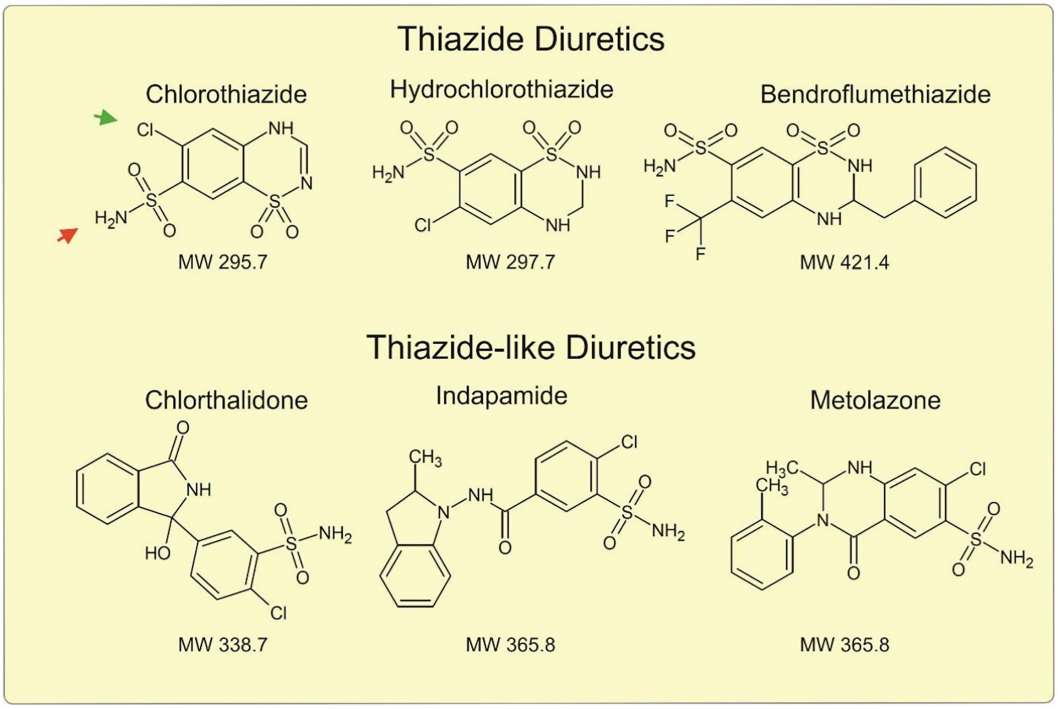



**FIGURE 2** Structure of thiazide diuretics and thiazide-like diuretics. Members of thiazides and thiazide-like diuretics are clinically used, including their molecular weight. For chlorothiazide, the sulfamoyl group ($H_2N$-$SO_2$, red arrow) and halogen (Chloro-) group (green arrow) are marked. All thiazides harbor a sulfamoyl group and halogen-like group and both are essential for their pharmacological activity. Differences between thiazides and thiazide-like diuretics mainly consist in the lack or incompleteness of the benzothiazidine ring in thiazide-like diuretics.

## 2 | THE $NA^+/CL^-$ COTRANSPORTER (NCC): THE CLASSICAL THIAZIDE TARGET

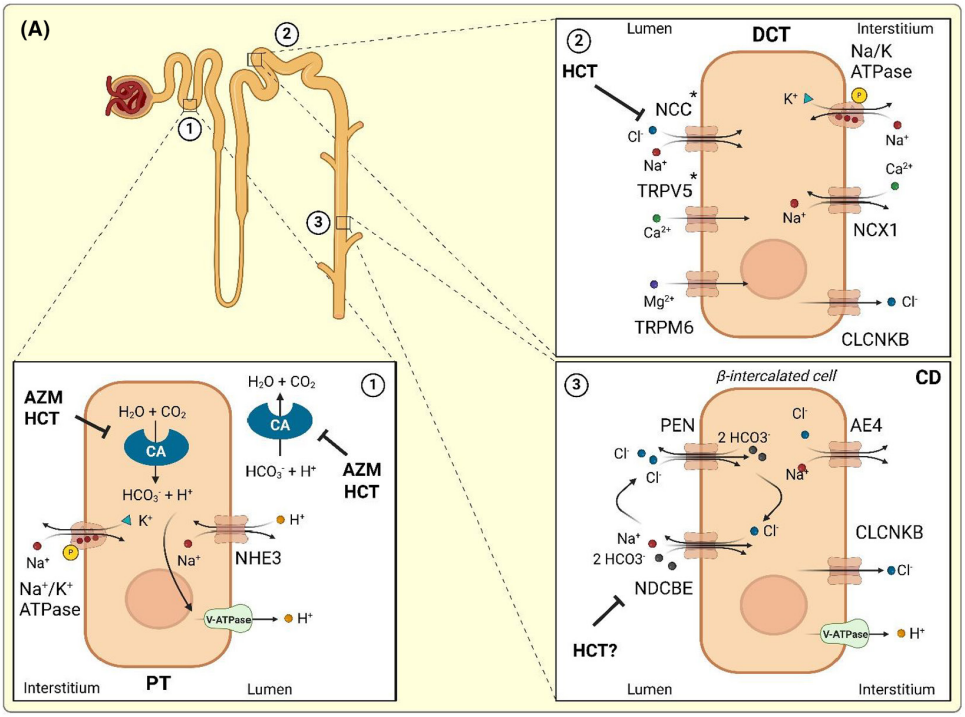
The principal target of thiazides is the $Na^+/Cl^-$ cotransporter NCC (also known as SLC12A3) in the distal convoluted tubules (DCT) of the kidney.[27] NCC inhibition by thiazides blocks $Na^+$ and $Cl^-$ reabsorption by the DCT. Inhibition of NCC in the DCT cannot be fully compensated by other tubular segments, thus resulting in a reduction of the extracellular volume and a drop in blood pressure. Through a still ill-defined mechanism, further described later in this review, thiazides also reduce urine calcium, which is exploited in the recurrence prevention of calcium-containing kidney stones.[28,29]

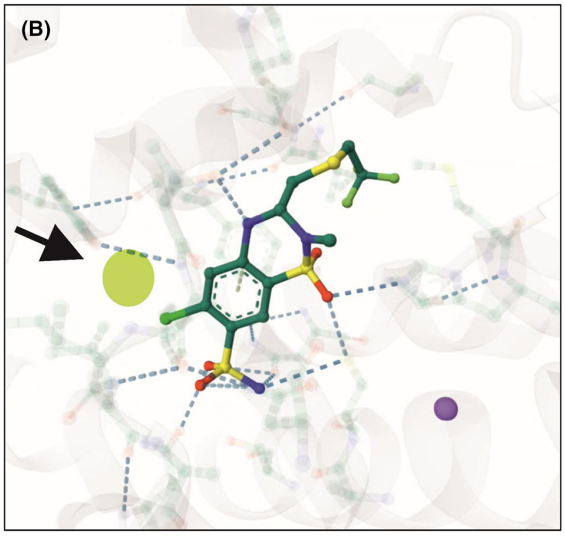
The interaction of thiazides with NCC has recently been solved at high resolution.[30] Polythiazide interacts with NCC in an outward-open conformation with its sulfamoyl group forming multiple hydrogen bonds deep in the binding pocket (Figures 2 and 3.B).[30] In addition, the chloro group of polythiazide competes with the $Cl^-$ binding site in NCC (Figure 3.B), as already proposed previously.[31] All thiazides likely bind to NCC in a similar fashion by competing with $Cl^-$ binding and by preventing the conformational switch from the outward-facing to the inward-facing state.[30]

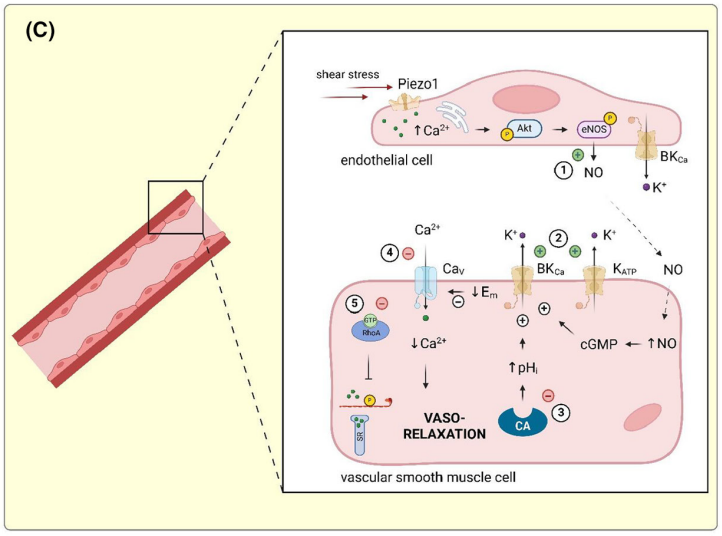
The phenotype of patients with homozygous inactivating mutations in the *NCC* gene (Gitelman's syndrome) or of mice with targeted disruption of the *Ncc* gene (NCC KO mice) is very similar to humans treated with thiazides.[27,32] Notable exceptions exist with regard to blood $K^+$ and blood pressure. Indeed, while hypokalemia is an almost universal finding in patients with Gitelman's syndrome or in patients treated with high doses of thiazides, NCC KO mice have no alterations of blood $K^+$ or blood pressure compared with WT mice under a normal rodent diet.[32,33] When challenged with a low $K^+$ diet, however, NCC KO mice displayed significantly reduced plasma $K^+$ concentrations compared with WT littermates.[34] Similarly, only when challenged with a $Na^+$-deficient diet, NCC KO mice display a reduction in blood pressure compared with WT mice.[32]

