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Opinion

Sodium and its manifold impact on our immune system

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The Western diet is rich in salt, and a high salt diet (HSD) is suspected to be a risk factor for cardiovascular diseases. It is now widely accepted that an experimental HSD can stimulate components of the immune system, potentially exacerbating certain autoimmune diseases, or alternatively, improving defenses against certain infections, such as cutaneous leishmaniasis. However, recent findings show that an experimental HSD may also aggravate other infections (e.g., pyelonephritis or systemic listeriosis). Here, we discuss the modulatory effects of a HSD on the microbiota, metabolic signaling, hormonal responses, local sodium concentrations, and their effects on various immune cell types in different tissues. We describe how these factors are integrated, resulting either in immune stimulation or suppression in various tissues and disease settings.

Highlights

A HSD can stimulate local tissue-specific accumulation of sodium in mice and humans.

Increases in sodium concentration can amplify inflammatory macrophage and T cell responses in mice and humans.

A HSD can affect systemic immune responses by altering the intestinal microbiome and its immunomodulatory metabolites in mice and humans.

A HSD can systemically suppress neutrophil-mediated immune responses by causing hyperglucorticoidism without diurnal rhythm in mice and humans.

The effect of a HSD on immunity is dependent on the tissue/organ affected, the type of immune cells implicated, and the presence and type of disease.

Salt (NaCl) used to be so rare and valuable that in the 17th century for instance, the Duchy of Bavaria and the city of Salzburg entered into war over it. Even the word 'salary' reflects the preciousness that salt once had [1]. Due to geotechnological advances, salt nowadays is neither rare nor expensive. We love it and, thus, we eat a lot of it. Typical diets in Western countries and China contain more than 10 g per day [2].

The high salt content of diets in industrialized countries is suspected to be a risk factor that drives mortality and disease burden [3]. The connection between elevated salt consumption and hypertension was first proposed more than 50 years ago [4], and remains intensively discussed, especially since recent work questioned this connection [5]. Furthermore, recent studies have uncovered new links of salt to our health, especially through the proinflammatory effects of sodium [6–14]. On the one hand, HSD can stimulate certain components of the immune system, namely, to combat pathogens more efficiently [10,11,15] to fight cancer [12,13], or to elicit more vigorous autoimmune responses [6,16,17]. Moreover, HSD can boost osteoclast activity and thereby facilitate orthodontic tooth movement [18] and augment proinflammatory activation of microglia, which can aggravate stroke injury [19]. Of note, **salt-losing tubulopathies** (see Glossary) that result in sodium loss, have enhanced susceptibility to mucosal infections in humans [20]. Likewise, treating renal transplant patients with **loop diuretics**, which causes the excretion of sodium, can increase the incidence of urinary tract infections in these patients [21]. This has led to the current view that sodium is generally immunostimulatory depending on the local microenvironment [22,23]. On the other hand, recent findings have uncovered the immunosuppressive effects of sodium [24,25]. Thus, a comprehensive theory about when and where sodium is immunostimulatory or suppressive is wholly lacking at present.

Here, we wish to draw a broader picture of the complex systemic immune regulatory circuits induced by dietary sodium and its local effects. We discuss sodium uptake and tissue storage, local effects on immune cells, systemic effects through the microbiota and endocrine alterations, as well as specific effects in immune-mediated diseases and infections, with a focus on **pyelonephritis**. We hypothesize that a bird's-eye view of immunology that encompasses anatomical, physiological, and

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microbiological factors, may allow to predict the effects of a HSD on certain diseases, and may also reconcile reports in the literature that are seemingly contradictory.

Dietary high salt and tissue sodium storage

Theoretically, a HSD may affect our organism via direct or indirect, local or systemic effects (Figure 1A,B). Specifically, preclinical studies with rodents using experimental sodium-rich diets revealed that sodium can accumulate in skin, thymus, liver, and spleen [13,26–28] and, in addition, sodium may be potentially stored in endothelial surface layers [29], and thus act locally on surrounding cells. Moreover, there is evidence in mice that the kidney and bone marrow can lose salt upon dietary experimental HSD [24,30], and consequently, these tissues display a lower sodium content upon HSD than low salt diet (LSD). In general, HSD does not uniformly result in sodium accumulation in all tissues. In addition, these responses may depend on the genetic background of animals and organisms studied [15]. These findings highlight the importance of quantifying tissue sodium concentrations, and ideally chloride, in further studies addressing the local effects of these electrolytes under high salt diet or LSDs.

