


## STATE-OF-THE-ART REVIEW

# Mechanisms of sodium-mediated injury in cardiovascular disease: old play, new scripts

Giacomo Rossitto<sup>1,2</sup> and Christian Delles<sup>1</sup> 

1 Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK

2 Department of Medicine (DIMED), University of Padua, Italy

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**Correspondence**

C. Delles, Institute of Cardiovascular and Medical Sciences, BHF Glasgow Cardiovascular Research Centre, University of Glasgow, 126 University Place, Glasgow G12 8TA, UK  
 Tel: +44 141 330 2749  
 E-mail: christian.delles@glasgow.ac.uk

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There is a strong association between salt intake and cardiovascular diseases, particularly hypertension, on the population level. The mechanisms that explain this association remain incompletely understood and appear to extend beyond blood pressure. In this review, we describe some of the ‘novel’ roles of Na<sup>+</sup> in cardiovascular health and disease: energetic implications of sodium handling in the kidneys; local accumulation in tissue; fluid dynamics; and the role of the microvasculature, with particular focus on the lymphatic system. We describe the interplay between these factors that involves body composition, metabolic signatures, inflammation and composition of the extracellular and intracellular milieus.

## Salt-mediated cardiovascular damage: an individualized look beyond blood pressure

A large and diverse body of evidence indicates a close association between salt and high blood pressure (BP), or hypertension, universally recognized as the biggest contributor to the global burden of cardiovascular disease (CVD) and disability [1–4]. Both observational/epidemiological studies [5,6] and randomized controlled interventional trials [7–9] led to the general agreement that a reduction in salt intake in a population translates into lower population-level BP and thereby into reduced mortality and disability-adjusted life-years [10]. The recommended extent of reduction remains, however, debated: some authors endorse a ‘the lower, the better’ approach [8], while others favour moderate over low intakes,

based on a reported J-shaped association with outcomes [11]. At least part of this uncertainty reflects the marked heterogeneity, and to a large extent unpredictability, of the BP response of single individuals to salt loading/unloading: a trait identified as ‘salt sensitivity of BP’ [12].

As per Guyton’s tenets, the concept of salt sensitivity of BP traditionally refers to the impairment in the primarily renal systems devoted to the excretion of salt, or more appropriately sodium (Na<sup>+</sup>) [13,14], requiring pressure natriuresis to preserve an even Na<sup>+</sup> balance [15–18]. Others rather point to a subnormal vasodilatory response to salt loading in the absence of any abnormally large increase in renal Na<sup>+</sup> retention

**Abbreviations**

BP, blood pressure; CVD, cardiovascular disease; EC, extracellular; EH, essential hypertension; GAGs, glycosaminoglycans; IC, intracellular; K<sup>+</sup>, potassium; MRI, magnetic resonance imaging; Na<sup>+</sup>, sodium; NO, nitric oxide; P, hydraulic pressure; PA, primary aldosteronism; TonEBP, tonicity-responsive enhancer binding protein; VEGF-C, vascular endothelial growth factor C; VEGFR3, vascular endothelial growth factor receptor 3; II, oncotic pressure.

[19–21]. As a result of these physiological, and genetic, environmental and clinical factors [14,22], the trait is normally distributed in humans and unravelled only by cumbersome and impractical loading tests with arbitrary diagnostic BP cut-offs [23].

Blood pressure is just one single and imperfect, although universally accessible and adopted, biomarker [24] to assess salt-related cardiovascular risk and disease, even among hypertensive patients. There is now direct preclinical and ultimately clinical [25] evidence, suggesting that excess  $\text{Na}^+$  can adversely affect cardiovascular health independent of BP. Others have already reviewed the topic [26–28] by discussing effects of  $\text{Na}^+$  on not only vascular function [29–33], stroke risk and cerebrovascular autoregulation [34–36], but also bone health and osteoporosis [37] or immune mechanisms [38].

Here, we will not systematically cover cardiocerebrorenovascular  $\text{Na}^+$ -mediated damage: we will rather highlight mechanisms of  $\text{Na}^+$  handling and  $\text{Na}^+$ -related injury, which emerged in the last few years, may vary widely across individuals and are not necessarily reflected by BP changes. While they could also impact on CVDs other than hypertension, their understanding is still preliminary and actively evolving.

