# WHY YOUR MOTHER WAS RIGHT: HOW POTASSIUM INTAKE REDUCES BLOOD PRESSURE

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## ABSTRACT

Low potassium intake, common in western diets, increases blood pressure and enhances salt-sensitivity. Most humans in "Westernized" countries also consume excess salt. In studies using mice, we found that a high-salt, low-potassium diet activates the thiazide-sensitive Na-Cl cotransporter in the kidney. This effect led to sodium retention and increased blood pressure, and was dependent on plasma potassium. We postulated that this effect was mediated by changes in intracellular chloride caused by changes in membrane voltage. We developed a model in cultured cells permitting us to confirm this hypothesis. We then confirmed, using urinary exosomes, that dietary changes in normal humans, affect the thiazide-sensitive Na-Cl cotransporter in the same way. These data show that dietary potassium deficiency increases blood pressure largely by stimulating salt reabsorption along the distal nephron. They suggest that global efforts should focus on increasing potassium intake, which will attenuate the effects of high-salt diets.

## INTRODUCTION

The majority of people in the world today consume a diet relatively high in salt (NaCl) and low in potassium ($K^+$) (1). Such a diet has been associated with hypertension, cardiovascular disease, and all-cause mortality. We focused on the distal convoluted tubule (DCT) and the thiazide-sensitive NaCl cotransporter, as this segment of the nephron has recently been shown to modulate potassium excretion. The DCT in most mammalian species is a heterogeneous segment, comprising a proximal portion, the DCT1, which primarily reabsorbs NaCl, and a distal portion, the DCT2, where electroneutral NaCl transport coexists with electrogenic $Na^+$ and $K^+$ transport pathways (2–5).

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The thiazide-sensitive NaCl cotransporter (NCC, gene symbol *SLC12A3*) is the predominant apical $Na^+$ entry pathway in DCT1 cells, cells in which electrogenic transport does not take place. Because DCT cells also control the delivery of NaCl into the connecting tubule, where the epithelial sodium channel (ENaC) mediates electrogenic $Na^+$ reabsorption and where $K^+$ is secreted (6), they appear to have a substantial, albeit indirect, role in $K^+$ secretion. The importance of the DCT in $K^+$ secretion became apparent when the molecular solution of FHHt led to the discovery of a molecular switch comprising the "with no lysine" (WNK) kinases, Ste20p-related proline alanine-rich kinase (SPAK) and oxidative stress response 1 kinase (OxSR1). FHHt-causing disease mutations appear to lock this switch in the "on" position, driving unrelenting NCC activity, leading to hyperkalemia and hypertension. Although this pathogenesis is clear, the nature of the physiological switch activator has remained elusive.

NCC is activated physiologically when dietary NaCl intake is reduced. Yet, NCC also responds to changes in dietary $K^+$ intake. High KCl intake suppresses (7−10), and low KCl intake increases NCC activity (9,11,12). These effects contribute to systemic $K^+$ homeostasis by altering $Na^+$ delivery to $K^+$ secretory segments.(9,13). We therefore sought to identify the factors modulating DCT function and NCC activity.

## MATERIALS AND METHODS

### Animals

All animal studies were approved by Oregon Health and Science University's Animal Care and Usage Committee (Protocol IS918). All mice were 12- to 24-weeks old, 25 to 30 g, and had a C57Bl/6 background except for $NCC^{-/-}$ mice, which were on a BALB/c background.

### Blood Pressure Measurement

Noninvasive systolic blood pressures were measured with tail-cuffs using volumetric pressure recording (CODA-16; Kent Scientific, Torrington, CT, USA). Mice were acclimated to the machine for 5 consecutive days before recording. In the dietary manipulation studies, mice were maintained on the baseline diet (high NaCl [6%]/normal $K^+$ [1%]) (Harlan Laboratories, Indianapolis, IN, USA, $K^+$-deficient diet [TD.88239] supplemented with NaCl and KCl) for 1 week before measurements were obtained. After blood pressures were recorded for 4 days on the baseline diet, diets were changed to high-NaCl/low-$K^+$

(0%). Animals were allowed to acclimate to the new diet for at least 4 days and data were recorded after this period.

