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Hold the salt: dietary sodium's effect on cardiovascular and kidney diseases

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Hold the salt: dietary sodium's effect on cardiovascular and kidney diseases

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Editorial: Hold the salt: dietary sodium's effect on cardiovascular and kidney diseases

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Editorial on the Research Topic
Hold the salt: dietary sodium's effect on cardiovascular and kidney diseases

Salt, or sodium chloride, has played a pivotal role in human history as a vital nutrient and as a commodity that shaped civilizations. Its importance dates back to prehistoric times, with archaeological evidence suggesting that early humans sought salt-rich environments to support their dietary needs. Salt was not only essential for survival but also became a key factor in the development of trade routes, influencing the rise and fall of empires. Ancient civilizations, such as the Egyptians, used salt for food preservation and mummification, showcasing its wide range of applications. As society evolved, salt became more than a nutritional element—it was central to religious rituals, cultural practices, and even currency systems (1).

The relationship between salt and health, however, has been more complex. Historically, salt was recognized as necessary for maintaining bodily functions, particularly in regulating fluid balance and nerve transmission. However, excessive salt consumption has been linked to adverse health outcomes, particularly cardiovascular and kidney diseases, which remain a global concern today. Numerous studies have established a strong relationship between high sodium intake and adverse cardiovascular outcomes, and consequently, public health guidelines recommend limiting sodium intake to reduce these risks. A meta-analysis demonstrated that excessive sodium intake is associated with elevated blood pressure, a well-known risk factor for cardiovascular diseases (CVD) such as myocardial infarction and stroke (2). The Global Burden of Disease Study found that high sodium consumption was responsible for 3 million deaths and 70 million disability-adjusted life years (DALYs) globally, highlighting its significant contribution to CVD (3). Furthermore, clinical studies such as the Dietary Approach to Stop Hypertension (DASH) study have shown that reducing salt intake can significantly lower blood pressure and decrease the risk of cardiovascular events (4). This body of evidence has led global health organizations, including the World Health Organization (WHO), to recommend limiting sodium intake to <2 g daily to mitigate cardiovascular risk.

In addition to cardiovascular risk, high sodium intake has detrimental effects on kidney function. Chronic high salt consumption is linked to the progression of

chronic kidney disease (CKD) and an increased risk of end-stage renal disease (ESRD) (5). Studies highlighted that high dietary salt exacerbates proteinuria and accelerates the decline in kidney function among patients with hypertension and CKD. Moreover, excessive sodium intake can induce glomerular hyperfiltration and promote kidney damage over time, particularly in individuals with pre-existing renal conditions (6–8). These findings underscore the importance of salt reduction not only for cardiovascular health but also for preventing and managing kidney disease.

Research into the effects of salt on the cardiovascular and kidney systems is essential for addressing rising global health challenges like hypertension, heart diseases, and CKD. Despite widespread awareness, gaps remain in our understanding of the specific mechanisms by which excess salt harms these systems. By offering fresh insights, our Research Topic fills this critical need, presenting new findings that highlight the nuanced impact of salt at clinical, epidemiological, and molecular levels.

A study on the North Indian population (Kaur et al.) found that sodium and salt consumption exceed recommended levels, while potassium intake remains below ideal. This imbalance is particularly concerning among individuals with CKD, suggesting the need for targeted dietary policies to mitigate CKD progression and improve overall public health outcomes. In a related study, high salt intake and over hydration among non-dialysis CKD patients were linked to an increased risk of cardiac structural and functional impairments (Duan et al.). An experimental study on rodents (Siddiqui et al.) revealed that diets high in fructose and salt led to cardiorenal dysfunctions. However, the chronic inhibition of the renin-angiotensin system (RAS) improved both cardiac and renal histopathological outcomes.

In the realm of the heart disease, a study on rheumatic heart disease (RHD; Zhang et al.) showed a decline in RHD-related mortality and DALYs over 30 years, with high systolic blood pressure identified as a key risk factor. This underscores the need for innovative, personalized interventions to reduce sodium intake and improve overall nutrient consumption, especially in light of cardiovascular and renal health goals. Moreover, research on hospitalized patients with dysnatremia (Liang et al.) found that sodium fluctuations are directly linked to increased mortality, emphasizing the importance of strict monitoring of sodium levels in clinical settings.

Investigations into potassium intake (Yuan et al.) demonstrated that higher potassium consumption is associated with a reduced risk of albuminuria, a marker of kidney disease. Another study (Xu et al.) revealed that the urinary sodium/potassium ratio (Na/K) correlates strongly with hypertension and blood pressure indices. This suggests that the Na/K ratio can serve as an effective tool for monitoring dietary sodium reduction and potassium increase. Further research into sodium-induced hypertension in the Black population of Sub-Saharan Africa (Masenga et al.) uncovered significant sex differences in sodium sensitivity. Women, in particular, exhibited a more pronounced disruption of the vascular endothelial glycocalyx following acute salt load, highlighting the importance of sex-specific intervention strategies for cardiovascular disease prevention.

Efforts to reduce sodium in food products are also advancing. For example, high-pressure processing in processed meats was shown to enhance saltiness perception and improve sensory acceptability without compromising taste (Bolumar et al.). Similarly, enzyme treatments in vegetable soups were found to increase saltiness and umami intensity, allowing for a 20% reduction in salt while maintaining clean-label requirements (Sakai et al.). Additionally, research on microencapsulated ingredients from aromatic plants and spices demonstrated their potential to significantly reduce salt content in sauces such as mayonnaise, mustard, and ketchup, permitting a reduction of up to 50%, without sacrificing taste (Serrano et al.). Finally, Kugler et al. study contributes to nutritional epidemiology by providing an extensive analysis of the salt content in bread sold in Slovenia, highlighting significant differences across bread types and retail environments. It underscores the need to include smaller bakeries in salt reduction efforts and reveals that, despite a modest decrease in bread salt content over a decade, WHO targets remain unmet, suggesting further reformulation strategies are necessary.

In light of these findings, we are undoubtedly at a pivotal moment in addressing the global health impacts of excessive sodium intake. The extensively body of evidence, including the studies presented in this Research Topic, leaves no room for hesitation: innovative, science-driven strategies to reduce sodium consumption are not just necessary—they are urgent. From targeted public health policies to advancements in food technologies, the solutions are within our grasp. However, realizing these solutions will require a unified effort from researchers, policymakers, and the food industry. As we move forward, the challenge lies in transforming this knowledge into concrete actions that prioritize both public health and the sustainability of our food systems.

Author contributions

MPB: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. MCS: Conceptualization, Investigation, Writing – review & editing. APM: Conceptualization, Investigation, Writing – review & editing. YL: Conceptualization, Investigation, Writing – review & editing.

Conflict of interest

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References

1. MacGregor GA, Wardener HEd. *Salt, Diet and Health: Neptune's Poisoned Chalice*. Cambridge: Cambridge University Press (1998).

2. Mozaffarian D, Fahimi S, Singh GM, Micha R, Khatibzadeh S, Engell RE, et al. Global sodium consumption and death from cardiovascular causes. *N Engl J Med.* (2014) 371:624–34. doi: 10.1056/NEJMoa1304127

3. Collaborators GBDD. Health effects of dietary risks in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* (2019) 393:1958–72. doi: 10.1016/S0140-6736(19)30041-8

4. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, et al. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *N Engl J Med.* (2001) 344:3–10. doi: 10.1056/NEJM200101043440101

5. Kang M, Kang E, Ryu H, Hong Y, Han SS, Park SK, et al. Measured sodium excretion is associated with CKD progression: results from the KNOW-CKD study. *Nephrol Dial Transplant.* (2021) 36:512–9. doi: 10.1093/ndt/gfaa107

6. Rossitto G, Maiolino G, Lerco S, Ceolotto G, Blackburn G, Mary S, et al. High sodium intake, glomerular hyperfiltration, and protein catabolism in patients with essential hypertension. *Cardiovasc Res.* (2021) 117:1372–81. doi: 10.1093/cvr/cvaa205

7. Mallamaci F, Leonardis D, Bellizzi V, Zoccali C. Does high salt intake cause hyperfiltration in patients with essential hypertension? *J Hum Hypertens.* (1996) 10:157–61.

8. Nomura K, Asayama K, Jacobs L, Thijs L, Staessen JA. Renal function in relation to sodium intake: a quantitative review of the literature. *Kidney Int.* (2017) 92:67–78. doi: 10.1016/j.kint.2016.11.032

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High-pressure processing enhances saltiness perception and sensory acceptability of raw but not of cooked cured pork loins—leveraging salty and umami taste

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The salt (NaCl) content in processed meats must be reduced because of its adverse effects on cardiovascular health. However, reducing salt in meat products typically leads to a lower taste intensity and, thus, consumer acceptability. Industry interventions must reduce salt content while maintaining taste, quality, and consumer acceptability. In this context, high-pressure processing (HPP) has been proposed to enhance saltiness perception, though there are contradictory reports to date. The present work aimed to conduct a targeted experiment to ascertain the influence of HPP (300/600 MPa) and cooking (71°C) on saltiness perception and sensory acceptability of meat products. HPP treatment (300/600 MPa) did enhance those two sensory attributes (approx. +1 on a 9-point hedonic scale) in raw (uncooked) cured pork loins but did not in their cooked counterparts. Further, the partition coefficient of sodium (P_{Na+}), as an estimate of Na^+ binding strength to the meat matrix, and the content of umami-taste nucleotides were investigated as potential causes. No effect of cooking (71°C) and HPP (300/600 MPa) could be observed on the P_{Na+} at equilibrium. However, HPP treatment at 300 MPa increased the inosine-5′-monophosphate (IMP) content in raw cured pork loins. Finally, hypothetical HPP effects on taste-mediating molecular mechanisms are outlined and discussed in light of boosting the sensory perception of raw meat products as a strategy to achieve effective salt reductions while keeping consumer acceptability.

KEYWORDS

high pressure processing, HPP, sensory perception, salt reduction, partition coefficient, umami, meat products

1 Introduction

High sodium (Na) intake is associated with high blood pressure, and reducing salt (NaCl) consumption to 5 g/day could mitigate cardiovascular diseases by 24% (1). Nearly 20% of Na-intake comes from processed meats (2). Hence, reformulating strategies such as the use of salt replacers (e.g., KCl), flavor enhancers and water binders' ingredients, and processing methods have been extensively researched in processed meats (3–7). However, the

multi-functionality of salt in the texture, flavor, and microbial shelf-life presents a challenge. Thus, there are still practical limitations to overcome, and one of the most remarkable is a weakening of the perception of saltiness and overall flavor intensity in salt-reduced products. This usually leads to reduced taste intensity and consumer acceptability, making its industrial implementation difficult (8).

Application of HPP serves as a cold pasteurization in meat products (9, 10) and has been proposed to compensate for the effects of salt reduction in ready-to-eat meat products (11). Moreover, the application of HPP to meat products has also been associated with an increased saltiness perception (12–15). However, contradictory results have been reported in the literature, with HPP treatment not affecting saltiness perception (6, 16–18). The weakening of the interaction between ions and muscle proteins may be a reason for that. HPP treatment will denature proteins, modifying electrostatic interactions between ions and muscle proteins. Consequently, releasing additional ions from the meat matrix (for instance, Na^+) could be favored, increasing this way saltiness perception. A relationship exists between sodium concentration in the saliva and perceived saltiness (19). The sodium concentration in the mouth should depend on sodium's binding strength to the meat matrix and its solubilization into the water phase. Determining the partition coefficient (P) can estimate this physicochemical interaction. The higher the P_{Na+}, the higher the proportion of salt in the water phase (saliva), potentially raising the perceived saltiness. The disassociation of NaCl in meat systems and the equilibrium established with ions bound to proteins and free ions are vital for the structure of the meat product and the perception of saltiness. The denaturation of proteins will change the native tridimensional structure of the proteins, whereby changing the molecular interactions of the polar and non-polar groups at the surface and interphases of the protein/−s with the water and the ions in the surrounding molecular system (20). Due to the thermal treatment applied to cooked meat products, muscle proteins are largely in a denatured state, and consequently, modification of protein structure in that case, for instance, using HPP, to affect the content of ions bound to proteins must be already minimal. In contrast, we hypothesized that meat products not undergoing a thermal treatment must be more susceptible to modification of their ionic equilibrium with the muscle proteins. Therefore, both raw and cooked cured pork loins were included in this study.

Besides, umami substances could act as flavor enhancers, especially relevant in salt-reduced foods. Nucleotides present in meat such as adenosine-5′-monophosphate (AMP), inosine-5′-monophosphate (IMP), and guanosine-5′-monophosphate (GMP) are umami-taste compounds (21), which also function as flavor enhancers (22, 23). They work synergistically with amino acids and intensify the taste sensation by binding to the same taste receptors (24, 25). HPP has recently been proven to modify the concentration of different nucleotides in meat by affecting endogenous enzyme activities under pressure conditions, potentially resulting in the accumulation of umami-taste compounds (26, 27).

The aim of this study was: firstly, to assess the effect of an HPP treatment at 300 and 600 MPa on the saltiness perception and overall acceptability of raw and cooked brine-immersed cured pork loins, and secondly, to investigate the molecular basis affecting the release of sodium and the presence of umami substances derived from the nucleotide breakdown in cured pork loins as affected by processing conditions such as cooking (71°C) and HPP treatment (300/600 MPa).

2 Materials and methods

2.1 Sensory study

2.1.1 Curing, cooking, and HPP treatment of pork loins

Pork loins, *m. longissimus* ($n = 6$) were collected from a local processing plant (Kulmbach slaughterhouse) and processed within 24–48 h post-mortem. Selected loins were within a pH range of 5.40–5.55. Loins were salted by immersion into an appropriate brine prepared using curing salt [99.5 (g/100 g) NaCl and 0.5 (g/100 g) $NaNO_2$] to reach an approximate concentration of 2 (g/100 g) NaCl in the final product. Similar meat products can be found commercially available, and microbiological safety is guaranteed and validated when working with good manufacturing practices. Loins were immersed in the brine for three weeks at 2°C. After salting, loins were split longitudinally into halves. Three left half-sides and three right half-sides were allocated to each raw or cooked group. The cooking was done by submersing the vacuum-packaged halves (PA/PE 90 bags) in a water bath at 74°C to an internal temperature of 71°C. The day after, all the loin halves were cut into slices of 2 mm, giving approximately 200 slices. Then, individual successive slices along the longitudinal direction of the loin half were distributed into three subgroups (i.e., each loin around 65 slices per treatment) for further processing, namely: no application of HPP treatment (untreated control at atmospheric pressure = 0.1 MPa), and application of HPP treatment at 300 MPa or 600 MPa for 5 min at room temperature (EPSI high-pressure system, Belgium). Packages with slices of cured pork loins allocated to the same sensory session were HPP-treated together in order to minimize the potential effect of the particular HPP conditions on product quality.

2.1.2 Sensory analysis

Six sensory sessions were conducted with a product of 3–5 storage days, along 3 consecutive days. Each sensory session included six different product samples, corresponding to two different loins (either raw or cooked), with each loin treated at three different pressure levels (0.1, 300, and 600 MPa). Three slices per loin and treatment were given to each panelist for sensory evaluation. Sensory evaluations of raw and cooked cured pork loins were done separately. Panelists wore red UV glasses (Solarium safety red UV glasses with elastic, 600,015-red, Wellnessprofi) in order to fade color differences between the various cured loins treated at different high-pressure levels, avoiding this way misleading sensory scores stemming from color differences of the product. A reference product, a counterpart with the same characteristics for both raw and cooked meat products, previously purchased in a local supermarket, was presented to the panelists before starting the sensory test, and a score for saltiness perception and overall acceptability for the reference sample was consensually agreed-upon testing of the reference sample among the participant panelists. Sensory evaluation was done using a scale ranging from 1 (no salty) to 9 (very salty) for saltiness perception and from 1 (extremely dislike) to 9 (extremely like) for overall acceptability. The overall acceptability was the pondering of the impressions of the product as a whole during its consumption (summing up the perceived sensations, mainly regarding taste (correct flavor and absence of off flavors), texture (bite and disruption of product in mouth), and juiciness of the product). Panelists were a group of people

($n = 10$) trained to assess meat products usually (10–20 times per year). The same group of panelists was used throughout the entire sensory evaluation.

2.2 Physicochemical molecular mechanisms study

In order to investigate potential physicochemical molecular mechanisms related to the increased saltiness perception and overall acceptability observed in raw cured pork loins after HPP, the partition coefficient of sodium (P_{Na+}) and the content of nucleotide compounds were studied along with a basic characterization of end-product quality parameters such as water binding and color.

2.2.1 Curing, cooking, and HPP treatment of pork loins

Pork loins ($n = 6$) were processed in the same way as described in the sensory study. Loins were collected from the same processing plant, and pH values were within a range of 5.40–5.55. After salting for three weeks at 2°C, loins were cut into six equivalent blocks, vacuum packaged, and randomly assigned to each treatment (six samples per treatment coming from six different loins of six independent animals). For each loin, three blocks were kept raw (R), and the other three were cooked (C) in a water bath at 74°C to an internal temperature of 71°C. After resting overnight, HPP treatment at 300 or 600 MPa for 5 min was applied to R and C loin blocks.

2.2.2 Process yield

Process yields were quantified throughout the process, from raw materials to salting, cooking, and HPP treatment, by weighting loins before and after each processing step.

2.2.3 Water content

Thirty grams of sea sand was weighed in evaporating glass dishes using an analytical scale (Sartorius CPA3202S, Göttingen; Germany) and pre-dried at 103°C overnight in an oven (WTC Binder ED240, Tuttlingen, Germany). Five grams of sample were weighed at room temperature (RT) in the evaporating dishes and mixed thoroughly with the sea sand. The dishes were dried for 10 min at 600 Watts in a microwave (Siemens HF23051, Germany) till no further weight loss could be observed. Before weighing the dried sample, the dishes were left to equilibrate in a desiccator at RT. The determination was carried out in duplicate. Water content was calculated by weight difference.

2.2.4 Water activity (a_w)

Samples were crushed for 5 s at 5000 rpm using a mixer GrindoMix (Retsch Gmbh, Haan, Germany). Subsequently, the minced sample was placed into a cylindrical plastic capsule (4 cm diameter × 1 cm height), avoiding air bubbles. The sample-filled capsule was inserted into an a_w-meter (SE Aw Lab, SE Schulz Electronic, Hoehenkirchen, Germany). The sample was let equilibrate inside the a_w-meter for 30 min at 25°C before reading the a_w-value.

2.2.5 Instrumental color

CIE L*-, a*- and b*-values and reflectance spectra were collected on day ten after HPP for objective measurement of color by using a portable spectrophotometer (CM-600d, Konica Minolta Sensing Europe, Munich, Germany) with a D65 illuminant and a 10° standard observer angle. After opening the vacuum packaging, samples were scanned at different locations in a freshly cut central area. Measurements were repeated ten times per sample, resulting in sixty scans per treatment. Calibration was carried out using a white standard plate ($L^* = 100$) and a light trap ($L^* = 0$). Hue angle and chroma (C^*) were calculated using the following Equations 1, 2. Reflectance was recorded simultaneously from 400 nm to 700 nm at 10 nm increments.

$$hue = \tan^{-1}\left(b^* / a^*\right) \tag{1}$$

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2} \tag{2}$$

2.2.6 Determination of partition coefficient (P_{Na+})

Methodologies were adapted from elsewhere (28–30). Cylindrical blocks (2.0 cm length × 1.6 cm of diameter, ~ 3.5 g) were cut from slices of 2 cm thickness of the different pork loins using a metallic punch. Individual cylinder samples were then placed in 50 mL plastic tubes and immersed in variable volumes of bi-distilled water. Firstly, a Na^+ selective electrode (Metrohm, Fildestadt, Germany) was used to estimate the equilibrium time (signal leveled off). Equilibrium was reached after approx. 60 h of incubation at 2°C (data not shown). Secondly, a test was carried out, including an incubation lasting for 12 days at 2°C. Equivalent meat cylinder blocks were submersed in 10 and 20 mL bi-distilled water, and individual tubes were incubated for 0, 1, 2, 5, 6, 7, and 12 days. Sodium content was determined in the product and the water phase at the specified time points using inductively coupled plasma – mass spectrometry (ICP-MS) (2.2.7). Incubation for 7 days at 2°C ensured the equilibrium was fully reached (data not shown). Thirdly, P_{Na+} was calculated by determining the concentration of sodium in the 3-day product at time zero (start of incubation) and in the water phase at equilibrium at four mass ratios (r) of the weight of the water phase (W_{Wa}) and weight of the product (W_P) ($r = W_{Wa}/W_P$) after incubation at 2°C for 7 days. The weights of cylindrical block samples were precisely weighed before and after incubation. The weight of water varied at 7, 10, 14, and 18 mL, corresponding to an r-value of 2.0, 2.9, 4.0, and 5.1, respectively. The weight of water at equilibrium was calculated according to a mass balance (Eq. 3).

$$\left(W_0^{\mathrm{P}} + \mathrm{W}_0^{\mathrm{Wa}}\right) = \left(\mathrm{W}_{\mathrm{eq}}^{\mathrm{P}} + W_{\mathrm{eq}}^{\mathrm{Wa}}\right) \tag{3}$$

The following mass balance (Eq. 4) was resolved for calculation:

$$\left(\left[\mathrm{Na}^+\right]_0^{\mathrm{P}} \mathrm{x}\, W_0^{\mathrm{P}}\right) + \left(\left[\mathrm{Na}^+\right]_0^{\mathrm{Wa}} \mathrm{x}\, \mathrm{W}_0^{\mathrm{Wa}}\right) = \left(\left[\mathrm{Na}^+\right]_{\mathrm{eq}}^{\mathrm{P}} \mathrm{x}\, \mathrm{W}_{\mathrm{eq}}^{\mathrm{P}}\right) + \left(\left[\mathrm{Na}^+\right]_{\mathrm{eq}}^{\mathrm{Wa}} \mathrm{x}\, W_{\mathrm{eq}}^{\mathrm{Wa}}\right) \tag{4}$$

where; W = weight, P = product, Wa = water phase, 0 = time zero, eq = time at equilibrium.

Assumptions:

$\left[Na^+\right]_0^{Wa} = 0$ the ultrapure water used in the experiment was analyzed by ICP-MS, and the sodium content was below the detection limit of 3.84 mg/L. For calculation purposes, it was assumed to be zero.

$\left[Na^+\right]_{eq}^{P}$ was calculated by resolving the Eq. 4.

P_{Na+} is defined as Eq. 5:

$$P = \frac{\left[Na^+\right]_{eq}^{Wa}}{\left[Na^+\right]_{eq}^{P}} \quad (5)$$

2.2.7 Sodium quantification

Sodium content was determined using ICP-MS. For analysis, 0.5 g of chopped meat sample was digested in duplicate after the addition of 4 mL ultrapure water, 6 mL 65% nitric acid (14.3 mol/L), and 1 mL 30% hydrochloric acid (9.4 mol/L) in a microwave (Start 1,500, MLS GmbH, Leutkirch, Germany) at 200°C for 30 min. Digests were made up to a final volume of 25 mL with ultrapure water and further diluted 1:10 with ultrapure water prior to analysis. For analysis of the water phase, liquid samples were filtered (0.45 µm, cellulose acetate, Sartorius Stedim Biotech GmbH, Göttingen, Germany) and diluted in duplicate 1:200 with 2% nitric acid solution (0.3 mol/L). Analysis of sodium was done using ICP-MS equipment (Agilent 7,800, Agilent Technologies, Waldbronn, Germany). Argon was used as a sample injection and plasma and aerosol dilution gas. Helium was used as collision gas. The acid matrix of the calibration standards was matched to the matrix of the digested meat samples and the diluted liquid samples, respectively. Internal standards were added online to the sample flow.

2.2.8 Nucleotide quantification

Umami-stimulating nucleotides IMP and GMP and the IMP precursor, AMP, were determined by liquid chromatography coupled to a mass spectrometer (LC–MS). For sample preparation, one g of frozen meat sample was extracted with 10 mL ice-cold 80:20 methanol:water (v/v) using a bead mill homogenizer (Bead Ruptor Elite, Omnilab, Germany). After homogenization, samples were incubated at −20°C for 30 min. The raw extract was centrifuged, and the pellet was re-extracted with 10 mL ice-cold 80:20 methanol:water (v/v) by repeating the first extraction step. Both supernatants were combined and mixed. One ml of the combined extract was again centrifuged (15,000 × g for 20 min, 4°C), and the supernatant was stored at −80°C until analysis. A DionexUltiMate 3,000 RS HPLC from Thermo Scientific (Waltham, USA) coupled to a maXis UHR-QToF system (Bruker Daltonik, Bremen, Germany) was used for the analysis. The column was a HILIC TSKgel Amide-80, 5 µm, 4.6 mm I.D. × 10 cm (Tosoh Bioscience GmbH, Griesheim, Germany). The column temperature was set at 40°C, and the injection volume was 15 µL. The mobile phase consisted of eluent A (95:5 water: acetonitrile with 20 mM ammonium acetate) and eluent B (90:10 acetonitrile:water with 20 mM ammonium acetate). The flow rate was 300 µL/min, and the step gradient was as follows: an initial 2 min at 10% A; 2–3 min to 60% A; elution of compounds during a 3–9 min isocratic step at 60% A; 9–10 min to 30% A for flushing and hold at 30% A from 10–13 min. After flushing, the gradient returned to starting conditions (10% A) from 13–16 min, and the column was re-equilibrated at 10% A for 5 min. Instrument settings for MS analysis were: positive ion mode $[M–H]^+$, capillary voltage 4.5 kV, nebulizer gas 5 bar, desolvation gas flow at 10 L/min, and a desolvation gas temperature of 250°C. MS data were acquired using Compass software 1.7 (Bruker Daltonik, Bremen, Germany). For data analysis, ion chromatograms using the exact compound masses were created, and peak areas were integrated for AMP (348.071), IMP (349.056), and GMP (364.065). Calibration was performed using triplicate runs of AMP, IMP, and GMP in the range [0.2–50 µmol/L].

2.3 Statistical analysis

ANOVA of three factors was conducted to assess the effect of panelist ($n = 10$), loin ($n = 6$), and pressure level ($n = 3$; 0.1, 300, and 600 MPa). Raw and cooked cured pork loins were analyzed separately for the variables, saltiness perception, and overall sensory acceptability. The statistical software package, JMP Software, from SAS was used. A one-way analysis of variance (ANOVA) with Tukey's pairwise test was used to analyze the statistical differences between processing treatments and storage times using the software Past version 3.2.

3 Results and discussion

3.1 Sensory study

Earlier sensory tests with cooked ham treated by HPP at 600 MPa showed no differences in the saltiness perception compared to a control (untreated) (data not shown). In the present study, both raw and cooked brine-immersed cured pork loins were included to investigate different extents of protein denaturation, which, in turn, likely have different electrostatic interactions. This electrostatic-interaction differential could result in different perceived saltiness. The test was conducted with loins cured by immersion (to minimize mechanical muscle disruption) and with no addition of phosphates (to avoid any other chemical interactions).

The salting process resulted in a product weight gain of 10.2 ± 0.6 (g/100 g). The storage of the raw cured pork loins for two days before slicing resulted in a weight loss of 2.8 ± 0.2 (g/100 g) due to water stabilization inside the loins. The cooking process resulted in a cooking loss of 27.5 ± 0.6 (g/100 g). Noteworthy, raw cured pork loins had a higher content of salt (NaCl) (~2.1 (g/100 g), namely 2.16, 2.10 and 2.08 for 0.1, 300 and 600 MPa, respectively) than cooked cured pork loins [~1.9 (g/100 g), namely 1.85, 1.88 and 1.90 for 0.1, 300 and 600 MPa, respectively]. The cooking process expelled water but also salts, so the resulting cooked products had a lower concentration of salt than the raw products ($p < 0.05$). No differences in the salt content were observed with pressure treatment and pressure level.

Figure 1 summarizes the sensory results regarding saltiness perception and overall acceptability of raw and cooked brine-immersed cured pork loins, and subsequently, subjected to HPP at 300 and 600 MPa. HPP treatment at 300 and 600 MPa increased the saltiness perception and overall acceptability of raw cured pork loins

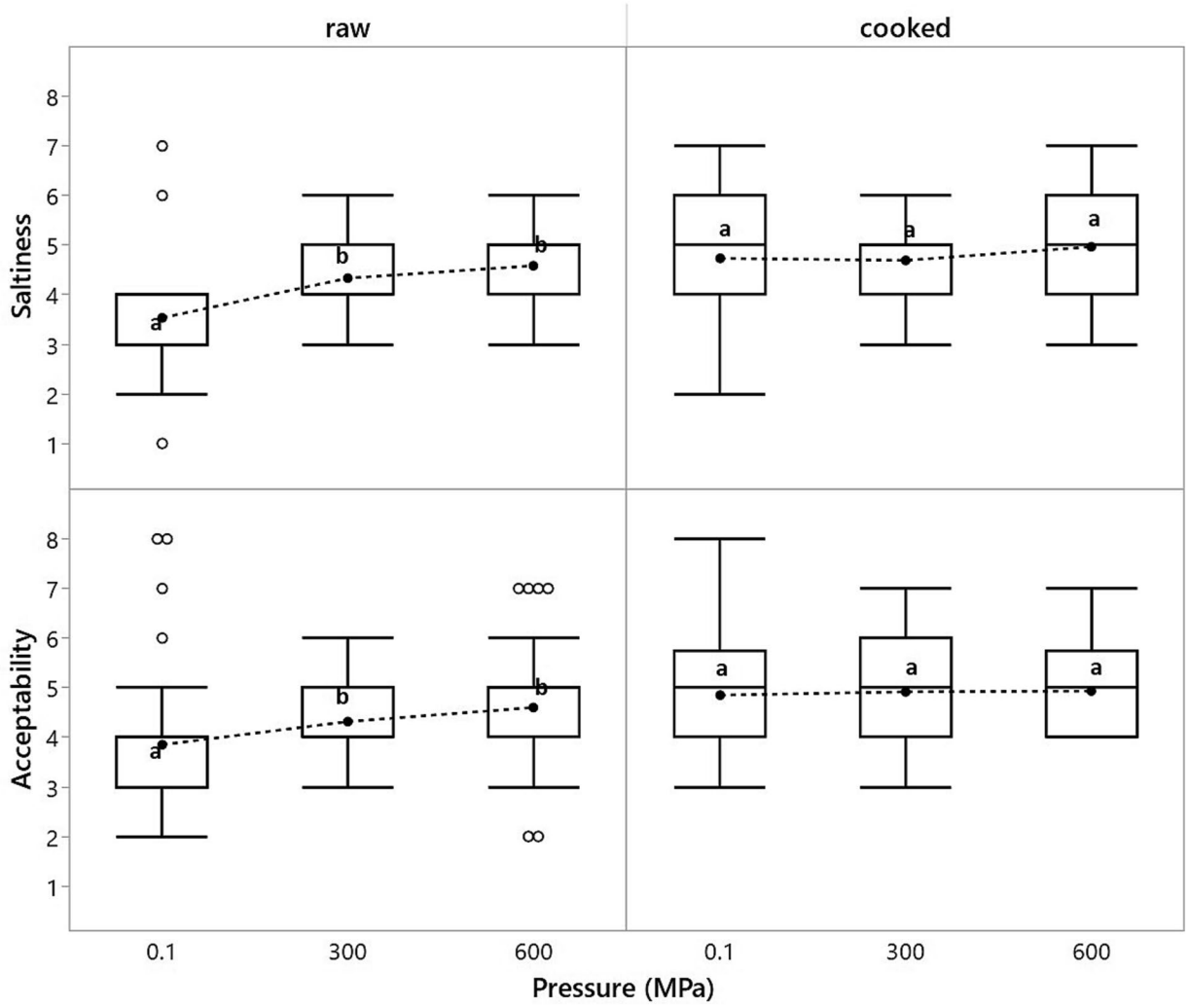

FIGURE 1
Effect of high-pressure treatment at 300 and 600 MPa for 5 min on saltiness perception and overall acceptability of raw (uncooked) and cooked brine-immersed cured pork loins ($n = 6$). Different superscripts within the same sensory trait and subjected to thermal processing or not (either raw or cooked) mean a significant difference at $p < 0.05$.

($p < 0.001$). Control raw pork loins that were not treated by HPP scored 3.53, whereas HPP treatment at 300 and 600 MP raised this score to 4.33 and 4.58, respectively (Figure 1). This fact was translated into higher overall acceptability of the HPP-treated raw cured pork loins, with a score of 4.31 and 4.60, for 300 and 600 MPa, respectively, compared to the control (not HPP-treated) that scored 3.85 (Figure 1). Despite the higher absolute value scored in saltiness perception and overall acceptability of the raw cured pork loins treated at the highest pressure (600 MPa), the differences between the two pressure levels (300/600 MPa) were statistically not significant. In contrast, no differences in saltiness perception and overall acceptability were observed in the cooked cured pork loins treated by HPP at 300 or 600 MPa compared to the control (not HP-treated) (Figure 1), as we had already observed in earlier tests.

It is noteworthy that cooked loins ranked a slightly higher score for both saltiness perception and overall acceptability than their raw counterparts (Figure 1). This fact would support the theory that cooked meat products are generally perceived as saltier than their raw counterparts even though cooked had indeed a significantly lower salt content [1.87 (g/100 g)] than raw cured pork loins [2.11 (g/100 g)], which agrees with the theory that cooked meat products do taste saltier than their raw counterparts (31). One of the reasons for this preference for cooked meat products over raw may stem from the fact that meat's taste develops with cooking (22, 32, 33).

The molecular mechanisms explaining these differences in saltiness perception are not well-established. However, the most probable cause may be the extent of muscle protein denaturation, which surely must be higher in cooked than in raw meat products. It is well established that heat and high pressures lead to the denaturation of proteins, and using differential scanning calorimetry (DSC), the T/P denaturation domains and level of affection have been quantified in meat products (34, 35). The application of HPP to raw meats would induce a level of protein denaturation, in effect changing the electrostatic interactions. In contrast, the HPP treatment would not result in further significant protein denaturation in a cooked product, as the muscle proteins would already be in a denatured state. Previous studies that showed increased saltiness perception after HPP treatment were primarily conducted with dry-cured ham (12–14), indeed, a raw

cured meat product. Whereas in studies with cooked meat products, no saltiness enhancement with HPP was observed (6, 16–18). That strongly agrees with our hypothesis that manipulating saltiness perception through HPP is more plausible in raw (uncooked) meat products than in cooked ones. However, to the best of our knowledge, no study has included cooked products and their raw counterparts in the same study and compared their perceived saltiness in a sensory analysis, as it does the present. Enhancing saltiness perception in raw, further-processed meat products through processing interventions such as an HPP treatment represents a novel, feasible approach that could allow lower salt content.

The following section describes an investigation regarding the potential molecular physicochemical mechanisms involved in the enhanced saltiness perception observed in raw cured pork loins after HPP, as opposed to cooked cured pork loins. Classical quality properties, such as water retention and color parameters, and specifically, additional tests looking into properties such as the P_{Na+} and the content of umami-taste nucleotides, were carried out.

3.2 Physicochemical molecular mechanisms study

3.2.1 Process yield, sodium content, and water binding

The cooking process resulted in a cooking loss of approx. 20 (g/100 g) (Table 1). This cooking loss also resulted in a loss of salt (NaCl) (Table 1). Again, cooked pork loins had a lower salt (NaCl) content [an average of 1.71 (g/100 g)] than their raw counterparts [an average of 1.85 (g/100 g)]. A similar pattern of water and salt losses was observed in the sensory study. HPP treatment of raw and cooked cured pork loins resulted in the expelling of exudates in the range of 1–2 (g/100 g). This value is in the same order of magnitude as the weight losses that occurred during storage of the control product (0.1 MPa) (Table 1). All cooked products had approximately 4–5 (g/100 g) less water content than their raw counterparts ($p < 0.05$) (Table 1). The a_w-values were within a tight range of 0.974–0.980 (Table 1).

3.2.2 Instrumental color

HPP impacted the color of meat products (Figure 2). In general, changes in L* (lightness) are observed already at pressures of 200 MPa, whereas a* (redness) decreases with pressures higher than 300–400 MPa (36). Several studies have reported a threshold of around 350–400 MPa for the lightness effect. Accordingly, above 400 MPa, no further increase in lightness can be measured (37). However, when the product is cured, nitrification protects myoglobin from oxidation, and changes of redness (a*) are minimal, with changes in lightness (L*) due to protein denaturation the most notorious effect (36). These theoretical patterns of HPP-induced color changes matched in a satisfactory manner to our results (Table 1), where the most remarkable color change was for the L*-value. Interestingly, the increase in L* followed the same pattern as the perceived saltiness intensity (in an increasing order; Raw-Control < Raw-HPP-treated < Cooked), indirectly pointing out a relationship between protein denaturation and perceived saltiness intensity.

Reflectance is the quantification of the radian energy reflecting on the surface. It relates to the L*-value in meat products and can also be associated with the protein denaturation extent. Figure 2 shows that increased pressure (600 MPa > 300 MPa > 0.1 MP) in raw cured pork loins increased reflectance. This observation is in line with the conclusions from Table 1 on color, particularly with the L*-value and

TABLE 1 Effect of cooking ($T_{internal\ core}$ = 71°C) and high-pressure treatment at 300 and 600 MPa for 5 min on processing yields[#], salt (NaCl) and water contents (g/100 g), water activity (a_w) and color parameters (L*, a*, b*, C*, and hue) of raw and cooked brine-immersed cured pork loins (mean ± standard error) (n = 6).

	Raw			Cooked		
	Control	HPP		Control	HPP	
	0.1 MPa	300 MPa	600 MPa	0.1 MPa	300 MPa	600 MPa
Cooking loss (g/100 g)	--	--	--	$-20.6^{A} \pm 1.2$	$-19.8^{A} \pm 1.7$	$-21.1^{A} \pm 1.0$
HPP loss (g/100 g)	$-1.2^{A} \pm 0.1$	$-1.2^{A} \pm 0.1$	$-1.6^{A} \pm 0.3$	$-2.0^{A} \pm 0.2$	$-1.4^{A} \pm 0.2$	$-1.9^{A} \pm 0.3$
NaCl (g/100 g)	$1.81^{B} \pm 0.14$	$1.92^{BC} \pm 0.05$	$1.83^{B} \pm 0.13$	$1.73^{B} \pm 0.14$	$1.63^{AB} \pm 0.10$	$1.77^{B} \pm 0.04$
Water content (g/100 g)	$73.7^{B} \pm 0.4$	$75.0^{B} \pm 0.3$	$73.3^{B} \pm 0.4$	$69.9^{A} \pm 0.7$	$69.2^{A} \pm 0.3$	$69.3^{A} \pm 0.9$
a_w	$0.978^{BC} \pm 0.001$	$0.978^{BC} \pm 0.001$	$0.980^{C} \pm 0.001$	$0.974^{AB} \pm 0.001$	$0.976^{B} \pm 0.001$	$0.978^{BC} \pm 0.001$
Color parameters						
L*	$56.1^{A} \pm 1.6$	$64.1^{B} \pm 0.9$	$69.5^{C} \pm 0.8$	$74.2^{C} \pm 0.8$	$73.1^{C} \pm 0.6$	$74.1^{CD} \pm 1.0$
a*	$7.1^{A} \pm 0.5$	$7.6^{A} \pm 0.3$	$7.1^{A} \pm 0.2$	$7.0^{A} \pm 0.3$	$7.6^{A} \pm 0.2$	$7.1^{A} \pm 0.5$
b*	$10.1^{A} \pm 0.4$	$9.1^{AB} \pm 0.4$	$8.9^{B} \pm 0.4$	$8.3^{B} \pm 0.1$	$8.7^{B} \pm 0.2$	$8.5^{B} \pm 0.2$
C*	$12.4^{A} \pm 0.5$	$11.9^{AB} \pm 0.5$	$11.4^{AB} \pm 0.3$	$10.9^{B} \pm 0.2$	$11.5^{AB} \pm 0.3$	$11.1^{AB} \pm 0.4$
Hue	$55.2^{A} \pm 2.0$	$50.1^{AB} \pm 1.2$	$51.2^{AB} \pm 1.2$	$50.1^{AB} \pm 0.8$	$48.8^{B} \pm 0.5$	$50.5^{AB} \pm 1.3$

Different superscripts within the same row mean a significant difference at $p < 0.05$. [#]The salting process had a process yield or brine uptake of 7.7 ± 0.39 (g/100 g) (mean ± standard error) (n = 6).

the saltiness perception order of R-600 MPa = R-300 MPa > R-0.1 MPa, where a higher reflectance can be associated with a higher saltiness perception. Furthermore, cooked products had higher reflectance than their raw counterparts (Figure 2). However, all cooked pork loins, non-HPP-treated (0.1 MPa) or HPP-treated at 300 or 600 MPa, had similar reflectance spectra. This fact would also support the absence of a different saltiness perception among the different cooked products, i.e., treated or not with HPP, and would support the hypothesis that HPP treatment of cooked pork loins will not induce a significant further protein denaturation, and consequently, no effect on the perceived saltiness.

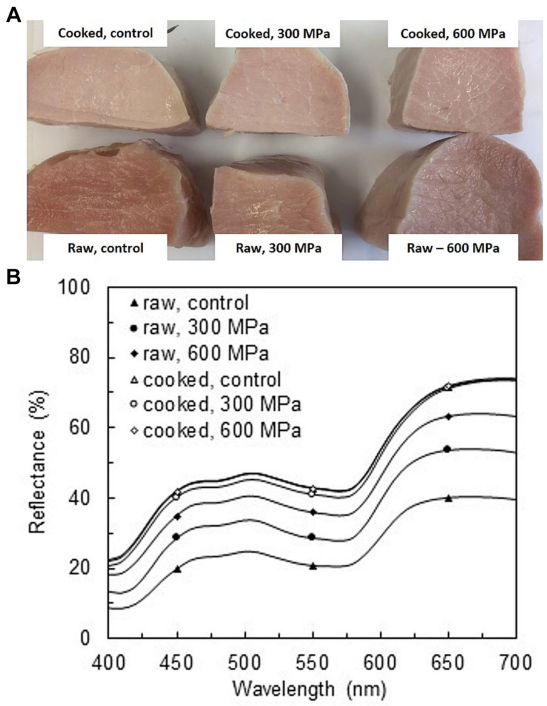

FIGURE 2
Illustrative picture **(A)** and corresponding reflectance spectra **(B)** of the raw (uncooked) and cooked brine-immersed pork cured loins, untreated (control) or subsequently treated by high-pressure processing (HPP) at 300 or 600 MPa ($n = 6$).

3.2.3 Partition coefficient (P_{Na+})

Table 2 shows the effect of HPP at 300 and 600 MPa for 5 min on the P_{Na+} from raw and cooked brine-immersed cured pork loins. The higher the P_{Na+}, the higher the proportion of salt in the water phase (saliva), potentially raising the perceived saltiness. No differences were observed for the value of the P_{Na+} among the different treatments assayed (i.e., raw or cooked, and application of HPP treatment at 300 or 600 MPa). No differences were observed even when the statistical comparison was made, grouping all the values into two main categories: raw ($P_{Na+} = 0.99 \pm 0.18$) and cooked cured pork loins ($P_{Na+} = 1.06 \pm 0.17$). Nonetheless, it can generally be stated that cooked cured pork loins were perceived as saltier than uncooked cured pork loins (Figure 1), despite actually having a lower salt (NaCl) content [2.11 (g/100 g) in raw versus 1.88 (g/100 g) in the cooked product]. A rule of thumb in meat science claims that cooked meat products are generally saltier than their raw counterparts (31). Although, no solid experimental data describe the responsible mechanisms. Ruusunen, Simolin, and Puolanne (38) showed that replacing lean pork meat with pork fat increased the sausage's perceived saltiness. When water was replaced with pork fat on an equal weight basis, the sausage's perceived saltiness did not change. These authors reported a strong negative correlation ($p < 0.01$) between perceived saltiness and protein content, thus suggesting a causative link between these two factors. The same effect was also described in another study where lowering fat and increasing meat proportion resulted in meat products that tasted less salty (39). Protein content and its ability to establish electrostatic interactions could be a major determinant of sodium binding within the meat matrix and the perceived saltiness. Because there was a significantly higher saltiness perception for the HPP-treated raw cured pork loins, a more plausible explanation would come from the kinetics of the release of Na^+ in the short time course of events (i.e., during mastication), which could likely be more determinant for the saltiness perception than the concentration of sodium at the equilibrium (as calculated for the P_{Na+}).

3.2.4 Nucleotide determination

ATP is rapidly metabolized upon slaughter into adenosine diphosphate (ADP) and AMP. Since its regeneration route is broken up, the pathway goes only downstream, forward the degradative direction, forming IMP, inosine, hypoxanthine, xanthine, and ending with uric acid (21, 22, 33).

Nucleotides quantified enhance the umami flavor in this order GMP > IMP > AMP (40). Table 3 shows the concentration of

TABLE 2 Effect of high-pressure treatment at 300 and 600 MPa for 5 min on the partition coefficient of sodium ion (P_{Na+}) of raw (uncooked) and cooked brine-immersed cured pork loins (mean ± standard error) ($n = 6$).

	Raw			Cooked		
	Control	HPP		Control	HPP	
r#	0.1 MPa	300 MPa	600 MPa	0.1 MPa	300 MPa	600 MPa
2.0	$1.00^{A} \pm 0.06$	$0.91^{A} \pm 0.06$	$0.92^{A} \pm 0.10$	$1.04^{A} \pm 0.08$	$1.08^{A} \pm 0.05$	$0.95^{A} \pm 0.07$
2.9	$1.14^{A} \pm 0.17$	$1.01^{A} \pm 0.32$	$0.98^{A} \pm 0.12$	$1.13^{A} \pm 0.12$	$1.31^{A} \pm 0.22$	$0.95^{A} \pm 0.10$
4.0	$0.92^{A} \pm 0.12$	$0.87^{A} \pm 0.19$	$1.15^{A} \pm 0.28$	$1.27^{A} \pm 0.16$	$1.23^{A} \pm 0.34$	$0.85^{A} \pm 0;06$
5.1	$1.11^{A} \pm 0.23$	$0.91^{A} \pm 0.27$	$0.95^{A} \pm 0.21$	$1.26^{A} \pm 0.24$	$0.85^{A} \pm 0.10$	$0.82^{A} \pm 0.15$
average	$1.05^{A} \pm 0.12$	$0.92^{A} \pm 0.19$	$1.00^{A} \pm 0.14$	$1.17^{A} \pm 0.13$	$1.12^{A} \pm 0.16$	$0.89^{A} \pm 0.08$

Different superscripts within the same row mean a significant difference at $p < 0.05$. $^{\#}r = \frac{\text{weight water phase}}{\text{weight product}}$.

TABLE 3 Effect of high-pressure treatment at 300 and 600 MPa for 5 min on the concentration (nmol/g of product) of umami-taste nucleotides (AMP, IMP, and GMP)[#] of raw (uncooked) and cooked brine-immersed cured pork loins (mean ± standard error) ($n = 6$).

	Storage time (days)	Raw			Cooked		
		Control	HPP		Control	HPP	
		0.1 MPa	300 MPa	600 MPa	0.1 MPa	300 MPa	600 MPa
AMP	3	$0.6^{A.a} \pm 0.01$	$0.7^{A.a} \pm 0.06$	$3.8^{B.a} \pm 0.42$	$7.3^{C.a} \pm 0.23$	$6.8^{C.a} \pm 0.56$	$7.1^{C.a} \pm 0.58$
	22	$0.5^{A.b} \pm 0.01$	$0.5^{A.b} \pm 0.01$	$0.5^{A.b} \pm 0.02$	$4.9^{B.b} \pm 0.27$	$5.00^{B.a} \pm 0.87$	$6.3^{B.b} \pm 0.30$
IMP	3	$29.9^{B.a} \pm 3.17$	$40.5^{A.a} \pm 2.24$	$28.6^{B.a} \pm 3.13$	$25.6^{B.a} \pm 1.06$	$25.3^{B.a} \pm 3.03$	$30.4^{B.a} \pm 4.09$
	22	$9.2^{A.b} \pm 0.81$	$11.9^{A.b} \pm 0.82$	$21.5^{B.a} \pm 2.14$	$27.1^{B.a} \pm 1.33$	$22.5^{B.a} \pm 4.41$	$27.1^{B.a} \pm 1.63$
GMP	3	$1.4^{A.a} \pm 0.03$	$1.3^{AB.a} \pm 0.03$	$1.2^{B.a} \pm 0.04$	$1.1^{BC.a} \pm 0.02$	$1.0^{BC.a} \pm 0.06$	$1.1^{B.a} \pm 0.04$
	22	$0.8^{A.b} \pm 0.04$	$0.8^{AB.b} \pm 0.02$	$0.9^{AB.b} \pm 0.03$	$1.1^{B.a} \pm 0.02$	$0.9^{AB.a} \pm 0.14$	$1.1^{B.a} \pm 0.03$

Significance of the comparisons between treatments (i.e., raw or cooked product with or without application of high-pressure treatment) is indicated by the first superscript (in capital letters), different superscripts within the same row, meaning a significant difference at $p < 0.05$. Significance of comparisons for the same treatment between the two storage times (3 and 22 days) is indicated by the second superscript after the comma (lowercase letters), different superscripts meaning a significant difference at $p < 0.05$. [#]AMP, adenosine-5′-monophosphate; IMP, inosine-5′-monophosphate; and GMP, guanosine-5′-monophosphate.

umami-taste nucleotides of raw and cooked brine-immersed cured pork loins. Cooking and HPP treatment at 600 MPa increased the content of AMP (Table 3) ($p < 0.05$). That could be due to thermal activation of the conversion from ADP to AMP, which would agree with the higher amount of ADP found in the raw cured pork loins that were not HPP-treated. Furthermore, AMP was degraded over storage time (day 3 to 22) (Table 3), suggesting that AMP-degrading enzymes are still active, at least partially, after cooking and HPP treatment.

Noteworthy, the highest IMP concentration was found in raw cured pork loins treated by HPP at 300 MPa (Table 3), which pinpoints a formation of IMP under these HP conditions. Huijuan et al. (26) found that the activity of adenosine monophosphate deaminase (AMPD), which is responsible for catalyzing the reaction from AMP to IMP, was maximized precisely under 300 MPa, promoting the formation of IMP to 1,250%. Furthermore, HPP at 150 and 300 MPa also improved the sensory quality of meat marinated in soy sauce by promoting the formation of different metabolites, including nucleotides (41). In contrast, treating raw pork loins at a higher pressure, 600 MPa, did not increase IMP compared to the control. Cooked samples did not show any increase in IMP, supposedly, as muscle enzymes responsible for the formation of IMP, namely the enzyme AMPD, must already have been inactive in cooked meat. Moreover, IMP was degraded over storage time in raw pork loins. However, it was not degraded when the raw cured pork loins were treated by HPP at 600 MPa or cooked, likely because these processing conditions resulted in enzyme inactivation of IMP degrading enzymes.

GMP was higher in control raw than in cooked cured pork loins, though the differences were relatively minor. The same explanation for the AMP could be valid here, as GMP was higher in the less processed samples (i.e., no HPP and/or cooking). In addition, GMP was reduced with storage time in raw cured pork loins, whereas it was not degraded in cooked cured pork loins.

Overall, cooking seemed to stop most of the nucleotide breakdown pathway activity, likely via the thermal inactivation of endogenous enzymes. The treatment of meat products by HPP, either raw or cooked, resulted in differences in the nucleotide profiles, and thus, this can affect flavor perception. Based on our results, the umami-taste substance, IMP, might be increased by applying HPP treatments at 300 MPa in raw meat products. The enzyme responsible for catalyzing this reaction is the AMPD, whose activity has been proven to be thermodynamically favored under pressure (26).

3.3 Overall discussion

Salt reduction comes with a reduction of flavor intensity that usually leads to reduced consumer acceptability (8). Therefore, the food industry should develop salt reduction strategies that guarantee adequate sensory eating quality. Taste perception is fundamentally formed at the interaction between food components and their docking with taste receptors on the tongue. A proper combination of salty and umami perception in savory products is paramount to defining overall acceptability. Salty taste is mediated via the epithelial sodium channel (ENaC), playing a major role in the perception of salts in saliva (42) (Figure 3). Umami, described as savory, meaty, or brothy, was initially identified by Kikunae Ikeda as the salt of L-glutamic acid in 1908 (44). The umami taste is elicited by the salts of two amino acids, L-glutamic acid (L-Glu) and L-aspartic acid (L-Asp), through binding to umami taste receptors T1R1 + T1R3 (45) and GluR4 (46). Monosodium glutamate (MSG) is widely used to impart umami flavor and enable sodium reductions (47). In addition, AMP, IMP, and GMP are meaty compounds that enhance umami taste by working together with amino acids by intensifying the taste sensation via binding to the same T1R1 + T1R3 receptors (24, 25, 42) (Figure 3).

The interaction of umami and salty perception affects the overall sensation of saltiness. Umami carriers, among others MSG and IMP, effectively enhance saltiness (48). The perception of odor and taste combined results in the overall flavor. Thus, an additional factor in saltiness perception, apart from the presence of specific ions and umami substances and their active concentration, is the interaction between aroma perception and taste sensation in the postnasal cavity. Substances with salty characteristics can significantly enhance sodium chloride perceived intensity and salty taste sensation (49). Therefore, another effective salt reduction strategy is using compounds with salty odor characteristics to enhance salty taste perception. Odor substances imparting salty characteristics enhance the perception of salty flavors. 3-(methylthio)propanal, 1-octen-3-ol, 2,5dimethylpyrazine, and 3-(methylthio) propanol isolated from soy sauce could enhance the salty taste perception (50). An integrative multifactorial

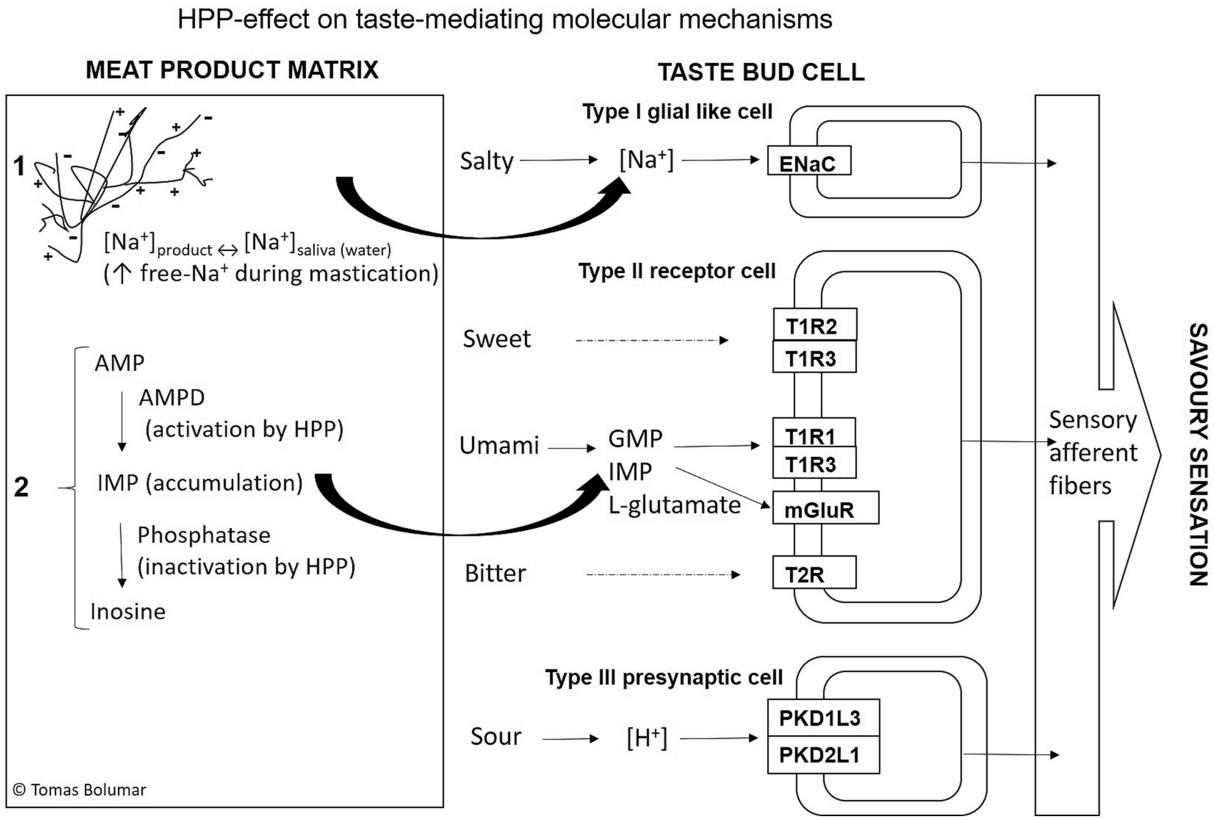

FIGURE 3
Schematic pathway of the five primary taste sensing transductions in humans [adapted from Kyoto Encyclopedia of Genes and Genomes (KEGG) (43)] and the two hypothetical molecular mechanisms suggested for manipulation of taste intensity through high-pressure processing (HPP) induced changes. Mechanism (1). Modifying electrostatics interactions between meat matrix, primarily proteins, and sodium ions (Na^+) leads to more free-Na^+ available to interact with salty-taste receptors. Mechanism (2). Nucleotide breakdown reactions in muscle result in the accumulation of IMP upon pressure treatment [adapted from Huijuan et al. (26)], with subsequent IMP docking on T1R1 and T1R3 umami-taste receptors. HPP, high-pressure processing; AMPD, adenosine monophosphate deaminase; AMP, adenosine-5′-monophosphate (AMP); IMP, inosine-5′-monophosphate; ENaC, epithelial sodium channel; Protein selected ion channels: PKD1L3 and PKDL.

saltiness-perception modulation considering (1) ions (salty), (2) umami substances, and (3) volatiles with salty enhancement ability could bring benefits when developing low-sodium foods.

Figure 3 depicts a schematic representation of the suggested hypothetical mechanisms behind the HPP-induced effect on saltiness perception and overall acceptability. First, HPP could induce enough protein denaturation in raw meat products to change electrostatics interactions, leading to more free-Na^+ for active interaction with the ENaC channel, particularly during mastication. That will intensify the saltiness perception. Second, umami-taste nucleotides like IMP, which can accumulate in HPP-treated products, will interact with T1R1 + T1R3 receptors. In combination with amino acids, this will boost the umami flavor.

The study was a first step to ascertain the HPP effect on the saltiness perception of commercially available meat products, and salt-reduced products beyond the standard levels could be assayed in further trials, where microbiological safety aspects would need to be evaluated. Yet, due to the large variability encountered in the salt content of meat products in food composition tables within a product category, with sausages 1.5–2.7 g/100 g, frankfurter 1.8–2.3 g/100 g and cooked hams 2.3–3.0 g/100 g [USDA Food National Database cited by Inguglia et al. (4)] and own data collected from the German market product monitoring [Max Rubner Institute (51), Table 6 for values of meat products], products with much higher salt content than the employed in this study are currently in the market. Hence, the HPP application already brings a salt reduction potential.

In this sense, however, it is difficult to reasonably predict what will occur under lower/higher salt content. One could forecast that the saltiness perception of products with higher salt content could be less influenced by HPP, as a higher salt concentration could bring about an excess of salt in saliva, giving rise to more salt interacting with taste receptors in the mouth and a possible saturated condition. Thus, the effect on the perception of saltiness could likely be more pronounced and relevant in lower salt-content meat products. In this sense, the salty taste elicited with table salt (NaCl) has a human detection threshold of about $1 \sim 30\,mmol^{-1}$ (i.e., 0.18 g NaCl/100 g). For reference, many commercially available soups contain NaCl at $100 \sim 200\,mmol^{-1}$ (i.e., 0.58–1.17 g NaCl/100 g) (52). Of course, the product's chemical and structural conditions at a micro- and macro-molecular level could influence these generic thresholds and would have to be investigated experimentally on a product-case basis.

The flavor industry utilizes extracts that simultaneously affect saltiness and umami perception, providing solutions tailored to salt-reduced meat products (53). Overall, the content of free-Na^+ and

umami-taste nucleotides in meat products target two different taste receptors, which are fundamental. A better molecular understanding of the thermodynamics and biochemical routes governing the concentration of compounds affecting both receptors can be used to develop meat products with reduced salt content while maintaining similar consumer acceptability and deserve further exploration.

4 Conclusion

HPP treatment at 300 and 600 MPa enhanced the perception of saltiness and overall sensory acceptability of raw (uncooked) cured pork loins but not of their cooked counterparts. This modulation of saltiness perception opens a new avenue to boost the taste perception of raw meat products by post-packaging HPP treatments. The causes behind this increase in saltiness perception are currently not well-established. No differences were detected in the P_{Na+}, which suggests that the kinetics of the release of Na^+ during mastication could be more related than at the equilibrium time point (as for the P_{Na+}). HPP at 300 MPa resulted in an increased content of IMP in raw cured pork loins that could also mediate to boost taste intensity. Overall, when reducing the salt (NaCl) content in meat products, HPP could be a technological tool for the industry to ensure food safety and shelf-life and manipulate and enhance product properties of importance, such as saltiness perception and sensory acceptability.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the studies involving humans because the sensory test was carried out on food products (cured loins) produced, stored and prepared under standard and approved food conditions for human consumption, which comply with current legislation in Germany. The meat to make the food products used in the sensory analysis were sorted and processed in establishments with governmental approval to produce foods for human consumption: the Kulmbach Municipality Slaughterhouse and the Pilot Plant of the Department of Safety and Quality of Meat of the Max Rubner Institute (MRI) in Kulmbach. In Bavaria (Germany), where the MRI's site in Kulmbach is located, sensory testing of meat and meat products approved for human consumption does not require formal advice from an ethics committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

TB: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. RL: Formal analysis, Investigation, Writing – review & editing. MP: Formal analysis, Investigation, Writing – review & editing. KT: Formal analysis, Investigation, Writing – review & editing. SM: Conceptualization, Formal analysis, Writing – review & editing. DB: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. World Health Organisation (WHO). (2013). *Mapping salt reduction initiatives in the WHO European region*. Copenhagen: WHO Regional Office for Europe, 1–51.