## Sodium and energetics

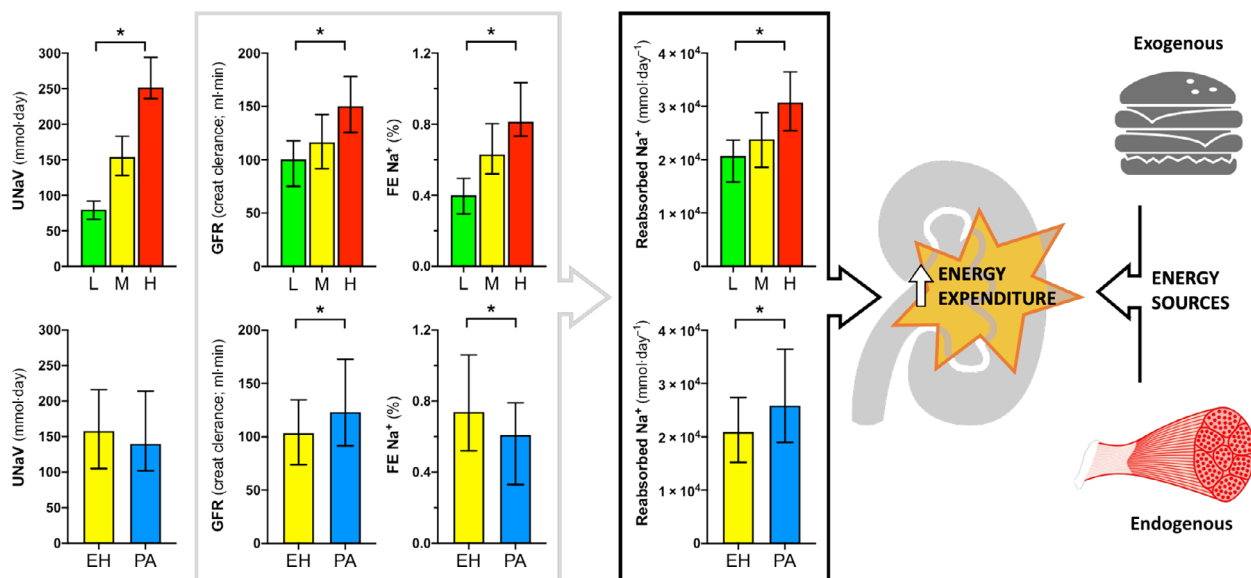
Recent work suggested a BP-independent link between high  $\text{Na}^+$  intake and a global shift in metabolism, driven by the ultimate need to preserve water upon this dietary habit. The original observation was made in a human long-term  $\text{Na}^+$  balance study during Russian spaceflight simulations [39]. The investigators modified the salt intake of ten healthy, young and male cosmonauts over 3.5–7 months and collected daily 24-h urine samples. Although the study was not devoid of limitations [40], the unique human experimental setting offered unprecedented control of diet and environment over a remarkably long period of time. At variance with previous human investigations, largely based on unphysiological short-term shifts from very low to high salt intakes, the authors reported large infradian changes in total body  $\text{Na}^+$  without parallel changes in body weight [39] and a decrease in water intake with high-salt diet, opposite to the expected increase [41]. Despite this, urine volumes remained unchanged. To reconcile the mismatch, the authors advocated surplus endogenous free water generation from exaggerated catabolic reactions and from enhanced renal accrual, which would make any extra exogenous water intake unnecessary. The hypothesis was tested in rodent models, where urea excess was found to be a key osmotic

force to minimize free water loss, via renal recycling and extrarenal generation [42]. The latter resulted from a salt-driven catabolic state, with muscle mass loss and protein breakdown as a source for urea, as well as from reprioritisation of global energy metabolism.

We have recently confirmed that water-preserving mechanisms are activated upon high  $\text{Na}^+$  intake in a large real-life cohort of patients with essential hypertension (EH). Patients exhibited glomerular hyperfiltration and excess  $\text{Na}^+$  load to the tubuli, resulting in increased tubular energy expenditure for its reabsorption [43] (Fig. 1, top). Preliminary data from patients with primary aldosteronism (PA), a prototypic form of salt-sensitive hypertension featuring glomerular hyperfiltration and excessive tubular  $\text{Na}^+$  reabsorption regardless of intake [44,45], point to similar conclusions (Fig. 1, bottom). In EH, these renal-specific changes were associated with peripheral metabolomics signatures suggestive of protein catabolism, from either endogenous or exogenous sources [43]. We therefore suggested that the kidneys are the primary determinants of the underlying energy imbalance, a proposal that is in keeping with the dependence of cardiovascular energy expenditure on renal sympathetic nerve activity in rats fed the high salt [46]. We cannot exclude, however, that additional mechanisms such as systemic hypercortisolism [42] also play a role.

Other authors conducted secondary analyses of the randomized DASH–sodium trial [7] and concluded, by reversing the investigational perspective, that low salt intake did not require reduced energy intake to keep weight stable [47]. In the study, however, weight did vary across study arms, and in the absence of calorimetry measures for body composition, unambiguous conclusions cannot be drawn. A conclusive randomized clinical trial to ‘end the salt wars’ [48] is still awaited.

Despite the need for further mechanistic confirmations, we consider the effect of high salt intake on metabolic pathways an important factor impacting cardiovascular health. In particular, metabolic effects of salt could significantly contribute to the prevalent clustering of diabetes, dyslipidaemia and obesity, with or without hypertension, in high-salt-consuming populations [49], even independent of energy intake [50]. Excess exploitation of endogenous proteins leading to lean mass loss (sarcopenia) or of exogenous (food) sources would ultimately result in insulin resistance [51] or excess fat deposition, regardless of food relative content [52] (Fig. 1). Such body changes, along with additional mechanisms described herein below, directly impact tissue microcomposition.