It is not completely clear why, when, or how sodium accumulates in tissues. Correlative analysis of glycosaminoglycan content, and its charge capacity in rodents [26,31,32] and humans [33], suggested that negatively charged glycosaminoglycans may at least partially contribute to tissue sodium accumulation by binding positively charged ions [26,31–33]. In addition, there is evidence from studies with rodents that this sodium storage in the skin generates a hypertonic environment [27]. How hypertonic fluid microdomains can be generated is unclear. One study modeled

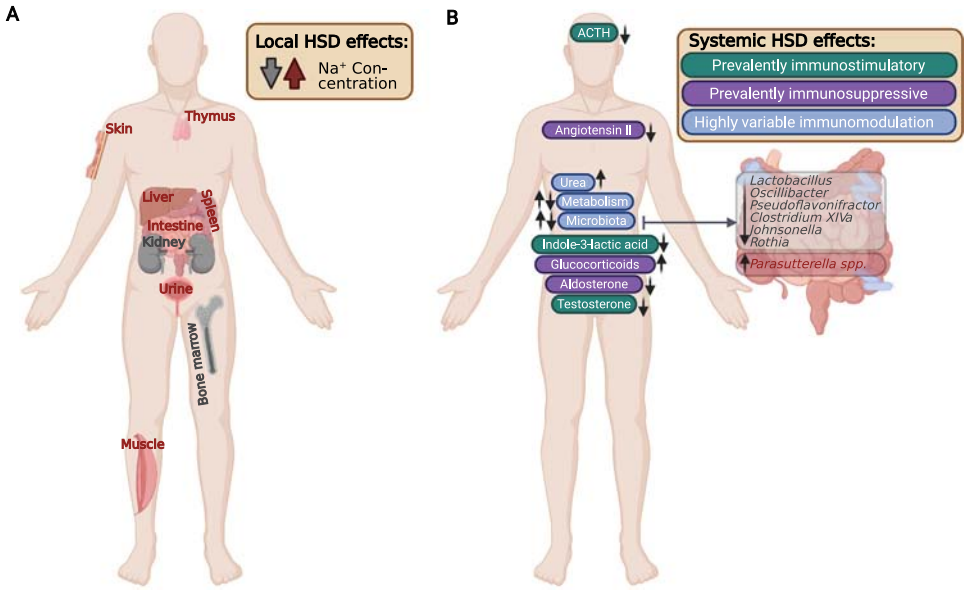

Figure 1. Direct and indirect changes induced by HSD. (A) HSD exposure leads to Na^+ increase in the skin, thymus, liver, spleen, and urine, but decreases in the bone marrow and kidneys [13,24,26–28,30,45]. HSD diminishes ACTH, angiotensin II, testosterone, and processes such as glycolysis, while elevating urea, glucocorticoids, ketone body production, and fatty acid oxidation [24,45,46]. Moreover, HSD consumption results in reshaping the microbiota composition by reducing indole-3 lactic acid concentrations in the gut [65]. All these changes might be either immunostimulatory or immunosuppressive, depending on the nature of the change and the immune cell type exposed to it. Some of the alterations shown here were demonstrated in mice. A human body is shown for convenience. The illustration was created with BioRender.com. Abbreviations: ACTH, adrenocorticotropic hormone; HSD, high salt diet.

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existing data and concluded that there might be a cutaneous countercurrent system similar to the kidney [34], allowing the generation of a hypertonic cutaneous fluid compartment rich in sodium. In this model, the lymphatics would represent a tubular/collecting duct-like system that drains sodium from the skin [34]. This model matches the findings that sodium content is substantially higher in cutaneous lymphatics of rodents compared to surrounding tissue [27]. In preclinical mouse and rat models, a HSD increased the compartment of circulating classical monocytes compared to a normal salt diet [35], and dermal macrophage numbers relative to LSD [26,27,36]. A mechanism was proposed in which cutaneous macrophages regulated the abundance of skin lymph capillaries [26,27]. Accordingly, osmoprotective signaling through the transcription factor nuclear factor of activated T cells 5 (NFAT5), also known as tonicity-responsive enhancer binding protein 5 (TonEBP) [37] in macrophages, removed excess sodium and prevented HSD-induced hypertension in these rodent models [26,27]. Of note, the exact role of NFAT5 and macrophages in local ionic balance has been reviewed elsewhere [22,26,38]. In humans, an experimental HSD (≥ approximately 12 g NaCl/day for 7–14 days) also resulted in the accumulation of Na^+ [39] and macrophages [40] in the skin, strongly suggesting that this system might be operative in humans as well.