2. Ruusunen M, Puolanne E. Reducing sodium intake from meat products. *Meat Sci*. (2005) 70:531–41. doi: 10.1016/j.meatsci.2004.07.016

3. Desmond E. Reducing salt: a challenge for the meat industry. *Meat Sci*. (2006) 74:188–96. doi: 10.1016/j.meatsci.2006.04.014

4. Inguglia ES, Zhang Z, Tiwari BK, Kerry JP, Burgess CM. Salt reduction strategies in processed meat products – a review. *Trends Food Sci Technol*. (2017) 59:70–8. doi: 10.1016/j.tifs.2016.10.016

5. Jaenke R, Barzi F, Mcmahon E, Webster J, Jaenke R, Barzi F, et al. Consumer acceptance of reformulated food products: a systematic review and meta-analysis of salt-reduced foods. *Crit Rev Food Sci Nutr*. (2017) 57:3357–72. doi: 10.1080/10408398.2015.1118009

6. Tamm A, Bolumar T, Bajovic B, Toepfl S, Heinz V. Salt (NaCl) reduction in cooked ham by a combined approach of high pressure treatment and the salt replacer KCl. *Innovative Food Sci Emerg Technol*. (2016) 36:294–302. doi: 10.1016/j.ifset.2016.07.010

7. Wang J, Huang XH, Zhang YY, Li S, Dong X, Qin L. Effect of sodium salt on meat products and reduction sodium strategies – a review. *Meat Sci*. (2023) 205:109296. doi: 10.1016/j.meatsci.2023.109296

8. Hoppu U, Hopia A, Pohjanheimo T, Rotola-Pukkila M, Mäkinen S, Pihlanto A, et al. Effect of salt reduction on consumer acceptance and sensory quality of food. *Food Secur*. (2017) 6:103. doi: 10.3390/foods6120103

9. Bajovic B, Bolumar T, Heinz V. Quality considerations with high pressure processing of fresh and value added meat products. *Meat Sci*. (2012) 92:280–9. doi: 10.1016/j.meatsci.2012.04.024

10. Bolumar T, Orlien V, Sikes A, Aganovic K, Bak KH, Guyon C, et al. High-pressure processing of meat: molecular impacts and industrial applications. *Compr Rev Food Sci Food Saf*. (2021) 20:332–68. doi: 10.1111/1541-4337.12670

11. Nuygen M, Arvaj L, Balamurugan S. The use of high pressure processing to compensate for the effects of salt reduction in ready-to-eat meat products. *Crit Rev Food Sci Nutr*. (2022) 1-15:1–15. doi: 10.1080/10408398.2022.2124398

12. Clariana M, Guerrero L, Sarraga C, Diaz I, Valero A, Garcia-Regueiro JA. Influence of high pressure application on the nutritional, sensory and microbiological characteristics of sliced vacuum packed dry-cured ham. Effects along the storage period. *Innovative Food Sci Emerg Technol*. (2011) 12:456–65. doi: 10.1016/j.ifset.2010.12.008

13. Fuentes V, Estevez M, Grebol N, Ventanas J, Ventanas S. Application of time-intensity method to assess the sensory properties of Iberian dry-cured ham: effect of fat content and high-pressure treatment. *Eur Food Res Technol*. (2014) 238:397–408. doi: 10.1007/s00217-013-2113-8

14. Fulladosa E, Serra X, Gou P, Arnau J. Effects of potassium lactate and high pressure on transglutaminase restructured dry-cured hams with reduced salt content. *Meat Sci*. (2009) 82:213–8. doi: 10.1016/j.meatsci.2009.01.013

15. Saccani G., Parolari G., Tanzi E., Rabbuti S. (2004). Sensory and microbiological properties of dried hams treated with high hydrostatic pressure. Proceedings of the 50th ICoMST, Helsinki, Finland.

16. Pietrasik Z, Gaudette NJ, Johnston SP. The impact of high hydrostatic pressure on the functionality and consumer acceptability of reduced sodium naturally cured wieners. *Meat Sci*. (2017) 129:127–34. doi: 10.1016/j.meatsci.2017.02.020

17. Zhou Y, Watkins P, Oiseth S, Cochet-Broch M, Sikes AL, Chen C, et al. High pressure processing improves the sensory quality of sodium-reduced chicken sausage formulated with three anion types of potassium salt. *Food Control*. (2021) 126:108008. doi: 10.1016/j.foodcont.2021.108008

18. Zhu Y, Yan Y, Yu Z, Wu T, Bennett LE. Effects of high pressure processing on microbial, textural and sensory properties of low-salt emulsified beef sausage. *Food Control*. (2022) 133:108596. doi: 10.1016/j.foodcont.2021.108596

19. Phan VA, Yven C, Lawrence G, Chabanet C, Reparet JM, Salles C. In vivo sodium release related to salty perception during eating model cheeses of different textures. *Int Dairy J*. (2008) 18:956–63. doi: 10.1016/j.idairyj.2008.03.015

20. Puolanne E, Halonen M. Theoretical aspects of water-holding in meat. *Meat Sci*. (2010) 86:151–65. doi: 10.1016/j.meatsci.2010.04.038

21. Aristoy M.C., Toldra F. (2009). Nucleotides and its derived compounds. Leo M. L. Nollet and Fidel Toldra, *Handbook of muscle foods analysis*. 279–298). Boca Raton, FL: CRC Press, Taylor and Francis.

22. Elmore JS, Mottram DS. Flavour development in meat In: JP Kerry and AD Ledward, editors. *Improving the sensory and nutritional quality of fresh meat*. Cambridge, England: Woodhead Publishing Limited (2009). 111–46.

23. Maga JA. Umami flavor of meat In: F Shahidi, editor. *Flavour of meat and meat products*. New York: Chapman and Hall (1994). 98–115.

24. Mouritsen OG, Khandelia H. Molecular mechanism of the allosteric enhancement of the umami taste sensation: dynamics of the umami receptor. *FEBS J*. (2012) 279:3112–20. doi: 10.1111/j.1742-4658.2012.08690.x

25. Zhang Y, Venkitasamy C, Pan Z, Wang W. Recent developments on umami ingredients of edible mushrooms – a review. *Trends Food Sci Technol*. (2013) 33:78–92. doi: 10.1016/j.tifs.2013.08.002

26. Huijuan Z, Jian P, Juan L, Xiaoxiao X. High-pressure effects on the mechanism of accumulated inosine 5′-monophosphate. *Innovative Food Sci Emerg Technol*. (2018) 45:330–4. doi: 10.1016/j.ifset.2017.12.005

27. Utama DT, Lee SG, Baek KH, Jang A, Pak JI, Lee SK. Effects of high-pressure processing on taste-related ATP breakdown compounds and aroma volatiles in grass-fed beef during vacuum aging. *Asian-Australasian J Anim Sci*. (2018) 31:1336–44. doi: 10.5713/ajas.17.0677

28. Boisard L, Andriot I, Martin C, Septier C, Boissard V, Salles C, et al. The salt and lipid composition of model cheeses modifies in-mouth flavour release and perception related to the free sodium ion content. *Food Chem*. (2013) 145:437–44. doi: 10.1016/j.foodchem.2013.08.049

29. Lauverjat C, De Loubens C, Déléris I, Tréléa IC, Souchon I. Rapid determination of partition and diffusion properties for salt and aroma compounds in complex food matrices. *J Food Eng*. (2009) 93:407–15. doi: 10.1016/j.jfoodeng.2009.02.003

30. Saint-eve A, Lauverjat C, Magnan C, Déléris I, Souchon I. Reducing salt and fat content: impact of composition, texture and cognitive interactions on the perception of flavoured model cheeses. *Food Chem*. (2009) 116:167–75. doi: 10.1016/j.foodchem.2009.02.027

31. Wirth F. Reducing the fat and sodium content of meat-products – what possibilities are there. *Fleischwirtschaft*. (1991) 71:294–7.

32. Flores M. The eating quality of meat: flavor In: F Toldra, editor. *Lawrie's meat science*. UK: Woodhead Publishing is an imprint of Elsevier (2017). 383–417.

33. Aaslyng, MD, Meinert, L. Meat flavour in pork and beef–From animal to meal. *Meat science* (2017). 132, 112–117.

34. Vaskoska R, Ha M, Ong L, Chen G, White J, Gras S, et al. Myosin sensitivity to thermal denaturation explains differences in water loss and shrinkage during cooking in muscles of distinct fibre types. *Meat Sci*. (2021) 179:108521. doi: 10.1016/j.meatsci.2021.108521

35. Zheng H-B, Xu B-C, Xu X-L, Li C, Bolumar T, Zhen Z-Y. Gelation of chicken batters during heating under high pressure. *Innov Food Sci Emerg Technol*. (2021) 74:102848. doi: 10.1016/j.ifset.2021.102848

36. Bak KH, Bolumar T, Karlsson AH, Lindahl G, Orlien V. Effect of high pressure treatment on the color of fresh and processed meats: a review. *Crit Rev Food Sci Nutr*. (2019) 59:228–52. doi: 10.1080/10408398.2017.1363712

37. Bak KH, Lindahl G, Karlsson AH, Orlien V. Effect of high pressure, temperature, and storage on the color of porcine *longissimus dorsi*. *Meat Sci*. (2012) 92:374–81. doi: 10.1016/j.meatsci.2012.02.002

38. Ruusunen M, Simolin M, Puolanne E. The effect of fat content and flavor enhancers on the perceived saltiness of cooked 'bologna-type' sausages. *J Muscle Foods*. (2001) 12:107–20. doi: 10.1111/j.1745-4573.2001.tb00303.x

39. Tobin BD, O'Sullivan MG, Hamill RM, Kerry JP. The impact of salt and fat level variation on the physiochemical properties and sensory quality of pork breakfast sausages. *Meat Sci*. (2013) 93:145–52. doi: 10.1016/j.meatsci.2012.08.008

40. Yamaguchi S, Yoshikawa T, Ikeda S, Ninomiya T. Measurement of the relative taste intensity of some L-α-amino acids and 5′-nucleotides. *J Food Sci*. (1971) 36:846–9. doi: 10.1111/j.1365-2621.1971.tb15541.x

41. Yang Y, Ye YF, Wang Y, Sun Y, Pan D, Cao JX. Effect of high pressure treatment on metabolite profile of marinated meat in soy sauce. *Food Chem*. (2018) 240:662–9. doi: 10.1016/j.foodchem.2017.08.006

42. Spaggiari G, Di Pizio A, Cozzini P. Sweet, umami and bitter taste receptors: state of the art of in silico molecular modeling approaches. *Trends Food Sci Technol*. (2020) 96:21–9. doi: 10.1016/j.tifs.2019.12.002

43. Kyoto Encyclopaedia of Genes and Genomes (KEGG). (2019). Taste transduction – *Homo sapiens* (human). Available at: https://www.genome.jp/kegg-bin/show_pathway?map=hsa04742&show_description=show (Accessed April 3, 2020)

44. Ikeda K. New seasonings. *Chem Senses*. (2002) 27:847–9. doi: 10.1093/chemse/27.9.847

45. Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJP, et al. An amino-acid taste receptor. *Nature*. (2002) 416:199–202. doi: 10.1038/nature726

46. Chaudhari N, Landin AM, Roper SD. A metabotropic glutamate receptor variant functions as a taste receptor. *Nat Neurosci*. (2000) 3:113–9. doi: 10.1038/72053

47. Maluly HDB, Arisseto-Bragotto AP, Reyes FGR. Monosodium glutamate as a tool to reduce sodium in foodstuffs: technological and safety aspects. *Food Sci Nutr*. (2017) 5:1039–48. doi: 10.1002/fsn3.499

48. Sun X, Zhong K, Zhang D, Shi B, Wang H, Shi J, et al. The enhancement of the perception of saltiness by umami sensation elicited by flavor enhancers in salt solutions. *Food Res Int*. (2022) 157:111287. doi: 10.1016/j.foodres.2022.111287

49. Vinitha K, Sethupathy P, Moses JA, Anandharamakrishnan C. Conventional and emerging approaches for reducing dietary intake of salt. *Food Res Int*. (2022) 152:110933. doi: 10.1016/j.foodres.2021.110933

50. Zhou T, Feng Y, Thomas D. Enhancement of saltiness perception by odorants selected from Chinese soy sauce: a gas chromatography/olfactometry associated taste study. *Food Chem*. (2020) 335:127664. doi: 10.1016/j.foodchem.2020.127664

51. Max Rubner Institute. (2020). Produktmonitoring 2020. Ergebnisbericht. Available at: https://www.mri.bund.de/fileadmin/MRI/Institute/EV/MRI-Produktmonitoring-2020_Ergebnisbericht-final.pdf (Accessed January 31, 2024)

52. Roper SD. Taste: mammalian taste bud physiology. *Reference Module Neurosci Biobehav Psychol*. (2017):887–93. doi: 10.1016/B978-0-12-809324-5.02908-4

53. Wang S, Tonnis BD, Wang ML, Zhang S, Adhikari K. Investigation of monosodium glutamate alternatives for content of umami substances and their enhancement effects in chicken soup compared to monosodium glutamate. *J Food Sci*. (2019) 84:3275–83. doi: 10.1111/1750-3841.14834

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Estimation of dietary intake of sodium, potassium, phosphorus and protein in healthy Indian population and patients with chronic kidney disease

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Introduction: Poor nutritious diet is a major risk element for non-communicable diseases (NCD), which are of considerable public health concern. Given the diverse dietary patterns in India, precise determination of nutrient consumption is crucial for disease management. The present study assessed the dietary intake of sodium, potassium, protein, and phosphorus among North Indians.

Methods: This cross-sectional study included healthy adults and adults with stage 2 to 4 chronic kidney disease (CKD). We analysed sodium, protein, potassium and phosphorus intakes using one-time 24-h urinary excretion. Dietary intake was also analysed in subgroups based on sex, body mass index, blood pressure and abdominal obesity. We evaluated the performance of various equations available to estimate sodium intake using a spot urine sample with respect to the sodium excretion measured in a 24-h urine sample. Descriptive statistics was used along with *t*-test for statistical significance.

Results: A total of 404 subjects (182 adult healthy subjects and 222 adults with CKD) with a mean age of 47.01 ± 11.46 years were studied. Mean dietary intakes of sodium, salt, potassium, protein and phosphorus were 2.94 ± 1.68 g/day, 7.42 ± 4.24 g/day, 1.43 ± 0.59 g/day, 47.67 ± 14.73 g/day and 0.86 ± 0.39 g/day, respectively. There were no differences in nutrient consumption between adults who were healthy and those with CKD. Consumption of sodium, salt, protein, potassium, and phosphorus among healthy population vs. those with CKD were 2.81 ± 1.60 vs. 3.05 ± 1.73 g/day ($p = 0.152$), 7.08 ± 4.04 vs. 7.70 ± 4.37 g/day ($p = 0.143$), 47.16 ± 14.59 vs. 48.08 ± 14.86 g/day ($p = 0.532$), 1.38 ± 0.59 vs. 1.48 ± 0.58 g/day ($p = 0.087$) and 0.86 ± 0.41 vs. 0.87 ± 0.37 g/day ($p = 0.738$), respectively. Men had higher consumption of these nutrients than women. Compared to non-hypertensives, hypertensive subjects had higher consumption of salt (8.23 ± 4.89 vs. 6.84 ± 3.59 g/day, $p = 0.002$) and potassium (1.51 ± 0.63 vs. 1.38 ± 0.55 g/day, $p = 0.024$), however, no difference were found in protein and phosphorus intakes. In terms of performance of equations used to estimate 24-h sodium intake from spot urinary sodium concentration against

the measured 24-h urinary sodium excretion, INTERSALT 2 equation exhibited the least bias [1.08 (95% CI, −5.50 to 7.66)].

Conclusion: The study shows higher-than-recommended salt and lower-than-recommended potassium intake in the north Indian population compared to those recommended by guidelines. The dietary protein intake is below the recommended dietary allowance. These findings help the development of targeted policies for dietary modification to reduce the risk of the development and progression of CKD.

KEYWORDS

urinary sodium excretion, salt intake, dietary protein, potassium intake, chronic kidney disease

Introduction

The burden of non-communicable diseases (NCDs) such as hypertension, diabetes mellitus, cardiovascular disease (CVD) and chronic kidney disease (CKD) is increasing in India. According to the World Health Organization (WHO), 63% of total deaths reported in 2016 in India were due to NCDs, with 27% attributed to cardiovascular diseases (CVD) (1). Similarly, the number of deaths due to CKD is rising in India. Unhealthy diets, lack of physical activity and use of alcohol and tobacco are major NCD risk factors (2, 3). The WHO guidelines recommend a daily dietary sodium intake of 2 g (corresponding to 5 g salt) and potassium consumption of at least 3.50 g (4). In the presence of chronic conditions such as kidney diseases and hypertension, sodium intake should not be more than 1.50 g per day (5). The National Academies of Sciences, Engineering, and Medicine Dietary Reference Intakes for Sodium and Potassium lists the maximum amount of sodium consumption as 2.30 grams per day for chronic disease risk reduction (6), and the Kidney Disease Global Outcomes (KDIGO) guidelines recommend restricting sodium intake to <2 g/day (7). According to the Institute of Medicine (The National Academy of Medicine), the recommended dietary allowance (RDA) for phosphorus and protein for healthy adults is 700 mg/day and 0.80 g/kg/day (8), respectively. The actual phosphorus and protein intake in the western populations, however, is 1,056–1,617 mg/day (9–11) and 1.30–1.40 g/kg/day (12), respectively. The biological availability of protein depends on the source, with vegetarian sources (biological value 56–74%) being less efficient than animal proteins (biological value 77–104%) (13). Normative data on nutrient intake is limited in Indian populations, and where available, is based on dietary recall, which indicates a gap in knowledge (2, 14–17).

Dietary manipulation is an important strategy in the management of patients with NCDs, including CKD. In addition to salt restriction, protein intake is recommended to not exceed beyond 0.80 g/kg/day (0.55–0.60 g/kg/day for CKD stage 3–5, 0.60–0.80 g/kg/day in case of diabetic patients) to slow the progression of CKD (18). For the Western societies, where the normal dietary protein intake is 1.30 g/kg/day (12), this is commonly expressed as 'limiting' protein intake. The appropriateness of this recommendation in other societies with substantially different food habits has not been examined. For example, the usual protein intake in predominantly vegetarian Indian societies is likely lower (39–57 g/day) (2, 14, 16). A systematic review also highlighted the diverse dietary patterns in India, including large variations across regions and over time. For example, sweets, snacks, meat or fish were the most prevalent diets in the Eastern and Southern parts of India whereas fruits, vegetables, rice and pulses were popular in the North and West (19). Accurate assessment of dietary intake is therefore essential to develop appropriate dietary advice for people with chronic conditions. The 24-h urinary excretion method is better than dietary recall methods as recall is a retrospective diet assessment method, where an individual needs to remember their food consumption during the preceding 24 h (20). Also, the recall method is prone to errors due to literacy demand, burden, and challenges with portion size estimation (21).

The dietary protein intake is best estimated by measuring the urea nitrogen appearance rate, which requires a 24-h urine collection (22). Similarly, the estimation of the 24-h urinary sodium excretion is the gold standard for estimating salt intake (23–26), and dietary potassium intake is correlated with the 24-h urinary excretion of potassium (27, 28). Dietary assessment has also been shown to be prone to errors in estimating dietary phosphorus intake, making 24-h urine collection the optimal method for this purpose as well (29).

Information on recommended dietary allowances and actual dietary intake of nutrients are important for development of personalized dietary plans in specific population with variable health issues. There are limited studies with small sample size on healthy individuals that have examined the dietary intake of nutrients. Most of these have focused on salt intake. In this study, we aimed to determine the daily intake of sodium, potassium, protein and phosphorus using 24-h urinary excretion method in a group of healthy subjects and those with mild-to-moderate CKD in north India. Given the importance of ascertaining the sodium intake, we also evaluated the performance of equations designed to estimate dietary sodium intake using sodium concentration in a spot urine sample to help identify the equation best suited for this purpose in Indian subjects. These equations have been derived in Caucasian, Japanese or Chinese populations and use different input parameters, for example, sex and potassium (30–35). None of these were developed for Indians.

Methods

Study design and population

This cross-sectional study was conducted at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, as a part of the study to measure the glomerular filtration rate

in Indian population between 2016 and 2020. We recruited adults over the age of 18 years of either sex with stage 2 to 4 CKD [estimated glomerular filtration rate (eGFR) 15–60 mL/min/1.73m^2 or eGFR >60 mL/min/1.73m^2 and proteinuria: ≥500 mg/day] (36) and healthy volunteers drawn from the families of those with CKD. Chronic Kidney Disease-Epidemiology Collaborative Group (CKD-EPI) creatinine equation 2009 (CKD-EPI GFR 2009) was used for eGFR estimation. All healthy volunteers were confirmed to be normotensive, and had a normal HbA1C, eGFR >60 mL/min/1.73 m2, and were normoalbuminuric. Other health conditions were excluded by self-reporting. Individuals with voiding problems, urinary incontinence, limb amputation, chronic liver disease, or cardiac disease were also excluded. Figure 1 shows the flow diagram for the study subjects. The study was approved by the Institutional Ethics Committee at PGIMER, Chandigarh, and all subjects provided written consent. All the methods used in this study were performed in accordance with relevant guidelines and regulations.

Study conduct

Urine sample collection

Written and verbal instructions for 24-h urine collection were provided to all subjects. Subjects were asked to discard the first void of the day of starting the urine collection and collect all subsequent urine voids over the next 24-h period. The volume of 24-h urine samples was measured at reporting. Subjects were asked to collect a second-morning spot urine sample in a 50 mL container. Both spot and 24-h urine samples were transported to the laboratory and aliquots were drawn. Complete urine collection of 24-h urine volume of ≥500 mL was considered for analysis. Urine collection compliance was confirmed by the following formula: collected urine volume/(body weight × 21) > 0.7 (37). For collections with values lower than 0.7, we checked the 24-h urine creatinine excretion. If it was lower than the reference value (< 670 mg for men or < 450 mg for women), the data were excluded from the analysis (37). Urine samples were centrifuged at 2000 rpm for 15 min, and supernatant was collected in 2 mL vials. A total of 4 aliquots of 2 mL were stored at -80°C, out of which one was given to the biochemistry laboratory for analysis. Estimation of dietary intake using 24-h urinary excretion has been considered a reliable tool provided the compliance of 24-h urine collection has been validated which we have done carefully (22).

Demographic, anthropometric and clinical measurements

Demographic details were recorded. Weight was measured in minimum clothing using calibrated scales. Height was measured as per standard protocols using a wall mount height measurement scale. The Body Mass index (BMI) was calculated as kg/m^2 and categorised as BMI ≥ 25 kg/m^2 and BMI <25 kg/m^2. The blood pressure was measured using a validated Omron BP monitor following WHO protocol (38). Hypertension was defined as blood pressure ≥ 140/90 mmHg or use of antihypertensive medications. The waist and hip circumferences were measured using standard methods (39). Abdominal obesity was defined as waist circumference ≥ 90 cm in men or ≥ 80 cm in women (40). All these anthropometric measurements, along with blood pressure, was measured three times by a trained study nurse, and the mean of three values was used for analysis.

Laboratory analyses

A total 5 mL blood sample was withdrawn from each subject and collected in BD Vacutainer® tubes and serum was separated for the analysis of serum creatinine (modified Jaffe's method traceable to isotope dilution mass spectrometry (IDMS) standard), urea, and albumin. 24-h urine samples were analysed for sodium, creatinine,

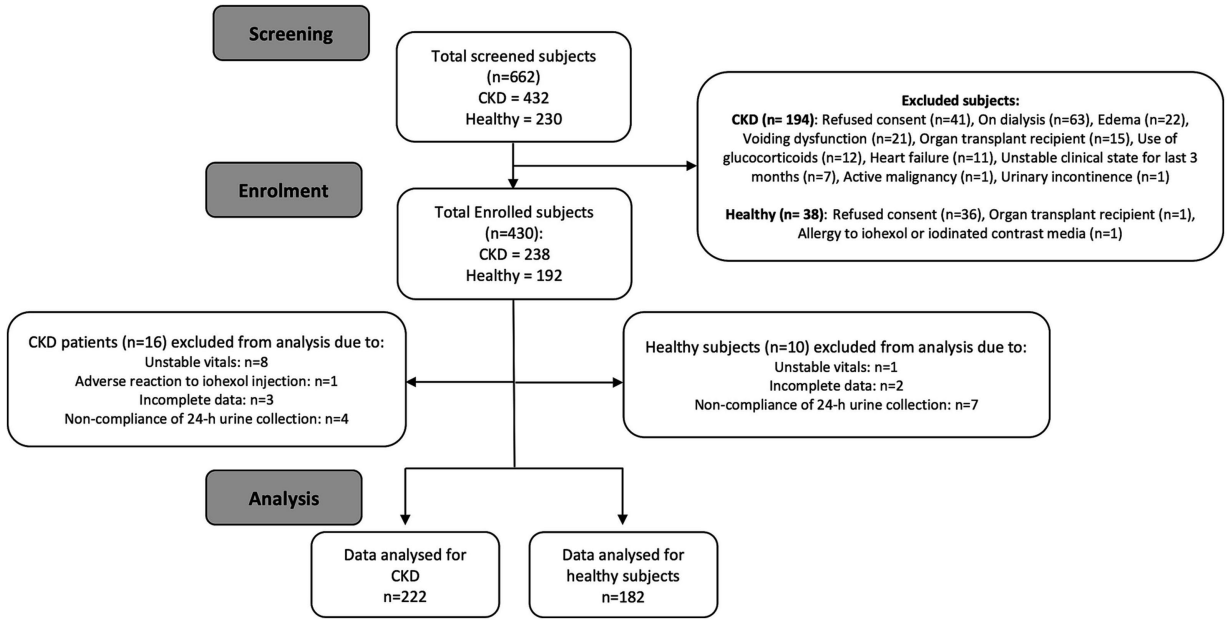

FIGURE 1
Consort flow diagram for the study subjects.

protein, urea, phosphorus and potassium. Urine spot samples were analysed for sodium excretion. All investigations were done at the Central Laboratory facility, PGIMER, Chandigarh using COBAS c702 auto-analyzer (Roche Diagnostic Limited, Rotkreuz, Switzerland). The sodium and potassium were measured using Ion-Selective Electrode method, and phosphorus and protein were measured using Molybdate UV (41) and Turbidimetric methods (42) respectively.

Dietary salt intake was calculated using 24-h urine sodium value using the formula (2):

$$\text{Amount of salt}\left(\text{g / day}\right) = \left(\text{Urinary sodium}\left(\text{mmol / day}\right) \times 0.023\right) / 0.397$$

The dietary protein intake per day was calculated by 24-h urine urea nitrogen method as follows.

$$\text{Protein intake}\left(\text{g / day}\right) = \left[\left(\text{urine urea nitrogen}\left(\text{g / day}\right) + \left(\text{weight}\left(\text{kg}\right) \times 0.031\right)\right] * 6.25\right)$$

(43, 44).

Potassium intake was calculated as:

$$\text{Potassium intake}\left(\text{mg / day}\right) = \text{Urinary potassium excretion}\left(\text{mmol / day}\right) \times 39\left(\text{molecular weight of potassium}\right)$$

(27, 28).

Phosphorus intake was calculated assuming an absorption rate of 65% (29).

$$\text{Dietary phosphorus intake}\left(\text{mg / day}\right) = \text{Urinary phosphorus excretion}\left(\text{mg / day}\right) / 0.65$$

Estimation of salt intake using spot urinary sodium concentration

We compared the measured 24 h urinary sodium excretion with those derived using seven estimation equations (Kawasaki, Tanaka, Toft, INTERSALT with and without potassium, Whitton and Mage) (30–35). Detailed formulae are presented in Appendix 1 in Supplementary Materials.

Statistical analyses

Data are presented as mean ± standard deviation or as median and 25th and 75th percentiles. The normality of data was tested using Shapiro–Wilk test. Continuous data were compared using independent t-test or Mann Whitney U test as appropriate. Categorical data were compared using the chi-square test. Correlation between variables was tested using Pearson's or Spearman's rho correlation as appropriate. Dietary intake of nutrients was also analysed in subgroups based on BMI, blood pressure and abdominal obesity. Mean bias for 24-h urinary sodium was computed as the difference between the measured and estimated values obtained from different equations and expressed as mean ± standard deviation (SD). The Bland–Altman Plot was used to estimate the bias and limits of agreement between sodium values obtained by 24 h urinary excretion method and sodium estimating equation methods. Limits of agreement were calculated as mean bias ±1.96 × SD. Precision was defined as 95% confidence interval of bias. Two-tailed p value <0.05 was considered significant. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software for Macintosh, version 26.0 (IBM Corp., Armonk, NY, United States).

Results

Overall, 662 individuals were screened, out of whom 430 were enrolled (Figure 1). Another 26 participants were excluded from analysis because of various reasons and finally, data from 404 participants (182 adult healthy volunteers and 222 adults with CKD) were analyzed (Figure 1). Among adults with CKD, 71 (32%) were in stage 4, 127 (57%) were in stage 3 and 24 (11%) in stage 2. The demographic characteristics of the participants are shown in Table 1. The mean age was 47.01 ± 11.46 years, with equal distribution of men and women. The mean eGFR of the study subjects was 71.10 ± 38.28 mL/min/1,73m^2, and the mean BMI was 24.94 ± 5.47 kg/m^2, with 121 (30%) being overweight (BMI ≥25 kg/m^2) and 51 (12.60%) were obese (BMI ≥30 kg/m^2).

Table 1 shows the daily dietary nutrient intake in the study population. The mean dietary intakes of sodium, salt, potassium, protein and phosphorus were 2.94 ± 1.68 g/day, 7.42 ± 4.24 g/day, 1.43 ± 0.59 g/day, 47.67 ± 14.73 g/day and 0.86 ± 0.39 g/day, respectively. Compared to healthy subjects, those with CKD had similar dietary intakes of sodium (2.81 ± 1.60 g/day vs., 3.05 ± 1.73 g/day, p = 0.136), salt (7.08 ± 4.04 g/day vs. 7.70 ± 4.37 g/day, p = 0.136) protein (47.16 ± 14.59 g/day vs. 48.08 ± 14.86 g/day, p = 0.531), potassium (1.38 ± 0.59 g/day vs1.48 ± 0.58 g/day, p = 0.095) and phosphorus (0.86 ± 0.41 mg/day vs. 0.87 ± 0.37 g/day, p = 0.741). However, protein intake per kilogram of body weight was more in healthy subjects (0.78 ± 0.25 g/kg/day vs 0.73 ± 0.20 g/kg/day, p = 0.015). The salt consumption was above the WHO recommended intake of 5 g/day in 118 (64.83%) of the healthy population and 150 (67.56%) of those with CKD.

In general, men consumed more dietary salt; 8.28 ± 4.44 vs. 6.55 ± 3.83 g/day (p < 0.001), protein; 51.43 ± 15.74 g/day vs. 43.84 ± 12.56 (p < 0.001), potassium; 1.52 ± 0.58 vs. 1.35 ± 0.58 g/day (p = 0.003) and phosphorus; 0.94 ± 0.39 mg/day vs. 0.79 ± 0.36 g/day (p < 0.011) as compared to women (Supplementary Table S1).

Stratification based on body mass index (BMI) and blood pressure was done as shown in Table 2. A total of 80 (44%) healthy volunteers and 92 (41%) subjects with CKD were overweight, whereas 168 (76%) of those with CKD were hypertensive. Consumption of salt, potassium, protein and phosphorus was higher among subjects with BMI ≥ 25 than those with BMI < 25, i.e., 8.38 ± 4.67 vs. 6.71 ± 3.73 g/day, p < 0.001 (salt), 1.57 ± 0.63 vs. 1.33 ± 0.53 g/day, p < 0.001 (potassium), 51.12 ± 13.77 vs. 45.11 ± 14.92 g/day, p < 0.001 (protein) and 0.93 ± 0.38 vs. 0.82 ± 0.38 g/day, p = 0.005 (phosphorus), respectively. Compared to normotensive study subjects, hypertensive individuals (blood pressure ≥ 140/90 mm Hg) consumed more salt and potassium, i.e., 8.23 ± 4.89 vs. 6.84 ± 3.59 g/day (p = 0.002) and 1.51 ± 0.63 vs. 1.38 ± 0.55 g/day (p = 0.024), respectively. No differences were seen in terms of protein and phosphorus intake.

Also, data were grouped based on the presence or absence of sex-specific abdominal obesity (waist circumference ≥ 90 cm in men or ≥ cm in women) (40). A total of 112 (62%) healthy individuals, and 137 (62%) of those with CKD had abdominal obesity. Overall, 115 (57%) men and 134 (68%) women had abdominal obesity. The protein

TABLE 1 Demographic and clinical characteristics of study population.

Parameter(s)	Total (n = 404)	CKD (n = 222)	Healthy volunteers (n = 182)	p-value
Age (years)	47.01 ± 11.46	47.82 ± 11.88	46.02 ± 10.88	0.115
Systolic blood pressure (mm Hg)	130 ± 18	135 ± 18	124 ± 17	<0.001
Diastolic blood pressure (mm Hg)	83 ± 11	85 ± 10	80 ± 12	<0.001
Waist/hip ratio	1.08 ± 0.11	1.07 ± 0.11	1.11 ± 0.10	<0.001
BMI (kg/m^2)	24.94 ± 5.47	25.16 ± 6.23	24.67 ± 4.39	0.364
Hypertension	168 (41.58)	168 (75.66)	—	—
Diabetes mellitus	56 (13.86)	56 (25.230)	—	—
CVD	7 (1.73)	7 (3.15)	—	—
Haemoglobin (g/dL)	12.56 ± 2.01	12.65 ± 2.30	12.45 ± 1.59	0.309
Serum creatinine (mg/dL)	1.46 ± 1.02	2.10 ± 0.95	0.68 ± 032	<0.001
eGFR (ml/min/1.73m^2)	71.10 ± 38.28	40.77 ± 19.27	107.92 ± 17.78	<0.001
Serum urea (mg/dL)	42.35 ± 27.71	59.04 ± 25.15	21.56 ± 12.50	<0.001
Serum albumin (g/dL)	4.25 ± 0.39	4.18 ± 0.41	4.34 ± 0.33	<0.001
24 h Urine creatinine (mg/day)	869.07 (704.00,1120.84)	934.44 (710.01, 1150.65)	836.92 (691.87, 1028.05)	0.137
24 h Urine protein (mg/day)	143.81 (81.69, 393.68)	303.40 (135.94, 1102.67)	88.50 (62.60, 131.63)	<0.001
Protein intake (g/day)	47.67 ± 14.73	48.09 ± 14.87	47.16 ± 14.59	0.531
Protein intake (g/kg/day)	0.75 ± 0.22	0.73 ± 0.20	0.78 ± 0.25	0.015
24 h urinary sodium (g/day)	2.94 ± 1.68	3.05 ± 1.73	2.81 ± 1.60	0.136
Salt intake (g/day)	7.42 ± 4.24	7.70 ± 4.37	7.08 ± 4.04	0.136
Potassium intake (g/day)	1.43 ± 0.59	1.48 ± 0.58	1.38 ± 0.59	0.095
Phosphorus intake (g/day)	0.86 ± 0.39	0.87 ± 0.37	86 ± 0.41	0.741

BMI: body mass index, eGFR: estimated glomerular filtration rate, CVD; cardiovascular disease expressed as mean ± standard deviation or median (25th and 75th quartiles).

intake in men and women with abdominal obesity was higher than in the non-obese (abdominal) individuals (54.33 ± 15.12 vs. 47.62 ± 15.98 g/day, p = 0.003 for men; and 45.68 ± 11.50 vs. 39.60 ± 14.05 g/day, p = 0.004 for women). However, weight-corrected protein intake was lower in men and women with abdominal obesity (0.72 ± 0.18 vs. 0.81 ± 0.28 g/kg/day, p = 0.014 for men and 0.71 ± 0.18 vs. 0.80 ± 0.27 g/kg/day, p = 0.019 for women). Intake of salt, potassium and phosphorus was significantly higher in both men and women with abdominal obesity (Table 3).

Table 4 and Figure 2 show the performance of equations used to estimate 24-h sodium intake from spot urinary sodium concentration against the measured 24-h urinary sodium excretion. The mean bias ranged from −47.82 (95% CI, −55.54 to −40.09) for the Kawasaki equation to 15.97 (95% CI, 9.24 to 22.69) for the Whitton formula. The INTERSALT2 formula had the least bias of all equations [1.08 (95% CI, −5.50 to 7.65)].

Discussion

This is the first study to provide a comprehensive assessment of the intake of multiple nutrients in the north Indian population that included healthy subjects and those with CKD. We document a higher-than-recommended dietary intake of salt and phosphorus and relatively low protein and potassium consumption.

The dietary salt intake was higher than recommended in about two-thirds of the study subjects, with no heterogeneity with regard to sex, or presence of hypertension or CKD (Figure 3). While this is the first study looking at salt intake in subjects with CKD, a similar finding has been reported in general population studies conducted in different parts of India. Johnson et al., using 24-h estimation, found the dietary salt intake to be higher among the population residing in Andhra Pradesh (9.46 g/day) compared to people of Delhi and Haryana (8.59 g/day) (17). A nationally representative cross-sectional survey conducted in 2017–18 in India estimated the salt intake to be 8 g/day using the INTERSALT equation on spot urine samples (45). The somewhat lower salt intake amongst subjects in this study compared to those from older studies could be attributed to increased awareness about the benefit of salt reduction and human health, in particular the targeted advice received by patients with CKD, which might have also influenced the consumption in other members of the family from whom the healthy population was drawn. Although the direction of movement is encouraging, a majority of the population (including those with CKD) is still consuming higher than recommended amounts of salt, indicating an unfinished public health agenda. This might also indicate the limits to which salt reduction is possible only with education. A study from China also reported the consumption of similar amounts of salt (7.40 g/day) among patients with CKD (46). Together, these findings raise the need to consider alternate interventions, such as low-sodium salt substitutes. Concerns are often raised about the high potassium content of salt substitutes. While these may be valid for those with advanced stages of CKD, large population-based studies (47) have not shown an increased risk of hyperkalemia. Given the remarkable ability of the kidneys to excrete potassium until the late stages of CKD, it is unlikely

TABLE 2 Daily dietary intake of nutrients per 24 h of urinary excretion as per BMI and blood pressure category in different subgroups.

	BMI (kg/m^2)			Blood pressure (mm Hg)		
	≥25	<25	*p* value	≥140/90	<140/90	*p* value
Total						
Number of subjects	172	232		168	236	
Salt (g/day)	8.38 ± 4.67	6.71 ± 3.73	<0.001	8.23 ± 4.89	6.84 ± 3.59	0.002
Protein (g/day)	51.12 ± 13.77	45.11 ± 14.92	<0.001	48.69 ± 14.32	46.94 ± 15.00	0.236
Protein (g/kg/day)	0.70 ± 0.16	0.79 ± 0.25	<0.001	0.75 ± 0.20	0.76 ± 0.24	0.594
Potassium (g/day)	1.57 ± 0.63	1.33 ± 0.53	<0.001	1.51 ± 0.63	1.38 ± 0.55	0.024
Phosphorus (g/day)	0.93 ± 0.38	0.82 ± 0.38	0.005	0.89 ± 0.39	0.85 ± 0.38	0.337
Men						
Number of subjects	76	128		92	112	
Salt (g/day)	9.80 ± 4.80	7.37 ± 3.97	<0.001	9.51 ± 5.16	7.27 ± 3.46	<0.001
Protein (g/day)	56.53 ± 15.06	48.39 ± 15.39	<0.001	53.07 ± 14.63	50.07 ± 16.53	0.172
Protein (g/kg/day)	0.71 ± 0.16	0.79 ± 0.26	0.004	0.75 ± 0.20	0.77 ± 0.26	0.702
Potassium (g/day)	1.75 ± 0.63	1.39 ± 0.52	<0.001	1.66 ± 0.65	1.41 ± 0.50	0.003
Phosphorus (g/day)	1.05 ± 0.40	0.87 ± 0.38	0.003	0.96 ± 0.39	0.91 ± 0.39	0.372
Women						
Number of subjects	96	104	0.013	76	124	0.701
Salt (g/day)	7.25 ± 4.27	5.90 ± 3.26		6.68 ± 4.08	6.46 ± 3.68	
Protein (g/day)	46.83 ± 10.96	41.07 ± 13.32	0.001	43.39 ± 12.05	44.11 ± 12.89	0.691
Protein (g/kg/day)	0.69 ± 0.16	0.79 ± 0.24	0.001	0.73 ± 0.20	0.75 ± 0.22	0.646
Potassium (g/day)	1.44 ± 0.60	1.27 ± 0.55	0.037	1.34 ± 0.56	1.35 ± 0.58	0.914
Phosphorus (g/day)	0.83 ± 0.34	0.75 ± 0.38	0.129	0.79 ± 0.37	0.79 ± 0.36	0.951

BMI; body mass index.

TABLE 3 Daily dietary intake of nutrients as per abdominal obesity in men and women.

Abdominal obesity			
Men			
Waist circumference (cm)	**≥90**	**<90**	***p* value**
Number of subjects	115	87	
Salt (g/day)	8.86 ± 4.88	7.57 ± 3.69	0.033
Protein (g/day)	54.33 ± 15.12	47.62 ± 15.98	0.003
Protein (g/kg/day)	0.72 ± 0.18	0.81 ± 0.28	0.014
Potassium (g/day)	1.62 ± 0.63	1.39 ± 0.49	0.005
Phosphorus (g/day)	0.98 ± 0.38	0.88 ± 0.40	0.091
Women			
Waist circumference (cm)	**≥80**	**<80**	***p* value**
Number of subjects	134	62	
Salt (g/day)	6.95 ± 3.95	5.63 ± 3.42	0.019
Protein (g/day)	45.68 ± 11.50	39.60 ± 14.05	0.004
Protein (g/kg/day)	0.71 ± 0.18	0.80 ± 0.27	0.019
Potassium (g/day)	1.41 ± 0.56	1.21 ± 0.59	0.025
Phosphorus (mg/day)	0.83 ± 0.33	0.70 ± 0.43	0.040

TABLE 4 Comparison of measured and estimated 24 h urinary sodium excretion in study subjects.

Method	Bias (Mean ± SD)	95% CI	LOA
Kawasaki	−47.82 ± 79.16	−55.54 to −40.09	107.33 to −202.97
Tanaka	−8.43 ± 69.16	−15.17 to −1.69	127.11 to −143.97
INTERSALT1	2.28 ± 67.40	−4.29 to 8.85	134.39 to −129.83
lNTERSALT2	1.08 ± 67.33	−5.50 to 7.65	133.06 to −130.89
Toft	−24.81 ± 68.48	−31.49 to −18.13	109.42 to −159.04
Whitton	15.97 ± 68.99	9.24 to 22.69	−119.24 to 151.18
Mage	15.68 ± 102.49	5.69 to 25.68	216.56 to −185.2

SD: standard deviation, CI: confidence interval, LOA: limit of agreement.

that use of salt substitutes will pose a substantial risk of hyperkalemia at a population level (48). Appropriate caution, however, should be exercised in those with advances stages of CKD.

Also striking was the low potassium intake in the study population, not only amongst those with CKD but also in the otherwise healthy population. The intake was substantially lower than 3.50 g/day recommended by the WHO for the healthy population and 2-4 g for those with early stages of CKD as per KDOQI 2020 guidelines (49). Given that nuts, green vegetables, and fruits are the main sources of potassium in the diet, the finding reflects low dietary diversity in our population (50). Our findings are consistent with the results of a 32-country INTERSTROKE study that found overall low urinary potassium excretion (1.68 g/day) in 32 countries, including India (15), and other Indian studies (50, 51). Studies from West Africa (1.8 g/day) (52) and China

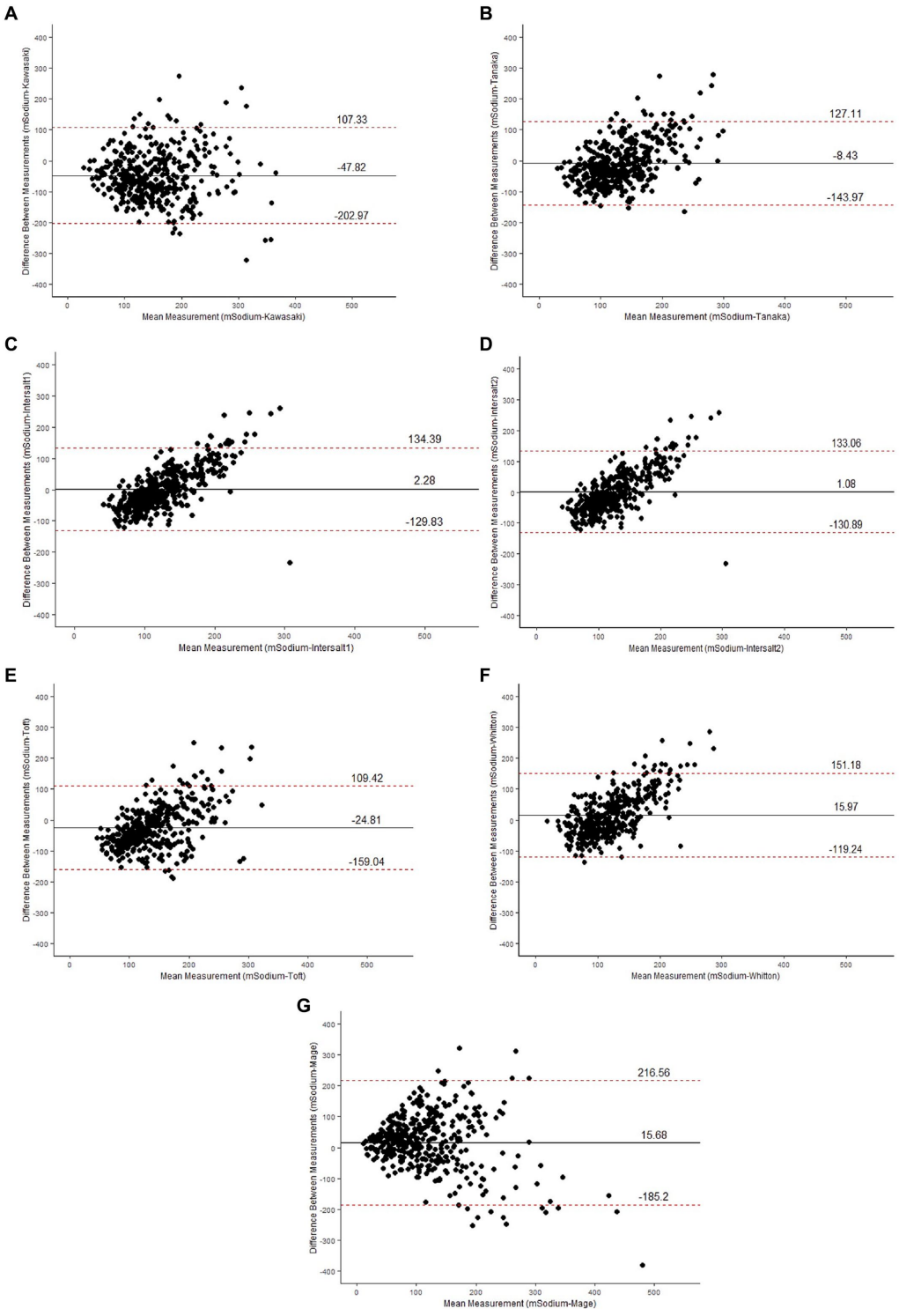

FIGURE 2
Bland–Altman plots for the difference in agreement between the measured sodium (mSodium) and estimated sodium using Kawasaki **(A)**, Tanaka **(B)**, INTERSALT1 **(C)**, INTERSALT1 **(D)**, Toft **(E)**, Whitton **(F)** and Mage **(G)** estimating equations shown by plotting the bias (measured sodium intake minus estimated sodium intake) against the mean of measured and estimated sodium intake. Central line represent the mean bias between measured and estimated methods and dotted line represent ±1.96 SD.

(1.5 g/day) (53) reported potassium intake similar to the current study. The combination of higher than recommended sodium and lower than recommended potassium collectively intake raises cardiovascular disease risk.

Another notable finding was the relatively low dietary protein intake, especially in the healthy individuals (0.78 g/kg). Previous studies from different parts of India that included healthy people and those with hypertension, CKD and diabetes have shown similar

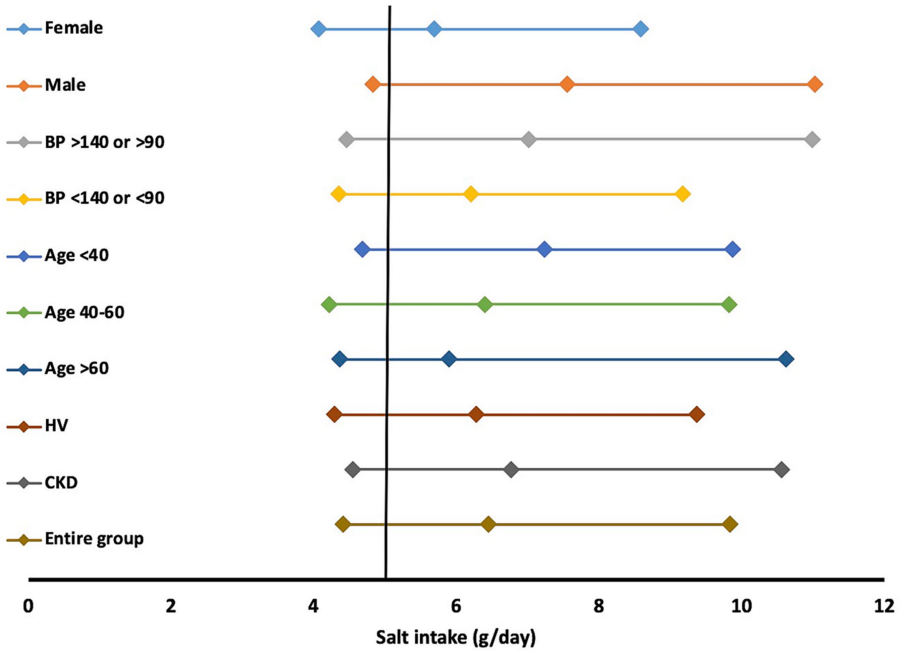

FIGURE 3
Salt intake per day in various subgroups. Data represented as median (25th, 75th percentile). HV; healthy volunteer, CKD; chronic kidney disease.

findings (Table 5). Protein intake measured using 24-h or 3-day dietary recall and or a validated Food Frequency Questionnaire (FFQ) in healthy individuals varies from 39.54 g/day to 67.5 g/day (2, 14, 16, 58), whereas protein intake in subjects with CKD was 39.05 g/day (2, 14, 16, 58). This finding is of relevance with regard to the CKD population. Current guidelines recommend restricting dietary protein intake to about 0.8 g/kg/day in those with CKD stage 1–2 and 0.55–0.60 g/kg/day in those with CKD Stage 3–5 (17) to retard disease progression. Data from Western countries show a wide variation in protein intake. A Danish survey reported protein intake ranging from 75.5–94.2 g/day among men and 59.1–69.7 g/day among women (59). A multinational (Denmark, Finland, Netherlands, United Kingdom, Spain, Bulgaria, Australia, New Zealand) randomised controlled trial in prediabetic obese individuals showed a protein intake of 105.8 ± 35.7 g/day (60, 61). The Nurses' Health Study from the US reported a mean protein intake of 76 grams both in subjects with normal renal function and with mild renal insufficiency (62). The EPIC-Oxford cohort of the general population, which included meat eaters, fish eaters, vegetarians, and vegans, also showed high protein intakes (0.99 to 1.28 g/kg/day) (63). Therefore in the Western context, restriction of dietary protein intake is advised. However, this advice is interpreted in other parts of the world without contextualizing the prevalent dietary practice. Further, excessive protein restriction is likely to lead to protein-energy wasting, a phenomenon shown to be associated with poorer outcomes in those with CKD (64).

Regarding phosphorus intake, the mean intake was above the recommended levels. Usually, the dietary phosphorus intake tracks with the protein in the western meat-based diets, but there may be a divergence in India, where dairy products are the main source of phosphorus and a reduced phosphorus intake in those with CKD could indicate preferential reduction of dairy products. Finally, doubts have been raised about the reliability of 24-h urinary phosphate for estimating dietary intake in the presence of CKD (65).

We have summarised findings from published studies on dietary intakes of multiple nutrients across India including healthy and individuals with various NCDs (Table 5) (2, 14, 16, 17, 45, 50, 54–57).

The salt, protein, potassium and phosphorus intakes were higher among subjects with higher BMI than those with normal BMI, consistent results with earlier findings (66–70), suggesting overall better nourishment. In line with results as per BMI, both men and women subjects with abdominal obesity has higher intake of all these nutrients. We also noted some sex-specific differences in dietary intake of salt, protein, potassium, and phosphorus. All of them were significantly lower in females. These findings are consistent with previous studies, and indicate a comparative nutritional disadvantage for females, except for salt.

Finally, we analyzed the performance of the estimated 24-h sodium consumption by comparing it with 24-h urine sodium excretion levels. Our data shows that the INTERSALT2 exhibited the least bias. Hence, we suggest the use of this equation to estimate sodium intake using spot urine samples. Whitton et al. also found the INTERSALT equation suitable for estimating sodium excretion in urban Asian populations (Singapore residents of Chinese, Malay, and Indian ethnicity) (30). One possible reason this equation performed better in our population is that it was developed using data from 32 countries that included Indian subjects in contrast to others that did not include Indian subjects. A recent study, however, found poor agreement between the actual sodium intake and the estimated intake using all equations, suggesting a high degree of variability and the need to use data from estimation equations with caution (71).

The strength of this study is the inclusion of a well-phenotyped population, including healthy subjects and those with CKD and the use of 24-h urine excretions for analysis of nutrient intake. Our study

TABLE 5 Studies showing the dietary intake of protein, salt, potassium and phosphorus in Indians.

Studies	Place	Number of subjects	Age group (years)	Men/ Women	Population	Method used	Protein (g/day)	Salt (g/day)	Potassium (g/day)	Phosphorus (g/day)
Mathur et al. (45)	India	2,266	18–69	1081/1185	General community population	INTERSALT equation with Potassium		8.00		
Thakur et al. (54)	Haryana	5,078	18–69	2294/2784	General community population	Diet and dietary salt questionnaire		8.00		
Johnson et al. (17)	Delhi, Haryana, and Andhra Pradesh	1,395	>20	704/691	General community population	24 h urinary sodium excretion		9.46 (Andhra Pradesh) 8.59 (Delhi and Haryana)		
Kumbla et al. (55)	Delhi, Mumbai, Calcutta, Bangalore and Chennai	466	18–75	254/192	Patients with HTN and dyslipidaemia	Three-day dietary recall		14.13 (Delhi) 9.81 (Mumbai) 10.12 (Calcutta) 9.38 (Bangalore and Chennai)		
Smina et al. (2)	Chennai	200	25–70	111/89	Healthy controls, patients with T2DM, HTN and CKD	24-h dietary recall method for protein intake, 24 h urinary sodium excretion for salt intake	39.54 (HC) 39.50 (T2DM) 42.9 (HTN) 39.05 (CKD)	12.60 (HC) 14.52 (T2DM) 11.85 (HTN) 13.37 (CKD)		
Radhika et al. (56)	Chennai	1902	>20	821/1081	Healthy controls and patients with HTN	Semi quantitative food frequency questionnaire		8.00 (HC) 9.90 (HTN)		
Anand et al. (50)	Delhi, Haryana	1,397	>20	624/773	General community population	24 h potassium excretion			1.14 (Rural) 1.08 (urban)	
Berkemeyer et al. (57)	Delhi	10	20–50		Healthy controls	2-day dietary record	67.90			1.79
Joshi et al. (16)	India	794	≥18	370/424	Gen population	3-day dietary recall, and a validated Food Frequency Questionnaire (FFQ)	57.89			
Kumar et al. (14)	Delhi	255	20–69	0/255	Healthy women	24-h dietary recall method	48.70			

CKD: chronic kidney disease, HC: healthy controls, HTN: Hypertension, T2DM: Type 2 diabetes.

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had a few limitations, including a relatively small sample size, lack of adjustments for dietary energy intake, lack of dietary assessment over a period of time and lack of a community-based study sample as the study was conducted at the hospital level. We also did not correct for the faecal loss of potassium and phosphorus. This limits the value of using urinary excretion as a surrogate for intake. Further, The enrolment of healthy subjects from amongst the family members of subjects with CKD might have introduced a bias since they could have altered their dietary habits as well. Therefore, our findings remain hypothesis-generating for the general population and should be confirmed in larger population-based samples.

In conclusion, the study shows that intake of several nutrients, including salt, potassium, phosphorus and protein, is either above or below the recommended levels, which might be one reason for increased NCD risk, including CKD. An improved understanding of dietary patterns and nutrient intakes will help provide tailored dietary advice to persons with various health conditions, including CKD. These data can help support the development of locally appropriate guidelines and implementation strategies such as public awareness, counselling at the individual level, and developing appropriate food policy.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by Postgraduate Institute of Medical Education and Research, Chandigarh, India. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

PK: Data curation, Methodology, Writing – original draft. AY: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. AP: Investigation, Methodology, Writing – review & editing. RJ: Investigation, Writing – review & editing. NuS: Methodology, Supervision, Resources, Writing – review & editing. NaS: Methodology, Data curation, Writing – review & editing. VK: Funding acquisition, Investigation, Data curation, Resources, Supervision, Writing – review & editing. VJ: Conceptualization, Data curation, Funding acquisition, Resources, Supervision, Writing – review & editing.

