

## REVIEW



# Sodium as an Important Regulator of Immunometabolism

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**ABSTRACT:** Salt sensitivity concerns blood pressure alterations after a change in salt intake (sodium chloride). The heart is a pump, and vessels are tubes; sodium can affect both. A high salt intake increases cardiac output, promotes vascular dysfunction and capillary rarefaction, and chronically leads to increased systemic vascular resistance. More recent findings suggest that sodium also acts as an important second messenger regulating energy metabolism and cellular functions. Besides endothelial cells and fibroblasts, sodium also affects innate and adaptive immunometabolism, immune cell function, and influences certain microbes and microbiota-derived metabolites. We propose the idea that the definition of salt sensitivity should be expanded beyond high blood pressure to cellular and molecular salt sensitivity. (*Hypertension*. 2024;81:426–435. DOI: 10.1161/HYPERTENSIONAHA.123.19489.) •

**Key Words:** macrophages ■ mitochondria ■ salts ■ sodium ■ T lymphocytes

## WHITHER SODIUM?

Earlier, all was clear.<sup>1</sup> The intracellular composition (muscle for instance) exhibited a sodium concentration of  $\pm 10$  mmol/L, potassium concentration of  $\pm 160$  mmol/L, magnesium concentration of  $\pm 35$  mmol/L, etc. Anions were protein and phosphate. In the extracellular plasma water, the corresponding concentrations were sodium,  $\pm 151$  mmol/L and potassium,  $\pm 4.3$  mmol/L. In humans, the electrolytes were distributed within the (about 40 L) total body water; two-thirds were inside cells and one-third outside cells. Sodium was mostly exchangeable and extracellular although some was deposited in bone, the fate of which was largely unclear but not so exchangeable. By the 1970s, the regulation of these constituents had been well worked out and balance concepts were sufficiently accepted to support pressure natriuresis of blood pressure regulation and our understanding of salt and water balance.<sup>2</sup> These ideas, and the supportive body of evidence, served us clinically well and

were convincing. A veritable clinical tool was the knowledge generated by Edelman et al<sup>3</sup> and Birkenfeld et al.<sup>4</sup> They studied the relationship between plasma sodium, exchangeable sodium, exchangeable potassium, and total body water. Their insights showed us that by calculating effective free water clearance, we clinicians could always predict whose plasma sodium was going up or down and even at what rate the changes would occur. As a result, we had a fairly clear understanding of total body water, electrolyte contents, plasma concentrations, and subsequent speculations on blood pressure regulation. Osmotic forces, being what they are, would dictate that gain of exchangeable components would increase water and vice versa. That these ideas (a standard model if you will) could have immunologic implications never occurred to us, and we did not need anything else.

Standard models work until the mathematical infrastructure becomes wobbly. Titze et al<sup>5</sup> studied only 3 people but those for weeks over time, in a Mars-flight simulation study. The subjects resided in a capsule over

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## Nonstandard Abbreviations and Acronyms

<b>CVD</b>	cardiovascular disease
<b>EAE</b>	experimental autoimmune encephalomyelitis
<b>HSD</b>	high-salt diet
<b>IL</b>	interleukin
<b>NCLX</b>	Na <sup>+</sup> /Ca <sup>2+</sup> exchanger
<b>Th17</b>	T helper 17
<b>VEGF-C</b>	vascular endothelial growth factor C

weeks. The sodium and all components of the diet were known. The investigators' findings that sodium accumulation ran largely independent of water accumulation (or vice versa) was unexpected and stressed the standard model. How do we fit these findings into our understanding of human physiology?

The next task was to discover where the not-so-exchangeable sodium could be hiding. This experiment required carcass ashing and electrolyte measurements with atomic absorption spectroscopy.<sup>6</sup> The skin proved to be an osmotically inactive, sodium accumulation site, an area that was also influenced by extremes of dietary salt intake or kidney function. Humans carry around about 5 kg of skin, and skin is the largest reservoir of extracellular fluid in the body. Skin is particularly rich in glycosaminoglycans, which because of their strong negative charge, in particular for sulphated glycosaminoglycans, became a prime candidate for a sodium interaction. Support for a role of glycosaminoglycans, in particular, sulphated glycosaminoglycans, was found in initial studies.<sup>7,8</sup> However, this notion has been contested in 2 more recent rodent studies showing either no change or a reduction in glycosaminoglycans in animals receiving a high-salt diet (HSD) or in the deoxycorticosterone acetate and high-salt diet (DOCA)-salt model, respectively.<sup>9,10</sup> Moreover, no indication of Na<sup>+</sup> binding to glycosaminoglycans was identified.<sup>10</sup> Thus, follow-up studies have to address this issue and to explain the discrepancies. Does sodium accumulation apply to man?

The development of magnetic resonance imaging for sodium allowed to visualize and quantify the tissue sodium content in humans.<sup>11</sup> Of note, sodium content appeared to be higher in skin than in muscle for men, while women tended to have higher muscle sodium.<sup>12</sup> Interestingly, hypertensive patients, patients with end-stage renal disease, as well as patients with autoimmune disease display an increased sodium content in this third space.<sup>13</sup> Sodium stores seem to be dynamic, since SGLT-2 inhibition in patients with type 2 diabetes,<sup>14</sup> diuretic treatment in patients with acute heart failure,<sup>15</sup> successful treatment of patients with primary aldosteronism,<sup>11</sup> hemodialysis,<sup>16</sup> and after kidney transplantation<sup>17</sup> show reduced tissue sodium content. In addition,

an experimental HSD study in humans triggered cutaneous Na<sup>+</sup> deposition as assessed by chemical analysis of punch biopsies.<sup>18</sup> These findings indicate that stored sodium can be mobilized. However, whether or not the sodium is readily exchangeable to immediately satisfy the Edelman equation is another issue.

These largely descriptive findings were enhanced by additional human balance studies in another Mars-simulation study. However, in this study, dietary salt intake could be manipulated. The subjects ingested diets differing only in 3 levels of sodium content. The investigators confirmed results from the earlier Mars-flight simulation study and found rhythmic sodium excretory and retention patterns that are independent of blood pressure or body water and occur independently of salt intake.<sup>19</sup> The idea that sodium can accumulate in skin and skeletal muscle is now generally accepted,<sup>20,21</sup> while how sodium influences blood pressure is not.<sup>21</sup> Taken together, these hosts of findings suggested that the standard model requires revision. More importantly, what local molecular mechanisms could help us further?