### Blood Analysis

Whole blood was collected via cardiac puncture, under anesthesia. Electrolytes were measured by iSTAT (Abbott Point of Care, Inc., Princeton, NJ, USA).

### Immunoblotting

Mice were maintained on indicated diets for 7 to 10 days or treated with amiloride (50 mg/L drinking water) for 5 to 7 days, after which kidneys were harvested and snap-frozen in liquid nitrogen. Kidneys were then homogenized on ice in chilled buffer containing protease and phosphatase inhibitors. Protein (20 to 80 $\mu$g) was separated on 4–12% (wt/vol) Bis-Tris gel (Invitrogen, Grand Island, NY, USA).

### Cell Culture

Flp-In NCC cells were generated as reported previously (14). HEK293T cells (American Type Culture Collection) were grown in Dulbecco's modified Eagle medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) in 5% $CO_2$ and 95% air at 37°C. Cells were grown to 50% to 70% confluence for transfection, and the corresponding cDNAs were simultaneously applied to the cells using TurboFect transfection reagent (Fermentas, Grand Junction, NY, USA). For the measurement of $K^+$ reversal potential, an Axon 200A patch-clamp amplifier was used, as reported (15).

### Human Studies

All human studies were approved by Oregon Health & Science University's Internal Review Board (Protocol IRB9934). Healthy human volunteers were asked to consume two different diets, a high-salt/low-$K^+$ diet and a high-salt/$K^+$-replete diet, each for a 4-day period. Volunteers were randomized to the order in which they consumed the two diets. On the morning of day 5 of each diet, urine was collected and spun in an ultracentrifuge according to a previously published protocol (16).

## RESULTS

In mice, we found that NCC and phosphorylated NCC (pNCC, as an index of activation) were more abundant in kidneys of mice consuming

a high-salt/low-$K^+$ (HS/LK) diet than a high-salt/normal-$K^+$ (HS/NK) diet (Fig. 1A). We then showed that mean arterial pressure, measured telemetrically, increased when mice were switched from HS/NK to HS/LK diet (Fig. 1B). The increase in arterial pressure on the LK diet was primarily during the active period, consistent with prior reports (11). To determine whether activation of NCC during LK diet contributed to the increase in blood pressure, we compared the effects of diet in *Slc12a3*$^{-/-}$ mice, in which NCC has been deleted genetically, and littermates. Note that *Slc12a3*$^{-/-}$ mice have normal blood pressure at baseline, as reported previously (17). In *Slc12a3*$^{-/-}$ mice, blood pressure did not increase during LK diet, whereas it did in littermates (Fig. 1B). The impact of genotype on response to LK diet was significant.

To determine whether humans exhibit a similar response to dietary $K^+$ intake, we analyzed pNCC in urinary exosomes (18,19) from volunteers who consumed an HS/LK diet for 4 days and then an HS/NK diet for 4 days. The abundance of pNCC was significantly greater after the HS/LK diet than after the HS/NK diet (Fig. 1C). The diets had similar $Na^+$ and calorie content, whereas dietary $K^+$ differed.

Plasma potassium was significantly lower in mice consuming an LK, compared with an NK diet (2.37 ± 0.13 vs. 3.38 ± 0.04). Yet some have