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Conflict of interest

VJ has received grant funding from GSK, Baxter Healthcare, and Biocon and honoraria from Bayer, AstraZeneca, Boeringer Ingelheim, NephroPlus and Zydus Cadilla, under the policy of all honoraria being paid to the organization.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1312581/full#supplementary-material

References

1. World Health Organization. Cardiovascular diseases Available at: https://www.who.int/india/health-topics/cardiovascular-diseases (Accessed on October 4, 2023)

2. Smina TP, Kumpatla S, Viswanathan V. Higher dietary salt and inappropriate proportion of macronutrients consumption among people with diabetes and other co morbid conditions in South India: estimation of salt intake with a formula. *Diabetes Metab Syndr*. (2019) 13:2863–8. doi: 10.1016/j.dsx.2019.07.059

3. World Health Organization. Global action plan for the prevention and control of NCDs 2013–2020 2013-2020 Available at: https://www.who.int/publications/i/item/9789241506236. (Accessed on October 4, 2023)

4. World Health Organization. WHO issues new guidance on dietary salt and potassium 2013 Available at: https://www.who.int/news/item/31-01-2013-who-issues-new-guidance-on-dietary-salt-and-potassium#:~:text=Adults%20should%20consume%20less%20than,guidelines%20issued%20by%20the%20WHO. (Accessed on October 4, 2023)

5. National Institute of Diabetes and Digestive and Kidney Diseases. Diet & Nutrition for adults with advanced chronic kidney Disease Available at: https://www.niddk.nih.gov/health-information/kidney-disease/chronic-kidney-disease-ckd/eating-nutrition/nutrition-advanced-chronic-kidney-disease-adults. (Accessed on October 4, 2023)

6. National Academies of sciences E, and medicine. Sodium and potassium dietary reference intake values updated in new report; introduces new category for sodium based on chronic Disease risk reduction 2019 Available at: https://www.nationalacademies.org/news/2019/03/sodium-and-potassium-dietary-reference-intake-values-updated-in-new-report#:~:text=For%20individuals%20ages%2014%20and,for%20children%20ages%201%2D13. (Accessed on October 4, 2023)

7. Kidney disease: improving global outcomes blood pressure work Group. KDIGO 2021 clinical practice guideline for the Management of Blood Pressure in chronic kidney Disease. *Kidney Int*. (2021) 99:S1–S87. doi: 10.1016/j.kint.2020.11.003

8. Institute of Medicine. Phosphorus Available at: https://ods.od.nih.gov/factsheets/Phosphorus-HealthProfessional/. (Accessed on October 4, 2023)

9. Itkonen ST, Karp HJ, Kemi VE, Kokkonen EM, Saarnio EM, Pekkinen MH, et al. Associations among total and food additive phosphorus intake and carotid intima-media thickness—a cross-sectional study in a middle-aged population in southern Finland. *Nutr J*. (2013) 12:94. doi: 10.1186/1475-2891-12-94

10. Olivo RE, Hale SL, Diamantidis CJ, Bhavsar NA, Tyson CC, Tucker KL, et al. Dietary phosphorus and ambulatory blood pressure in African Americans: the Jackson heart Study. *Am J Hypertens*. (2019) 32:94–103. doi: 10.1093/ajh/hpy126

11. Gutierrez OM, Porter AK, Viggeswarapu M, Roberts JL, Beck GR Jr. Effects of phosphorus and calcium to phosphorus consumption ratio on mineral metabolism and cardiometabolic health. *J Nutr Biochem*. (2020) 80:108374. doi: 10.1016/j.jnutbio.2020.108374

12. Mariotti F, Gardner CD. Dietary Protein and Amino Acids in Vegetarian Diets-A Review. *Nutrients*. (2019) 11. doi: 10.3390/nu11112661

13. Berrazaga I, Micard V, Gueugneau M, Walrand S. The role of the anabolic properties of plant- versus animal-based protein sources in supporting muscle mass maintenance: a critical review. *Nutrients*. (2019) 11. doi: 10.3390/nu11081825

14. Kumar A, Mittal S, Orito S, Ishitani K, Ohta H. Impact of dietary intake, education, and physical activity on bone mineral density among north Indian women. *J Bone Miner Metab*. (2010) 28:192–201. doi: 10.1007/s00774-009-0118-y

15. Judge C, O'Donnell MJ, Hankey GJ, Rangarajan S, Chin SL, Rao-Melacini P, et al. Urinary sodium and potassium, and risk of ischemic and hemorrhagic stroke (INTERSTROKE): a case-control Study. *Am J Hypertens*. (2021) 34:414–25. doi: 10.1093/ajh/hpaa176

16. Joshi SR, Bhansali A, Bajaj S, Banzal SS, Dharmalingam M, Gupta S, et al. Results from a dietary survey in an Indian T2DM population: a STARCH study. *BMJ Open*. (2014) 4:e005138. doi: 10.1136/bmjopen-2014-005138

17. Johnson C, Mohan S, Rogers K, Shivashankar R, Thout SR, Gupta P, et al. Mean dietary salt intake in urban and rural areas in India: a population survey of 1395 persons. *J Am Heart Assoc*. (2017) 6. doi: 10.1161/JAHA.116.004547

18. Ikizler TA, Burrowes JD, Byham-Gray LD, Campbell KL, Carrero JJ, Chan W, et al. KDOQI clinical practice guideline for nutrition in CKD: 2020 update. *Am J Kidney Dis*. (2020) 76:S1–S107. doi: 10.1053/j.ajkd.2020.05.006

19. Green R, Milner J, Joy EJ, Agrawal S, Dangour AD. Dietary patterns in India: a systematic review. *Br J Nutr*. (2016) 116:142–8. doi: 10.1017/S0007114516001598

20. Raina SK. Limitations of 24-hour recall method: micronutrient intake and the presence of the metabolic syndrome. *N Am J Med Sci*. (2013) 5:498. doi: 10.4103/1947-2714.117329

21. Block G. Human dietary assessment: methods and issues. *Prev Med*. (1989) 18:653–60. doi: 10.1016/0091-7435(89)90036-4

22. Abdel-Nabey M, Saint-Jacques C, Boffa JJ, Frochot V, Livrozet M, Daudon M, et al. 24-h urine collection: a relevant tool in CKD nutrition evaluation. *Nutrients*. (2020) 12. doi: 10.3390/nu12092615

23. Campbell NRC, He FJ, Tan M, Cappuccio FP, Neal B, Woodward M, et al. The international consortium for Quality research on dietary sodium/salt (TRUE) position statement on the use of 24-hour, spot, and short duration (<24 hours) timed urine collections to assess dietary sodium intake. *J Clin Hypertens (Greenwich)*. (2019) 21:700–9. doi: 10.1111/jch.13551

24. Ji C, Sykes L, Paul C, Dary O, Legetic B, Campbell NR, et al. Systematic review of studies comparing 24-hour and spot urine collections for estimating population salt intake. *Rev Panam Salud Publica*. (2012) 32:307–15. doi: 10.1590/S1020-49892012001000010

25. Lucko AM, Doktorchik C, Woodward M, Cogswell M, Neal B, Rabi D, et al. Percentage of ingested sodium excreted in 24-hour urine collections: a systematic review and meta-analysis. *J Clin Hypertens (Greenwich)*. (2018) 20:1220–9. doi: 10.1111/jch.13353

26. Cogswell ME, Maalouf J, Elliott P, Loria CM, Patel S, Bowman BA. Use of urine biomarkers to assess sodium intake: challenges and opportunities. *Annu Rev Nutr*. (2015) 35:349–87. doi: 10.1146/annurev-nutr-071714-034322

27. Dennis B, Stamler J, Buzzard M, Conway R, Elliott P, Moag-Stahlberg A, et al. INTERMAP: the dietary data—process and quality control. *J Hum Hypertens*. (2003) 17:609–22. doi: 10.1038/sj.jhh.1001604

28. Taylor EN, Stampfer MJ, Mount DB, Curhan GC. DASH-style diet and 24-hour urine composition. *Clin J Am Soc Nephrol*. (2010) 5:2315–22. doi: 10.2215/CJN.04420510

29. Morimoto Y, Sakuma M, Ohta H, Suzuki A, Matsushita A, Umeda M, et al. Estimate of dietary phosphorus intake using 24-h urine collection. *J Clin Biochem Nutr*. (2014) 55:62–6. doi: 10.3164/jcbn.14-15

30. Whitton C, Gay GM, Lim RB, Tan LW, Lim WY, van Dam RM. Evaluation of equations for predicting 24-hour urinary sodium excretion from casual urine samples in Asian adults. *J Nutr*. (2016) 146:1609–15. doi: 10.3945/jn.116.232108

31. Toft U, Cerqueira C, Andreasen AH, Thuesen BH, Laurberg P, Ovesen L, et al. Estimating salt intake in a Caucasian population: can spot urine substitute 24-hour urine samples? *Eur J Prev Cardiol*. (2014) 21:1300–7. doi: 10.1177/2047487313485517

32. Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H, et al. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. *J Hum Hypertens*. (2002) 16:97–103. doi: 10.1038/sj.jhh.1001307

33. Mage DT, Allen RH, Kodali A. Creatinine corrections for estimating children's and adult's pesticide intake doses in equilibrium with urinary pesticide and creatinine concentrations. *J Expo Sci Environ Epidemiol*. (2008) 18:360–8. doi: 10.1038/sj.jes.7500614

34. Kawasaki T, Itoh K, Uezono K, Sasaki H. A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. *Clin Exp Pharmacol Physiol*. (1993) 20:7–14. doi: 10.1111/j.1440-1681.1993.tb01496.x

35. Brown IJ, Dyer AR, Chan Q, Cogswell ME, Ueshima H, Stamler J, et al. Estimating 24-hour urinary sodium excretion from casual urinary sodium concentrations in Western populations: the INTERSALT study. *Am J Epidemiol*. (2013) 177:1180–92. doi: 10.1093/aje/kwt066

36. Chapter 1: Definition and classification of CKD. *Kidney Int Suppl (2011)*. (2013) 3:19–62. doi: 10.1038/kisup.2012.64

37. Blackburn GL, Bistrian BR, Maini BS, Schlamm HT, Smith MF. Nutritional and metabolic assessment of the hospitalized patient. *JPEN J Parenter Enteral Nutr*. (1977) 1:11–21. doi: 10.1177/014860717700100101

38. World Health Organization. *WHO technical specifications for automated non-invasive blood pressure measuring devices with cuff 2020* Available at: https://www.who.int/docs/default-source/searo/indonesia/who-tech-spec-for-automated-non-invasive-blood-pressure-measuring-devices-with-cuff.pdf?sfvrsn=b112be47_2. (Accessed on October 4, 2023)

39. World Health Organization. Waist circumference and waist–hip ratio: Report of a WHO expert consultation 2008 Available at: http://apps.who.int/iris/bitstream/handle/10665/44583/9789241501491_eng.pdf;jsessionid=929929DB3B3D25FCAB721EA4272618A3?sequence=1. (Accessed on October 4, 2023)

40. Pradeepa R, Anjana RM, Joshi SR, Bhansali A, Deepa M, Joshi PP, et al. Prevalence of generalized & abdominal obesity in urban & rural India—the ICMR-INDIAB Study (phase-I) [ICMR-NDIAB-3]. *Indian J Med Res*. (2015) 142:139–50. doi: 10.4103/0971-5916.164234

41. Henry RJ. *Clinical chemistry: principles and technics*. 2nd ed. Hagerstown, MD: Harper and Row (1974). 723 p.

42. Iwata J, Nishikaze O. New micro-turbidimetric method for determination of protein in cerebrospinal fluid and urine. *Clin Chem*. (1979) 25:1317–9. doi: 10.1093/clinchem/25.7.1317

43. Maroni BJ, Steinman TI, Mitch WE. A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int*. (1985) 27:58–65. doi: 10.1038/ki.1985.10

44. Masud T, Manatunga A, Cotsonis G, Mitch WE. The precision of estimating protein intake of patients with chronic renal failure. *Kidney Int*. (2002) 62:1750–6. doi: 10.1046/j.1523-1755.2002.00606.x

45. Mathur P, Kulothungan V, Leburu S, Krishnan A, Chaturvedi HK, Salve HR, et al. National noncommunicable disease monitoring survey (NNMS) in India: estimating risk factor prevalence in adult population. *PLoS One*. (2021) 16:e0246712. doi: 10.1371/journal.pone.0246712

46. Sun L, Duan S, Zuo C, Sun Z, Nie G, Zhang C, et al. Characteristics of urinary sodium excretion in patients with chronic kidney disease in Jiangsu. *China J Clin Hypertens (Greenwich)*. (2021) 23:1372–9. doi: 10.1111/jch.14307

47. Neal B, Wu Y, Feng X, Zhang R, Zhang Y, Shi J, et al. Effect of salt substitution on cardiovascular events and death. *N Engl J Med*. (2021) 385:1067–77. doi: 10.1056/NEJMoa2105675

48. Greer RC, Marklund M, Anderson CAM, Cobb LK, Dalcin AT, Henry M, et al. Potassium-enriched salt substitutes as a means to lower blood pressure: benefits and risks. *Hypertension*. (2020) 75:266–74. doi: 10.1161/HYPERTENSIONAHA.119.13241

49. Kidney Disease Outcomes Quality I. K/DOQI clinical practice guidelines on hypertension and antihypertensive agents in chronic kidney disease. *Am J Kidney Dis*. (2004) 43:11–3. doi: 10.1053/j.ajkd.2004.03.003

50. Anand S, Shivashankar R, Kondal D, Garg V, Khandelwal S, Gupta R, et al. Potassium intake in India: opportunity for mitigating risks of high-sodium diets. *Am J Prev Med*. (2020) 58:302–12. doi: 10.1016/j.amepre.2019.09.017

51. Jan RA, Shah S, Saleem SM, Waheed A, Mufti S, Lone MA, et al. Sodium and potassium excretion in normotensive and hypertensive population in Kashmir. *J Assoc Physicians India*. (2006) 54:22–6.

52. Mizehoun-Adissoda C, Houinato D, Houehanou C, Chianea T, Dalmay F, Bigot A, et al. Dietary sodium and potassium intakes: data from urban and rural areas. *Nutrition*. (2017) 33:35–41. doi: 10.1016/j.nut.2016.08.007

53. Du X, Fang L, Xu J, Chen X, Bai Y, Zhong J. Association between 24-h urinary sodium and potassium excretion and blood pressure among Chinese adults aged 18-69 years. *Sci Rep*. (2021) 11:3474. doi: 10.1038/s41598-021-83049-8

54. Thakur JS, Jeet G, Nangia R, Singh D, Grover S, Lyngdoh T, et al. Non-communicable diseases risk factors and their determinants: a cross-sectional state-wide STEPS survey, Haryana, North India. *PLoS One*. (2019) 14:e0208872. doi: 10.1371/journal.pone.0208872

55. Kumbla D, Dharmalingam M, Dalvi K, Ray S, Shah MK, Gupta S, et al. A Study of salt and fat consumption pattern in regional Indian diet among hypertensive and dyslipidemic PaTients-SCRIPT study. *J Assoc Physicians India*. (2016) 64:47–54.

56. Radhika G, Sathya RM, Sudha V, Ganesan A, Mohan V. Dietary salt intake and hypertension in an urban south Indian population—[CURES-53]. *J Assoc Physicians India*. (2007) 55:405–11.

57. Berkemeyer S, Bhargava A, Bhargava U. Urinary phosphorus rather than urinary calcium possibly increases renal stone formation in a sample of Asian Indian, male stone-formers. *Br J Nutr*. (2007) 98:1224–8. doi: 10.1017/S0007114507778686

58. Swaminathan S, Vaz M, Kurpad AV. Protein intakes in India. *Br J Nutr*. (2012) 108:S50–8. doi: 10.1017/S0007114512002413

59. Kristensen NB, Madsen ML, Hansen TH, Allin KH, Hoppe C, Fagt S, et al. Intake of macro- and micro-nutrients in Danish vegans. *Nutr J*. (2015) 14:115. doi: 10.1186/s12937-015-0103-3

60. Moller G, Rikardt Andersen J, Ritz C, PS M, Navas-Carretero S, Jalo E, et al. Higher protein intake is not associated with decreased kidney function in pre-diabetic older adults following a one-year intervention-a preview sub-Study. *Nutrients*. (2018) 10. doi: 10.3390/nu10010054

61. Cirillo M, Lombardi C, Chiricone D, De Santo NG, Zanchetti A, Bilancio G. Protein intake and kidney function in the middle-age population: contrast between cross-sectional and longitudinal data. *Nephrol Dial Transplant*. (2014) 29:1733–40. doi: 10.1093/ndt/gfu056

62. Knight EL, Stampfer MJ, Hankinson SE, Spiegelman D, Curhan GC. The impact of protein intake on renal function decline in women with normal renal function or mild renal insufficiency. *Ann Intern Med*. (2003) 138:460–7. doi: 10.7326/0003-4819-138-6-200303180-00009

63. Sobiecki JG, Appleby PN, Bradbury KE, Key TJ. High compliance with dietary recommendations in a cohort of meat eaters, fish eaters, vegetarians, and vegans: results from the European prospective investigation into Cancer and nutrition-Oxford study. *Nutr Res*. (2016) 36:464–77. doi: 10.1016/j.nutres.2015.12.016

64. Kopple JD, Levey AS, Greene T, Chumlea WC, Gassman JJ, Hollinger DL, et al. Effect of dietary protein restriction on nutritional status in the Modification of Diet in Renal Disease Study. *Kidney Int*. (1997) 52:778–91. doi: 10.1038/ki.1997.395

65. Stremke ER, McCabe LD, McCabe GP, Martin BR, Moe SM, Weaver CM, et al. Twenty-four-hour urine phosphorus as a biomarker of dietary phosphorus intake and absorption in CKD: a secondary analysis from a controlled Diet balance Study. *Clin J Am Soc Nephrol*. (2018) 13:1002–12. doi: 10.2215/CJN.00390118

66. Ravi S, Bermudez OI, Harivanzan V, Kenneth Chui KH, Vasudevan P, Must A, et al. Sodium intake, blood pressure, and dietary sources of sodium in an adult south Indian population. *Ann Glob Health*. (2016) 82:234–42. doi: 10.1016/j.aogh.2016.02.001

67. Nair S, Bandyopadhyay S. Sodium intake pattern in west Indian population. *Indian J Community Med*. (2018) 43:67–71. doi: 10.4103/ijcm.IJCM_116_17

68. Menyanu EK, Corso B, Minicuci N, Rocco I, Russell J, Ware LJ, et al. Salt and potassium intake among adult Ghanaians: WHO-SAGE Ghana wave 3. *BMC Nutr*. (2020) 6:54. doi: 10.1186/s40795-020-00379-y

69. Sari DW, Noguchi-Watanabe M, Sasaki S, Sahar J, Yamamoto-Mitani N. Estimation of sodium and potassium intakes assessed by two 24-hour urine collections in a city of Indonesia. *Br J Nutr*. (2021) 126:1537–48. doi: 10.1017/S0007114521000271

70. Yamamoto KT, Robinson-Cohen C, de Oliveira MC, Kostina A, Nettleton JA, Ix JH, et al. Dietary phosphorus is associated with greater left ventricular mass. *Kidney Int*. (2013) 83:707–14. doi: 10.1038/ki.2012.303

71. Lobene AJ, Stremke ER, McCabe GP, Moe SM, Moorthi RN, Hill Gallant KM. Spot urine samples to estimate Na and K intake in patients with chronic kidney Disease and healthy adults: a secondary analysis from a controlled feeding Study. *J Ren Nutr*. (2021) 31:602–10. doi: 10.1053/j.jrn.2020.09.007

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Erythrocyte glycocalyx sensitivity to sodium is associated with salt sensitivity of blood pressure in women but not men

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Background: While salt sensitivity of blood pressure (SSBP) is a risk factor for hypertension, end-organ damage and death, most studies are conducted in western countries and in White people. We previously found that the prevalence of SSBP in Blacks living in Sub-Saharan Africa is as high as 75–80% like what has been reported in the west. Erythrocyte glycocalyx sensitivity to sodium (eGCSS), a marker of sodium-induced damage to the erythrocyte and vascular endothelial glycocalyx is thought to be related to blood pressure perturbations associated with salt intake. We hypothesized that SSBP correlates with eGCSS differently in men and women in Black people.

Methods: We conducted a cross sectional study using data from our recent clinical trial from Livingstone University Teaching Hospital among 117 normotensive young adults. We used a "salt blood test" to determine eGCSS and an immediate pressor response to oral salt (IPROS) for the diagnosis of SSBP.

Results: The proportion of males were equal to females and the median age (interquartile range) of the participants was 29 (22–45) years. The eGCSS scores were higher in salt-resistant females compared to salt-sensitive females and males. eGCSS correlated negatively with SSBP (AOR 0.98, 95% CI 0.97–0.99, $p = 0.008$), however, this relationship was driven by female sex and abrogated by male sex. Although blood pressure elevations exhibited a sustained bimodal pattern in both sexes, in males, systolic and diastolic blood pressure never returned to baseline during the time course as it did in females.

Conclusion: In this study, eGCSS correlated negatively with SSBP in black women but not in black men and the pressor response to dietary salt was significantly higher in men compared to women. These results suggest that women tend to have a higher disruption of the vascular endothelial glycocalyx by an acute salt load, implying that acute changes in blood pressure may not be driven directly by the endothelial glycocalyx. Our findings suggest a novel mechanism linking eGCSS and SSBP with potential implications for sex differences in salt-induced cardiovascular disease.

Clinical trial registration: https://clinicaltrials.gov/, identifier [NCT04844255].

KEYWORDS

erythrocyte glycocalyx sensitivity to sodium, salt sensitivity of blood pressure, immediate pressor response to dietary salt, blood pressure, systolic blood pressure, diastolic blood pressure, sodium chloride, salt

1 Introduction

Excess dietary salt is associated with future development of hypertension, cardiovascular disease and higher mortality (1–3). Although the pressor effect of long term excess in salt intake has implications for a chronic blood pressure elevation, we have previously demonstrated an immediate pressor response to oral dietary salt (IPROS) in salt sensitivity of blood pressure (SSBP), in a young population of normotensive individuals (4). Previously, it has been suggested that the contribution of excess dietary salt to hypertension is mediated by salt's direct damaging effect on the endothelial and erythrocyte glycocalyx resulting in the extravasation of fluids and sodium, inflammation and hypertension (5). We have reported recently that high salt intake was associated with poor vascular sodium buffering capacity or high erythrocyte glycocalyx sensitivity to sodium (eGCSS) in a cohort of Black people in Zambia (6). Previous studies have reported sex differences in blood pressure regulation (7–10) including SSBP (11). Although SSBP is prevalent in both women and men, there is more evidence showing that premenopausal women have higher prevalence compared to men and menopause increases this prevalence and severity (12–15). However, the role of eGCSS in sex-specific responses to an acute salt-load or SSBP remains to be known. Moreover, most studies on SSBP and eGCSS were conducted in White populations whereas Black populations, particularly in Sub-Sahara Africa have not been studied. Thus, the goal of this study was to determine the sex differences in the relationship between eGCSS and SSBP in a young black population from sub-Saharan Africa (SSA).

2 Materials and methods

2.1 Study design and setting

This was a cross sectional study, where we used secondary data from a time series clinical trial conducted at Livingstone University Teaching Hospital among a young normotensive black population (4).

2.2 Study procedure and recruitment

The recruitment, study procedures and demographic characteristics of this population have been described in detail previously (4). Briefly, we selected only participants from the dataset of 127 participants (4) who had complete information on all the variables (employment status, marital status, HIV status, ankle brachial index) including eGCSS ($n = 117$). A total of 117 participants were studied who had undergone a salt loading procedure to diagnose SSBP based on an IPROS (4) after an overnight fast of at least 8 hrs. Participants rested for 40 min prior to receiving 2 g of oral salt (~788 mg or 34 mmol of sodium) and blood pressure was monitored and recorded for 120 min in 10-min intervals.

Blood pressure was measured using an automated validated sphygmomanometer (Omron HEM-7120; Omron Healthcare Co., Limited, Kyoto, Japan) while adhering to accurate blood pressure monitoring procedures (4). Sociodemographic characteristics were collected prior to the intervention. The ankle-brachial index was recorded with the aid of a Sonotrax Vascular Doppler (Ultrasonic pocket doppler; Shanghai International Holding Corp. GmbH, Hamburg, Germany), a vascular probe and a sphygmomanometer with attached gauge.

The eGCSS determination was described in detail by Oberleithner and Wilhelmi (16) and we recently described it (6), as well as others (17). Briefly, 50 µL of capillary blood is used to determine erythrocyte sedimentation for 60 min in a hematocrit tube. eGCSS was calculated as a percentage relative to the standard values of 21.4 and 26.1 mm for males and females, respectively (16).

2.3 Definitions and outcomes

We diagnosed SSBP based on our previous clinical trial study described in detail (4). Briefly, SSBP was diagnosed as mean arterial pressure (MAP) difference of ≥10 mmHg between the baseline and the highest MAP during the 120-min time course after salt loading. To compute the *ankle-brachial* index, we used the higher of two systolic blood pressure (SBP) from the dorsalis pedis and posterior tibial arteries in the foot and divided by the higher brachial SBP in the participant's arms. The measurements were taken with the participant in supine position.

2.4 Data analysis

We used IBM SPSS ver. 22.0 (IBM Corp., Armonk, NY, United States) and GraphPad Prism version 9.5.1 for all descriptive and inferential statistical analyses and graphs. We used Mann–Whitney *U* test to compare age, body mass index, ankle brachial index, fasting blood sugar, eGCSS and red blood cell count between salt sensitive and salt resistant individuals. To compare systolic and diastolic means between males and females, we used ANOVA, Welch's test with multiple comparisons. To compare distributions between categorical variables and SSBP, we used the Chi-square test. To determine correlates of SSBP we used univariable and multivariable logistic regression models. Age, sex and body mass index have been reported in multiple studies to influence blood pressure responses to salt intake (14, 18–21). We therefore included these three variables in the multiple logistic regression model to control for confounding.

2.5 Ethical approval

We received ethics clearance and approval from the University of Zambia Biomedical Research Ethics committee (IRB00001131 of IORG0000774) and the National Health Research Ethics Board under reference number 981–2020. All participants signed an Institutional Review Board-approved consent form after a thorough explanation of the protocol before being included in the study. The study was conducted in accordance with the Declaration of Helsinki.

3 Results

3.1 Study characteristics

The median age (interquartile range, IQR) of participants was 29 years (22–45 years) with 1:1 male-to-female ratio (Supplementary Table S1).

Most participants were single (55.6%), unemployed (74.4%), HIV negative (96%), salt sensitive (61.5%), and had high eGCSS (64.1%) with a normal ankle-brachial index (94.9%), red blood cell count, fasting blood sugar and body mass index. Apart from eGCSS, all variables were comparable between salt sensitive and salt resistant groups.

3.2 Sex differences in blood pressure response to dietary salt

Although the proportion of males and females did not differ between salt sensitive and salt resistant groups, we found significant sex differences in systolic and diastolic blood pressure response to dietary salt (Figure 1). Males had higher systolic blood pressure (SBP) than females at baseline, which was sustained throughout the 120 min after ingesting salt (Figure 1A). Diastolic blood pressure (DBP) for males and females were similar at baseline (time 0) but significant differences were noted at 30, 40, 70, 80, and 100–120 min with males recording higher DBP (Figure 1B). Overall, males had higher SBP (Figure 1C) and DBP (Figure 1D) increase compared to females.

To determine the blood pressure variability and degree of change in all participants, females and males, respectively, we compared the differences between the final and baseline blood pressure changes (Figure 2). We found that SBP, DBP and mean arterial pressure but not pulse pressure (PP), were significantly higher in all participants and for each sex following salt loading compared to baseline values. This suggested the presence of an immediate pressor response to oral salt.

3.3 Erythrocyte glycocalyx sensitivity to sodium and salt-sensitivity of blood pressure

We compared eGCSS among salt sensitive and salt resistant participants and found that the salt resistant had a higher median (IQR) eGCSS [156% (128, 172, IQR)] when compared to the salt sensitive group [129% (100, 156)] (Figure 3A). We have previously compared the proportions of participants with SSBP to eGCSS tertile and found a significant relationship between the two characteristics (4). Compared to the salt resistant group, participants with SSBP were more in the low and average eGCSS tertile (Figure 3B) (4). We therefore compared eGCSS scores by sex between salt sensitive and salt resistant participants and found that eGCSS was higher in salt resistant females compared to salt sensitive females and males (Figure 3C).

To further explore this relationship by sex, we then compared correlations between eGCSS and changes in blood pressure between baseline and highest change (delta) and found that SBP change correlated negatively with eGCSS in the whole group (Figure 4A), females (Figures 4B,D) and males (Figures 4C,D) but the relationship was not significant ($p > 0.05$). DBP change in the whole group (Figure 4E) correlated negatively with eGCSS and the correlation was significant ($r = -0.21$, $p = 0.017$) but not so when segregated by sex (Figures 4F–H). MAP correlated negatively with eGCSS in the whole group (Figure 4I) ($r = -0.20$, $p = 0.023$) and among the females (Figure 4J) ($r = -0.32$, $p = 0.013$) but not among the males (Figures 4K,L) ($r = -0.06$, $p = 0.605$) suggesting a specific sex-driven contribution. Pulse pressure (PP) correlated positively with eGCSS in

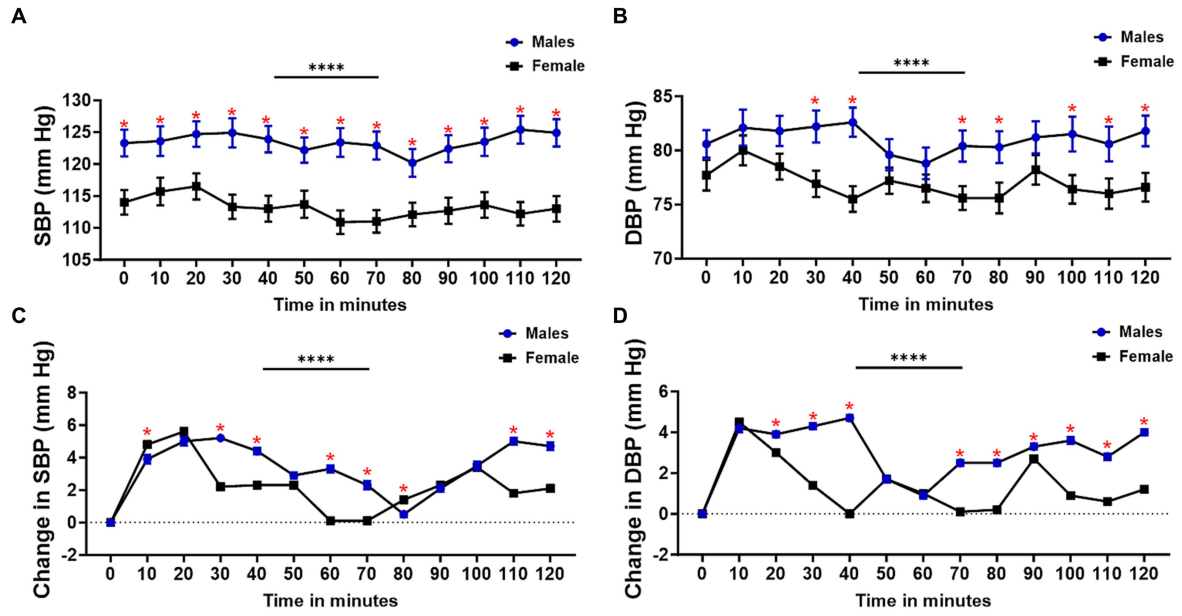

FIGURE 1
Sex differences in blood pressure response to dietary salt. **(A)** Systolic blood pressure (SBP), **(B)** diastolic blood pressure (DBP), **(C)** change in SBP, and **(D)** change in DBP from baseline (0 min) for males and females. Welch's test was used to compare mean blood pressure between males and females **(A,B)**. Overall BP changes and changes at each interval were compared using a 2-way ANOVA with Šídák's multiple comparisons test **(C,D)**. Asterisks indicate a time point with significant differences in blood pressure between males and females. *Interval with significant differences between blood pressure ($p < 0.05$); ****$p < 0.0001$.

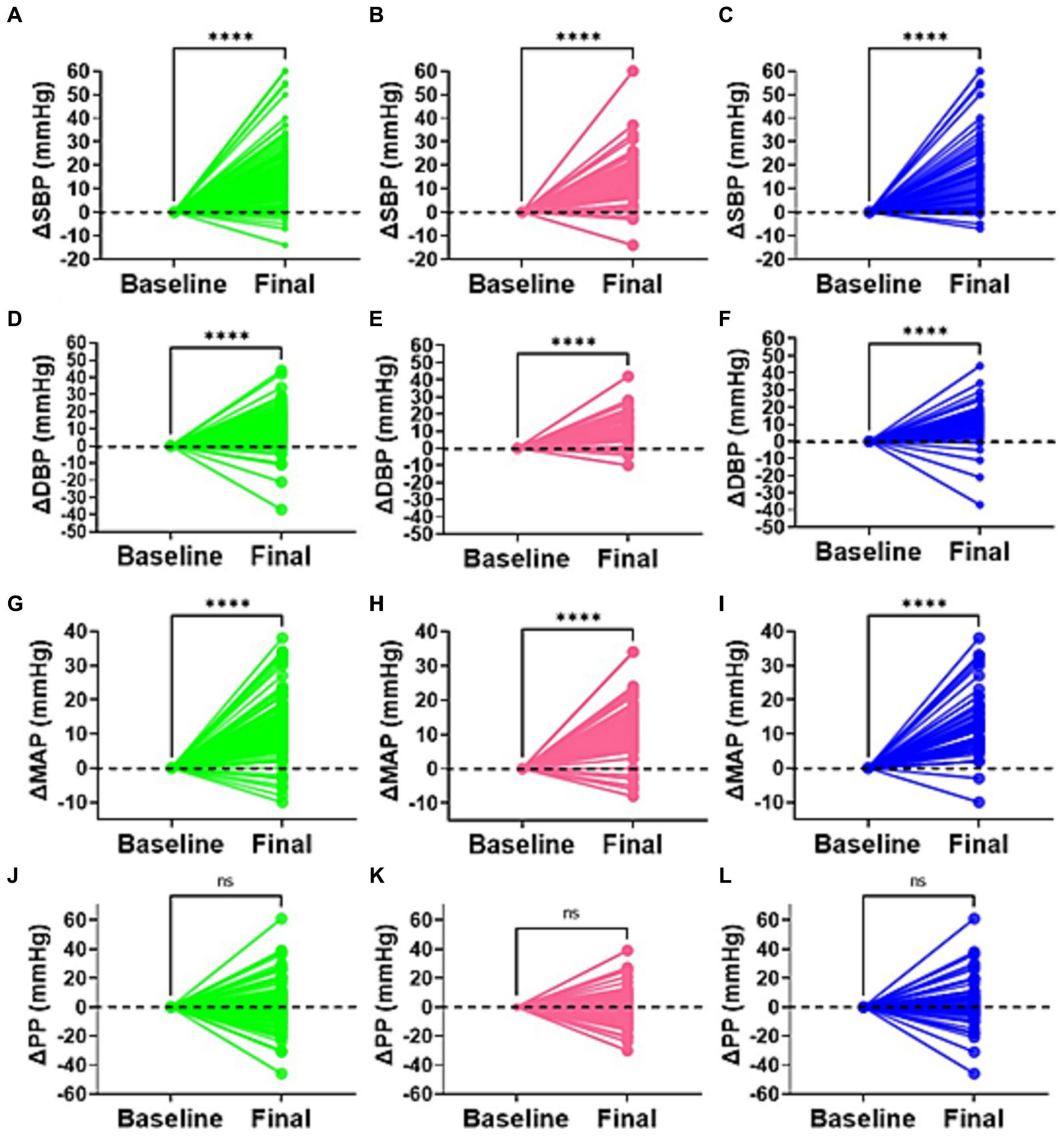

FIGURE 2
Delta difference in blood pressure. Changes in blood pressure between baseline and highest point (final) for all participants (green), females (pink), and males (blue) is indicated for **(A–C)** systolic blood pressure (SBP), **(D–F)** diastolic blood pressure (DBP), **(G–I)** mean arterial pressure (MAP), and **(J–L)** pulse pressure. ****$p < 0.0001$. ns, not statistically significant ($p > 0.05$).

the whole group (Figure 4M), in females (Figure 4N), and males (Figures 4O,P) but the relationship was not significant.

3.4 Sex differences in the association between erythrocyte glycocalyx sensitivity to sodium and salt-sensitivity of blood pressure

To determine the direction, magnitude and tertile of eGCSS associated with SSBP, we performed a logistic regression model and adjusted eGCSS for age, sex and body mass index (Table 1). We found that eGCSS correlated negatively with SSBP. In order to determine the contribution of each eGCSS category in predicting SSBP, we repeated the regression model using eGCSS tertile and found that participants with average eGCSS were 5.9 times more likely to have an SSBP compared to participants with high eGCSS. We repeated the model with eGCSS dichotomized and found that participants with low-to-average eGCSS were 4.4 times more likely to have SSBP compared to those with high eGCSS.

To account for sex differences, we segregated the regression model in Table 1 by sex and found that the negative significant association between eGCSS and SSBP was driven by female sex (Table 2), given that the statistical significance was lost in the males (Table 3).

4 Discussion

The goal of our study was to determine the sex differences in the relationship between eGCSS and SSBP and other variables in young normotensive black adults. There is currently no standard protocol for the diagnosis of SSBP and the current methods are still not feasible in

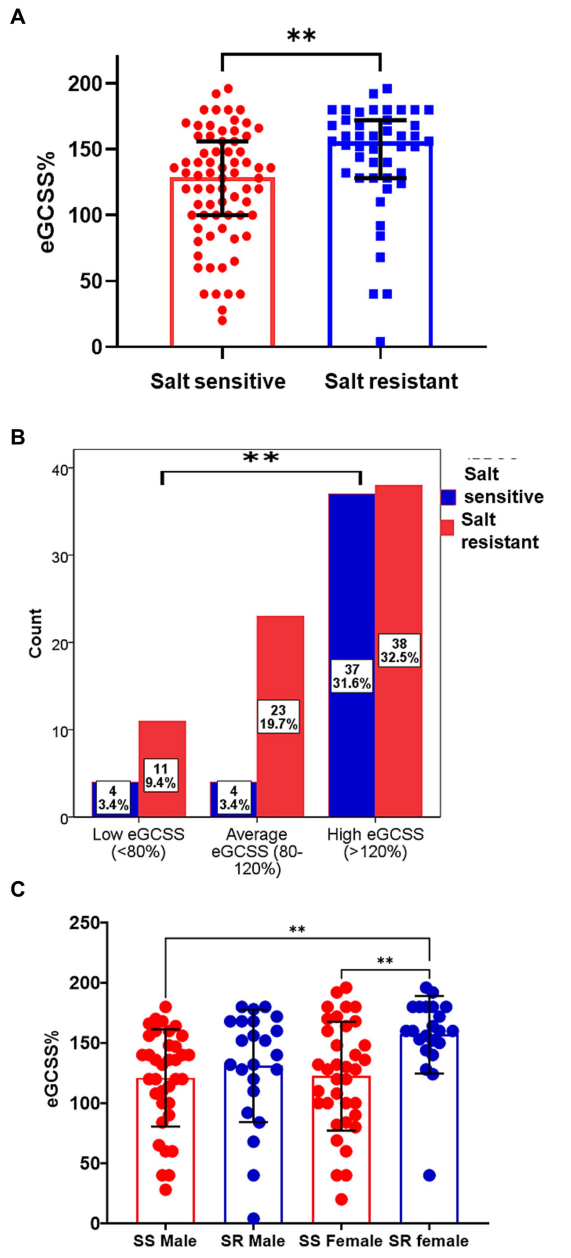

FIGURE 3
Erythrocyte glycocalyx sensitivity to sodium among salt sensitive and salt resistant groups. **(A)** The median (IQR) eGCSS among the salt sensitive [129% (100, 156,)] was lower compared to the salt resistant group[156% (128, 172, IQR)]. Mann–Whitney *U* test used. **(B)** SSBP status Stratification to eGCSS tertile Chi-square used. **(C)** Comparison of eGCSS between Salt sensitive (SS) and salt resistant (SR) males and females. eGCSS was higher in SR female compared to SS female and SS males; Brown-Forsythe and Welch ANOVA with Dunnett's T3 multiple comparisons test. eGCSS, erythrocyte glycocalyx sensitivity to sodium; IQR, interquartile range; SSBP, salt sensitivity of blood pressure. $^{**}p < 0.01$.

clinical settings. We used an acute protocol to identify an immediate pressor response to an oral dose of NaCl to define SSBP. This is the first study known to us to demonstrate that eGCSS correlated negatively with SSBP in a sex-specific manner.

The erythrocyte and vascular endothelial glycocalyces have negatively charged heparan sulfate residues that inhibit erosion on each other by repelling effects and selectively bind sodium (Figure 5A) such that in excess sodium intake, the glycocalyx's buffering capacity is decreased resulting in erosion and damage to the glycocalyces (Figure 5B) (22, 23). We recently found that high salt intake correlates with a high eGCSS (6). The eGCSS is a reflection and marker of cell membrane damage in hypertension due to sodium retention characterized by a low-renin state as demonstrated recently by McNally et al. (17).

Chronic high salt intake is well recognized as a risk factor for future hypertension and mortality (1, 2), but the acute effects of salt and its relation to the status of the erythrocyte and endothelial glycocalyx are not well studied. A study by Dickinson et al. demonstrated that salt intake can have immediate effects on endothelial function assessed by brachial artery flow-mediated dilatation (FMD) (24). Compared to a low salt meal, consuming a high salt meal containing 65 mmol of Na^+ suppressed FMD at 30 and 60 min postprandial without any significant differences in blood pressure and reactive hyperemia index. However, in a similar study by Wahab et al. (25) where participants consumed a high salt diet containing 4 g of salt equivalent to 68 mmol of Na^+, they found that FMD increased along with plasma sodium, correlating with carotid pulsatility index. In both studies, they did not measure eGCSS in order to make comparisons with glycocalyx health. Our study also did not assess FMD or carotid pulsatility index to relate with endothelial function. However, evidence for the effect of salt on endothelial function is abundant (24, 26–30). Excess sodium acts on the endothelium and cells of the immune system resulting in oxidative stress, increased endothelial cell stiffness, damage to the endothelial glycocalyx and ultimately, endothelial dysfunction (31–34).

We found that eGCSS correlated negatively with SSBP, especially in individuals with average eGCSS but the mechanism is not known and is beyond the scope of this current study. It is conceivable that in individuals with high eGCSS, the damage to the glycocalyx and endothelium, which is associated with arterial stiffness and poor conduit artery elasticity may inhibit early detection of an acute response to salt. In contrast, in individuals with low-to-average eGCSS, arterial function and elasticity is preserved, and it can be detected early. Further follow-up studies are required to ascertain this relationship.

Although blood pressure was significantly higher in both males and females during the time course compared to baseline, systolic and diastolic blood pressure were consistently higher in males than in females. We found that blood pressure changes from baseline and several time points (SBP at 10, 30, 40, 60 70, 80, 110, and 120 and DBP at 20, 30, 40, and 70–120 min) were higher in males than in females. It is well known that males tend to have higher blood pressure compared to premenopausal females (9, 35, 36). While eGCSS was associated with SSBP, this relationship was abrogated by the male sex. Why the significant association was sustained in females remains to be explored and is beyond the scope of this study. However, emerging evidence now indicates that women in premenopausal age are more salt sensitive compared to men (37). Thus, women's vascular function and response to salt may be physiologically deranged in SSBP compared to men. It is important to also emphasize that black individuals are more salt sensitive compared to white individuals and this difference may be determined by a lot of factors such as disparities in historical and contemporary socioeconomic status, genetic susceptibility, sodium handling by the

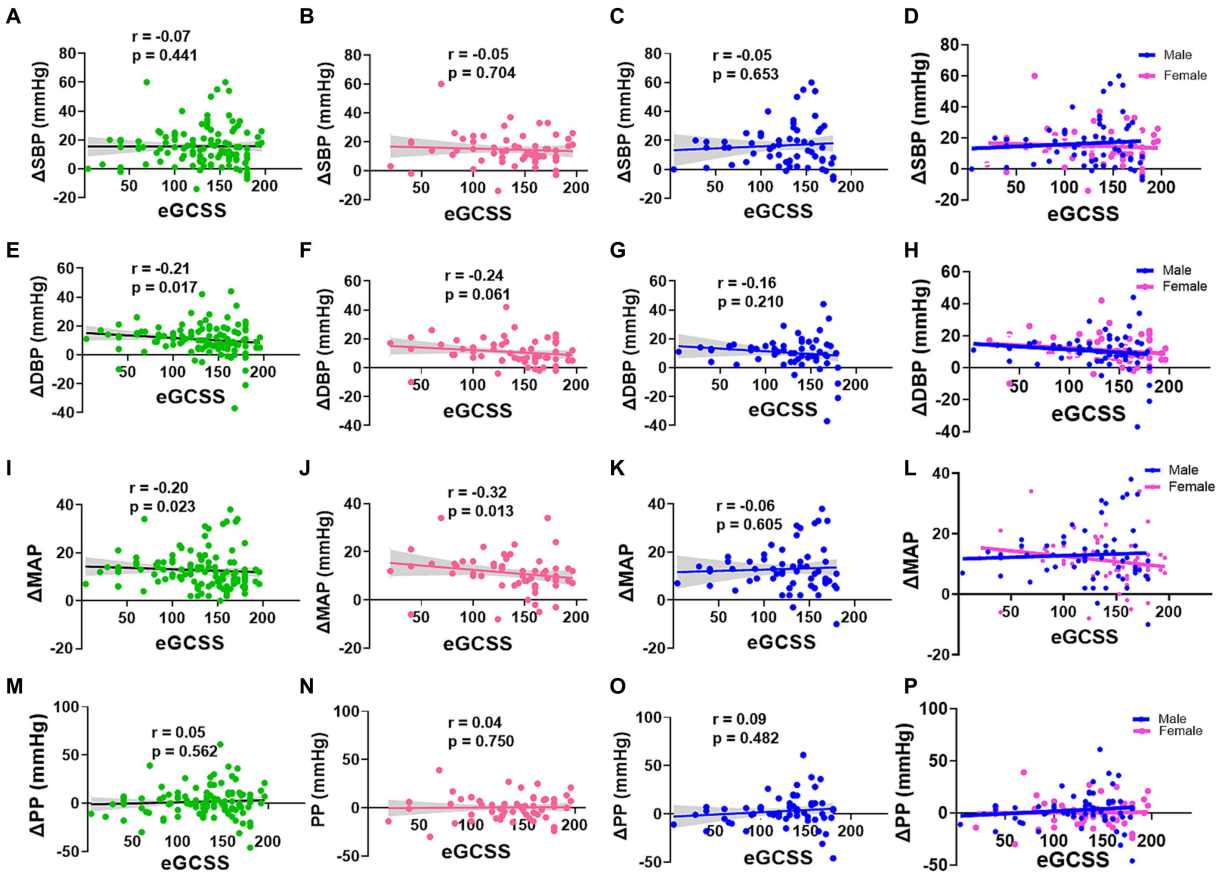

FIGURE 4
Correlation between Blood pressure change and Erythrocyte glycocalyx sensitivity to sodium by sex. SBP, DBP, MAP, and PP change (Δ) in all participants **(A,E,I,M)**, females **(B,F,J,N)**, males **(C,G,K,O)**, and males and females superinterposed **(D,H,L,P)** respectively, was performed using spearman's correlation. SBP, systolic blood pressure; DBP, diastolic blood pressure (DBP); MAP, mean arterial pressure; PP, pulse pressure; eGCSS, erythrocyte glycocalyx sensitivity to sodium.

TABLE 1 **Predictors of SSBP in logistic regression (all participants).**

Variable	Odds ratio (OR) (95% CI)	*p*-value	Adjusted odds ratio AOR (95%CI)	*p*-value
Age, years	0.99 (0.97, 1.02)	0.844	0.99 (0.96, 1.02)	0.597
Sex				
Females	1		1	
Males	0.95 (0.45, 2.01)	0.907	0.82 (0.36, 1.83)	0.633
Body mass index (kg/m^2)	1.00 (0.94, 1.06)	0.834	1.01 (0.95, 1.08)	0.629
*eGCSS scores	0.98 (0.97, 0.99)	**0.010**	0.98 (0.97, 0.99)	**0.008**
*eGCSS tertile (%)				
High (>120)	1		1	
Low (<80)	2.67 (0.78, 9.16)	0.117	2.89 (0.82, 10.14)	0.097
Average (80–120)	5.59 (1.76, 17.75)	**0.003**	5.99 (1.86, 19.28)	**0.003**
*eGCSS category (%)				
High (>120)	1		1	
Low-to-average (≤120)	4.13 (1.69, 10.11)	**0.002**	4.46 (1.78, 11.13)	**0.001**

EGCSS, erythrocyte glycocalyx sensitivity to sodium. *Adjusted for age, sex and body mass index in the model. Bold values delineate *p* value <0.05.

TABLE 2 Predictors of SSBP among females in logistic regression.

Variable	Odds ratio (OR) (95% CI)	*p*-value	Adjusted odds ratio AOR (95%CI)	*p*-value
Age, years	0.99 (0.95, 1.03)	0.786	0.98 (0.94, 1.02)	0.398
Body mass index (kg/m^2)	1.04 (0.96, 1.13)	0.307	1.03 (0.93, 1.13)	0.508
*eGCSS	0.97 (0.95, 0.99)	**0.007**	0.97 (0.95, 0.99)	**0.008**
*eGCSS tertile (%)				
High (> 120)	1		1	
Low (< 80)	5.52 (0.59, 51.64)	0.134	5.23 (0.52, 52.17)	0.159
Average (80–120)	22.06 (0.57, 98.84)	0.970	23.06 (0.53, 102.96)	0.990
*eGCSS category (%)				
High (> 120)	1		1	
Low-to-average (≤120)	18.78 (2.27, 154.99)	**0.006**	21.09 (2.45, 181.65)	**0.006**

EGCSS, erythrocyte glycocalyx sensitivity to sodium. *Adjusted for age, and body mass index in the model. Bold values delineate *p* value <0.05.

TABLE 3 Predictors of SSBP among males in logistic regression.

Variable	Odds ratio (OR) (95% CI)	*p*-value	Adjusted odds ratio AOR (95%CI)	*p*-value
Age, years	1.00 (0.96, 1.03)	0.982	1.00 (0.96, 1.05)	0.748
Body mass index (kg/m^2)	0.93 (0.84, 1.04)	0.237	0.93 (0.82, 5.64)	0.317
*eGCSS tertile	0.99 (0.98, 1.01)	0.380	0.99 (0.98, 1.00)	0.544
*eGCSS tertile (%)				
High (> 120)	1		1	
Low (< 80)	1.68 (0.36, 7.83)	0.506	1.39 (0.27, 6.96)	0.688
Average (80–120)	2.31 (0.61, 8.70)	0.214	2.06 (0.53, 7.96)	0.292
*eGCSS category (%)				
High (> 120)	1		1	
Low-to-average (≤120)	2.04 (0.67, 6.16)	0.204	1.78 (0.56, 5.64)	0.323

EGCSS, erythrocyte glycocalyx sensitivity to sodium. *Adjusted for age, and body mass index in the model.

kidney, the renin-angiotensin-aldosterone system and the autonomic nervous system's response to salt, and environmental factors (38–42). However, our study was limited to black individuals and therefore could not determine racial disparities.

4.1 Clinical implications

4.1.1 Clinical implications of high eGCSS

Most participants had high eGCSS. From what we know so far about eGCSS, it is a novel test that reflects endothelial and erythrocyte surface damage but its utility in clinical practice has not yet been established. Results from multiple studies found that high eGCSS was associated with low renin levels and high albumin-creatinine ratio in hypertension (17), correlated with high salt intake in a similar population from Zambia (6), and was associated with hypertension despite use of antihypertensive medication (43). One study also found that about one third of healthy individuals exhibit a high eGCSS (16) suggesting that eGCSS can be influenced by environmental factors such as dietary salt (44). An experimental study by Oberleithner et al. (22) demonstrated that sodium renders endothelial cells sticky to red blood cells suggesting a potential contribution to thrombotic events. The sex differences in correlates of eGCSS have not been studied and future studies are needed to determine mechanisms.

4.1.2 Clinical implications of an acute SSBP and ABI

The bimodal pattern of sustained SBP and DBP changes during the 2-h time course in this study is interesting and akin to the broader 24-h ambulatory blood pressure changes that were described by Degaute et al. (45) three decades ago. However, the bimodal pattern of blood pressure change in our study was slightly different in men and women. In men, the highest SBP were recorded at 30- and 110-min and the highest DBP at 40- and 120-min post salt load. In women the highest SBP were recorded at 20 and 100-min while the DBP were highest at 10- and 90-min. This suggests that women respond earlier to the pressor effect of salt than men. Our data also demonstrates that men have a more severe increase in blood pressure with blood pressure elevations that do not return to baseline within 2 h compared to women. In women, SBP and DBP returned to baseline twice and thrice, respectively, during the 2-h time course. This may suggest that the stimulus responsible for the initial elevation in SBP and DBP in men does not dissipate quickly in men compared to women. However, more studies are required to validate this.

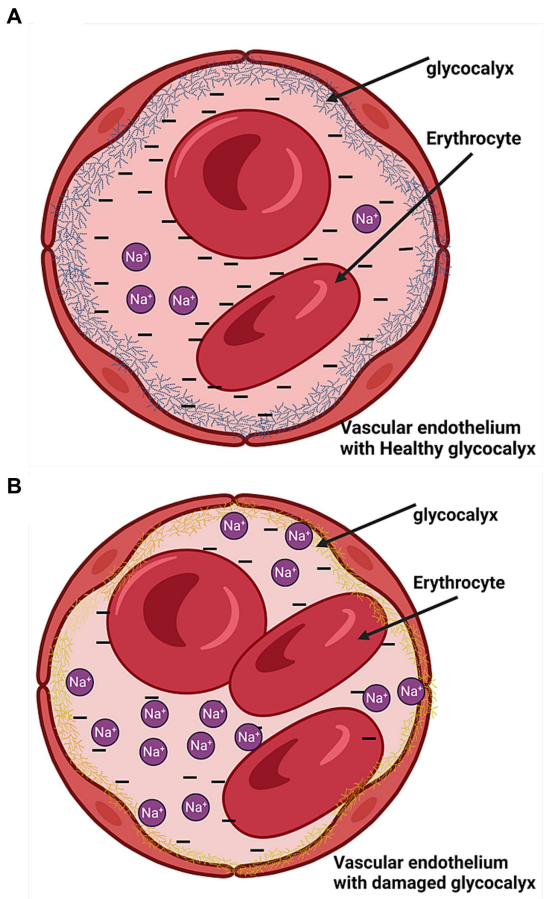

FIGURE 5
Endothelial cells with the glycocalyx. **(A)** Health glycocalyx. **(B)** Damaged glycocalyx (yellow).

A tendency to have significant perturbations in blood pressure may contribute to acute or chronic blood–brain barrier disruptions depending on the magnitude and duration of the perturbation (46). Generally, it is normal for blood pressure to fluctuate during the day but significant and chronic perturbations arising from dietary salt may be associated with elevated 24-h ambulatory blood pressure, worsening of non-dipping blood pressure and increase the risk for hypertension (47). A randomized, double-blind crossover study by Tzemos et al. (48) found that 5-day acute salt loading impaired endothelial function, electric repolarization and left ventricular mechanical relaxation in young healthy normotensives. We used a one-dose salt load to monitor its impact on blood pressure in young normotensive black individuals who were seated for 2 h and whose blood pressure increase was sustained for the entire follow up period in both sexes. Our study suggests two aspects of clinical significance. First, with three routine meals per day customary for most Zambians that have similar or higher salt load, blood pressure increases are likely to be more significant in increasing the 24-h ambulatory blood pressure. With chronic high salt habits, this may likely increase the risk for the development of hypertension. The second implication is that if blood pressure is significantly raised in a sedentary sitting position, higher blood pressure perturbations are likely to occur with routine activity and consequentially increase vascular stress on blood vessels and hypertensive tendencies. However, these hypotheses need to be validated in future studies.

It was also interesting to find that 5.1% of this young population studied had evidence of peripheral artery disease or narrowing of peripheral arteries as evidenced from a higher ABI. Sub-analysis of this data (not shown) indicated that none of the participants with peripheral artery disease were living with HIV and the distribution by sex was comparable. However, the number of participants with abnormal ABI was not sufficient to draw any conclusions.

4.2 Strengths and limitations

To our knowledge, this is the first study to report sex dimorphism mediating the relationship between eGCSS and SSBP in a young population of normotensive black individuals. In the main prior study, we could not find a significant relationship between eGCSS and SSBP because we only included eGCSS tertiles in the multivariable analysis and adjusted for more variables that were not significant at univariable analysis (4). However, in this sub study, we used three models with fewer variable adjustments (adjusting only for variables that influence the outcome) to avoid overfitting the regression model. In the first instance, eGCSS was added to the logistic regression model as a continuous variable, then we repeated the analysis replacing the continuous variable eGCSS with eGCSS tertiles and lastly, we dichotomized eGCSS into high and low-to-average categories (Table 3).

The experimental nature of the study to determine SSBP is one of the strengths of this study. Although there are different protocols used to define SSBP with variations in blood pressure responses (49, 50), the choice of using an oral salt loading protocol to define SSBP is appropriate in the context of studies focused on the immediate effects of salt on blood pressure. However, acute salt loading protocols may underestimate the burden of SSBP. Protocols involving longer periods of salt loading are more accurate and have higher sensitivity in determining SSBP status. eGCSS is a potentially useful marker of sodium sensitivity to damage induced by dietary salt at the cellular level (17) but requires further validation studies to ascertain whether it can be used in clinical settings. For studies assessing sex differences within subcategories of eGCSS, this study may not have been adequately powered. To generalize and validate these findings, a study with a larger sample size is required. Another limitation is that there was no information collected regarding the stage of the menstrual cycle during the time the women participated in the study. Variations in blood pressure in women that reflects the stage of menstrual cycle has been reported in literature (51, 52).

Although eGCSS was associated with SSBP and driven by female sex, there are several markers of endothelial function and factors that influence eGCSS such as previous habitual intake of salt that were not measured in this study. This is a major limitation. It was going to be important to also report on additional factors that influence salt handling such as glomerular filtration rate, plasma levels of insulin, renin, aldosterone, and angiotensin II. These data were not available in this study and will be the focus of future studies. However, renal and kidney function tests were measured to exclude participants with any kidney or liver abnormality but have not been presented as they were not intended for statistical analyses prioli. It is possible that because we only included a younger population in this study, variances in both

eGCSS scores and SSBP changes which are affected by age could not be elaborated. The level of physical activity may likely affect eGCSS and was not determined in this study. The findings from this study should therefore be interpreted in the context of the specific characteristics of this population. We are currently studying dietary salt effects in our laboratory, and we plan to have additional robust techniques to assess endothelial function from the effects of dietary salt in future. Finally, this was a cross sectional study and does not implicate causality.

5 Conclusion

The eGCSS correlated negatively with SSBP in females but not in males in this study and the immediate pressor response to dietary salt was significantly higher in males compared to females. The implication of our findings suggests that there could be a novel underlying mechanism linking eGCSS with acute salt loading. However, more studies are required to elucidate this link and robust markers of endothelial function are required to validate the role that eGCSS plays in SSBP.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the University of Zambia Biomedical Research Ethics committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. BH: Data curation, Validation, Writing – review & editing. KP: Validation, Visualization, Writing – review & editing. AK: Formal analysis, Funding acquisition, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1334853/full#supplementary-material

References

1. Subasinghe AK, Arabshahi S, Businge D, Evans RG, Walker KZ, Riddell MA, et al. Association between salt and hypertension in rural and urban populations of low to middle income countries: a systematic review and meta-analysis of population based studies. *Asia Pac J Clin Nutr*. (2016) 25:402–13. doi: 10.6133/apjcn.2016.25.2.25

2. Graudal N, Jürgens G, Baslund B, Alderman MH. Compared with usual sodium intake, low- and excessive-sodium diets are associated with increased mortality: a meta-analysis. *Am J Hypertens*. (2014) 27:1129–37. doi: 10.1093/ajh/hpu028

3. Masenga SK, Kirabo A. Salt and gut microbiota in heart failure. *Curr Hypertens Rep*. (2023) 25:173–84. doi: 10.1007/s11906-023-01245-5

4. Masenga SK, Pilic L, Hamooya BM, Nzala S, Heimburger DC, Mutale W, et al. Immediate pressor response to oral salt and its assessment in the clinic: a time series clinical trial. *Clin Hypertens*. (2022) 28:25. doi: 10.1186/s40885-022-00209-2

5. Oberleithner H, Peters W, Kusche-Vihrog K, Korte S, Schillers H, Kliche K, et al. Salt overload damages the glycocalyx sodium barrier of vascular endothelium. *Pflugers Arch*. (2011) 462:519–28. doi: 10.1007/s00424-011-0999-1

6. Masenga SK, Pilic L, Malumani M, Hamooya BM. Erythrocyte sodium buffering capacity status correlates with self-reported salt intake in a population from Livingstone, Zambia. *PLoS One*. (2022) 17:e0264650. doi: 10.1371/journal.pone.0264650

7. Reckelhoff JF. Gender differences in the regulation of blood pressure. *Hypertension*. (2001) 37:1199–208. doi: 10.1161/01.HYP.37.5.1199

8. Gillis EE, Sullivan JC. Sex differences in hypertension: recent advances. *Hypertension*. (2016) 68:1322–7. doi: 10.1161/HYPERTENSIONAHA.116.06602

9. Connelly PJ, Currie G, Delles C. Sex differences in the prevalence, outcomes and management of hypertension. *Curr Hypertens Rep*. (2022) 24:185–92. doi: 10.1007/s11906-022-01183-8

10. Masenga SK, Povia JP, Mutengo KH, Hamooya BM, Nzala S, Heimburger DC, et al. Sex differences in hypertension among people living with HIV after initiation of antiretroviral therapy. *Front Cardiovasc Med*. (2022) 9:1006789. doi: 10.3389/fcvm.2022.1006789

11. Sahinoz M, Elijovich F, Ertuglu LA, Ishimwe J, Pitzer A, Saleem M, et al. Salt-sensitivity of blood pressure in blacks and women: a role of inflammation, oxidative stress and ENaC. *Antioxid Redox Signal*. (2021) 35:1477–93. doi: 10.1089/ars.2021.0212

12. He J, Gu D, Chen J, Jaquish CE, Rao DC, Hixson JE, et al. Gender difference in blood pressure responses to dietary sodium intervention in the GenSalt study. *J Hypertens*. (2009) 27:48–54. doi: 10.1097/HJH.0b013e328316bb87

13. Wesseling S, Koeners MP, Joles JA. Salt sensitivity of blood pressure: developmental and sex-related effects1234. *Am J Clin Nutr*. (2011) 94:S1928–32. doi: 10.3945/ajcn.110.000901

14. Faulkner JL, Belin de Chantemèle EJ. Female sex, a major risk factor for salt-sensitive hypertension. *Curr Hypertens Rep*. (2020) 22:99. doi: 10.1007/s11906-020-01113-6

15. Kim J-M, Kim T-H, Lee H-H, Lee SH, Wang T. Postmenopausal hypertension and sodium sensitivity. *J Menopausal Med*. (2014) 20:1–6. doi: 10.6118/jmm.2014.20.1.1

16. Oberleithner H, Wilhelmi M. Salt sensitivity determined from capillary blood. *Ren Physiol Biochem*. (2016) 41:355–64. doi: 10.1159/000443438

17. McNally RJ, Morselli F, Farukh B, Chowienczyk PJ, Faconti L. A pilot study to evaluate the erythrocyte glycocalyx sensitivity to sodium as a marker for cellular salt sensitivity in hypertension. *J Hum Hypertens*. (2023) 37:286–91. doi: 10.1038/s41371-022-00683-z

18. Hoffmann IS, Alfieri AB, Cubeddu LX. Salt-resistant and salt-sensitive phenotypes determine the sensitivity of blood pressure to weight loss in overweight/obese patients. *J Clin Hypertens*. (2008) 10:355–61. doi: 10.1111/j.1751-7176.2008.07609.x

19. Sougawa Y, Miyai N, Morioka I, Utsumi M, Takeda S, Miyashita K, et al. The combination of obesity and high salt intake are associated with blood pressure elevation among healthy Japanese adolescents. *J Hum Hypertens*. (2020) 34:117–24. doi: 10.1038/s41371-019-0239-2

20. Overlack A, Ruppert M, Kolloch R, Kraft K, Stumpe KO. Age is a major determinant of the divergent blood pressure responses to varying salt intake in essential hypertension. *Am J Hypertens*. (1995) 8:829–36. doi: 10.1016/0895-7061(95)00213-9

21. Huang L, Trieu K, Yoshimura S, Neal B, Woodward M, Campbell NRC, et al. Effect of dose and duration of reduction in dietary sodium on blood pressure levels: systematic review and meta-analysis of randomised trials. *BMJ*. (2020) 368:m315. doi: 10.1136/bmj.m315

22. Oberleithner H, Wälte M, Kusche-Vihrog K. Sodium renders endothelial cells sticky for red blood cells. *Front Physiol*. (2015) 6:188. doi: 10.3389/fphys.2015.00188

23. Kusche-Vihrog K, Schmitz B, Brand E. Salt controls endothelial and vascular phenotype. *Pflugers Arch Eur J Physiol*. (2015) 467:499–512. doi: 10.1007/s00424-014-1657-1

24. Dickinson KM, Clifton PM, Keogh JB. Endothelial function is impaired after a high-salt meal in healthy subjects1–3. *Am J Clin Nutr*. (2011) 93:500–5. doi: 10.3945/ajcn.110.006155

25. Abdel Wahab MAK. Vascular effects of a single high salt meal. *Egypt Heart J*. (2016) 68:159–64. doi: 10.1016/j.ehj.2015.12.003

26. Boegehold MA. The effect of high salt intake on endothelial function: reduced vascular nitric oxide in the absence of hypertension. *J Vasc Res*. (2013) 50:458–67. doi: 10.1159/000355270

27. Bragulat E, de la Sierra A. Salt intake, endothelial dysfunction, and salt-sensitive hypertension. *J Clin Hypertens*. (2007) 4:41–6. doi: 10.1111/j.1524-6175.2002.00503.x

28. Vinaiphat A, Pazhanchamy K, JebaMercy G, Ngan SC, Leow MK-S, Ho HH, et al. Endothelial damage arising from high salt hypertension is elucidated by vascular bed systematic profiling. *Arterioscler Thromb Vasc Biol*. (2023) 43:427–42. doi: 10.1161/ATVBAHA.122.318439

29. Baqar S, Kong YW, Chen AX, O'Callaghan C, MacIsaac RJ, Bouterakos M, et al. Effect of salt supplementation on sympathetic activity and endothelial function in salt-sensitive type 2 diabetes. *J Clin Endocrinol Metabol*. (2020) 105:e1187–200. doi: 10.1210/clinem/dgz219

30. He FJ, Tan M, Ma Y, MacGregor GA. Salt reduction to prevent hypertension and cardiovascular disease: JACC state-of-the-art review. *J Am Coll Cardiol*. (2020) 75:632–47. doi: 10.1016/j.jacc.2019.11.055

31. Edwards DG, Farquhar WB. Vascular effects of dietary salt. *Curr Opin Nephrol Hypertens*. (2015) 24:8–13. doi: 10.1097/MNH.0000000000000089

32. Patik JC, Lennon SL, Farquhar WB, Edwards DG. Mechanisms of dietary sodium-induced impairments in endothelial function and potential countermeasures. *Nutrients*. (2021) 13:270. doi: 10.3390/nu13010270

33. Masenga SK, Hamooya BM, Nzala S, Kwenda G, Heimburger DC, Mutale W, et al. HIV, immune activation and salt-sensitive hypertension (HISH): a research proposal. *BMC Res Notes*. (2019) 12:424. doi: 10.1186/s13104-019-4470-2

34. Masenga SK, Kabwe LS, Chakulya M, Kirabo A. Mechanisms of oxidative stress in metabolic syndrome. *Int J Mol Sci*. (2023) 24:7898. doi: 10.3390/ijms24097898

35. Maranon R, Reckelhoff JF. Sex and gender differences in control of blood pressure. *Clin Sci*. (2013) 125:311–8. doi: 10.1042/CS20130140

36. Alhawari HH, Al-Shelleh S, Alhawari HH, Al-Saudi A, Aljbour Al-Majali D, Al-Faris L, et al. Blood pressure and its association with gender, body mass index, smoking, and family history among university students. *Int J Hypertens*. (2018) 2018:e4186496. doi: 10.1155/2018/4186496

37. Barris CT, Faulkner JL, Belin de Chantemèle EJ. Salt sensitivity of blood pressure in women. *Hypertension*. (2023) 80:268–78. doi: 10.1161/HYPERTENSIONAHA.122.17952

38. Lindhorst J, Alexander N, Blignaut J, Rayner B. Differences in hypertension between blacks and whites: an overview. *Cardiovasc J Afr*. (2007) 18:241–7.