## 3 | NONCLASSICAL THIAZIDE TARGETS

While NCC is the primary site of thiazide action, other thiazide targets have been described. Despite considerable chemical alterations of the original AZM scaffold, all thiazides have retained the ability to inhibit CA.[14,35] However, there is considerable heterogeneity between the different thiazides with respect to their inhibitory potency toward the




currently known CA isoforms.[36] Clinically, inhibition of CA isoforms by thiazides has been proposed to contribute to the reduction of blood pressure by induction of vasorelaxation and to enhance the (distal tubular) diuretic effect by an additional, direct proximal tubular effect.[37,38]

Eladari and colleagues described an additional thiazide target in the cortical collecting duct (CCD), the $Na^+$-driven $Cl^-$/bicarbonate exchanger NDCBE (also known as SLC4A8).[39,40] Microperfusion studies on isolated CCD segments indicated that a significant fraction of $Na^+$ reabsorption in this segment is amiloride-independent (i.e., independent of the epithelial $Na^+$ channel ENaC) but thiazide-sensitive.[39] This concept was further supported by the finding that HCT increased renal $Na^+$ and $Cl^-$ excretion even in NCC KO mice.[39] Genetic ablation of NDCBE abolished the thiazide-sensitive $Na^+$ transport in the CCD and supported the notion that NDCBE is indeed a direct thiazide target,[39] although another group did not detect NDCBE in the mCCD, the OMCD/IMCD and failed to detect differences in acid–base status or blood electrolytes.[41] Hence, in addition to the DCT and proximal tubule, thiazides may also inhibit a $Na^+$ and $Cl^-$ transport pathway in the CCD. The role of NDCBE in sodium homeostasis is most striking in conditions, where NCC is inhibited or (genetically) absent and seems to be important for the prevention of hypokalemia in this setting. Inhibition of NDCBE by thiazides has therefore been implicated in the development of thiazide-induced hypokalemia.[39,42] The clinical relevance of this finding in the context of usual pharmacological thiazide

**FIGURE 3** (A) Action of thiazides along the nephron. Along the nephron, thiazides such as hydrochlorothiazide (HCT) mainly act in the distal convoluted tubule (DCT) by blocking the sodium chloride cotransporter (NCC). However, as structural analogs of carbonic anhydrase (CA) inhibitors, here exemplified by acetazolamide (AZM), they retain inhibitory capacity on CA. Recently, an additional target in β-intercalated cells of the collecting duct (CD), $Na^+$-driven $Cl^-$/bicarbonate exchanger (NDCBE) has been proposed. AE4: anion exchanger, isoform 4, CD: collecting duct, CLCNKB: chloride voltage-gated channel Kb, NHE3: sodium hydrogen exchanger, isoform 3. NCX1: $Na^+/Ca^{2+}$ exchanger isoform 1, PEN: pendrin (Chloride bicarbonate exchanger), PT: proximal tubule, TRPV5: transient receptor potential cation channel subfamily V member 5, TRPM6: transient receptor potential cation channel, subfamily M, member 6, V-ATPase: vacuolar $H^+$-ATPase, *In box 2: NCC is only co-expressed with TRPV5 in the more distal part of the DCT transitioning to the connecting tubules (CNT), called DCT2. (B) Position of polythiazide in the binding pocket of its main target, NCC. Polythiazide binds NCC in its outward-open conformation. The sulfamoyl group (bottom, red, arrow) is essential as it allows multiple hydrogen bonds with residues of the binding pocket of NCC. The halogen/Chloride group (green) is localized close to the space usually occupied by a $Cl^-$ ion (yellow dot/arrow). The $Na^+$ ion present is seen as a violet dot. Image created using Mol*[166] with and according to the NCC structure[167] and publication[30] of Zhang, Fan, Feng, et al. (PDB ID: 8FHO) deposited at RCSB PDB.[168] (C) Proposed mechanisms of direct vascular effect by thiazides leading to blood pressure reduction. Multiple pathways were proposed to mediate the long-term blood pressure reduction of thiazide diuretics. The proposed pathways all lead to relaxation of the vasculature, either by direct action to increase nitric oxide (NO) production via stimulation of endothelial nitric oxide synthase (eNOS) in endothelial cells (1) or by direct action on vascular smooth muscle cells (2–5). Proposed action of thiazides on vascular smooth muscle cells (VSMC) are direct inhibition of $Ca^{2+}$- and voltage-activated $K^+$ channels ($BK_{Ca}$, 2), leading to hyperpolarization with attenuation of $Ca^{2+}$ entry into cells via $Ca^{2+}$ channels and consequently vasodilation due to reduced intracellular $Ca^{2+}$. Other mechanisms proposed are direct inhibition of voltage-gated $Ca^{2+}$ channels (4), inhibition of carbonic anhydrase (CA, 3), leading to higher pH due to reduced CA function and consequently increased open probability of $BK_{Ca}$. Another mechanism proposed is reduction of Rho and Rho Kinase (5) and consequently reduced $Ca^{2+}$-sensitization of the myofilaments of VSMCs and resulting vasodilation. cGMP: cyclic guanosine monophosphate, $E_m$, membrane equilibrium potential. Created with Biorender.com.

doses, however, is currently unknown. Mechanistically, the chloride bicarbonate exchanger pendrin (SLC26A4) seems to be coupled to NDCBE to enable NaCl reabsorption (see Figure 3.A, panel 3).[39] Single genetic deletions of NCC,[32] pendrin,[43,44] or NDCBE[39] in rodents do not lead to excessive salt wasting, likely due to compensatory effect by the remaining transport proteins. The severe salt-wasting phenotypes of NCC/Pendrin[45]- and NCC/NDCBE[42]-double KO mice support this concept.

## 4 | COMMON SIDE EFFECTS OF THIAZIDES

The most frequent side effects of thiazides are electrolyte disturbances, notably hypokalemia, hyponatremia, and hypomagnesemia. These electrolyte disturbances are not off-target effects but rather expected, secondary changes due to inhibition of renal $Na^+$ and $Cl^-$ transport. Thiazide-induced high distal tubular $Na^+$ delivery and hypovolemia-mediated activation of the renin–angiotensin–aldosterone (RAA) system synergistically stimulate renal $K^+$ secretion. Especially multimorbid elderly patients[46] and patients receiving high doses of thiazides[47] with a high $Na^+$ intake are prone to develop hypokalemia.[48] Another common side effect of thiazides is hyponatremia.[49] This side effect is equally dose-dependent and typically encountered in elderly patients with low solute intake, impaired osmoregulation, and limited urinary dilution capacity.[50] Several mechanisms have been discussed such as the combination of urinary $Na^+$ and $K^+$ losses with enhanced ADH-mediated water reabsorption, and ADH-independent activation of water reabsorption in the inner medullary collecting duct.[51,52] Rodent and human data also suggest a role for renal prostaglandins in thiazide-induced hyponatremia.[49,52–54] Another conundrum is the paradoxical antidiuretic effect of thiazides, which is therapeutically exploited in the treatment of patients with nephrogenic diabetes insipidus. The most commonly followed explanation is that the mild volume depletion induced by inhibition of NCC activates $Na^+$ and water reabsorption in the proximal tubule.[55] Another proposed mechanism involves a direct upregulation of aquaporin-2 in the distal collecting duct.[56] An antiaquaretic effect due to inhibition of proximal tubular CA with activation of tubuloglomerular feedback has also been postulated.[57] Hypomagnesemia due to renal $Mg^{2+}$ wasting, another electrolyte abnormality frequently observed with thiazide therapy, is likely a consequence of morphological and functional changes directly in the DCT when NCC is chronically absent or inhibited.[28,33] Other classical side effects of thiazides due to volume contraction include hyperuricemia/gout and (reversible) worsening of renal function.

## 5 | DIRECT THIAZIDE EFFECTS ON BLOOD VESSELS

While the initial reduction in blood pressure is mediated by volume contraction and leads to transiently increased

vascular resistance induced by counterregulatory pathways,[58] the long-term antihypertensive effect of thiazides is mediated by multifaceted reduction in total peripheral vascular resistance, but persisting slight volume contraction.[59–61] Many different pathways and molecular targets have been proposed to mediate this reduction in vascular tone (Figure 3.C), which is likely due to direct and indirect actions on vascular smooth muscle cells.[62,63] One possible contribution is inhibition of CA (mainly isoform I) in vascular smooth muscle cells (and potentially also endothelial cells),[37] leading to intracellular alkalinization and activation of $Ca^{2+}$-activated $K^+$ channels, consequently resulting in vasodilation. Other proposed mechanisms include direct activation of $Ca^{2+}$-activated $K^+$ channels ($BK_{Ca}$)[64] in vascular smooth muscle or endothelial cells,[65] $Ca^{2+}$-desensitization in vascular smooth muscle cells mediated via Rho-kinases,[66] and $Ca^{2+}$-antagonism-like effects (only indapamide), likely via a different mechanism than classical $Ca^{2+}$-antagonists[67–70] (Figure 3.C).

A major limitation of most of these studies is the supratherapeutic thiazide doses used in experiments.[62,71–73] It is currently unclear, whether mechanisms exist that increase the local concentration of thiazides in endothelial or vascular smooth muscle cells, similar to that observed in red blood cells due to binding of thiazides to CA.[26,62,74] Clearly, more research is needed to determine the exact molecular mechanisms responsible for long-term blood pressure reduction mediated by thiazides.