**Fig. 1.** Excess  $\text{Na}^+$  load to the tubuli, as a result of (top) high  $\text{Na}^+$  intake and secondary glomerular hyperfiltration in patients with EH (panels modified from Rossitto *et al.* [43]), or (bottom) of PA, featuring glomerular hyperfiltration (middle) and enhanced distal  $\text{Na}^+$  reabsorption leading to lower fraction (FE) in comparison with EH (right) despite similar intake (left), translates into a higher absolute amount of reabsorbed  $\text{Na}^+$  (black-contoured panels) and requires excess energy consumption. This energy can be generated by catabolism of exogenous (via excess food intake) or endogenous (leading to relative sarcopenia) sources. uNaV, urinary  $\text{Na}^+$  excretion, as an estimate of  $\text{Na}^+$  intake; GFR, glomerular filtration rate; FE, fractional excretion; L: low  $\text{Na}^+$  intake ( $< 2.3 \text{ g Na}^+ \cdot \text{day}^{-1}$ ); M: medium  $\text{Na}^+$  intake (2.3–5  $\text{g Na}^+ \cdot \text{day}^{-1}$ ); and H: high  $\text{Na}^+$  intake ( $> 5 \text{ g Na}^+ \cdot \text{day}^{-1}$ ). Bottom panels depict unpublished data from the same original study cohort of Rossitto *et al.* [43], collected in PA with the same methodology already reported for EH patients. All data are shown as median and interquartile range; intergroup comparisons by Tukey's or Dunn's *post hoc* tests for top panels [43] and Student's *t*-test or Mann–Whitney *U* test for bottom panels, as appropriate; \* $P < 0.05$ .

## Sodium balance and interstitium

### Implications of long-term (im)balance studies and the nature of tissue sodium accumulation

Physiology classically advocates the ultimate achievement of a balance between salt intake and excretion [53], with  $\text{Na}^+$  closely paralleled by commensurate water to maintain body fluid tonicity homeostasis [54]. At variance, the aforementioned 'spaceflight' and other previous studies [55] identified large changes in total body  $\text{Na}^+$  without parallel changes in body weight [39], a surrogate of water. This was interpreted in the context of 'osmotically inactive'  $\text{Na}^+$  accumulation. Similar salt-loading studies, conducted in rodent models of normotension, salt-resistant and salt-sensitive hypertension, revealed differences in total body  $\text{Na}^+$  and  $\text{Na}^+$ -associated volumes across groups and an apparent dissociation of  $\text{Na}^+$  and water tissue contents [56]. The analytical separation of body compartments suggested the existence of a skin-specific hypertonic  $\text{Na}^+$  depot [57,58], identified in the extracellular matrix and particularly in the negatively charged glycosaminoglycans (GAGs) network, which increased in

content and sulfation upon salt loading [59]. A local self-regulatory system triggered by the osmo sensor tonicity-responsive enhancer binding protein (TonEBP) would facilitate interstitial  $\text{Na}^+$  clearance (see next section) [60–62], as its disruption led to excess skin  $\text{Na}^+$  accumulation in the animals and, remarkably, salt-sensitive hypertension.

In humans, similar evidence of skin  $\text{Na}^+$  excess has been found in many CVDs or associated risk factors, such as older age and hypertension [63,64]; diabetes [65]; chronic kidney disease [66]; acute heart failure [67]; and sclerodermic [68] or infected skin [69], by means of  $^{23}\text{Na}$  magnetic resonance imaging (MRI). However, in most of these MRI studies also skeletal muscle, and more recently myocardium in patients with PA [70], showed high  $\text{Na}^+$  signal. This questions the originally proposed skin specificity of the phenomenon; moreover, the dual osmotically active and inactive nature of interstitial  $\text{Na}^+$ , driving TonEBP-mediated signalling while simultaneously eluding parallel and commensurate water accrual, appeared somehow equivocal. In fact, our recent whole-body composition study identified excess tissue  $\text{Na}^+$  not only in skin but also in other organs of  $\text{Na}^+$ -loaded rats,

without evidence of hypertonic  $\text{Na}^+$  accumulation [71]. The results demonstrated that changes in whole-tissue  $\text{Na}^+$  concentration do not necessarily reflect hypertonicity: in keeping with a ‘histochemical deductive approach’ of the 1940s biochemists [72–74],  $\text{Na}^+$  and  $\text{K}^+$  concentrations in the whole tissue are *per se* function of the tissue extracellular (EC)-to-intracellular (IC) volume ratio [75], being  $\text{Na}^+$  the most abundant cation in the EC and  $\text{K}^+$  in the IC space, respectively. Unless their sum ( $\text{Na}^+ + \text{K}^+$ ) is increased above physiological levels, the one is increased and the other reduced by simple changes in EC relative volume. In many studies investigating the topic, and certainly those relying on the signal from a single isotope, like  $^{23}\text{Na}$ -MRI studies, assessment of this sum is lacking.