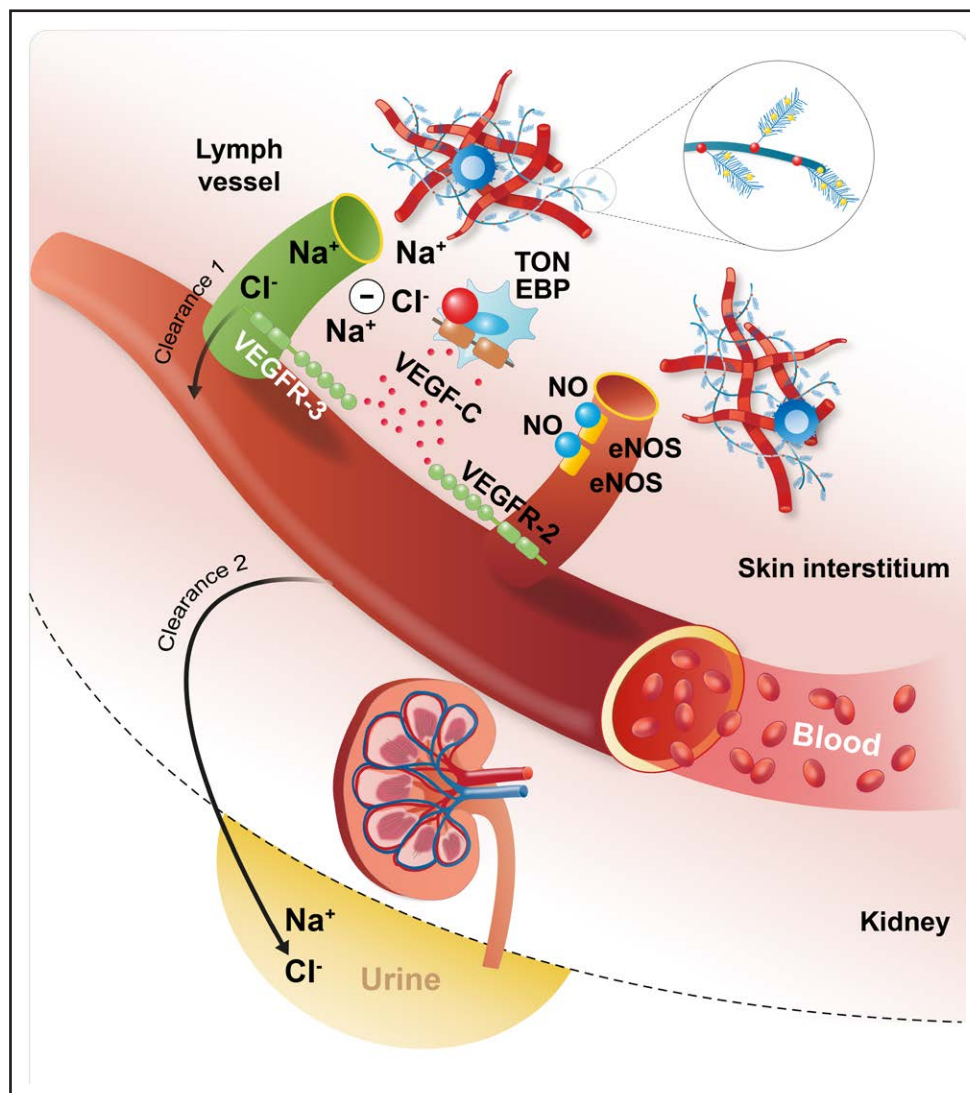
The answers lay in basic research. Parallel to the above reports, the Titze group focused on addressing the mechanisms involved. They were aware that TonEBP (tonicity-responsive enhancer-binding protein; NFAT5 [nuclear factor of activated T-cells 5])—a rel-like protein that activates transcription in response to hypertonicity—can detect even minimal changes in osmolarity.<sup>22</sup> HSD led to interstitial hypertonic sodium accumulation in skin, resulting in increased density and hyperplasia of the lymph-capillary network. Mechanistically, the NFAT5–VEGF-C (vascular endothelial growth factor C) axis in mononuclear phagocytes infiltrating the interstitium of the skin was the underlying mechanism for these effects on skin lymphatics. Mononuclear phagocyte depletion or VEGF-C trapping by soluble VEGFR-3 (vascular endothelial growth factor receptor-3) blocked VEGF-C signaling, augmented interstitial hypertonic volume retention, decreased endothelial NO synthase expression, and elevated blood pressure in response to HSD.<sup>23</sup> These preclinical findings were the first to connect immune cells with salt balance, which was also observed in humans in later follow-up studies.<sup>24,25</sup> Obvious calls were issued to confirm these results that were based on the uncomfortable assumption that differences in osmolality can exist between cell surfaces in a nanometer range. Thus, the experiments were repeated. The subsequent results demonstrated that the skin harbors a hypertonic fluid compartment in which mononuclear phagocytes modify local cutaneous electrolyte clearance via NFAT5 and VEGF-C/VEGFR-3–mediated modulation of cutaneous lymphatic capillary function. Thereby, mononuclear phagocytes carry out a homeostatic and blood pressure–regulatory function (Figure 1).<sup>26</sup> In this study, several confirmatory methods were used to investigate

the osmolar differences at the cellular level.<sup>26,27</sup> Subsequently, these ideas were translated to an infectious disease and inflammatory skin diseases, incorporating both magnetic resonance imaging for sodium and molecular data.<sup>13,28–30</sup> Most recently, a link between inflammation, metabolic dysfunction in obesity, and tissue sodium levels was established.<sup>31</sup> These ideas, particularly those concerning osmolar gradients in the nanometer range, understandably caused skepticism.<sup>32</sup> However, as discussed earlier, the methods available at the present time did not give sufficient detailed spatial information to allow resolving these questions. Methodological differences could be responsible. For instance, the precision ashing of tissues and atomic absorption spectroscopy (rats) to measure cation concentrations was not performed in all studies. These methods require expensive and not distributable facilities. Recent data

from the Pravenec laboratory, which measured such precautions, supported the notion that sodium accumulation and blood capillary rarefaction in the skin predisposes to hypertension in a rat model.<sup>27</sup> Nonetheless, we argue that immune cells are involved in electrolyte metabolism, even at the most local of levels. We are convinced that this argument is compelling.

## SODIUM SHIFTS THE IMMUNE CELL BALANCE TOWARD INFLAMMATION

Immune cells patrol throughout our body, change location from gut or bone marrow to the circulation, spleen, lymph nodes, and to target organs to fulfill their diverse functions. On their way, they sense and integrate diverse environmental signals, translate them into intracellular



**Figure 1.** A schematic diagram of how local (nanometer domain) sodium gradients influence osmotic sensors, effectors, and immune cells.

eNOS indicates endothelial NO synthase; TonEBP, tonicity-responsive enhancer-binding protein; VEGF-C, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor-2; and VEGFR-3, vascular endothelial growth factor receptor-3.

information to permit adaption to a particular inflammatory environment for the maintenance of immune homeostasis. In the 1990s, investigators aimed to study the mechanism of saturated potassium iodide to treat infections. They used increases in NaCl as a control and found that increases by 40 mM, which is similar to increases found in skin stores upon dietary and inflammatory stimuli, could provoke proinflammatory responses in peripheral blood mononuclear cells.<sup>33</sup> These findings were among the first studies to show that ionic signals are able to profoundly impact immune cell responses. Since then, the literature clearly demonstrates that high-salt conditions favor proinflammatory macrophage and T helper 17 (Th17) cell activation and curtail the regulatory and anti-inflammatory activation of these cells.<sup>34,35</sup> In macrophages, the NCLX (Na<sup>+</sup>/Ca<sup>2+</sup> exchanger) is critically involved in sensing ionic sodium balance changes<sup>36</sup> and triggers enhanced activation of osmoprotective signaling pathways, such as NFAT5. Of utmost interest, these high salt responses contain profound metabolic adaption of macrophages, which includes triggering increased stabilization of hypoxia-inducible factor 1 $\alpha$  and autophagy.<sup>37</sup> This process helps to fight infections.<sup>27,36–40</sup> Increased local sodium conditions, therefore, represent a local microenvironmental cue, which can strengthen barrier surfaces.<sup>27,40,41</sup>

In 2013, 2 landmark studies<sup>42,43</sup> connected high salt intake as a potential environmental factor contributing to experimental autoimmunity. These investigations showed that high NaCl conditions could boost the differentiation of naive CD4<sup>+</sup> T cells in humans and mice into pathogenic Th17 cells. IL (interleukin)-17A and IL-23R (IL-23 receptor) expression under Th17 differentiation conditions was enhanced. This regulatory mechanism involved p38/MAPK (mitogen-activated protein kinase), NFAT5, and SGK1.<sup>34</sup> SGK1 inactivates FOXO1 (forkhead box O1), thereby promoting enhanced ROR $\gamma$ t (retinoic acid receptor-related orphan receptor gamma t) and IL-23R expression.<sup>43</sup> In vivo, an HSD increases the number of Th17 cells in gut-associated lymphoid tissue and the central nervous system, resulting in exacerbating experimental autoimmune encephalomyelitis (EAE), a mouse model mimicking many aspects of multiple sclerosis.<sup>42,43</sup> CD4<sup>+</sup> T cell-specific SGK1 (serum/glucocorticoid regulated kinase 1) deletion in mice leads to resistance to EAE, owing to a defect in maintaining the Th17 phenotype.<sup>43</sup> Both in vitro and in vivo evidence in EAE suggests that Th17 activation by high NaCl is not mediated through dendritic cells but rather through a separate pathway or direct impact on Th17 cells.<sup>44</sup> Besides the NFAT5 axis, high salt also affects SGK1 via the upstream long noncoding RNA (Lnc)-SGK1.<sup>45</sup> Furthermore, aside from its pivotal role in T cells, SGK1 in dendritic cells is important for salt-sensitive hypertension, vascular function, and renal inflammation.<sup>46</sup> Translating these experimental findings to humans, a 10-day HSD (15 g NaCl/

day, about 300 mmol Na<sup>+</sup>/day) in healthy nonsmoking male volunteers resulted in increased Th17 population in the peripheral blood, which was reversed after switching the men to normal-salt diet.<sup>47</sup> Further, also a rather moderate 14-day dietary high-salt challenge (6 g of NaCl/day in addition to accustomed diets) increased circulating Th17 frequencies.<sup>48</sup> Of note, in humans, potassium also affects high salt-induced Th17 polarization by a thus far unknown mechanism.<sup>49</sup> Circulating IL-17 levels were significantly increased after switching from 3 to 18 g of NaCl for 7 days, while the addition of 4.5 g KCl to the HSD completely reversed the IL-17 levels.<sup>49</sup> A dichotomous role for NaCl on Th17-like cells has also been reported, where the impact of NaCl on CD4<sup>+</sup> T cells may be context dependent and could differ depending on stimulation and cytokine environment.<sup>50</sup>