## 6 | THIAZIDES IN THE PREVENTION OF KIDNEY STONE RECURRENCE

Nephrolithiasis is a common condition in the general population, with increasing prevalence and incidence all over the world.[75] Notably, a substantial proportion of first-time kidney stone formers, approximately 35%, are predisposed to recurrent episodes.[76] The socioeconomic ramifications of nephrolithiasis are profound, encompassing not only the healthcare costs but also the loss of productivity due to work absenteeism, cumulatively exceeding $10 billion annually in the United States alone.[77] Predominantly, kidney stones are composed of calcium oxalate (CaOx), calcium phosphate (CaP), or a mixture of both, and to a lesser extent, uric acid.[78] From a physicochemical perspective, the phenomenon of lithogenic salt formation is mainly explained by the concept of urine supersaturation. The latter is acknowledged as the pivotal driver for the nucleation and subsequent growth of crystalline structures.[79–81] The assessment of urinary supersaturation relative to the crystalline phases of stone formation is achievable through computational analyses using programs such as EQUIL2, JESS, and LithoRisk.[82–84] These methodologies evaluate the ionic equilibrium of lithogenic salts and confront these findings against established thermodynamic solubility constants to approximate supersaturation metrics. Specifically, the supersaturation states of CaOx and CaP drive the crystallization of the corresponding salts in the urines and are primarily influenced by the urinary concentrations of calcium, oxalate, phosphate, magnesium, citrate, and urine pH. Urine supersaturations are highly correlated with kidney stone composition and well-established proxies for the risk of recurrent stone formation.[80,85–88] Interventions that reduced stone events closely correlated with reductions in urine supersaturations.[85,86,89,90]

High urine calcium is the most common metabolic abnormality encountered in patients with calcium-containing kidney stones and drives both CaOx and CaP supersaturations.[76,81,85] Epidemiological data demonstrated the association between urinary calcium and risks of stone formation. Across genders, the lower 95% confidence interval (CI) for a relative risk of stone formation exceeding 1 occurs at a urinary calcium excretion of ~200 mg (=5 mmol)/24 h.[91–93] Therefore, strategies aimed at reducing urinary calcium are the mainstay of the management of calcium nephrolithiasis.

Thiazide diuretics reduce urine calcium and are widely employed to mitigate the risk of kidney stone recurrence, but large studies recently challenged this approach.[25,94] Several mechanisms have been proposed for the thiazide-induced reduction of urine calcium, with the most compelling evidence for a proximal tubular effect. In a small prospective study involving individuals treated with a 6-month regimen of chlorthalidone at a daily dose of 25 mg, the thiazide-like diuretic chlorthalidone led to a decrease in urine calcium, as well as reductions in both the fractional excretion of calcium and lithium.[28] These findings suggest a decreased delivery of calcium from the proximal tubule to more distal sections of the nephron.[28,95] In support of these results, micropuncture experiments in mice demonstrated increased reabsorption of $Na^+$ and $Ca^{2+}$ in the proximal tubule during chronic thiazide treatment, whereas $Ca^{2+}$ reabsorption in the distal convolution appeared unaffected. Second, chronic thiazide administration still induced hypocalciuria in transient receptor potential channel subfamily V, member 5 (TRPV5) KO mice, in which active distal $Ca^{2+}$ reabsorption is abolished due to inactivation of the epithelial $Ca^{2+}$ channel TRPV5.[28] Still, NCC inhibition may lead to augmented distal tubular $Ca^{2+}$ reabsorption and thereby contribute to thiazide-induced hypocalciuria,[29] at least with acute thiazide treatment,[96] and increased expression of TRPV5 and calbindin have been observed in kidneys on mRNA and (for TRPV5) protein level with thiazide treatment.[97] Additionally, crosstalk

between $Ca^{2+}$ and $Mg^{2+}$ transport may play a role in increased distal tubular $Ca^{2+}$ reabsorption.[98]

Although thiazides are associated with a variable but still significant reduction in urine calcium excretion, this positive effect might be counteracted by one of its main and common adverse effects, hypokalemia.[19,99–101] The thiazide-induced hypokalemia in turn causes a deleterious decrease in urinary citrate excretion, which counteracts the beneficial effect of urinary calcium reduction.[102,103] Hypokalemia predominantly arises from augmented distal $Na^+$ delivery and activation of the RAA system, which facilitates $K^+$ secretion in principal cells of the collecting duct.[104] Hypokalemia induces an intracellular acidosis in proximal tubular cells,[105] which stimulates proximal tubular citrate reabsorption and metabolism.[106–108]

As a result of all these factors and including difficulties in achieving a sustained and long-term reduction in salt intake, thiazides were observed to have a variable effect on urine supersaturations for CaOx and CaP. Indeed, the recent state-of-the-art, randomized double-blind placebo-controlled trial NOSTONE found no consistent changes in urine supersaturation for CaOx and CaP with any HCT dosage compared to placebo, accompanied by a tendency to lower urinary citrate in patients assigned to HCT compared to placebo. Reduction of urine calcium among patients receiving HCT in NOSTONE was modest (9–17% compared to baseline, 15–16% compared to placebo), without any dose–response effect. Although sodium intake was similar across all groups, it exceeded the recommended level despite repeated dietary instructions by experienced professionals. This may have mitigated the hypocalciuric effect of HCT to some extent, but sodium intake in NOSTONE was comparable to that of previous thiazide trials for the prevention of kidney stones.[109,110] Consistent with these biochemical proxies of stone formation risk in the urine, NOSTONE failed to demonstrate any benefit of HCT at doses up to 50 mg daily in patients with calcium-containing kidney stones at a high risk of recurrence compared to placebo. These findings align with a very recent large observational study reporting a benefit with alkali treatment but not with thiazides in preventing clinically significant symptomatic stone events in patients with kidney stones.[94] In contrast, a recent Mendelian randomization study encompassing 1′079′657 individuals found that genetic proxies of thiazide diuretics were associated with a 15% reduced odds for kidney stones.[111] A previously not addressed limitation of this study is that analyzed genetic proxies were selected by their association with systolic blood pressure and focused only on *SLC12A3* (the gene encoding NCC) and its regulating regions but did not include additional thiazide loci.[111] While these findings at the population level provide a rationale for targeting NCC to prevent kidney stone formation, the recent outcome trial NOSTONE and a large contemporary observational study indicate that the currently used strategy of prescribing thiazides has limited clinical efficacy.[25,94]

Since thiazide-like diuretics, such as indapamide and chlorthalidone, are more potent and have a significantly longer half-life compared to HCT,[110,112] it might be presumed they can achieve more effective prevention of stone recurrence. However, this assertion lacks robust randomized evidence and no head-to-head comparison of different thiazides for kidney stone recurrence prevention has been conducted so far.

Available data on long-acting thiazides indapamide and chlorthalidone are scarce, showing heterogeneous and variable effects on urine composition and relative supersaturations. Indeed, no data are available on the effects of chlorthalidone on urine supersaturation for CaOx and CaP in humans. For indapamide, a single randomized prospective study with outdated dietary recommendations showed a 54% reduction in the relative supersaturation for CaOx and a 22% reduction in relative supersaturation for CaP compared to baseline.[110] With respect to urine calcium, indapamide at doses of 1.5 or 2.5 mg reduced urine calcium by 20%–55% compared with baseline,[110,113,114] whereas chlorthalidone achieved similar reduction rates with doses of 25–100 mg daily.[112,115,116] With respect to urine citrate, no change up to a reduction of 20%–30% has been reported for both indapamide and chlorthalidone compared with baseline.[110,115,117,118] Based on recent trial data, it seems prudent to recommend the use of alkali such as potassium citrate as first-line treatment for pharmacologic recurrence prevention of kidney stones.[23,119–124] In patients with calcium kidney stones that are not responsive, or intolerant to citrate supplementation, potent long-acting thiazides such as chlorthalidone or indapamide may be tried, despite the lack of data showing an advantage of these thiazide-like compounds over HCT for kidney stone recurrence prevention. Collectively, these data underscore the unmet medical need for additional studies, including head-to-head comparison of different thiazides for kidney stone recurrence prevention or for the established proxies of recurrence risk, urine RSRs. A crossover trial comparing hydrochlorothiazide, indapamide, and chlorthalidone (INDAPACHLOR trial; NCT06111885) that addresses this critical knowledge gap has recently been initiated.