39. Wenner MM, Paul EP, Robinson AT, Rose WC, Farquhar WB. Acute NaCl loading reveals a higher blood pressure for a given serum sodium level in African American compared to Caucasian adults. *Front Physiol*. (2018) 9:1354. doi: 10.3389/fphys.2018.01354

40. Robinson AT, Wenner MM, Charkoudian N. Differential influences of dietary sodium on blood pressure regulation based on race and sex. *Auton Neurosci*. (2021) 236:102873. doi: 10.1016/j.autneu.2021.102873

41. Pilic L, Mavrommatis Y. Genetic predisposition to salt-sensitive normotension and its effects on salt taste perception and intake. *Br J Nutr*. (2018) 120:721–31. doi: 10.1017/S0007114518002027

42. Pilic L, Lubasinski NJ, Berk M, Ward D, Graham CA-M, Da Silva AV, et al. The associations between genetics, salt taste perception and salt intake in young adults. *Food Qual Prefer*. (2020) 84:103954. doi: 10.1016/j.foodqual.2020.103954

43. Oberleithner H, Wilhelmi M. Vascular glycocalyx sodium store – determinant of salt sensitivity? *Blood Purif*. (2015) 39:7–10. doi: 10.1159/000368922

44. Sembajwe LF, Ssekandi AM, Namaganda A, Muwonge H, Kasolo JN, Kalyesubula R, et al. Glycocalyx-sodium interaction in vascular endothelium. *Nutrients*. (2023) 15:2873. doi: 10.3390/nu15132873

45. Degaute JP, van de Borne P, Linkowski P, Van Cauter E. Quantitative analysis of the 24-hour blood pressure and heart rate patterns in young men. *Hypertension*. (1991) 18:199–210. doi: 10.1161/01.hyp.18.2.199

46. Zhao N, Chung TD, Guo Z, Jamieson JJ, Liang L, Linville RM, et al. The influence of physiological and pathological perturbations on blood-brain barrier function. *Front Neurosci*. (2023) 17:1289894. doi: 10.3389/fnins.2023.1289894

47. Masenga SK, Kirabo A, Hamooya BM, Nzala S, Kwenda G, Heimburger DC, et al. HIV-positive demonstrate more salt sensitivity and nocturnal non-dipping blood pressure than HIV-negative individuals. *Clin Hypertens*. (2021) 27:2. doi: 10.1186/s40885-020-00160-0

48. Tzemos N, Lim PO, Wong S, Struthers AD, MacDonald TM. Adverse cardiovascular effects of acute salt loading in young normotensive individuals. *Hypertension*. (2008) 51:1525–30. doi: 10.1161/HYPERTENSIONAHA.108.109868

49. Elijovich F, Weinberger MH, Anderson CAM, Appel LJ, Bursztyn M, Cook NR, et al. Salt sensitivity of blood pressure: a scientific statement from the American Heart Association. *Hypertension*. (2016) 68:e7–e46. doi: 10.1161/HYP.0000000000000047

50. Kurtz TW, DiCarlo SE, Pravenec M, Morris RC. An appraisal of methods recently recommended for testing salt sensitivity of blood pressure. *J Am Heart Assoc*. (2017) 6:e005653. doi: 10.1161/JAHA.117.005653

51. Dunne FP, Barry DG, Ferriss JB, Grealy G, Murphy D. Changes in blood pressure during the normal menstrual cycle. *Clin Sci*. (1991) 81:515–8. doi: 10.1042/cs0810515

52. Chung H-F, Ferreira I, Mishra GD. The association between menstrual symptoms and hypertension among young women: a prospective longitudinal study. *Maturitas*. (2021) 143:17–24. doi: 10.1016/j.maturitas.2020.08.006

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High sodium intake and fluid overhydration predict cardiac structural and functional impairments in chronic kidney disease

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Background: High sodium intake and fluid overhydration are common factors of and strongly associated with adverse outcomes in chronic kidney disease (CKD) patients. Yet, their effects on cardiac dysfunction remain unclear.

Aims: The study aimed to explore the impact of salt and volume overload on cardiac alterations in non-dialysis CKD.

Methods: In all, 409 patients with CKD stages 1–4 (G1–G4) were enrolled. Daily salt intake (DSI) was estimated by 24-h urinary sodium excretion. Volume status was evaluated by the ratio of extracellular water (ECW) to total body water (TBW) measured by body composition monitor. Recruited patients were categorized into four groups according to DSI (6g/day) and median ECW/TBW (0.439). Echocardiographic and body composition parameters and clinical indicators were compared. Associations between echocardiographic findings and basic characteristics were performed by Spearman's correlations. Univariate and multivariate binary logistic regression analysis were used to determine the associations between DSI and ECW/TBW in the study groups and the incidence of left ventricular hypertrophy (LVH) and elevated left ventricular filling pressure (ELVFP). In addition, the subgroup effects of DSI and ECW/TBW on cardiac abnormalities were estimated using Cox regression.

Results: Of the enrolled patients with CKD, the median urinary protein was 0.94 (0.28–3.14) g/d and estimated glomerular filtration rate (eGFR) was 92.05 (IQR: 64.52–110.99) mL/min/1.73m^2. The distributions of CKD stages G1–G4 in the four groups was significantly different (p=0.020). Furthermore, compared to group 1 (low DSI and low ECW/TBW), group 4 (high DSI and high ECW/TBW) showed a 2.396-fold (95%CI: 1.171–4.902; p=0.017) excess risk of LVH and/or ELVFP incidence after adjusting for important CKD and cardiovascular disease risk factors. Moreover, combined with eGFR, DSI and ECW/TBW could identify patients with higher cardiac dysfunction risk estimates with an AUC of 0.704 (sensitivity: 75.2%, specificity: 61.0%). The specificity increased to 85.7% in those with nephrotic proteinuria (AUC=0.713). The magnitude of these associations was consistent across subgroups analyses.

Conclusion: The combination of high DSI (>6 g/d) and high ECW/TBW (>0.439) independently predicted a greater risk of LVH or ELVFP incidence in non-dialysis CKD patients. Moreover, the inclusion of eGFR and proteinuria improved the risk stratification ability of DSI and ECW/TBW in cardiac impairments in CKD.

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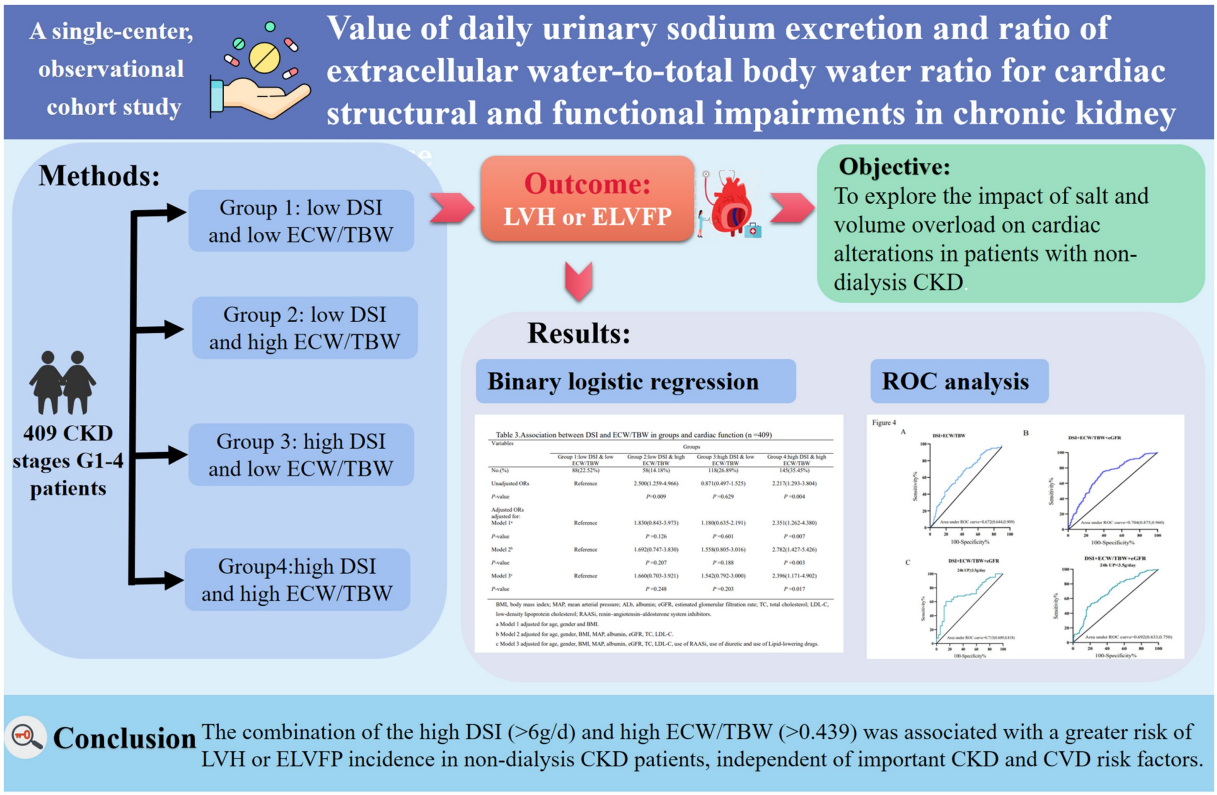

GRAPHICAL ABSTRACT

KEYWORDS

chronic kidney disease, total body water, extracellular water, daily salt intake, left ventricular hypertrophy, elevated left ventricular filling pressure

Highlights

- High sodium intake and fluid overhydration are common in CKD patients and strongly associated with adverse outcomes, yet effects on cardiac dysfunction remain unclear.
- The combination of the high daily salt intake (DSI) (>6g/d) and high extracellular water (ECW)/total body water (TBW) (>0.439) is associated with a greater risk of of cardiac structural and functional impairments in non-dialysis CKD patients, independent of important CKD and CVD risk factors.
- Adding eGFR and proteinuria into consideration would improve the ability of DSI and ECW/TBW for risk stratification of cardiac impairments in CKD patients.
- Salt and volume overload may be important mechanisms contributing to the CVD morbidity and mortality observed in non-dialysis CKD. Timely modifying the salt and fluid status may be benefit for achieving better CVD outcomes and therefore decreasing the burden of mortality in CKD.

1 Introduction

Chronic kidney disease (CKD) has emerged as an increasingly important risk factor contributing to cardiovascular diseases (CVD), which is currently the leading cause of morbidity and mortality worldwide (1, 2). Numerous epidemiological studies have reported a strong association between deteriorating renal function and ultimate outcomes of CVD, and severe CV events accounted for almost 50% of all deaths in the population with kidney disease (2–4). According to the 2019 Global Burden of Disease study, 15–20% of the world's population is affected by CKD, which is ranked the 12th leading risk factor affecting disability-adjusted life-years (5, 6). Thus, predicting CV risk and strategies for preventing the development of CV events in CKD would not only improve CKD outcomes but also give rise to significant amelioration of the heavy burden in public health and medical expenses.

Diastolic filling of the left ventricle (LV) is a highly complex process dependent on LV relaxation, LV compliance, and left atrial pressure, which is contributing to exercise capacity and quality of life (7). LV-filling pressure (LVFP), is recognized as a potential factor contributing to pulmonary congestion (8). Besides, the severity of CKD has been reported as the most independent predictors of

elevated LVFP (ELVFP) (8). Among the echocardiographic parameters, the ratio of early diastolic mitral inflow velocity (E) to early diastolic mitral annular velocity (e') has been proven to be a useful parameter for assessing LVFP (9–11). Recent European guidelines on heart failure (HF) proposed a resting average E/e' > 9 as an objective criterion of ELVFP (12). Moreover, E/e' > 9 showed an association with increased risk of CV and all-cause mortality (7). However, a huge body of evidence indicated that LVH was the most common structural abnormality associated with CKD patients, the prevalence of which ranged from 34 to 78%, with increasing values correlating with the worsening in renal function (13, 14). One of the main mechanisms involving LV hypertrophy (LVH) is a physiological response to pressure and volume overload (8, 15). Hence, exploring the risk factors of LVH and ELVFP not only provides a more in-depth understanding of the underlying mechanisms of the major complication but also highlights opportunities for potential therapeutic intervention with the aim to improve the CVD outcomes of CKD in clinical practice.

Salt and volume overload are key factors contributing to LVH, LV dilatation, and adverse CVD outcomes in CKD (14, 16). However, how to monitor salt and fluid balance in patients with non-dialysis CKD remains largely unclear in clinical practice. High salt intake as measured by 24-h urinary sodium excretion is associated with hypertension, increased urinary protein, occurrence of CVD, and kidney outcome in CKD (17–19). Volume overload is the consequence of excessive sodium loading by reduced glomerular filtration, and in turn contributes to hypertension in CKD patients (20). Moreover, volume overload has shown great promise as a potential modifiable risk factor of CKD progression and CVD (21). Hence, our primary aim was to analyze the effects of both daily salt intake (DSI) (by detecting 24-h urinary sodium excretion) and volume status as assessed by the ratio of extracellular water (ECW) to total body water (TBW) (measured using a body composition monitor) on cardiac abnormalities in patients with non-dialysis CKD. Additionally, we explored the specific group of patients with comorbid cardiac structural and functional impairments who may most benefit from water and sodium restriction.

2 Materials and methods

2.1 Subjects

This was a observational cohort study with patients recruited from the Department of Nephrology in the First Affiliated Hospital of Nanjing Medical University from December 2019 to April 2022. The inclusion criteria were as follows: (1) patients diagnosed with CKD, which was defined as abnormalities of kidney structure or function that persist for ≥3 months based on the Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guidelines (22); (2) patients cooperating with body composition analysis; (3) intact echocardiography data; and (4) estimated glomerular filtration rate (eGFR) > 15 mL/min/1.73 m^2. The exclusion criteria were as follows: (1) lack of body composition index and echocardiography information or clinic data; (2) age < 18 years; (3) clinical state affecting body composition, such as liver cirrhosis, active infectious disease, or acute CV events within 3 months before screening for inclusion; and (4) a 24-h urine sample either <500 mL or > 5,000 mL. Eventually, 409 patients were eligible for the analysis (Figure 1). This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (No. 2023-SR-178) and conducted in accordance with the guidelines of the Declaration of Helsinki.

2.2 Clinical and laboratory parameters

The complete clinical and laboratory information of enrolled patients was collected during hospitalization from medical records and included age, sex, blood pressure, history of diabetes mellitus (DM), history of hypertension, body mass index (BMI), hemoglobin (Hb), albumin (ALb), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), uremic acid (UA), serum creatine (Scr), eGFR, blood urea nitrogen (BUN), 24-h urinary protein excretion (24-h UP), and 24-h urinary sodium. All 24-h urine samples were collected from the day of admission to the following day. In addition, we also recorded the use of drugs such as renin–angiotensin–aldosterone system inhibitors (RAASi), diuretics, and lipid-lowering drugs.

Furthermore, DSI was estimated from 24-h urinary sodium excretion (19, 23, 24). The calculation was performed with the following formula: DSI (g/d) = urinary sodium concentration (mmol/L) × 24-h urine volume (L) × 0.05842 (g/mmol). Based on it, patients were categorized into two groups according to DSI with a cut-off level of 6 g/day, which is recommended on the basis of evidence-based clinical practice guidelines for CKD (19, 25–28).

2.3 Measurement of volume status

All patients were analyzed using the body composition monitor (Fresenius Medical Care, Bad Homburg, Germany), by a designated technician according to the manufacturer's instructions. Hydration status including TBW, ECW, intracellular water (ICW), and overhydration (OH) was recorded (29, 30). ECW/TBW has been recognized as a notable indicator of adverse outcomes of a composite of volume overload and protein-energy wasting (31, 32). Accordingly, patients were then divided into two groups as per the median ECW/TBW.

2.4 Divisions

Four groups were divided according to both DSI and ECW/TBW as previously reported (19): Group 1, low DSI (<6 g/day) and low ECW/TBW (<median value 0.439); Group 2, low DSI (<6 g/day) and high ECW/TBW (≥median value 0.439); Group 3, high DSI (≥6 g/day) and low ECW/TBW (<median value 0.439); and Group 4, high DSI (≥6 g/day) and high ECW/TBW (≥median value 0.439).

2.5 Echocardiographic measurements

Echocardiographic data was acquired by an ultrasound machine (Vivid E9; GE Vingemed Ultrasound AS, Horten, Norway) with a 2.5-MHz transducer. All individuals were assessed for diastolic function measurement including mitral E wave velocity, mitral A wave

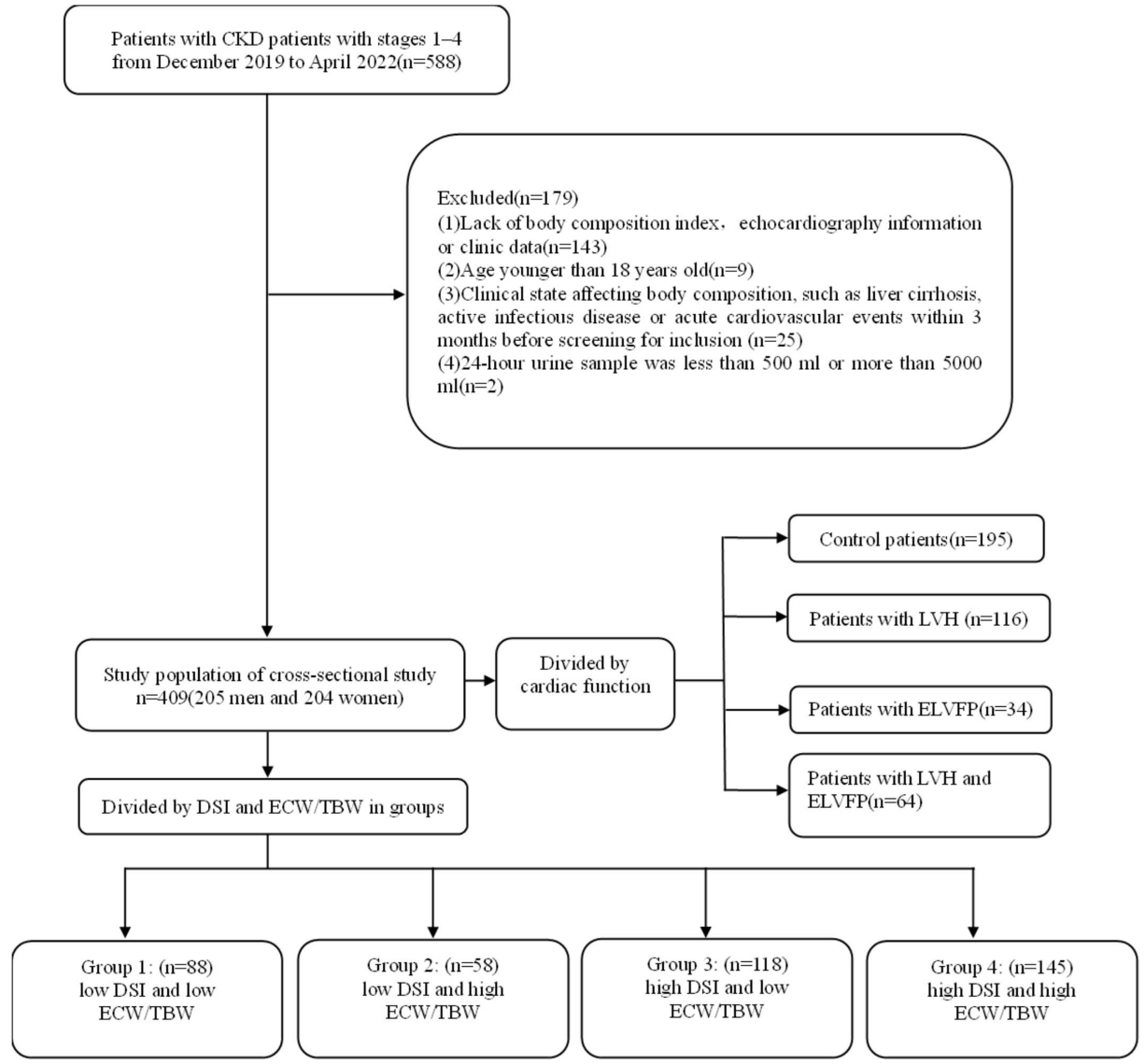

FIGURE 1
Flowchart of study participants. CKD, chronic kidney disease; TBW, total body water; ECW, extracellular water; DSI, daily salt intake; LVH, left ventricular hypertrophy; ELVFP, elevated left ventricular filling pressure.

velocity, mitral E:A ratio, septal or lateral mitral annular e' velocity (e' velocity), and E:e' ratio. The value of E/e' was helpful to diagnose the diastolic dysfunction of the left ventricle (15). E/e' > 9 was associated with increased risk of cardiovascular and all-cause mortality and was also used as an indicator to define ELVFP (7, 10, 11, 15).

Cardiac chamber quantification was performed in accordance with the American Society of Echocardiography guidelines (33). The dimensions of the interventricular septal thickness (IVST), left ventricular end-diastolic dimension (LVDd), left ventricular posterior wall thickness (PWT), left atrial dimension (LAD), and left ventricular ejection fraction (LVEF) using the biplane Simpson's method were measured. IVST and PWT were measured at end-diastole. Left ventricular hypertrophy (LVH) was defined as an LVMI>115 g/m^2 in male and > 95 g/m^2 in female patients (33).

The cardiac data was calcuated using the following formula: Left ventricular mass = $0.8 \times 1.04 \times [(IVST+LVDd+PWT)^3 - LVDd^3] + 0.6$ (g). Body surface area (BSA) = {([height (cm) × weight (kg)]/3600)$^{\times 1/2}$)} m^2. Left ventricular mass index (LVMI) = LVM/BSA (g/m^2). Relative wall thickness (RWT) = 2 × PWT/LVDd.

2.6 Statistical analysis

Categorical variables were reported in percentage and continuous variables were calculated for mean ± SD or medians and interquartile ranges with or without normal distribution, respectively. Comparisons of categorical variables among the four groups were performed by chi-squared test. Mann–Whitney test and Kruskal–Wallis test were used to compare continuous variables as appropriate.

The correlations between echocardiographic parameters and clinical indicators were assessed using Spearman's correlation analysis. Univariate and multivariate binary logistic regression

analysis were performed to determine the associations between DSI and ECW/TBW in groups and cardiac dysfunction. Multiple covariables were adjusted. In the fully adjusted model, factors included but not limited to age, sex, BMI, and cardiovascular health indicators were used for adjustment. Subgroup analyses were performed for serum albumin, eGFR, BMI, 24-h urinary protein, and history of hypertension and DM in the association between DSI, ECW/TBW, and cardiac impairments.

A p value of less than 0.05 was considered to indicate statistically significant differences between groups. And the analyses were performed using SPSS version 27.0 (IBM Corporation, Armonk, NY, USA).

3 Results

3.1 Basic characteristics

A total of 409 patients (205 men and 204 women) with stages G1-G4 CKD (53.05, 25.18, 17.60, and 4.16% at different stages respectively) were recruited (Figure 1; Table 1). The median age of patients was 45.00 (IQR: 32.00–56.00) years, with a mean BMI of $24.59 \pm 3.74\,kg/m^2$. The median urinary protein was 0.94 (0.28–3.14) g/d and eGFR was 92.05 (IQR: 64.52–110.99) mL/min/1.73 m^2. In terms of echocardiographic parameters, the median LVMI, E/e', and LVEF were 97.76 (82.21–115.38) g/m^2, 7.20 (6.00–8.80), and 64.00 (62.40–65.80) %, respectively. The DSI estimated from 24-h urinary sodium excretion was 7.13 (IQR: 5.04–9.65) g, and the median ECW/TBW was 0.439 (IQR: 0.421–0.468). DSI was at the lowest level in stage G3b, followed by CKD G4 (G3b vs. G1: $p < 0.001$; G3b vs. G2: $p < 0.05$; G3b vs. G3a: $p < 0.01$) (Figure 2A). Moreover, there was a significant increase in ECW/TBW at stage G3a (G3a vs. G1: $p < 0.01$; G3a vs. G2: $p < 0.05$; G3a vs. G3b: $p < 0.05$) (Figure 2A).

Next, based on DSI estimated from 24-h urinary sodium excretion and ECW/TBW, all enrolled patients were categorized into four groups (Table 1). Patients with both low level of DSI and low level of ECW/TBW were in group 1 and those with both high level of DSI and high level of ECW/TBW were in group 4. Patients with high ECW/TBW (groups 2 and 4) were significantly older, female, and had a higher level of BMI and MAP than those with ECW/TBW below the median value (groups 1 and 3) regardless of the DSI level ($p < 0.05$ for all four groups). Echocardiographic parameters including LAD, LVDd, LVMI, and E/e' were significantly different among the four groups (all $p < 0.05$). Patients in group 2, with low DSI and high ECW/TBW, had the highest values of LVMI and E/e', which was 104.63 (81.91–120.91) g/m^2 and 7.95 (6.48–9.33), respectively. With respect to bioelectrical impedance data, OH, ECW, and OH/ECW were highest in group 4 and lowest in group 1 ($p < 0.05$ among four groups). Meanwhile, TBW and ICW were highest in group 3 and lowest in group 2 ($p < 0.05$ among four groups). The levels of Hb, Alb, and LDL-C were higher; those of TC and TG were lower in groups 1 and 3 than in groups 2 and 4; furthermore, groups 1 and 3 required less frequent use of diuretics than groups 2 and 4($p < 0.05$ among four groups). Kidney function was worst in group 2, with the lowest level of eGFR (73.15 [48.83–101.04] ml/min/1.73 m^2) and highest level of Scr (88.85 [61.53–117.65] umol/L) ($p < 0.05$ among four groups). Besides, patients in group 4 had the highest level of 24-h UP (2.43 [0.64–5.93] g/d), followed by those in group 2 (1.95 [0.69–7.35] g/d). As displayed in Figure 2B, the distributions of CKD stages G1–G4 in the four groups was significantly different ($p = 0.020$). Patients in group 3 had the highest proportion of CKD stages G1 and G2 (85%) along with the lowest proportion of G3 and G4 (15%). Additionally, the highest ratio of stages G3 and G4 (36%) were observed in group 2.

3.2 Comparisons of baseline parameters according to echocardiographic indicators

According to cardiac function, 195 patients were categorized as controls, 116 patients had LVH, 34 patients had ELVFP, and 64 patients had LVH + ELVFP (Table 2). Compared to control patients, those in other three groups were significantly older (vs. control patients, $p < 0.01$ for each). BMI was significantly different among all four groups ($p < 0.001$), and the LVH group had the lowest BMI ($p < 0.01$). There were significant differences in echocardiographic parameters including LAD, LVDd, LVMI, and E/e' among the four groups (all $p < 0.001$). All bioelectrical impedance parameters including OH, TBW, ECW, ICW, ECW/ICW, OH/ECW, and ECW/TBW showed statistically significant differences. More specifically, the ELVFP group had the highest level of OH, TBW, ECW, and OH/ECW, and the LVH + ELVFP group had the highest level of ECW/ICW and ECW/TBW (vs. control patients, $p < 0.01$). Meanwhile, the LVH group had the lowest level of TBW, ECW, and ICW (vs. control patients, $p < 0.01$). With respect to laboratory parameters, the LVH group had significantly decreased levels of Hb and Alb, but increased levels of HDL-C; whereas, the ELVFP group had significant lower Alb levels (vs. control patients, $p < 0.01$; $p < 0.05$ among four groups). Kidney function was significantly different among all four groups, and the LVH + ELVFP group had the lowest level of eGFR, followed by the ELVFP group ($p < 0.05$ among four groups). Besides, the most severe proteinuria was observed in the ELVFP group with 3.86 g/d (IQR: 1.00–7.01), followed by the LVH + ELVFP group with a level of 2.10 g/d (IQR: 0.61–4.68) ($p < 0.05$ among four groups). However, DSI showed no significant difference among the four groups ($p > 0.05$). Medications of RAASi and lipid-lowering drugs were found to be significantly different among the four groups ($p < 0.05$).

3.3 Correlations between echocardiographic findings and basic parameters

The correlations between echocardiographic parameters, body composition parameters, and clinical indicators were assessed using Spearman's correlation analysis (Figure 3). ECW/TBW was found to be positively correlated with DSI ($r = 0.131$, $p = 0.008$); MAP ($r = 0.134$, $p = 0.007$); and lipid metabolic parameters including TC ($r = 0.331$, $p < 0.001$); LDL-C ($r = 0.301$, $p < 0.001$); but negatively correlated with renal function indicators including albumin ($r = -0.622$, $p < 0.001$) and eGFR ($r = -0.116$, $p = 0.019$). While DSI was positively correlated with eGFR ($r = 0.143$, $p = 0.004$), and fluid status indicators including OH ($r = 0.160$, $p = 0.001$); TBW ($r = 0.240$, $p < 0.001$); ECW ($r = 0.292$, $p < 0.001$); ICW ($r = 0.184$, $p < 0.001$); ECW/ICW ($r = 0.130$, $p = 0.008$); and OH/ECW ($r = 0.165$, $p < 0.001$); ECW/TBW ($r = 0.131$, $p = 0.008$), but negatively correlated with albumin ($r = -0.107$, $p = 0.030$).

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TABLE 1 Baseline characteristics stratified by DSI and ECW/TBW categories ($n = 409$).

Variables	Total ($n = 409$)	DSI				p value
		Low		High		
		ECW/TBW				
		Low (group 1:$n = 88$)	High (group 2:$n = 58$)	Low (group 3:$n = 118$)	High (group 4:$n = 145$)	
Age (years)	45.00 (32.00–56.00)	35.50 (29.00–50.00)	53.50 (39.75–66.00)	38.00 (30.75–50.00)	51.00 (40.50–59.00)	$p < 0.001$
Gender (male/female)	205/204	42/46	21/37	77/41	65/80	$p = 0.043$
Clinical parameter						
BMI(kg/m^2)	24.59 ± 3.74	23.12 ± 3.02	24.48 ± 3.20	24.25 ± 3.98	25.80 ± 3.77	$p < 0.001$
MAP(mmHg)	98.67 (88.67–106.83)	97.33 (87.08–104.00)	100.50 (90.00–106.67)	95.33 (86.92–106.25)	100.67 (90.83–109.00)	$p = 0.020$
ECHO						
LAD (mm)	32.00 (30.00–36.00)	31.00 (28.00–32.75)	32.50 (28.75–37.00)	32.00 (30.00–35.00)	34.00 (31.50–38.00)	$p < 0.001$
LVDd (mm)	46.00 (43.00–48.00)	44.50 (42.00–47.00)	45.00 (42.00–48.00)	46.50 (44.00–49.00)	47.00 (44.00–49.00)	$p < 0.001$
LVMI (g/m^2)	97.76 (82.21–115.38)	94.63 (80.54–111.34)	104.63 (81.91–120.91)	93.28 (80.88–106.93)	101.06 (86.45–121.81)	$p = 0.021$
E/e'	7.20 (6.00–8.80)	6.50 (5.60–7.77)	7.95 (6.48–9.33)	6.70 (5.90–8.00)	7.80 (6.55–9.65)	$p < 0.001$
LVEF (%)	64.00 (62.40–65.80)	64.40 (63.08–66.03)	64.00 (62.10–66.50)	64.40 (63.00–65.90)	63.70 (62.10–65.40)	$p = 0.076$
Bioelectrical impedance						
OH (L)	0.20(−0.60–1.70)	−0.50(−1.08–0.10)	0.90 (0.10–5.40)	−0.45(−1.20–0.20)	1.40 (0.50–3.60)	$p < 0.001$
TBW (L)	35.30 (30.00–41.95)	34.20 (30.00–39.88)	30.95 (26.55–41.28)	37.70 (33.15–44.20)	35.30 (30.55–41.40)	$p < 0.001$
ECW (L)	15.60 (13.35–18.55)	14.10 (12.60–16.58)	14.15 (12.30–20.05)	15.70 (13.90–18.53)	17.40 (14.40–19.65)	$p < 0.001$
ICW (L)	19.80 (16.40–23.50)	20.05 (17.20–23.58)	15.65 (13.98–20.20)	21.90 (18.98–26.18)	18.70 (16.00–21.95)	$p < 0.001$
ECW/ICW	0.78 (0.72–0.88)	0.73 (0.68–0.76)	0.93 (0.84–1.00)	0.73 (0.69–0.75)	0.88 (0.83–0.97)	$p < 0.001$
OH/ECW ($\times 10^{-2}$)	1.32(−4.29–9.46)	−3.08(−7.49–0.81)	6.94 (0.85–26.47)	−3.03(−7.29–1.24)	9.03 (2.93–19.94)	$p < 0.001$
ECW/TBW	0.439 (0.421–0.468)	0.421 (0.405–0.432)	0.478 (0.454–0.502)	0.421 (0.407–0.430)	0.466 (0.454–0.493)	$p < 0.001$
Laboratory parameter						
Hb (g/L)	129.88 ± 21.03	135.43 ± 18.82	124.16 ± 23.42	137.68 ± 19.02	122.46 ± 19.76	$p < 0.001$
ALb (g/L)	36.70 (28.40–40.05)	38.60 (36.35–41.60)	29.65 (21.73–37.83)	39.60 (36.00–42.30)	30.70 (22.35–36.95)	$p < 0.001$
TC (mmol/L)	4.86 (4.16–6.03)	4.67 (3.98–5.58)	5.20 (4.02–7.14)	4.65 (3.93–5.38)	5.35 (4.47–6.50)	$p < 0.001$
TG (mmol/L)	1.50 (1.01–2.23)	1.23 (0.90–1.83)	1.93 (1.26–2.70)	1.38 (0.90–2.00)	1.65 (1.11–2.39)	$p < 0.001$
HDL-C (mmol/L)	1.14 (0.96–1.41)	1.13 (0.96–1.40)	1.22 (0.99–1.57)	1.08 (0.93–1.34)	1.18 (0.99–1.38)	$p = 0.051$
LDL-C (mmol/L)	3.03 (2.45–3.73)	2.91 (2.30–3.51)	3.05 (2.32–4.09)	2.86 (2.36–3.46)	3.31 (2.74–4.11)	$p < 0.001$
eGFR (ml/min/1.73 m2)	92.05 (64.52–110.99)	91.47 (70.48–112.13)	73.15 (48.83–101.04)	97.08 (77.79–114.61)	95.62 (60.98–110.90)	$p = 0.003$
CKD stage (1/2/3a/3b/4)	217/103/42/30/17	47/24/2/10/5	19/18/11/6/4	69/32/11/4/2	82/29/18/10/6	$p = 0.002$
Scr (umol/L)	79.10 (60.50–105.30)	81.35 (66.33–106.15)	88.85 (61.53–117.65)	79.00 (63.23–100.83)	74.40 (57.50–97.50)	$p = 0.072$
24 h UP (g/d)	0.94 (0.28–3.14)	0.48 (0.17–1.06)	1.95 (0.69–7.35)	0.39 (0.16–1.26)	2.43 (0.64–5.93)	$p < 0.001$
24 h urinary sodium (mmol/d)	122.00 (86.30–165.15)	77.25 (63.13–88.43)	76.30 (59.08–87.90)	145.35 (120.53–191.40)	154.40 (127.35–194.60)	$p < 0.001$
DSI (g/d)	7.13 (5.04–9.65)	4.51 (3.69–5.17)	4.46 (3.45–5.14)	8.49 (7.04–11.18)	9.02 (7.44–11.37)	$p < 0.001$
Medications						
RAASi (%)	218 (53.30)	46 (52.27)	35 (60.34)	61 (51.69)	76 (52.41)	$p = 0.715$
Diuretic (%)	21 (5.13)	1 (1.14)	11 (18.97)	1 (0.84)	8 (5.51)	$p < 0.001$
Lipid-lowering drugs (%)	67 (16.38)	10 (11.36)	15 (25.86)	15 (12.71)	27 (18.62)	$p = 0.068$

BMI, body mass index; MAP, mean arterial pressure; ECHO, Echocardiography; LAD, left atrial dimension; LVDd, left ventricular end-diastolic dimension; LVMI, left ventricular mass index; LVEF, left ventricular ejection fraction; OH, overhydration; TBW, total body water; ECW, extracellular water; ICW, intracellular water; Hb, hemoglobin; ALb, albumin; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; Scr, serum creatine; UP, urinary protein; DSI, daily salt intake; RAASi, renin–angiotensin–aldosterone system inhibitors.

Data were presented as the mean ± SD, the median with interquartile range or counts, and percentages. A two-tailed $p < 0.05$ was considered statistically significant.

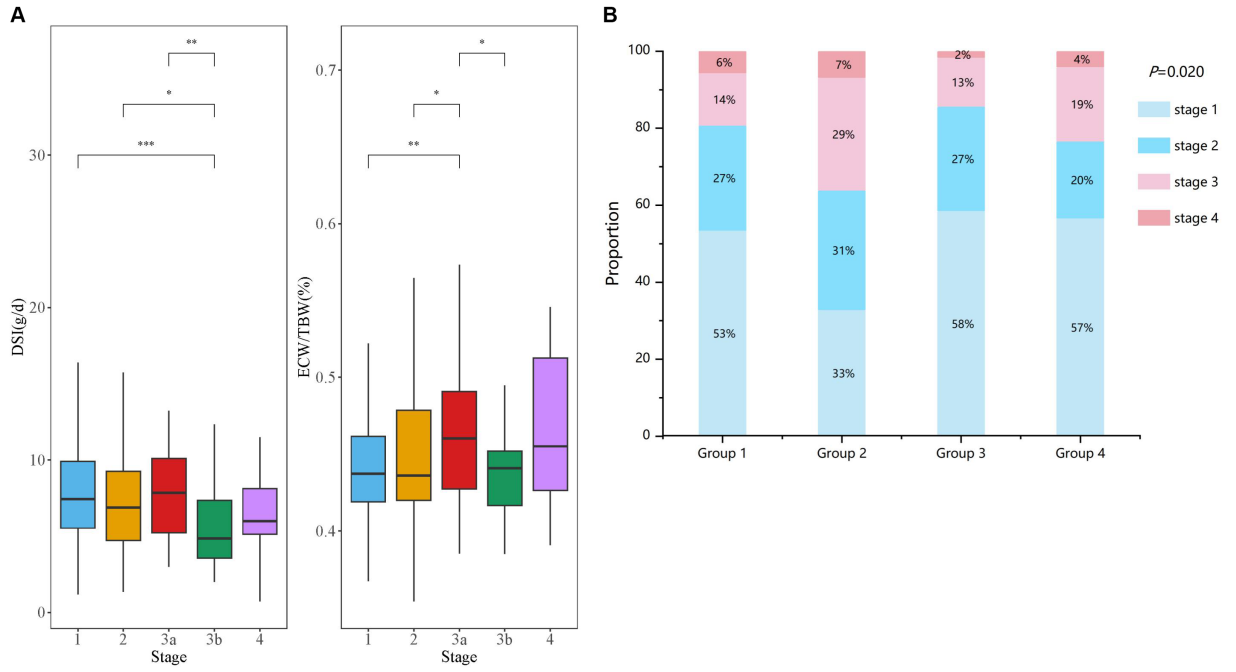

FIGURE 2
Comparison of DSI and ECW/TBW in CKD patients with different stages. **(A)** Comparisons of the levels of DSI and ECW/TBW among all enrolled patients are shown at stages G1–G4 of CKD. **(B)** The four groups were divided according to both DSI and ECW/TBW. Proportion of CKD stages G1–G4 are displayed in groups 1–4 **(B)**.

Moreover, this showed that LVMI was negatively correlated with renal function parameters albumin ($r = -0.189$, $p < 0.001$) and eGFR ($r = -0.291$, $p < 0.001$); fluid status indicators including TBW ($r = -0.212$, $p < 0.001$); ECW ($r = -0.138$, $p = 0.005$); and ICW ($r = -0.247$, $p < 0.001$) and positively correlated with OH ($r = 0.208$, $p < 0.001$); ECW/ICW ($r = 0.178$, $p < 0.001$); OH/ECW ($r = 0.205$, $p < 0.001$); ECW/TBW ($r = 0.176$, $p < 0.001$); MAP ($r = 0.134$, $p = 0.007$) and 24-h UP ($r = 0.171$, $p < 0.001$). Meanwhile, E/e' showed negative correlations with albumin ($r = -0.256$, $p < 0.001$) and eGFR ($r = -0.307$, $p < 0.001$), but positive correlations with MAP ($r = 0.213$, $p < 0.001$); OH ($r = 0.281$, $p < 0.001$); ECW ($r = 0.142$, $p = 0.004$); ECW/ICW ($r = 0.310$, $p < 0.001$); OH/ECW ($r = 0.281$, $p < 0.001$); ECW/TBW ($r = 0.311$, $p < 0.001$); and 24-h UP ($r = 0.250$, $p < 0.001$).

3.4 Association between DSI, ECW/TBW, and cardiac abnormalities

As shown in Table 3, the association between DSI and ECW/TBW among the study groups and the risks for the incidence of LVH or ELVFP was determined by binary logistic regression models. In the unadjusted model group, group 2 (low DSI & high ECW/TBW) and group 4 (high DSI & high ECW/TBW) had a 2.5-times and 2.217-times risk, respectively, for the diagnosis of LVH or ELVFP than group 1 (low DSI & low ECW/TBW) (group 2: OR = 2.500, 95%CI = 1.259–4.966, $p = 0.009$; group 4: OR = 2.217, 95%CI: 1.293–3.804, $p = 0.004$). After adjusting for age, sex, and BMI, the association between group 4 and an increased risk for the incidence of LVH or ELVFP was still significant with an OR of 2.351 (95%CI: 1.262–4.380, $p = 0.007$, model 1 in Table 3). This increased risk remained in group 4, even after extensive adjustment for model 1 plus MAP, albumin, eGFR, TC, and LDL-C (OR = 2.782, 95%CI = 1.427–5.426, $p = 0.003$, model 2 in Table 3). Additionally, the findings remained in a fully adjusted model including model 2 plus the use of RAASi, diuretics, and lipid-lowering drugs, in which group 4 was associated with a 2.396-times higher likelihood of LVH or ELVFP occurring, as compared to group 1 (OR = 2.396, 95%CI = 1.171–4.902, $p = 0.017$, model 3 in Table 3).

3.5 Prediction performance of DSI and ECW/TBW on cardiac abnormalities

We used ROC analysis to evaluate the predictive value of DSI and ECW/TBW on the incidence of LVH or ELVFP. A combination of DSI and ECW/TBW showed an AUC of 0.673 (sensitivity = 71.0%, specificity = 54.9%) for predicting the occurrence of LVH or ELVFP. Moreover, adding DSI and ECW/TBW to eGFR boosted the predictive ability on the incidence of LVH or ELVFP with an AUC of 0.704 (sensitivity = 75.2%, specificity = 61.0%). According to different levels of 24-h UP (<3.5 or ≥ 3.5 g/day), the combination of DSI, ECW/TBW, and eGFR showed an AUC of 0.713 (sensitivity = 60.3%, specificity = 85.7%) for the incidence of LVH or ELVFP in patients with nephrotic proteinuria (Figure 4).

3.6 Subgroup analysis

To evaluate the modification effects of subgroups on the relationship between DSI, ECW/TBW, and cardiac dysfunction,

TABLE 2 Baseline characteristics stratified by cardiac function categories ($n = 409$).

Variables	Control patients ($n = 195$)	LVH ($n = 116$)	ELVFP ($n = 34$)	LVH + ELVFP ($n = 64$)	p-value
Age (years)	36.00 (29.00–50.00)	46.50 (36.25–55.00)[a]	53.00 (41.00–65.50)[a]	56.00 (47.25–65.00)[a]	$p < 0.001$
Gender (male/female)	135/60	21/95	28/6	21/43	$p < 0.001$
Clinical parameter					
BMI (kg/m^2)	25.13 ± 3.78	23.43 ± 3.30[a]	25.77 ± 4.23	24.41 ± 3.61	$p < 0.001$
MAP (mmHg)	98.33 (88.33–105.33)	98.00 (88.17–106.00)	99.33 (87.25–111.00)	99.50 (90.58–111.75)	$p = 0.227$
ECHO					
LAD (mm)	32.00 (29.00–35.00)	32.00 (29.00–35.00)	35.00 (32.00–37.25)[a]	37.00 (32.00–40.00)[a]	$p < 0.001$
LVDd (mm)	45.00 (42.00–48.00)	46.00 (44.00–48.00)	47.00 (44.00–48.00)	48.00 (45.00–50.00)[a]	$p < 0.001$
LVMI (g/m^2)	84.35 (73.42–92.97)	112.57 (103.55–123.22)[a]	89.08 (78.65–97.85)[b]	136.29 (114.57–149.78)[a]	$p < 0.001$
E/e'	6.40 (5.70–7.30)	7.00 (6.00–7.80)[a]	10.35 (9.50–11.30)[a]	10.70 (9.50–12.35)[a]	$p < 0.001$
LVEF (%)	64.40 (62.70–65.60)	64.00 (62.78–65.80)	64.00 (61.90–64.85)	64.40 (61.90–65.80)	$p = 0.629$
Bioelectrical impedance					
OH (L)	0.00(−0.70–0.90)	0.20(−0.70–1.17)	1.35 (0.43–4.35)[a]	1.05 (0.00–3.65)[a]	$p < 0.001$
TBW (L)	38.90 (33.40–44.80)	30.95 (27.53–35.23)[a]	41.05 (36.4–47.85)	33.45 (28.60–38.60)[a]	$p < 0.001$
ECW(L)	16.60 (14.30–19.10)	13.70 (12.30–15.68)[a]	19.55 (16.83–22.88)[a]	15.15 (13.73–19.08)	$p < 0.001$
ICW (L)	22.10 (18.50–25.20)	17.05 (15.10–20.00)[a]	20.95 (18.33–24.85)	17.55 (15.10–20.38)[a]	$p < 0.001$
ECW/ICW	0.76 (0.70–0.83)	0.79 (0.74–0.87)[a]	0.86 (0.76–1.02)[a]	0.87 (0.77–1.02)[a]	$p < 0.001$
OH/ECW ($\times 10^{-2}$)	0.00(−4.65–4.82)	1.52(−5.57–8.43)	8.28 (2.17–20.48)[a]	6.94 (0.00–21.32)[a]	$p < 0.001$
ECW/TBW	0.432 (0.413–0.455)	0.441 (0.425–0.464)[a]	0.461 (0.433–0.505)[a]	0.466 (0.436–0.506)[a]	$p < 0.001$
Laboratory parameter					
Hb (g/L)	137.35 ± 19.26	124.95 ± 16.79[a]	132.00 ± 23.70	114.94 ± 21.58[a]	$p < 0.001$
ALb (g/L)	38.10 (33.20–41.00)	35.95 (28.48–39.20)[a]	29.55 (18.35–37.98)[a]	32.90 (25.23–37.60)[a]	$p < 0.001$
TC (mmol/L)	4.76 (4.06–5.94)	4.94 (4.15–6.26)	5.24 (4.45–7.65)	4.90 (4.39–6.21)	$p = 0.266$
TG(mmol/L)	1.47 (1.01–2.23)	1.50 (1.04–2.21)	1.81 (1.05–2.52)	1.43 (0.90–2.25)	$p = 0.733$
HDL-C (mmol/L)	1.09 (0.93–1.36)	1.24 (1.06–1.45)[a]	1.06 (0.93–1.45)	1.14 (0.96–1.46)	$p = 0.010$
LDL-C (mmol/L)	3.02 (2.42–3.62)	3.08 (2.37–3.87)	3.22 (2.62–4.16)	3.02 (2.69–3.62)	$p = 0.759$
eGFR (ml/min/1.73 m2)	98.01 (78.18–116.07)	90.88 (63.22–111.07)[b]	81.97 (53.06–106.14)[b]	67.12 (46.35–96.67)[a]	$p < 0.001$
CKD stage (1/2/3a/3b/4)	122/46/14/9/4	59/30/9/12/6	14/10/7/3/0	22/17/12/6/7	$p < 0.001$
Scr (umol/L)	80.00 (66.40–100.50)	68.85 (55.33–101.28)[b]	90.85 (64.78–125.93)	83.85 (62.85–115.90)	$p = 0.017$
24 h UP (g/d)	0.61 (0.25–1.96)	0.85 (0.20–2.53)	3.86 (1.00–7.01)[a]	2.10 (0.61–4.68)[a]	$p < 0.001$
24 h urinary sodium (mmol/d)	121.20 (86.40–171.00)	118.75 (80.15–163.53)	150.10 (104.53–192.10)	121.70 (87.90–144.83)	$p = 0.068$
DSI (g/d)	7.08 (5.05–9.99)	6.94 (4.68–9.55)	8.77 (6.11–11.22)	7.11 (5.14–8.46)	$p = 0.068$
Medications					
RAASi (%)	98 (50.25)	54 (46.55)	18 (52.94)	48 (75.00)	$p = 0.002$
Diuretic (%)	11 (5.64)	4 (3.45)	2 (5.88)	4 (6.25)	$p = 0.762$
Lipid-lowering drugs (%)	26 (13.33)	19 (16.38)	4 (11.76)	18 (28.13)	$p = 0.040$

BMI, body mass index; MAP, mean arterial pressure; ECHO, Echocardiography; LAD, left atrial dimension; LVDd, left ventricular end-diastolic dimension; LVMI, left ventricular mass index; LVEF, left ventricular ejection fraction; OH, overhydration; TBW, total body water; ECW, extracellular water; ICW, intracellular water; Hb, hemoglobin; ALb, albumin; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; DSI, daily salt intake; RAASi, renin–angiotensin–aldosterone system inhibitors.

Data were presented as the mean ± SD, the median with interquartile range or counts, and percentages. A two-tailed $p < 0.05$ was considered statistically significant.

[a]Two-tailed $p < 0.01$(compared to the control patients); [b] Two-tailed $p < 0.05$(compared to the control patients).

we performed subgroup analyses in subgroups stratified by serum albumin (≤30 or > 30 g/L), eGFR (≤60 or > 60 mL/min/1.73 m^2), BMI (≤25 kg/m^2 or > 25 kg/m^2), 24-h UP (<3.5 or ≥ 3.5 g/day), history of hypertension (yes or no), and history of DM (yes or no). Hig DSI and ECW/TBW (group 4) was strongly correlated with higher risks of the incidence of LVH or ELVFP (OR = 1.151, 95%CI = 1.003–1.321, $p = 0.046$). Moreover, the p values for interactions were > 0.05 for the subgroups by serum albumin, eGFR, BMI, 24-h UP, and history of

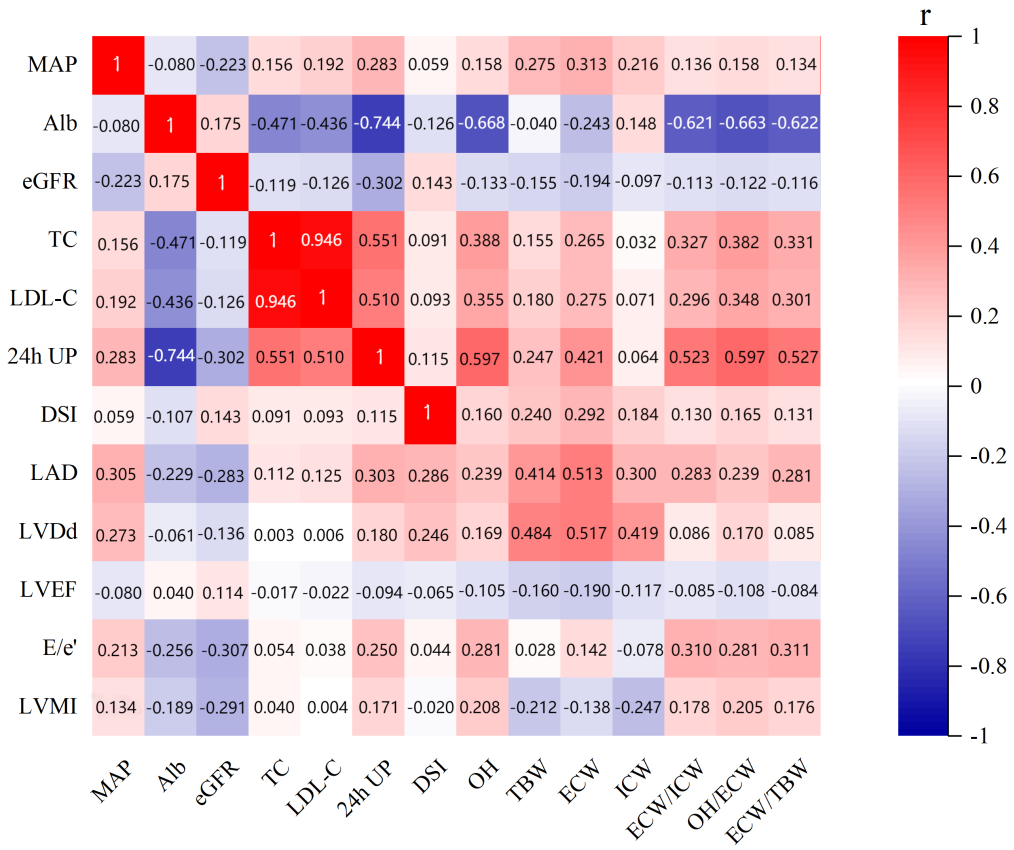

FIGURE 3
Correlations between echocardiographic parameters, body composition parameters, and clinical indicators. MAP, mean arterial pressure; LAD, left atrial dimension; LVDd, left ventricular end-diastolic dimension; LVMI, left ventricular mass index; LVEF, left ventricular ejection fraction; OH, overhydration; TBW, total body water; ECW, extracellular water; ICW, intracellular water; ALb, albumin; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; DSI, daily salt intake; 24-h UP, 24-h urinary protein.

hypertension and DM, suggesting that the increased risk of diastolic dysfunction associated with higher DSI and ECW/TBW was evident regardless of these factors (Table 4).

4 Discussion

The present study strengthens the association between a combination of sodium excretion and volume overload and risks of cardiac structural and functional impairments in a population with non-dialysis CKD. These analyses document a significantly increased risk of LVH or ELVFP occurrence in individuals with both high DSI (≥6 g/d) and high ECW/TBW (≥median value 0.439), independent of important CKD and CVD risk factors. Compared to group 1 (low DSI and low ECW/TBW), group 4 (high DSI and high ECW/TBW) showed a 2.396-fold (95%CI: 1.171–4.902; $p = 0.017$) high risk of LVH or ELVFP incidence in a fully adjusted model. Furthermore, ROC analysis confirmed the predictive ability with an AUC of 0.673 with a high sensitivity but only fair specificity. More importantly, combined with eGFR, DSI and ECW/TBW could identify patients with higher cardiac dysfunction risk estimates with a satisfactory accuracy (AUC = 0.704, sensitivity = 75.2%, specificity = 61.0%). The specificity was improved to 85.7% in those with nephrotic proteinuria (AUC = 0.713). These findings were consistent across subgroups that strongly supported the impact of the combination of DSI and ECW/TBW on cardiac structural and functional impairments in CKD patients.

The ECW/TBW ratio which is considered an "edema index," served as a significant predictor of poorer outcomes indicating a composite of overhydration and protein-energy wasting (31, 34). It evaluated extracellular fluid (ECF) status by body composition monitor based on cell membrane resistance to frequency currents (35). ECF excess is a proven common characteristic of advanced ongoing-dialysis CKD and has a close association with all-cause mortality and CV morbidity (34–36). Renal function loss resulted in reduced excretion of sodium and water, thereby resulting in ECF excess (37). Consistently, our results revealed that ECW/TBW was negatively correlated with eGFR. Moreover, ECW/TBW was found to be significantly correlated with conventional CV risk factors as indicated by MAP, TC, and LDL-C values, which further support that ECF excess contributed to hypertension, HF, and pulmonary vascular congestion in CKD patients (37, 38). Previous studies have shown that in the presence of ECF excess, urinary excretion of sodium and fluid was increased through a negative feedback mechanism to maintain fluid homeostasis. Disruption of water and salt homeostasis and a reduction in the ability to perform natriuresis were classic features and the major underlying pathogenesis of CKD that are often coupled with the expansion of the extracellular water (ECW) compartment (39).

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TABLE 3 Association between DSI and ECW/TBW in groups and cardiac function ($n = 409$).

Variables	Groups			
	Group 1:low DSI & low ECW/TBW	Group 2:low DSI & high ECW/TBW	Group 3:high DSI & low ECW/TBW	Group 4:high DSI & high ECW/TBW
No. (%)	88 (22.52%)	58 (14.18%)	118 (26.89%)	145 (35.45%)
Unadjusted ORs	Reference	2.500 (1.259–4.966)	0.871 (0.497–1.525)	2.217 (1.293–3.804)
p-value		$p = 0.009$	$p = 0.629$	$p = 0.004$
Adjusted ORs adjusted for:				
Model 1[a]	Reference	1.830 (0.843–3.973)	1.180 (0.635–2.191)	2.351 (1.262–4.380)
p-value		$p = 0.126$	$p = 0.601$	$p = 0.007$
Model 2[b]	Reference	1.692 (0.747–3.830)	1.558 (0.805–3.016)	2.782 (1.427–5.426)
p-value		$p = 0.207$	$p = 0.188$	$p = 0.003$
Model 3[c]	Reference	1.660 (0.703–3.921)	1.542 (0.792–3.000)	2.396 (1.171–4.902)
p-value		$p = 0.248$	$p = 0.203$	$p = 0.017$

BMI, body mass index; MAP, mean arterial pressure; ALb, albumin; eGFR, estimated glomerular filtration rate; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; RAASi, renin–angiotensin–aldosterone system inhibitors.

[a]Model 1 adjusted for age, gender and BMI.

[b]Model 2 adjusted for age, gender, BMI, MAP, albumin, eGFR, TC, LDL-C.

[c]Model 3 adjusted for age, gender, BMI, MAP, albumin, eGFR, TC, LDL-C, use of RAASi, use of diuretic and use of Lipid-lowering drugs.

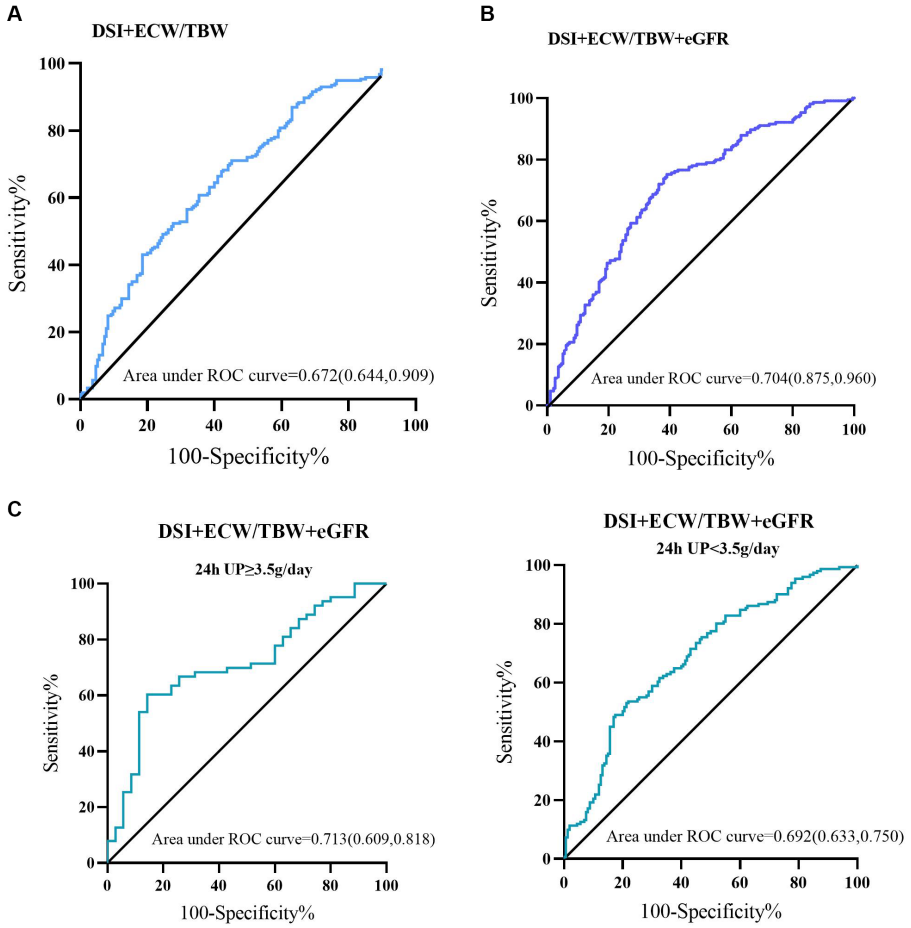

FIGURE 4
Predictive performances of DSI and ECW/TBW for the incidence of LVH or ELVFP evaluated by ROC curves. ROC curves of **(A)** DSI and ECW/TBW, **(B)** adding eGFR to DSI and ECW/TBW for the occurrence of LVH or ELVFP, and in those with nephrotic or non-nephrotic proteinuria **(C)**. AUC, area under the ROC curve; ROC, receiver operating characteristic; TBW, total body water; ECW, extracellular water; DSI, daily salt intake; LVH, left ventricular hypertrophy; ELVFP, elevated left ventricular filling pressure.

Accordingly, ECW/TBW was positively correlated with DSI in our study. Our study added evidence to the potential relationship of ECW/TBW and DSI in different CKD stages. As has been suggested in previous literatures, when renal function deteriorates, urinary excretion of sodium and fluid is reduced along with triggering of the intrarenal RAAS, leading to persistent and exacerbated ECF excess (34, 40).