IL-17 plays a critical role in infections, autoimmune diseases, and inflammatory diseases. Of note, hypertensive humans show significantly increased circulating IL-17 levels.<sup>51</sup> Physiologically, IL-17 is not only involved in host defense but also may promote inflammatory tissue damage. IL-17A knockout mice initially increase the blood pressure similarly to controls upon angiotensin II infusion, whereas over time, they develop a less hypertensive state.<sup>51</sup> In addition, human prototype IL-17-related diseases such as psoriasis, periodontal disease, and rheumatoid arthritis are also associated with hypertension.<sup>52–54</sup>

High salt is further linked to an increased risk of cerebrovascular diseases and dementia. A recent study focusing on the gut-brain axis described how high salt initiates a Th17 response in the gut resulting in a marked increase in plasma IL-17, which subsequently suppresses resting cerebral blood flow and endothelial function, leading to neurovascular dysregulation and cognitive impairment.<sup>55</sup> Endothelial dysfunction and cognitive impairment are mediated via inhibitory Rho kinase-dependent phosphorylation of endothelial NO synthase with reduced NO production of cerebral endothelial cells.<sup>55</sup>

Lupus-prone MRL/lpr mice under HSD showed a significant increase in the ratio of splenic Th1/Th2 and Th17/Regulatory T (Treg) cells, accompanied with reduced survival.<sup>56,57</sup> In BALB/c mice, HSD induced tubular proteinuria and a profibrotic phenotype with increased renal cortical Th1 and Th17 and reduced Treg cells.<sup>58</sup> HSD also primed follicular helper T-cell differentiation and promoted autoimmunity by hydroxytransferase TET2 (Ten-Eleven Translocation 2)-induced DNA demethylation. In vitro, TET2 silencing reduced NaCl-induced follicular helper T-cell polarization. In models of colitis, several reports have established the relationship between high sodium and Th17 cells. In human intestinal lamina propria mononuclear cells, IL-17A, IL-23R, TNF- $\alpha$ , and ROR $\gamma$ t significantly increased after NaCl exposure.<sup>59</sup> Mice on HSD developed severe 2,4,6-trinitrobenzenesulfonic acid (TNBS)- or dextran sulfate

sodium (DSS)-induced colitis compared with control mice, which could be ameliorated by p38 MAPK inhibition.<sup>59</sup> Besides Th17 cells, IL-17-producing type 3 innate lymphoid cells may also be affected by HSD in this context. When mice were fed an HSD, an intestinal inflammatory response associated with increased IL-23 production, neutrophil mobilization, and frequency of IL-17-producing type 3 innate lymphoid cells in the colon was observed. Since intestinal inflammation was not observed in IL-17 knockout mice but albeit to a lesser degree in recombination-activating genes (RAG)-deficient mice, which lack B and T cells but have ILC, IL-17 production by both type 3 innate lymphoid cells and Th17 cells likely contributes to the inflammatory response.<sup>60</sup> Altogether, these data suggest that a high salt intake may prime various T-cell subsets, promote IL-17 production with consequences for the pathogenesis of cardiovascular diseases (CVDs) and autoimmune diseases. Whether or not reducing salt intake to currently recommended levels would reduce this state of affairs remains to be seen.

## SODIUM AFFECTS THE MICROBIOME AND INTESTINAL INFLAMMATION

Recent research has further been focusing on indirect roles of NaCl regulating immunity through changing the gut microbiota.<sup>34</sup> High salt intake impacts the gut microbiota in mice and humans, particularly by depleting *Lactobacillus* spp.<sup>48,61–63</sup> Along with changes in the gut microbiota, HSD increases the frequency of Th17 cells in the intestines, spleen, and spinal cord along with the blood pressure and aggravated disease courses of EAE mice.<sup>48</sup> Replenishment of live *Lactobacillus murinus* to HSD-fed mice ameliorated the HSD-mediated exacerbation of EAE and salt-sensitive hypertension by regulating Th17 cells, likely through increased levels of the bacterial tryptophan metabolite indole-3 lactic acid. Mechanistically, indole-3 lactic acid suppressed Th17 cell polarization, suggesting that tryptophan metabolites of bacterial origin may act as inhibitors of the high salt-induced Th17 increase of the host.<sup>48</sup> Concordantly, a meta-analysis demonstrated that probiotics (mostly containing *Lactobacillus* spp.) may reduce blood pressure in hypertensive subjects.<sup>64</sup> Kirabo et al<sup>65</sup> also have shown that a high salt intake is associated with changes in the gut microbiome, as well as higher blood pressures in humans. Further, blood pressures in salt-treated mice were elevated when the mice received suppressor doses of Ang II (angiotensin II).<sup>65</sup> HSD was associated with changes in an increase in the genera *Firmicutes*, *Proteobacteria*, and *Prevotella* bacteria and a decrease in the genera of lactic acid-producing bacteria.<sup>65</sup> Adaptive fecal microbial transfer from mice fed an HSD predisposes germ-free recipients to inflammation and hypertension.<sup>65</sup> Further evidence for microbiota-dependent effects of an HSD on inflammation is the observation that an HSD

promotes experimental DSS- and dinitrobenzenesulfonic acid-induced colitis in mice by reducing the presence of intestinal *Lactobacilli* and butyrate production. This effect was not observed in germ-free mice.<sup>62</sup>

## SODIUM BLUNTS ANTI-INFLAMMATORY REGULATORY T-CELL FUNCTION

Regulatory FOXP3<sup>+</sup> (forkhead box P3) T cells (Tregs) play an essential role for the maintenance of peripheral tolerance and immune cell homeostasis.<sup>66,67</sup> Depending on the microenvironment and tissue, Tregs show the ability to suppress innate and adaptive immune cells by the secretion of anti-inflammatory cytokines, such as IL-10, or by cell-cell contact-dependent mechanism involving costimulatory receptors like cytotoxic T-lymphocyte-associated protein 4.<sup>66,67</sup> In humans, mutations in the *FOXP3* gene, a master regulator of Tregs, lead to dysfunctional Tregs with fatal autoimmunity. Mutations have been linked to multiple sclerosis, type 1 diabetes, systemic lupus erythematosus, chronic infections, or inflammation like rheumatoid arthritis.<sup>66–69</sup> Further, differential expression of *FOXP3* splice variants related to Treg function was associated with unstable plaques in patients with atherosclerosis.<sup>70</sup> Experimental and clinical studies confirmed that Tregs are important mediators to contain chronic inflammation in CVD such as atherosclerosis,<sup>71</sup> hypertensive target organ damage,<sup>72</sup> or wound healing after myocardial infarction.<sup>73</sup> However, importantly, reduced Treg numbers and a dysfunctional Treg phenotype similar to autoimmunity have been reported in atherosclerosis, heart failure, and myocardial infarction and are associated with progression of disease.<sup>74–77</sup> Furthermore, HSD also negatively affected the regulatory balance of T cells in transplantation and precipitates rejection.<sup>78</sup> Of note, the deleterious effect of an HSD in the absence of SGK1 on CD4<sup>+</sup> T cells in transplanted recipients was diminished.<sup>78</sup>

Mechanistically, dysfunctional Tregs found in several inflammatory diseases are characterized by a proinflammatory Th1-like phenotype with high expression levels of IFN- $\gamma$  (interferon- $\gamma$ ) and lower levels of IL-10.<sup>66,79–81</sup> Several reports suggested that SGK1 not only plays a pivotal role in high salt-induced Th17 cell polarization<sup>29,42,43</sup> but also in Tregs.<sup>50,78,79,82–84</sup> In vitro and in vivo, salt-SGK1 signaling axis enables Treg cells to acquire a Th17-like phenotype, thus establishing salt as a nonimmune factor that affects Treg functional adaptation.<sup>85</sup> In vitro, high salt was sufficient to induce ROR $\gamma$ t expression in both thymic Treg and inducible Tregs without IL-17A production.<sup>85</sup> A moderate decrease in FOXP3 expression in thymic high salt-induced ROR $\gamma$ t<sup>+</sup> Treg cells was not associated with a loss of Treg identity and function most likely due to still sufficient FOXP3 levels. In contrast, IL-23<sup>84</sup> or IL-1 $\beta$ <sup>86</sup> can readily induce IL-17A<sup>+</sup>ROR $\gamma$ t<sup>+</sup>FOXP3<sup>+</sup> inducible Treg cells into inflammatory Th17-like Treg cells. These data suggest that salt-induced Treg dysfunction could