## 7 | THIAZIDE-INDUCED GLUCOSE INTOLERANCE

The association of thiazide use with worsened glucose tolerance or new-onset diabetes mellitus has been recognized almost immediately after their introduction in clinical medicine 60 years ago.[125–128] Numerous observational studies and randomized trials have since confirmed these initial observations.[129–134] Also, the recent NOSTONE kidney stone trial

reported a higher rate of adverse events, including newly diagnosed diabetes mellitus, in patients receiving HCT compared with patients assigned to the placebo.[135] A large network meta-analysis of 22 large clinical trials involving 143′153 patients treated with antihypertensive drugs found that thiazides are associated with the highest risk of incident diabetes mellitus.[136] Interestingly, patients with Gitelman's syndrome were also reported to exhibit higher fasting glucose, higher levels of markers of insulin resistance, and at increased risk for the development of type 2 diabetes compared to heterozygous carriers or healthy noncarriers.[137,138] The persistent concern regarding the metabolic side effects of thiazides has led numerous physicians to discontinue the use of these inexpensive, readily available, and effective medications. The underlying molecular mechanisms remain unclear,[139] but several hypotheses have been put forth to explain thiazide-induced glucose intolerance. The most popular one involves thiazide-induced potassium depletion resulting in decreased insulin secretion from β cells. Indeed, there is a correlation between the degree of thiazide-induced hypokalemia and the increase in blood glucose, and evidence that prevention of hypokalemia by potassium supplementation or co-administration of potassium-sparing diuretics attenuates the thiazide-induced increase in blood glucose.[100] However, the evidence supporting the concept that hypokalemia in the range observed in patients treated with thiazides (typically 0.2–0.6 mmol/L[19,140,141]) actually decreases insulin secretion and thereby promotes hyperglycemia is incomplete.[139] Further, a well-designed randomized controlled trial revealed no significant correlation between changes in plasma potassium and blood glucose or plasma insulin in patients treated with HCT, although a significant increase of blood glucose was noted in participants receiving HCT.[133] Similarly, the profound hypokalemia observed in patients with chronic hyperaldosteronism is not associated with hyperglycemia.[142,143] Other potential mechanisms proposed include direct thiazide-mediated inhibition of insulin secretion in β cells[144,145] and thiazide-induced decreases of peripheral[146–148] or hepatic insulin sensitivity.[149,150]

The functional role of the thiazide targets NCC or NDCBE in β cells had not been studied, yet previous studies demonstrated that AZM or thiazides at high concentrations attenuated insulin secretion in vitro, suggesting that inhibition of CA or NDCBE may play a role in thiazide-induced glucose intolerance.[151–153]

## 8 | IDENTIFICATION OF THE MOLECULAR THIAZIDE TARGET IN β CELLS

We set out to further investigate the molecular mechanisms underlying thiazide-induced glucose intolerance. By employing intraperitoneal glucose and insulin tolerance tests, we observed that the administration of HCT to mice resulted in an acute glucose intolerance accompanied by a reduction in circulating insulin. However, insulin sensitivity and plasma potassium concentrations remained unchanged. Consistent with these findings, thiazides frequently employed in clinical practice, such as bendroflumethiazide, chlorthalidone, indapamide, metolazone, and benzoflumethiazide, and HCT, attenuated insulin secretion dose-dependently in mouse islets and the mouse β-cell line Min6 within a pharmacologically relevant, submicromolar range. The inhibitory effect of thiazides on insulin secretion was $CO_2/HCO_3^-$-dependent, not additive to unselective CA inhibition with AZM, and independent of extracellular potassium. In contrast, although we discovered that both NCC and NDCBE are expressed in islets and Min6 cells, islets isolated from NDCBE and NCC KO mice displayed no insulin secretion deficit. Furthermore, deletion of NCC in mice was not associated with altered glucose tolerance in vivo, and HCT attenuated insulin secretion to a similar degree in islets of NCC KO mice as in islets of WT mice. Collectively, these results suggested that (i) thiazides target one or several CA isoform(s) that are critical for insulin secretion and (ii) NCC and NDCBE are dispensable for insulin secretion in β cells. Subsequent experiments with individual KO of CA isoforms expressed in Min6 cells demonstrated that cytosolic CA2 and mitochondrial CA5b are critical for insulin secretion in Min6 cells. However, CA expression analysis of purified murine β cells revealed that primary β cells only express two isoforms, mitochondrial CA5b and CA10, a catalytically inactive and secreted CA isoform. These results strongly indicated that mitochondrial CA5b is the probable target of thiazides in β cells.[154] Consonant with such a mechanism, thiazides are known to efficiently cross lipid bilayer membranes and to exhibit high affinity for CA, resulting in the intracellular accumulation of thiazides at sites of CA expression (e.g. erythrocytes).[155,156] In addition, HCT and other thiazides are potent inhibitors of purified human CA isoforms (including CA5b) in vitro with inhibitor constants in the nanomolar range, and co-crystallization studies revealed direct binding of thiazides to CA isoforms.[157,158]

## 9 | ROLE OF MITOCHONDRIAL CA IN INSULIN SECRETION

Mitochondria are impermeant to $HCO_3^-$, yet a number of biosynthetic enzymes within mitochondria are highly reliant on $HCO_3^-$, including pyruvate carboxylase (PC), which converts pyruvate and $HCO_3^-$ into oxaloacetate (OAA).[159] To this end $CO_2$, which freely diffuses into mitochondria, is used by the two mitochondrial CA isoforms, CA5a and CA5b, to generate $HCO_3^-$ (Figure 4). CA5a and CA5b

exhibit a different tissue distribution, and β cells exclusively express CA5b.[152,154,159] β cells exhibit high PC activity, and a significant proportion of pyruvate that enters mitochondria is converted to OAA. OAA is a key metabolite in nutrient-induced insulin secretion, and PC activity correlates strongly with insulin secretion (Figure 4).[160] First, OAA synthesis by PC (anaplerosis) fuels the tricarboxylic acid cycle (TCA) leading to an increase of TCA intermediates, which then exit from mitochondria to the cytoplasm (cataplerosis). Second, phosphoenolpyruvate (PEP) synthesis via the mitochondrial GTP-dependent enzyme PCK2 is initiated from OAA.[160–163] PEP produced from OAA is transported to the cytosol, where pyruvate kinase (PK) converts ADP and PEP into ATP and pyruvate, leading to closure of $K_{ATP}$ channels and initiation of insulin secretion.[164,165] The third OAA-dependent pathway contributing to nutrient-stimulated insulin secretion is the pyruvate/malate shuttle, which results in the generation of cytosolic NADPH via malic enzyme.[160,161]

## 10 | THIAZIDES ATTENUATE OXALOACETATE SYNTHESIS IN B CELLS

KO of CA5b or treatment with HCT severely reduced OAA levels in Min6 cells. This reduction in OAA levels was not additive to the reduction of OAA levels observed with PC inhibition by phenylacetic acid or in $CO_2$/$HCO_3^-$-free conditions. The decrease in OAA is closely correlated with the decrease in insulin secretion experiments observed under these conditions. We then performed insulin secretion experiments with islets isolated from WT and CA5b KO mice. HCT significantly attenuated secretagogue-induced insulin secretion in islets of WT mice. CA5b KO islets displayed increased basal but greatly impaired secretagogue-induced insulin secretion compared with WT islets. In addition, HCT did not affect insulin secretion of CA5b KO islets. A similar pattern was observed when we quantified OAA levels in WT and CA5b KO islets. In glucose tolerance tests, HCT-treated WT but not CA5b KO mice displayed higher blood glucose and lower serum insulin compared to vehicle-treated mice. Acid–base parameters and electrolytes were similar in both groups of mice, and insulin tolerance tests revealed no differences in insulin sensitivity between WT and CA5b KO mice. Thus, CA5b KO mice are resistant to thiazide-induced glucose intolerance, and thiazides do not affect insulin secretion of CA5b KO islets in vitro.[154] Together, these results indicate that thiazides induce glucose intolerance by attenuation of insulin secretion in β cells through inhibition of mitochondrial CA5b. Acute thiazide effects observed occurred in the absence

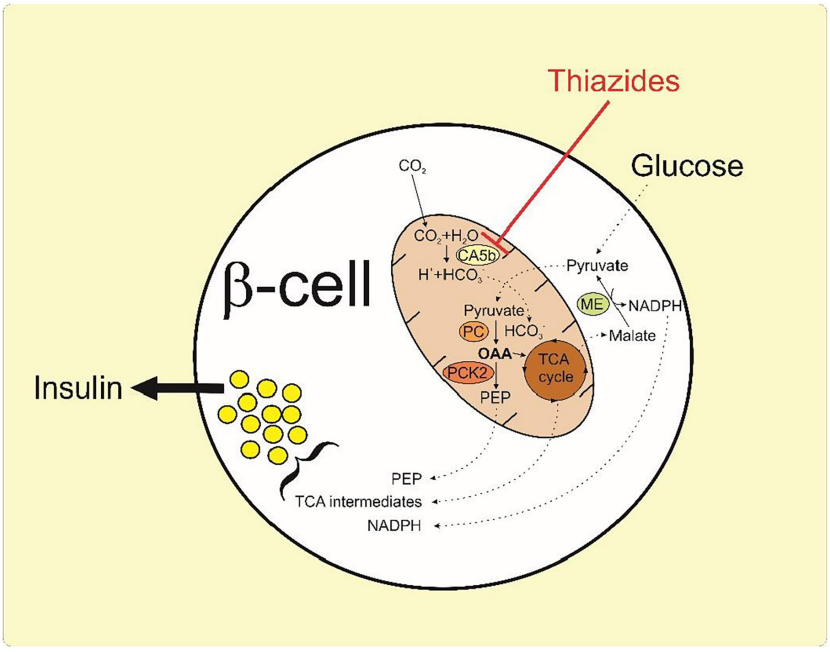



**FIGURE 4** Thiazides attenuate insulin secretion in β cells (from [154]). Carbonic anhydrase type 5b (CA5b) furnishes $HCO_3^-$ which is used by pyruvate carboxylase (PC) to generate oxaloacetate (OAA). Thiazides inhibit CA5b, thereby attenuating OAA synthesis. OAA is a crucial metabolite in nutrient-induced insulin secretion. OAA synthesis by PC (anaplerosis) fuels the tricarboxylic acid cycle (TCA) resulting in increased TCA intermediate export to the cytoplasm, and activation of the pyruvate/malate shuttle, which yields cytosolic NADPH via malic enzyme (ME). In addition, OAA also supports phosphoenolpyruvate (PEP) synthesis via the mitochondrial GTP-dependent enzyme PCK2.

of changes in extracellular potassium. Yet, it is possible that other mechanisms, such as potassium depletion or a decrease in insulin sensitivity, contribute to thiazide-induced glucose intolerance during chronic administration of thiazides. Hence, long-term studies with WT and CA5b KO mice, treated with thiazides or vehicles under a range of different potassium diets are needed to investigate this further. Similarly, a conditional CA5b KO mouse model would be desirable in these studies to exclude the possibility that pathways outside the β cells contribute to thiazide-induced glucose intolerance.