Human skin, particularly in the context of hypertensive ageing, makes no exception [71], despite the suggested parallels between specific subepidermal anatomy and physiology with renal medulla [76]. Given the lack of conclusive evidence for this analogy and the enormous energetic cost that generation and maintenance of local hypertonicity via active  $\text{Na}^+$  transport would require, we believe that the findings of water-parallelled accumulation are simply more plausible. This conclusion is supported by independent reports [77], including those of isotonic skin interstitial fluid even upon salt loading [78]. It is appreciated, however, that precise measurement of tissue water and electrolyte content is extremely challenging and different conclusions can be the result of different methodological approaches.

### The ‘site’ of accumulation

The originally proposed site for  $\text{Na}^+$  accumulation was the interstitial GAG network [59]. This prompts a couple of considerations. First, both chemical ‘destructive’ and  $^{23}\text{Na}$ -MRI approaches give a ‘whole-tissue’ readout, which cannot discriminate EC from IC space [75]. Whether excess EC  $\text{Na}^+$  is mechanistically linked to an increase also in IC  $\text{Na}^+$  depends on the activity of multiple channels and pumps and largely remains an unexplored question. Based on established [79] and novel evidence [80–82] of high intracellular  $\text{Na}^+$  impacting on cellular metabolism, this closely links to the global metabolic changes discussed in the previous section. Second, the argument of a high-salt diet-induced modulation of skin GAGs [59] or their correlation with tissue  $\text{Na}^+$  content [83] lends *per se* no support to a water-free binding of  $\text{Na}^+$  to their negative moieties: GAGs have prominent hygroscopic properties, and they normally bind water molecules, in addition to  $\text{Na}^+$ , to guarantee cartilage function in joints. As for

$\text{Na}^+$  [83], a strong association was reported for skin GAGs and water content in patients with heart failure [84]. In fact, to the best of our knowledge, no measured  $\text{Na}^+$ -to-water stoichiometry of binding to tissue GAGs is available for any human CVD. What is clearly established, on the contrary, is that GAGs are key components of the EC matrix [85] and accumulation of interstitial  $\text{Na}^+$  in the isotonic form of oedema, by exerting biophysical forces, may well affect local mechanosensing, the phenotype of surrounding cells and, ultimately, the remodelling of matrix itself [86,87]. Arthur Guyton foresaw such EC plasticity when alluded to ‘tissue fluids, pressures and gels’ [13].

Collectively, studies conducted in the macroscopic scale of whole-tissue analysis cannot unequivocally exclude the occurrence of hypertonic accumulation of  $\text{Na}^+$  in microscopic ‘niches’ (see next section), but the macroscopic and highly prevalent excess revealed in human tissues by  $^{23}\text{Na}$ -MRI signal should be generally regarded as a measure of EC volume expansion and oedema. Its clinical relevance is suggested by the strong association with target organ damage, for example left ventricular hypertrophy in the typically salt-sensitive population of patients with chronic kidney disease [66].