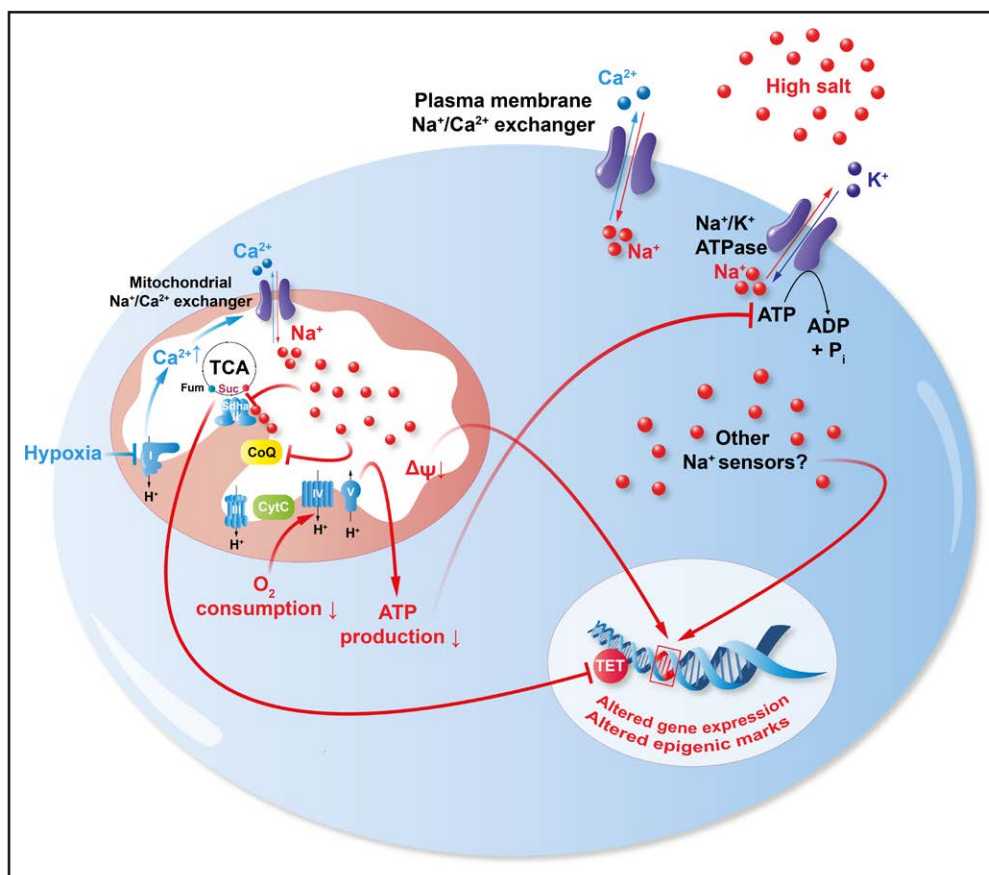
accelerate not only the pathogenesis of autoimmune disease but also the progression of CVD.

## SODIUM INHIBITS MITOCHONDRIAL FUNCTION

As mentioned above, sodium is the most abundant cation in the extracellular space, kept in low concentrations intracellularly. This asymmetrical distribution builds up a high electrochemical gradient across the plasma membrane and allows for a fast and substantial sodium influx. The latter is mediated by different channels and transporters, such as the NHE (sodium-proton exchanger), the NKCC (sodium-potassium-chloride cotransporter), the NCLX, the TRPM4 (transient receptor potential melastatin 4), and the amiloride-sensitive sodium channel.<sup>87</sup> Sodium influx and efflux not only affect cell membrane potential, excitation, and electrochemical conductivity but also intracellular pH and concentration of other ions and metabolites.

Interestingly, during ischemia, sodium not only accumulates in myocardial tissue but was also shown to

invade the intracellular space and accumulate in subcellular compartments, more concretely, in the mitochondria. The contraction of healthy cardiac myocytes consumes large quantities of ATP, which is produced by mitochondrial oxidative phosphorylation.<sup>88</sup> During cardiac ischemia, intracellular and mitochondrial  $\text{Na}^+$  levels increase along with a reduction in mitochondrial respiration with reduced ATP production (Figure 2), which might, in part, explain the imbalance of the energy demand in the failing heart.<sup>88</sup> Also studies in patients with type 2 diabetes have shown that the myocardium under these conditions display reduced mitochondrial respiration and higher oxidative stress, further linking mitochondrial dysfunction with the pathophysiology of heart failure.<sup>89</sup> Besides its effect on oxidative phosphorylation, the  $\text{Na}^+$  overload can cause an imbalance in  $\text{Ca}^{2+}$  homeostasis by reverting the calcium-sodium exchange process and thus accelerate heart failure.<sup>90,91</sup> These studies highlight how HSD and imbalances in the ionic microenvironment can be detrimental to a plethora of different diseases and how cellular metabolic shifts are key in regulating cellular function and overall homeostasis.



**Figure 2. Sodium and mitochondrial metabolism.**

Pictogram of a cell under hypoxic or hypertonic saline conditions. Sodium enters the cell and mitochondria (at least, in part) via the NCLX ( $\text{Na}^+/\text{Ca}^{2+}$  exchanger) and inhibits the electron transfer chain at the level of succinate dehydrogenase complex flavoprotein subunit A complex II (SdhA, II)/ubiquinone (CoQ). Subsequently, mitochondrial respiration, membrane potential ( $\Delta\Psi$ ), and ATP production are reduced. In several immune cells, a proinflammatory gene expression is observed. CytC indicates cytochrome C; Fum, fumarate; Suc, succinate; TCA, tricarboxylic acid cycle; and TET, ten-eleven translocation.

It was shown that sodium could impact circulating and tissue-invading monocytes, M1/M2 macrophages, dendritic cells,<sup>87</sup> and as outlined before in detail, Th17 cells and Tregs, as well as vascular endothelial cell stiffness.<sup>34</sup> Interestingly, many of these phenotypes are mediated by NCLX, both at the plasma and the inner mitochondrial membrane. With these findings, there has been a strong focus on how sodium affects cellular bioenergetics.

Cellular metabolism and specifically its plasticity are undoubtedly of outmost importance for cell function and adaptation. Metabolic reprogramming is not just a hallmark of cancer but also emerged as critical regulator of immune cell activation.<sup>92,93</sup> Sodium was shown to have several metabolic targets. In ischemic myocardial tissue, mononuclear phagocytes, and Tregs, sodium reduces mitochondrial oxidative phosphorylation. Sodium could enter the mitochondrial matrix via NCLX and interacts with phospholipids in the inner mitochondrial membrane, reducing the membrane fluidity and the diffusion of ubiquinone between complex II and complex III of the electron transport chain.<sup>94</sup> In M1/M2 macrophages<sup>38</sup> and Tregs,<sup>95</sup> salt directly inhibits complex II/III of the electron transport chain. Treg cell-specific ablation of mitochondrial respiratory chain complex III in mice resulted in loss of function and subsequent development of fatal inflammatory disease early in life, without altering Treg cell proliferation and survival.<sup>96</sup> Recent data demonstrated that high salt mirrored the metabolic and gene expression signatures and functional phenotype observed after complex III inhibition.<sup>95</sup> The loss of mitochondrial function after short-term engagement in high-salt environments *in vitro* provoked a long-term loss of function of human and murine Tregs *in vivo*.<sup>95</sup> Xenogeneic graft versus host disease in immunodeficient mice is a model for *in vivo* analysis of human Treg functionality.<sup>83,97,98</sup> Adoptive transfer of high salt-treated or complex III inhibited human Tregs in xenogeneic graft versus host disease or murine high salt-treated Tregs to EAE similarly have long-term consequences *in vivo*.<sup>95</sup> Interestingly, inhibition of CII/III is accompanied by an accumulation of succinate, which in turn inhibits TET2-mediated DNA demethylation (Figure 2). This accumulation could be a potential mechanism by which HSD induces altered epigenetic markers and thereby produces long-term effects despite a relatively short-term high-salt stimulation. Besides electron transport chain complex II/III, in cancer and macrophage cell lines, salt induces aerobic glycolysis via a pyruvate dehydrogenase kinase-mediated activation of pyruvate dehydrogenase, with reduced tricarboxylic acid cycling and oxidative phosphorylation. Interestingly, sodium was recently also identified as a regulator of the liquidity of intracellular condensates, affecting protein-protein interactions (at least partially by electrostatic shielding of the proteins) and thus protein aggregation under hypertonic stress.<sup>99</sup> However, the exact extend of salt-induced metabolic remodeling in various different cell types remains

ill defined. And there are so far only limited data available in respect to time resolution of salt-induced metabolic remodeling in different cell types under different metabolic states. The integration and investigation of in-parallel-occurring signaling events will be crucial to understand these processes in more detail.