Accumulating evidence indicated that high intake of dietary salt was an established risk factor for not only renal function loss but also CVD incidence in CKD (19, 41). Owing to impaired tubuloglomerular feedback and increased renal sodium reabsorption, blood pressure was even more sensitive to high sodium intake in CKD (42). One prospective cohort study of CKD patients from the Chronic Renal Insufficiency Cohort Study suggested that higher urinary sodium excretion was associated with increased risk of CVD (17). Another study revealed that 24-h urinary sodium excretion was associated with CKD progression and all-cause mortality among 3,757 patients with CKD in the Chronic Renal Insufficiency Cohort Study (43). In addition, higher DSI (>6 g/day) was significantly associated with renal outcome than lower DSI (≤6 g/day), and strict salt restriction has reportedly shown an association with improvements in hypertension and urinary protein reduction overload (44, 45). However, the association of salt intake and CKD progression is still controversial. Fan et al. found that when DSI was under 3 g/day, higher urinary sodium was associated with increased risk of kidney failure in those with baseline proteinuria under 1 g/day and with lower risk of kidney failure than in those with baseline proteinuria ≥1 g/day (46). Another study revealed that in patients with advanced CKD (eGFR<30 mL/min/1.73 m^2), sodium intake does not impact the CKD progression (47). In addition, low sodium intake was also associated with kidney failure in those with macroalbuminuria (48). In the current study, DSI was positively correlated with eGFR. Patients in group 2 (low DSI and high ECW/TBW) but not in group 4 (high DSI and high ECW/TBW) had the most severe renal function and the highest ratio of CKD stages G3 and G4. S A possible explanation for the discrepancy was the inaccuracy of the 24-h urine sample collections and small sample sizes. Combined with our results, low DSI may be because of impaired renal function in advanced CKD, resulting in reduced urine volume and decreased urinary sodium excretion. Taken together, it may be more appropriate to take the CKD stages into account when analyzing the effect of DSI on CVD in CKD patients. The addition of eGFR to DSI and ECW/TBW which resulted in improvements of predictive performance of LVH or ELVFP incidence supported this hypothesis.

LVH was not only the most frequent structural cardiac abnormality in patients with CKD but also an important risk factor for adverse CV outcomes in patients with CKD (21, 49). Cerasola et al. reported a progressive increase of LVH prevalence with decreasing renal function and an inverse association between GFR and LVM, independent of potential confounders (50). Additionally, several studies suggested that the risk of LVH was significantly associated with CKD severity, with increasing prevalence correlating with a deterioration in renal function (4, 13). Besides, high albumin excretion was independently related to LVH (51). Consistent with previous studies, our results indicated that LVMI was negatively correlated with albumin and eGFR, but positively correlated with MAP and 24-h UP, which reiterated the necessity and importance of early detection of LVH and LV dysfunction in CKD patients. Furthermore, diastolic LV filling is complex and hence, the echocardiographic assessment of the value of E/e' was a robust marker of LVFP and helpful to diagnose LV diastolic dysfunction (7, 15). Elevated E/e' ratio has been reported to be associated with increased mortality in a range of CVDs including HF with reduced EF, mitral and aortic regurgitation, aortic stenosis, and hypertension (7, 15). E/e' remained a marker of increased mortality with a pivot point of increased mortality at ~9, which was also the diagnostic cut-off value to define ELVFP (7, 10, 12). In the current study, E/e' showed significant correlations with renal function and proteinuria. In our study, those in the ELVFP group had the lowest eGFR and highest level of 24-h UP, further indicating the association between CKD and increased risk of CV morbidity and mortality. Therefore, the identification of novel risk factors in combination with traditional risk factors might enable extensive documentation and analysis of patients at high risk of CVD incidence, thereby preventing CV diseases in CKD patients in an opportune and efficient manner to improve survival.

The primary finding of the study was an association of the combination of higher DSI and ECW/TBW with an increased risk of incidence of LVH or ELVFP in CKD, highlighting the critical role of sodium and fluid overload in CV morbidity future mortality in CKD patients. A high-salt diet could induce sodium retention in CKD patients, leading to fluid overload which, in turn, can cause pressure overload contributing to CVD risk (44, 45). Direct effects of DSI on RAAS activity were also suggested in the association of DSI and CVD (52). Moreover, the findings reported here are independent of adjustment for conventional important CVD risk factors such as MAP; albumin; eGFR; TC; and LDL-C and the use of RAASi, diuretics, and lipid-lowering drugs, suggesting that other potential mechanisms likely play a role in the effect of sodium and fluid overload on CVD in CKD patients. Hung et al. suggested that fluid retention in patients or animals was associated with renal inflammation with macrophage infiltration and tumor necrosis factor-α overexpression, glomerular sclerosis, and cardiac fibrosis (21). The other potential mechanisms involved in the direct effects of DSI on CVD included endothelial dysfunction, increased oxidative stress leading to vascular damage, and insulin resistance (17). In addition, several research data have shown that CKD is involved in many pathophysiological pathways that potentially contribute to the elevated risk of CVD observed in patients with CKD, and the relative contributions of these mechanisms varied across CKD severity (14). Our findings confirmed these observations that DSI and ECW/TBW as non-traditional risk factors which were also related to CKD progression, broadened group of CV risk factors and the prevalence increases with declining eGFR. More importantly, although the effect of DSI and ECW/TBW on CVD was independent of adjusting eGFR, adding DSI and ECW/TBW to eGFR could greatly boost the prediction ability of LVH or ELVFP incidence in CKD patients. They showed high specificity especially in those with nephrotic proteinuria. These results not only reflected a shared sodium and fluid overload linking CKD to CVD but also suggested the need for timely targeting of the risk factors on the CV system even in the early stages of the disease.

4.1 Limitations

This study has some limitations. First, as a cross-sectional observational analysis, this study could not establish the causality of

TABLE 4 Subgroup analysis.

Characteristics	Events, %	Group 1 OR (95%CI)	Group 2 OR (95%CI), *p*	Group 3 OR (95%CI), *p*	Group 4 OR (95%CI), *p*
All patients	214 (52.3)	1(Reference)[a]	1.092 (0.93, 1.282), 0.286[a]	1.069 (0.946, 1.208), 0.285[a]	1.151 (1.003, 1.321), 0.046[a]
Serum albumin					
>30 g/L	135 (63.1)	1(Reference)	1.038 (0.846, 1.273), 0.723	1.018 (0.892, 1.161), 0.796	1.123 (0.954, 1.322), 0.165
≤30 g/L	79 (36.9)	1(Reference)	1.46 (0.918, 2.322), 0.113	1.647 (0.995, 2.727), 0.055	1.485 (0.948, 2.324) 0.087
p for interaction		Reference	0.552	0.915	0.275
eGFR					
>60.ml.min.1.73m^2	152 (71.0)	1(Reference)	1.094 (0.904, 1.324), 0.356	1.074 (0.937, 1.231), 0.304	1.129 (0.963, 1.324), 0.136
≤60.ml.min.1.73m^2	62 (29.0)	1(Reference)	1.063 (0.755, 1.495), 0.729	1.031 (0.746, 1.424), 0.855	1.168 (0.855, 1.597), 0.332
p for interaction		Reference	0.213	0.903	0.503
BMI					
≤25 kg/m2	129 (60.3)	1(Reference)	1.137 (0.925, 1.398), 0.224	1.143 (0.979, 1.335), 0.092	1.187 (0.98, 1.437), 0.081
>25 kg/m2	85 (39.7)	1(Reference)	1.065 (0.806, 1.408), 0.657	0.927 (0.747, 1.15), 0.49	1.073 (0.861, 1.337), 0.53
p for interaction		Reference	0.162	0.073	0.478
Proteinuria					
<3.5 g/d	151 (70.6)	1(Reference)	1.083 (0.895, 1.312), 0.412	1.061 (0.931, 1.209), 0.373	1.147 (0.981, 1.34), 0.087
≥3.5 g/d	63 (29.4)	1(Reference)	1.181 (0.698, 1.998), 0.537	1.104 (0.637, 1.912), 0.725	1.174 (0.71, 1.942), 0.533
p for interaction		Reference	0.724	0.935	0.789
History of hypertension					
No	126 (58.9)	1	1.405 (0.342, 5.997), 0.639	1.625 (0.503, 5.375), 0419	1.349 (0.41, 4.406), 0.619
Yes	88 (41.1)	1	1.603 (0.482, 5.387), 0.44	1.181 (0.486, 2.927), 0.715	2.715 (0.962, 7.959), 0.063
p for interaction			1	0.017	0.987
History of DM					
No	171 (79.9)	1	1.823 (0.706, 4.833), 0.219	1.415 (0.708, 2.867), 0.33	2.136 (0.958, 4.829), 0.065
Yes	43 (20.1)	1	1.184 (0.034, 47.386), 0.925	5.586 (0.227, 207.392),0.31	2.557 (0.121, 59.439), 0.541
p for interaction			0.718	0.965	0.846

[a]Adjusted for age, gender, BMI, MAP, albumin, Hb, eGFR, TC, LDL-C, use of ACEI/ARB, use of diuretic and use of Lipid-lowering drugs.
BMI, body mass index; MAP, mean arterial pressure; ECHO, Echocardiography; Hb, hemoglobin; ALb, albumin; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; DM, diabetes mellitus.

the relationship between sodium and volume overload and outcomes. Second, DSI and ECW/TBW were measured at enrollment and not serially. Thus, the results of single-point measurements may not clearly reflect the impact of fluid and sodium changes over time or the time-averaged exposure on CVD incidence. Third, it lacked follow-up data concerning future CV events which limits the study's predictive validity. Hence, further large, well-designed, prospective studies with multiple-measurements during follow-up are warranted to ascertain the short- and long-term efficacy of DSI and ECW/TBW on CVD in CKD patients.

5 Conclusion

We translate salt and volume status into clinical practice. The combination of high DSI (>6 g/d) and high ECW/TBW (>0.439) was associated with a greater risk of LVH or ELVFP incidence in non-dialysis CKD patients, independent of age, sex, BMI, MAP, albumin, eGFR, TC, LDL-C and the use of RAASi, diuretics, and lipid-lowering drugs. Moreover, adding well-accepted and widely-used parameters eGFR and proteinuria into consideration improved the diagnostic ability of DSI and ECW/TBW for risk stratification of cardiac impairments in CKD patients.

Our findings suggest that the salt and volume overload may be important mechanisms contributing to the CVD morbidity and mortality observed in non-dialysis CKD. Moreover, the findings establish salt and volume status reference ranges for risk stratification of future CVD in CKD. Timely modification of the salt and fluid status in non-dialysis CKD patients may be beneficial to achieve better CVD outcomes and therefore decrease the burden of mortality.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of the First Affiliated Hospital of Nanjing Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants or his/her legal guardians/next of kin.

Author contributions

SD: Writing – review & editing, Writing – original draft, Conceptualization. YM: Writing – original draft, Formal analysis, Data curation. FL: Writing – review & editing, Software, Formal analysis. CZ: Writing – original draft, Software, Investigation. HG: Writing – review & editing, Methodology, Data curation. MZ: Writing – review & editing, Data curation. BS: Writing – review & editing, Data curation. YY: Writing – review & editing, Data curation. CX: Writing – review & editing. HM: Writing – review & editing, Supervision. BZ: Validation, Writing – review & editing, Writing – original draft, Supervision, Funding acquisition.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. (2004) 351:1296–305. doi: 10.1056/NEJMoa041031

2. Stopic B, Medic-Brkic B, Savic-Vujovic K, Davidovic Z, Todorovic J, Dimkovic N. Biomarkers and predictors of adverse cardiovascular events in different stages of chronic kidney disease. *Dose Response*. (2022) 20:15593258221127568. doi: 10.1177/15593258221127568

3. Heywood JT, Fonarow GC, Costanzo MR, Mathur VS, Wigneswaran JR, Wynne J. High prevalence of renal dysfunction and its impact on outcome in 118,465 patients hospitalized with acute decompensated heart failure: a report from the adhere database. *J Card Fail*. (2007) 13:422–30. doi: 10.1016/j.cardfail.2007.03.011

4. Maqbool S, Shafiq S, Ali S, Rehman MEU, Malik J, Lee KY. Left ventricular hypertrophy (Lvh) and left ventricular geometric patterns in patients with chronic kidney disease (Ckd) stage 2-5 with preserved ejection fraction (Ef): a systematic review to explore Ckd stage-wise Lvh patterns. *Curr Probl Cardiol*. (2023) 48:101590. doi: 10.1016/j.cpcardiol.2023.101590

5. Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, et al. Association of Estimated Glomerular Filtration Rate and Albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative Meta-analysis. *Lancet*. (2010) 375:2073–81. doi: 10.1016/s0140-6736(10)60674-5

6. GBD 2019 Risk Factors Collaborators. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the global burden of disease study 2019. *Lancet*. (2020) 396:1223–49. doi: 10.1016/s0140-6736(20)30752-2

7. Playford D, Strange G, Celermajer DS, Evans G, Scalia GM, Stewart S, et al. Diastolic dysfunction and mortality in 436 360 men and women: the National Echo Database Australia (Neda). *Eur Heart J Cardiovasc Imaging*. (2021) 22:505–15. doi: 10.1093/ehjci/jeaa253

8. Ogawa T, Nitta K. Clinical impact of left ventricular diastolic dysfunction in chronic kidney disease. *Contrib Nephrol*. (2018) 195:81–91. doi: 10.1159/000486938

9. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*. (2009) 10:165–93. doi: 10.1093/ejechocard/jep007

10. Li C, Zhang J, Zhou C, Huang L, Tang H, Rao L. Will simultaneous measurement of E/E' index facilitate the non-invasive assessment of left ventricular filling pressure in patients with non-Valvular atrial fibrillation? *Eur J Echocardiogr*. (2010) 11:296–301. doi: 10.1093/ejechocard/jep218

11. Chen SM, He R, Li WH, Li ZP, Chen BX, Feng XH. Relationship between exercise induced elevation of left ventricular filling pressure and exercise intolerance in patients with atrial fibrillation. *J Geriatr Cardiol*. (2016) 13:546–51. doi: 10.11909/j.issn.1671-5411.2016.06.016

12. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. Corrigendum to: 2021 esc guidelines for the diagnosis and treatment of acute and chronic heart failure: developed by the task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (esc) with the special contribution of the heart failure association (Hfa) of the esc. *Eur Heart J*. (2021) 42:4901. doi: 10.1093/eurheartj/ehab670

13. Vallianou NG, Mitesh S, Gkogkou A, Geladari E. Chronic kidney disease and cardiovascular disease: is there any relationship? *Curr Cardiol Rev*. (2019) 15:55–63. doi: 10.2174/1573403x14666180711124825

14. Matsushita K, Ballew SH, Wang AY, Kalyesubula R, Schaeffner E, Agarwal R. Epidemiology and risk of cardiovascular disease in populations with chronic kidney disease. *Nat Rev Nephrol*. (2022) 18:696–707. doi: 10.1038/s41581-022-00616-6

15. Romejko K, Rymarz A, Szamotulska K, Bartoszewicz Z, Rozmyslowicz T, Niemczyk S. Left ventricular diastolic dysfunction in chronic kidney disease patients not treated with Dialysis. *Nutrients*. (2022) 14:4664. doi: 10.3390/nu14214664

16. Cai QZ, Lu XZ, Lu Y, Wang AY. Longitudinal changes of cardiac structure and function in Ckd (Cascade study). *J Am Soc Nephrol*. (2014) 25:1599–608. doi: 10.1681/asn.2013080899

17. Mills KT, Chen J, Yang W, Appel LJ, Kusek JW, Alper A, et al. Sodium excretion and the risk of cardiovascular disease in patients with chronic kidney disease. *JAMA*. (2016) 315:2200–10. Epub 2016/05/25. doi: 10.1001/jama.2016.4447

18. Lambers Heerspink HJ, Holtkamp FA, Parving HH, Navis GJ, Lewis JB, Ritz E, et al. Moderation of dietary sodium potentiates the renal and cardiovascular protective effects of angiotensin receptor blockers. *Kidney Int*. (2012) 82:330–7. doi: 10.1038/ki.2012.74

19. Kohatsu K, Shimizu S, Shibagaki Y, Sakurada T. Association between daily urinary sodium excretion, ratio of extracellular water-to-Total body water ratio, and kidney outcome in patients with chronic kidney disease. *Nutrients*. (2021) 13:650. doi: 10.3390/nu13020650

20. Mok Y, Ballew SH, Matsushita K. Chronic kidney disease measures for cardiovascular risk prediction. *Atherosclerosis*. (2021) 335:110–8. Epub 2021/09/25. doi: 10.1016/j.atherosclerosis.2021.09.007

21. Hung SC, Lai YS, Kuo KL, Tarng DC. Volume overload and adverse outcomes in chronic kidney disease: clinical observational and animal studies. *J Am Heart Assoc*. (2015) 4:e001918. doi: 10.1161/JAHA.115.001918

22. Kdigo 2021 clinical practice guideline for the management of glomerular diseases. *Kidney Int*. (2021) 100:S1–s276. doi: 10.1016/j.kint.2021.05.021

23. McLean RM. Measuring population sodium intake: a review of methods. *Nutrients*. (2014) 6:4651–62. doi: 10.3390/nu6114651

24. Olde Engberink RHG, van den Hoek TC, van Noordenne ND, van den Born BH, Peters-Sengers H, Vogt L. Use of a single baseline versus multiyear 24-hour urine collection for estimation of long-term sodium intake and associated cardiovascular and renal risk. *Circulation*. (2017) 136:917–26. doi: 10.1161/circulationaha.117.029028

25. Essential points from evidence-based clinical practice guidelines for chronic kidney disease 2018. *Clin Exp Nephrol*. (2019) 23:1–15. doi: 10.1007/s10157-018-1648-1

26. Evidence-based practice guideline for the treatment of Ckd. *Clin Exp Nephrol*. (2009) 13:537–66. doi: 10.1007/s10157-009-0237-8

27. Xu X, Zeng L, Jha V, Cobb LK, Shibuya K, Appel LJ, et al. Potassium-enriched salt substitutes: a review of recommendations in clinical management guidelines. *Hypertension*. (2024) 81:400–14. doi: 10.1161/hypertensionaha.123.21343

28. Chinese clinical practice guideline for the management of "Ckd-Peridialysis"-the periods Prior to and in the early-stage of initial Dialysis. *Kidney Int Rep*. (2022) 7:S531–58. doi: 10.1016/j.ekir.2022.10.001

29. Sun L, Li Q, Sun Z, Duan S, Nie G, Dong J, et al. Impact of Overhydration on left ventricular hypertrophy in patients with chronic kidney disease. *Front Nutr*. (2022) 9:761848. doi: 10.3389/fnut.2022.761848

30. Wu B, Peng Y, Zhang S, Yan W, Gao M, Ge Y, et al. Body composition analysis as a predictor of prognosis for patients with acute kidney injury requiring kidney replacement therapy. *J Ren Nutr*. (2021) 31:560–8.e2. doi: 10.1053/j.jrn.2020.12.011

31. Sukackiene D, Laucyte-Cibulskiene A, Vickiene A, Rimsevicius L, Miglinas M. Risk stratification for patients awaiting kidney transplantation: role of bioimpedance derived edema index and nutrition status. *Clin Nutr*. (2020) 39:2759–63. doi: 10.1016/j.clnu.2019.12.001

32. Zhang J, Zhang N, Du S, Liu S, Ma G. Effects of water restriction and water replenishment on the content of body water with bioelectrical impedance among young adults in Baoding, China: a randomized controlled trial (Rct). *Nutrients*. (2021) 13:3645. doi: 10.3390/nu13103645

33. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European association of, cardiovascular imaging. *Eur Heart J Cardiovasc Imaging*. (2016) 17:412. doi: 10.1093/ehjci/jew041

34. Park S, Lee CJ, Jhee JH, Yun HR, Kim H, Jung SY, et al. Extracellular fluid excess is significantly associated with coronary artery calcification in patients with chronic kidney disease. *J Am Heart Assoc*. (2018) 7:e008935. doi: 10.1161/jaha.118.008935

35. Kim EJ, Choi MJ, Lee JH, Oh JE, Seo JW, Lee YK, et al. Extracellular fluid/intracellular fluid volume ratio as a novel risk Indicator for all-cause mortality and cardiovascular disease in hemodialysis patients. *PLoS One*. (2017) 12:e0170272. doi: 10.1371/journal.pone.0170272

36. Tsai YC, Chiu YW, Tsai JC, Kuo HT, Hung CC, Hwang SJ, et al. Association of Fluid Overload with cardiovascular morbidity and all-cause mortality in stages 4 and 5 Ckd. *Clin J Am Soc Nephrol*. (2015) 10:39–46. doi: 10.2215/cjn.03610414

37. Low S, Pek S, Liu YL, Moh A, Ang K, Tang WE, et al. Higher extracellular water to Total body water ratio was associated with chronic kidney disease progression in type 2 diabetes. *J Diabetes Complicat*. (2021) 35:107930. doi: 10.1016/j.jdiacomp.2021.107930

38. Stegmayr BG. Ultrafiltration and dry weight-what are the cardiovascular effects? *Artif Organs*. (2003) 27:227–9. doi: 10.1046/j.1525-1594.2003.07205.x

39. Liu AYL, Pek S, Low S, Moh A, Ang K, Tang WE, et al. Association of Overhydration and Serum Pigment Epithelium-Derived Factor with Ckd progression in diabetic kidney disease: a prospective cohort study. *Diabetes Res Clin Pract*. (2021) 174:108754. doi: 10.1016/j.diabres.2021.108754

40. Fontecave-Jallon J, Thomas SR. Implementation of a model of bodily fluids regulation. *Acta Biotheor*. (2015) 63:269–82. Epub 2015/05/04. doi: 10.1007/s10441-015-9250-3

41. D'Elia L, Rossi G, Schiano di Cola M, Savino I, Galletti F, Strazzullo P. Meta-analysis of the effect of dietary sodium restriction with or without concomitant renin-angiotensin-aldosterone system-inhibiting treatment on albuminuria. *Clin J Am Soc Nephrol*. (2015) 10:1542–52. doi: 10.2215/cjn.09110914

42. Sen T, Heerspink HJL. A kidney perspective on the mechanism of action of sodium glucose co-transporter 2 inhibitors. *Cell Metab*. (2021) 33:732–9. doi: 10.1016/j.cmet.2021.02.016

43. He J, Mills KT, Appel LJ, Yang W, Chen J, Lee BT, et al. Urinary sodium and potassium excretion and Ckd progression. *J Am Soc Nephrol*. (2016) 27:1202–12. doi: 10.1681/asn.2015010022

44. Campbell KL, Johnson DW, Bauer JD, Hawley CM, Isbel NM, Stowasser M, et al. A randomized trial of sodium-restriction on kidney function, fluid volume and Adipokines in Ckd patients. *BMC Nephrol*. (2014) 15:57. doi: 10.1186/1471-2369-15-57

45. Hwang JH, Chin HJ, Kim S, Kim DK, Kim S, Park JH, et al. Effects of intensive Low-salt diet education on albuminuria among nondiabetic patients with hypertension treated with Olmesartan: a single-blinded randomized, controlled trial. *Clin J Am Soc Nephrol*. (2014) 9:2059–69. doi: 10.2215/cjn.01310214

46. Fan L, Tighiouart H, Levey AS, Beck GJ, Sarnak MJ. Urinary sodium excretion and kidney failure in nondiabetic chronic kidney disease. *Kidney Int*. (2014) 86:582–8. doi: 10.1038/ki.2014.59

47. Mazarova A, Molnar AO, Akbari A, Sood MM, Hiremath S, Burns KD, et al. The Association of Urinary Sodium Excretion and the need for renal replacement therapy in advanced chronic kidney disease: a cohort study. *BMC Nephrol*. (2016) 17:123. doi: 10.1186/s12882-016-0338-z

48. Thomas MC, Moran J, Forsblom C, Harjutsalo V, Thorn L, Ahola A, et al. The association between dietary sodium intake, Esrd, and all-cause mortality in patients with type 1 diabetes. *Diabetes Care*. (2011) 34:861–6. doi: 10.2337/dc10-1722

49. Cerasola G, Nardi E, Palermo A, Mulè G, Cottone S. Epidemiology and pathophysiology of left ventricular abnormalities in chronic kidney disease: a review. *J Nephrol*. (2011) 24:1–10. doi: 10.5301/jn.2010.2030

50. Cerasola G, Nardi E, Mulè G, Palermo A, Cusimano P, Guarneri M, et al. Left ventricular mass in hypertensive patients with mild-to-moderate reduction of renal function. *Nephrology*. (2010) 15:203–10. doi: 10.1111/j.1440-1797.2009.01178.x

51. Wachtell K, Olsen MH, Dahlöf B, Devereux RB, Kjeldsen SE, Nieminen MS, et al. Microalbuminuria in hypertensive patients with electrocardiographic left ventricular hypertrophy: the life study. *J Hypertens*. (2002) 20:405–12. doi: 10.1097/00004872-200203000-00015

52. Frohlich ED. The salt conundrum: a hypothesis. *Hypertension*. (2007) 50:161–6. doi: 10.1161/hypertensionaha.107.088328

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The potassium puzzle: exploring the intriguing connection to albuminuria

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Background: Studies have revealed a relationship between dietary potassium intake and albuminuria, despite the fact that the human body needs a lot of potassium. Our study concentrated on the link between dietary potassium intake and albuminuria.

Methods: This study used subgroup analysis and weighted multivariate regression analysis. Data from the National Health and Nutrition Examination Survey (NHANES) were examined to determine the urinary albumin-to-creatinine ratio (ACR) and participant age (20 years or older). ACR >30 mg/g was the threshold for albuminuria.

Results: 7,564 individuals in all were included in the study. The link between the two was significant in both our original model (OR = 0.99; 95% CI, 0.98–0.99, $p < 0.0001$) and the minimum adjusted model (OR = 0.99; 95% CI, 0.98–0.99, $p < 0.0001$). A fully adjusted model did not change the significance of the negative correlation between potassium consumption and albuminuria (OR = 0.99; 95% CI, 0.98–1.00, $p = 0.0005$), indicating that each unit increase in potassium intake was related with a 1% decrease in the chance of developing albuminuria. The negative correlation between potassium intake and albuminuria was not significantly influenced by sex, age, BMI, hypertension, diabetes, or smoking, according to interaction tests (p for interaction >0.05).

Conclusion: Reduced risk of albuminuria was linked to higher dietary potassium intake. The particular mechanism linking the two still has to be explained by several inventive and prospective studies.

KEYWORDS

albuminuria, potassium, dietary element intake, NHANES, cross-sectional study

1 Introduction

Albuminuria is the amount of albumin in the urine that exceeds the normal range and is often an important indicator of kidney disease. The kidney is one of the important organs in the human body, which is mainly responsible for excreting waste and regulating water, electrolyte and acid–base balance in the body. Albumin is an important plasma protein synthesized by the liver, reabsorbed by the renal tubules after glomerular filtration, and finally excreted from the body. When the glomerular filtration membrane is damaged or the renal tubule reabsorption function is reduced, it causes albumin to leak out of the urine, forming albuminuria. Potassium is one of the essential minerals in the human body and plays an

important role in maintaining the normal function of the heart, muscles and nervous system. The kidney is one of the main organs regulating the balance of potassium in the human body, and maintains the stability of potassium in the body through excretion and reabsorption. When kidney function is impaired, potassium metabolism is disrupted, which in turn affects the normal function of the heart, muscles and nervous system. Recent studies have found that there is a certain relationship between potassium and albuminuria (1). Some studies have found that moderate potassium intake can reduce the risk of albuminuria, possibly because potassium promotes the reabsorption of albumin by the renal tubules, reducing albumin leakage from the urine. In addition, other studies have found that excessive potassium intake may increase the risk of albuminuria, possibly because excess potassium can cause a heavier burden on the kidneys, which in turn affects the function of the glomerular filtration membrane. In summary, there is a relationship between potassium and albuminuria, and moderate potassium intake may reduce the risk of albuminuria, but excessive potassium intake may increase the risk of albuminuria. Therefore, it is recommended that people according to their health conditions and eating habits, moderate intake of potassium. If you have kidney disease or other health problems, dietary adjustments and treatment should be made under the guidance of your doctor.

Albuminuria independently predicts cardiovascular disease (2) and chronic kidney disease (3). The ratio of urine albumin to creatinine (ACR), which is used to determine whether or not a person has albuminuria (increased excretion of urinary albumin), must be greater than 30 mg/g (4). Albuminuria can be detected using a variety of techniques, such as standard urine testing, protein qualitative analysis, urine microalbumin detection, etc. ACR detection is currently a relatively quick and accurate process, nevertheless (5). Multiple studies have found that albuminuria has become a serious public health problem, with The median prevalence of ACR ≥30 mg/g across cohorts was 32.1% in diabetes and 21.8% in hypertension (6). Therefore, research into albuminuria is crucial.

Potassium is a chemical element, located in the fourth period of the periodic table (7). Potassium is very active chemically and is a white metal with a soft texture, low boiling point, and melting point, and lower density than water (8). Potassium intake is very important to the body (9), serving as an electrolyte, balancing body fluids, and controlling muscles (10). A lack of potassium at the same time can lead to irregular heartbeats (11), insomnia (9), muscle cramps (12), and fatigue (13). In community-dwelling Japanese people, Kabasawa et al. discovered that dietary potassium intake was linked to higher albuminuria (14). According to Sharma et al., higher sodium and potassium intake is associated with a lower incidence of CKD in U.S. adults. These results should be confirmed by longitudinal studies and specially designed clinical trials to examine the effects of dietary sodium and potassium intake on kidney disease and its progression (15). It is possible to include dietary potassium consumption as an exposure variable because the association between potassium intake and albuminuria has been studied in Japan.

Dietary potassium intake and albuminuria have a complex relationship. CKD progression was found to be substantially faster in hyperkaluric people than in hypokaluric people in a study of patients with CKD stages 2–4 and the association between urine potassium and CKD progression and all-cause mortality (16). Another study discovered that the glomerular filtration rate was unrelated to the substantial association between each 1,000 mg increase in daily dietary potassium intake and a 29% reduction in the probability of developing proteinuria (17). Higher levels of the dietary protein-to-potassium ratio (NEAP) were linked to albuminuria and inversely connected with potassium consumption, according to research by Kabasawa et al. (18). However, we discovered contradictory findings from Kabasawa et al. twice about the link between albuminuria and potassium consumption.

So, we looked into the connection between albuminuria and potassium consumption. Additionally, it was proposed that a higher incidence of albuminuria was linked to a decreased potassium intake.

2 Materials and methods

2.1 Data sources and participants

The NHANES study evaluates children's and adults' nutritional and health status in the US. Children and adults were excluded from this study at the age of 20, and the evaluation study comprised data from questionnaire interviews, tests, and laboratory measurements (19). The board of the NCHS Research Ethics Review has approved the NHANES program of the National Center for Health Statistics. Legal guardians of survey participants under the age of 16 have additionally given written informed consent.

In this study, two cycles of NHANES, 2017–2018 and 2019–2020, were selected. In this study, the 2017–2018 cycle and the 2019–2020 cycle are selected, which are the two most recent cycles with new data. At the same time, this study selects two cycles of data volume to meet the requirements of data statistics. Not only these two cycles included complete dietary potassium intake and albuminuria data, but also the National Center for Health Statistics (NCHS) had merged data for these two cycles, and the data were reliable.

A flowchart of the research population selection is shown in Figure 1. According to the inclusion and exclusion criteria, this study excluded 6,328 people under the age of 20 from the 15,560 participants, excluded 913 people with missing creatinine ratio data, excluded 755 people with missing dietary potassium intake data, and finally included 7,564 people in the study.

2.2 Definition of dietary potassium intake and definition of albuminuria

It was selected from the total nutrient intake on day 1 from diet interview data. Interviews were conducted in a fixed environment set up by NHANE, and subjects were provided with a set of test guidelines for reporting food intake. Dietary potassium intake in this study was set as the exposure variable in milligrams.

In this study, the variable URDACT was selected from laboratory data. The urine albumin/creatinine ratio in mg/g (URDACT) was calculated by dividing the urine albumin by the urine creatinine value and multiplying it by 100 (rounded to 2 decimal places). The simultaneous measurement of human urinary albumin is a solid-phase fluorescent immunoassay. Albuminuria was defined as URDACT >30 mg/g (20). Albuminuria was set as an outcome variable in this study.

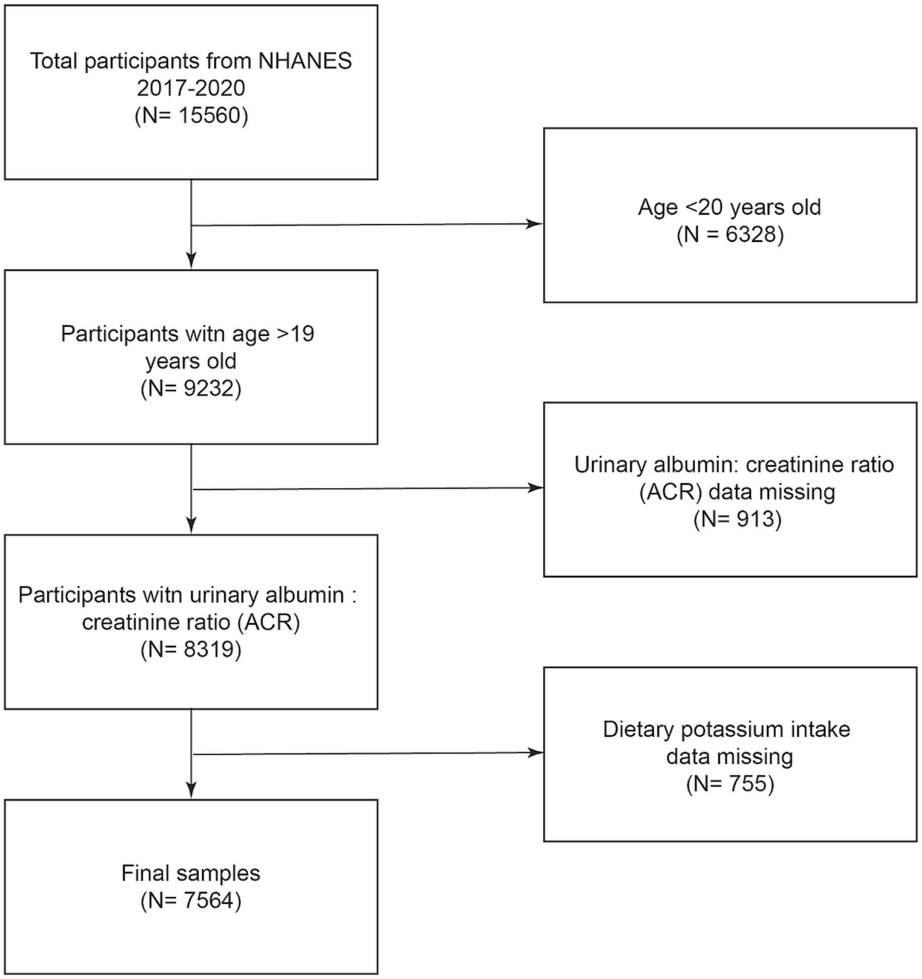

FIGURE 1
NHANES participant selection flowchart for 2017–2020.

2.3 Covariates

In this investigation, factors that could influence the inverse relationship between dietary potassium intake and albuminuria were included. Covariates include age, poverty-income ratio, total energy intake, total protein intake, dietary carbohydrate intake, dietary sugar intake, dietary total fat intake, dietary cholesterol, dietary calcium intake, caffeine intake, DBP, BMI, fasting blood glucose (21), serum creatinine (22), globulin, Triglycerides (23), uric acid (24), gender, race, education, marital status, drinking level, Hypertensive (25), exercise level, smoking level, etc. BMI, systolic and diastolic blood pressure are important indicators in health assessment, and they were used to analyze the health status of participants in the NHANES study. First of all, BMI (Body Mass Index) is a value obtained by dividing weight (kilograms) by the square of height (meters), used to assess the weight status of an individual. In NHANES, BMI is used to determine whether adults and children are in a healthy weight range, or whether they are underweight, overweight or obese. Second, Systolic Blood Pressure (SBP) refers to the highest pressure in the arteries when the heart contracts, often referred to as "high pressure." When the heart contracts to pump blood into the arteries, the pressure inside the arteries rises and the blood pressure measured is called systolic. In NHANES, a measurement of systolic blood pressure is used to assess the risk of cardiovascular disease, and it is generally believed that a systolic blood pressure greater than or equal to 140 mmHg in adults may indicate an increased risk of hypertension. Finally, Diastolic Blood Pressure (DBP) is the lowest pressure in the arteries when the heart diastates, also known as "low pressure." When the heart diastates and blood flows back to the heart, the pressure in the arteries decreases, and the blood pressure measured at this time is called diastolic pressure. In NHANES, diastolic blood pressure measurements are also used to assess the risk of cardiovascular disease, and it is generally believed that diastolic blood pressure greater than or equal to 90 mmHg in adults may indicate an increased risk of hypertension. These indicators are important for understanding the health status of the U.S. population, and they can help public health policymakers develop targeted health promotion programs and interventions. Through these data, we can monitor the prevalence trend of chronic diseases such as hypertension and obesity in the population, so as to provide a scientific basis for the prevention and control of these diseases. The income-to-poverty ratio in NHANES is a measure used to assess participants' economic status and standard of living. Data on the income-to-poverty ratio is one of the important socioeconomic variables in the NHANES survey. These data are often used to analyze

the relationship between health outcomes and economic status, as economic level has a direct impact on the health status of individuals and families. Alcohol consumption levels were assessed as to whether subjects had ever drank 4 to 5 drinks or more per day during their life, 4 drinks or more for women and 5 drinks or more for men. The exercise status questionnaire was set as follows: Does the profession require exerting high levels of effort for at least 10 min at a time, such as lifting or carrying heavy objects continuously, digging, or building work? Selecting smoking status, using a questionnaire, have you ever smoked at least 100 cigarettes in your life, including other tobacco use? 9th through 11th grade (including 12th grade without a diploma). Systolic blood pressure and diastolic blood pressure are, respectively, selected from the average value of three consecutive blood pressure measurement data.

2.4 Statistic analysis

Statistical studies were done using the R language (version 4.1.0) and EmpowerStats (version: 4.0). The baseline table of the research population was counted by dietary potassium groups, continuous variables were represented by means and positive and negative standard deviations, and weighted linear regression models were used to describe them.

Different models of the relationship between dietary potassium intake and albuminuria were tested using multivariate logistic regression. Race, age, and gender factors were adjusted in Model 2 but not in Model 1, Model 3 adjusted covariates poverty income ratio, total dietary energy, protein, dietary carbohydrate, sugar, fat, dietary cholesterol intake, dietary calcium intake, caffeine intake, DBP, BMI, fasting blood glucose, serum creatinine, globulin, Triglycerides, uric acid, gender, race, education, marital status, drinking level, hypertensive, exercise level, smoking level. In addition, stratification criteria for the subgroup analysis of the association between dietary potassium intake and albumin birds include gender, educational attainment, smoking status, diabetes, and hypertension. For continuous variables, the median was used as a replacement, and for categorical variables, the mode. Statistical significance is defined as $p < 0.05$.

3 Results

3.1 Baseline characteristic

According to the inclusion and exclusion criteria, there were 7,564 participants over the age of 20 in this study, and the average age of the participants was 50.62 ± 17.33 years old. The subjects in this study were 48.69% male, 51.31% female, 11.63% Mexican American, 26.72% non-Hispanic black, 35.75% non-Hispanic white, 10.23% other Hispanic, and 15.67% other races. The average dietary potassium intake was 2519.81 ± 1271.07 mg.

Table 1 presents the baseline characteristics of the dietary potassium intake tertile pairs. Among the tertile of dietary potassium intake, poverty-income ratio, total energy, marital status, total protein, gender, carbohydrates, education level, total sugar, total fat, dietary cholesterol, dietary calcium, race, caffeine, DBP, BMI, uric acid, fasting blood glucose, serum creatinine, globulin, Triglycerides, drinking level, hypertensive, exercise level, smoking level, and albuminuria were all statistically significant ($p < 0.05$). At the same time, 13.59% of subjects were classified as having increased urinary albumin excretion, which decreased with increasing dietary potassium intake. Overall, the third quartile of dietary potassium intake and the part with higher intake are more likely to have a low poverty-income ratio, high dietary total energy intake, high protein intake, high carbohydrate intake, high total sugar intake, high total fat intake, high cholesterol intake, high calcium intake, high caffeine intake, low DBP, low BMI, high serum creatinine, low globulin, high Triglycerides, high uric acid, male, non-Hispanic white, Some college or AA degree, Married/Living with Partner, non-drinking, non-hypertensive, non-participating in strenuous exercise, non-smoking, non-albuminuric subjects (see Table 2).

3.2 Relationship between albuminuria and dietary potassium intake

According to our research, a higher dietary potassium intake was linked to a lower risk of albuminuria. In our original model ($p < 0.0001$) and minimally adjusted model ($p < 0.0001$), there was a strong correlation between the two. This negative association between potassium intake and albuminuria remained significant in a fully adjusted model ($p = 0.0005$), indicating that each unit increase in potassium intake was associated with a 1% reduction in the risk of developing albuminuria. After conducting sensitivity analysis, it was discovered that patients in the third quartile had a statistically significant 26% lower incidence of albuminuria than those in the first quartile ($p = 0.0016$).

Table 3 is the multivariate logistic regression model for albuminuria. In fully adjusted models, age, education, smoking level, exercise level, BMI, SBP, DBP, hypertension, diabetes, uric acid, Triglycerides, total dietary protein intake, serum creatinine, and cholesterol were significantly ($p < 0.05$) associated with albuminuria. Subjects with 9-11th grade, High school graduate/GED or similar. Some college or AA degree, College graduate or above, and Subjects with Less Than 9th Grade Education were 35.3, 45.2, 48.4, and 63.2% Less Likely to Have Albuminuria, respectively. When compared to participants who engaged in vigorous activity regularly, those who did not had a 33.3% higher risk of developing albuminuria. Nonsmokers were 18.7% less likely to have albuminuria than smokers. At the same time, the possibility of non-hypertensive and non-diabetic albuminuria was reduced by 69.3 and 80.7%. For each unit increase of SBP and DBP, the possibility of albuminuria increased by 3.1 and 1.8%.

3.3 Subgroup analysis

Figure 2 displays a subgroup analysis of the relationship between dietary potassium intake and albuminuria. We discovered erratic relationships between dietary potassium intake and a potential decline in albuminuria prevalence. By sex, age, and smoking, potassium intake was significantly associated with albuminuria in each subgroup. Among BMI subgroups, the association of potassium intake with albuminuria was significant for normal weight and overweight subgroups, but not statistically significant for lower weight and obese subgroups. Simultaneous interaction tests indicated that gender, age,

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TABLE 1 Baseline characteristics of dietary potassium intake by quantile for the study population.

Dietary potassium intake	Tertile 1 (n = 2,515)	Tertile 2 (n = 2,526)	Tertile 3 (n = 2,523)	p-value
Age (years)	49.98 ± 17.61	51.02 ± 17.42	50.85 ± 16.93	0.110
Income of poverty ratio	2.32 ± 1.56	2.73 ± 1.62	2.82 ± 1.66	<0.001
Energy (kcal)	1411.49 ± 565.16	2065.69 ± 668.15	2905.50 ± 1121.18	<0.001
Protein (gm)	49.03 ± 20.98	76.45 ± 26.43	112.38 ± 48.01	<0.001
Carbohydrate (gm)	167.38 ± 80.16	237.40 ± 92.11	332.47 ± 141.21	<0.001
Total sugars (gm)	73.39 ± 54.95	101.42 ± 63.15	142.58 ± 89.96	<0.001
Total fat (gm)	56.91 ± 29.20	85.39 ± 36.99	118.19 ± 58.28	<0.001
Cholesterol (mg)	213.01 ± 192.92	311.74 ± 222.01	424.86 ± 302.00	<0.001
Calcium (mg)	574.15 ± 337.20	869.45 ± 403.83	1254.05 ± 669.72	<0.001
Caffeine (mg)	95.28 ± 114.44	136.05 ± 151.53	193.68 ± 291.78	<0.001
SBP (mmHg)	125.09 ± 20.46	123.89 ± 18.64	124.57 ± 17.81	0.265
DBP (mmHg)	75.61 ± 11.93	74.44 ± 11.26	74.31 ± 11.12	<0.001
BMI (kg/m2)	30.81 ± 8.01	30.18 ± 7.46	29.36 ± 7.01	<0.001
Glycohemoglobin (%)	5.89 ± 1.19	5.86 ± 1.12	5.83 ± 1.06	0.678
Fasting glucose (mmol/L)	6.21 ± 2.00	6.34 ± 2.26	6.33 ± 2.05	0.002
Creatinine, refrigerated serum (umol/L)	79.42 ± 42.33	78.15 ± 37.49	80.22 ± 34.84	<0.001
Globulin (g/L)	31.43 ± 4.38	30.97 ± 4.49	30.43 ± 4.17	<0.001
Cholesterol, refrigerated serum (mmol/L)	4.80 ± 1.06	4.81 ± 1.05	4.84 ± 1.07	0.295
Triglycerides (mmol/L)	1.50 ± 1.17	1.55 ± 0.98	1.67 ± 1.47	<0.001
Uric acid (umol/L)	318.73 ± 89.15	318.80 ± 87.20	326.21 ± 86.18	0.001
Gender (%)				<0.001
Male	37.26	45.37	63.42	
Female	62.74	54.63	36.58	
Race (%)				<0.001
Mexican American	9.50	11.44	13.95	
Other Hispanic	9.98	9.74	10.98	
Non-Hispanic White	31.37	38.08	37.77	
Non-Hispanic Black	34.83	24.98	20.37	
Other race	14.31	15.76	16.92	
Education level (%)				<0.001
Less than 9th grade	6.96	6.25	7.41	
9-11th grade	12.13	9.78	10.34	
High school graduate/GED or equivalent	29.38	22.84	20.13	
Some college or AA degree	33.64	34.60	31.83	
College graduate or above	17.89	26.53	30.29	
Marital status (%)				<0.001
Married/living with partner	51.41	60.06	62.47	
Widowed/divorced/separated	25.09	21.77	20.41	
Never married	23.5	18.17	17.12	
Drinking status (%)				<0.001
Yes	13.36	13.57	19.42	
No	86.64	86.43	80.58	

(Continued)

TABLE 1 (Continued)

Dietary potassium intake	Tertile 1 (n = 2,515)	Tertile 2 (n = 2,526)	Tertile 3 (n = 2,523)	p-value
Hypertensive (%)				0.001
Yes	40.91	39.07	35.24	
No	59.09	60.93	64.76	
Pain or discomfort in chest (%)				0.479
Yes	31.33	30.47	29.38	
No	68.67	69.53	70.61	
Diabetes (%)				0.066
Yes	16.42	15.56	13.75	
No	83.58	84.44	86.25	
Vigorous work activity (%)				0.003
Yes	24.06	25.22	28.42	
No	75.94	74.78	71.58	
Smoke status				0.026
Yes	42.54	40.22	44.43	
No	57.46	59.78	55.57	
Albuminuria (%)				0.002
Yes	14.99	14.13	11.65	
No	85.01	85.87	88.35	

The mean ± SD of continuous variables: p-value is calculated using a weighted linear regression model categorical variable: p-value is calculated by weighted chi-square test.

TABLE 2 Association between dietary potassium intake and albuminuria.

	OR[a] (95% CI[b]), p-value		
	Crude model (model 1)[c]	Minimally adjusted model (model 2)[d]	Fully adjusted model (model 3)[e]
Continuous	0.99 (0.98, 0.99) <0.0001	0.99 (0.98, 0.99) <0.0001	0.99 (0.98, 1.00) 0.0005
Categories			
Tertile 1	Reference	Reference	Reference
Tertile 2	0.93 (0.80, 1.09), 0.3884	0.92 (0.78, 1.08), 0.3153	0.91 (0.76, 1.09), 0.2967
Tertile 3	0.75 (0.64, 0.88), 0.0005	0.73 (0.62, 0.87), 0.0004	0.74 (0.61, 0.89), 0.0016
p for trend	0.0004	0.0004	0.0014

In the sensitivity analysis, dietary potassium intake is converted from a continuous variable to a categorical variable (quantile), where we set the third place.
[a]OR: odds ratio.
[b]95%CI: 95% confidence interval.
[c]Model 1: no covariates were adjusted.
[d]Model 2: adjusted for gender, age, and race.
[e]Model 3: adjusted for gender, age, race, income of poverty ratio, energy, protein, carbohydrate, total sugars, total fat, cholesterol, calcium, caffeine, SBP, DBP, BMI, triglyceride, total Cholesterol, glycohemoglobin, fasting Glucose, creatinine, refrigerated serum, cholesterol, refrigerated serum, triglycerides, uric acid, education level, marital status, drinking status, hypertensive, pain, diabetes, vigorous work activity, smoke status, and albuminuria. Because the results were not obvious, we amplified dietary potassium intake by 100 times to study.

BMI, hypertension, diabetes, and smoking had no significant dependence on the negative association between potassium intake and albuminuria.

4 Discussion

Based on the inclusion and exclusion criteria, 7,564 people participated in the study throughout the National Health and Nutrition Examination Survey (NHANES) cycle from 2017 to 2020. We discovered that people who consumed more potassium through their diets had a lower likelihood of having albuminuria. Interaction tests and subgroup analysis suggest that this link may hold across populations. Our findings suggest that consuming a diet rich in potassium may raise the likelihood of developing albuminuria.

Potassium is one of the important minerals for maintaining normal physiological functions of the human body and is also very important for kidney health. Proteinuria refers to the amount of protein in the urine that exceeds the normal range and is often an important indicator of kidney disease. Current studies have been inconsistent on the effect of potassium intake on the likelihood of proteinuria. Some studies have

TABLE 3 Multivariate logistic regression model of albuminuria.

Variables	OR[a] (95%CI[b])	*p*-value
Dietary potassium intake	0.988 (0.983, 0.994)	0.00004
Age (year)	1.035 (1.031, 1.040)	<0.00001
Female (versus male)	0.950 (0.833, 1.083)	0.44197
Race (versus Mexican American)		
Other Hispanic	0.773 (0.579, 1.032)	0.08063
Non-Hispanic White	0.865 (0.695, 1.076)	0.19339
Non-Hispanic Black	1.068 (0.855, 1.335)	0.56034
Other race	0.864 (0.671, 1.114)	0.25960
Education level (versus less than 9th grade)		
9-11th grade	0.647 (0.491, 0.854)	0.00210
High school graduate/GED or equivalent	0.548 (0.429, 0.699)	<0.00001
Some college or AA degree	0.516 (0.408, 0.652)	<0.00001
College graduate or above	0.368 (0.286, 0.475)	<0.00001
Smoke status (no versus yes)	0.813 (0.712, 0.928)	0.00210
Vigorous work activity (no versus yes)	1.333 (1.137, 1.562)	0.00039
BMI (kg/m2)	1.022 (1.013, 1.030)	<0.00001
SBP (mmHg)	1.031 (1.028, 1.035)	<0.00001
DBP (mmHg)	1.018 (1.012, 1.024)	<0.00001
Hypertensive (no versus yes)	0.307 (0.268, 0.352)	<0.00001
Diabetes (no versus yes)	0.193 (0.167, 0.224)	<0.00001
Uric acid (umol/L)	1.002 (1.002, 1.003)	<0.00001
Triglycerides (mmol/L)	1.140 (1.087, 1.195)	<0.00001
Total Protein (g/L)	1.035 (1.019, 1.051)	<0.00001
Creatinine (umol/L)	1.016 (1.013, 1.018)	<0.00001
Cholesterol (mmol/L)	0.885 (0.828, 0.945)	0.00030

Total protein (g/L) is the amount of total protein in the serum. The unit of the continuous variable and the reference value of the non-continuous variable are moved next to the variable. In the continuous variable, the OR of proteinuria increased per unit, compared to the reference value in the categorical variable.
[a]OR: odds ratio.
[b]95%CI: 95% confidence interval.

found that moderate potassium intake can reduce the risk of proteinuria because potassium can help maintain normal blood pressure and kidney function. For example, a study published in the Journal of the American College of Cardiology found that people who consumed 2,000 to 4,000 milligrams of potassium per day had a 30 percent lower risk of proteinuria compared to those who consumed less than 2,000 milligrams of potassium per day. However, some studies have found that excessive potassium intake may increase the risk of proteinuria. For example, a study published in the American Journal of Clinical Nutrition found that people who consumed more than 5,000 mg of potassium per day had a 40 percent increased risk of proteinuria compared to those who consumed less than 2,000 mg of potassium per day. Overall, moderate potassium intake is beneficial for maintaining kidney health and reducing the risk of proteinuria. However, excessive potassium intake may increase the risk of proteinuria. Therefore, it is recommended that people according to their health conditions and dietary habits, moderate intake of potassium. If you have kidney disease or other health problems, you should adjust your potassium intake under the guidance of your doctor.

According to certain research, inadequate dietary potassium consumption may cause a decline in renal function, which raises the risk of albuminuria. This is about the impact of dietary potassium intake on renal function (26). According to Wu et al., Kir5.1, which is encoded by Kcnj16, may modulate the connection between dietary potassium intake and renal potassium excretion (27). In this study, the authors demonstrate that deleting Kir5.1 eliminates the inhibitory effect of high dietary potassium intake on NCC and impairs the kidney's ability to excrete potassium when dietary potassium intake is increased. Their findings suggest that Kir5.1, like Kir4.1, is also an important component of the potassium sensing mechanism in the distal convoluted tubules, and that Kir5.1 is essential for regulating renal potassium excretion and maintaining potassium homeostasis. Claudino et al. (28) concluded that chronic kidney disease allows for small potassium intakes. The presence of residual renal function is an important factor affecting potassium excretion, and a low-restriction diet can be adopted. Potassium can affect blood pressure in addition to renal function. Increased potassium intake, according to Lawrence J. Appel, may lower blood pressure (29). High potassium intake is good for the human body, including hypertension, urolithiasis, and osteoporosis, according to research by Penton et al. on the relationship between dietary potassium intake and renal sodium and potassium processing (30). Dietary potassium is protective (31). Therefore, in the selection of covariates, we chose blood pressure. Beyond that, potassium intake may be linked to cognitive performance. Nowak et al. found that higher potassium intake may lead to cognitive decline in the elderly (24). In a Korean cohort study conducted by Mun et al., glomerular filtration rate was linked to dietary potassium and a lower risk of developing chronic kidney disease. Adequate potassium intake has also been shown to be beneficial in the treatment of hypertension (32). Blanch et al. (33) found that increasing potassium intake was effective in improving vascular function. Many studies have suggested that high potassium intake is beneficial, however, Virojanawat et al. (34) found that low potassium intake may be the key factor for hypokalemia in chronic peritoneal dialysis patients in Thailand, rather than potassium conversion in cells or potassium excretion. Due to the kidney's crucial role in the body's potassium homeostasis, low potassium levels will cause the glomerular filtration rate to drop, which will influence the excretion of albumin. Like in most research, albuminuria can be decreased by minor increases in dietary potassium intake.

Effect of albuminuria on dietary potassium intake, Kabasawa et al. found a positive correlation between dietary sodium-potassium ratio and albuminuria (14) but did not study the relationship between potassium and albuminuria. This study found that potassium may be a key factor affecting albuminuria. Our study is meaningful. There is no study investigating the relationship between the two from NHANES. Kabasawa et al. (14) discovered a potential inverse link between potassium intake and albuminuria in another investigation. The research results of Burnier et al. (35) and Elfassy et al. (36) are opposite, and our research results are consistent with Burnier et al., increasing potassium intake may reduce the incidence of albuminuria. Influence of Other Factors on Dietary Potassium Intake and Albuminuria in addition to renal function and albuminuria, many other factors may affect dietary potassium intake and the incidence of albuminuria. For example, factors such as age, sex, body mass index (37), diabetes (38), etc. may have an impact on these indicators. In addition, different cultural backgrounds and

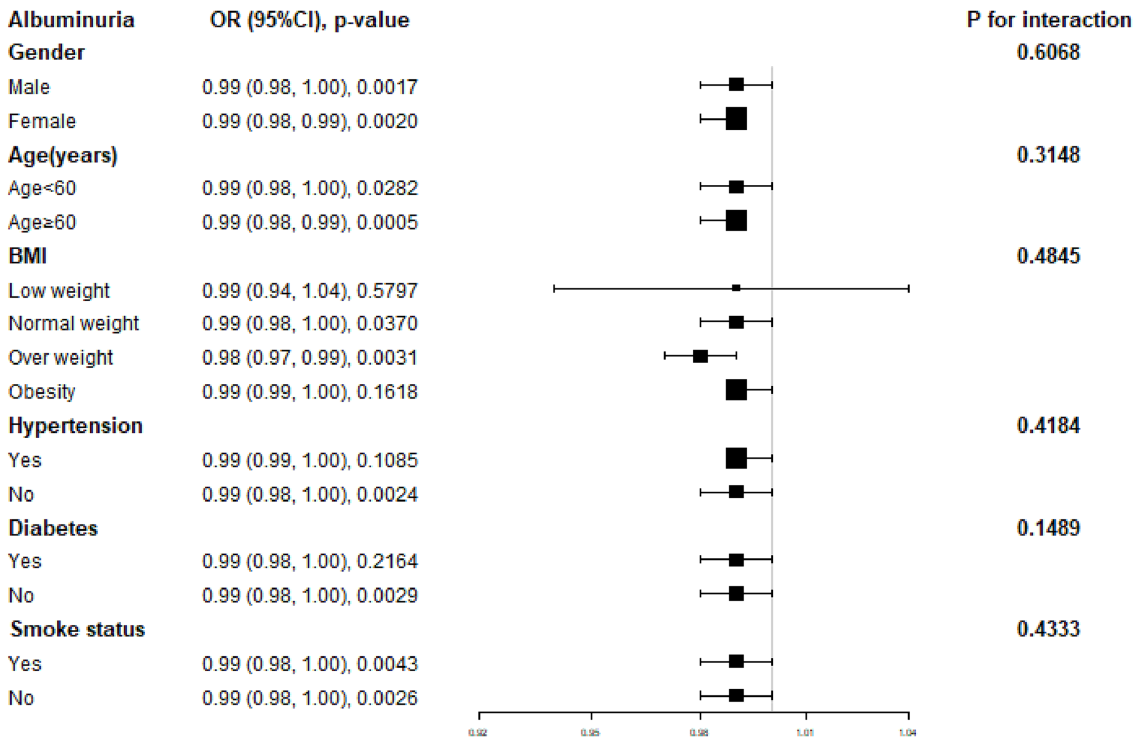

FIGURE 2
Subgroup analysis of association between dietary potassium intake and albuminuria.

dietary habits can also affect dietary potassium intake. As a result, while analyzing the connection between dietary potassium intake and albuminuria, the combined impact of these factors must be taken into account. The development of this study is crucial because it has a wide range of clinical application value and market potential. Because previous studies on the association between potassium intake and albuminuria were inconsistent, it was difficult to draw firm conclusions.

No mechanism is fully understood to explain the link between dietary potassium intake and albuminuria. However, some studies have shown that this link may be related to the following aspects: the regulation of potassium by the kidney (39), an important organ for maintaining potassium homeostasis in the body, which can regulate the concentration of potassium in the blood through mechanisms such as glomerular filtration, renal tubular secretion, and reabsorption. Changes in cell membrane permeability (40). Albumin is an important plasma protein whose presence can affect cell membrane permeability. Several studies have suggested (41) that cell membrane permeability may be altered in patients with albuminuria, resulting in reduced renal potassium reabsorption. Therefore, an appropriate increase in dietary potassium intake can help improve cell membrane permeability, thereby reducing the incidence of albuminuria.

The mechanism of the influence of dietary potassium on renal function and proteinuria mainly has the following aspects: Maintain normal blood pressure: Potassium can help vasodilate and lower blood pressure, thereby reducing the burden on the kidneys and reducing the risk of proteinuria. Promote sodium excretion: Potassium can promote the excretion of sodium by the kidneys, thus reducing the amount of sodium in the body and lowering the risk of blood pressure and proteinuria. Tubule protection: Potassium protects tubule epithelium and reduces tubule damage and inflammation, thereby reducing the risk of proteinuria. Regulating acid–base balance: Potassium can help maintain acid–base balance in the body, which reduces the burden on the kidneys and reduces the risk of proteinuria.

This study has the advantage of using trustworthy NHANES data and a sizable sample size. Second, we looked into previous research and included all necessary factors in this study. Because this study is cross-sectional, there is no causal association between the variables, which is a drawback. The effects of dietary potassium on renal function and proteinuria have certain limitations, as follows: The effects of potassium intake vary from person to person: different people have different kidney function and metabolism, and different dietary potassium requirements. Therefore, for some people, consuming too much dietary potassium may be a burden on the kidneys and increase the risk of proteinuria. Sources and absorption of dietary potassium: Sources of dietary potassium include fruits, vegetables, nuts, legumes and other foods, some of which also contain other beneficial nutrients. In addition, the absorption of dietary potassium is also affected by intestinal flora. Therefore, simply increasing dietary potassium intake is not necessarily effective in improving kidney function and reducing the risk of proteinuria. Influence of other factors: The occurrence and development of renal function and proteinuria are affected by many factors, including hypertension, diabetes, obesity and so on. Therefore, relying solely on dietary potassium to improve kidney function and reduce the risk of proteinuria may not be effective enough, and treatment considering other factors is also needed. The causal

relationship between factors must therefore be explained by numerous prospective studies.