It is unclear why sodium concentrations can be decreased in the bone marrow upon HSD exposure in mice [30]. Perhaps processes of proper hematopoiesis and cell proliferation might require lower sodium concentrations than immune cell activation, given that higher tissue osmolality may be encountered by infiltrating immune cells such as dendritic cells (DC) and T cells in secondary lymphatics [13,41]. In line with this, relative to controls, increased tonicity in cancer cell lines has augmented the effectiveness of cytotoxic activity of death receptors [42] which are involved in hematopoiesis as well [43]. Of note, mice consuming a LSD did not show higher sodium in the bone marrow if myeloid cells did not express NFAT5 [30], suggesting that this transcription factor might contribute to mediating sodium accumulation in this organ. However, as these possibilities remain speculative, further studies are needed to understand regulatory circuits involving sodium in the bone marrow.

By contrast, a sodium loss in the kidneys of mice fed a HSD might be explained by available knowledge; under normal conditions, the kidney uses sodium and urea to create an osmotic gradient between the kidney cortex and the medulla, to reabsorb water from the glomerular ultrafiltrate [44]. Under a HSD, the kidneys excrete excess sodium by switching off sodium reabsorption [45,46]. Instead, in this situation, the kidney preferentially uses organic osmolytes to establish the osmotic gradient, especially urea, a process referred to as 'urea-dependent water conservation' [45]. Thus, during a HSD, the renal medulla is rich in urea, while sodium concentrations decrease [24]. However, mechanistic insight for sodium storage in other tissues remains poorly understood.

Diet-independent sodium accumulation and impact on immune cells

Sodium tissue accumulation can also occur in a diet-independent manner. Human long-lasting sodium balance studies demonstrate that under resting conditions, total body sodium concentrations follow an infradian rhythmicity that is under hormonal control [47]. Infection and inflammation can cause sodium build-up in afflicted human and mouse skin tissues in a diet-independent manner [10,48,49]. Increased sodium concentrations have been found in brain lesions of multiple sclerosis patients [50], fibrotic skin in patients with **diffuse cutaneous systemic sclerosis** [51], and in the skin of patients with **lipedema** [52] relative to healthy controls. Furthermore, there is evidence from noninvasive ^{23}Na-MRI imaging that sodium concentrations in cancerous human brain and breast tissue are increased as well, relative to controls [53,54]. Overall, the mechanisms contributing to diet-independent local sodium accumulation are ill-defined. It is reasonable to speculate that sodium

Glossary

Autolysosome: degradative organelle established by fusion of autophagosomes with lysosomes.
Autophagy: regulated cellular catabolic mechanism triggered by stress; removes unnecessary or dysfunctional components; allows degradation and recycling of cellular components.
Diffuse cutaneous systemic sclerosis: potentially life-threatening chronic multiorgan disease, whose key elements are vascular dysfunction and gradually increasing fibrosis, not limited to the skin but including internal organs such as the lung, kidney, and heart.
Ketogenesis: biochemical process that produces ketone bodies, which can supply energy during caloric restriction.
Lipedema: chronic condition of subcutaneous fat deposition resulting in disproportional enlargement of extremities.
Loop diuretics: act by preventing the ability of Henle's loop to build the intrarenal osmotic gradient for water reabsorption, such as furosemide.
Pyelonephritis: Infection of the kidney due to ascension of bacteria from the bladder into the kidney. Complications include sepsis, kidney scarring, (chronic) renal insufficiency, and potentially, renal carcinoma.
Resting membrane potential: transmembrane potential under resting conditions dependent on the differential permeability of the plasma membrane to ions.
Salt-losing tubulopathies: diseases compromising the ability of renal tubules to reabsorb electrolytes filtered in renal glomeruli.
Short-chain fatty acid: bear less than six carbon atoms; usually derived from intestinal microbial fermentation of food that can be used as nutrients by various cell types.

changes reflect an inflammation-triggered reprogramming of the extracellular tissue matrix [34,38,55]. In addition to local tissue sodium accumulation, there is evidence in a model of isolated guinea pig trachea that hypertonic conditions exist in the airways on mucosal surface liquids due to water evaporation under steady state conditions [56]. Therefore, alveolar macrophages might be especially well adapted to operate in sodium-rich environments. Overall, these findings demonstrate that in addition to dietary factors, inflammatory conditions and local anatomical peculiarities might also affect the local sodium balance.