## OUTLOOK

Accumulating evidence suggests that the blood pressure-centric definition of salt sensitivity could be broadened to cellular and metabolic sodium sensitivity. While the actions of other cations such as  $\text{Ca}^{2+}$  as important intracellular messengers are widely recognized, evidence continues to accumulate highlighting various unexpected roles of sodium in the regulation of cellular function. Overall, it is becoming clearer that sodium modulates broader bodily functions besides fluid homeostasis and regulates various cell functions, particularly in cells of the innate and adaptive immune system. Given the relevance of immune function for CVD and cardiometabolic disease, it is tempting to speculate that these findings may have important implications and are not only relevant for autoimmunity. However, it remains elusive as to where, why, and how sodium is compartmentalized, both on a tissue/supracellular and organelle/subcellular level and whether all cells are similarly sodium responsive. More research is needed to understand how tissue or cellular salinization is linked to health or disease states, age, and gender, as well as nutrition, and how metabolic plasticity on both the cellular and body level is affected.

Recent investigations (and studies well back in the 20th century) showed that the substitution of  $\text{Na}^+$  with  $\text{K}^+$  (in terms of intake) showed significantly lower rates of stroke, major adverse cardiovascular events, and death from any cause.<sup>100</sup> The driving force for this potassium-protection idea were the seminal studies and observations from the Tobian group.<sup>101</sup> With potassium augmentation, better systolic blood pressure lowering was observed,<sup>102</sup> compared with those subjects who received a regular salt intake. Whether or not salt (NaCl) reduction or salt substitution by KCl can affect the tissue/supracellular and organelle/subcellular micromilieu, signaling, and immunometabolism should be addressed in future studies. By understanding the dependencies for these microenvironmental changes and the corresponding environmental sensing mechanisms, we envision targeted approaches to fine-tune immune cell, myocardial, fibroblast, and endothelial cell function and thus better control inflammation and CVD.

## ARTICLE INFORMATION

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### Disclosures

M. Kleinewietfeld is listed inventor on a pending patent related to mitochondrial metabolism and immunomodulation.

### REFERENCES

- Pitts RF. *Physiology of the Kidney and Body Fluids*. Year Book Medical Publishers Inc; 1974;3:11–34.
- Andersson B. Regulation of body fluids. *Annu Rev Physiol*. 1977;39:185–200. doi: 10.1146/annurev.ph.39.030177.001153
- Edelman IS, Leibman J, O'Meara MP, Birkenfeld LW. Interrelations between serum sodium concentration, serum osmolarity and total exchangeable sodium, total exchangeable potassium and total body water. *J Clin Invest*. 1958;37:1236–1256. doi: 10.1172/JCI103712
- Birkenfeld LW, Leibman J, O'Meara MP, Edelman IS. Total exchangeable sodium, total exchangeable potassium, and total body water in edematous patients with cirrhosis of the liver and congestive heart failure. *J Clin Invest*. 1958;37:687–698. doi: 10.1172/JCI103655
- Titze J, Maillet A, Lang R, Gunja HC, Johannes B, Gauquelin-Koch G, Kihm E, Larina I, Gharib C, Kirsch KA. Long-term sodium balance in humans in a terrestrial space station simulation study. *Am J Kidney Dis*. 2002;40:508–516. doi: 10.1053/ajkd.2002.34908
- Titze J, Lang R, Iliès C, Schwind KH, Kirsch KA, Dietsch P, Luft FC, Hilgers KF. Osmotically inactive skin Na<sup>+</sup> storage in rats. *Am J Physiol Renal Physiol*. 2003;285:F1108–F1117. doi: 10.1152/ajprenal.00200.2003
- Titze J, Shakibaei M, Schaffhuber M, Schulze-Tanzil G, Porst M, Schwind KH, Dietsch P, Hilgers KF. Glycosaminoglycan polymerization may enable osmotically inactive Na<sup>+</sup> storage in the skin. *Am J Physiol Heart Circ Physiol*. 2004;287:H203–H208. doi: 10.1152/ajpheart.01237.2003
- Schaffhuber M, Volpi N, Dahlmann A, Hilgers KF, Maccari F, Dietsch P, Wagner H, Luft FC, Eckardt KU, Titze J. Mobilization of osmotically inactive Na<sup>+</sup> by growth and by dietary salt restriction in rats. *Am J Physiol Renal Physiol*. 2007;292:F1490–F1500. doi: 10.1152/ajprenal.00300.2006
- Thowsen IM, Reikvam T, Skogstrand T, Samuelsson AM, Muller DN, Tenstad O, Alitalo K, Karlén T, Wiig H. Genetic engineering of lymphangiogenesis in skin does not affect blood pressure in mouse models of salt-sensitive hypertension. *Hypertension*. 2022;79:2451–2462. doi: 10.1161/HYPERTENSIONAHA.122.19777
- Thowsen IM, Karlén T, Nikpey E, Haslene-Hox H, Skogstrand T, Randolph GJ, Zinselmeyer BH, Tenstad O, Wiig H. Na<sup>+</sup> is shifted from the extracellular to the intracellular compartment and is not inactivated by glycosaminoglycans during high salt conditions in rats. *J Physiol*. 2022;600:2293–2309. doi: 10.1113/JP282715
- Kopp C, Linz P, Wachsmuth L, Dahlmann A, Horbach T, Schofl C, Renz W, Santoro D, Niendorf T, Muller DN, et al. <sup>23</sup>Na magnetic resonance imaging of tissue sodium. *Hypertension*. 2012;59:167–172. doi: 10.1161/HYPERTENSIONAHA.111.183517
- Wang P, Deger MS, Kang H, Ikizler TA, Titze J, Gore JC. Sex differences in sodium deposition in human muscle and skin. *Magn Reson Imaging*. 2017;36:93–97. doi: 10.1016/j.mri.2016.10.023
- Maifeld A, Wild J, Karlén T, Rakova N, Wistorf E, Linz P, Jung R, Birukov A, Gimenez-Rivera VA, Wilck N, et al. Skin sodium accumulates in psoriasis and reflects disease severity. *J Invest Dermatol*. 2022;142:166–178.e8. doi: 10.1016/j.jid.2021.06.013
- Karg MV, Bosch A, Kannenkeril D, Striepe K, Ott C, Schneider MP, Boehme-Zelch F, Linz P, Nagel AM, Titze J, et al. SGLT-2-inhibition with dapagliflozin reduces tissue sodium content: a randomised controlled trial. *Cardiovasc Diabetol*. 2018;17:5. doi: 10.1186/s12933-017-0654-z
- Hammon M, Grossmann S, Linz P, Kopp C, Dahlmann A, Garlachs C, Janka R, Cavallaro A, Luft FC, Uder M, et al. <sup>23</sup>Na magnetic resonance imaging of the lower leg of acute heart failure patients during diuretic treatment. *PLoS One*. 2015;10:e0141336. doi: 10.1371/journal.pone.0141336
- Dahlmann A, Dorfelt K, Eicher F, Linz P, Kopp C, Mossinger I, Horn S, Buschges-Seraphin B, Wabel P, Hammon M, et al. Magnetic resonance-determined sodium removal from tissue stores in hemodialysis patients. *Kidney Int*. 2015;87:434–441. doi: 10.1038/ki.2014.269
- Dahlmann A, Linz P, Zucker I, Haag V, Jantsch J, Dienemann T, Nagel AM, Neubert P, Rosenhauer D, Rauh M, et al. Reduction of tissue Na<sup>+</sup> accumulation after renal transplantation. *Kidney Int Rep*. 2021;6:2338–2347. doi: 10.1016/j.ekir.2021.06.022
- Selvarajah V, Maki-Petaja KM, Pedro L, Bruggraber SFA, Burling K, Goodhart AK, Brown MJ, McEniery CM, Wilkinson IB. Novel mechanism for buffering dietary salt in humans: effects of salt loading on skin sodium, vascular endothelial growth factor C, and blood pressure. *Hypertension*. 2017;70:930–937. doi: 10.1161/HYPERTENSIONAHA.117.10003
- Rakova N, Juttner K, Dahlmann A, Schroder A, Linz P, Kopp C, Rauh M, Goller U, Beck L, Agureev A, et al. Long-term space flight simulation reveals infradian rhythmicity in human Na<sup>+</sup> balance. *Cell Metab*. 2013;17:125–131. doi: 10.1016/j.cmet.2012.11.013
- Bie P. Mechanisms of sodium balance: total body sodium, surrogate variables, and renal sodium excretion. *Am J Physiol Regul Integr Comp Physiol*. 2018;315:R945–R962. doi: 10.1152/ajpregu.00363.2017
- Ellison DH, Welling P. Insights into salt handling and blood pressure. Reply. *N Engl J Med*. 2022;386:e18. doi: 10.1056/NEJMc2119480
- Miyakawa H, Woo SK, Dahl SC, Handler JS, Kwon HM. Tonicity-responsive enhancer binding protein, a rel-like protein that stimulates transcription in response to hypertonicity. *Proc Natl Acad Sci USA*. 1999;96:2538–2542. doi: 10.1073/pnas.96.5.2538
- Machnik A, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, Machura K, Park JK, Beck FX, Muller DN, Derer W, et al. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med*. 2009;15:545–552. doi: 10.1038/nm.1960
- Zhang MZ, Yao B, Wang Y, Yang S, Wang S, Fan X, Harris RC. Inhibition of cyclooxygenase-2 in hematopoietic cells results in salt-sensitive hypertension. *J Clin Invest*. 2015;125:4281–4294. doi: 10.1172/JCI81550
- Wenstedt EF, Verberk SG, Kroon J, Neele AE, Baardman J, Claessen N, Pasaoglu OT, Rademaker E, Schrooten EM, Wouda RD, et al. Salt increases monocyte CCR2 expression and inflammatory responses in humans. *JCI Insight*. 2019;4:e130508. doi: 10.1172/jci.insight.130508
- Wiig H, Schroder A, Neuhofer W, Jantsch J, Kopp C, Karlén T, Voschmann M, Goss J, Bry M, Rakova N, et al. Immune cells control skin lymphatic electrolyte homeostasis and blood pressure. *J Clin Invest*. 2013;123:2803–2815. doi: 10.1172/JCI60113
- Silhavy J, Mlejnek P, Simakova M, Liska F, Kubovciak J, Sticova E, Pravenec M. Sodium accumulation and blood capillary rarefaction in the skin predispose spontaneously hypertensive rats to salt sensitive hypertension. *Biomedicines*. 2022;10:376. doi: 10.3390/biomedicines10020376
- Jantsch J, Schatz V, Friedrich D, Schroder A, Kopp C, Siegert I, Maronna A, Wendelborn D, Linz P, Binger KJ, et al. Cutaneous Na<sup>+</sup> storage