5 Conclusion

Reduced risk of albuminuria was linked to higher dietary potassium intake. The relationship between the two still needs further prospective investigations, though.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the National Center for Health Statistics Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

WYua: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. TW: Conceptualization, Data curation, Formal analysis, Funding acquisition, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. WYue: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Imamura S, Hirata K, Orii M, Shimamura K, Shiono Y, Ishibashi K, et al. Relation of albuminuria to coronary microvascular function in patients with chronic kidney disease. *Am J Cardiol*. (2014) 113:779–85. doi: 10.1016/j.amjcard.2013.11.026

2. Souweine J-S, Corbel A, Rigothier C, Roque CD, Hadjadj S, Cristol JP, et al. Interest of albuminuria in nephrology, diabetology and as a marker of cardiovascular risk. *Ann Biol Clin*. (2019) 77:26–35. doi: 10.1684/abc.2018.1402

3. Neuen BL, Ohkuma T, Neal B, Matthews DR, de Zeeuw D, Mahaffey KW, et al. Effect of Canagliflozin on renal and cardiovascular outcomes across different levels of albuminuria: data from the CANVAS program. *J Am Soc Nephrol*. (2019) 30:2229–42. doi: 10.1681/ASN.2019010064

4. Eknoyan G, Hostetter T, Bakris GL, Hebert L, Levey AS, Parving H-H, et al. Proteinuria and other markers of chronic kidney disease: a position statement of the national kidney foundation (NKF) and the national institute of diabetes and digestive and kidney diseases (NIDDK). *Am J Kidney Dis*. (2003) 42:617–22. doi: 10.1016/S0272-6386(03)00826-6

5. Baldan-Martin M, Rodríguez-Sánchez E, González-Calero L, Ruilope LM, Alvarez-Llamas G, Barderas MG, et al. Translational science in albuminuria: a new view of de novo albuminuria under chronic RAS suppression. *Clin Sci*. (2018) 132:739–58. doi: 10.1042/CS20180097

6. Shin J-I, Chang AR, Grams ME, Coresh J, Ballew SH, Surapaneni A, et al. Albuminuria testing in hypertension and diabetes: an individual-participant data meta-analysis in a global consortium. *Hypertension*. (2021) 78:1042–52. doi: 10.1161/HYPERTENSIONAHA.121.17323

7. D'Elia L, Masulli M, Cappuccio FP, Zarrella AF, Strazzullo P, Galletti F. Dietary potassium intake and risk of diabetes: a systematic review and meta-analysis of prospective studies. *Nutrients*. (2022) 14:4785. doi: 10.3390/nu14224785

8. Ginos BNR, Engberink RHGO. Estimation of sodium and potassium intake: current limitations and future perspectives. *Nutrients*. (2020) 12:3275. doi: 10.3390/nu12113275

9. Narasaki Y, Okuda Y, Kalantar SS, You AS, Novoa A, Nguyen T, et al. Dietary potassium intake and mortality in a prospective hemodialysis cohort. *J Ren Nutr*. (2021) 31:411–20. doi: 10.1053/j.jrn.2020.05.008

10. Potassium and fiber: a controversial couple in the nutritional management of children with chronic kidney disease | SpringerLink. Available at: https://link.springer.com/article/10.1007/s00467-021-05365-5 (Accessed July 23, 2023)

11. St-Jules DE, Clegg DJ, Palmer BF, Carrero J-J. Can novel potassium binders liberate people with chronic kidney disease from the low-potassium diet?: A cautionary tale. *Clin J Am Soc Nephrol*. (2022) 17:467–72. doi: 10.2215/CJN.09660721

12. Cheng C-J, Kuo E, Huang C-L. Extracellular potassium homeostasis: insights from hypokalemic periodic paralysis. *Semin Nephrol*. (2013) 33:237–47. doi: 10.1016/j.semnephrol.2013.04.004

13. Bernier-Jean A, Wong G, Saglimbene V, Ruospo M, Palmer SC, Natale P, et al. Dietary potassium intake and all-cause mortality in adults treated with hemodialysis. *Clin J Am Soc Nephrol*. (2021) 16:1851–61. doi: 10.2215/CJN.08360621

14. Kabasawa K, Takachi R, Nakamura K, Sawada N, Tsugane S, Ito Y, et al. Associations of urinary and dietary sodium-to-potassium ratios with albuminuria in community-dwelling Japanese adults: A cross-sectional study. *Kidney Blood Press Res*. (2022) 47:576–85. doi: 10.1159/000526277

15. Sharma S, McFann K, Chonchol M, de Boer IH, Kendrick J. Association between dietary sodium and potassium intake with chronic kidney disease in US adults: A cross-sectional study. *Am J Nephrol*. (2013) 37:526–33. doi: 10.1159/000351178

16. Mamenko M, Boukelmoune N, Tomilin V, Zaika O, Jensen VB, O'Neil RG, et al. The renal TRPV4 channel is essential for adaptation to increased dietary potassium. *Kidney Int*. (2017) 91:1398–409. doi: 10.1016/j.kint.2016.12.010

17. Wang M-X, Gallardo CC, Su X-T, Wu P, Gao Z-X, Lin D-H, et al. Potassium (K+) intake modulates NCC activity via the K+ channel, Kir4.1. *Kidney Int*. (2018) 93:893–902. doi: 10.1016/j.kint.2017.10.023

18. Kabasawa K, Hosojima M, Takachi R, Nakamura K, Ito Y, Saito A, et al. Association of estimated dietary acid load with albuminuria in Japanese adults: a cross-sectional study. *BMC Nephrol*. (2019) 20:194. doi: 10.1186/s12882-019-1352-8

19. Mirel LB, Mohadjer LK, Dohrmann SM, Clark J, Burt VL, Johnson CL, et al. National Health and nutrition examination survey: estimation procedures, 2007-2010. *Vital Health Stat*. (2013) 2:1–17.

20. Cho Y-T, Chen C-W, Chen M-P, Hu J-L, Su H, Shiea J, et al. Diagnosis of albuminuria by tryptic digestion and matrix-assisted laser desorption ionization/time-of-flight mass spectrometry. *Clin Chim Acta*. (2013) 420:76–81. doi: 10.1016/j.cca.2012.12.016

21. American Diabetes Association. Erratum. Classification and diagnosis of diabetes. Sec. 2. In standards of medical Care in Diabetes-2016. *Diabetes Care*. (2016) 39:S13–22. doi: 10.2337/dc16-er09

22. Gritter M, Wouda RD, Yeung SMH, Wieërs MLA, Geurts F, de Ridder MAJ, et al. Effects of short-term potassium chloride supplementation in patients with CKD. *J Am Soc Nephrol*. (2022) 33:1779–89. doi: 10.1681/ASN.2022020147

23. Wieërs MLAJ, Mulder J, Rotmans JI, Hoorn EJ. Potassium and the kidney: a reciprocal relationship with clinical relevance. *Pediatr Nephrol*. (2022) 37:2245–54. doi: 10.1007/s00467-022-05494-5

24. Nowak KL, Fried L, Jovanovich A, Ix J, Yaffe K, You Z, et al. Dietary sodium/potassium intake does not affect cognitive function or brain imaging indices. *Am J Nephrol*. (2018) 47:57–65. doi: 10.1159/000486580

25. Wei K-Y, Gritter M, Vogt L, de Borst MH, Rotmans JI, Hoorn EJ. Dietary potassium and the kidney: lifesaving physiology. *Clin Kidney J*. (2020) 13:952–68. doi: 10.1093/ckj/sfaa157

26. Akbar SR, Ahmed US, Iqbal HI. Review of hyperkalemia in end stage renal disease. *W V Med J*. (2016) 112:34–8.

27. Wu P, Gao Z-X, Zhang D-D, Su X-T, Wang W-H, Lin D-H. Deletion of Kir5.1 impairs renal ability to excrete potassium during increased dietary potassium intake. *J Am Soc Nephrol*. (2019) 30:1425–38. doi: 10.1681/ASN.2019010025

28. Claudino G, Ramos CI, de Andrade LS, Pereira NBF, Teixeira RR, Muniz GAS, et al. The role of residual renal function on potassium intake and excretion in patients on peritoneal dialysis. *Int Urol Nephrol*. (2023) 55:1549–56. doi: 10.1007/s11255-023-03462-y

29. Appel LJ. The effects of dietary factors on blood pressure. *Cardiol Clin*. (2017) 35:197–212. doi: 10.1016/j.ccl.2016.12.002

30. Penton D, Czogalla J, Loffing J. Dietary potassium and the renal control of salt balance and blood pressure. *Pflugers Arch—Eur J Physiol*. (2015) 467:513–30. doi: 10.1007/s00424-014-1673-1

31. Gritter M, Vogt L, SMH Y, Wouda RD, CRB R, de Borst MH, et al. Rationale and Design of a Randomized Placebo-Controlled Clinical Trial Assessing the Renoprotective effects of potassium supplementation in chronic kidney disease. *Nephron*. (2018) 140:48–57. doi: 10.1159/000490261

32. Mun KH, Yu GI, Choi BY, Kim MK, Shin M-H, Shin DH. Association of Dietary Potassium Intake with the development of chronic kidney disease and renal function in patients with mildly decreased kidney function: the Korean multi-rural communities cohort study. *Med Sci Monit*. (2019) 25:1061–70. doi: 10.12659/MSM.913504

33. Blanch N, Clifton PM, Keogh JB. A systematic review of vascular and endothelial function: effects of fruit, vegetable and potassium intake. *Nutr Metab Cardiovasc Dis*. (2015) 25:253–66. doi: 10.1016/j.numecd.2014.10.001

34. Virojanawat M, Puapatanakul P, Chuengsaman P, Boonyakrai C, Buranaosot S, Katavetin P, et al. Hypokalemia in peritoneal dialysis patients in Thailand: the pivotal role of low potassium intake. *Int Urol Nephrol*. (2021) 53:1463–71. doi: 10.1007/s11255-020-02773-8

35. Burnier M. Increasing potassium intake to prevent kidney damage: a new population strategy? *Kidney Int*. (2020) 98:59–61. doi: 10.1016/j.kint.2020.04.014

36. Elfassy T, Zhang L, Raij L, Bibbins-Domingo K, Lewis CE, Allen NB, et al. Results of the CARDIA study suggest that higher dietary potassium may be kidney protective. *Kidney Int*. (2020) 98:187–94. doi: 10.1016/j.kint.2020.02.037

37. Snelson M, Clarke RE, Coughlan MT. Stirring the pot: can dietary modification alleviate the burden of CKD? *Nutrients*. (2017) 9:265. doi: 10.3390/nu9030265

38. Kho M, Smith JA, Verweij N, Shang L, Ryan KA, Zhao W, et al. Genome-wide association Meta-analysis of individuals of European ancestry identifies suggestive loci for sodium intake, potassium intake, and their ratio measured from 24-hour or half-day urine samples. *J Nutr*. (2020) 150:2635–45. doi: 10.1093/jn/nxaa241

39. Murillo-de-Ozores AR, Gamba G, Castañeda-Bueno M. Chapter nine—molecular mechanisms for the regulation of blood pressure by potassium In: SN Orlov, editor. *Current topics in membranes. Membrane transporters in the pathogenesis of cardiovascular and lung disorders*: Academic Press (2019). 285–313.

40. Roy A, Al-bataineh MM, Pastor-Soler NM. Collecting duct intercalated cell function and regulation. *Clin J Am Soc Nephrol*. (2015) 10:305–24. doi: 10.2215/CJN.08880914

41. Bankir L, Bouby N, Blondeau B, Crambert G. Glucagon actions on the kidney revisited: possible role in potassium homeostasis. *Am J Physiol Renal Physiol*. (2016) 311:F469–86. doi: 10.1152/ajprenal.00560.2015

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Association between spot urinary sodium-to-potassium ratio and blood pressure among Chinese adults aged 18–69 years: the SMASH study

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Background: Excessive sodium and low potassium intake are involved in the development of hypertension. Growing evidence showed that the sodium-to-potassium ratio (Na/K) was significantly associated with blood pressure (BP). However, studies on the dose-response relationship of spot urinary Na/K ratio with hypertension and BP in the general population are scarce, especially in the Chinese population.

Materials and methods: Data from the post-intervention survey of the Shandong Ministry of Health Action on Salt and Hypertension (SMASH) project was analyzed. Associations between Na/K molar ratio and hypertension prevalence and between Na/K molar ratio and BP indices were analyzed using multivariable logistic and linear regression, respectively, followed by subgroup analysis and interaction analysis. The restricted cubic spline model was used to explore the dose–response relationship. Informed by existing literature, we adjusted for potential confounding factors, including temperature and renal function, to assess the association and dose–response relationship.

Results: There was a non-linear positive association between Na/K and hypertension (OR:1.09, 95%CI: 1.08–1.11) and a linear positive association between Na/K and systolic BP, diastolic BP, and mean arterial pressure (β 0.53, 95%CI: 0.45–0.60; β 0.36, 95%CI: 0.31–0.41; and β 0.42, 95%CI: 0.36–0.47, respectively). The association was stronger in individuals with hypertension, female patients, those in the 50–59-year age group, and those who were obese. Environmental temperatures had little impact on associations.

Conclusion: Our findings provide further evidence that the spot urinary Na/K ratio is a simple, useful, and convenient indicator for monitoring salt reduction and potassium increase, which could be used in clinical and public health practices.

KEYWORDS

sodium-to-potassium ratio, spot urine, hypertension, blood pressure, Chinese

1 Introduction

Cardiovascular disease (CVD) is a major public health concern and remains the leading cause of global mortality (1). Excessive sodium intake is considered a significant risk factor for CVD, contributing to ~1.89 million CVD deaths in 2019 (2). The World Health Organization (WHO) listed salt reduction as one of the five priority intervention strategies for non-communicable disease prevention in 2007 (3) and proposed a global target of a 30% reduction in sodium intake by 2025 in 2013 (4). Nevertheless, none of the WHO Member States has yet reached the target (5). Evidence suggests that a reduction in dietary sodium intake alone is challenging to achieve (6). Potassium ions, antagonistically and synergistically interacting with sodium ions to maintain homeostasis, are important for life. Growing evidence indicates that high potassium intake can reduce blood pressure (BP), and this BP-lowering effect may attenuate the BP-raising effect of excessive sodium intake (7–10). Therefore, increasing potassium intake with salt reduction may be more beneficial than salt reduction alone.

China is one of the countries with the highest daily salt consumption, with an average salt intake per capita of 9.3 g/day (11), nearly twice the WHO recommendation (12). Excessive salt intake was one of the top three risk factors for death and life-years lost in China, accounting for more than 5% of disability-adjusted life years (DALYs) (13). In addition, the average potassium intake is 1,417 mg/day, <½ of the recommended amount of 3,500 mg/day (14). Shandong province, the second most populous province in China, has the highest death rate from hypertension and the second highest death rate from CVD in the country (15). In 2011, the Shandong Ministry of Health Action on Salt and Hypertension (SMASH) project was launched by the People's Government of Shandong Province and the National Health Commission of the People's Republic of China (formerly the Ministry of Health) (16). After 5 years of large-scale population salt reduction action, urinary sodium excretion decreased to 4,013 mg/day and potassium excretion increased to 1,850 mg/day (17). While effective progress has been demonstrated, a large gap still exists between the current intake and the recommended intake of sodium and potassium.

To improve the dietary consumption of high sodium and low potassium, restricting sodium intake and simultaneously increasing potassium intake are urgently needed in China. Currently, the sodium-to-potassium ratio has been regarded as a better monitoring indicator than the ion alone (17–21). Clarifying the relationship between the urinary Na/K ratio and BP will aid in developing effective public health strategies. Numerous studies based on 24-h urine collection have shown that a higher urinary Na/K ratio is associated with higher BP levels, regardless of the relationship between sodium intake and BP (21–26). In our previous research, we found an insignificant association between sodium intake and BP but a positive association between 24-h urinary Na/K ratio and BP, which was only observed in overweight/obese individuals (27). However, the evidence for the relationship between spot urinary Na/K ratio and BP or hypertension is limited in China, especially in different population subgroups. We analyzed data from the post-intervention survey of SMASH—a provincial representative cross-sectional survey to address this research gap. We aimed to examine the association between spot urinary Na/K ratio and BP or hypertension, assess variations among population subgroups, and identify the key populations for tailored intervention.

2 Materials and methods

2.1 Study design

Data for this study were obtained from the post-intervention survey of the SMASH program—a cross-sectional survey conducted from 20th June 2016 to 29th August 2016. This survey used a multistage stratified sampling method to select a provincially representative sample of the adult population aged 18–69 years. A total of 16,490 eligible participants from 156 villages or communities in 8 urban districts and 12 rural counties were investigated through face-to-face interviews to administer questionnaires and collect spot urine (SU) samples. A subsample of 2,019 participants was selected from 52 randomly chosen villages or communities across the aforementioned districts and counties, and they were asked to collect 24-h urine samples. Detailed methods have been published elsewhere (28). The ethical committee of the Shandong Center for Disease Control and Prevention approved the post-intervention survey. All respondents provided written informed consent.

2.2 Urine collection and estimation of 24-h urinary sodium and potassium excretion

Trained health professionals informed participants of the detailed procedure for SU and 24-h urine collection. All participants were asked to collect a fasting morning urine sample at home and take the collection tube to the survey site. A total of 2019 subjects were provided a 5 L urine container and a 2 L urine container to collect 24-h urine samples. The participants collecting 24-h urine samples were instructed to empty their bladders at the survey site and record the time of their morning's micturition as the start time of 24-h urine collection. All urine samples over the following 24-h were collected. Trained health professionals recorded the start and finish duration of 24-h urine collections and asked for all missed collections carefully. The total volume was measured and recorded. If the urine volume was <0.5 L or more than two omissions were documented, urine collections were discarded due to incompletion. Finally, a 5-ml urine aliquot of SU or 24-h urine sample was extracted and frozen at −20°C for further analysis.

All urine aliquots were shipped to a certified laboratory for further analysis. Urinary sodium and potassium were detected by the ion-selective electrode method, whereas urinary creatinine was measured with the enzyme method. To ensure the accuracy and reliability of laboratory test results, standard quality control samples at both high and low concentration levels were employed for quality control purposes. The coefficient of variation (CV) for the low and high concentration levels of sodium, potassium, and creatinine resulted in values of 1.97 and 1.17%, 1.18 and 1.95%, and 2.41 and 2.88%, respectively. All of these values fell within the maximum allowable CV specified by the Ministry of

Health's industry standards, which are 2.8% for sodium, 3.43% for potassium, and 5% for creatinine.

Urine samples were regarded as complete when 24-h urinary creatinine excretion was in the sex-specific mean ± 2 standard deviation (SD). Estimated excretion values of sodium and potassium were calculated using the Tanaka equation-based SU sample. The Tanaka equation is as follows (29): (1) predicted 24-h urinary creatinine excretion ($PrUCr_{24\text{-}h}$, mg/day) = 14.89 × weight (kg) + 16.14 × height (cm) − 2.04 × age (years) − 2,244.45; (2) estimated 24-h urinary sodium excretion (24-h eUNa, mmol/day) = 21.98 × (spot urinary sodium (mmol/L)/spot urinary creatinine (mg/L) × $PrUCr_{24\text{-}h}$ [mg/day)]$^{0.392}$; and (3) estimated 24-h urinary potassium excretion (24-h eUK, mmol/day) = 7.59 × (spot urinary potassium (mmol/L)/spot urinary creatinine (mg/L) × $PrUCr_{24\text{-}h}$ [mg/day)]$^{0.431}$.

2.3 Collection and definition of other variables

Sociodemographic information, history of non-communicable diseases, knowledge, attitudes, and behaviors (KABs) related to dietary sodium intake and hypertension, and lifestyle information were obtained using a questionnaire. We identified three primary KAB indicators in the present study, including "As salt intake decreases, so does blood pressure," "Approval of a low salt diet," and "Have taken action to reduce dietary salt intake." These indicators were defined based on positive responses, indicating agreement or the enactment of the stated behavior. Regular physical exercise was defined as any physical activity performed at least three times a week and lasting 30 min or more each time.

A fasting blood specimen was collected for the measurement of glucose, lipid profiles, and creatinine. Glomerular filtration rate (eGFR) was estimated from blood creatinine by the chronic kidney disease epidemiology collaboration (CKD–EPI) equation (30). Other measurements, including height, weight, waist circumference, and blood pressure, were also obtained by trained health professionals. BP was measured three times using a validated electronic sphygmomanometer (HEM-7071, Omron Corporation, Dalian, China) at 1-min intervals, and the average was used. All participants are classified into three groups based on their BP levels: the normal group, the prehypertension group, and the hypertension group. Hypertension was defined as the average systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, or self-reported diagnosis of hypertension in hospitals at the township level and above, or taking antihypertensive medicine in the last 2 weeks. Prehypertension was defined as the SBP of 120–139 mmHg and/or the DBP of 80–89 mmHg, and normotensives were participants with a BP < 120/80 mmHg. We also calculated mean arterial pressure (MAP) by using (SBP + 2*DBP)/3.

The season of urine collection can potentially affect the urinary excretion of cations due to varying sweat excretion rates throughout different seasons. To mitigate this bias, we have also collected data on environmental temperature for adjustment. Environmental temperature data from the day before the on-site investigation were obtained from the National Meteorological Science Data Sharing Center.

2.4 Statistical analysis

We used R software (version 4.3.1) to analyze the data. A p-value of < 0.05 was considered to be statistically significant.

We first excluded participants without spot urine specimens ($n = 310$). Outliers of Na/K and estimated 24-h urinary sodium and potassium excretion [exceeding three times the interquartile range (IQR)], were also excluded ($n = 134$). After the exclusion criteria were applied, 16,046 participants were included in the analytic sample. Categorical variables were expressed in the form of percentages. Normally distributed variables were described as mean ± standard deviation, while non-normally distributed variables were presented as median (IQR). Differences between groups were compared using the t-test, the χ^2-test, and the non-parametric test.

Multivariate logistic regression models and linear regression models were used to explore the associations of Na/K with hypertension prevalence and blood pressure levels, respectively. Spot urinary Na/K was analyzed as a categorical variable (grouped by quartiles) and as a continuous variable in the above analyses. In these multivariate analyses, variables including age, gender, region, residence, education, occupation, income level, body mass index (BMI), regular physical exercise, smoking, alcohol consumption, history of diabetes, history of stroke, history of coronary heart disease (CHD), and KABs of dietary sodium intake and hypertension, eGFR and average temperature were adjusted. Antihypertensive medication use was also adjusted in the linear regression models and in the analysis specific to participants in the hypertension groups. Then, we used restricted cubic spline (RCS) models to clarify the dose–response relationship between the Na/K ratio and hypertension prevalence or blood pressure. Moreover, further subgroup analysis was performed to explore the interactive effects of age, gender, BMI, smoking, alcohol use, and physical exercise on the Na/K ratio.

We also carried out sensitivity analysis using the 24-h urinary indices. Among the 2019 participants who collected 24-h urine samples, 344 were excluded because of incomplete collection, and four were excluded due to a lack of spot urine specimens. To evaluate the potential influence of different urine specimens on studied associations, we performed logistic regression, linear regression, and the RCS model in the remaining 1,671 participants who collected both 24-h urine and spot urine samples. Other sensitivity analyses were also conducted as follows: (1) excluding participants who used anti-hypertension medicines; (2) excluding participants with stroke or CHD; (3) removing alcohol use from adjusted variables; (4) removing regular physical exercise from adjusted variables; (5) removing occupation and income levels from adjusted variables; and (6) removing KABs of salt and hypertension from adjusted variables.

3 Results

3.1 Descriptive analysis of the studied population

Among 16,046 (50.33% female) participants, the mean age was 42.21 ± 13.37 years. More than 30% of participants had prehypertension, and 20.11% of participants had hypertension. The age and gender distribution, residence, region, education,

occupation, income, lifestyle status, prevalence of diabetes, stroke and CHD, and BMI were all significantly different in the different BP categories ($P < 0.05$; Table 1). Participants with hypertension had better salt reduction knowledge and behavior. Both estimated excretion from spot urine specimens and measured excretion from 24-h urine samples showed that participants with hypertension had significantly higher Na/K molar ratio and sodium excretion.

3.2 Distribution of the primary spot urinary Na/K molar ratio in the studied population

The distribution of spot urinary Na/K ratio was summarized in Supplementary Table 1. Significant differences in the Na/K ratio among different genders, regions, educations, and occupations were observed ($P < 0.05$). The spot urine Na/K rose significantly with age and BMI, respectively ($P < 0.05$). Participants who smoked, consumed alcohol, exercised irregularly, had high blood pressure, or had a history of stroke exhibited a higher Na/K ratio compared to their counterparts ($P < 0.05$). Respondents who were aware of the relationship between salt intake and blood pressure favored low salt diets and had already taken action toward salt reduction had a lower Na/K ratio ($P < 0.05$).

3.3 Spot urinary Na/K ratio with hypertension prevalence and blood pressure levels

As shown in Tables 2, 3, the spot urinary Na/K ratio was positively associated with the risk of hypertension and blood pressure levels. In the fully adjusted model, the third and fourth quartile groups of the Na/K ratio had a higher prevalence of hypertension than the first quartile group (third: OR 1.22, 95% CI 1.08–1.39; fourth: OR 1.78, 95% CI 1.57–2.01), and there was a linear trend ($P < 0.001$; Table 2). The third and fourth quartile groups of the Na/K ratio were significantly correlated with SBP (third: β 2.21, 95% CI 1.57–2.84; and fourth: β 3.88, 95% CI 3.23–4.53) and MAP (third: β 1.63, 95% CI 1.16–2.10; and fourth: β 2.99, 95% CI 2.51–3.47) levels, while the highest quartile of the Na/K ratio had a significant correlation with DBP levels (β 2.55, 95% CI 2.11–2.98; Table 3). The linear trend across different quartiles was also significant (P for trend test < 0.0001).

Figure 1 shows the dose–response relationship of Na/K with the risk of hypertension and blood pressure levels. There was a non-linear positive association between Na/K and hypertension prevalence (Figure 1A, P for non-linearity < 0.0001). A linear positive dose–response relationship of Na/K with SBP, DBP, and MAP levels was also observed (Figures 1B–D). The p-values for non-linearity were 0.556, 0.145, and 0.266, respectively. The confounding factors adjusted in the RCS models were the same as those adjusted in the logistic or linear regression model 3. As shown in Table 4, the risk of hypertension increased by 9% for each Na/K ratio unit increase, and with each unit increase in Na/K ratio, the SBP, DBP, and MAP increased by 0.53, 0.36, and 0.42 mmHg, respectively. The results changed little after adjusting for average temperature. Moreover, the magnitude of the statistically significant associations with BP indices varied across the different BP groups, except for the fact that the relationship of the Na/K ratio with SBP in participants in the prehypertension group did not reach statistical significance (Table 4). Compared to participants in the normal group and prehypertension group, participants in the hypertension group had a steeper regression slope.

3.4 Interaction of gender, age, BMI, smoking, alcohol consumption, regular physical exercise, and spot urinary Na/K ratio on hypertension prevalence and blood pressure indices

We analyzed the relationship of spot urinary Na/K ratio with hypertension and blood pressure levels stratified by gender, age, and BMI (Supplementary Table 2). This association was stronger in female patients and the 50–59 age group. Regarding BMI, the association between the Na/K ratio and hypertension was the strongest among underweight participants, while the associations with blood pressure indices were the strongest in those classified as obese.

Figure 2 presents the interaction effects of the spot urinary Na/K ratio and other variables on hypertension and blood pressure levels. Age and Na/K ratio had a significant interaction with hypertension. The interactive effect of age and Na/K ratio, BMI, and Na/K ratio were significant on blood pressure levels. There were no significant interactions for gender, smoking, alcohol consumption, regular physical exercise, or the Na/K ratio.

3.5 Sensitivity analysis

We used a subsample of participants who had both spot and 24-h urine specimens to conduct the sensitivity analysis. The mean of 24-h urinary sodium and potassium excretions were 176.34 ± 79.08 mmol/day (4.06 ± 1.82 g/day) and 47.26 ± 21.81 mmol/day (1.84 ± 0.85 g/day), respectively. The median 24-h urinary Na/K molar ratio was 3.76 (IQR: 2.68–5.30; Table 1). The Pearson correlation coefficient of spot urinary Na/K and 24-h urinary Na/K was 0.48. Estimates for risk of hypertension by 24-h urine were mostly consistent with those using spot urine (Supplementary Table 3). A stronger effect on blood pressure was found in 24-h urine samples compared to SU (Supplementary Table 3). As shown in Supplementary Figure 1, we found that, when the 24-h urinary Na/K ratio increased, the risk of hypertension and blood pressure increased gradually. The relationships were consistent with those based on SU samples. The results of other sensitivity analyses remained robust (Supplementary Table 4).

4 Discussion

In the present study, we found that the urinary Na/K ratio based on spot urine samples was positively associated with the risk of hypertension, SBP, DBP, and MAP. There was also evidence of a dose–response relationship between them, that is,

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TABLE 1 Characteristics of the 16,046 participants in the SMASH post-intervention survey.

Variables	All ($n = 16,046$)	Normal ($n = 7,597$)	Prehypertension ($n = 4,902$)	Hypertension ($n = 3,547$)	*P*-value
Age (years, mean ± SD)	42.21 ± 13.37	38.19 ± 12.66	42.63 ± 13.08	50.24 ± 11.38	<0.0001
Age group [*n* (%)]					<0.0001
18–29	3,717 (23.16)	2,433 (32.03)	1,052 (21.46)	232 (6.54)	
30–39	3,148 (19.62)	1,836 (24.17)	926 (18.89)	386 (10.88)	
40–49	4,138 (25.79)	1,810 (23.83)	1,370 (27.95)	958 (27.01)	
50–59	3,122 (19.46)	1,019 (13.41)	984 (20.07)	1,119 (31.55)	
60–69	1,921 (11.97)	499 (6.57)	570 (11.63)	852 (24.02)	
Gender [*n* (%)]					<0.0001
Man	7,970 (49.67)	3,060 (40.28)	3,013 (61.46)	1,897 (53.48)	
Woman	8,076 (50.33)	4,537 (59.72)	1,889 (38.54)	1,650 (46.52)	
Ethnicity [*n* (%)]					0.304
Han	16,000 (99.71)	7,570 (99.64)	4,891 (99.78)	3,539 (99.77)	
Others	46 (0.29)	27 (0.36)	11 (0.22)	8 (0.23)	
Residence [*n* (%)]					0.0001
Urban	4,971 (30.98)	2,478 (32.62)	1,449 (29.56)	1,044 (29.43)	
Rural	11,075 (69.02)	5,119 (67.38)	3,453 (70.44)	2,503 (70.57)	
Region [*n* (%)]					<0.0001
East	3,995 (24.90)	1,645 (21.65)	1,397 (28.5)	953 (26.87)	
Middle and South	6,151 (38.33)	3,015 (39.69)	1,909 (38.94)	1,227 (34.59)	
Northwest	5,900 (36.77)	2,937 (38.66)	1,596 (32.56)	1,367 (38.54)	
Education [*n* (%)]					<0.0001
Primary, middle school, and under	11,762 (73.30)	5,248 (69.09)	3,639 (74.24)	2,875 (81.05)	
High school and above	4,283 (26.69)	2,348 (30.91)	1,263 (25.76)	672 (18.95)	
Occupation [*n* (%)]					<0.0001
(Hard physical work) Farmer/ Peasant/Manual worker	9,272 (57.78)	4,002 (52.69)	3,002 (61.24)	2,268 (63.94)	
(Light physical work) Service/Administrative/Technical/ Professionals/Others	5,921 (36.90)	3,246 (42.74)	1,660 (33.86)	1,015 (28.62)	
Underemployment/Retired	851 (5.30)	347 (4.57)	240 (4.90)	264 (7.44)	
Annual household income [*n* (%)]					<0.0001
First tertile	5,336 (34.19)	2,343 (31.79)	1,604 (33.56)	1,389 (40.17)	
Second tertile	5,119 (32.80)	2,481 (33.66)	1,584 (33.14)	1,054 (30.48)	
Third tertile	5,154 (33.02)	2,547 (34.55)	1,592 (33.31)	1,015 (29.35)	
Lifestyle status [*n* (%)]					
Smoking	4,525 (28.21)	1,779 (23.43)	1,642 (33.51)	1,104 (31.13)	<0.0001
Alcohol consumption	5,392 (33.62)	2,060 (27.13)	1,962 (40.04)	1,370 (38.64)	<0.0001
Regular physical exercise	3,025 (18.85)	1,274 (16.77)	918 (18.73)	833 (23.48)	<0.0001
Disease status [*n* (%)]					
Diabetes	1,325 (8.26)	259 (3.41)	429 (8.75)	637 (17.96)	<0.0001
Stroke	189 (1.18)	22 (0.29)	32 (0.65)	135 (3.81)	<0.0001

(Continued)

TABLE 1 (Continued)

Variables	All (n = 16,046)	Normal (n = 7,597)	Prehypertension (n = 4,902)	Hypertension (n = 3,547)	P-value
Coronary heart disease	411 (2.56)	93 (1.22)	83 (1.69)	235 (6.63)	<0.0001
KABs of salt and hypertension					
As salt intake decreases, so does blood pressure	10,995 (68.53)	5,051 (66.50)	3,341 (68.17)	2,603 (73.39)	<0.0001
Approval of a low-salt diet	14,773 (92.08)	6,978 (91.88)	4,514 (92.10)	3,281 (92.50)	0.523
Has taken action to reduce dietary salt intake	10,167 (63.37)	4,746 (62.49)	3,042 (62.07)	2,379 (67.07)	<0.0001
BMI (kg/m^2, mean ± SD)	25.18 ± 4.12	23.76 ± 3.62	25.76 ± 3.88	27.43 ± 4.27	<0.0001
SBP (mmHg, mean ± SD)	120.85 ± 17.19	107.55 ± 7.70	125.81 ± 6.38	142.51 ± 16.50	<0.0001
DBP (mmHg, mean ± SD)	76.99 ± 11.41	68.79 ± 6.14	79.70 ± 5.81	90.79 ± 10.82	<0.0001
MAP (mmHg, mean ± SD)	91.61 ± 12.76	81.71 ± 6.01	95.07 ± 4.66	108.03 ± 11.54	<0.0001
Spot urine indices					
Sodium concentration (mmol/L, mean ± SD)	137.89 ± 62.27	133.83 ± 62.28	141.08 ± 62.32	142.2 ± 61.66	<0.0001
Potassium concentration (mmol/L, mean ± SD)	35.00 ± 23.68	36.05 ± 24.25	35.38 ± 23.42	32.26 ± 22.56	<0.0001
Creatinine concentration (mmol/L, mean ± SD)	10.20 ± 6.05	10.62 ± 6.16	10.59 ± 6.17	8.76 ± 5.40	<0.0001
Sodium/potassium ratio [median (IQR)]	4.51 (2.93–6.70)	4.19 (2.75–6.27)	4.53 (3.04–6.66)	5.27 (3.28–7.63)	<0.0001
Estimated sodium excretion[a] (*g/day*, mean ± SD)	3.83 ± 0.90	3.63 ± 0.82	3.87 ± 0.87	4.20 ± 0.99	<0.0001
Estimated potassium excretion[a] (*g/day*, mean ± SD)	1.45 ± 0.33	1.42 ± 0.33	1.47 ± 0.34	1.52 ± 0.33	<0.0001
Predicted creatinine excretion[b] (g/day, mean ± SD)	1.31 ± 0.30	1.24 ± 0.26	1.37 ± 0.31	1.36 ± 0.32	<0.0001
eGFR[c] (ml/min per 1.73 m^2, mean ± SD)	106.37 ± 13.42	109.72 ± 12.43	106.1 ± 12.96	99.56 ± 13.45	<0.0001
24-h urine indices*					
Urine volume (L)	1,545.19 ± 599.96	1,532.38 ± 585.85	1,506.27 ± 596.26	1,614.21 ± 624.81	0.019
Sodium concentration (mmol/L, mean ± SD)	123.81 ± 56.97	118.8 ± 54.96	129.93 ± 59.54	125.67 ± 56.79	0.003
Potassium concentration (mmol/L, mean ± SD)	33.65 ± 17.75	33.15 ± 17.48	35.57 ± 17.86	32.27 ± 17.95	0.013
Creatinine concentration (mmol/L, mean ± SD)	7.73 ± 3.89	7.53 ± 3.76	8.47 ± 4.12	7.22 ± 3.72	<0.0001
Sodium/potassium ratio [median (IQR)]	3.76 (2.68–5.30)	3.69 (2.67–5.04)	3.74 (2.68–5.25)	4.02 (2.74–6.03)	0.016
Measured sodium excretion (*g/day*, mean ± SD)	4.06 ± 1.82	3.85 ± 1.66	4.13 ± 1.78	4.35 ± 2.08	<0.0001
Measured potassium excretion (*g/day*, mean ± SD)	1.84 ± 0.85	1.81 ± 0.83	1.89 ± 0.81	1.85 ± 0.92	0.262
Measured creatinine excretion (g/day, mean ± SD)	1.19 ± 0.40	1.15 ± 0.38	1.27 ± 0.41	1.17 ± 0.43	<0.0001

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; eGFR, estimated glomerular filtration rate; IQR, interquartile range.

[a]Estimated excretion was calculated using the Tanaka equation from a fasting morning urine specimen.

[b]Estimated creatinine excretion was calculated using a formula that was also used in the Tanaka equation to estimate sodium and potassium excretion.

[c]Estimated from blood creatinine by the CKD-EPI formula.

*Results for the subsample with 24-h urine collection (n = 1,671).

TABLE 2 Associations of Na/K (categorical variable) with the risk of hypertension.

Outcome	Quartile of urinary sodium-to-potassium ratio			
	Q1 (<2.93)	Q2 (2.93–4.51)	Q3 (4.51–6.70)	Q4 (>6.70)
Hypertension				
Crude[a]	1.00 (Ref.)	0.98 (0.87, 1.09)	1.38 (1.24, 1.54)*	1.93 (1.74, 2.14)*
Model 1[b]	1.00 (Ref.)	0.92 (0.81, 1.03)	1.28 (1.14, 1.43)*	1.89 (1.69, 2.11)*
Model 2[c]	1.00 (Ref.)	0.92 (0.81, 1.05)	1.25 (1.11, 1.42)*	1.83 (1.62, 2.08)*
Model 3[d]	1.00 (Ref.)	0.91 (0.80, 1.04)	1.22 (1.08, 1.39)*	1.78 (1.57, 2.01)*

Data are odds ratio (OR) and its 95% CI, *P < 0.05.
[a]Crude model adjusted for none.
[b]Model 1 included adjustment of gender and age.
[c]Model 2 included adjustment of gender, age, region, residence, education, occupation, income level, BMI, regular physical exercise, smoking, alcohol consumption, KABs of dietary sodium intake and hypertension, history of diabetes, history of stroke, history of CHD, and eGFR.
[d]Model 3: model 2 plus adjustment for average temperature.

TABLE 3 Associations of Na/K (categorical variable) with SBP, DBP, and MAP levels.

Outcome	Quartile of urinary sodium-to-potassium ratio			
	Q1 (<2.93)	Q2 (2.93-4.51)	Q3 (4.51-6.70)	Q4 (>6.70)
SBP				
Crude[a]	1.00 (Ref.)	0.97 (0.22, 1.72)	3.21 (2.47, 3.96)*	5.75 (5.01, 6.50)*
Model 1[b]	1.00 (Ref.)	0.52 (−0.17, 1.21)	2.21 (1.52, 2.89)*	4.62 (3.93, 5.31)*
Model 2[c]	1.00 (Ref.)	0.78 (0.15, 1.42)	2.27 (1.64, 2.91)*	3.97 (3.32, 4.61)*
Model 3[d]	1.00 (Ref.)	0.74 (0.11, 1.38)	2.21 (1.57, 2.84)*	3.88 (3.23, 4.53)*
DBP				
Crude[a]	1.00 (Ref.)	0.43 (−0.07, 0.92)	1.96 (1.46, 2.45)*	3.74 (3.25, 4.24)*
Model 1[b]	1.00 (Ref.)	0.21 (−0.27, 0.68)	1.46 (0.99, 1.93)	3.19 (2.71, 3.66)*
Model 2[c]	1.00 (Ref.)	0.29 (−0.14, 0.71)	1.43 (1.00, 1.85)*	2.66 (2.23, 3.09)*
Model 3[d]	1.00 (Ref.)	0.23 (−0.19, 0.66)	1.34 (0.91, 1.77)	2.55 (2.11, 2.98)*
MAP				
Crude[a]	1.00 (Ref.)	0.61 (0.06, 1.16)	2.38 (1.82, 2.93)*	4.41 (3.86, 4.96)*
Model 1[b]	1.00 (Ref.)	0.31 (−0.21, 0.83)	1.71 (1.19, 2.23)*	3.66 (3.15, 4.18)*
Model 2[c]	1.00 (Ref.)	0.45 (−0.01, 0.92)	1.71 (1.24, 2.18)*	3.10 (2.62, 3.57)*
Model 3[d]	1.00 (Ref.)	0.40 (−0.06,0.87)	1.63 (1.16,2.10)*	2.99 (2.51, 3.47)*

Data are regression coefficient (β) and its 95% CI, *P < 0.05.
[a]Crude model adjusted for none.
[b]Model 1 included adjustment of gender and age.
[c]Model 2 included adjustment of gender, age, region, residence, education, occupation, income level, BMI, regular physical exercise, smoking, alcohol consumption, KABs of dietary sodium intake and hypertension, history of diabetes, history of stroke, history of CHD, eGFR, and use of antihypertensive drugs.
[d]Model 3: model 2 plus adjustment for average temperature.

as the urinary Na/K ratio increased, the risk of hypertension and blood pressure levels increased significantly. Our findings also indicate that environmental temperature had little impact on their associations. In addition, sensitivity analysis showed similar associations and dose–response relationships between them when using 24-h urine samples. These findings indicate that spot urinary Na/K was a suitable alternative measurement in population-based epidemiological studies, which is consistent with previous research in diverse populations (19).

Our study demonstrated that one unit of Na/K molar ratio increased SBP and DBP by 0.53 and 0.36 mmHg, respectively, which was consistent with the previous cross-sectional study (1.16 mmHg SBP and 0.84 mmHg DBP increase per 3-units of Na/K) (31). Our findings were also consistent with those of Yin et al., who found that SBP and DBP increased by 4.33 and 1.67 mmHg for a one-unit increase in the gram-to-gram Na/K ratio, respectively (32). Although the strength of the association reported above differed from the results of studies based on 24-h urine samples, the direction of the associations was consistent (22, 23, 25, 27, 33, 34). Furthermore, the use of the Na/K molar ratio (concentration ratios of these two ions) would be more convenient than those conversion values, such as the gram-to-gram ratio of sodium excreted to potassium excreted. It is well-known that accurate estimation of sodium intake can be challenging due to both random

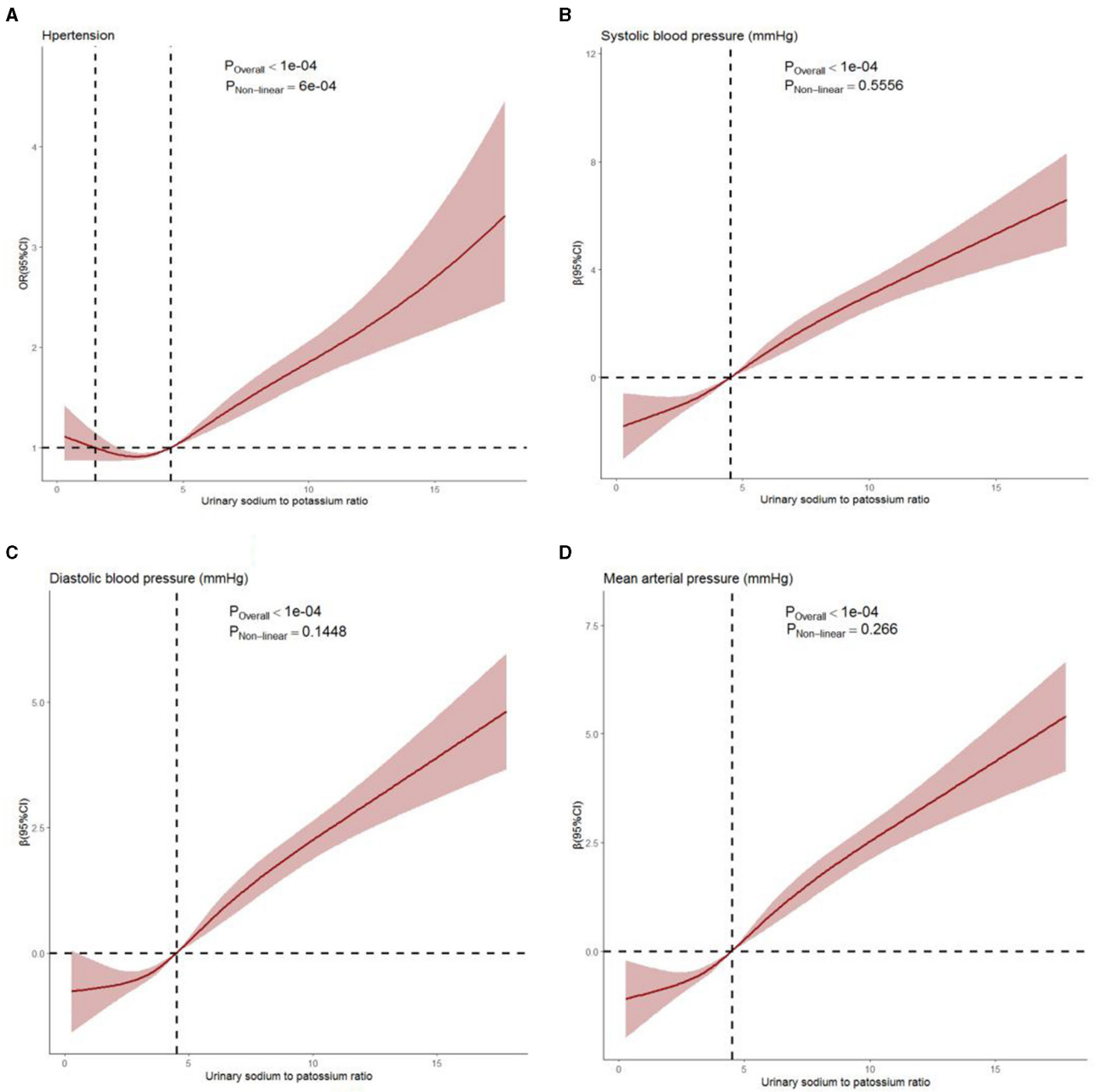

FIGURE 1
Dose–response relationship between Na/K ratio and hypertension prevalence **(A)**, systolic blood pressure **(B)**, diastolic blood pressure **(C)**, and mean arterial pressure **(D)** levels. Age, gender, region, residence, education, occupation, income level, BMI, regular physical exercise, smoking, alcohol consumption, history of diabetes, history of stroke, history of CHD, eGFR, average temperature, and use of antihypertensive drugs were adjusted. The solid line and shaded region represent the OR or β-value and its 95% confidence intervals (CIs).

and systematic errors. Spot urine samples tend to overestimate or underestimate salt intake at different levels of salt consumption derived from the estimation equations (35, 36). The Na/K ratio has been reported as a surrogate index that is resistant to systematic errors (19). Therefore, spot urinary Na/K is expected to be a useful and convenient indicator in clinical and public health practices, as it is significantly easier to obtain (19).

Several factors, such as gender, age, BMI, renal function, and salt restriction, affect the spot urinary Na/K ratio. We found that female participants, older adults, and those classified as obese had a much stronger association between the Na/K ratio and blood pressure. We additionally found significant interactions between age and Na/K and BMI, and Na/K on blood pressure. The 2018 China Chronic Disease and Risk Factor Surveillance also revealed that BMI played an important role in the relationship between urinary sodium and potassium and blood pressure, although the Na/K ratio showed no significant predominance over sodium or potassium alone (37). The Japanese Nagahama cohort study

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TABLE 4 Associations of Na/K (continuous variable) with the risk of hypertension, SBP, DBP, and MAP levels.

Outcome	Crude[c]	Model 1[d]	Model 2[e]	Model 3[f]
Total population				
Hypertension[a]	1.10 (1.08, 1.11)*	1.10 (1.08, 1.11)*	1.09 (1.08, 1.11)*	1.09 (1.08, 1.11)*
SBP[b]	0.77 (0.68, 0.85)*	0.64 (0.56, 0.71)*	0.54 (0.46, 0.61)*	0.53 (0.45, 0.60)*
DBP[b]	0.51 (0.46, 0.57)*	0.45 (0.39, 0.50)*	0.38 (0.33, 0.43)*	0.36 (0.31, 0.41)*
MBP[b]	0.60 (0.54, 0.66)*	0.51 (0.45, 0.57)*	0.43 (0.37, 0.49)*	0.42 (0.36, 0.47)*
Normotensive group				
SBP[b]	0.13 (0.07, 0.19)*	0.10 (0.04, 0.16)*	0.11 (0.05, 0.17)*	0.11 (0.05, 0.17)*
DBP[b]	0.09 (0.04, 0.14)*	0.09 (0.04, 0.14)*	0.11 (0.06, 0.15)*	0.10 (0.05, 0.15)*
MBP[b]	0.11 (0.06, 0.15)*	0.09 (0.05, 0.14)*	0.11 (0.06, 0.15)*	0.10 (0.06, 0.15)*
Prehypertensive group				
SBP[b]	0.05 (−0.01, 0.11)	0.03 (−0.03, 0.09)	0.04 (−0.02, 0.10)	0.04 (−0.02, 0.10)
DBP[b]	0.10 (0.04, 0.15)*	0.11 (0.05, 0.16)*	0.10 (0.05, 0.15)*	0.09 (0.04, 0.15)*
MBP[b]	0.08 (0.04, 0.12)*	0.08 (0.04, 0.13)*	0.08 (0.04, 0.12)*	0.08 (0.03, 0.12)*
Hypertensive group				
SBP[b]	0.40 (0.22, 0.54)*	0.37 (0.21, 0.53)*	0.54 (0.38, 0.70)*	0.54 (0.38, 0.71)*
DBP[b]	0.26 (0.15, 0.36)*	0.26 (0.16, 0.36)*	0.34 (0.24, 0.44)*	0.33 (0.20, 0.43)*
MBP[b]	0.30 (0.19, 0.41)*	0.29 (0.18, 0.40)*	0.40 (0.29, 0.51)*	0.40 (0.29, 0.51)*

[a]Multivariate logistic regression analysis was performed.
[b]Multivariate linear regression analysis was performed.
[c]Crude model adjusted for none.
[d]Model 1 included adjustment of gender and age.
[e]Model 2 included adjustment of gender, age, region, residence, education, occupation, income level, BMI, regular physical exercise, smoking, alcohol consumption, KABs of dietary sodium intake and hypertension, history of diabetes, history of stroke, history of CHD, eGFR, and use of antihypertensive drugs (only in hypertension group).
[f]Model 3 included model 2 plus adjustment for average temperature. *$P < 0.05$.

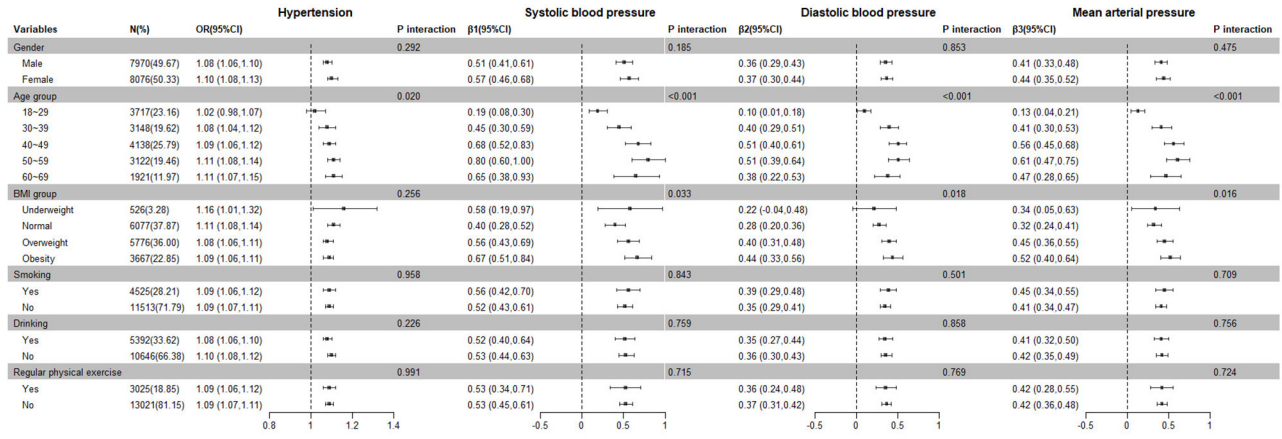

FIGURE 2
Subgroup and interaction analysis of the relationship between the Na/K ratio and hypertension prevalence and blood pressure levels.

presented that the association was more significant in older groups (18). The possible reason may be attributed to salt sensitivity. Studies have shown that being female, being of older age, and being obese might increase salt sensitivity of blood pressure (38, 39). Increasing potassium intake might attenuate the frequency and severity of salt sensitivity, particularly in participants whose dietary potassium intake is deficient (40). Therefore, implementing salt reduction strategies should be accompanied by actions to increase potassium intake, especially for those key populations.

The spot urinary Na/K ratio may be influenced by the season of urine collection. Compared with other seasons, the excretion of sodium and potassium in urine decreased in the summer due to elevated excretion via sweat (41). The sodium excreted in sweat has been estimated to be between 0.3 and 2.7 g/L (41). In our study,

we were unable to calculate the excretion of sodium through sweat. Therefore, we adjusted for environmental temperature to minimize the bias. However, the temperature had minimal influence on the associations, which may be because our study was conducted during the summer when temperatures did not vary significantly from day to day. Further studies are needed to clarify the impact of sweat or temperature on the associations. We also found that the slope for associations between Na/K ratio and blood pressure was steeper in participants in the hypertension group, which is in line with previous studies. It might be because those in the hypertension group had a higher age and BMI than those in the prehypertension and normal groups. On the other hand, 28–74% of those in the hypertension group are salt sensitive, so they have a high BP response to high sodium intake (38). Participants in the hypertension group will, therefore, have stronger benefits from salt reduction strategies combined with potassium supplementation.

Substantial evidence exists demonstrating the impact of excessive sodium and low potassium intake on the development of hypertension (42, 43). However, few studies have reported the relationship between the spot urinary Na/K ratio and the risk of hypertension in the Chinese general population. Our results showed a positive association between them, with an odds ratio of 1.09 (95% CI: 1.08–1.11) for one unit increment of Na/K molar ratio. In addition, the effect size was larger in the third and fourth quartiles. A cross-sectional study conducted in Dallas also found the same results with an OR of 1.12 (95% CI: 1.02–1.22) for a three-unit increase of Na/K (31). The adjusted OR of hypertension in the highest quartile in our study was 1.78 (95% CI: 1.57–2.01), consistent with findings of a study using 24-h urine samples (OR 1.71, 95% CI: 1.16–2.51) (25). Our findings further confirmed that spot urinary Na/K ratio might be a better predictor of hypertension and elevated blood pressure (18). Future studies are needed to further elucidate its long-term effects and explore whether spot urinary Na/K ratio monitoring can be used as a strategy for blood pressure management.

There are several limitations to the present study. First, as a cross-sectional study, the present study does not elucidate the causality between spot urinary Na/K molar ratio and hypertension. Although we further elucidated the dose–response relationship of Na/K with hypertension prevalence and blood pressure levels, it cannot clarify the long-term effect of spot urinary Na/K on hypertension and blood pressure. Second, we used only a single fasting morning urine specimen for analysis. We found a weak-to-moderate correlation between spot urinary Na/K ratio and 24-h urinary Na/K ratio ($r = 0.48$), which corroborated the findings of Iwahori et al. (44). They also demonstrated that estimation of the urinary Na/K ratio using six random specimens of daytime casual urine collected on different days served as a good substitute for the traditional 2-day 24-h urine collection. Other studies have also confirmed that, despite a higher correlation between casual Na/K and individual casual sodium or potassium levels, the mean Na/K of 4–7 repeated measurements of casual urine is better for minimizing systematic errors due to diurnal and day-to-day variations (45, 46). The relationship between the mean Na/K of multiple spot urine collections and blood pressure requires further research. Additionally, the current study did not take into account the impact of within-person variability in spot urine sodium-to-potassium ratio, which might obscure the relationship with blood pressure and other health outcomes (47). It also needs to be addressed in future studies by repeated measurements of spot urine samples. Third, spot urinary Na/K is affected by multiple clinical and environmental factors. Therefore, we adjusted as many covariates as possible to minimize the potential effects of confounding factors. We adjusted for renal function using the formula-derived estimates of eGFR, but whether the CKD-EPI equation was appropriate for Chinese was uncertain. Although we adjusted the use of antihypertensive medication, different kinds of antihypertensive drugs could not be assessed. Some evidence suggested that the correlation between spot urinary Na/K and 24-h urinary Na/K was stronger when using calcium channel blockers, angiotensin 2 receptor blockers, and thiazide diuretics (45, 48). This aspect needs to be taken into consideration in future studies.

5 Conclusion

This cross-sectional study using data from the SMASH project found that spot urinary Na/K molar ratio had a statistically significant dose–response relationship with hypertension prevalence and blood pressure, and the associations were more pronounced among those with hypertension, female participants, older adults, and those classified as obese. The primary utility of the findings from our study further demonstrates the usefulness of a simple spot urine Na/K ratio as a convenient surrogate index for the more burdensome 24-h urine assessment. Further studies are needed to clarify the long-term effect of spot urinary Na/K on hypertension and blood pressure and address whether it could be used as an intervention indicator in salt reduction action and blood pressure management.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethical Committee of the Shandong Center for Disease Control and Prevention. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CX: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. JD: Methodology, Investigation, Writing – original draft. DL: Methodology, Investigation, Writing – original draft. JX: Project administration, Writing – original draft. BZ: Data curation, Investigation, Writing – original draft. ZL: Data curation, Investigation, Writing – original draft. LW: Project administration, Writing – original draft. JT: Investigation, Writing – original draft. XZ: Project

administration, Writing – original draft. JR: Investigation, Writing – original draft. XY: Formal analysis, Writing – original draft. RG: Formal analysis, Writing – original draft. XG: Writing – review & editing, Conceptualization, Methodology, Supervision. JW: Conceptualization, Methodology, Supervision, Writing – review & editing. JM: Conceptualization, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1383243/full#supplementary-material

References

1. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study. *J Am Coll Cardiol.* (2020) 76:2982–3021. doi: 10.1016/j.jacc.2020.11.010

2. GBD 2019 Risk Factors Collaborators. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. (2020) 396:1223–49. doi: 10.1016/S0140-6736(20)30752-2

3. World Health Organization. *Reducing Salt Intake in Populations: Report of a WHO Forum and Technical Meeting*. Geneva: World Health Organization (2007). p. 109–16.

4. World Health Organization. *Global Action Plan for the Prevention and Control of NCDs 2013–2020*. (2013). Available online at: http://apps.who.int/iris/bitstream/10665/94384/1/9789241506236_eng.pdf

5. World Health Organization. *WHO Global Report on Sodium Intake Reduction*. (2023). Available online at: https://iris.who.int/bitstream/handle/10665/366393/9789240069985-eng.pdf?sequence=1

6. Mercado CI, Cogswell ME, Perrine CG, Gillespie C. Diet quality associated with total sodium intake among US adults aged ≥18 years-national health and nutrition examination survey, 2009-2012. *Nutrients.* (2017) 9:1164. doi: 10.3390/nu9111164

7. Mente A, O'Donnell MJ, Rangarajan S, McQueen MJ, Poirier P, Wielgosz A, et al. Association of urinary sodium and potassium excretion with blood pressure. *N Engl J Med.* (2014) 371:601–11. doi: 10.1056/NEJMoa1311989

8. Grimes CA, Riddell LJ, Campbell KJ, Beckford K, Baxter JR, He FJ, et al. Dietary intake and sources of sodium and potassium among Australian schoolchildren: results from the cross-sectional Salt and Other Nutrients in Children (SONIC) study. *Br Med J Open.* (2017) 7:e16639. doi: 10.1136/bmjopen-2017-016639

9. Imamura M, Sasaki H, Hayashi K, Shibata S. Mid-point of the active phase is better to achieve the natriuretic effect of acute salt load in mice. *Nutrients.* (2023) 15:1679. doi: 10.3390/nu15071679

10. Wouda RD, Boekholdt SM, Khaw KT, Wareham NJ, de Borst MH, Hoorn EJ, et al. Sex-specific associations between potassium intake, blood pressure, and cardiovascular outcomes: the EPIC-Norfolk study. *Eur Heart J.* (2022) 43:2867–75. doi: 10.1093/eurheartj/ehac313

11. Commission CNHA. *Report on the Nutrition and Chronic Disease Status of Chinese Residents*. Beijing: People's Medical Publishing House (2020).

12. World Health Organization. *Global Status Report on Non-communicable Diseases*. Geneva: World Health Organization (2010).