For several years, interest has been growing to understand the impact of these ionic tissue signals on immune cell responses. Indeed, immune cells are not only equipped to adjust to these sodium-rich microdomains, but salt conditions can also profoundly influence the functions of immune cells [22,23,38]. For instance, high salt conditions enhance the proinflammatory activation of mouse macrophages, while limiting their anti-inflammatory potential [6–9,12–14]. Likewise, sodium-rich environments have been reported to favor the development of inflammatory IL-17-producing $CD4^+$ T cells (Th17) under Th17 polarizing experimental conditions in mice and humans [16,17,25,49] and to block the immunosuppressive activity of human regulatory T cells [57]. In the absence of proinflammatory Th17-skewing conditions, however, high sodium can favor the development of T helper 2 responses from human naïve and memory T cells [49] or able to induce regulatory outputs in human and mouse Th17 cells [25]. These findings suggest that sodium-induced effects on T cells are contextual. These various sodium-triggered T cell responses can involve osmoprotective signaling, including NFAT5 and/ or serum and glucocorticoid regulated kinase 1-dependent signal transduction [16,17,25,49,57]. Therefore, the molecular underpinnings driving these divergent T cell responses require further molecular investigation.

The mechanisms of how immune cells can detect increased sodium concentrations are largely elusive. In contrast to epithelial cells, immune cells lack an apical-basal axis orientation, indicating that their ability to handle changes in the local ionic composition will differ from the mechanisms in epithelial cells. In addition, in macrophages for instance, the **resting membrane potential** is not as negative as in epithelial cells [58,59], which has implications on the activity and thermodynamics of channels, exchangers, and transporters. Our group reported that the Na^+/Ca^{2+} exchanger plays an important role in the detection of increases in local extracellular sodium levels and contributes to amplifying proinflammatory mouse macrophage responses [58]. However, sodium handling ensuing cellular responses remain undetermined and we do not know how other immune cells might detect and/or handle excess sodium amounts. Of note, in juvenile Japanese eels (*Anguilla japonica*), a sodium-binding system exists that allows the organism to adapt to acute sodium-rich environments [60]. Whether similar systems exist in mammals requires further investigation.

In mouse macrophages, local increases in sodium do not only boost their inflammatory potential but also enhance their antimicrobial activity. While HS-augmented defenses against the protozoan parasite *Leishmania major* hinged on the increased NFAT5-dependent production of leishmanicidal nitric oxide (NO) in a mouse model of cutaneous leishmaniasis [10], HS-boosted antibacterial responses in mouse macrophages required **autophagy** and targeting of intracellular bacteria to acidic **autolysosomes** [61]. While NFAT5 coordinated the subcellular targeting of bacteria to autolysosomal structures in mouse macrophages, enhanced expression of components of the autophagy machinery depends on the transcription factor hypoxia-inducible factor 1α (HIF1α) (Figure 2) [61]. Autophagy and HIF1α-dependent signaling are both linked to metabolism, and a recent study reported an important role of sodium in mitochondrial energy generation in bovine aortic endothelial cells and mouse embryonic fibroblasts [62]. Therefore, this suggested that sodium might reprogram metabolic and cellular energetics, which might ultimately impact inflammatory outputs and the handling of sodium. Given that metabolic signaling is recognized as