- strengthens the antimicrobial barrier function of the skin and boosts macrophage-driven host defense. *Cell Metab*. 2015;21:493–501. doi: 10.1016/j.cmet.2015.02.003
29. Kopp C, Beyer C, Linz P, Dahlmann A, Hammon M, Jantsch J, Neubert P, Rosenhauer D, Muller DN, Cavallaro A, et al. Na<sup>+</sup> deposition in the fibrotic skin of systemic sclerosis patients detected by <sup>23</sup>Na-magnetic resonance imaging. *Rheumatology (Oxford)*. 2017;56:556–560. doi: 10.1093/rheumatology/kew371
  30. Matthias J, Maul J, Noster R, Meinel H, Chao YY, Gerstenberg H, Jeschke F, Gasparoni G, Welle A, Walter J, et al. Sodium chloride is an ionic checkpoint for human T(H)2 cells and shapes the atopic skin microenvironment. *Sci Transl Med*. 2019;11:eau0683. doi: 10.1126/scitranslmed.aau0683
  31. Ertuglu LA, Sahinoz M, Alsouqi A, Deger SM, Guide A, Stewart TG, Pike M, Robinson-Cohen C, Akwo E, Pridmore M, et al. High tissue-sodium associates with systemic inflammation and insulin resistance in obese individuals. *Nutr Metab Cardiovasc Dis*. 2023;33:1398–1406. doi: 10.1016/j.numecd.2023.03.024
  32. Rossitto G, Mary S, Chen JY, Boder P, Chew KS, Neves KB, Alves RL, Montezano AC, Welsh P, Petrie MC, et al. Tissue sodium excess is not hypertonic and reflects extracellular volume expansion. *Nat Commun*. 2020;11:4222. doi: 10.1038/s41467-020-17820-2
  33. Shapiro L, Dinarello CA. Osmotic regulation of cytokine synthesis in vitro. *Proc Natl Acad Sci U S A*. 1995;92:12230–12234. doi: 10.1073/pnas.92.26.12230
  34. Muller DN, Wilck N, Haase S, Kleinewietfeld M, Linker RA. Sodium in the microenvironment regulates immune responses and tissue homeostasis. *Nat Rev Immunol*. 2019;19:243–254. doi: 10.1038/s41577-018-0113-4
  35. Jobin K, Muller DN, Jantsch J, Kurts C. Sodium and its manifold impact on our immune system. *Trends Immunol*. 2021;42:469–479. doi: 10.1016/j.it.2021.04.002
  36. Neubert P, Homann A, Wendelborn D, Bar AL, Krampert L, Trum M, Schroder A, Ebner S, Weichselbaum A, Schatz V, et al. NXC1 represents an ionic Na<sup>+</sup> sensing mechanism in macrophages. *PLoS Biol*. 2020;18:e3000722. doi: 10.1371/journal.pbio.3000722
  37. Neubert P, Weichselbaum A, Reitingner C, Schatz V, Schroder A, Ferdinand JR, Simon M, Bar AL, Brochhausen C, Gerlach RG, et al. HIF1A and NFAT5 coordinate Na<sup>(+)</sup>-boosted antibacterial defense via enhanced autophagy and autolysosomal targeting. *Autophagy*. 2019;15:1899–1916. doi: 10.1080/15548627.2019.1596483
  38. Geisberger S, Bartolomaeus H, Neubert P, Willebrand R, Zasada C, Bartolomaeus T, McParland V, Swinnen D, Geuzens A, Maifeld A, et al. Salt transiently inhibits mitochondrial energetics in mononuclear phagocytes. *Circulation*. 2021;144:144–158. doi: 10.1161/CIRCULATIONAHA.120.052788
  39. Binger KJ, Gebhardt M, Heinig M, Rintisch C, Schroeder A, Neuhofer W, Hilgers K, Manzel A, Schwartz C, Kleinewietfeld M, et al. High salt reduces the activation of IL-4- and IL-13-stimulated macrophages. *J Clin Invest*. 2015;125:4223–4238. doi: 10.1172/JCI80919
  40. Berry MR, Mathews RJ, Ferdinand JR, Jing C, Loudon KW, Wlodek E, Dennison TW, Kuper C, Neuhofer W, Clatworthy MR. Renal sodium gradient orchestrates a dynamic antibacterial defense zone. *Cell*. 2017;170:860–874.e19. doi: 10.1016/j.cell.2017.07.022
  41. Evans RDR, Antonelou M, Sathiananthamoorthy S, Rega M, Henderson S, Ceron-Gutierrez L, Barcenas-Morales G, Muller CA, Doffinger R, Walsh SB, et al. Inherited salt-losing tubulopathies are associated with immunodeficiency due to impaired IL-17 responses. *Nat Commun*. 2020;11:4368. doi: 10.1038/s41467-020-18184-3
  42. Kleinewietfeld M, Manzel A, Titze J, Kvakana H, Yosef N, Linker RA, Muller DN, Hafler DA. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. *Nature*. 2013;496:518–522. doi: 10.1038/nature11868
  43. Wu C, Yosef N, Thalhammer T, Zhu C, Xiao S, Kishi Y, Regev A, Kuchroo VK. Induction of pathogenic TH17 cells by inducible salt-sensing kinase SGK1. *Nature*. 2013;496:513–517. doi: 10.1038/nature11984
  44. Jorg S, Kissel J, Manzel A, Kleinewietfeld M, Haghikia A, Gold R, Muller DN, Linker RA. High salt drives Th17 responses in experimental autoimmune encephalomyelitis without impacting myeloid dendritic cells. *Exp Neurol*. 2016;279:212–222. doi: 10.1016/j.expneurol.2016.03.010
  45. Yao Y, Jiang Q, Jiang L, Wu J, Zhang Q, Wang J, Feng H, Zang P. Lnc-SGK1 induced by *Helicobacter pylori* infection and high salt diet promote Th2 and Th17 differentiation in human gastric cancer by SGK1/Jun B signaling. *Oncotarget*. 2016;7:20549–20560. doi: 10.18632/oncotarget.7823
  46. Van Beusecum JP, Barbaro NR, McDowell Z, Aden LA, Xiao L, Pandey AK, Itani HA, Himmel LE, Harrison DG, Kirabo A. High salt activates CD11c(+) antigen-presenting cells via SGK (serum glucocorticoid kinase) 1 to promote renal inflammation and salt-sensitive hypertension. *Hypertension*. 2019;74:555–563. doi: 10.1161/HYPERTENSIONAHA.119.12761