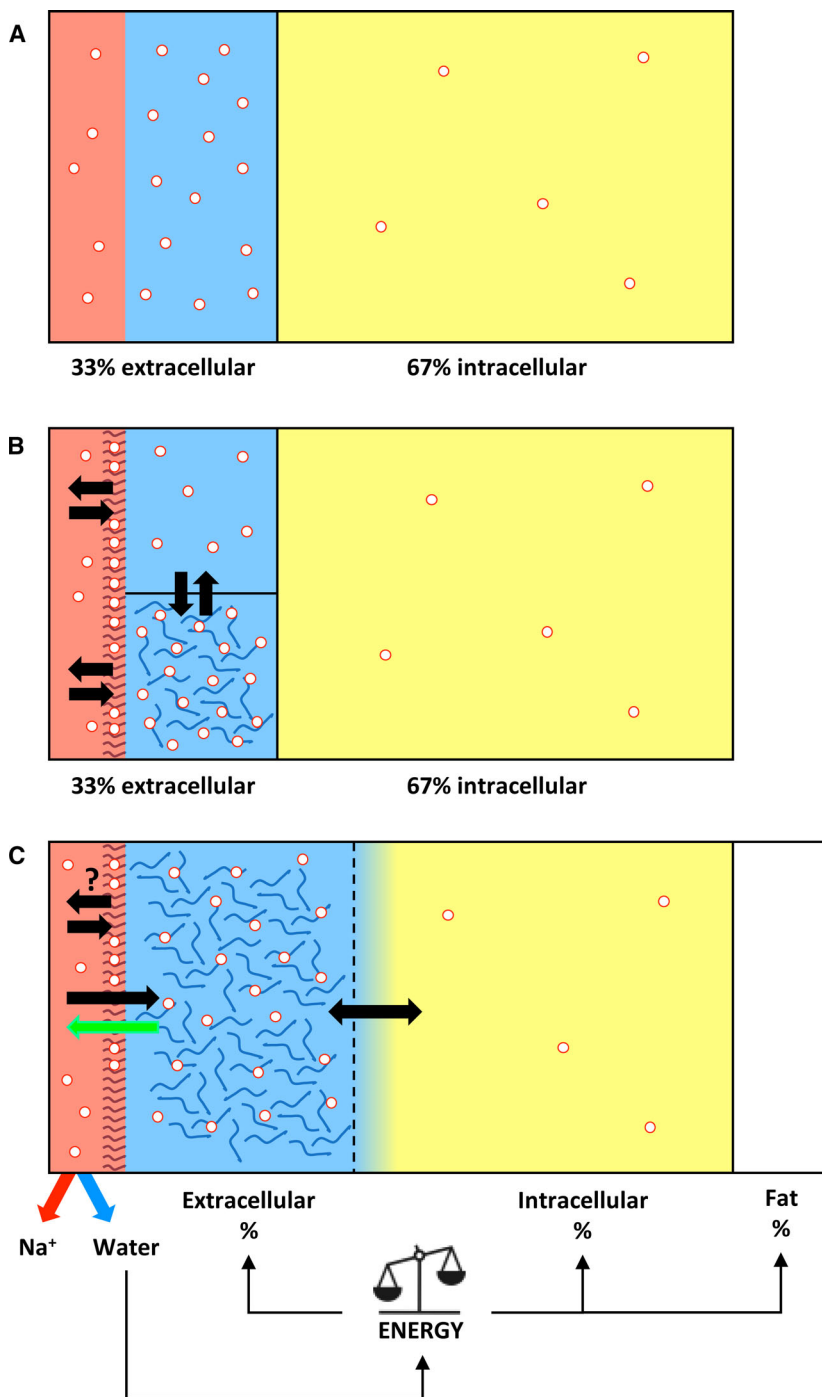
### Tissue sodium as a measure of EC volume

The above considerations by no means detract value from the novel concept of tissue  $\text{Na}^+$  accumulation [88], which challenged the dogma of even salt balance [53]. In the short-term, experimental evidence of a body weight gain ‘commensurate to iso-osmolar water retention’ in salt-sensitive compared with salt-resistant subjects [89] already supports our contention. In the long term, future assessment of this balance will probably have to take into consideration the limited sensitivity of water compared with  $\text{Na}^+$  in the identification of oedema [75]; the dissociation of their renal handling upon conditions of depletion/excess [41–43]; commonly overlooked nonrenal routes for their elimination [90–92]; and, most importantly, dynamic changes in the EC-to-IC composition of tissues. With regard to the latter, a change in EC-to-IC volume proportions could be an active adaptive mechanism to ‘accommodate’  $\text{Na}^+$  space and preserve osmolality without necessarily keeping excess water on board, as per Cannon’s homeostasis [53]. Involvement of TonEBP, a master regulator of cellular responses to osmotic stress [93,94], is speculative at the moment. On the other hand, it could be the passive result of a  $\text{Na}^+$ -related catabolic state, reducing ‘cellularity’ in different tissues and changing many reference parameters involved in or

affecting the balance. In other words, whichever the active or passive reason,  $\text{Na}^+$  retention with isotonic expansion of the EC volume does not necessarily require a positive water balance, if the 'cellular mass' is reduced (Fig. 2).

Of note, EC volume expansion is not synonymous with intravascular volume, and in patients with

hypertension [95,96], or more overtly in conditions such as heart failure [97,98], it may well preferentially involve the interstitial compartment. While speculations on a direct impact on organ function are tempting, for example in the myocardium [99] or the vascular wall [100,101], the actual biophysical, molecular and ultimately clinical implications of such



**Fig. 2.** (A) 'Classic' distribution of fluids and  $\text{Na}^+$  (O) in the intracellular and extracellular space, including intravascular (light red) and extravascular (light blue) compartments. (B) Model accounting for hypertonic (water-independent) accumulation of  $\text{Na}^+$  in the interstitium and in the endothelial surface layer (glycocalyx; adapted from Olde Engberink *et al.* [145]). (C) Model reconciling an excess tissue  $\text{Na}^+$  content and concentration with overall ECV isotonicity; microscopic niches of water-independent accumulation (i.e. in the glycocalyx) cannot be excluded ('?' in the panel). ICV-to-ECV ratio, which translates into tissue  $\text{Na}^+$  content, is modulated passively by energy balance and metabolic status ( $\pm$  fat deposition; see the section on energetics) and/or actively by cellular plasticity (double arrow) to accommodate unexcreted excess body  $\text{Na}^+$ . Such response could involve cellular TonEBP signalling. Excess isotonic  $\text{Na}^+$  in the interstitium, as a result of excess extravasation (black rightward arrow), triggers anatomical and functional lymphatic expansion for removal (green leftward arrow; see next session). Dissociation of  $\text{Na}^+$  from water excretion (red and blue arrows) via renal and nonrenal routes, although energetically demanding, preserves whole-body water homeostasis.

expansion largely remain unexplored. For sure, EC volume expansion is known to portend ominous prognosis when overt, as in heart failure [98].