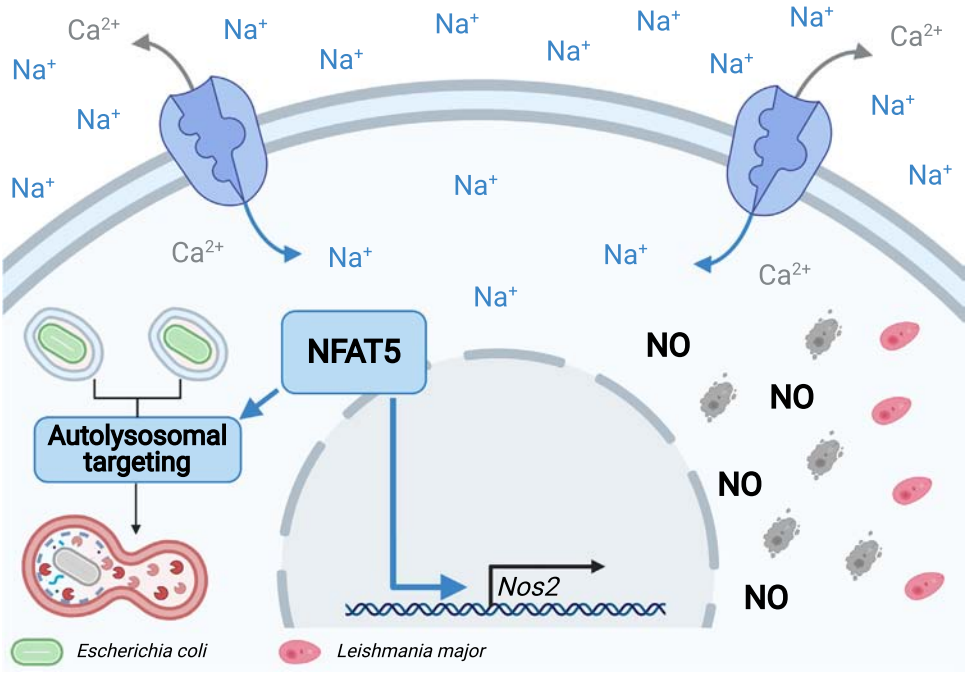

Figure 2. The effect of sodium on the antimicrobial properties of murine macrophages. Na^+/Ca^{2+} exchanger 1 (NCX1) plays an important role in the detection of extracellular Na^+ concentrations by macrophages [58]. NCX1-facilitated Na^+ entry triggers a Ca^{2+} loss and enhances NFAT5 accumulation [58]. NFAT5 boosts the expression of *Nos2*, which ultimately results in the increased production of leishmanicidal NO in a murine model of skin leishmaniasis [10]. In addition, sodium-triggered NFAT5 facilitates autolysosomal targeting of intracellular bacteria, thereby contributing to their destruction [61]. The illustration was created with BioRender.com. Abbreviations: NFAT5, nuclear factor of activated T cells 5; NO, nitric oxide.

an important immune regulator, it will be interesting to further investigate any potential interactions between sodium handling and immunometabolism.

A HSD can affect the gut microbiome and microbiota-derived tryptophan metabolites

Only recently have we started to understand how the intestinal microbiota and its metabolites impact the host and contribute to health or disease. The gut microbiota is now regarded as an endocrine organ generating metabolites affecting host physiology, and triggering responses in the local microenvironment or distant target organs [63]. While the impact of high fat and sugar on the gut microbiome has been extensively studied, the effect of high salt was only recently described: in mice, a high fat diet can lead to a profound shift in the composition of the gut microbiome [64], and a HSD modifies the gut microbiome to a much lesser extent, particularly when depleting *Lactobacillus spp* [65]. In addition, a HSD can also decrease microbial populations across a variety of genera including *Oscillibacter, Pseudoflavonifractor, Clostridium* XIVa, *Johnsonella,* and *Rothia* [65]. With a HSD, other genera such as *Parasutterella spp.* are increased in the intestine [65]. Along with an altered gut microbiome, a HSD increases systolic blood pressure in mice, as well as clinical symptoms (paralysis score) of experimental autoimmune encephalomyelitis (EAE), and also the frequency of Th17 cells in the gut, spleen, and spinal cord [65]. Besides its pivotal role in the generation of autoimmunity, Th17 cells also play a role in hypertension [66]. Moreover, *Lactobacilli* are able to metabolize the essential amino acid tryptophan to indole metabolites [67], and thus, it might not be surprising that a HSD, beside its suppressive action

on *Lactobacillus* abundance, can also reduce fecal indole metabolites [65]. Conversely, probiotic *Lactobacillus* treatment in mice increases fecal indole-3 metabolites, together with a reduction in Th17 numbers, systolic blood pressure, and experimental autoimmunity in this EAE model. Mechanistically, indole-3 lactic acid inhibited Th17 polarization, suggesting that tryptophan metabolites might act as Th17 inhibitors [65]. In line with these data, in another study, a HSD also accelerated experimental colitis in mice by decreasing intestinal *Lactobacillus* abundance and **short-chain fatty acid** butyrate production [68]. Of note, a modest reduction in oral sodium intake in therapy-naïve hypertensive humans has resulted in an increase in serum short-chain fatty acids, which are also associated with reduced blood pressure and arterial stiffness [69]. Altogether, high dietary sodium intake can alter the gut microbiota and consequently, the concentration of microbiota-derived metabolites. As these altered metabolites are absorbed into the mucosa micromilieu, they may locally affect gut immune homeostasis. This in turn, may have consequences for the host and influence disease pathogenesis in certain inflammatory, autoimmune, and cardiovascular diseases.