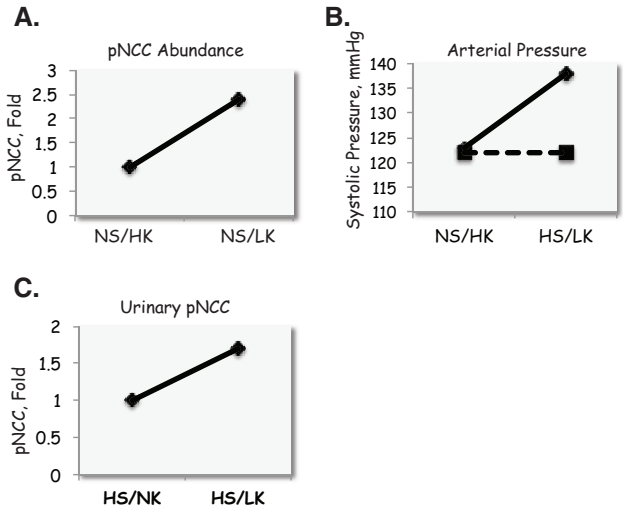


FIG. 1. Effects of dietary potassium intake on NCC abundance. **(A)** Effects of high-salt/high-potassium (HS/HK) versus high-salt/low-potassium (HS/LK) diet on the abundance of phosphorylated NCC (pNCC), measured in arbitrary units. **(B)** Effects of the same diets on arterial pressure in wild type mice (*solid line*) and NCC$^{-/-}$ mice (*dashed line*). **(C)** Effect of similar diets on pNCC in the uriine of human volunteers.

suggested that $K^+$ must be ingested orally to alter NCC (8). To test the role of plasma [$K^+$] without changing $K^+$ intake, we manipulated plasma [$K^+$] pharmacologically with the $K^+$-sparing diuretic amiloride. Amiloride directly inhibits the ENaC, leading to $K^+$ retention, but it does not affect NCC directly (20). Amiloride treatment of mice caused substantial hyperkalemia and (Fig. 2A), and, as suggested by a higher hematocrit, (Fig. 2B), extracellular fluid (ECF) volume depletion. Despite the volume depletion, which would be expected to stimulate NCC, amiloride reduced both NCC and pNCC, suggesting that the $K^+$ signal overrides the ECF signal (Fig. 2C). Because amiloride might have effects unrelated to the changes in plasma [$K^+$], we treated mice with an LK diet along with amiloride to maintain [$K^+$] in the normal range. This prevented hyperkalemia (Fig. 2A), alterations in hematocrit (Fig. 2B), and alterations in NCC and pNCC (Fig. 2C). Together these results suggest that dietary $K^+$ intake affects NCC through effects on plasma or total body $K^+$.

We next tested whether NCC is modulated directly by extracellular [$K^+$], using cultured Flp-In NCC cells (14). Cells cultured in LK medium had greater pNCC abundance than cells cultured in NK (Fig. 3A). Because LK medium would be expected to hyperpolarize cells (21), we determined the effects of changing membrane potential

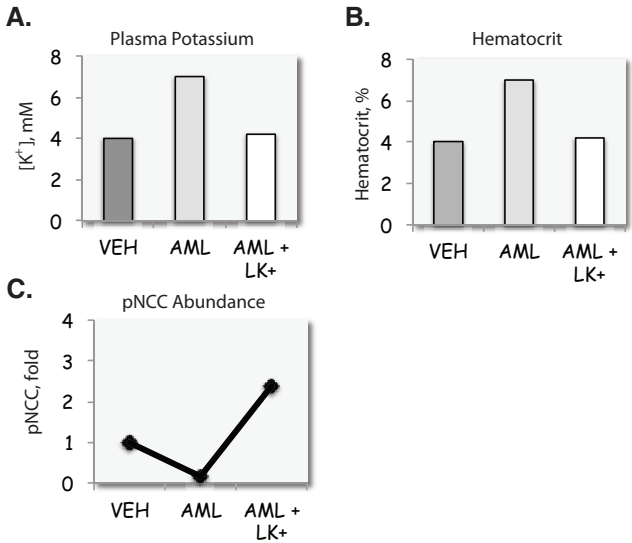


FIG. 2. Effects of plasma potassium on NCC abundance. **(A)** Plasma potassium concentration in mice treated with vehicle (VEH), amiloride (AML), or amiloride plus low potassium diet (AML+LK). **(B)** Hematocrit values in the same mice. **(C)** Abundance of pNCC in the kidney of the same mice.