In summary, in this brief review, we outline the pharmacology and mechanism of action of thiazides, highlight their usefulness in the treatment of volume overload states and arterial hypertension, and discuss recent trial evidence on the utility of thiazides for kidney stone recurrence prevention. We examine potential harms associated with thiazide treatment, such as new-onset diabetes and hypokalemia/hypocitraturia, and review recent insights into the molecular mechanisms of thiazide-induced glucose intolerance. Although thiazides come of age, many questions surrounding their use in clinical medicine, their mode of action, and the origin of their off-target effects remain unanswered and necessitating additional research.

## AUTHOR CONTRIBUTIONS

**Matteo Bargagli:** Writing – original draft; data curation; writing – review and editing; methodology; investigation. **Manuel A. Anderegg:** Data curation; writing – review and editing; visualization; writing – original draft; investigation. **Daniel G. Fuster:** Conceptualization; writing – original draft; methodology; validation; visualization; writing – review and editing; supervision; resources; data curation; project administration; investigation; funding acquisition.

## ACKNOWLEDGMENTS

We thank all NOSTONE trial participants and the study team for their contributions, D. Eladari and J. Loffing for transgenic mice and P. Halban for Min6 cells. Open access funding provided by Inselspital Universitatsspital Bern.

## FUNDING INFORMATION

This work was supported by the Swiss National Science Foundation (grants # 33IC30_166785, 31003A_172974), the Swiss National Centers of Competence in Research (NCCR TransCure and NCCR Kidney.CH), the Novartis Research Foundation and Inselspital, Bern University Hospital, University of Bern, Switzerland.

## CONFLICT OF INTEREST STATEMENT

The authors have declared that no conflict of interest exists.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

## ORCID

*Manuel A. Anderegg* https://orcid.org/0000-0002-8449-0232
*Daniel G. Fuster* https://orcid.org/0000-0001-7220-1803

## REFERENCES

1. Bhadoriya SS, Ganeshpurkar A, Narwaria J, Rai G, Jain AP. Tamarindus indica: extent of explored potential. *Pharmacogn Rev*. 2011;5(9):73-81.
2. Riddle JM. One Plant, One Chapter. In: *Dioscorides on Pharmacy and Medicine*. New York, USA: University of Texas Press; 1985. p.25-93. https://doi.org/10.7560/715448-005
3. Ebers G, Stern L. Papyros Ebers: das Hermetische Buch über die Arzneimittel der alten Ägypter in hieratischer Schrift. 1875.
4. Totelin L. Hippocratic Recipes: Oral and Written Transmission of Pharmacological Knowledge in Fifth-and Fourth-Century Greece. *Hippocratic Recipes*. Brill; 2008.
5. Eknoyan G. A history of edema and its management. *Kidney Int Suppl*. 1997;59:S118-S126.
6. Stringham JS. On the diuretic effects of mercury in a case of syphilis. *Med Phys J*. 1807;18(104):331-333.
7. Mann T, Keilin D. Sulphanilamide as a specific inhibitor of carbonic anhydrase. *Nature*. 1940;146(3692):164-165.
8. Southworth H, Long PH, White F. Acidosis associated with the administration of para-amino-benzene-sulfonamide (Prontylin). *Proc Soc Exp Biol Med*. 1937;36(1):58-61.
9. Strauss MB, Southworth H. Urinary changes due to sulfanilamide administration. *Bull Johns Hopkins Hosp*. 1938;63:41.
10. Moser M, Feig PU. Fifty years of thiazide diuretic therapy for hypertension. *Arch Intern Med*. 2009;169(20):1851-1856.
11. Roblin RO, Clapp JW. The preparation of heterocyclic sulfonamides1. *J Am Chem Soc*. 1950;72:4890-4892.
12. Hanley T, Platts MM. Acetazolamide (diamox) in the treatment of congestive heart-failure. *Lancet*. 1956;267(6919):357-359.
13. Counihan TB, Evans BM, Milne MD. Observations on the pharmacology of the carbonic anhydrase inhibitor diamox. *Clin Sci*. 1954;13(4):583-598.
14. Novello FC, Sprague JM. Benzothiadiazine dioxides as novel diuretics. *J Am Chem Soc*. 1957;79:2028-2029.
15. Beyer KH. The mechanism of action of chlorothiazide. *Ann N Y Acad Sci*. 1958;71(4):363-379.
16. Beyer KH Jr, Baer JE, Russo HF, Noll R. Electrolyte excretion as influenced by chlorothiazide. *Science*. 1958;127(3290):146-147.
17. Reilly RF, Peixoto AJ, Desir GV. The evidence-based use of thiazide diuretics in hypertension and nephrolithiasis. *Clin J Am Soc Nephrol*. 2010;5(10):1893-1903.
18. James PA, Oparil S, Carter BL, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). *JAMA*. 2014;311(5):507-520.
19. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: the Antihypertensive and Lipid-Lowering

Treatment to Prevent Heart Attack Trial (ALLHAT). *JAMA*. 2002;288(23):2981-2997.

20. Appel LJ. The verdict from ALLHAT—thiazide diuretics are the preferred initial therapy for hypertension. *JAMA*. 2002;288(23):3039-3042.
21. Lamberg BA, Kuhlback B. Effect of chlorothiazide and hydrochlorothiazide on the excretion of calcium in urine. *Scand J Clin Lab Invest*. 1959;11:351-357.
22. Lichtwitz A, Parlier R, Hioco D, Miravet L. The hypocalciuric effect of diuretic sulfonamides. *Sem Med Prof Med Soc*. 1961;37:2350-2362.
23. Fink HA, Wilt TJ, Eidman KE, et al. Medical management to prevent recurrent nephrolithiasis in adults: a systematic review for an American College of Physicians Clinical Guideline. *Ann Intern Med*. 2013;158(7):535-543.
24. Li DF, Gao YL, Liu HC, Huang XC, Zhu RF, Zhu CT. Use of thiazide diuretics for the prevention of recurrent kidney calculi: a systematic review and meta-analysis. *J Transl Med*. 2020;18(1):106.
25. Dhayat NA, Bonny O, Roth B, et al. Hydrochlorothiazide and prevention of kidney-stone recurrence. *N Engl J Med*. 2023;388(9):781-791.
26. Duarte JD, Cooper-DeHoff RM. Mechanisms for blood pressure lowering and metabolic effects of thiazide and thiazide-like diuretics. *Expert Rev Cardiovasc Ther*. 2010;8(6):793-802.
27. Simon DB, Nelson-Williams C, Bia MJ, et al. Gitelman's variant of Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. *Nat Genet*. 1996;12(1):24-30.
28. Nijenhuis T, Vallon V, van der Kemp AW, Loffing J, Hoenderop JG, Bindels RJ. Enhanced passive $Ca^{2+}$ reabsorption and reduced $Mg^{2+}$ channel abundance explains thiazide-induced hypocalciuria and hypomagnesemia. *J Clin Invest*. 2005;115(6):1651-1658.
29. Gesek FA, Friedman PA. Mechanism of calcium transport stimulated by chlorothiazide in mouse distal convoluted tubule cells. *J Clin Invest*. 1992;90(2):429-438.
30. Fan M, Zhang J, Lee CL, Zhang J, Feng L. Structure and thiazide inhibition mechanism of the human Na-Cl cotransporter. *Nature*. 2023;614(7949):788-793.
31. Beaumont K, Vaughn DA, Fanestil DD. Thiazide diuretic drug receptors in rat kidney: identification with [3H]metolazone. *Proc Natl Acad Sci USA*. 1988;85(7):2311-2314.
32. Schultheis PJ, Lorenz JN, Meneton P, et al. Phenotype resembling Gitelman's syndrome in mice lacking the apical $Na^+$-$Cl^-$ cotransporter of the distal convoluted tubule. *J Biol Chem*. 1998;273(44):29150-29155.
33. Loffing J, Vallon V, Loffing-Cueni D, et al. Altered renal distal tubule structure and renal Na(+) and Ca(2+) handling in a mouse model for Gitelman's syndrome. *J Am Soc Nephrol*. 2004;15(9):2276-2288.
34. Morris RG, Hoorn EJ, Knepper MA. Hypokalemia in a mouse model of Gitelman's syndrome. *Am J Physiol Renal Physiol*. 2006;290(6):F1416-F1420.
35. Temperini C, Innocenti A, Scozzafava A, Supuran CT. Carbonic anhydrase activators: kinetic and X-ray crystallographic study for the interaction of D- and L-tryptophan with the mammalian isoforms I–XIV. *Bioorg Med Chem*. 2008;16(18):8373-8378.
36. Bua S, Nocentini A, Supuran CT. Chapter 14—carbonic anhydrase inhibitors as diuretics. In: Supuran CT, Nocentini A, eds. *Carbonic Anhydrases*. Academic Press; 2019:287-309.
37. Pickkers P, Garcha RS, Schachter M, Smits P, Hughes AD. Inhibition of carbonic anhydrase accounts for the direct vascular effects of hydrochlorothiazide. *Hypertension*. 1999;33(4):1043-1048.
38. Kunau RT Jr, Weller DR, Webb HL. Clarification of the site of action of chlorothiazide in the rat nephron. *J Clin Invest*. 1975;56(2):401-407.
39. Leviel F, Hubner CA, Houillier P, et al. The $Na^+$−dependent chloride-bicarbonate exchanger SLC4A8 mediates an electroneutral $Na^+$ reabsorption process in the renal cortical collecting ducts of mice. *J Clin Invest*. 2010;120(5):1627-1635.
40. Eladari D, Hubner CA. Novel mechanisms for NaCl reabsorption in the collecting duct. *Curr Opin Nephrol Hypertens*. 2011;20(5):506-511.
41. Xu J, Barone S, Zahedi K, Brooks M, Soleimani M. Slc4a8 in the kidney: expression, subcellular localization and role in salt reabsorption. *Cell Physiol Biochem*. 2018;50(4):1361-1375.
42. Sinning A, Radionov N, Trepiccione F, et al. Double knockout of the $Na^+$-driven Cl−/HCO3− exchanger and $Na^+/Cl^-$ cotransporter induces hypokalemia and volume depletion. *J Am Soc Nephrol*. 2017;28(1):130-139.
43. Royaux IE, Wall SM, Karniski LP, et al. Pendrin, encoded by the Pendred syndrome gene, resides in the apical region of renal intercalated cells and mediates bicarbonate secretion. *Proc Natl Acad Sci USA*. 2001;98(7):4221-4226.
44. Wall SM, Kim YH, Stanley L, et al. NaCl restriction upregulates renal Slc26a4 through subcellular redistribution: role in Cl− conservation. *Hypertension*. 2004;44(6):982-987.
45. Soleimani M, Barone S, Xu J, et al. Double knockout of pendrin and Na-Cl cotransporter (NCC) causes severe salt wasting, volume depletion, and renal failure. *Proc Natl Acad Sci USA*. 2012;109(33):13368-13373.
46. Makam AN, Boscardin WJ, Miao Y, Steinman MA. Risk of thiazide-induced metabolic adverse events in older adults. *J Am Geriatr Soc*. 2014;62(6):1039-1045.
47. Sharabi Y, Illan R, Kamari Y, et al. Diuretic induced hyponatraemia in elderly hypertensive women. *J Hum Hypertens*. 2002;16(9):631-635.
48. Lin Z, Wong LYF, Cheung BMY. Diuretic-induced hypokalaemia: an updated review. *Postgrad Med J*. 2022;98(1160):477-482.
49. Ware JS, Wain LV, Channavajjhala SK, et al. Phenotypic and pharmacogenetic evaluation of patients with thiazide-induced hyponatremia. *J Clin Invest*. 2017;127(9):3367-3374.
50. Liamis G, Filippatos TD, Elisaf MS. Thiazide-associated hyponatremia in the elderly: what the clinician needs to know. *J Geriatr Cardiol*. 2016;13(2):175-182.
51. Ashraf N, Locksley R, Arieff AI. Thiazide-induced hyponatremia associated with death or neurologic damage in outpatients. *Am J Med*. 1981;70(6):1163-1168.
52. Cesar KR, Magaldi AJ. Thiazide induces water absorption in the inner medullary collecting duct of normal and Brattleboro rats. *Am J Phys*. 1999;277(5):F756-F760.
53. Clark BA, Shannon RP, Rosa RM, Epstein FH. Increased susceptibility to thiazide-induced hyponatremia in the elderly. *J Am Soc Nephrol*. 1994;5(4):1106-1111.