A HSD has systemic endocrine effects that can indirectly affect immunity

A HSD not only alters systemic immunity by modifying microbiota-derived metabolites but can also influence the endocrine system. Hormones of the renin-angiotensin aldosterone system are downregulated during a HSD, and facilitate excess sodium excretion by the kidney [70]. Some of these hormones have immunoregulatory properties. Thus, angiotensin II has been reported to increase natural killer (NK) cell cytotoxic activity, monocyte, and neutrophil chemotaxis, as well as human DC and T helper cell functions [71]. In addition, aldosterone promoted the activation of proinflammatory macrophages, resulting in elevated reactive oxygen species (ROS) production, increased Th17 polarization, and boosted $CD8^+$ T cell activation *in vitro* [72–75]. Moreover, aldosterone has stimulated human neutrophil degranulation and release of myeloperoxidase [76,77]. Given that a HSD can reduce aldosterone concentrations, it is reasonable to hypothesize that its effects on immunity might be reduced during a HSD intake, but to our knowledge, this has not been tested, and whether this effect on aldosterone has consequences for the induction of immune-mediated diseases or anti-infectious defense is unknown.

From another angle, glucocorticoids are increased during a HSD in mice and humans [24,45,46]. Studies in mice have revealed this to be a direct consequence of the downregulation of aldosterone synthase [24], which causes the accumulation of corticosterone, an aldosterone precursor with glucocorticoid functionality [78]. Of note, corticosterone concentrations did not follow the diurnal rhythm that glucocorticoids normally display, and this rhythm has been shown to influence immune cells in a complex manner [79]. Moreover, corticosterone stimulates **ketogenesis** in the liver and promotes the production of urea [45]. As mentioned previously, during a HSD, urea establishes a renal osmotic gradient for water retention, since excess sodium needs to be excreted [45,46].

Specifically, glucocorticoids can affect immune cells differently; for instance, they inhibit neutrophil-mediated phagocytosis (pathogen clearance), but stimulate mononuclear phagocyte-mediated phagocytosis (e.g., dead neutrophil clearance) [80]. Furthermore, glucocorticoids suppress bacterial digestion by neutrophils, but not by macrophages *in* vitro [24]. Therefore, despite the general anti-inflammatory properties of glucocorticoids, these modulators may differentially influence immune responses carried out primarily by macrophages or neutrophils.

Similarly to sodium, glucocorticoids can augment NFAT5 expression [24]. Thus, the increase of NFAT5 expression often seen under a HSD, might be at least partially be a consequence of hyperglucocorticoidism, although this remains speculative. However, if true, it might help to explain why NFAT5 increases in tissues that do not accumulate sodium under a HSD, such as the kidney [24].

In summary, a HSD can decrease the concentrations of hormones with proinflammatory properties, such as angiotensin, aldosterone, and adrenocorticotropic hormone (ACTH), while in some cases, causing immunosuppressive hyperglucocorticoidism. As the effects appear to be cell type-dependent, the outcome of a HSD may hinge on the immune cell type involved in a given physiologic process, which may subsequently influence the outcome of a disease, or not.

Local and systemic effects of a HSD on pyelonephritis

Urinary bacterial infections occur frequently in daily clinical practice [81]. Ascending urinary tract infections can cause pyelonephritis, a life-threatening kidney infection [82]. Neutrophils are key in innate antimicrobial defense against urinary tract infections; they phagocytose and clear uropathogenic *Escherichia coli* (UPEC), while mononuclear phagocytes such as macrophages or DCs play an important role in neutrophil attraction and activation [81,83,84].