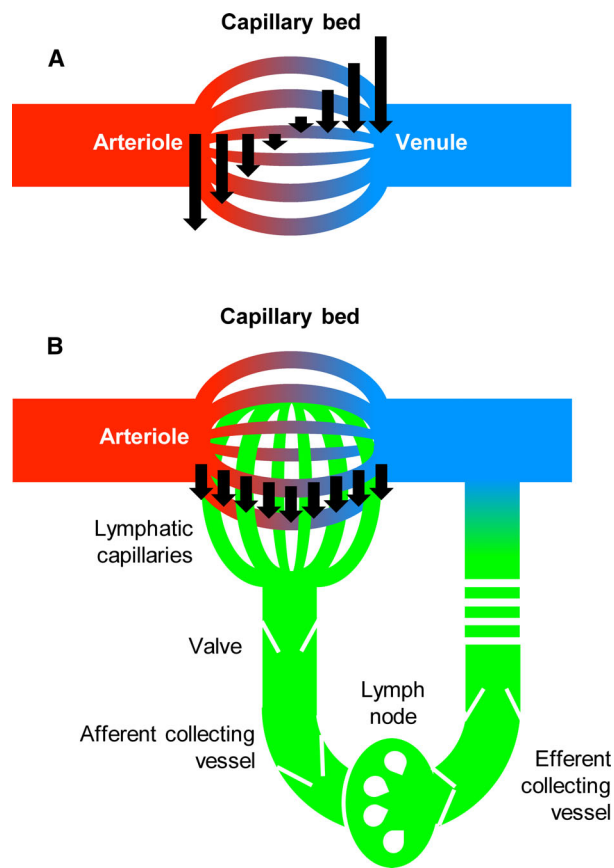
## Sodium, microvessels and interstitial fluid dynamics

### Reappraisal of microvascular dynamics

If tissue  $\text{Na}^+$  has to be primarily regarded as a surrogate of EC volume, a relevant focus of research should be on microcirculation, where the exchange of fluids between plasma and the perivascular interstitial space takes place. For more than a century, this phenomenon has been interpreted according to the Starling–Landis principle, whereby the rate of filtration is proportional to the hydraulic pressure gradient between plasma and interstitium ( $P_p - P_i$ ) minus the opposing oncotic gradient ( $\Pi_p - \Pi_i$ ) [102]. Contemporary evidence, based on direct measurements and appreciation of the structural complexity of the microvascular wall, revealed that the net sum of forces favours filtration over absorption along the entire length of the microvessels [103], rather than a sustained reabsorption of interstitial fluid at the venous end of the capillary bed (Fig. 3).

### Glycocalyx and endothelium

The first key layer encountered by fluids and solutes in the process of extravasation at the capillary-to-interstitium interface is the endothelial surface layer, or glycocalyx. It is composed of glycosamino- and proteoglycans, regulated in length and composition by the shear forces of the flowing blood [104–106]. As such, it modulates many endothelial functions, including transition from quiescence to activation towards leucocyte adhesion/extravasation, as well as nitric oxide (NO) production in response to transduced biomechanical signals [107]. In addition, it crucially controls filtration: conditions such as diabetes, end-stage kidney disease or even sepsis, where the thickness and integrity of the glycocalyx is affected [108–110], are associated with excess vascular permeability. In keeping with the speculations regarding interstitial GAGs, some authors suggested that the endothelial glycocalyx could bind excess  $\text{Na}^+$  and serve as a first  $\text{Na}^+$  buffer [111], protecting from interstitial accumulation. As for the interstitial space, the exact stoichiometry of local  $\text{Na}^+$ -to-water dynamics within and across this ultrathin layer remains unclear. In this case, even macroscopic evidence of preserved isotonicity in tissues cannot exclude a ‘microscopic’ hypertonicity exerting specific



**Fig. 3.** (A) Traditional arteriovenocentric understanding of Starling equilibrium, assuming negligible interstitial forces and reabsorption of fluids at the venous end of the microvascular bed, accordingly. (B) Reappraisal of the above, accounting for the role of glycocalyx in the control of fluid filtration and for directly measured interstitial forces, which result in a constantly positive sum of outward forces along the entire capillary bed [103]; the filtered fluid is returned to the central venous blood by the lymphatic system.

biological actions on circulating and adhering immune or vascular endothelial cells.