54. Hoorn EJ, Wetzels JFM. Prostaglandins in thiazide-induced hyponatraemia: do they hold water? *Nat Rev Nephrol*. 2017;13(11):665-666.
55. Earley LE, Orloff J. The mechanism of antidiuresis associated with the administration of hydrochlorothiazide to patients with vasopressin-resistant diabetes insipidus. *J Clin Invest*. 1962;41(11):1988-1997.
56. Kim GH, Lee JW, Oh YK, et al. Antidiuretic effect of hydrochlorothiazide in lithium-induced nephrogenic diabetes insipidus is associated with upregulation of aquaporin-2, Na-Cl co-transporter, and epithelial sodium channel. *J Am Soc Nephrol*. 2004;15(11):2836-2843.
57. Sinke AP, Kortenoeven ML, de Groot T, et al. Hydrochlorothiazide attenuates lithium-induced nephrogenic diabetes insipidus independently of the sodium-chloride cotransporter. *Am J Physiol Renal Physiol*. 2014;306(5):F525-F533.
58. Wilson IM, Freis ED. Relationship between plasma and extracellular fluid volume depletion and the antihypertensive effect of chlorothiazide. *Circulation*. 1959;20:1028-1036.
59. Bennett WM, McDonald WJ, Kuehnel E, Hartnett MN, Porter GA. Commentary. *Clin Pharmacol Ther*. 1977;22(5part1):499-504.
60. Tarazi RC, Dustan HP, Frohlich ED. Long-term thiazide therapy in essential hypertension. Evidence for persistent alteration in plasma volume and renin activity. *Circulation*. 1970;41(4):709-717.
61. Priddle WW, Liu SF, Breithaupt DJ. Management of hypertension—further sodium and potassium studies. *J Am Geriatr Soc*. 1970;18(11):861-892.
62. Hughes AD. How do thiazide and thiazide-like diuretics lower blood pressure? *J Renin-Angio-Aldo Syst*. 2004;5(4):155-160.
63. Tamargo J, Segura J, Ruilope LM. Diuretics in the treatment of hypertension. Part 1: thiazide and thiazide-like diuretics. *Expert Opin Pharmacother*. 2014;15(4):527-547.
64. Calder JA, Schachter M, Sever PS. Potassium channel opening properties of thiazide diuretics in isolated Guinea pig resistance arteries. *J Cardiovasc Pharmacol*. 1994;24(1):158-164.
65. Colas B, Slama M, Collin T, Safar M, Andrejak M. Mechanisms of methyclothiazide-induced inhibition of contractile responses in rat aorta. *Eur J Pharmacol*. 2000;408(1):63-67.
66. Zhu Z, Zhu S, Liu D, Cao T, Wang L, Tepel M. Thiazide-like diuretics attenuate agonist-induced vasoconstriction by calcium desensitization linked to rho kinase. *Hypertension*. 2005;45(2):233-239.
67. Calder JA, Schachter M, Sever PS. Ion channel involvement in the acute vascular effects of thiazide diuretics and related compounds. *J Pharmacol Exp Ther*. 1993;265(3):1175-1180.
68. De Wildt DJ, Van de Kuil A, Hillen FC, De Groot G. Further study of possible direct vascular actions of indapamide in the conduit and renal arteriolar vessels of spontaneously hypertensive rats. *Eur J Pharmacol*. 1988;147(3):327-334.
69. Del Rio M, Chulia T, Gonzalez P, Tejerina T. Effects of indapamide on contractile responses and 45Ca2+ movements in various isolated blood vessels. *Eur J Pharmacol*. 1993;250(1):133-139.
70. Mironneau J, Savineau JP, Mironneau C. Compared effects of indapamide, hydrochlorothiazide and chlorthalidone on electrical and mechanical activities in vascular smooth muscle. *Eur J Pharmacol*. 1981;75(2–3):109-113.
71. Rapoport RM, Soleimani M. Mechanism of thiazide diuretic arterial pressure reduction: the search continues. *Front Pharmacol*. 2019;10:815.
72. Pickkers P, Hughes AD, Russel FG, Thien T, Smits P. Thiazide-induced vasodilation in humans is mediated by potassium channel activation. *Hypertension*. 1998;32(6):1071-1076.
73. Overbeck HW, Molnar JI, Haddy FJ. Resistance to blood flow through the vascular bed of the dog forelimb: local effects of sodium, potassium, calcium, magnesium, acetate, hypertonicity and hypotonicity. *Am J Cardiol*. 1961;8(4):533-541.
74. Swenson ER. New insights into carbonic anhydrase inhibition, vasodilation, and treatment of hypertensive-related diseases. *Curr Hypertens Rep*. 2014;16(9):467.
75. Scales CD Jr, Smith AC, Hanley JM, Saigal CS. Urologic diseases in America P. Prevalence of kidney stones in the United States. *Eur Urol*. 2012;62(1):160-165.
76. Coe FL, Worcester EM, Evan AP. Idiopathic hypercalciuria and formation of calcium renal stones. *Nat Rev Nephrol*. 2016;12(9):519-533.
77. Kirkali Z, Rasooly R, Star RA, Rodgers GP. Urinary stone disease: Progress, status, and needs. *Urology*. 2015;86(4):651-653.
78. Worcester EM, Coe FL. New insights into the pathogenesis of idiopathic hypercalciuria. *Semin Nephrol*. 2008;28(2):120-132.
79. Asplin J, Parks J, Lingeman J, et al. Supersaturation and stone composition in a network of dispersed treatment sites. *J Urol*. 1998;159(6):1821-1825.
80. Parks JH, Coward M, Coe FL. Correspondence between stone composition and urine supersaturation in nephrolithiasis. *Kidney Int*. 1997;51(3):894-900.
81. Coe FL, Wise H, Parks JH, Asplin JR. Proportional reduction of urine supersaturation during nephrolithiasis treatment. *J Urol*. 2001;166(4):1247-1251.
82. Rodgers A, Allie-Hamdulay S, Jackson G. Therapeutic action of citrate in urolithiasis explained by chemical speciation: increase in pH is the determinant factor. *Nephrol Dial Transplant*. 2006;21(2):361-369.
83. Marangella M, Petrarulo M, Vitale C, Daniele P, Sammartano S. LITHORISK.COM: the novel version of a software for calculating and visualizing the risk of renal stone. *Urolithiasis*. 2021;49(3):211-217.
84. Werness PG, Brown CM, Smith LH, Finlayson B. EQUIL2: a BASIC computer program for the calculation of urinary saturation. *J Urol*. 1985;134(6):1242-1244.
85. Ferraro PM, Ticinesi A, Meschi T, et al. Short-term changes in urinary relative supersaturation predict recurrence of kidney stones: a tool to guide preventive measures in urolithiasis. *J Urol*. 2018;200(5):1082-1087.
86. Borghi L, Meschi T, Amato F, Briganti A, Novarini A, Giannini A. Urinary volume, water and recurrences in idiopathic calcium nephrolithiasis: a 5-year randomized prospective study. *J Urol*. 1996;155(3):839-843.
87. Prochaska M, Taylor E, Ferraro PM, Curhan G. Relative supersaturation of 24-hour urine and likelihood of kidney stones. *J Urol*. 2018;199(5):1262-1266.
88. Rodriguez A, Cunha TDS, Rodgers AL, Gambaro G, Ferraro PM. Comparison of supersaturation outputs from different programs and their application in testing correspondence with kidney stone composition. *J Endourol*. 2021;35(5):687-694.
89. Siener R, Glatz S, Nicolay C, Hesse A. Prospective study on the efficacy of a selective treatment and risk factors for