The functionality of mononuclear phagocytes differs in the kidney cortex and medulla [85], and the high medullary osmolarity is thought to be a responsible factor [82,86]. Medullary mononuclear phagocytes exposed to high sodium microenvironments have been reported to skew cells towards an anti-inflammatory state in human transplant rejection [87], and to reduce their antigen presentation capacity [88]. By contrast, based on the general view that sodium might stimulate these immune cells via NFAT5, it has been proposed that this ion might establish an intrarenal antibacterial defense zone in the renal medulla, thereby promoting defense against pyelonephritis [9]. This conclusion was supported by experiments demonstrating aggravated pyelonephritis in mice treated with diuretics to disrupt the osmotic gradient or after systemic genetic deletion of NFAT5 [9]. However, the particular diuretics used, tolvaptan and demeclocycline, interfered with water retention in the collecting duct, and whether they disrupted the intrarenal osmotic gradient was not tested [9]. Moreover, NFAT5 is required for cellular resistance against osmotic stress [89], and the hyperosmolar medullary environment might render NFAT5-deficient kidney cells more vulnerable to bacterial infections [9]. The authors suggested that this sodium-rich zone in the kidney might be beneficial in situations of dehydration, when the kidney increases the corticomedullary osmotic gradient, for example by accumulating sodium. Also this has not been experimentally tested, but if true, it might strengthen renal antimicrobial activity by macrophages [9]. However, the key immune effectors in pyelonephritis are neutrophils whose antimicrobial function, in contrast to macrophages, is not boosted by sodium [24]. Thus, increasing sodium concentrations in the renal medulla might not stimulate the type of immunity needed against pyelonephritis. In particular, it must be emphasized that achieving this increase by restricting fluid intake [9] might be counterproductive, because it would compromise the flushing of bacteria from the kidney. Indeed, we argue that the traditional medical advice to drink much fluid during pyelonephritis is still valid, and this opinion is supported by many clinical studies [84,87,90,91].

During consumption of a HSD, the intrarenal microenvironment changes dramatically [24]. Consequently, a state of pyelonephritis can be aggravated, as shown in mice under a HSD. This exacerbation was shown to be due to two effects that led to suppressed neutrophil effector functions [24]. First, the HSD-induced downregulation of the renin–angiotensin–aldosterone system (RAAS) reduced the sodium concentrations in the renal medulla, where instead the chaotropic compound urea accumulated [45]. Urea in turn inhibited neutrophil function, presumably by interfering with the actin skeleton [92]. Second, the glucocorticoids produced because of downregulated aldosterone synthesis suppressed neutrophil functions directly, both locally in a murine pyelonephritis model and systemically in a *Listeria monocytogenes* mouse infection model. Suppressed neutrophil functions were also noted in humans consuming a HSD for only one week [24] (Figure 3).

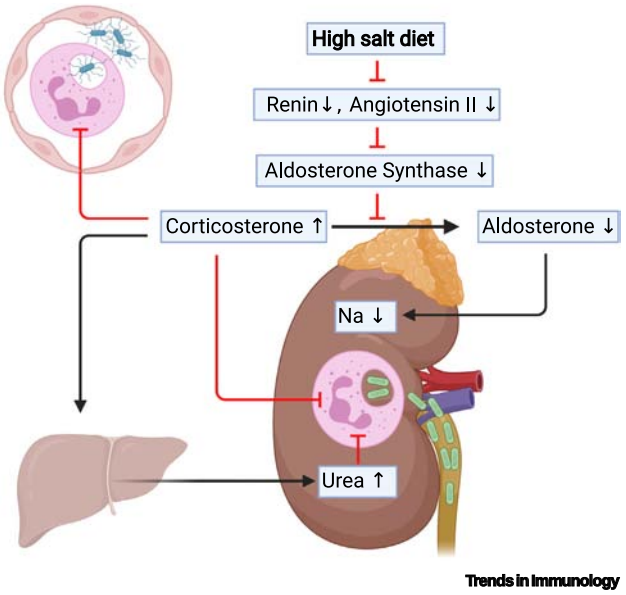

Figure 3. HSD-induced adrenal and renal changes in mice and humans A HSD reduces renin and angiotensin II levels, which downregulates aldosterone synthase, so that aldosterone production ceases at the expense of accumulation of glucocorticoids that suppress neutrophil antibacterial activity in mice and humans [24]. Additionally, an aldosterone decrease intensifies sodium excretion to the urine; the kidney in response, builds an osmotic gradient using urea instead, whose production in the liver is stimulated by glucocorticoids; this suppresse neutrophils in a murine model of pyelonephritis [24]. The illustration was created with BioRender.com. Abbreviations: HSD, high salt diet; PMNs, *polymorphonuclear* leukocytes.