Preservation of glycocalyx thickness limits the extravasation of fluids for biophysical reasons, including the microscopic modulation of oncotic gradients, as extensively reviewed elsewhere [102,103]. On the contrary, exposure to high  $\text{Na}^+$  conditions *in vitro* makes the glycocalyx stiffer and thinner, thus facilitating the entry of  $\text{Na}^+$  into the endothelium [112]. The biophysical properties (increased stiffness) and function (reduced NO release) of this second layer, encountered by extravasating fluids immediately after the glycocalyx, are similarly affected by such  $\text{Na}^+$  excess [113]. Overall, this suggests intravascular mechanisms of protection from excess  $\text{Na}^+$  and fluid filtration,

which may be impaired in conditions of  $\text{Na}^+$ -related disease by a self-sustaining vicious circle.

### Lymphatic vessels

Once the endothelial surface and cellular layers have been trespassed, the lack of sustained reabsorption of fluid from the venous capillaries in most clinical/anatomical conditions [102] implicates other routes for drainage, offered by the lymphatic system (Fig. 3). By transporting lymph back to the central veins, lymphatic vessels maintain tissue volume homeostasis. To do so, they not only are ubiquitous [114] but they also show a highly sophisticated anatomy and function. Lymphatic vessels are organized into series of lymphatic capillaries, precollecting vessels and collecting vessels that ultimately drain into the thoracic or right lymphatic duct and the subclavian veins. The specific anchoring with the matrix and the buttonlike junctions in the blind-ended capillaries (opened and closed by interstitial-to-intravascular pressure gradients for the controlled entry of solutes, macromolecules and cells), the contractile activity of the peri-endothelial lymphatic muscle cells in collectors and the one-way valves separating the in-series functional units (each called 'lymphangion') confer unidirectionality [115,116]. Of note, the contractile activity remarkably shares functional aspects with the myocardium, including pacemaker-generated action potentials for coordination of vascular systole and diastole and the sensitivity of the contractile machinery to external stimuli, for example preload [115,117,118]. Therefore, it is no surprise that the accumulation of 'salty' interstitial fluid potentiates its drainage by lymphatics: excess tissue  $\text{Na}^+$  upon salt loading has been shown to induce the plastic expansion of the lymphatic vascular network [60,61] and to increase lymph flow [62]. The lymphangiogenic response is dependent on TonEBP activation in resident mononuclear phagocytic cells, with downstream signalling involving VEGF-C secretion and VEGFR3 activation: *in vivo* depletion of mononuclear cells [60], conditional phagocyte-specific deletion of TonEBP [61] and VEGF receptor blockade [61,119] led to hypoplastic capillaries and excess  $\text{Na}^+$  accumulation. The exact mechanisms of TonEBP activation, confirmed in rodent models even in the context of a 'macroscopically' isotonic ECV [71], still require clarification; however, they may involve changes in the biophysical properties of the interstitium upon oedema accumulation, as reported for mechanically stretched vascular smooth muscle cells [120].

After the first reports of  $\text{Na}^+$ -induced expansion [60,61], enhanced cardiac lymphangiogenesis by

VEGF-C was shown to reduce myocardial fibrosis and macrophage infiltration, decrease BP and preserve myocardial function in a salt-sensitive rat model of hypertension, while VEGF-C blockade produced opposite effects [121]; similarly, an inducible genetic model of kidney-specific lymphangiogenesis proved resistant to the development of hypertension induced by high-salt diet [122], as well as by multiple other prohypertensive stimuli [123,124]. In the broader context of CVD at large, increased lymphatic flow was also shown to facilitate resolution of postischaemic myocardial oedema and improve cardiac function in rodent models [125]; similar roles for lymphatics are emerging also in obesity, diabetes and atherosclerosis, as reviewed elsewhere [115,116,126–128].

Overall, all such evidence led to the appreciation of lymphatics as key regulators of local  $\text{Na}^+$  and fluid homeostasis in a broad range of conditions where tissue oedema occurs [129]. However, this pathophysiological appreciation contrasts with a still scant evidence of a lymphangiogenic or lymphatic defect in human  $\text{Na}^+$  excess-associated disease. Recently, our group demonstrated a defective lymphatic reserve in patients with heart failure compared with healthy controls of similar age and sex [130]. This led to an earlier onset of tissue fluid accumulation when venous pressure was increased. Similar investigations, aimed at dissecting the anatomical and functional complexity of lymphatics in conditions where tissue  $\text{Na}^+$  and fluid excess is more subtle, will better clarify the role of this frequently neglected arm of the circulation in human CVD. Based on the established role played by inflammation in CVD, these investigations should not ignore the immunomodulatory – in addition to hydraulic – function that these vessels locally exert.

### Local sodium excess and inflammatory cells

Once  $\text{Na}^+$  accumulates in tissues, either because of exposure to an excessive load or because of defective drainage, it does produce biological effects. We have previously alluded to the possibility of a secondary EC matrix remodelling, which would perpetuate EC volume expansion and would likely impact the function of the more 'noble' IC component of tissues, although related mechanisms and the extent of such impact remain largely unexplored. On the contrary, it is now well established that excess  $\text{Na}^+$  intake and accumulation can modulate the function of immune cells [131]. We will just briefly touch on it for what pertains aspects discussed above, and refer the reader to another recent review on the complexity of the topic

[38]; at variance with 'hypertonic tissue environments', we just see therein discussed 'diet-dependent and diet-independent'  $\text{Na}^+$  accumulations as reflective of ECV expansion (with or without oedema).