  47. Luo T, Ji WJ, Yuan F, Guo ZZ, Li YX, Dong Y, Ma YQ, Zhou X, Li YM. Th17/Treg imbalance induced by dietary salt variation indicates inflammation of target organs in humans. *Sci Rep*. 2016;6:26767. doi: 10.1038/srep26767
  48. Wilck N, Matus MG, Kearney SM, Olesen SW, Forslund K, Bartolomaeus H, Haase S, Mahler A, Balogh A, Marko L, et al. Salt-responsive gut commensal modulates TH17 axis and disease. *Nature*. 2017;551:585–589. doi: 10.1038/nature24628
  49. Wen W, Wan Z, Ren K, Zhou D, Gao Q, Wu Y, Wang L, Yuan Z, Zhou J. Potassium supplementation inhibits IL-17A production induced by salt loading in human T lymphocytes via p38/MAPK-SGK1 pathway. *Exp Mol Pathol*. 2016;100:370–377. doi: 10.1016/j.jyxmp.2016.03.009
  50. Matthias J, Heink S, Picard F, Zeitrag J, Kolz A, Chao YY, Soll D, de Almeida GP, Glasmacher E, Jacobsen ID, et al. Salt generates anti-inflammatory Th17 cells but amplifies pathogenicity in proinflammatory cytokine microenvironments. *J Clin Invest*. 2020;130:4587–4600. doi: 10.1172/JCI137786
  51. Madhur MS, Lob HE, McCann LA, Iwakura Y, Blinder Y, Guzik TJ, Harrison DG. Interleukin 17 promotes angiotensin II-induced hypertension and vascular dysfunction. *Hypertension*. 2010;55:500–507. doi: 10.1161/HYPERTENSIONAHA.109.145094
  52. Panoulas VF, Douglas KM, Milionis HJ, Stavropoulos-Kalinglou A, Nightingale P, Kita MD, Tselios AL, Metsios GS, Elisaf MS, Kitis GD. Prevalence and associations of hypertension and its control in patients with rheumatoid arthritis. *Rheumatology (Oxford)*. 2007;46:1477–1482. doi: 10.1093/rheumatology/kem169
  53. Neimann AL, Shin DB, Wang X, Margolis DJ, Troxel AB, Gelfand JM. Prevalence of cardiovascular risk factors in patients with psoriasis. *J Am Acad Dermatol*. 2006;55:829–835. doi: 10.1016/j.jaad.2006.08.040
  54. Czesnikiewicz-Guzik M, Osmenda G, Siedlinski M, Nosalski R, Pelka P, Nowakowski D, Wilk G, Mikolajczyk TP, Schramm-Luc A, Furtak A, et al. Causal association between periodontitis and hypertension: evidence from Mendelian randomization and a randomized controlled trial of non-surgical periodontal therapy. *Eur Heart J*. 2019;40:3459–3470. doi: 10.1093/eurheartj/ehz646
  55. Faraco G, Brea D, Garcia-Bonilla L, Wang G, Racchumi G, Chang H, Buendia I, Santesteban MM, Segarra SG, Koizumi K, et al. Dietary salt promotes neurovascular and cognitive dysfunction through a gut-initiated TH17 response. *Nat Neurosci*. 2018;21:240–249. doi: 10.1038/s41593-017-0059-z
  56. Yang X, Yao G, Chen W, Tang X, Feng X, Sun L. Exacerbation of lupus nephritis by high sodium chloride related to activation of SGK1 pathway. *Int Immunopharmacol*. 2015;29:568–573. doi: 10.1016/j.intimp.2015.09.027
  57. Wu H, Huang X, Qiu H, Zhao M, Liao W, Yuan S, Xie Y, Dai Y, Chang C, Yoshimura A, et al. High salt promotes autoimmunity by TET2-induced DNA demethylation and driving the differentiation of Tfh cells. *Sci Rep*. 2016;6:28065. doi: 10.1038/srep28065
  58. Teixeira DE, Peruchetti DB, Souza MC, das Gracias Henriques MG, Pinheiro AAS, Caruso-Neves C. A high salt diet induces tubular damage associated with a pro-inflammatory and pro-fibrotic response in a hypertension-independent manner. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866:165907. doi: 10.1016/j.bbdis.2020.165907
  59. Monteleone I, Marafini I, Dinallo V, Di Fusco D, Troncone E, Zorzi F, Laudisi F, Monteleone G. Sodium chloride-enriched diet enhanced inflammatory cytokine production and exacerbated experimental colitis in mice. *J Crohns Colitis*. 2017;11:237–245. doi: 10.1093/ecco-jcc/jjw139
  60. Aguiar SLF, Miranda MCG, Guimaraes MAJ, Santiago HC, Queiroz CP, Cunha PDS, Cara DC, Foureaux G, Ferreira AF, Cardoso VN, et al. High-salt diet induces IL-17-dependent gut inflammation and exacerbates colitis in mice. *Front Immunol*. 2017;8:1969. doi: 10.3389/fimmu.2017.01969
  61. Zhang Z, Zhao J, Tian C, Chen X, Li H, Wei X, Lin W, Zheng N, Jiang A, Feng R, et al. Targeting the gut microbiota to investigate the mechanism of lactulose in negating the effects of a high-salt diet on hypertension. *Mol Nutr Food Res*. 2019;63:e1800941. doi: 10.1002/mnfr.201800941
  62. Miranda PM, De Palma G, Serkis V, Lu J, Louis-Auguste MP, McCarville JL, Verdu EF, Collins SM, Bercik P. High salt diet exacerbates colitis in mice by decreasing *Lactobacillus* levels and butyrate production. *Microbiome*. 2018;6:57. doi: 10.1186/s40168-018-0433-4
  63. Hamad I, Cardilli A, Corte-Real BF, Dyczko A, Vangronsveld J, Kleinewietfeld M. High-salt diet induces depletion of lactic acid-producing bacteria in murine gut. *Nutrients*. 2022;14:1171. doi: 10.3390/nu14061171
  64. Khalesi S, Sun J, Buys N, Jayasinghe R. Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. *Hypertension*. 2014;64:897–903. doi: 10.1161/HYPERTENSIONAHA.114.03469