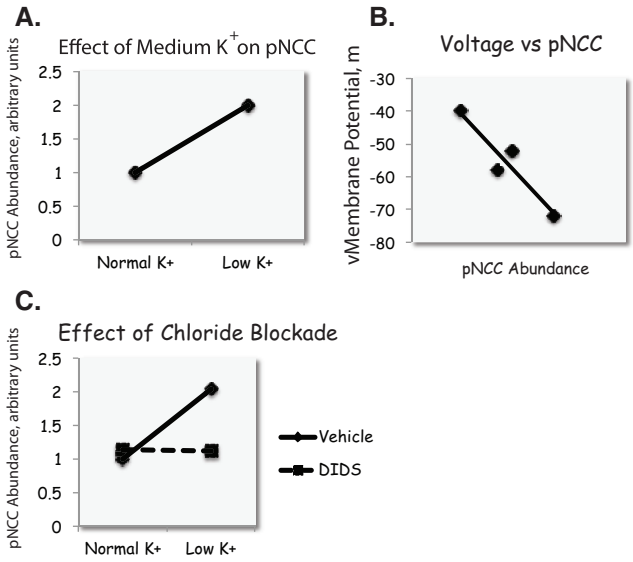


FIG. 3. Effects of potassium and membrane voltage on pNCC in cultured cells. **(A)** Effect of low $K^+$ medium on pNCC abundance in cultured Human Embryonic Kidney 293 cells. **(B)** Regression of pNCC abundance versus membrane voltage in HEK cells. **(C)** Effect of blocking chloride channels with DIDS on the effects of low $K^+$ medium in HEK cells.

on pNCC. We found a close correlation between membrane voltage and pNCC abundance (Fig. 3B). We then showed that effects of $K^+$ on pNCC could be blocked by the chloride channel blocking drug 4,4′-Diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS) (Fig. 3C).

## DISCUSSION

These results show the dominant role that plasma [$K^+$] plays in regulating NCC, and indicate that extracellular [$K^+$] affects NCC indirectly by modulating intracellular [$Cl^-$]. Because the DCT lies upstream of important $K^+$ secretory nephron segments, NCC activity will have important effects on $K^+$ excretion. The results suggest that the health benefits of dietary $K^+$ supplementation are highly dependent on NCC.

The current results confirm that dietary $K^+$ intake modulates pNCC abundance, and show that dietary $K^+$ intake is more powerful in this regard than ECF volume. These effects on NCC lead to blood pressure changes, which require modulation of NCC activity. They are mediated largely by changes in plasma [$K^+$], as the effects were reproduced when plasma [$K^+$] was altered using drugs or diet, a

conclusion consistent with $K^+$ infusion experiments recently reported (7).

The results indicate that extracellular [$K^+$], independent of hormonal effects, directly modulates the WNK-SPAK/OxSR1-NCC axis. Our cell model suggested that changes in membrane voltage are involved, as maneuvers that alter membrane voltage produced changes in pNCC abundance consistent with voltage dependence. The evidence also suggests that the effects of potassium are mediated by changes in intracellular chloride concentration.

It is widely accepted that a low NaCl diet stimulates NCC activity, contributing to physiologically beneficial NaCl retention, through the actions of hormones including angiotensin II and aldosterone (22,23). The current work adds to a growing body of evidence showing that NCC is also activated by an LK diet, and that the effects of an LK diet on NCC activity occur, and are enhanced, by HS intake. While this conclusion seems counterintuitive, it corroborates recent results by another group (11). Further, it likely accounts for the observation that dietary $K^+$ has a greater effect to lower pressure in humans during high- compared with low-NaCl intake (24). As the effects of an HS/LK diet on arterial pressure are absent when NCC has been deleted genetically (Fig. 2C), NCC plays an essential and non-redundant role in the antihypertensive effects of dietary $K^+$. Because we documented that dietary $K^+$ deprivation in humans also stimulates NCC, the results are relevant to human health, where an HS/LK diet is widely considered to contribute to the global epidemic of hypertension (1,25,26).

The results suggest that the DCT acts as a renal $K^+$ sensor, working in concert with the adrenal gland to preserve $K^+$ homeostasis. In this respect, DCT cells and adrenal cells of the zona glomerulosa both sense the same signal, membrane voltage, but respond in opposite directions. An increase in plasma [$K^+$] stimulates aldosterone production and release by the adrenal gland, whereas an increase in plasma [$K^+$] inhibits NCC. To produce kaliuresis, events in adrenal and DCT must occur *pari passu*. This is assured by having the same signal, extracellular [$K^+$], regulate both cell types. As adrenal cells respond to the ECF volume signal angiotensin II, DCT cells likely do too. In this case, however, the signals in the two organs are concordant.