Outstanding questions

What are the concentrations of ions and osmolytes in tissues that have not been closely examined (e.g., skin or kidney) in health, disease, and upon dietary challenges? A comprehensive picture of these electrolyte changes would be a valuable resource for future studies in the field.

Which mechanisms account for the regulation of local sodium balance in tissues? Recent work uncovered that this regulation is more complex than previously thought and a complete theory of the underlying mechanisms might also improve our understanding of sodium regulatory effects on immunity.

Can local Na^+ cell/tissue storage be affected by reducing dietary salt intake? This is an obvious question which remains unresolved.

How are sodium concentrations sensed and interpreted by immune cells? A mechanism for macrophages has been shown, but other immune cells may use other mechanisms.

How do HSD-altered mineralocorticoid and glucocorticoid concentrations affect the progression of inflammatory diseases mediated by immune cells sensitive to these hormones, such as gout?

How quickly can HSD-induced systemic changes and their repercussions normalize after salt intake reduction?

What are the effects of combining a HSD with high sugar, fat, and protein diet on immune cells? A typical Western diet contains too many of these nutrients and in combination, might potentially have stronger or distinct effects on immunity.

These changes in sodium and glucocorticoids seemed to provide an explanation, at least in part, as to why intake of a HSD had diametric effects on immunity against two infections, cutaneous *Leishmania* and bacterial pyelonephritis. On the one hand, sodium accumulation in the skin boosted activation of dermal macrophages in mice, important for fighting leishmaniasis [93,94], while neutrophils could be detrimental [95]. In the kidney, sodium was decreased and urea increased, and the latter suppressed neutrophils, important for fighting pyelonephritis [24]. On the other hand, the HSD-induced hyperglucocorticoidism suppressed bactericidal neutrophil activity, and thereby defenses against pyelonephritis, but did not suppress macrophages, and hence, the defense against *Leishmania* was not compromised [10]. Thus, by integrating the local and cell-type specific effects of sodium versus glucocorticoids, it may be possible to reconcile the seemingly contradictory effects of HSD consumption on the defense against an infection such as leishmaniosis (in the skin), versus one such as pyelonephritis (in the kidney).

Concluding remarks

Here, we have discussed potential inhibition and activation mechanisms as well as outcomes for consuming a HSD, and its effects on cells of the immune system, and ultimately, on tissue/organ homeostasis. The effects of a HSD on immune responses depends on several factors, for example, the organ or tissue examined. In mammals, a HSD can increase sodium in the skin, but reduce it in the kidney and bone marrow, with obvious consequences for cells that are responsive to sodium. A second factor is the target immune cell being affected by a HSD. Macrophages and T cells, but not neutrophils, can be activated by sodium. Thus far, the literature has shown that a HSD can stimulate certain branches of innate or adaptive immunity, for example, to fight diseases such as parasite infections, in which mononuclear phagocytes are crucial, but in which neutrophils may not be important or as relevant. Until now, we have no comprehensive picture on the importance of the microbiome on salt-induced immunomodulation. Hygiene regimes of different laboratories might crucially affect the response of sodium-rich diets on the murine immune system. In line with this, research in wildling mice, diverse in the microbial makeup compared to 'clean' laboratory mice, may mimic some human disease phenotypes better than current experimental models [96]. In addition, the content of sodium in the chow and drinking water

under regular (so-called normal salt) conditions is not monitored regularly and might also introduce additional experimental variation. A third factor are glucocorticoids, which are systemically elevated during a HSD. Some immune effector cells, like neutrophils, are suppressed by glucocorticoids [97], whereas some functions of macrophages are even stimulated [98]. A fourth factor concerns the alterations that occur in gut microbiome-derived metabolites under a HSD, as these molecules may possess immunoregulatory properties, affecting the physiological homeostasis (or disease state) of an organism in a context-, organ-, and immune cell type-specific manner.

We posit that the effects of a HSD in one specific disease cannot be simply extrapolated to other conditions with other immune cells or other anatomical sites involved. However, when the anatomical, physiological, and microbiological conditions are known, it is possible to predict whether a HSD might aggravate or attenuate a disease state for specific pathologies and to reconcile conflicting data in the literature. Much information to this end is available already, but many questions remain that need to be answered (see Outstanding questions).

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Declaration of interests

No interests are declared.

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