65. Ferguson JF, Aden LA, Barbaro NR, Van Beusecum JP, Xiao L, Simmons AJ, Warden C, Pasic L, Himmel LE, Washington MK, et al. High dietary salt-induced dendritic cell activation underlies microbial dysbiosis-associated hypertension. *JCI Insight*. 2019;5:e126241. doi: 10.1172/jci.insight.126241
66. Kleinewietfeld M, Hafler DA. Regulatory T cells in autoimmune neuroinflammation. *Immunol Rev*. 2014;259:231–244. doi: 10.1111/immr.12169
67. Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3+ regulatory T cells in the human immune system. *Nat Rev Immunol*. 2010;10:490–500. doi: 10.1038/nri2785
68. Arroyo Hornero R, Hamad I, Córte-Real B, Kleinewietfeld M. The impact of dietary components on regulatory T cells and disease. *Front Immunol*. 2020;11:253. doi: 10.3389/fimmu.2020.00253
69. Kleinewietfeld M, Hafler DA. The plasticity of human Treg and Th17 cells and its role in autoimmunity. *Semin Immunol*. 2013;25:305–312. doi: 10.1016/j.smim.2013.10.009
70. Joly AL, Seitz C, Liu S, Kuznetsov NV, Gertow K, Westerberg LS, Paulsson-Berne G, Hansson GK, Andersson J. Alternative splicing of FOXP3 controls regulatory T cell effector functions and is associated with human atherosclerotic plaque stability. *Circ Res*. 2018;122:1385–1394. doi: 10.1161/CIRCRESAHA.117.312340
71. Ait-Oufella H, Salomon BL, Potteaux S, Robertson AK, Gourdy P, Zoll J, Merval R, Esposito B, Cohen JL, Fisson S, et al. Natural regulatory T cells control the development of atherosclerosis in mice. *Nat Med*. 2006;12:178–180. doi: 10.1038/nm1343
72. Kvakani H, Kleinewietfeld M, Qadri F, Park JK, Fischer R, Schwarz I, Rahn HP, Plehm R, Wellner M, Elitok S, et al. Regulatory T cells ameliorate angiotensin II-induced cardiac damage. *Circulation*. 2009;119:2904–2912. doi: 10.1161/CIRCULATIONAHA.108.832782
73. Weirather J, Hofmann UD, Beyersdorf N, Ramos GC, Vogel B, Frey A, Ertl G, Kerkauf T, Frantz S. Foxp3+ CD4+ T cells improve healing after myocardial infarction by modulating monocyte/macrophage differentiation. *Circ Res*. 2014;115:55–67. doi: 10.1161/CIRCRESAHA.115.303895
74. Meng X, Yang J, Dong M, Zhang K, Tu E, Gao Q, Chen W, Zhang C, Zhang Y. Regulatory T cells in cardiovascular diseases. *Nat Rev Cardiol*. 2016;13:167–179. doi: 10.1038/nrcardio.2015.169
75. Baardman J, Lutgens E. Regulatory T cell metabolism in atherosclerosis. *Metabolites*. 2020;10:279. doi: 10.3390/metabo10070279
76. Saigusa R, Winkels H, Ley K. T cell subsets and functions in atherosclerosis. *Nat Rev Cardiol*. 2020;17:387–401. doi: 10.1038/s41569-020-0352-5
77. Tang TT, Ding YJ, Liao YH, Yu X, Xiao H, Xie JJ, Yuan J, Zhou ZH, Liao MY, Yao R, et al. Defective circulating CD4CD25+Foxp3+CD127(low) regulatory T-cells in patients with chronic heart failure. *Cell Physiol Biochem*. 2010;25:451–458. doi: 10.1159/000303050
78. Safa K, Othori S, Borges TJ, Uehara M, Batal I, Shimizu T, Magee CN, Belizaire R, Abdi R, Wu C, et al. Salt accelerates allograft rejection through serum- and glucocorticoid-regulated kinase-1-dependent inhibition of regulatory T cells. *J Am Soc Nephrol*. 2015;26:2341–2347. doi: 10.1681/ASN.2014090914
79. Sumida T, Lincoln MR, Ukeje CM, Rodriguez DM, Akazawa H, Noda T, Naito AT, Komuro I, Dominguez-Villar M, Hafler DA. Activated  $\beta$ -catenin in Foxp3(+) regulatory T cells links inflammatory environments to autoimmunity. *Nat Immunol*. 2018;19:1391–1402. doi: 10.1038/s41590-018-0236-6
80. Dominguez-Villar M, Hafler DA. Regulatory T cells in autoimmune disease. *Nat Immunol*. 2018;19:665–673. doi: 10.1038/s41590-018-0120-4
81. Bansal SS, Ismail MA, Goel M, Zhou G, Rokosh G, Hamid T, Prabhu SD. Dysfunctional and proinflammatory regulatory T-lymphocytes are essential for adverse cardiac remodeling in ischemic cardiomyopathy. *Circulation*. 2019;139:206–221. doi: 10.1161/CIRCULATIONAHA.118.036065
82. Luo Y, Xue Y, Wang J, Dang J, Fang Q, Huang G, Olsen N, Zheng SG. Negligible effect of sodium chloride on the development and function of TGF-beta-induced CD4(+) Foxp3(+) regulatory T cells. *Cell Rep*. 2019;26:1869–1879.e3. doi: 10.1016/j.celrep.2019.01.066
83. Hernandez AL, Kitz A, Wu C, Lowther DE, Rodriguez DM, Vudattu N, Deng S, Herold KC, Kuchroo VK, Kleinewietfeld M, et al. Sodium chloride inhibits the suppressive function of FOXP3+ regulatory T cells. *J Clin Invest*. 2015;125:4212–4222. doi: 10.1172/JCI81151
84. Wu C, Chen Z, Xiao S, Thalhammer T, Madi A, Han T, Kuchroo V. SGK1 governs the reciprocal development of Th17 and regulatory T cells. *Cell Rep*. 2018;22:653–665. doi: 10.1016/j.celrep.2017.12.068
85. Yang YH, Istomine R, Alvarez F, Al-Aubodah TA, Shi XQ, Takano T, Thornton AM, Shevach EM, Zhang J, Piccirillo CA. Salt sensing by serum/glucocorticoid-regulated kinase 1 promotes Th17-like inflammatory adaptation of Foxp3(+) regulatory T cells. *Cell Rep*. 2020;30:1515–1529.e4. doi: 10.1016/j.celrep.2020.01.002
86. Alvarez F, Istomine R, Shourian M, Pavey N, Al-Aubodah TA, Qureshi S, Fritz JH, Piccirillo CA. The alarmins IL-1 and IL-33 differentially regulate the functional specialisation of Foxp3(+) regulatory T cells during mucosal inflammation. *Mucosal Immunol*. 2019;12:746–760. doi: 10.1038/s41385-019-0153-5
87. Barbaro NR, Foss JD, Kryshal DO, Tsyba N, Kumaresan S, Xiao L, Mernaugh RL, Itani HA, Loperena R, Chen W, et al. Dendritic cell amiloride-sensitive channels mediate sodium-induced inflammation and hypertension. *Cell Rep*. 2017;21:1009–1020. doi: 10.1016/j.celrep.2017.10.002
88. Tian R, Colucci WS, Arany Z, Bachschmid MM, Ballinger SW, Boudina S, Bruce JE, Busija DW, Dikalov S, Dorn GW II, et al. Unlocking the secrets of mitochondria in the cardiovascular system: path to a cure in heart failure—a report from the 2018 National Heart, Lung, and Blood Institute Workshop. *Circulation*. 2019;140:1205–1216. doi: 10.1161/CIRCULATIONAHA.119.040551
89. Montaigne D, Marechal X, Coisne A, Debry N, Modine T, Fayad G, Potelle C, El Arid JM, Mouton S, Sebt Y, et al. Myocardial contractile dysfunction is associated with impaired mitochondrial function and dynamics in type 2 diabetic but not in obese patients. *Circulation*. 2014;130:554–564. doi: 10.1161/CIRCULATIONAHA.113.008476
90. Mattiello JA, Margulies KB, Jeevanandam V, Houser SR. Contribution of reverse-mode sodium-calcium exchange to contractions in failing human left ventricular myocytes. *Cardiovasc Res*. 1998;37:424–431. doi: 10.1016/s0008-6363(97)00271-x
91. Lai L, Qiu H. The physiological and pathological roles of mitochondrial calcium uptake in heart. *Int J Mol Sci*. 2020;21:7689. doi: 10.3390/ijms21207689
92. van Teijlingen Bakker N, Pearce EJ. Cell-intrinsic metabolic regulation of mononuclear phagocyte activation: findings from the tip of the iceberg. *Immunol Rev*. 2020;295:54–67. doi: 10.1111/immr.12848
93. Geltink RIK, Kyle RL, Pearce EL. Unraveling the complex interplay between T cell metabolism and function. *Annu Rev Immunol*. 2018;36:461–488. doi: 10.1146/annurev-immunol-042617-053019
94. Hernansanz-Agustin P, Choya-Foces C, Carregal-Romero S, Ramos E, Oliva T, Villa-Pina T, Moreno L, Izquierdo-Alvarez A, Cabrera-Garcia JD, Cortes A, et al. Na(+) controls hypoxic signalling by the mitochondrial respiratory chain. *Nature*. 2020;586:287–291. doi: 10.1038/s41586-020-2551-y
95. Corte-Real BF, Hamad I, Arroyo Hornero R, Geisberger S, Roels J, Van Zeebroeck L, Dyczko A, van Gisbergen MW, Kurniawan H, Wagner A, et al. Sodium perturbs mitochondrial respiration and induces dysfunctional Tregs. *Cell Metab*. 2023;35:299–315.e8. doi: 10.1016/j.cmet.2023.01.009
96. Weinberg SE, Singer BD, Steinert EM, Martinez CA, Mehta MM, Martinez-Reyes I, Gao P, Helmin KA, Abdala-Valencia H, Sena LA, et al. Mitochondrial complex III is essential for suppressive function of regulatory T cells. *Nature*. 2019;565:495–499. doi: 10.1038/s41586-018-0846-z
97. Hahn SA, Bellinghausen I, Trinschek B, Becker C. Translating Treg therapy in humanized mice. *Front Immunol*. 2015;6:623. doi: 10.3389/fimmu.2015.00623
98. Kleinewietfeld M, Starke M, Di Mitri D, Borsellino G, Battistini L, Rotzschke O, Falk K. CD49d provides access to “untouched” human Foxp3+ Treg free of contaminating effector cells. *Blood*. 2009;113:827–836. doi: 10.1182/blood-2008-04-150524
99. Morishita K, Watanabe K, Naguro I, Ichijo H. Sodium ion influx regulates liquidity of biomolecular condensates in hyperosmotic stress response. *Cell Rep*. 2023;42:112315. doi: 10.1016/j.celrep.2023.112315
100. Neal B, Wu Y, Feng X, Zhang R, Zhang Y, Shi J, Zhang J, Tian M, Huang L, Li Z, et al. Effect of salt substitution on cardiovascular events and death. *N Engl J Med*. 2021;385:1067–1077. doi: 10.1056/NEJMoa2105675
101. Tobian L. Protecting arteries against hypertensive injury. *Clin Exp Hypertens A*. 1992;14:35–43. doi: 10.3109/10641969209036169
102. Yuan Y, Jin A, Neal B, Feng X, Qiao Q, Wang H, Zhang R, Li J, Duan P, Cao L, et al. Salt substitution and salt-supply restriction for lowering blood pressure in elderly care facilities: a cluster-randomized trial. *Nat Med*. 2023;29:973–981. doi: 10.1038/s41591-023-02286-8