The initial report of skin resident mononuclear phagocytic cell activation upon salt loading [60] was followed by many others, describing the activation of pathogenic immune-inflammatory cells upon culture in suprphysiological concentrations of  $\text{Na}^+$ . In particular, hypertonic extracellular  $\text{Na}^+$  was found to stimulate proinflammatory [69,132,133] and to inhibit anti-inflammatory [134,135] macrophages and T cells, respectively; dendritic cells, presenting antigens and coactivating T cells, are similarly affected [136,137]. In physiological conditions, these mechanisms appear to play an important role in host defence: in the renal medulla, where hypertonic  $\text{Na}^+$  gradients are actively maintained, hypersalinity enhances mononuclear phagocytes bactericidal activity and cytokine production to generate a zone of defence; patients with urinary concentrating defects are susceptible to kidney infections [138]. However, it remains unclear whether and how a similar  $\text{Na}^+$ -induced overactivation of immune cells occurs *in vivo* in tissues or conditions where such hypertonicity seems lacking. Tissue biophysical changes or microscopic niches, as discussed, may well play a role. In this sense, even microgradients impacting appropriate sensors [139] may induce spatial and phenotypic polarization of cells, which may explain the increased leucocyte adhesion to the vascular endothelium under high  $\text{Na}^+$  conditions; alternatively, enhanced transvascular migration of immune cells could directly result from the biophysical stiffening or rarefaction/thinning of the glycocalyx [112,140–142].

Importantly, the established dysregulation of immune cells upon conditions of salt excess, including the possible modulation by epigenetic changes [143], may involve mechanisms other than the local environment: these would entail diet-induced modulation of the microbiome, neuro-hormonal axes [38,144] and, we contend, all the long-term compensatory responses to them.

All in all,  $\text{Na}^+$ -induced overactivation of immune cells has the potential to promote an inflammatory status that is known to underlie organ damage in hypertension and many other cardiovascular conditions; in humans, the exact mechanisms of this activation still lack precise characterization.

## Conclusions

In this review, we described some of the 'novel' roles of  $\text{Na}^+$  in cardiovascular health and disease, focussing on

energetics implications, local accumulation, fluid dynamics and the role of the microvasculature with particular focus on the lymphatic system. The suggested changes in metabolism may directly or indirectly impact on body composition, and therefore not only on aspects such as insulin sensitivity but also on systemic IC-to-EC volume ratio, which links to interstitial  $\text{Na}^+$ . The interplay between the latter, the microvasculature and inflammatory responses contributes to systemic, as well as local, inflammatory implications of salt excess in organ damage. This complexity of  $\text{Na}^+$  homeostasis explains why simple cut-offs for or 'safe' ranges of  $\text{Na}^+$  intake cannot be easily applied to different disease scenarios. Accordingly, 'salt sensitivity' appears to be a complex phenotype, regulated by a multitude of factors, and BP response cannot remain the sole clinical readout to assess the susceptibility of an individual to the effects of  $\text{Na}^+$  excess if other factors including microvascular changes and inflammatory response are neglected. Most of these aspects remain incompletely elucidated, particularly in relation to human disease. However, they identify  $\text{Na}^+$  as a *fil rouge* linking derangements in metabolic, renal, vascular and ultimately interstitial function, even independent of BP values, in the frequently comorbid population of patients with CVD. Appreciation of the prevalence and broad biological relevance of isotonic tissue  $\text{Na}^+$  accumulation in tissues should prompt its identification in these patients at earlier stages of disease, long before it results in obvious 'congestion' like in heart failure or end-stage CKD.

To conclude, the play of  $\text{Na}^+$  in CVD is clearly a very old one, but the last years of research, with occasional waves of enthusiasms along with others of neglect, have changed the scripts. They now include the following: new acts, on the energetic implications of  $\text{Na}^+$  handling, in relation to renal function and to energy sources availability/access; *coupes de théâtre*, with the disproof of a long-term even balance in relation to  $\text{Na}^+$  accumulation in tissues, regarded by us as a measure of qualitative and quantitative changes in systemic EC volume; new sceneries, moving the story of  $\text{Na}^+$  control down to the depths of capillary interface and interstitium; and new characters, like the dedicated and duty-driven but only apparently tireless sweepers played by lymphatics, or the two-face immune cells, friends or foes at varying times. *Ça va sans dire*, we are excited to see the drama unfolding.

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## Conflict of interest

The authors declare no conflict of interest.

## Author contributions

CD and GR both substantially contributed to the conception and design of the article and interpreting the relevant literature. GR drafted the article. CD revised it critically for important intellectual content.

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