13. Zhou M, Wang H, Zeng X, Yin P, Zhu J, Chen W, et al. Mortality, morbidity, and risk factors in China and its provinces, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* (2019) 394:1145–58. doi: 10.1016/S0140-6736(19)30427-1

14. Tan M, He FJ, Wang C, MacGregor GA. Twenty-four-hour urinary sodium and potassium excretion in China: a systematic review and meta-analysis. *J Am Heart Assoc.* (2019) 8:e012923. doi: 10.1161/JAHA.119.012923

15. Pu H, Li J, Wang P, Cui L, Wang H. The death of the circulatory system diseases in China: provincial socioeconomic and environmental perspective. *Environ Sci Pollut Res Int.* (2017) 24:10381–90. doi: 10.1007/s11356-017-8677-2

16. Bi Z, Liang X, Xu A, Wang L, Shi X, Zhao W, et al. Hypertension prevalence, awareness, treatment, and control and sodium intake in Shandong Province, China: baseline results from Shandong-Ministry of Health Action on Salt Reduction and Hypertension (SMASH), 2011. *Prev Chronic Dis.* (2014) 11:E88. doi: 10.5888/pcd11.130423

17. Higo Y, Nagashima S, Tabara Y, Setoh K, Kawaguchi T, Takahashi Y, et al. Association of the spot urine sodium-to-potassium ratio with blood pressure is independent of urinary Na and K levels: the Nagahama study. *Hypertens Res.* (2019) 42:1624–30. doi: 10.1038/s41440-019-0276-9

18. Abe T, Endo T, Hamano T, Okuyama K, Yano S. Changes in the urinary sodium-to-potassium ratio are associated with blood pressure change in older Japanese adults: a 7-year longitudinal study. *J Clin Med.* (2022) 11:5093. doi: 10.3390/jcm11175093

19. Iwahori T, Miura K, Ueshima H. Time to consider use of the sodium-to-potassium ratio for practical sodium reduction and potassium increase. *Nutrients.* (2017) 9:700. doi: 10.3390/nu9070700

20. Kogure M, Nakaya N, Hirata T, Tsuchiya N, Nakamura T, Narita A, et al. Sodium/potassium ratio change was associated with blood pressure change: possibility of population approach for sodium/potassium ratio reduction in health checkup. *Hypertens Res.* (2021) 44:225–31. doi: 10.1038/s41440-020-00536-7

21. Ndanuko RN, Ibrahim R, Hapsari RA, Neale EP, Raubenheimer D, Charlton KE. Association between the urinary sodium to potassium ratio and blood pressure in adults: a systematic review and meta-analysis. *Adv Nutr.* (2021) 12:1751–67. doi: 10.1093/advances/nmab036

22. Zhao L, Stamler J, Yan LL, Zhou B, Wu Y, Liu K, et al. Blood pressure differences between northern and southern Chinese: role of dietary factors: the International Study on Macronutrients and Blood Pressure. *Hypertension.* (2004) 43:1332–7. doi: 10.1161/01.HYP.0000128243.06502.bc

23. Xu J, Chen X, Ge Z, Liang H, Yan L, Guo X, et al. Associations of usual 24-hour sodium and potassium intakes with blood pressure and risk of hypertension among adults in China's Shandong and Jiangsu Provinces, *Kidney Blood Press Res.* (2017) 42:188–200. doi: 10.1159/000475486

24. Tian HG, Nan Y, Shao RC, Dong QN, Hu G, Pietinen P, et al. Associations between blood pressure and dietary intake and urinary excretion of electrolytes in a Chinese population. *J Hypertens.* (1995) 13:49–56. doi: 10.1097/00004872-199501000-00009

25. Du X, Fang L, Xu J, Chen X, Bai Y, Zhong J. Association between 24-h urinary sodium and potassium excretion and blood pressure among Chinese adults aged 18-69 years. *Sci Rep.* (2021) 11:3474. doi: 10.1038/s41598-021-83049-8

26. He J, Tell GS, Tang YC, Mo PS, He GQ. Relation of electrolytes to blood pressure in men. The Yi people study. *Hypertension.* (1991) 17:378–85. doi: 10.1161/01.HYP.17.3.378

27. Yan L, Bi Z, Tang J, Wang L, Yang Q, Guo X, et al. Relationships between blood pressure and 24-hour urinary excretion of sodium and potassium by body mass index status in Chinese adults. *J Clin Hypertens.* (2015) 17:916–25. doi: 10.1111/jch.12658

28. Xu A, Ma J, Guo X, Wang L, Wu J, Zhang J, et al. Association of a province-wide intervention with salt intake and hypertension in Shandong Province, China, 2011-2016. *JAMA Intern Med.* (2020) 180:877–86. doi: 10.1001/jamainternmed.2020.0904

29. Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H, et al. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. *J Hum Hypertens.* (2002) 16:97–103. doi: 10.1038/sj.jhh.1001307

30. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AR, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006

31. Hedayati SS, Minhajuddin AT, Ijaz A, Moe OW, Elsayed EF, Reilly RF, et al. Association of urinary sodium/potassium ratio with blood pressure: sex and racial differences. *Clin J Am Soc Nephrol.* (2012) 7:315–22. doi: 10.2215/CJN.02060311

32. Yin L, Deng G, Mente A, Sun Y, Liu X, Zhang X, et al. Association patterns of urinary sodium, potassium, and their ratio with blood pressure across various levels of salt-diet regions in China. *Sci Rep.* (2018) 8:6727. doi: 10.1038/s41598-018-25097-1

33. Stamler J, Chan Q, Daviglus ML, Dyer AR, Van Horn L, Garside DB, et al. Relation of dietary sodium (salt) to blood pressure and its possible modulation by other dietary factors: the INTERMAP study. *Hypertension.* (2018) 71:631–7. doi: 10.1161/HYPERTENSIONAHA.117.09928

34. Xie J, Liu L, Kesteloot H. Blood pressure and urinary cations in a low-fat intake Chinese population sample. *Acta Cardiol.* (2001) 56:163–8. doi: 10.2143/AC.56.3.2005636

35. Huang L, Crino M, Wu JH, Woodward M, Barzi F, Land MA, et al. Mean population salt intake estimated from 24-h urine samples and spot urine samples: a systematic review and meta-analysis. *Int J Epidemiol.* (2016) 45:239–50. doi: 10.1093/ije/dyv313

36. Santos JA, Li KC, Huang L, Mclean R, Petersen K, Di Tanna GL, et al. Change in mean salt intake over time using 24-h urine versus overnight and spot urine samples: a systematic review and meta-analysis. *Nutr J.* (2020) 19:136. doi: 10.1186/s12937-020-00651-8

37. Guo X, Zhang M, Li C, Zhang X, Zhao Z, Huang Z, et al. Association between urinary sodium and potassium excretion and blood pressure among non-hypertensive adults—China, 2018–2019. *China CDC Wkly.* (2022) 4:522–6. doi: 10.46234/ccdcw2022.066

38. He J, Gu D, Chen J, Jaquish CE, Rao DC, Hixson JE, et al. Gender difference in blood pressure responses to dietary sodium intervention in the GenSalt study. *J Hypertens.* (2009) 27:48–54. doi: 10.1097/HJH.0b013e328316bb87

39. Liu Y, Shi M, Dolan J, He J. Sodium sensitivity of blood pressure in Chinese populations. *J Hum Hypertens.* (2020) 34:94–107. doi: 10.1038/s41371-018-0152-0

40. Morris RJ, Sebastian A, Forman A, Tanaka M, Schmidlin O. Normotensive salt sensitivity: effects of race and dietary potassium. *Hypertension.* (1999) 33:18–23. doi: 10.1161/01.HYP.33.1.18

41. Holbrook JT, Patterson KY, Bodner JE, Douglas LW, Veillon C, Kelsay JL, et al. Sodium and potassium intake and balance in adults consuming self-selected diets. *Am J Clin Nutr.* (1984) 40:786–93. doi: 10.1093/ajcn/40.4.786

42. Huang L, Trieu K, Yoshimura S, Neal B, Woodward M, Campbell N, et al. Effect of dose and duration of reduction in dietary sodium on blood pressure levels: systematic review and meta-analysis of randomised trials. *Br Med J.* (2020) 368:m315. doi: 10.1136/bmj.m315

43. Haddy FJ, Vanhoutte PM, Feletou M. Role of potassium in regulating blood flow and blood pressure. *Am J Physiol Regul Integr Comp Physiol.* (2006) 290:R546–R52. doi: 10.1152/ajpregu.00491.2005

44. Iwahori T, Ueshima H, Miyagawa N, Ohgami N, Yamashita H, Ohkubo T, et al. Six random specimens of daytime casual urine on different days are sufficient to estimate daily sodium/potassium ratio in comparison to 7-day 24-h urine collections. *Hypertens Res.* (2014) 37:765–71. doi: 10.1038/hr.2014.76

45. Iwahori T, Miura K, Ueshima H, Chan Q, Dyer AR, Elliott P, et al. Estimating 24-h urinary sodium/potassium ratio from casual ('spot') urinary sodium/potassium ratio: the INTERSALT study. *Int J Epidemiol.* (2017) 46:1564–72. doi: 10.1093/ije/dyw287

46. Iwahori T, Ueshima H, Torii S, Saito Y, Fujiyoshi A, Ohkubo T, et al. Four to seven random casual urine specimens are sufficient to estimate 24-h urinary sodium/potassium ratio in individuals with high blood pressure. *J Hum Hypertens.* (2016) 30:328–34. doi: 10.1038/jhh.2015.84

47. Re F, Hammami I, Littlejohns TJ, Arnold M, Lewington S, Clarke RJ, et al. Effects of within-person variability in spot urinary sodium measurements on associations with blood pressure and cardiovascular disease. *Hypertension.* (2021) 78:1628–36. doi: 10.1161/HYPERTENSIONAHA.120.16549

48. Li Y, Zhang P, Wu J, Ma J, Xu J, Zhang X, et al. Twenty-four-hour urinary sodium and potassium excretion and their associations with blood pressure among adults in China: baseline survey of action on salt China. *Hypertension.* (2020) 76:1580–8. doi: 10.1161/HYPERTENSIONAHA.120.15238

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Sodium fluctuation as a parameter in predicting mortality in general hospitalized patients

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Background: Dysnatremia is the most common electrolyte disorder in hospitalized patients. Sodium fluctuation level may be a better parameter in dysnatremia management. We aimed to examine the association between sodium fluctuation level during hospitalization and mortality and to evaluate its value in predicting poor prognosis among general hospitalized patients.

Methods: Data were collected from patients admitted to Peking Union Medical College Hospital. The generalized estimated equation (GEE) was used to examine the relationship between sodium fluctuation level and mortality. Receiver-operating characteristic (ROC) curve analysis was performed to calculate the optimal cutoff value and the area under the ROC curve (AUC).

Results: Sodium fluctuation level showed a dose-dependent association with increased mortality in general hospitalized patients. After adjusting age, sex, length of hospital stay, and Charlson comorbidity index, the ORs of group G2 to G6 were 5.92 (95% CI 5.16–6.79), 26.45 (95% CI 22.68–30.86), 50.71 (95% CI 41.78–61.55), 104.38 (95% CI 81.57–133.58), and 157.64 (95% CI 112.83–220.24), respectively, p trend <0.001. Both normonatremia and dysnatremia patients on admission had the dose-dependent associations similar to general hospitalized patients. The AUC of sodium fluctuation level was 0.868 (95% CI 0.859–0.877) in general hospitalized patients, with an optimal cutoff point of 7.5 mmol/L, a sensitivity of 76.5% and a specificity of 84.2%.

Conclusion: We determined that sodium fluctuation level had a dose-dependent association with increased mortality in general hospitalized patients. Sodium fluctuation level could be used to develop a single parameter system in predicting mortality in general hospitalized patients with acceptable accuracy, sensitivity, and specificity.

KEYWORDS

dysnatremia, sodium fluctuation level, prediction, single parameter system, mortality

1 Introduction

Dysnatremia is the most common electrolyte disorder in hospitalized patients. Dysnatremia is classified into hyponatremia and hypernatremia. Hyponatremia is defined as a serum sodium level below 135 mmol/L and is observed in 14–22% hospitalized patients (1, 2). Hypernatremia is regarded as a serum sodium level above 145 mmol/L and is found in 21–26% hospitalized patients (3, 4). Previous studies have shown that both hyponatremia and hypernatremia were independently associated with poor prognosis (3, 5). Severe hyponatremia may cause cerebral edema (6). While hypernatremia contributes to cerebral dehydration, resulting in epileptic seizures, coma, or respiratory arrest (7). Therefore, accurate identification of high-risk dysnatremia patients is crucial for precise management.

In recent years, several studies noted that hyponatremia and hypernatremia frequently occurred in the same patient within a short period; this condition was defined as mixed dysnatremia (8). Patients with mixed dysnatremia were associated with an increased risk of mortality (9), suggesting that sodium fluctuation level during hospitalization could be more accurate in reflecting disease severity than serum sodium level. In addition, patients with serum sodium levels between reference ranges (135 mmol/L-145 mmol/L) are regarded as low-risk patients. However, recent studies suggested that sodium fluctuations were associated with mortality even within sodium reference ranges (10). Therefore, considering the clinical significance of mixed dysnatremia and sodium fluctuations within the normal range, sodium fluctuation level during hospitalization may be a better parameter in dysnatremia management.

The poor prognostic impact of sodium fluctuation during hospitalization has been evaluated in patients with normonatremia on admission (11–13). However, these studies ignored patients with dysnatremia on admission. Patients admitted with dysnatremia frequently combined with chronic hyponatremia or hypernatremia. Thus, when these patients experience additional sodium fluctuations during hospitalization, the management of dysnatremia will be more difficult, leading to an increased risk of mortality. We hypothesized that sodium fluctuation during hospitalization was also associated with poor prognosis in patients with dysnatremia on admission. Therefore, this study included patients with normonatremia and dysnatremia on admission to evaluate the value of sodium fluctuation level during hospitalization in predicting mortality in general hospitalized patients.

The aim of our study is (i) to determine the association between sodium fluctuation level during hospitalization and the risk of mortality; (ii) to evaluate the value of sodium fluctuation level in predicting prognosis among general hospitalized patients.

2 Materials and methods

2.1 Study design

The single-center retrospective cohort study was conducted at Peking Union Medical College Hospital (Beijing, China). Patients admitted between 1 January 2015 and 9 August 2020 were included in this study. Both data collection and follow-up were from the Electronic Medical Record (EMR), and the data collection stopped after the patients were discharged.

The study was approved by the Ethics Committee of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences (approval number: S-k1272, approval date: 9 October 2020). This study was reported according to The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) (14).

2.2 Participants

Participants were from Peking Union Medical College Hospital, Chinese Academy of Medical Sciences. Patients were included if they (i) were over 18 years of age; (ii) had at least two serum sodium measurements during hospitalization. Patients were excluded if they (i) had incomplete diagnostic codes; (ii) were without serum sodium measurement within 24 h after admission; or (iii) had conflict serum sodium levels in the same laboratory test. The inclusion and exclusion of participants were based on the records of EMR.

2.3 Study variables

All variables were collected through EMR, including age, sex, diagnosis codes, laboratory tests, and intensive care unit (ICU) transfer. General hospitalized patients were classified into 2 groups according to their serum sodium level on admission: (i) normonatremia on admission (serum sodium level between 135 mmol/L and 145 mmol/L on admission); (ii) dysnatremia on admission (serum sodium level below 135 mmol/L or above 145 mmol/L on admission). The minimum serum sodium level was defined as the lowest record of serum sodium measurements during a single hospitalization. The maximum serum sodium level was defined as the highest record of serum sodium measurements during a single hospitalization. Sodium fluctuation level was defined as difference between the maximum and minimum serum sodium levels during a single hospitalization.

According to previous studies, sodium fluctuation level below 6.0 mmol/L was treated as normal and was associated with a relatively low risk of death (10). Therefore, we divided patients into 6 groups to examine the dose-dependent relationship between sodium fluctuation level and mortality: G1 group (sodium fluctuation level < 6.0 mmol/L), G2 group (sodium fluctuation level between 6.0 mmol/L and 12.0 mmol/L), G3 group (sodium fluctuation level between 12.0 mmol/L and 18.0 mmol/L), G4 group (sodium fluctuation level between 18.0 mmol/L and 24.0 mmol/L), G5 group (sodium fluctuation level between 24.0 mmol/L and 30.0 mmol/L), and G6 group (sodium fluctuation level > 30.0 mmol/L).

We further categorized the primary diagnoses according to the International Classification of Diseases, 10th Revision, and calculated the Charlson comorbidity index (CCI) to measure comorbidity (15). The diagnostic codes were shown in Supplementary Table S1.

2.4 Outcomes

Our primary outcome was mortality, which was extracted from EMR. Several patients discharged against medical advice (AMA) had poor prognoses. However, they chose to leave the hospital due to various reasons (16). Considering the high mortality rate in AMA

discharged patients in this study center, we defined mortality as in-hospital mortality and AMA discharged.

2.5 Statistical analysis

Categorical variables were reported as count (%), and continuous variables were reported as mean with standard deviation (SD). The Cochran-Armitage test was used to assess trends between categorical variables.

The primary analysis was the GEE. We chose GEE because our study comprised 20,164 re-hospitalized patients. In our analysis, the sodium fluctuation level was defined as the difference between the maximum and minimum serum sodium levels observed during a single hospitalization. Consequently, the readmitted patients had multiple measurements of sodium fluctuation levels. Prior studies have also utilized GEE to analyze repeated measurements (17, 18). Hence, we opted for GEE to model the relationship between sodium fluctuations, the number of hospitalizations, and mortality, and to test the main effect of sodium fluctuation on mortality. The GEE approach identified the repeated measurements by patient ID, and an exchangeable matrix was used as the working matrix. The odds ratio (OR) with 95% confidence interval (CI) were calculated to examine (i) the association and overall trends between sodium fluctuation level and mortality; and (ii) the association between sodium fluctuation level during hospitalization, minimum serum sodium level, maximum serum sodium level and mortality. The variance inflation factor test was performed to avoid the collinearity of the variables included in the models. Sensitivity analysis was further used to adjust the impact of AMA discharge on patients' outcomes.

We used receiver-operating characteristic (ROC) curve analysis to investigate the ability of a single laboratory value, such as sodium fluctuation level during hospitalization, minimum serum sodium level, or maximum serum sodium level to predict mortality. The patient-specific predicted survival values and optimal cutoff value were derived from the ROC curve analysis. AUC was calculated based on original value of sodium fluctuation level during hospitalization, minimum serum sodium level, or maximum serum sodium level. The Delong test was used to compare the difference between AUCs of sodium fluctuation level during hospitalization, minimum serum sodium level, and maximum serum sodium level. The Brier score and calibration metrics were derived from logistic regression model.

All analyses were conducted with R (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria, 2020[1]).

3 Results

3.1 Demographic and characters

A total of 390,116 patients admitted to Peking Union Medical College Hospital between 1 January 2015 and 9 August 2020 were screened for inclusion. After excluding non-eligible cases, 135,482 patients were included in this study, as shown in Figure 1. The baseline characters of patients were summarized in Table 1. Patients had a mean age of 54.15 years (SD 15.95), with a mean CCI of 2.11 (SD 2.52). The cohort had a similar number of men and women, and 70,037 (51.7%) of 135,482 patients were female. The average of hospital stays was 13.24 days (SD 13.26) in general hospitalized patients, 12.77 days (SD 12.21) in normonatremia patients on admission, and 18.33 days (SD 20.95) in dysnatremia patients on admission. 1912 (1.4%) patients died in hospital or had an AMA discharge.

Among these mortality cases, 824 (7.2%) patients had dysnatremia on admission, and 1,088 (0.9%) patients were normonatremia on admission.

3.2 Impact of sodium fluctuation level during hospitalization on outcomes

Sodium fluctuation level during hospitalization showed a dose-dependent association with increased mortality in general hospitalized patients. The mortality rate of group G1 to G6 were 372 (0.4%), 554 (2.4%), 475 (10.2%), 274 (17.7%), 159 (29.3%), and 85 (26.6%), respectively, p trend <0.001, as shown in Table 2. As Supplementary Table S2 showed that sodium fluctuation level was moderately correlated with length of hospital stays ($r = 0.45$, $p < 0.001$), and was weakly correlated with age ($r = 0.09$, $p < 0.001$) and CCI ($r = 0.13$, $p < 0.001$). Therefore, we adjusted age, sex, CCI, and length of hospital stays in model 1. The ORs of group G2 to G6 were 5.92 (95% CI 5.16–6.79), 26.45 (95% CI 22.68–30.86), 50.71 (95% CI 41.78–61.55), 104.38 (95% CI 81.57–133.58), and 157.64 (95% CI 112.83–220.24), respectively, p trend <0.001. Moreover, both normonatremia patients and dysnatremia patients on admission showed the dose-dependent associations similar to general hospitalized patients. After adjusting age, sex, CCI, and length of hospital stay, the ORs of group G2 to G6 in normonatremia patients on admission were 6.90 (95% CI 5.48–7.68), 34.26 (95% CI 28.42–41.29), 71.85 (95% CI 56.23–91.81), 121.87 (95% CI 85.26–174.18), and 226.55 (95% CI 136.54–375.90), respectively, p trend <0.001; the ORs of group G2 to G6 in dysnatremia patients on admission were 2.06 (95% CI 1.63–2.60), 5.34 (95% CI 4.15–6.88), 8.66 (95% CI 6.43–11.66), 20.77 (95% CI 14.70–29.34), and 26.02 (95% CI 16.62–40.73), respectively, p trend <0.001.

Figure 2 depicts the dose-dependent association between sodium fluctuation level during hospitalization and mortality in general hospitalized patients, normonatremia patients on admission, and dysnatremia patients on admission. The results of the sensitivity analysis were shown in Supplementary Tables S3, S4, which showed no significant difference with Table 2. We further examined the association between sodium fluctuation level divided by cutoffs of 3 and 10 and mortality. The results were consistent with the results of sodium fluctuation divided by cutoffs of 6, which were summarized in Supplementary Tables S5, S6, Supplementary Figures S1, S2.

3.3 The association between sodium fluctuation level during hospitalization, minimum serum sodium level, maximum serum sodium level and mortality

Table 3 demonstrated that sodium fluctuation level during hospitalization was associated with mortality in general hospitalized

1 https://www.R-project.org/

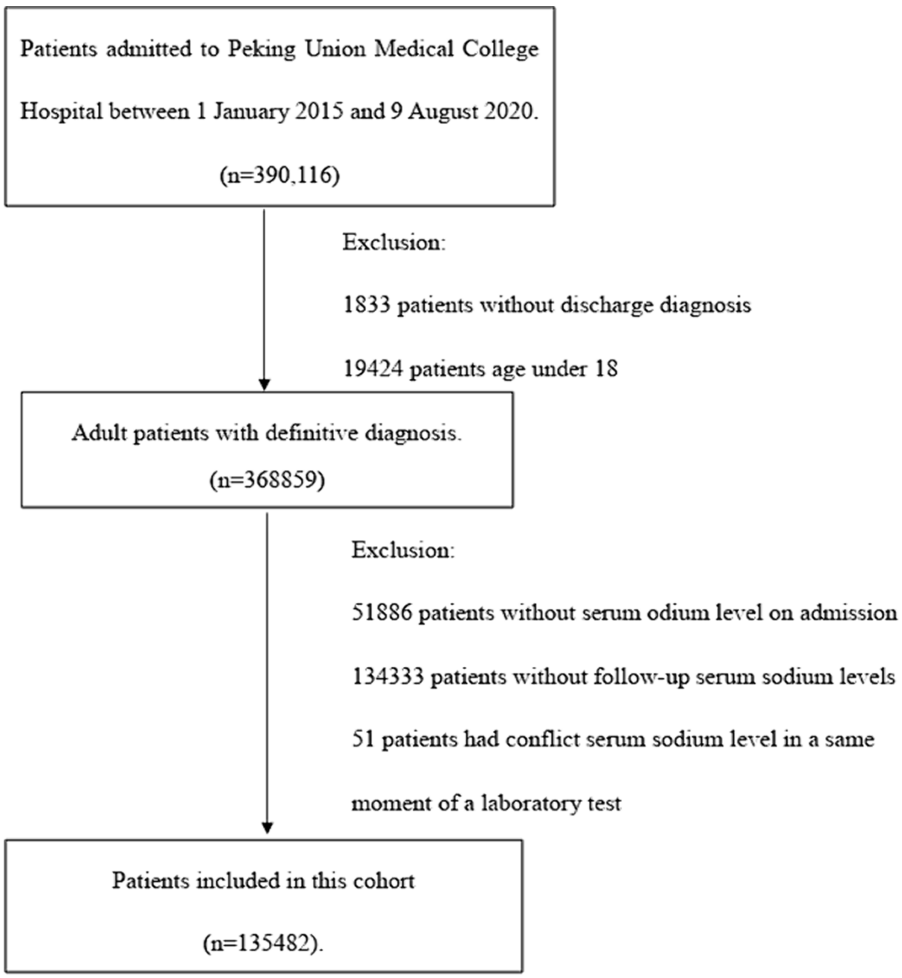

FIGURE 1
Flow chart of inclusion and exclusion of study cohort.

patients, normonatremia patients on admission, and dysnatremia patients on admission. The ORs of sodium fluctuation level increased per 1 mmol/L were 1.21 (95% CI 1.20–1.22), 1.24 (95% CI 1.23–1.26), and 1.12 (95% CI 1.10–1.14), respectively, after adjusting age, sex, length of hospital stays, and CCI. Additionally, an increase in the maximum serum sodium level was associated with increased mortality in general hospitalized patients (OR 1.21, 95% CI 1.20–1.22), normonatremia patients on admission (OR 1.27, 95% CI 1.25–1.29), and dysnatremia patients on admission (OR 1.11, 95% CI 1.10–1.13), after the adjustment of age, sex, length of hospital stays, and CCI. However, minimum serum sodium level was associated with mortality in only general hospitalized patients and normonatremia patients on admission. After the adjustment of age, sex, length of hospital stays, and CCI, the ORs of minimum serum sodium level decreased per 1 mmol/L were 1.39 (95% CI 1.37–1.41) and 1.46 (95% CI 1.43–1.49), respectively. The sensitivity analysis yielded similar results, as shown in Supplementary Tables S7, S8.

3.4 Sodium fluctuation level during hospitalization predict mortality

ROC curve analysis was performed to calculate the optimal cutoff values and the AUCs of minimum serum sodium level, maximum serum sodium level, and sodium fluctuation level during hospitalization in general hospitalized patients, as shown in Figure 3A. The AUC of sodium fluctuation level during hospitalization was 0.868 (95% CI 0.859–0.877), which was significantly higher than minimum serum sodium level (AUC 0.750, 95% CI 0.736–0.764, $p < 0.001$) and maximum serum sodium level (AUC 0.705, 95% CI 0.688–0.721, $p < 0.001$). In addition, the AUCs of sodium fluctuation level were 0.868 (95% CI 0.855–0.881) in normonatremia patients on admission and 0.728 (95% CI 0.709–0.747) in dysnatremia patients on admission, which were also significantly higher than the AUCs of minimum and maximum serum sodium level in both normonatremia and dysnatremia patients on admission.

The optimal cutoff point of sodium fluctuation level during hospitalization was 7.5 mmol/L in general hospitalized patients, with a sensitivity of 76.5% and a specificity of 84.2%. The cutoff points of minimum and maximum serum sodium levels were 134.5 mmol/L and 144.5 mmol/L, respectively. These cutoff points were further examined in normonatremia patients and dysnatremia patients on admission, as depicted in Figures 3B,C. The cutoff point of sodium fluctuation level (7.5 mmol/L) had a sensitivity of 73.9%, a specificity of 87.3% in normonatremia patients on admission, and a sensitivity of 48.2%, a specificity of 80.0% in dysnatremia patients on admission.

TABLE 1 Baseline characters and outcomes of normonatremia patients on admission and dysnatremia patients on admission.

		General hospitalized patients	Normonatremia patients on admission	Dysnatremia patients on admission
Demographic				
N, %		135,482	124,056	11,426
Age, years		54.15 (15.95)	53.82 (15.82)	57.69 (16.94)
Age, *n* (%)				
	18–65 years	100,795 (74.4)	93,391 (75.3)	7,404 (64.8)
	66–75 years	23,416 (17.3)	21,077 (17.0)	2,339 (20.5)
	> 75 years	11,271 (8.3)	9,588 (7.7)	1,683 (14.7)
Sex, *n* (%)				
	Female	70,037 (51.7)	64,855 (52.3)	5,182 (45.4)
	Male	65,445 (48.3)	59,201 (47.7)	6,244 (54.6)
Comorbidities				
Myocardial infarction, *n* (%)		2,675 (2.0)	2,311 (1.9)	364 (3.2)
Congestive heart failure, *n* (%)		10,591 (7.8)	9,149 (7.4)	1,442 (12.6)
Peripheral vascular disease, *n* (%)		13,874 (10.2)	12,597 (10.2)	1,277 (11.2)
Cerebrovascular disease, *n* (%)		11,069 (8.2)	9,794 (7.9)	1,275 (11.2)
Dementia, *n* (%)		351 (0.3)	282 (0.2)	69 (0.6)
Chronic pulmonary disease, *n* (%)		7,686 (5.7)	6,781 (5.5)	905 (7.9)
Connective tissue disease, *n* (%)		8,324 (6.1)	7,165 (5.8)	1,159 (10.1)
Ulcer disease, *n* (%)		2,544 (1.9)	2,145 (1.7)	399 (3.5)
Diabetes				
	Without end organ damage, *n* (%)	18,127 (13.4)	16,059 (12.9)	2,068 (18.1)
Hemiplegia	With end organ damage, *n* (%)	998 (0.7)	894 (0.7)	104 (0.9)
		383 (0.3)	325 (0.3)	58 (0.5)
Tumor				
	Tumor without metastasis, *n* (%)	35,919 (26.5)	33,248 (26.8)	2,671 (23.4)
	Metastatic solid tumor, *n* (%)	13,411 (9.9)	11,805 (9.5)	1,606 (14.1)
Leukemia, *n* (%)		1,202 (0.9)	1,048 (0.8)	154 (1.3)
Lymphoma, *n* (%)		3,624 (2.7)	2,907 (2.3)	717 (6.3)
Liver disease				
	Mild liver disease, *n* (%)	14,476 (10.7)	13,150 (10.6)	1,326 (11.6)
	Moderate or severe liver disease, *n* (%)	1,505 (1.1)	1,162 (0.9)	343 (3.0)

(Continued)

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TABLE 1 (Continued)

		General hospitalized patients	Normonatremia patients on admission	Dysnatremia patients on admission
Moderate or severe renal disease, *n* (%)		13,397 (9.9)	11,140 (9.0)	2,257 (19.8)
Acquired immune deficiency syndrome, *n* (%)		107 (0.1)	84 (0.1)	23 (0.2)
Charlson Comorbidities Index		2.11 (2.52)	2.04 (2.48)	2.89 (2.86)
Average length of hospital stays, day		13.24 (13.26)	12.77 (12.21)	18.33 (20.95)
Re-hospitalization, *n* (%)		20,164 (14.9)	17,638 (14.2)	2,526 (22.1)
Average times of re-hospitalization, times		1.24 (0.76)	1.23 (0.73)	1.38 (0.98)
ICU transferal, *n* (%)		10,844 (8.0)	9,118 (7.3)	1,726 (15.1)
Serum sodium level on admission, mmol/L		139.41 (3.48)	139.93 (2.23)	133.81 (7.43)
Average serum sodium level, mmol/L		139.25 (2.73)	139.55 (2.15)	135.98 (5.13)
Minimum serum sodium level, mmol/L		136.91 (3.71)	137.45 (2.99)	131.14 (5.46)
Maximum serum sodium level, mmol/L		141.58 (3.36)	141.66 (2.78)	140.66 (7.01)
Fluctuation range of serum sodium level, mmol/L		4.66 (4.28)	4.21 (3.68)	9.52 (6.69)
Outcome				
Mortality, *n* (%)		1,912 (1.4)	1,088 (0.9)	824 (7.2)
	In-hospital mortality	1,412 (1.0)	785 (0.6)	627 (5.5)
	AMA discharge	500 (0.4)	303 (0.2)	197 (1.7)

Categorical variables are presented as counts and percentages; continuous variables are presented as mean ± SD. AMA, against medical advice; ICU, intensive care unit.

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TABLE 2 The association between sodium fluctuation level during hospitalization and mortality based on generalized estimated equations.

	G1 group	G2 group	G3 group	G4 group	G5 group	G6 group	*P* trends
Sodium fluctuation level during hospitalization, mmol/L	0–6.0	6.0–12.0	12.0–18.0	18.0–24.0	24.0–30.0	> 30.0	
General hospitalized patients							
N, *n*	105,808	22,702	4,676	1,545	519	232	
Mortality, *n* (%)	372 (0.4)	554 (2.4)	475 (10.2)	274 (17.7)	152 (29.3)	85 (26.6)	<0.001
Model 1[a]	1.00	5.92 (5.16–6.79)	26.45 (22.68–30.86)	50.71 (41.78–61.55)	104.38 (81.57–133.58)	157.64 (112.83–220.24)	<0.001
Model 2[b]	1.00	6.32 (5.50–7.26)	26.76 (22.89–31.29)	45.40 (37.39–55.12)	83.42 (564.51–107.87)	112.55 (79.67–159.01)	<0.001
Normonatremia patients on admission							
N, *n*	101,471	18,394	3,031	843	228	89	
Mortality, *n* (%)	239 (0.2)	331 (1.8)	279 (9.2)	147 (17.4)	58 (25.4)	34 (38.2)	<0.001
Model 1[a]	1.00	6.90 (5.48–7.68)	34.26 (28.42–41.29)	71.85 (56.23–91.81)	121.87 (85.26–174.18)	226.55 (136.54–375.90)	<0.001
Model 2[b]	1.00	7.09 (5.97–8.40)	35.44 (29.28–42.90)	63.81 (49.78–81.80)	92.05 (63.07–134.33)	147.95 (86.64–252.66)	<0.001
Dysnatremia patients on admission							
N, *n*	4,337	4,308	1,645	702	291	143	
Mortality, *n* (%)	133 (3.1)	223 (5.2)	196 (11.9)	127 (18.1)	94 (32.3)	51 (35.7)	<0.001
Model 1[a]	1.00	2.06 (1.63–2.60)	5.34 (4.15–6.88)	8.66 (6.43–11.66)	20.77 (14.70–29.34)	26.02 (16.62–40.73)	<0.001
Model 2[b]	1.00	1.89 (1.50–2.40)	4.62 (3.58–5.96)	6.36 (3.66–8.67)	13.56 (9.40–19.54)	14.89 (9.27–23.91)	<0.001

Categorical variables are presented as counts and percentages. Results of models are presented in Odds Ratio and 95% Confidence Interval. Cochran-Armitage test was used to examine trends among categorical variables and odds ratios. [a]Adjusted with age, sex, length of hospital stays, and Charlson Comorbidities Index. [b]Adjusted with age, sex, length of hospital stays, myocardial infarction, chronic pulmonary disease, moderate or severe liver disease, moderate or severe renal disease, metastatic solid tumor, serum sodium level on admission and average serum sodium level during hospitalization.

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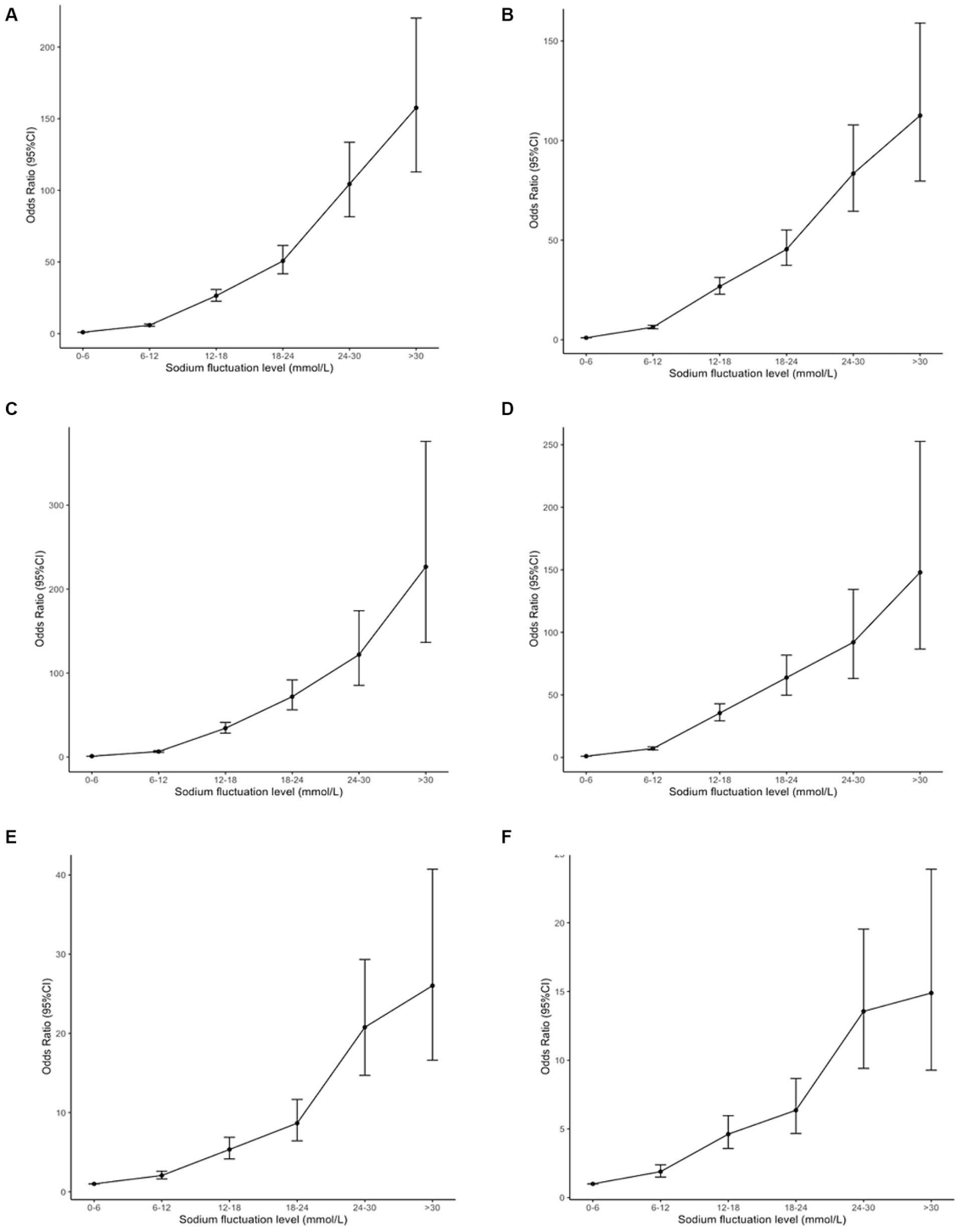

FIGURE 2
The association between serum sodium fluctuation level during hospitalization (cutoff = 6 mmol/L) and mortality. The Odds Ratios (OR) and 95% Confidence Intervals (CI) were generated based on generalized estimated equations. **(A,C,E)** Showed the OR and 95% CI of sodium fluctuation level during hospitalization (cutoff = 6 mmol/L) after adjustment of age, sex, length of hospital stays, and Charlson Comorbidities Index in general hospitalized patients, normonatremia patients on admission, and dysnatremia patients on admission. **(B,D,F)** Showed the OR and 95% CI of sodium fluctuation level during hospitalization (cutoff = 6 mmol/L) after adjustment of age, sex, length of hospital stays, myocardial infarction, chronic lung disease, moderate-to-severe liver failure, moderate-to-severe kidney failure, metastatic solid tumor, serum sodium level on admission and average serum sodium level during hospitalization in general hospitalized patients, normonatremia patients on admission, and dysnatremia patients on admission. CI, confidence interval.

Compared to patients with sodium fluctuation level below 7.5 mmol/L, patients with sodium fluctuation level higher than 7.5 mmol/L had a significantly increased mortality rate in general hospitalized patients (0.4% vs. 6.5%, $p < 0.001$), normonatremia patients on admission (0.3% vs. 4.9%, $p < 0.001$), and dysnatremia patients on admission (3.1% vs. 10.7%, $p < 0.001$), as shown in Table 4.

TABLE 3 The association between sodium fluctuation level during hospitalization, minimum serum sodium level, maximum serum sodium level and mortality based on generalized estimated equations.

	Minimum serum sodium level, decrease per 1 mmol/L	*P*-value	Maximum serum sodium level, increase per 1 mmol/L	*P*-value	Sodium fluctuation level during hospitalization, increase per 1 mmol/L	*P*-value
General hospitalized patients						
Model 1[a]	1.39 (1.37–1.41)	<0.001	1.36 (1.34–1.39)	<0.001	1.20 (1.19–1.21)	<0.001
Model 2[b]	1.15 (1.14–1.16)	<0.001	1.21 (1.20–1.22)	<0.001	1.21 (1.20–1.22)	<0.001
Normonatremia patients on admission						
Model 1[a]	1.46 (1.43–1.49)	<0.001	1.42 (1.39–1.45)	<0.001	1.24 (1.22–1.25)	<0.001
Model 2[b]	1.19 (1.17–1.21)	<0.001	1.27 (1.25–1.29)	<0.001	1.24 (1.23–1.26)	<0.001
Dysnatremia patients on admission						
Model 1[a]	1.18 (1.15–1.21)	<0.001	1.18 (1.16–1.21)	<0.001	1.10 (1.09–1.12)	<0.001
Model 2[b]	0.99 (0.97–1.01)	0.203	1.11 (1.10–1.13)	<0.001	1.12 (1.10–1.14)	<0.001

Categorical variables are presented as counts and percentages. Results of models are presented in Odds Ratio and 95% Confidence Interval. [a]Adjusted with age, sex, length of hospital stays, myocardial infarction, chronic pulmonary disease, moderate or severe liver disease, moderate or severe renal disease, and metastatic solid tumor. [b]Adjusted with age, sex, length of hospital stays, and Charlson Comorbidities Index.

The sensitivity analysis of ROC curves demonstrated that the AUC of sodium fluctuation was higher than that of minimum and maximum sodium levels in general hospitalized patients, normonatremia patients on admission, and dysnatremia patients on admission. The results of the sensitivity analysis are presented in Supplementary Figures S3, S4, which align with the findings in Figure 3. Furthermore, the sensitivity analysis of the optimal cutoff value showed that, compared to patients with a sodium fluctuation level below 7.5 mmol/L, patients with a sodium fluctuation level higher than 7.5 mmol/L had a significantly increased mortality rate in general hospitalized patients, normonatremia patients on admission, and dysnatremia patients on admission. These results are presented in Supplementary Tables S9, S10, which are consistent with the findings in Table 4. The result of Briers score and its sensitivity analysis were shown in Supplementary Tables S11–S13. The result of calibration metric and its sensitivity analysis were shown in Supplementary Figures S5–S7.

4 Discussion

In this study, we examined the dose-dependent association between sodium fluctuation level during hospitalization and mortality in general hospitalized patients, normonatremia patients on admission, and dysnatremia patients on admission. We evaluated the value of sodium fluctuation level during hospitalization in dysnatremia management, which could be used as a marker to develop a single parameter system for predicting adverse outcomes in general hospitalized patients with acceptable accuracy, sensitivity, and specificity.

The study was conducted in a large retrospective cohort. Dysnatremia is the most common electrolyte disorder and is independently associated with adverse outcomes (3, 5). The serum sodium level is the most commonly used parameter in dysnatremia management. Previous studies suggested that compared to patients with simple hyponatremia or simple hypernatremia, patients with mixed dysnatremia had a higher risk of mortality (9). In addition, patients with serum sodium level between reference ranges (135 mmol/L-145 mmol/L) are regarded as low-risk patients. However, recent studies have suggested that sodium fluctuations were associated with mortality even within sodium reference ranges (10). Therefore, using serum sodium level as a management parameter has several limitations in identifying high-risk patients and providing precise management.

Sodium fluctuation level during hospitalization may be a better parameter in dysnatremia management, considering the clinical significance of mixed dysnatremia and sodium fluctuations within the normal range. Sodium fluctuation level during hospitalization was the difference between the maximum and minimum serum sodium levels (12, 13). Previous studies indicated that sodium fluctuation level below 6.0 mmol/L was treated as safe and was associated with a relatively low risk of death (10). Therefore, we divided patients into 6 groups (group G1-G6) to examine the dose-dependent relationship between sodium fluctuation level during hospitalization and mortality. After multivariable analysis, the ORs of group G2 to G6 in general hospitalized patients were 5.92 (95% CI 5.16–6.79), 26.45 (95% CI 22.68–30.86), 50.71 (95% CI 41.78–61.55), 104.38 (95% CI 81.57–133.58), and 157.64 (95% CI 112.83–220.24), respectively, p trend <0.001. The results were similar in normonatremia patients on admission. Patients admitted with dysnatremia also demonstrated a similar dose-dependent association. However, the ORs of group G2 to G6 were lower in these patients due to the independent association between dysnatremia on admission and poor prognosis. Our results suggested that sodium fluctuation level during hospitalization had a dose-dependent association with increased mortality in general hospitalized patients. Therefore, sodium fluctuation level should be paid more attention in clinical practice for accurate identification of high-risk patients.

We evaluated the impact of sodium fluctuation level during hospitalization, minimum and maximum serum sodium levels on adverse outcomes, and further compared the accuracy of the three parameters in predicting mortality. In general hospitalized patients, minimum serum sodium level (OR 1.39, 95% CI 1.37–1.41),

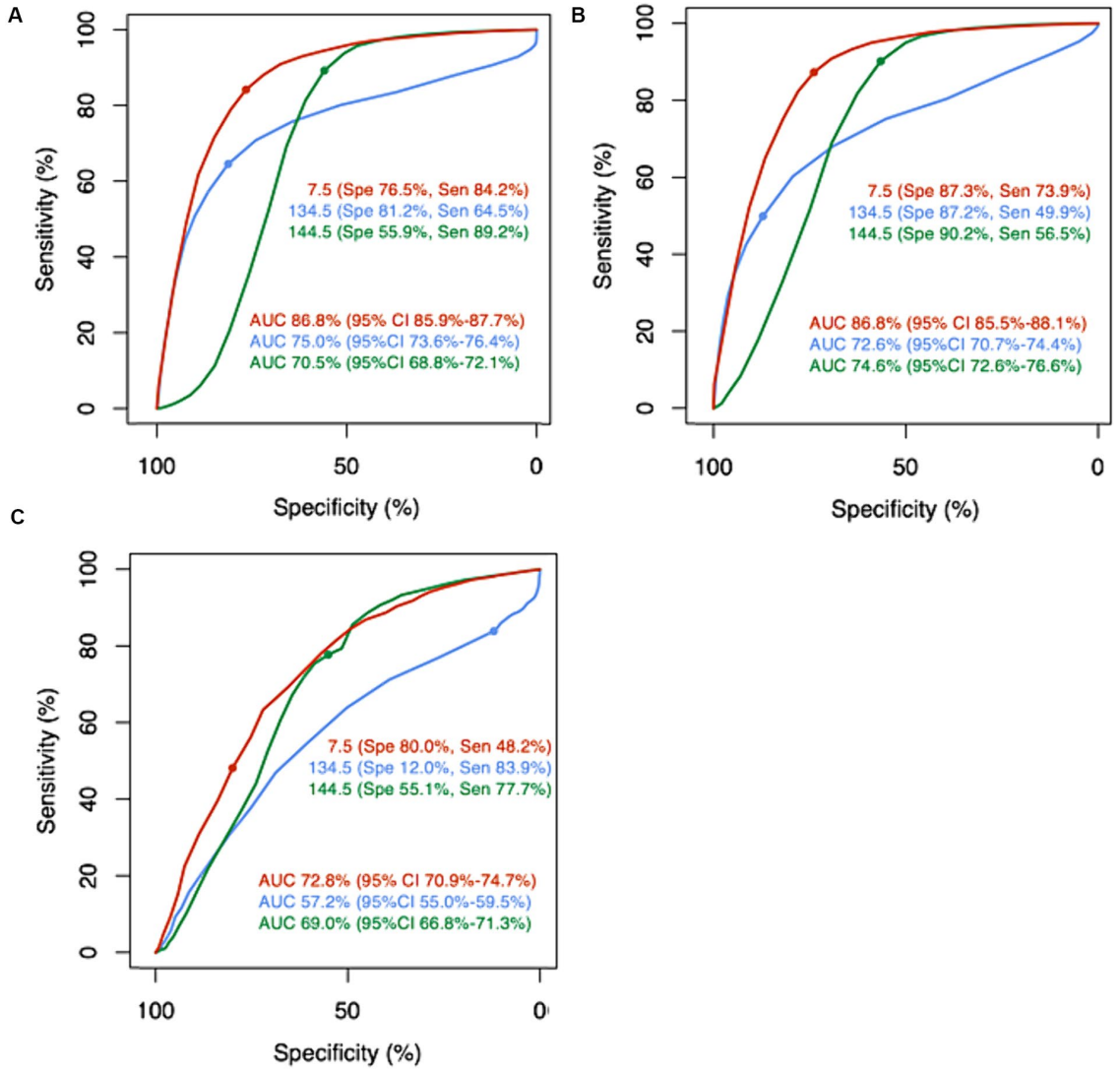

FIGURE 3
The receiver operating character curve of minimum serum sodium level (blue curve), maximum serum sodium level (green curve), and sodium fluctuation level during hospitalization (red curve) in predicting mortality. The cutoffs of minimum serum sodium level, maximum serum sodium level, and sodium fluctuation level during hospitalization were determined based on the optimal cutoffs in general hospitalized patients. **(A–C)** Showed the Receiver Operating Character Curves in general hospitalized patients, normonatremia patients on admission, and dysnatremia patients on admission, respectively. AUC, area under curve; CI, confidence internal; Sen, sensitivity; Spe, specificity.

maximum serum sodium level (OR 1.21, 95% CI 1.20–1.22), and sodium fluctuation level during hospitalization (OR 1.21, 95% CI 1.20–1.22) were associated with mortality. The ROC results indicated that the AUC of sodium fluctuation level during hospitalization in predicting mortality was significantly higher than the AUC of minimum and maximum serum sodium levels in generalized hospitalized patients, normonatremia patients on admission, and dysnatremia patients on admission. Our results suggested that sodium fluctuation level may better reflect the disease progression (19), which provides more accurate risk stratification in general hospitalized patients.

Our study suggested that sodium fluctuation was another kind of dysnatremia. Previous studies demonstrated that sodium fluctuation was independently associated with increased mortality in normonatremia patients on admission (12, 13, 20, 21). However, the prognostic impact of sodium fluctuation has not been evaluated in dysnatremia patients on admission. Our results showed that sodium fluctuation level during hospitalization had a dose-dependent relationship with increased mortality in general hospitalized patients, normonatremia patients on admission, and dysnatremia patients on admission. Therefore, we suggested that the management of sodium fluctuation level during hospitalization was necessary for decreasing adverse outcomes among general hospitalized patients. Moreover, our study illustrated that sodium fluctuation level could provide a more thorough evaluation in dysnatremia patients as sodium fluctuation level represents the degree of neurohumoral activation (19, 22), which indicates the severity of underlying diseases.

Sodium fluctuation level during hospitalization could be used as a marker to develop a single parameter system for predicting

TABLE 4 Outcomes of patients divided by cutoffs of sodium fluctuation level during hospitalization recognized by receiver operator characteristic curves in general hospitalized patients.

	General hospitalized patients			Normonatremia patients on admission			Dysnatremia patients on admission		
	< 7.5 mmol/L	> 7.5 mmol/L	*P*-value	< 7.5 mmol/L	> 7.5 mmol/L	*P*-value	< 7.5 mmol/L	> 7.5 mmol/L	*P*-value
N	112,905	22,577		107,634	16,422		5,271	6,155	
Mortality, *n* (%)	449 (0.4)	1,463 (6.5)	<0.001	284 (0.3)	804 (4.9)	<0.001	165 (3.1)	659 (10.7)	<0.001
In-hospital mortality, *n* (%)	265 (0.2)	1,147 (5.1)	<0.001	143 (0.1)	642 (3.9)	<0.001	122 (2.3)	505 (8.2)	<0.001
AMA discharge, *n* (%)	184 (0.2)	316 (1.4)	<0.001	141 (0.1)	162 (1.0)	<0.001	43 (0.8)	154 (2.5)	<0.001

Categorical variables are presented as counts and percentages. Results of models are presented in Odds Ratio and 95% Confidence Interval. AMA, against medical advice.

adverse outcomes in general hospitalized patients with acceptable accuracy, sensitivity, and specificity. An important concern of precise management is to prevent clinical deterioration and adverse events in hospitalized patients (23). The ROC analysis revealed that the AUC of sodium fluctuation level in predicting mortality was 0.868 in generalized hospitalized patients. The optimal cut-off point was 7.5 mmol/L with a sensitivity of 76.5% and a specificity of 84.2%. The single parameter system using sodium fluctuation level as the marker performed better than previously reported single parameter systems and multiple parameter weighting systems (24, 25). Our results suggested that the clinical significance of sodium fluctuation should be emphasized in clinical practice. Sodium fluctuation level during hospitalization is a reliable marker for predicting mortality in patients with normonatremia on admission, which provides the basis for precise management in dysnatremia.

Our study has several limitations. First, the retrospective cohort study collected data from a single medical center. The medical center may differ from other hospitals concerning disease spectrum and treatment routine. Therefore, the validity and generalizability of our results need further validation in external cohorts. Second, as this study is an observational study, iatrogenic factors related to sodium fluctuations such as fluid management were not included. Third, sodium fluctuation level during hospitalization was defined as the difference between the maximum and minimum serum sodium levels. However, due to the lack of data on treatment, we were unable to identify sodium fluctuations caused by the correction of dysnatremia or the deterioration of diseases. The clinical significance of sodium fluctuation level during hospitalization needs further validation in patients with treatment data.

5 Conclusion

In summary, we determined that sodium fluctuation level during hospitalization had a dose-dependent association with an increased mortality rate in general hospitalized patients, normonatremia patients on admission, and dysnatremia patients on admission. Sodium fluctuation level during hospitalization patients could be used as a marker to develop a single parameter system for predicting adverse outcomes in general hospitalized patients with acceptable accuracy, sensitivity, and specificity.

Data availability statement

The datasets used in this study are available from the corresponding author on reasonable request.

Ethics statement

The studies involving humans were approved by Ethics Committee of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences (approval number: S-k1272, approval date: 9 October 2020). The studies were conducted in accordance with the local legislation and institutional

requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

SL: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. LS: Conceptualization, Writing – review & editing, Data curation, Formal analysis, Methodology, Writing – original draft. YZ: Conceptualization, Methodology, Writing – review & editing. QZ: Conceptualization, Data curation, Writing – review & editing. NJ: Conceptualization, Data curation, Writing – review & editing. HZ: Conceptualization, Supervision, Writing – review & editing. SC: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. HP: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

1. Al Mawed S, Pankratz VS, Chong K, Sandoval M, Roumelioti ME, Unruh M. Low serum sodium levels at hospital admission: outcomes among 2.3 million hospitalized patients. *PLoS One*. (2018) 13:e0194379. doi: 10.1371/journal.pone.0194379

2. Hao J, Li Y, Zhang X, Pang C, Wang Y, Nigwekar SU, et al. The prevalence and mortality of hyponatremia is seriously underestimated in Chinese general medical patients: an observational retrospective study. *BMC Nephrol*. (2017) 18, 18:328. doi: 10.1186/s12882-017-0744-x

3. Akirov A, Diker-Cohen T, Steinmetz T, Amitai O, Shimon I. Sodium levels on admission are associated with mortality risk in hospitalized patients. *Eur J Intern Med*. (2017) 46:25–9. doi: 10.1016/j.ejim.2017.07.017

4. Tsipotis E, Price LL, Jaber BL, Madias NE. Hospital-associated hypernatremia Spectrum and clinical outcomes in an unselected cohort. *Am J Med*. (2018) 131:72–82.e1. doi: 10.1016/j.amjmed.2017.08.011

5. Waikar SS, Mount DB, Curhan GC. Mortality after hospitalization with mild, moderate, and severe hyponatremia. *Am J Med*. (2009) 122:857–65. doi: 10.1016/j.amjmed.2009.01.027

6. Sterns RH. Disorders of plasma sodium — causes, consequences, and correction. *N Engl J Med*. (2015) 372:55–65. doi: 10.1056/NEJMra1404489

7. Adrogué HJ, Madias NE. Hypernatremia. *N Engl J Med*. (2000) 342:1493–9. doi: 10.1056/nejm200005183422006

8. Funk GC, Lindner G, Druml W, Metnitz B, Schwarz C, Bauer P, et al. Incidence and prognosis of dysnatremias present on ICU admission. *Intensive Care Med*. (2010) 36:304–11. doi: 10.1007/s00134-009-1692-0

9. Stelfox HT, Ahmed SB, Khandwala F, Zygun D, Shahpori R, Laupland K. The epidemiology of intensive care unit-acquired hyponatraemia and hypernatraemia in medical-surgical intensive care units. *Crit Care*. (2008) 12:R162. doi: 10.1186/cc7162

10. Thongprayoon C, Cheungpasitporn W, Yap JQ, Qian Q. Increased mortality risk associated with serum sodium variations and borderline hypo-and hypernatremia in hospitalized adults. *Nephrol Dial Transplant*. (2020) 35:1746–52. doi: 10.1093/ndt/gfz098

11. Liang S, Chen S, Zhang Y, Zhu H, Pan H. Sodium fluctuation, a novel single parameter to predict hospital mortality. *Eur J Intern Med*. (2021) 85:124–6. doi: 10.1016/j.ejim.2020.11.013

12. Sakr Y, Rother S, Ferreira AM, Ewald C, Dünisch P, Riedemmann N, et al. Fluctuations in serum sodium level are associated with an increased risk of death in surgical ICU patients. *Crit Care Med*. (2013) 41:133–42. doi: 10.1097/CCM.0b013e318265f576

13. Topjian AA, Stuart A, Pabalan AA, Clair A, Kilbaugh TJ, Abend NS, et al. Greater fluctuations in serum sodium levels are associated with increased mortality in children with externalized ventriculostomy drains in a PICU. *Pediatr Crit Care Med*. (2014) 15:846–55. doi: 10.1097/pcc.0000000000000223

14. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet*. (2007) 370:1453–7. doi: 10.1016/s0140-6736(07)61602-x

15. Charlson ME, Sax FL, MacKenzie CR, Fields SD, Braham RL, Douglas RG Jr. Resuscitation: how do we decide? A prospective study of physicians' preferences and the clinical course of hospitalized patients. *JAMA*. (1986) 255:1316–22. doi: 10.1001/jama.1986.03370100110027

16. Yong TY, Fok JS, Hakendorf P, Ben-Tovim D, Thompson CH, Li JY. Characteristics and outcomes of discharges against medical advice among hospitalised patients. *Intern Med J*. (2013) 43:798–802. doi: 10.1111/imj.12109

17. Guasch-Ferré M, Hu FB, Martínez-González MA, Fitó M, Bulló M, Estruch R, et al. Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED study. *BMC Med*. (2014) 12:78. doi: 10.1186/1741-7015-12-78

18. Haltmeier T, Inaba K, Durso J, Khan M, Siboni S, Cheng V, et al. Transthyretin at admission and over time as a marker for clinical outcomes in critically ill trauma patients: a prospective single-center study. *World J Surg*. (2020) 44:115–23. doi: 10.1007/s00268-019-05140-6

19. Hoorn EJ, Zietse R. Hyponatremia and mortality: moving beyond associations. *Am J Kidney Dis*. (2013) 62:139–49. doi: 10.1053/j.ajkd.2012.09.019

20. Lombardi G, Ferraro PM, Calvaruso L, Naticchia A, D'Alonzo S, Gambaro G. Sodium fluctuations and mortality in a general hospitalized population. *Kidney Blood Press Res*. (2019) 44:604–14. doi: 10.1159/000500916

21. Oliva-Damaso N, Baamonde-Laborda E, Oliva-Damaso E, Payan J, Marañes A, Vega-Diaz N, et al. Fluctuation of pre-hemodialysis serum sodium. *Clin Nephrol*. (2018) 90:396–403. doi: 10.5414/cn109355

22. Ginès P, Guevara M. Hyponatremia in cirrhosis: pathogenesis, clinical significance, and management. *Hepatology*. (2008) 48:1002–10. doi: 10.1002/hep.22418

23. National Institute for Health and Care Excellence: Guidelines. Acutely ill patients in hospital: recognition of and response to acute illness in adults in hospital. London: National Institute for Health and Clinical Excellence (NICE) (2007).

24. Peelen RV, Eddahchouri Y, Koeneman M, van de Belt TH, van Goor H, Bredie SJ. Algorithms for prediction of clinical deterioration on the general wards: a scoping review. *J Hosp Med*. (2021) 16:612–9. doi: 10.12788/jhm.3630

25. Wang Y, Hu J, Geng X, Zhang X, Xu X, Lin J, et al. A novel scoring system for assessing the severity of electrolyte and acid-base disorders and predicting outcomes in hospitalized patients. *J Investig Med*. (2019) 67:750–60. doi: 10.1136/jim-2018-000900

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Umami and saltiness enhancements of vegetable soup by enzyme-produced glutamic acid and branched-chain amino acids

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Introduction: One major challenge of reducing salt content in food is the risk of the overall taste becoming bland. Enhancing saltiness is an effective strategy for salt reduction, and the development of salt-reduced foods using these saltiness-enhancing flavorants as food additives is underway. However, an increasing number of consumers demand a reduction in additives in clean-label foods.

Objective: Enzyme processing of food is an attractive strategy for developing clean-label foods because enzymes are not considered additives. We aimed to improve the saltiness and umami intensity of vegetable soups by enzyme treatment while meeting clean-label requirements. We first optimized the enzymatic reaction conditions of a protease and glutaminase blend and then investigated the synergistic effects of this enzyme blend on the taste of vegetable soup.

Results: Sensory evaluations indicated that the reaction products (e.g., protein hydrolysates or amino acids) could enhance the umami, kokumi, and saltiness intensity of vegetable soup supplemented with 0.5% NaCl. Notably, the saltiness intensity ratio of the enzyme-treated soup with 0.50, 0.45, and 0.40% NaCl were increased by 1.31-, 1.16-, and 0.99-fold, respectively, when this ratio for the control soup with 0.50% NaCl was set to 1.0. This indicates a 20% salt reduction rate can be achieved by enzyme treatment. Moreover, we found that these enhancements were synergically caused by enzyme-produced glutamic acid and branched-chain amino acids.

Conclusion: Our findings suggest that using enzyme blends of bacterial and fungal proteases and glutaminase is an effective approach to enhancing the saltiness levels of vegetable soups while meeting clean-label requirements.

KEYWORDS

protease, glutaminase, saltiness enhancer, glutamic acid, branched-chain amino acid

1 Introduction

Saltiness is the taste conferred by sodium and other ions in food. It not only contributes to delectability but is also routinely used to emphasize the taste of food (1). However, higher sodium intake is often associated with an increased risk of high blood pressure (2, 3). People in developed countries consume 9–12 g of NaCl per day, which far exceeds the recommended

amount of 5 g/day (4, 5). Thus, reducing salt intake is essential for preventing the onset and aggravation of hypertension and improving longevity (6–9). However, one of the major challenges of salt reduction is that the taste of the food can become bland.

The saltiness enhancement effect (taste–taste and odor–taste interactions) by adding certain flavorants (tastants and odorants) is an effective strategy for salt reduction. Various saltiness-enhancing tastants have been reported: amino acids (e.g., acidic amino acids, basic amino acids, and branched-chain amino acids), peptides (e.g., arginylproline), nucleotides (e.g., guanosine monophosphate and inosine monophosphate), sour organic acids (e.g., tartaric acid), and some spicy substances (e.g., spilanthol) (10–15). Also, several saltiness-enhancing odorants have been reported: pyrazine compounds (e.g., 2-ethyl-3,5-dimethyl pyrazine), fatty alcohols (e.g., 1-octen-3-ol), fatty aldehydes (e.g., 3-(methylthio) propanal), and sulfur-containing compounds (e.g., 1-(2-furyl)ethanethiol, 2-furfuryl mercaptan) (16–19). The development of salt-reduced foods using these saltiness-enhancing flavorants as food additives is currently in progress. In particular, glutamic acid and yeast extract have been extensively studied and commercialized as food additives that enhance saltiness in various foods (20). Although this is an effective strategy, the increasing consumer demand for clean-label food necessitates the reduction of additives (21). To meet these demands, the scientific community and food industry are attempting to develop more acceptable strategies for reducing salt content while maintaining saltiness levels.

Various industrial enzymes have been used in food processing and ingredient production to improve the physical, nutritional, and/or sensory properties of food (22–25). Recently, enzyme catalysis in food processing has received significant attention as an effective strategy for producing clean-label foods because enzymes in a denatured or inactivated form in the final product are not considered as additives (21, 25–28). Therefore, taste modification using enzymes is an attractive approach for increasing the umami or saltiness levels of clean-label foods. Various previous studies reported amino acids and peptides with umami and enzymes synthesizing them: glutamic acid synthesized by glutaminase (EC 3.5.1.2), γ-glutamyl peptides synthesized by γ-glutamyltranspeptidase (EC 2.3.2.2), lactoyl-peptides synthesized by lactase (EC 3.2.1.108) or other endogenous enzymes derived from microorganisms, and succinyl amino acids synthesized by transsuccinylase (EC 2.3.1.109) (29). Whereas, relatively few previous studies reported saltiness or saltiness-enhancing amino acids and peptides and enzymes synthesizing them: protein hydrolysates synthesized by proteases (EC 3.4.-.-), peptide-based Maillard reaction products by proteases and heat treatment, γ-glutamyl peptides synthesized by γ-glutamyltranspeptidase (30–32). However, the effects of these enzymes and their products in possibly enhancing the umami or saltiness of actual foods are largely unclear.