relapse in recurrent calcium oxalate stone patients. *Eur Urol*. 2003;44(4):467-474.
90. Borghi L, Schianchi T, Meschi T, et al. Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. *N Engl J Med*. 2002;346(2):77-84.
91. Moe OW. Kidney stones: pathophysiology and medical management. *Lancet*. 2006;367(9507):333-344.
92. Taylor EN, Curhan GC. Demographic, dietary, and urinary factors and 24-h urinary calcium excretion. *Clin J Am Soc Nephrol*. 2009;4(12):1980-1987.
93. Curhan GC, Taylor EN. 24-h uric acid excretion and the risk of kidney stones. *Kidney Int*. 2008;73(4):489-496.
94. Arivoli K, Valicevic AN, Oerline MK, et al. Preventive Pharmacological Therapy and Risk of Recurrent Urinary Stone Disease. *Clin J Am Soc Nephrol*. 2024;9900:1-8. doi:10.2215/CJN.0000000000000428. Epub ahead of print.
95. Bergsland KJ, Worcester EM, Coe FL. Role of proximal tubule in the hypocalciuric response to thiazide of patients with idiopathic hypercalciuria. *Am J Physiol Renal Physiol*. 2013;305(4):F592-F599.
96. Costanzo LS, Windhager EE. Calcium and sodium transport by the distal convoluted tubule of the rat. *Am J Phys*. 1978;235(5):F492-F506.
97. Lee CT, Shang S, Lai LW, Yong KC, Lien YH. Effect of thiazide on renal gene expression of apical calcium channels and calbindins. *Am J Physiol Renal Physiol*. 2004;287(6):F1164-F1170.
98. Lee J, Cha S-K, Sun T-J, Huang C-L. PIP2 activates TRPV5 and releases its inhibition by intracellular $Mg^{2+}$. *J General Physiol*. 2005;126(5):439-451.
99. Rodenburg EM, Visser LE, Hoorn EJ, et al. Thiazides and the risk of hypokalemia in the general population. *J Hypertens*. 2014;32(10):2092-2097. discussion 2097.
100. Zillich AJ, Garg J, Basu S, Bakris GL, Carter BL. Thiazide diuretics, potassium, and the development of diabetes: a quantitative review. *Hypertension*. 2006;48(2):219-224.
101. Liamis G, Rodenburg EM, Hofman A, Zietse R, Stricker BH, Hoorn EJ. Electrolyte disorders in community subjects: prevalence and risk factors. *Am J Med*. 2013;126(3):256-263.
102. Pak CY, Peterson R, Sakhaee K, Fuller C, Preminger G, Reisch J. Correction of hypocitraturia and prevention of stone formation by combined thiazide and potassium citrate therapy in thiazide-unresponsive hypercalciuric nephrolithiasis. *Am J Med*. 1985;79(3):284-288.
103. Zuckerman JM, Assimos DG. Hypocitraturia: pathophysiology and medical management. *Rev Urol*. 2009;11(3):134-144.
104. Ellison DH, Loffing J. Thiazide effects and adverse effects: insights from molecular genetics. *Hypertension*. 2009;54(2):196-202.
105. Adler S, Fraley DS. Potassium and intracellular pH. *Kidney Int*. 1977;11(6):433-442.
106. Soleimani M, Bergman JA, Hosford MA, McKinney TD. Potassium depletion increases luminal $Na^+/H^+$ exchange and basolateral $Na^+$:CO3=:HCO3− cotransport in rat renal cortex. *J Clin Invest*. 1990;86(4):1076-1083.
107. Levi M, McDonald LA, Preisig PA, Alpern RJ. Chronic K depletion stimulates rat renal brush-border membrane Na-citrate cotransporter. *Am J Phys*. 1991;261(5 Pt 2):F767-F773.
108. Moe OW, Preisig PA. Dual role of citrate in mammalian urine. *Curr Opin Nephrol Hypertens*. 2006;15(4):419-424.
109. Scholz D, Schwille PO, Sigel A. Double-blind study with thiazide in recurrent calcium lithiasis. *J Urol*. 1982;128(5):903-907.
110. Borghi L, Meschi T, Guerra A, Novarini A. Randomized prospective study of a nonthiazide diuretic, indapamide, in preventing calcium stone recurrences. *J Cardiovasc Pharmacol*. 1993;22(Suppl 6):S78-S86.
111. Triozzi JL, Hsi RS, Wang G, et al. Mendelian randomization analysis of genetic proxies of thiazide diuretics and the reduction of kidney stone risk. *JAMA Netw Open*. 2023;6(11):e2343290.
112. Ettinger B, Citron JT, Livermore B, Dolman LI. Chlorthalidone reduces calcium oxalate calculous recurrence but magnesium hydroxide does not. *J Urol*. 1988;139(4):679-684.
113. Lemieux G. Treatment of idiopathic hypercalciuria with indapamide. *CMAJ*. 1986;135(2):119-121.
114. Borghi L, Elia G, Trapassi MR, et al. Acute effect of indapamide on urine calcium excretion in nephrolithiasis and human essential hypertension. *Pharmacology*. 1988;36(5):348-355.
115. Wolfgram DF, Gundu V, Astor BC, Jhagroo RA. Hydrochlorothiazide compared to chlorthalidone in reduction of urinary calcium in patients with kidney stones. *Urolithiasis*. 2013;41(4):315-322.
116. Coe FL, Parks JH, Bushinsky DA, Langman CB, Favus MJ. Chlorthalidone promotes mineral retention in patients with idiopathic hypercalciuria. *Kidney Int*. 1988;33(6):1140-1146. doi: 10.1038/ki.1988
117. Martins MC, Meyers AM, Whalley NA, Margolius LP, Buys ME. Indapamide (Natrilix): the agent of choice in the treatment of recurrent renal calculi associated with idiopathic hypercalciuria. *Br J Urol*. 1996;78(2):176-180.
118. Alonso D, Pieras E, Piza P, Grases F, Prieto RM. Effects of short and long-term indapamide treatments on urinary calcium excretion in patients with calcium oxalate dihydrate urinary stone disease: a pilot study. *Scand J Urol Nephrol*. 2012;46(2):97-101.
119. Barcelo P, Wuhl O, Servitge E, Rousaud A, Pak CY. Randomized double-blind study of potassium citrate in idiopathic hypocitraturic calcium nephrolithiasis. *J Urol*. 1993;150(6):1761-1764.
120. Ettinger B, Pak CY, Citron JT, Thomas C, Adams-Huet B, Vangessel A. Potassium-magnesium citrate is an effective prophylaxis against recurrent calcium oxalate nephrolithiasis. *J Urol*. 1997;158(6):2069-2073.
121. Phillips R, Hanchanale VS, Myatt A, Somani B, Nabi G, Biyani CS. Citrate salts for preventing and treating calcium containing kidney stones in adults. *Cochrane Database Syst Rev*. 2015;2015(10):CD010057.
122. Pearle MS, Goldfarb DS, Assimos DG, et al. Medical management of kidney stones: AUA guideline. *J Urol*. 2014;192(2):316-324.
123. Ljungberg B, Albiges L, Bedke J, et al. EAU guidelines, edn. *Presented at the EAU Annual Congress Milan 2021*. EAU Guidelines Office; 2021 http://uroweborg/guidelines/compilations-of-all-guidelines
124. Qaseem A, Dallas P, Forciea MA, Starkey M, Denberg TD. Clinical Guidelines Committee of the American College of Physicians. Dietary and pharmacologic management to prevent recurrent nephrolithiasis in adults: a clinical practice guideline from the American College of Physicians. *Ann Intern Med*. 2014;161(9):659-667. doi: 10.7326/M13-2908