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## DISCUSSION

**Wolf, Boston**: What is the effect of low and high magnesium on your cells, because magnesium affects almost all the transport channels you demonstrated?

**Ellison, Portland**: I don't know from a big picture standpoint. If you perfuse DCT segments or distill nephron segments with low magnesium in the bath, the cells hyperpolarize. So you might get effects. And certainly hypomagnesemia can make it difficult to correct hypokalemia and may contribute to that as well. So I need to think about that.

**Wolf, Boston**: There is some epidemiologic evidence that diets high in magnesium are associated with low or high blood pressure and lower cardiovascular risk.

**Ellison, Portland**: That's a great question that I need to spend some time thinking about.

**Zeidel, Boston**: Just to clarify, you have a cell in the distal convoluted tubule that does have an electroneutral sodium chloride entry, and it's having an impact on potassium. The thought is that there is reduced delivery; if it absorbs more salt, there is less sodium getting into the collecting duct. Therefore, it reduces the excretion of potassium. Have you measured chloride levels in the distal convoluted tubal cells? Have you started doing those kinds of measurements?

**Ellison, Portland**: We haven't measured it in DCT cells *in vivo*; we're hoping to do that. We have measured it in our cell model, and those are clearly affected in the way that we predict. And we have also shown that those low chloride concentrations in the cells are required for activation of NCC. We are working to move that into a more physiologic model, but as you know that is not so easy.

**Zeidel, Boston**: When you reduce the outside potassium level and you hyperpolarize the cell, does that hyperpolarization persist in a chronic state as opposed to what you do

in the culture where you abruptly lower the potassium? I would think that the cell would adapt, but maybe I am wrong.

**Ellison, Portland**: We always think of it as just the potassium setting the resting potential of cells, which makes me think it is more chronic. Certainly in the zona glomerulosa this is believed to be the main mechanism for potassium regulation of aldosterone secretion. I think it is assumed that it stays stable, but I don't know data to support that.

**Schuster, New York**: Are there activating mutations in the distal convoluted tubule K-channel that would mimic this effect?

**Ellison, Portland**: The mutations that we put into the cells are mutations that cause Epilepsy, Ataxia, Sensorineural Deafness and Tubulopathy Syndrome (called EAST or Seizures, Sensorineural Deafness, Ataxia, Mental Retardation, and Electrolyte Imbalance, called SeSAME syndrome) which is a sensory neuroepilepsy syndrome. But they look like Gitelman syndrome. Those mutations do have an effect that resembles Gitelman syndrome. They depolarize the cells and therefore turn off pNCC. They are not activating mutations in those channels that I am aware of.

**Blantz, San Diego**: We know a lot of humans who, in spite of high sodium/low potassium intake, do not become hypertensive. Do you have any kind epidemiologic studies in rodents that give you an example of why some are non-responders to this dietary formula?

**Ellison, Portland**: I think that is consistent with the model postulated by Rick Clifton where he showed heterozygous carrier states for the NCC or the NKCC2 appear to prevent the development of — or reduce the risk of developing — hypertension over the lifetime. Those would potentially be people who could eat a ton of salt and never get hypertensive.

**Blantz, San Diego**: Do you have a gene that your epidemiologic or outcomes colleagues could find that would direct you towards obviating their dietary indiscretions?

**Ellison, Portland**: We have a grant that we submitted together with Howard Pratt from Indiana University to try and look at some of those things, but I don't know that it has been done yet.

**Luke, Cincinnati**: There is evidence that on a low-salt diet potassium changes have much less effect on blood pressure than on a high-salt diet. When you're using a low-salt diet, the potassium load doesn't produce so much effect on blood pressure, which continues to stress the importance of salt in terms of extracellular volume. So what we should be focusing on is a high-potassium/low-salt diet. Do you agree with that?

**Ellison, Portland**: Yes.

**Luke, Cincinnati**: And we don't know how low the salt should go. I mean that is quite clear. The American heart may have gone too far with 1.5 grams. But I don't think we should forget the story, and maybe we need to emphasize potassium a bit more, and the DASH diet does that. Sodium chloride loading is very different from sodium bicarbonate loading, which again I think supports the hypothesis.

**Ellison, Portland**: Yes. Thank you for those comments.