125. Amery A, Berthaux P, Bulpitt C, et al. Glucose intolerance during diuretic therapy. Results of trial by the European Working Party on Hypertension in the Elderly. *Lancet*. 1978;1(8066):681-683.
126. Goldner MG, Zarowitz H, Akgun S. Hyperglycemia and glycosuria due to thiazide derivatives administered in diabetes mellitus. *N Engl J Med*. 1960;262:403-405.
127. Hollis WC. Aggravation of diabetes mellitus during treatment with chlorothiazide. *JAMA*. 1961;176:947-949.
128. Samaan N, Dollery CT, Fraser R. Diabetogenic action of benzothiadiazines: serum-insulin-like activity in diabetes worsened or precipitated by thiazide diuretics. *Lancet*. 1963;2(7320):1244-1246.
129. Taylor EN, Hu FB, Curhan GC. Antihypertensive medications and the risk of incident type 2 diabetes. *Diabetes Care*. 2006;29(5):1065-1070.
130. Bengtsson C, Blohme G, Lapidus L, et al. Do antihypertensive drugs precipitate diabetes? *Br Med J (Clin Res Ed)*. 1984;289(6457):1495-1497.
131. Bengtsson C, Blohme G, Lapidus L, Lissner L, Lundgren H. Diabetes incidence in users and non-users of antihypertensive drugs in relation to serum insulin, glucose tolerance and degree of adiposity: a 12-year prospective population study of women in Gothenburg, Sweden. *J Intern Med*. 1992;231(6):583-588.
132. Bakris G, Molitch M, Hewkin A, et al. Differences in glucose tolerance between fixed-dose antihypertensive drug combinations in people with metabolic syndrome. *Diabetes Care*. 2006;29(12):2592-2597.
133. Smith SM, Anderson SD, Wen S, et al. Lack of correlation between thiazide-induced hyperglycemia and hypokalemia: subgroup analysis of results from the pharmacogenomic evaluation of antihypertensive responses (PEAR) study. *Pharmacotherapy*. 2009;29(10):1157-1165.
134. Barzilay JI, Davis BR, Cutler JA, et al. Fasting glucose levels and incident diabetes mellitus in older nondiabetic adults randomized to receive 3 different classes of antihypertensive treatment: a report from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *Arch Intern Med*. 2006;166(20):2191-2201.
135. Dhayat NA, Faller N, Bonny O, et al. Efficacy of standard and low dose hydrochlorothiazide in the recurrence prevention of calcium nephrolithiasis (NOSTONE trial): protocol for a randomized double-blind placebo-controlled trial. *BMC Nephrol*. 2018;19(1):349.
136. Elliott WJ, Meyer PM. Incident diabetes in clinical trials of antihypertensive drugs: a network meta-analysis. *Lancet*. 2007;369(9557):201-207.
137. Ren H, Qin L, Wang W, et al. Abnormal glucose metabolism and insulin sensitivity in Chinese patients with Gitelman syndrome. *Am J Nephrol*. 2013;37(2):152-157.
138. Blanchard A, Vallet M, Dubourg L, et al. Resistance to insulin in patients with Gitelman syndrome and a subtle intermediate phenotype in heterozygous carriers: a cross-sectional study. *J Am Soc Nephrol*. 2019;30(8):1534-1545.
139. Carter BL, Einhorn PT, Brands M, et al. Thiazide-induced dysglycemia: call for research from a working group from the national heart, lung, and blood institute. *Hypertension*. 2008;52(1):30-36.
140. Lakshman MR, Reda DJ, Materson BJ, Cushman WC, Freis ED. Diuretics and beta-blockers do not have adverse effects at 1 year on plasma lipid and lipoprotein profiles in men with hypertension. Department of Veterans Affairs Cooperative Study Group on Antihypertensive Agents. *Arch Intern Med*. 1999;159(6):551-558.
141. Savage PJ, Pressel SL, Curb JD, et al. Influence of long-term, low-dose, diuretic-based, antihypertensive therapy on glucose, lipid, uric acid, and potassium levels in older men and women with isolated systolic hypertension: the Systolic Hypertension in the Elderly Program. SHEP Cooperative Research Group. *Arch Intern Med*. 1998;158(7):741-751.
142. Catena C, Lapenna R, Baroselli S, et al. Insulin sensitivity in patients with primary aldosteronism: a follow-up study. *J Clin Endocrinol Metab*. 2006;91(9):3457-3463.
143. Corry DB, Tuck ML. The effect of aldosterone on glucose metabolism. *Curr Hypertens Rep*. 2003;5(2):106-109.
144. Pickkers P, Schachter M, Hughes AD, Feher MD, Sever PS. Thiazide-induced hyperglycaemia: a role for calcium-activated potassium channels? *Diabetologia*. 1996;39(7):861-864.
145. Sandstrom PE. Inhibition by hydrochlorothiazide of insulin release and calcium influx in mouse pancreatic beta-cells. *Br J Pharmacol*. 1993;110(4):1359-1362.
146. Pollare T, Lithell H, Berne C. A comparison of the effects of hydrochlorothiazide and captopril on glucose and lipid metabolism in patients with hypertension. *N Engl J Med*. 1989;321(13):868-873.
147. Eriksson JW, Jansson PA, Carlberg B, et al. Hydrochlorothiazide, but not candesartan, aggravates insulin resistance and causes visceral and hepatic fat accumulation: the mechanisms for the diabetes preventing effect of candesartan (MEDICA) study. *Hypertension*. 2008;52(6):1030-1037.
148. Cooper-DeHoff RM, Wen S, Beitelshees AL, et al. Impact of abdominal obesity on incidence of adverse metabolic effects associated with antihypertensive medications. *Hypertension*. 2010;55(1):61-68.
149. Harper R, Ennis CN, Sheridan B, Atkinson AB, Johnston GD, Bell PM. Effects of low dose versus conventional dose thiazide diuretic on insulin action in essential hypertension. *BMJ*. 1994;309(6949):226-230.
150. Rooney DP, Neely RD, Ennis CN, et al. Insulin action and hepatic glucose cycling in essential hypertension. *Metabolism*. 1992;41(3):317-324.
151. Sener A, Jijakli H, Zahedi Asl S, et al. Possible role of carbonic anhydrase in rat pancreatic islets: enzymatic, secretory, metabolic, ionic, and electrical aspects. *Am J Physiol Endocrinol Metab*. 2007;292(6):E1624-E1630.
152. Parkkila AK, Scarim AL, Parkkila S, Waheed A, Corbett JA, Sly WS. Expression of carbonic anhydrase V in pancreatic beta cells suggests role for mitochondrial carbonic anhydrase in insulin secretion. *J Biol Chem*. 1998;273(38):24620-24623.
153. Boquist L, Backman AM, Stromberg C. Effects of acetazolamide on insulin release, serum glucose and insulin, glucose tolerance, and alloxan sensitivity of mice. *Med Biol*. 1980;58(3):169-173.
154. Kucharczyk P, Albano G, Deisl C, et al. Thiazides attenuate insulin secretion through inhibition of mitochondrial carbonic anhydrase 5b in β-islet cells in mice. *J Am Soc Nephrol*. 2023;34:1179-1190. doi:10.1681/ASN.0000000000000122
155. Matsumoto K, Miyazaki H, Fujii T, Hashimoto M. Binding of sulfonamides to erythrocytes and their components. *Chem Pharm Bull (Tokyo)*. 1989;37(7):1913-1915.

156. Dieterle W, Wagner J, Faigle JW. Binding of chlorthalidone (Hygroton®) to blood components in man. *Eur J Clin Pharmacol*. 1976;10:37-42.
157. Temperini C, Cecchi A, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Interaction of indapamide and related diuretics with 12 mammalian isozymes and X-ray crystallographic studies for the indapamide-isozyme II adduct. *Bioorg Med Chem Lett*. 2008;18(8):2567-2573.
158. Temperini C, Cecchi A, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Comparison of chlorthalidone, indapamide, trichloromethiazide, and furosemide X-ray crystal structures in adducts with isozyme II, when several water molecules make the difference. *Bioorg Med Chem*. 2009;17(3):1214-1221.
159. Shah GN, Rubbelke TS, Hendin J, et al. Targeted mutagenesis of mitochondrial carbonic anhydrases VA and VB implicates both enzymes in ammonia detoxification and glucose metabolism. *Proc Natl Acad Sci USA*. 2013;110(18):7423-7428.
160. Merrins MJ, Corkey BE, Kibbey RG, Prentki M. Metabolic cycles and signals for insulin secretion. *Cell Metab*. 2022;34:947-968.
161. MacDonald MJ. Feasibility of a mitochondrial pyruvate malate shuttle in pancreatic islets. Further implication of cytosolic NADPH in insulin secretion. *J Biol Chem*. 1995;270(34):20051-20058.
162. Xu J, Han J, Long YS, Epstein PN, Liu YQ. The role of pyruvate carboxylase in insulin secretion and proliferation in rat pancreatic beta cells. *Diabetologia*. 2008;51(11):2022-2030.
163. Prentki M, Matschinsky FM, Madiraju SR. Metabolic signaling in fuel-induced insulin secretion. *Cell Metab*. 2013;18(2):162-185.
164. Abulizi A, Cardone RL, Stark R, et al. Multi-tissue acceleration of the mitochondrial phosphoenolpyruvate cycle improves whole-body metabolic health. *Cell Metab*. 2020;32(5):751-766 e711.
165. Lewandowski SL, Cardone RL, Foster HR, et al. Pyruvate kinase controls signal strength in the insulin secretory pathway. *Cell Metab*. 2020;32(5):736-750 e735.
166. Sehnal D, Bittrich S, Deshpande M, et al. Mol* viewer: modern web app for 3D visualization and analysis of large biomolecular structures. *Nucleic Acids Res*. 2021;49(W1):W431-W437.
167. Cryo-EM structure of human NCC (class 1). 2022.
168. Berman HM, Westbrook J, Feng Z, et al. The protein data bank. *Nucleic Acids Res*. 2000;28(1):235-242.

**How to cite this article:** Bargagli M, Anderegg MA, Fuster DG. Effects of thiazides and new findings on kidney stones and dysglycemic side effects. *Acta Physiol*. 2024;00:e14155. doi:10.1111/apha.14155