The present study aimed to develop modification methods using food-grade enzymes to improve the saltiness levels of vegetable soup while meeting clean-label requirements. Three types of enzymes were used in this study: bacterial and fungal proteases and glutaminase. Extracellular proteases catalyze the breakdown of proteins into smaller polypeptides or single amino acids (33). Bacterial proteases mainly contain *endo*-peptidases, which degrade proteins to larger peptides, while fungal proteases mainly contain *exo*-peptidases, which degrade larger peptides to smaller peptides and amino acids (33). One possible adverse effect using proteases in food industry is the bitterness development especially plant-derived peptides might display bitterness owing to the higher proportion of hydrophobic amino acids in plant proteins (34–36). In addition, glutaminase is an enzyme that catalyzes the deamidation of glutamine (Gln) to glutamic acid (Glu) and is widely used for improving the umami taste of foods (37). In general, umami enhancement by Glu molecule can mask the intensity of bitterness in foods (38). We optimized the ratio of these three enzymes and their reactions to produce glutamic acid and then investigated the synergistic effects and salt reduction rate of the enzyme blend in enhancing the umami and saltiness levels of vegetable soup. Subsequently, we analyzed the enzymatically generated amino acids and evaluated their saltiness-enhancing effects by taste–taste interactions. Finally, we investigated the ability of the reaction products by proteases and glutaminase to mask the certain bitterness of potassium chloride (KCl) as a salt replacer.

2 Materials and methods

2.1 Materials

Concentrated vegetable soup (Kagome Co., Ltd., Tokyo, Japan) and salt were obtained from a local supermarket in Nagoya (Japan). The nutritional content of the soup was 161 kcal, 1.9% protein, 0.4% lipid, and 37.5% carbohydrate. Soy protein isolate was purchased from Fuji Oil Co., Ltd. (Osaka, Japan). Bacterial and fungal proteases and bacterial glutaminase (Amano Enzyme Inc., Nagoya, Japan) were commercially available food-grade products (Thermoase PC10FA, Protease "Amano" A, and Glutaminase SD-C100S).

2.2 Preparation of vegetable soup

In this study, we selected commercially available concentrated vegetable soups to minimize the experimental errors caused by the variety, quality, harvest time, and storage conditions of vegetables and the cooking methods of the soup. This soup is made from a mixture of onions, carrots, celery, and broccoli. According to the manufacturer's recipe, an appropriate concentration of vegetable soup was prepared by diluting the concentrated soup with water (1:20 mass-to-volume ratio). We optimized three enzyme ratios to maximize the produced Glu amounts by enzymes (Supplementary Figure S1). Enzyme assays were performed in the mixtures containing 50 mM phosphate buffer (pH 6.0), 5% soy protein isolate, and various concentrations of each enzyme. Enzymatic reactions were incubated at 50°C for 1 h. As a preliminary result, combination ratios of 30–40 U/g-protein bacterial protease and 16,000–20,000 U/g-protein fungal protease maximized the total amounts of Gln + Glu at approximately 2 mg/g-protein (Supplementary Figures S1A,B). Then, as a result of adding various concentrations of glutaminases to the two optimized protease blends, 0.03–0.09 U/g-protein glutaminase maximized the amount of produced Glu amounts (Supplementary Figure S1C). Based on these preliminary results, the prepared soup was treated with bacterial and fungal proteases (30 and 1,600 U/g-protein, respectively) and glutaminase (0.03 U/g-protein) at 50°C for 1 h with agitation. The enzyme activities were referred to as product information. When evaluating the effect of the enzyme blends on the taste of soup containing NaCl or KCl, at the same time as the enzyme addition,

NaCl was added to the soup at the final concentrations of 0.35–0.50%, or KCl was added to the soup at the final concentrations of 0.30% or 0.50%. After its reaction, the enzyme-treated soup was heated at 90°C for 10 min to deactivate the enzymes. It was cooled to 50°C before being used for further analyses. Moreover, when investigating the effects of additional amino acids on saltiness enhancement through taste–taste interactions, these amino acids were added to the soup at the following concentrations: 20.9 μmol/L His, 122.8 μmol/L Asp, 205.8 μmol/L Glu, 146.7 μmol/L Ser, 225.6 μmol/L Ala, 75.4 μmol/L Gly, 93.3 μmol/L Val, 76.1 μmol/L Leu, and/or 68.7 μmol/L Ile. The soup was incubated at 50°C for 1 h with agitation and was finally heated at 90°C for 10 min.

2.3 Residual activity of enzymes after deactivation process

The residual activity of protease was assayed in 6 mL mixtures containing 50 mM phosphate buffer (pH 6.0), 5% soy protein isolate, and enzyme-contained soup after the deactivation process. This reaction solution was incubated at 50°C 4 h and added to 5 mL 0.44 mol/L trichloroacetic acid solution. After the incubation at 37°C for 30 min, the TCA-treated solution was centrifuged at 15,000 × g for 10 min. Then, 5 mL 0.55 mol/L sodium carbonate solution and 1 mL Folin & Ciocalteu's Phenol Reagent (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) were added to 2 mL supernatant and incubated at 37°C for 30 min. The absorbance of the reaction solution was measured at 660 nm. One unit of protease activity was defined as the amount of enzyme required to produce 1 μg of amino acids per minute.

The residual activity of glutaminase was assayed in 2 mL mixtures containing 50 mM phosphate buffer (pH 6.0), 2% Gln, and enzyme-contained soup after the deactivation process. This reaction solution was incubated at 37°C 4 h. Produced Glu amounts were measured by L-Glutamate KIT YAMASA NEO according to the manufacturer's instructions (Yamasa Corporation, Chiba, Japan). One unit of glutaminase activity was defined as the amount of enzyme required to produce 1 μmol of Glu molecule per minute.

2.4 Amino acid analysis

The amino acids produced by the enzymes were quantified using an Agilent 1,100 HPLC system (Agilent Technologies, Santa Clara, CA, United States) equipped with a fluorescence detector (excitation 230 nm and emission 450 nm PMT 9 or excitation 266 nm and emission 305 nm PMT 7). The supernatant was treated with *o*-phthalaldehyde and 9-fluorenylmethylchloroformate and separated on a Zorbax Eclipse-AAA column (4.6 × 150 mm, Agilent) with a gradient of 20 mM Na_2HPO_4 buffer (pH 8.2) and a 45:45:10 methanol:acetonitrile:H_2O mixture for 20 min at a flow rate of 0.65 mL/min (39).

2.5 Peptide analysis

To precipitate protein molecules, 990 μL enzyme-treated and control vegetable soups were treated with 10 μL 10% trichloroacetic acid solution and these soups with a final concentration of 0.1% TCA were centrifuged at 15,000 × g for 10 min. The enzyme-produced peptides in the supernatants were analyzed using HPLC (Nexera X2, Shimadzu, Kyoto, Japan) equipped with a UV detector (220 nm). Each injection volume was 10 μL. The peptides were separated using the TSKgel G2000SWXL (300 × 7.8 mm I.D., Tosoh Corporation, Tokyo, Japan) with isocratic elution of 45% acetonitrile containing 0.1% trifluoroacetic acid for 25 min at a flow rate of 0.7 mL/min and 40°C.

2.6 Free amino nitrogen analysis

Enzyme-treated and control vegetable soups were centrifuged at 15,000 × *g* for 10 min, and their supernatants were deproteinized with a Nanosep® Centrifugal Device (Pall Corporation, Port Washington, NY, United States) with a molecular weight cut-off of 30,000 Da. The amount of free amino nitrogen (FAN) was measured using the ninhydrin method (40).

2.7 Sodium ion analysis

Sodium ions were quantified using a LAQUAtwin-Na-11 pocket meter (Horiba Ltd., Kyoto, Japan). To quantify sodium concentration, standard curves were generated using 0.01, 0.1, 1, 2.0, and 2.5% NaCl solution at 25°C. Subsequently, the sodium ion concentration in the vegetable soup was spiked and analyzed under the same conditions.

2.8 Color analysis

The color of the vegetable soup was determined using a colorimeter (Chroma Meter CM-700d/600d; Konica Minolta, Tokyo, Japan) (41). The color analysis results were expressed according to the Commission International de l'Eclairage (CIE) system and reported as Hunter L^* (lightness), a^* (redness), and b^* (yellowness).

2.9 Sensory tests

The sensory taste of the vegetable soup was evaluated by 12 employees (seven men and five women aged 25–55 years) of Amano Enzyme Inc., trained in sensory evaluation. These sensory panelists were selected based on their ability to perceive five basic tastes (umami, saltiness, sweetness, bitterness, and sourness) according to ISO 8586:2023 (42). The terms of five basic tastes were defined in this standard. Whereas the term kokumi was defined as a perceived thickness, mouthfulness, continuity, and depth, with reference to the previous report (43). These vocabularies are defined in ISO 8586:2023 or ISO 5492:2008 (44). For the tastes of vegetable soup, the samples were evaluated for four attributes (umami, saltiness, bitterness, and kokumi). The samples were scored on a structured scale from −2 (weak) to +2 (strong) relative to the control soup (non-treated soup), which was scored as 0. For the smell of vegetable soup, the samples were evaluated for only one attribute (smell). The samples were similarly scored on a structured scale from −2 (unpleasant odor) to +2 (pleasant fragrance). The Umami and saltiness ratio of the enzyme-treated vegetable soup was scored on an unstructured scale relative to the control soup (non-treated soup), in which the ratio was 1. To evaluate this test more accurately, the sensory panelists were trained using NaCl solution in 0.05% increments.

2.10 Statistical analysis

Vegetable soup in five different lots was used in this study. Data are presented as the mean ± standard error of five independent experiments to evaluate the effects of different sample formulations and preparations. Statistical differences among multiple groups were determined by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Statistical differences between the curves were analyzed using a two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Tukey's test at significance levels of 95% ($p < 0.05$) and 99% ($p < 0.01$) was used to determine the significant differences in the results. Heatmap and clustering analysis were performed using Heatplus in the Bioconductor package (version 3.19).

3 Results and discussion

3.1 Characteristics of enzyme-treated vegetable soup

To maximize the umami intensity of the vegetable soup, glutamic acid (Glu) content was determined as an indicator (Figure 1A). The vegetable soup was treated with an optimized blend of protease and glutaminase for 60 min. The Glu content in the control soup remained constant at 40.0 μmol/L, which was below the panelists' threshold Glu concentration (90 μmol/L) that our panelists required for sensing umami taste. In general, the Glu threshold for humans to perceive umami is 63 μmol/L (45). In contrast, Glu content in the enzyme-treated soup increased in a time-dependent manner and reached a plateau at 250 μmol/L. However, soups tended to become bitter when the reaction time exceeded 30 min in simple sensory tests. Generally, owing to the higher proportion of hydrophobic amino acids in plant-derived proteins, the peptides produced by enzymatic hydrolysis can elicit bitterness (34–36). Therefore, in the subsequent experiments, the reaction time for the enzyme blend was set to 30 min. The activities of proteases and glutaminase in the vegetable soup were not detected after the deactivation process. Furthermore, the reactivity of the enzyme blend did not differ among the five lots of vegetable soup.

Figure 1B shows the appearance of control and enzyme-treated soups. Other possible adverse effect of processing vegetable soup using proteases is color change via the Maillard reaction caused by free amino acids. However, both soups were visually similar in color. To investigate the color properties in detail, the objective color attributes of the proteins were characterized using $L^*a^*b^*$ coordinates. The L^* (whiteness), a^* (redness), and b^* (yellowness) values of the control soup were 48.10 ± 0.19, 3.93 ± 0.01, and 18.76 ± 0.10, whereas those of the enzyme-treated soup were 48.22 ± 0.18, 3.93 ± 0.01, and 18.83 ± 0.15. This finding indicated that both soups have similar color properties.

3.2 Protein hydrolysates in enzyme-treated vegetable soup

To investigate the protein hydrolysates produced by the blend of proteases and glutaminase, free amino acids and peptides in the vegetable soup were measured using HPLC analysis (Figures 1C,D). Figure 1C shows the heatmap and cluster analysis of the increased rate of enzyme-treated soup compared with the amino acid content in the control soup. The relative free amino acid compositions were clustered into five different groups according to Euclidean distance: (a) significantly lower cluster, (b) no change cluster, (c) slightly higher cluster, (d) significantly higher cluster, and (e) significantly highest cluster. As evident from the clustering, most amino acids were enhanced. Total amino acid amounts of control and enzyme-treated soup were 730.4 ± 24.2 and 1252.8 ± 87.3 μmol/L, respectively. Interestingly, amino acids classified into cluster (d) showed that the amounts of branched-chain amino acids (BCAAs) such as Ile, Leu, and Val and other umami acidic amino acids such as Asp were increased by enzymatic reactions. Moreover, the Glu content in vegetable soup significantly increased with a decrease in Gln content. Figure 1D shows the results of size exclusion chromatography for separating peptides from vegetable soup by molecular weight. As evident from the chromatography results, enzyme treatment substantially enhanced the amount of total peptides in the vegetable soup. The same trend was observed for the FAN amounts, which control and enzyme-treated soup had 11.0 ± 0.3 and 39.9 ± 2.0 mg/L, respectively. Nine peaks were detected in vegetable soup hydrolyzed by protease, and the estimated molecular weights from peaks A to H were 7.6, 7.1, 6.0, 5.6, 4.8, 4.4, 3.6, 1.2, and 0.6 kDa, respectively. The estimated molecular weight of peak H was equal to at least the size of a tri-to-pentapeptide. These findings indicate that proteases substantially produce specific amino acids and lower molecular weight peptides from proteins in vegetable soup, and glutaminase converts free Gln into Glu with an umami taste.

The type and amount of free amino acids are important factors affecting human nutrition and food palatability (45–47). Regarding nutrition, five of the nine essential amino acids (Trp, Thr, Ile, Leu, and Val) were increased by enzyme treatment (Figure 1C). BCAAs are proteinogenic amino acids that account for 35% of the essential amino acids in muscle proteins and 40% of preformed amino acids required by mammals (48). Because not all ingested food proteins can be degraded into amino acids (49, 50), increasing the content of essential amino acids in food by enzyme treatment can potentially boost the amount of amino acids metabolized as nutrients. Regarding taste, of the 14 amino acids increased by the enzyme blend, nine (Asn, Tyr, Trp, Thr, Arg, His, Val, Leu, and Ile) have bitter taste, three (Ser, Ala, and Gly) have sweet taste, and two (Asp and Glu) have umami taste (45). However, the concentrations of all amino acids except Glu were lower than the threshold for sensing bitter, sweet, and umami tastes. Therefore, only Glu contributed to the umami taste of the soup.

3.3 Sensory evaluation of enzyme-treated vegetable soup with added sodium chloride

The effects on the sensory properties of vegetable soup were assessed using sensory evaluation. As shown in Figure 2A, the umami and kokumi levels of the enzyme-treated vegetable soup were significantly higher ($p < 0.01$) and the saltiness and smell of the enzyme-treated soup were slightly higher ($p > 0.01$) than those of the control soup. Here, regardless of enzyme addition, NaCl contents remained unchanged, which control and enzyme-treated soup had 0.33 ± 0.02 and 0.34 ± 0.04%, respectively. Although one possible adverse effect of using protease is bitterness development, the bitterness score of both vegetable soups was the same. Thus, the

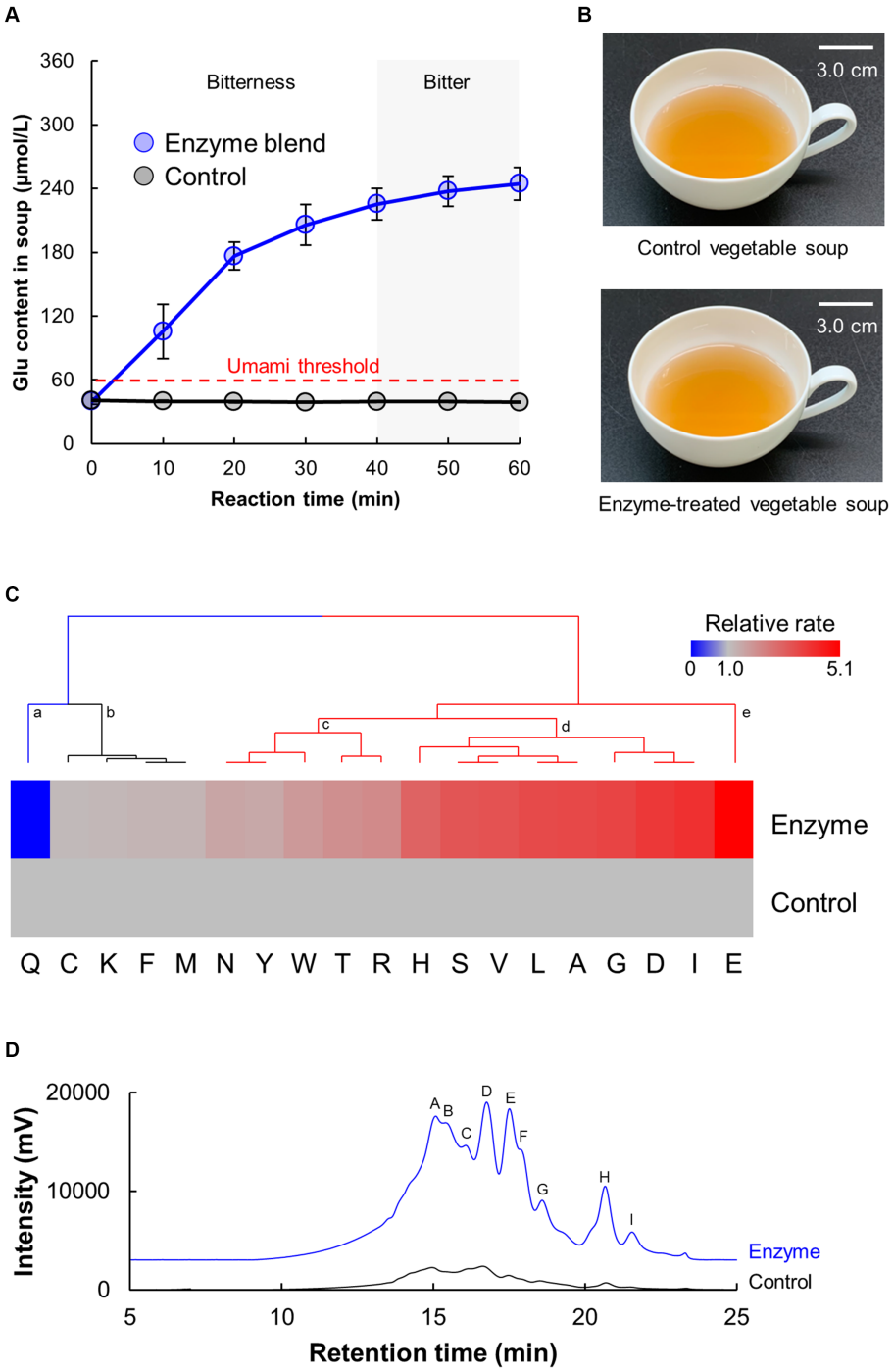

FIGURE 1
Vegetable soup produced by a blend of proteases and glutaminase. **(A)** Time-course of the Glu content produced by the enzyme blend. **(B)** Appearance of control and enzyme-treated vegetable soup. **(C,D)** Composition analysis of protein hydrolysates produced by the enzyme blend. Free amino acid **(C)** and peptide **(D)** profiles analyzed using HPLC. **(C)** Heatmap and cluster analysis of free amino acid profile in vegetable soup. **(D)** Size exclusion chromatography of free peptides in vegetable soup.

proteases and glutaminase blend could enhance the umami and kokumi tastes of the vegetable soup without causing bitterness.

Next, because the saltiness score of the enzyme-treated soup was slightly higher than that of the control soup, the ability of enzyme treatment to enhance the saltiness intensity of NaCl was evaluated. As shown in Figure 2B, 0.5% NaCl supplementation significantly increased the umami and kokumi levels in the enzyme-treated vegetable soup compared with the control vegetable soup ($p < 0.01$); however, bitterness was not significantly different between the two groups ($p > 0.05$). This was consistent with the results obtained for vegetable soup without NaCl addition (Figure 2A). Interestingly, in the presence of 0.5% NaCl, the saltiness of the enzyme-treated vegetable soup was significantly higher ($p < 0.01$) than that of the control soup. Thus, reaction products such as protein hydrolysates or

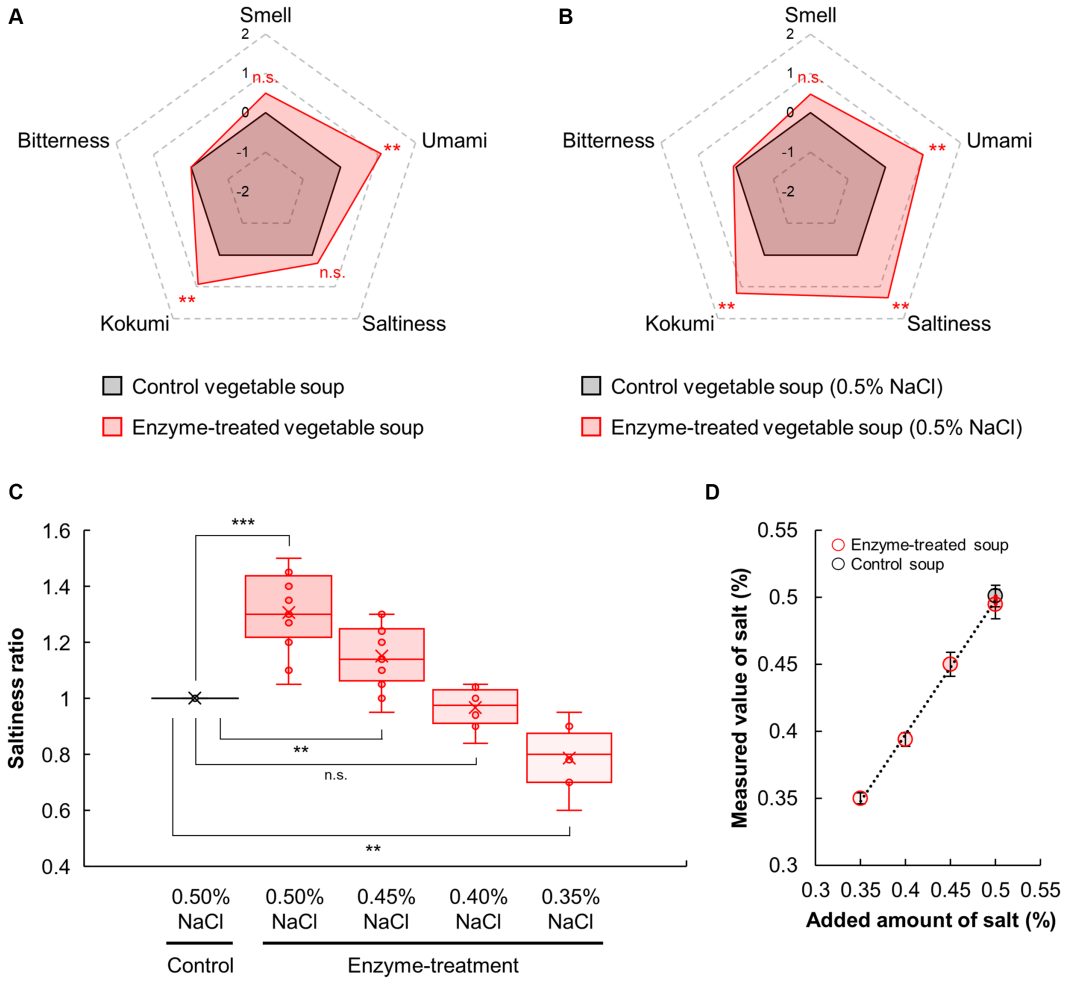

FIGURE 2
Sensory profiles of vegetable soup after NaCl addition. Sensory evaluations of control and enzyme-treated vegetable soups conducted in the absence **(A)** and presence **(B)** of 0.5% NaCl final concentration. **(C)** Salt reduction rate of vegetable soup. Sensory evaluations of enzyme-treated vegetable soup in the presence of 0.35, 0.40, 0.45%, and −0.50% NaCl compared with the saltiness of the control soup. **(D)** NaCl contents in the vegetable soup.

amino acids produced by a protease and glutaminase blend can enhance the saltiness of NaCl in vegetable soups.

Furthermore, we investigated the salt reduction rate of the enzyme-treated vegetable soup compared with the control soup following NaCl supplementation (Figure 2C). The saltiness intensity ratio of the control soup supplemented with 0.50% NaCl was set to 1.0, and compared with this, the ratio of the enzyme-treated soup supplemented with 0.50, 0.45, 0.40, and 0.35% NaCl were 1.31, 1.16, 0.99, and 0.79, respectively in the sensory evaluation. Compared with the control soup with 0.50% NaCl, the saltiness intensity of the enzyme-treated soup with 0.50 and 0.45% NaCl was significantly higher ($p < 0.01$); however, the saltiness intensity of the enzyme-treated soup with 0.40% NaCl was comparable to that of the control soup. As expected, regardless of enzyme addition, NaCl content in vegetable soup remained unchanged (Figure 2D). Interestingly, the effects of the enzyme blend on saltiness and umami enhancement were more pronounced when the temperature of the vegetable soup was increased (Figure 3). This finding suggests that enzymatic treatment with protease and glutaminase may be an effective tool for achieving a 20% salt reduction rate.

The rate of salt reduction by saltiness-enhancing flavorants is an important indicator for the practical development of salt-reduced food products. Some previous studies have reported salt reduction rates equal to or greater than 20% (Figure 2C) using enzymes: 60% reduction using Glu (1), 30% reduction using octapeptides derived from *Agaricus bisporus* (51), 25% reduction using proteases derived from *Lentinula edodes* (52), 24% reduction using disodium succinate (14), and 25–30% reduction using pyroglutamyl tripeptides and organic acids (53). While adding these enhancers to actual final foods can be effective strategy, the feasibility may be constrained by factors such as their concentration levels, manufacturing and purification costs, taste, and regulatory considerations as food additives. Notably, such strategies are unable to meet the recent clean-label demands to reduce the amount and number of food additives, and an increasing number of consumers tend to avoid food products containing additives (21). However, the enzyme blend and their reaction products in this study are not considered as additives because the enzymes were deactivated during the processing of the vegetable soup. Therefore, enzyme blends of proteases and glutaminases could be a novel tactic to enhance the

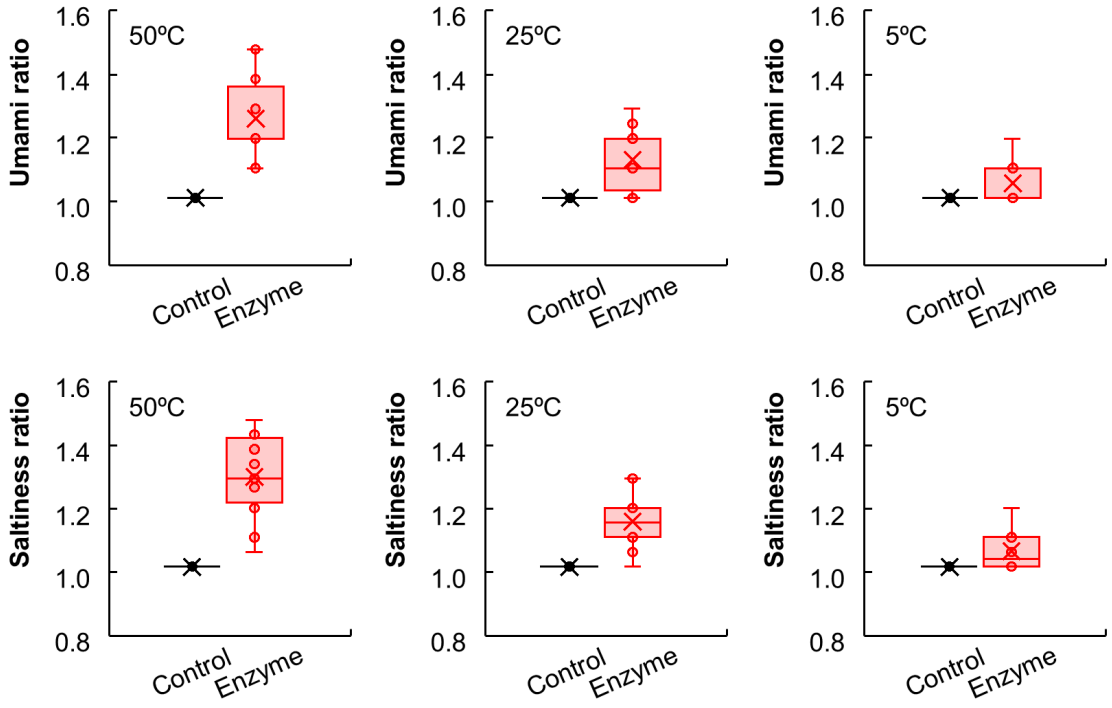

FIGURE 3
The effects of temperature on umami and saltiness of vegetable soup. Sensory evaluations conducted on control and enzyme-treated vegetable soups at 50°C, 25°C, and 5°C. Umami and saltiness scores of enzyme-treated vegetable soups compared to control soup.

saltiness levels of final food products while meeting clean-label requirements.

3.4 Identifying the saltiness-enhancing tastants in enzyme-treated vegetable soup with added sodium chloride

Next, we explored the saltiness enhancement tastants in enzyme-treated vegetable soup (Figure 4; Supplementary Figure S2). As shown in Figures 1C,D, the protease and glutaminase blend produced amino acids and peptides. Because fractionation and collection of peptides in the soup were difficult, we evaluated the effect of taste–taste interactions by amino acids (His, Ser, Val, Ala, Leu, Gly, Asp, Ile, and Glu) that were significantly increased by enzyme treatment (Figure 1C). Glu addition significantly enhanced the umami intensity of the control soup after 0.5% NaCl supplementation, and this intensity was comparable to that of the enzyme-treated soup containing 0.5% NaCl (Figure 4A). Although Glu addition significantly enhanced the saltiness of the control soup containing 0.5% NaCl, this intensity was lower than that of the enzyme-treated soup containing 0.5% NaCl (Figure 4B). Interestingly, after adding all nine amino acids increased by the enzyme treatment to the control soup containing NaCl, the saltiness levels were significantly increased compared with the control soup containing NaCl and Glu (Supplementary Figure S2A). These findings suggest that amino acids other than Glu molecules that can enhance saltiness exist in enzyme-treated vegetable soups.

The interaction of amino acids expressing the five basic tastes with taste receptors generally depends on the structure (size, charge, and polarity) of their side chain (45). Thus, amino acids increased by enzyme treatment were classified according to their chemical and taste properties, and we searched for other amino acids that have a saltiness enhancement effect of NaCl through taste–taste interactions, except for Glu. Accordingly, nine amino acids were classified into five groups: (i) polar basic amino acid (bitter), His; (ii) polar acidic amino acid (umami), Asp and Glu; (iii) polar neutral amino acid (sweet), Ser; (iv) non-polar amino acid (sweet), Ala and Gly; and (v) nonpolar amino acid (bitter), Val, Leu, and Ile (Supplementary Figure S2B). Considering various combinations of these groups, soup containing a combination of BCAAs (Val, Leu, and Ile) and Glu had a higher saltiness intensity of NaCl (Supplementary Figure S2A; Figure 4). BCAA addition did not affect the umami taste of the control soup containing 0.5% NaCl irrespective of the presence of Glu (Figure 4A). Similarly, BCAA addition did not affect the saltiness of the control soup containing 0.5% NaCl. However, compared with Glu or BCAA addition alone, adding both BCAA and Glu significantly enhanced the saltiness of control soup with 0.5% NaCl (Figure 4B). These findings indicate that the combination of BCAA and Glu could be an important factor for enhancing the saltiness intensity of NaCl.

Saltiness is perceived by several receptors and ion channels, especially epithelial sodium channels, transient receptor potential vanillic acid, and transmembrane channel-like 4 (30, 51, 52). However, our knowledge regarding the receptor structures and interactions between saltiness-enhancing flavorants and receptors is limited, and hence, there is a lack of research on saltiness-enhancing flavorants at the molecular level (30). Some previous studies, using an electronic tongue evaluation method, have reported that certain amino acids, such as Glu or BCAA, can enhance saltiness (10, 54). Recently, a study using molecular docking simulation and electronic tongue evaluation

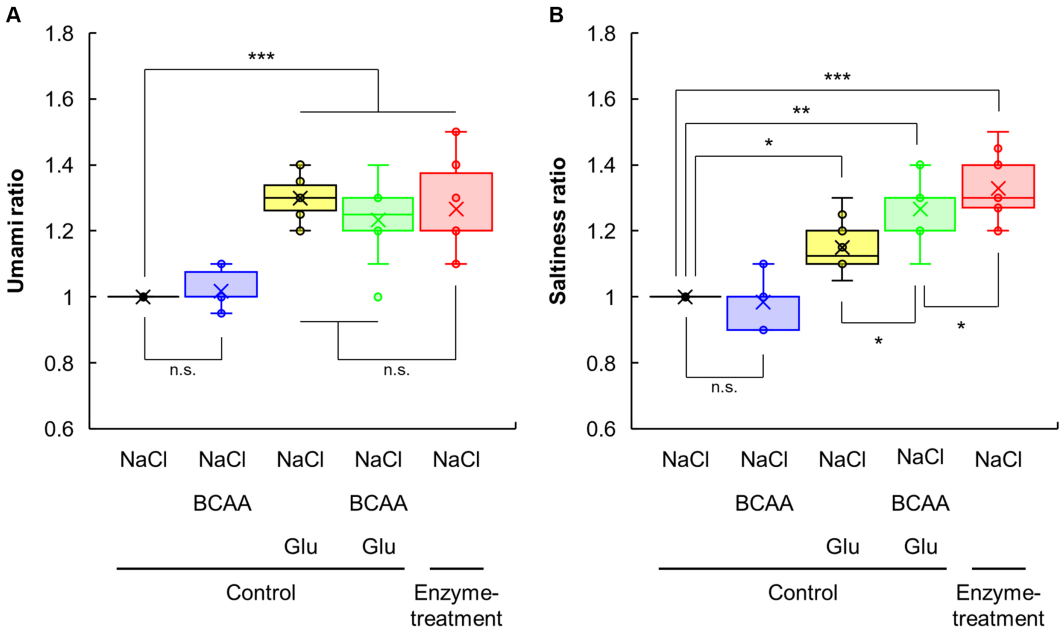

FIGURE 4
The effects of amino acid addition on umami and saltiness of vegetable soup. Sensory evaluations of enzyme-treated vegetable soup in the presence of NaCl, Glu, and/or BCAA, compared with the umami **(A)** and saltiness **(B)** of the control soup.

reported that Glu molecules under neutral conditions have a negative charge and can bind to the transmembrane channel-like 4 receptor, thereby enhancing saltiness intensity (55). Another recent study using molecular docking simulation and sensory evaluation has reported that Glu and constituent amino acid residues are important for binding to the transmembrane channel-like 4 receptor and enhancing salty taste in saltiness-enhancing peptides (DIQPEER, DEPLIVW, and LPDEPSR) identified from mushrooms (52). Based on these findings, as well as the present study's results, we suggest that Glu and BCAAs released by enzyme blends can interact with saltiness receptors, increasing the saltiness intensity of NaCl in vegetable soups. In contrast, the saltiness intensities of control soups containing 0.5% NaCl supplemented with all nine amino acids or Glu + BCAA were similar but significantly lower than those of the enzyme-treated soup containing 0.5% NaCl. Thus, components other than BCAA and Glu that enhance the salty taste may exist in enzyme-treated vegetable soups. However, further research is required to identify saltiness-enhancing peptides through taste–taste interactions because the peptides produced by enzyme blends are difficult to isolate (Figure 1D).

3.5 Sensory evaluation of enzyme-treated vegetable soup with added potassium chloride

Another approach for reducing the salt content is the addition of salt replacers other than saltiness-enhancing flavorants. Among salt replacers, potassium chloride (KCl) is the most popular choice as a feasible salt substitute (56). In particular, salt reduction obtained by the proportional replacement of NaCl with KCl is the easiest method for manufacturers. However, consumer acceptance is limited owing to its pronounced bitter or metallic taste and aftertaste (57). Generally, the umami taste of Glu is able to mask the bitterness intensity in food (38). Thus, we investigated the ability of the Glu molecules released by the blend of proteases and glutaminase to reduce KCl bitterness intensity (Figure 5). Compared with each control soup, 0.3% KCl addition to the enzyme-treated soup slightly enhanced the saltiness intensity ($p > 0.05$), and 0.5% KCl addition significantly enhanced ($p < 0.01$). Compared with each control soup, enzyme treatment significantly reduced the bitterness intensity caused by 0.3% KCl ($p < 0.05$) but did not affect the bitterness intensity of 0.5% KCl. These findings indicate that treatment with enzyme blends can suppress the characteristic bitterness of KCl, when its concentration is approximately ≤0.3%.

Many studies have reported that Glu molecules decrease bitterness levels (29, 38, 58–60). However, some studies also have reported results that contradict these results and this is potentially caused by the differences in bitter substances, their concentrations, and test methods in the sensory test (61, 62). Of them, the intensities and combinations of Glu and bitter substances might be important factors. One recent study reported that Glu molecules did not enhance or suppress other tastes when other tastes were displayed at relatively stronger intensities (62). Generally, the thresholds for KCl bitterness are 0.06% in an aqueous solution and 0.10% in an emulsion state similar to that found in food (63), and bitterness intensity of KCl tends to further increase depending on the concentration (64). As shown in Figure 5, the bitterness of 0.3% KCl relatively close to the bitter threshold was suppressed by Glu presence but the bitterness of 0.5% KCl bitterness was not. Therefore, it is suggested that the ability of Glu to mask KCl bitterness might be affected by the KCl concentration (bitterness intensity). Although there are few reports about the masking effects of Glu on the bitter tastes of food containing NaCl partially replaced by KCl, one previous study reported that bitterness induced by 0.15–0.35% KCl could be suppressed by Glu addition (65). Nevertheless, there is currently limited research and knowledge on the

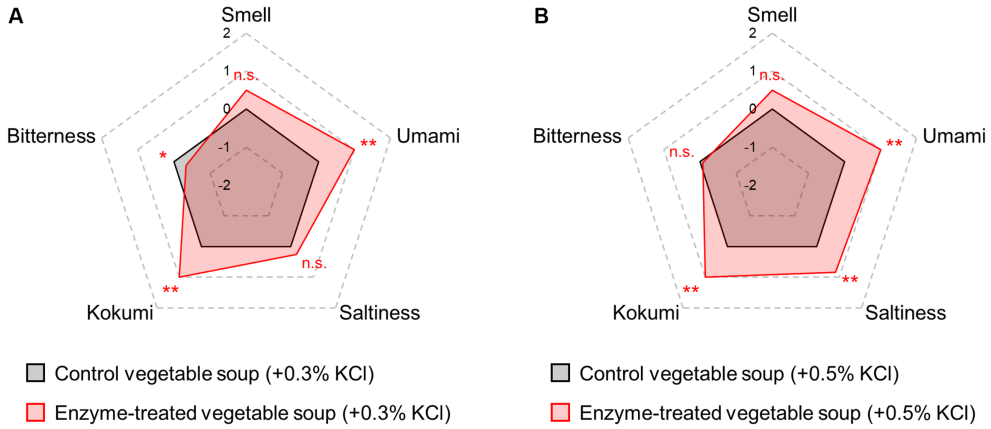

FIGURE 5
Sensory profiles of vegetable soup added with KCl. Sensory evaluations of control and enzyme-treated vegetable soups conducted in the presence of 0.3% **(A)** and 0.5% **(B)** KCl final concentration.

range of KCl bitterness intensity (or its concentration) that the Glu molecule can decrease.

4 Conclusion

In this study, we optimized the enzymatic reaction conditions for a mixture of bacterial and fungal proteases and glutaminase and then investigated the synergistic effects of this enzyme blend on the umami and saltiness levels of vegetable soup. The activities of proteases and glutaminase in the vegetable soup were not detected after deactivation. Sensory evaluations indicated that the reaction products (e.g., protein hydrolysates or amino acids) enhanced the umami, kokumi, and saltiness of vegetable soups containing 0.5% NaCl. Notably, the saltiness intensity ratio of the enzyme-treated soup with 0.50, 0.45, and 0.40% NaCl was 1.31-, 1.16-and 0.99-fold higher compared with that of control soup, achieving a 20% salt reduction rate. Moreover, the enzyme-produced Glu and BCAA synergistically enhanced the saltiness intensity of NaCl. These findings suggest that enzyme blends of bacterial and fungal proteases and glutaminase can be used to enhance the saltiness of vegetable soups while meeting clean-label requirements.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Research Commission of Innovation Divisions at Amano Enzyme, Inc. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

KS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MO: Supervision, Writing – review & editing. SY: Supervision, Writing – review & editing.

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Conflict of interest

KS, MO, and SY were employed by Amano Enzyme Inc.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1436113/full#supplementary-material

References

1. Hayabuchi H, Morita R, Ohta M, Nanri A, Matsumoto H, Fujitani S, et al. Validation of preferred salt concentration in soup based on a randomized blinded experiment in multiple regions in Japan-influence of umami (L-glutamate) on saltiness and palatability of low-salt solutions. *Hypertens Res*. (2020) 43:525–33. doi: 10.1038/s41440-020-0397-1

2. Mente A, O'Donnell MJ, Rangarajan S, McQueen MJ, Poirier P, Wielgosz A, et al. Association of urinary sodium and potassium excretion with blood pressure. *N Engl J Med*. (2014) 371:601–11. doi: 10.1056/NEJMoa1311989

3. Chan Q, Stamler J, Griep LMO, Daviglus ML, Horn LV, Elliott P. An update on nutrients and blood pressure. *J Atheroscler Thromb*. (2016) 23:276–89. doi: 10.5551/jat.30000

4. Jiménez-Colmenero F, Carballo J, Cofrades S. Healthier meat and meat products: their role as functional foods. *Meat Sci*. (2001) 59:5–13. doi: 10.1016/S0309-1740(01)00053-5

5. World Health Organization (WHO). Diet, nutrition and the prevention of chronic diseases. *3. Global and regional food consumption patterns and trends 3rd ed*. (2003) 1–149.

6. Turnbull F. Managing cardiovascular risk factors: the gap between evidence and practice. *PLoS Med*. (2005) 2:374–5. doi: 10.1371/journal.pmed.0020131

7. Strazzullo P, D'Elia L, Kandala NB, Cappuccio FP. Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. *BMJ*. (2009) 339:b4567. doi: 10.1136/bmj.b4567

8. Tuomilehto J, Jousilahti P, Rastenyte D, Moltchanov V, Tanskanen A, Pietinen P, et al. Urinary sodium excretion and cardiovascular mortality in Finland: a prospective study. *Lancet*. (2001) 357:848–51. doi: 10.1016/S0140-6736(00)04199-4

9. Cook NR, Appel LJ, Whelton PK. Sodium intake and all-cause mortality over 20 years in the trials of hypertension prevention. *J Am Coll Cardiol*. (2016) 68:1609–17. doi: 10.1016/j.jacc.2016.07.745

10. Nakatani F, Ienaga T, Wu X, Tahara Y, Ikezaki H, Sano H, et al. Development of a sensor with a lipid/polymer membrane comprising Na^+ ionophores to evaluate the saltiness enhancement effect. *Sensors*. (2019) 19:5251. doi: 10.3390/s19235251

11. Manabe M. Saltiness enhancement by the characteristic flavor of dried bonito stock. *J Food Sci*. (2008) 73:S321–5. doi: 10.1111/j.1750-3841.2008.00844.x

12. Tamura M, Seki T, Kawasaki Y, Tada M, Kikuchi E, Okai H. An enhancing effect on the saltiness of sodium chloride of added amino acids and their esters. *Agric Biol Chem*. (1989) 53:1625–33. doi: 10.1080/00021369.1989.10869536

13. Schindler A, Dunkel A, Stähler F, Backes M, Ley J, Meyerhof W, et al. Discovery of salt taste enhancing arginyl dipeptides in protein digests and fermented fish sauces by means of a sensomics approach. *J Agric Food Chem*. (2011) 59:12578–88. doi: 10.1021/jf2041593

14. Sun X, Zhong K, Zhang D, Shi B, Wang H, Shi J, et al. The enhancement of the perception of saltiness by umami sensation elicited by flavor enhancers in salt solutions. *Food Res Int*. (2022) 157:111287. doi: 10.1016/j.foodres.2022.111287

15. Xu J, Lewandowski BC, Miyazawa T, Shoji Y, Yee K, Bryant BP. Spilanthol enhances sensitivity to sodium in mouse taste bud cells. *Chem Senses*. (2019) 44:91–103. doi: 10.1093/chemse/bjy069

16. Gao Y, Wang W, Zhang H, Chen H, Wang S, Sun B. The enhancement of the perception of saltiness by odorants selected from Chinese Douchi in salt solution. *Foods*. (2022) 11:2260. doi: 10.3390/foods11152260

17. Zhou T, Feng Y, Thomas-Danguin T, Zhao M. Enhancement of saltiness perception by odorants selected from Chinese soy sauce: a gas chromatography/olfactometry-associated taste study. *Food Chem*. (2021) 335:127664. doi: 10.1016/j.foodchem.2020.127664

18. Lopez J, Kerley T, Jenkinson L, Luckett CR, Munafo JP Jr. Odorants from the thermal treatment of hydrolyzed mushroom protein and cysteine enhance saltiness perception. *J Agric Food Chem*. (2019) 67:11444–53. doi: 10.1021/acs.jafc.9b04153

19. Chen YP, Wang M, Fang X, Liya A, Zhang H, Blank I, et al. Odorants identified in Chinese dry-cured ham contribute to salty taste enhancement. *J Agric Food Chem*. (2024) 72:613–24. doi: 10.1021/acs.jafc.3c05848

20. Inguglia ES, Zhang Z, Tiwari BK, Kerry JP, Burgess CM. Salt reduction strategies in processed meat products—a review. *Trends Food Sci Technol*. (2017) 59:70–8. doi: 10.1016/j.tifs.2016.10.016

21. Asioli D, Aschemann-Witzel J, Caputo V, Vecchio R, Annunziata A, Næs T, et al. Making sense of the "clean label" trends: a review of consumer food choice behavior and discussion of industry implications. *Food Res Int*. (2017) 99:58–71. doi: 10.1016/j.foodres.2017.07.022

22. Bangar SP, Ashogbon AO, Singh A, Chaudhary V, Whiteside WS. Enzymatic modification of starch: a green approach for starch applications. *Carbohydr Polym*. (2022) 287:119265. doi: 10.1016/j.carbpol.2022.119265

23. Vasić K, Knez Ž, Leitgeb M. Transglutaminase in foods and biotechnology. *Int J Mol Sci*. (2023) 24:12402. doi: 10.3390/ijms241512402

24. Sakai K, Okada M, Yamaguchi S. Protein-glutaminase improves water-/oil-holding capacity and beany off-flavor profiles of plant-based meat analogs. *PLoS One*. (2023) 18:e0294637. doi: 10.1371/journal.pone.0294637

25. Sakai K, Sato Y, Okada M, Yamaguchi S. Cyclodextrins produced by cyclodextrin glucanotransferase mask beany off-flavors in plant-based meat analogs. *PLoS One*. (2022) 17:e0269278. doi: 10.1371/journal.pone.0269278

26. Dai Y, Tyl C. A review on mechanistic aspects of individual versus combined uses of enzymes as clean label-friendly dough conditioners in breads. *J Food Sci*. (2021) 86:1583–98. doi: 10.1111/1750-3841.15713

27. Park S, Kim YR. Clean label starch: production, physicochemical characteristics, and industrial applications. *Food Sci Biotechnol*. (2021) 30:1–17. doi: 10.1007/s10068-020-00834-3

28. Scarton M, Ganancio JRC, de Avelar MHM, Clerici MTPS, Steel CJ. Lime juice and enzymes in clean label pan bread: baking quality and preservative effect. *J Food Sci Technol*. (2021) 58:1819–28. doi: 10.1007/s13197-020-04693-y

29. Zhang J, Sun-Waterhouse D, Su G, Zhao M. New insight into umami receptor, umami/umami-enhancing peptides and their derivatives: a review. *Trends Food Sci Technol*. (2019) 88:429–38. doi: 10.1016/j.tifs.2019.04.008

30. Le B, Yu B, Amin MS, Liu R, Zhang N, Soladoye OP, et al. Salt taste receptors and associated salty/salt taste-enhancing peptides: a comprehensive review of structure and function. *Trends Food Sci Technol*. (2022) 129:657–66. doi: 10.1016/j.tifs.2022.11.014

31. Yang F, Lv S, Liu Y, Bi S, Zhang Y. Determination of umami compounds in edible fungi and evaluation of salty enhancement effect of Antler fungus enzymatic hydrolysate. *Food Chem*. (2022) 387:132890. doi: 10.1016/j.foodchem.2022.132890

32. Cho Y, Lee J, Lee MY, Cho HY, Choi MJ. Effects of hydrolyzed animal protein on the enhancement of saltiness and quality characteristics of white pan bread. *Food Sci Biotechnol*. (2019) 12:1832–41. doi: 10.1007/s11947-019-02332-9

33. Song P, Zhang X, Wang S, Xu W, Wang F, Fu R, et al. Microbial proteases and their applications. *Front Microbiol*. (2023) 14:1236368. doi: 10.3389/fmicb.2023.1236368

34. Sun X, Zheng J, Liu B, Huang Z, Chen F. Characteristics of the enzyme-induced release of bitter peptides from wheat gluten hydrolysates. *Front Nutr*. (2022) 9:1022257. doi: 10.3389/fnut.2022.1022257

35. Lei F, Hu C, Zhang N, He D. The specificity of an aminopeptidase affects its performance in hydrolyzing peanut protein isolate and zein. *LWT*. (2019) 102:37–44. doi: 10.1016/j.lwt.2018.10.041

36. Yu Z, Wang Y, Zhao W, Li J, Shuian D, Liu J. Identification of *Oncorhynchus mykiss* nebulin-derived peptides as bitter taste receptor TAS2R14 blockers by *in silico* screening and molecular docking. *Food Chem*. (2022) 368:130839. doi: 10.1016/j.foodchem.2021.130839

37. Martínez YB, Ferreira FV, Musumeci MA. L-glutamine-, peptidyl-and protein-glutaminases: structural features and applications in the food industry. *World J Microbiol Biotechnol*. (2022) 38:204. doi: 10.1007/s11274-022-03391-5

38. Wang W, Zhou X, Liu Y. Characterization and evaluation of umami taste: a review. *Trends Anal Chem*. (2020) 127:115876. doi: 10.1016/j.trac.2020.115876

39. Sakai K, Okada M, Yamaguchi S. Decolorization and detoxication of plant-based proteins using hydrogen peroxide and catalase. *Sci Rep*. (2022) 12:22432. doi: 10.1038/s41598-022-26883-8

40. Sakai K, Sato Y, Okada M, Yamaguchi S. Improved functional properties of meat analogs by laccase catalyzed protein and pectin crosslinks. *Sci Rep*. (2021) 11:16631. doi: 10.1038/s41598-021-96058-4

41. Sakai K, Sato Y, Okada M, Yamaguchi S. Synergistic effects of laccase and pectin on the color changes and functional properties of meat analogs containing beet red pigment. *Sci Rep*. (2022) 12:1168. doi: 10.1038/s41598-022-05091-4

42. ISO 8586:2023. Sensory analysis—selection and training of sensory assessors. Warsaw, Poland: The Polish Committee for Standardization (2023).

43. Yamamoto T, Inui-Yamamoto C. The flavor-enhancing action of glutamate and its mechanism involving the notion of kokumi. *npj Science of Food*. (2023) 7:3. doi: 10.1038/s41538-023-00178-2

44. ISO 5492:2008. Sensory analysis—vocabulary. Geneva, Switzerland: International Organization for Standardization (ISO) (2008).

45. Delompré T, Guichard E, Briand L, Salles C. Taste perception of nutrients found in nutritional supplements: a review. *Nutrients*. (2019) 11:2050. doi: 10.3390/nu11092050

46. Diepeveen J, Moerdijk-Poortvliet TCW, van der Leij FR. Molecular insights into human taste perception and umami tastants: a review. *J Food Sci*. (2022) 87:1449–65. doi: 10.1111/1750-3841.16101

47. Dai Z, Zheng W, Locasale JW. Amino acid variability, tradeoffs and optimality in human diet. *Nat Commun*. (2022) 13:6683. doi: 10.1038/s41467-022-34486-0

48. Shimomura Y, Murakami T, Nakai N, Nagasaki M, Harris RA. Exercise promotes BCAA catabolism: effects of BCAA supplementation on skeletal muscle during exercise. *J Nutr*. (2004) 134:1583S–7S. doi: 10.1093/jn/134.6.1583S

49. Brodkorb A, Egger L, Alminger M, Alvito P, Assunção R, Ballance S, et al. INFOGEST static *in vitro* simulation of gastrointestinal food digestion. *Nat Protoc*. (2019) 14:991–1014. doi: 10.1038/s41596-018-0119-1

50. Huang S, Wang LM, Sivendiran T, Bohrer BM. Review: amino acid concentration of high protein food products and an overview of the current methods used to determine protein quality. *Crit Rev Food Sci Nutr*. 58:2673–8. doi: 10.1080/10408398.2017.1396202

51. Wang Z, Cheng Y, Muhoza B, Sun M, Feng T, Yao L, et al. Discovery of peptides with saltiness-enhancing effects in enzymatic hydrolyzed *Agaricus bisporus* protein and evaluation of their salt-reduction property. *Food Res Int*. (2024) 177:113917. doi: 10.1016/j.foodres.2023.113917

52. Chen D, Chen W, Wu D, Zhang Z, Liu P, Li W, et al. Saltiness enhancing peptides isolated from enzymolysis extract of *Lentinula edodes* and their taste enhancing action mechanisms. *LWT*. (2023) 188:115430. doi: 10.1016/j.lwt.2023.115430

53. Sahni O, Didzbalis J, Munafo JP Jr. Saltiness enhancement through the synergism of pyroglutamyl peptides and organic acids. *J Agric Food Chem*. (2024) 72:625–33. doi: 10.1021/acs.jafc.3c05911

54. Nishimura T, Goto S, Miura K, Takakura Y, Egusa AS, Wakabayashi H. Umami compounds enhance the intensity of retronasal sensation of aromas from model chicken soups. *Food Chem*. (2016) 196:577–83. doi: 10.1016/j.foodchem.2015.09.036

55. Xie X, Dang Y, Pan D, Sun Y, Zhou C, He J, et al. The enhancement and mechanism of the perception of saltiness by umami peptide from *Ruditapes philippinarum* and ham. *Food Chem*. (2023) 405:134886. doi: 10.1016/j.foodchem.2022.134886

56. van der Klaauw NJ, Smith DV. Taste quality profiles for fifteen organic and inorganic salts. *Physiol Behav*. (1995) 58:295–306. doi: 10.1016/0031-9384(95)00056-O

57. Sinopoli DA, Lawless HT. Taste properties of potassium chloride alone and in mixtures with sodium chloride using a check all that apply method. *J Food Sci*. (2012) 77:S319–22. doi: 10.1111/j.1750-3841.2012.02862.x

58. Noguchi M, Yamashita M, Arai S, Fujimaki M. On the bitter-masking activity of a glutamic acid-rich oligopeptide fraction. *J Food Sci*. (1975) 40:367–9. doi: 10.1111/j.1365-2621.1975.tb02203.x

59. Kim MJ, Son HJ, Kim Y, Misaka T, Rhyu MR. Umami-bitter interactions: the suppression of bitterness by umami peptides via human bitter taste receptor. *Biochem Biophys Res Commun*. (2015) 456:586–90. doi: 10.1016/j.bbrc.2014.11.114

60. Rhyu MR, Kim Y, Misaka T. Suppression of hTAS2R16 signaling by umami substances. *Int J Mol Sci*. (2020) 21:7045. doi: 10.3390/ijms21197045

61. Kemp SE, Beauchamp GK. Flavor modification by sodium chloride and monosodium glutamate. *J Food Sci*. (1994) 59:682–6. doi: 10.1111/j.1365-2621.1994.tb05592.x

62. Wang S, Dermiki M, Methven L, Kennedy OB, Cheng Q. Interactions of umami with the four other basic tastes in equi-intense aqueous solutions. *Food Qual Prefer*. (2022) 98:104503. doi: 10.1016/j.foodqual.2021.104503

63. Torrico DD, Sae-Eaw A, Sriwattana S, Boeneke C, Prinyawiwatkul W. Oil-in-water emulsion exhibits bitterness-suppressing effects in a sensory threshold study. *J Food Sci*. (2015) 80:S1404–11. doi: 10.1111/1750-3841.12901

64. Ben Abu N, Harries D, Voet H, Niv MY. The taste of KCl—what a difference a sugar makes. *Food Chem*. (2018) 255:165–73. doi: 10.1016/j.foodchem.2018.01.175

65. Walker JC, Dando R. Sodium replacement with KCl and MSG: attitudes, perception and acceptance in reduced salt soups. *Foods*. (2023) 12:2063. doi: 10.3390/foods12102063

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Impact of inhibition of the renin-angiotensin system on early cardiac and renal abnormalities in Sprague Dawley rats fed short-term high fructose plus high salt diet

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Introduction: The combination of a high fructose and high salt diet typical of western diet induces high blood pressure, aortic stiffening, left ventricular (LV) diastolic dysfunction and impaired renal function in rodents. Despite an activated renin-angiotensin system (RAS) in rats fed high fructose and high salt, acute inhibition of the RAS pathway does not improve cardiac and vascular parameters. It may well be that longer term treatment is required to permit remodeling and improve cardiovascular function. Thus, we hypothesized that chronic RAS inhibition fructose+high salt-fed rats to restore blood pressure (BP) to levels similar to glucose plus normal salt-fed controls will improve cardiorenal function and histopathology.

Methods: Male and female Sprague Dawley rats monitored by hemodynamic telemetry were fed 0.4% NaCl chow during baseline, then changed to chow containing either 20% glucose+0.4% NaCl (G) or 20% fructose+4% NaCl (F) and treated with vehicle, enalapril (Enal, 4 mg/kg/d) or losartan (Los, 8 mg/kg/d) by osmotic minipump for 25–26 days.

Results: BP was elevated in the fructose+high salt groups of both sexes ($P < 0.05$) and restored to control levels by Enal or Los. Pulse wave velocity (PWV) was lower in female F+Los rats and cardiac output higher in female F+Enal rats. GFR was not changed by diet or treatment. Fructose+high salt groups of both sexes displayed higher albuminuria that was decreased by Enal in male rats. Cardiac fibrosis and mesangial hypercellularity were greater in fructose+high salt-fed rats of both sexes and improved with either Los or Enal.

Discussion: Thus, inhibition of the RAS improves early changes in cardiac and renal histopathology in both sexes and albuminuria in male rats fed high fructose and high salt diet. Functional improvements in cardiorenal parameters may require longer treatment.

KEYWORDS

albuminuria, cardiac fibrosis, renal function, fructose, salt, renin angiotensin system

1 Introduction

The presence of cardiac and renal dysfunction is a worldwide phenomenon observed in the United State, Europe, Asia and Africa (1–3). The close interaction between cardiac and renal function has been recognized for more than 200 years (4, 5). The heart and kidneys play crucial roles in the regulation of blood volume, blood pressure and tissue perfusion. For example, the prevalence of left ventricular hypertrophy and diastolic dysfunction is increased in individuals with chronic kidney disease as is the risk of a major adverse cardiovascular event or mortality (6–8). Conversely, up to one-third of individuals with heart failure develop worsening renal function (9, 10) with the greatest risk observed in individuals with heart failure and preserved ejection fraction (11). Often it is unclear which organ was affected first in cardiorenal disease patients (2), and it is altogether possible that cardiac and renal damage occur concurrently due to a common etiology (12).

Traditional risk factors such as diabetes, obesity, smoking and hypertension account for only approximately 40% of individuals with cardiac and renal disease (13, 14) particularly in individuals with reduced access to nutritious food due to socioeconomic stressors (15). Neurohormonal dysregulation, abnormal metabolism and oxidative stress may all contribute to worsening cardiac and renal function (14, 16). Studies suggest that the increase in consumption of fructose, spurred by the use of high-fructose corn syrup in beverages and processed foods, together with high salt intake strongly contributes to the development of cardiometabolic and renal disease (17, 18). Preclinical data in rats indicate that the concurrent ingestion of fructose limits urinary sodium excretion and raises blood pressure. Thus, fructose intake can induce salt sensitivity of blood pressure (19–21). Notably, salt sensitivity of blood pressure is associated with increased cardiovascular mortality even in normotensive individuals (22).

Arterial stiffness (23–25) and insulin resistance (26, 27) are recognized as independent risk factors for the development of cardiovascular and renal disease in humans. Male rats ingesting 20% fructose (w/v) in the drinking water and 4% NaCl in the rat chow for 3 weeks display normal serum glucose levels but exhibit insulin resistance, elevated blood pressure, reduced aortic compliance and elevated pulse wave velocity (PWV), whereas blood pressure, aortic compliance and left ventricular function are not altered in fructose and high salt-fed female rats (28–30). In a murine model, ingestion of much higher fructose (60% w/v) for 12 weeks induces left ventricular hypertrophy and cardiac fibrosis (31) thereby impairing cardiac function (32). Limited data are available on renal histopathology, but renal mesangial cell hyperplasia has been demonstrated with a fructose-rich diet (33). In Dahl salt-sensitive rats, fructose (60% w/v) diet for 12 weeks results in glomerular sclerosis, afferent arteriolar thickening and interstitial fibrosis (34). Glomerular filtration rate (GFR) is significantly lower and urine albumin excretion is higher in male rats ingesting rat chow with 20% caloric intake as fructose plus 4% NaCl for 12 weeks compared with rats on a 20% glucose with 0.4% NaCl diet.

Fructose-fed male rats exhibit higher plasma renin activity, hepatic angiotensinogen and plasma angiotensin II levels (Ang II) (35). Expression of both angiotensin converting enzyme (ACE) and angiotensin type 1 receptor (AT_1R) mRNA is upregulated in cardiac and aortic tissues with high fructose feeding in male but not female rats (36). Concurrent intake of high fructose and high salt diet augments renal sympathetic nerve activity (28) and prevents the suppression of renin despite higher arterial pressure and expanded extracellular volume (20). Renal denervation normalizes blood pressure, decreases renin and Ang II, and improves insulin resistance (28). Acute administration of the central sympathetic inhibitor clonidine improves aortic stiffness in the male rats. Despite a decline in arterial pressure comparable to that observed with clonidine, acute blockade of the renin-angiotensin system (RAS) with either losartan or enalapril does not improve PWV (30). It is not known if inhibition of the RAS system requires a more prolonged treatment to prevent cardiac, vascular and/or renal histopathology and function. Moreover, there are scant data on the functional impact of fructose and high salt diet on cardiorenal parameters in female rats.

Thus, we hypothesized that interruption of RAS activity for 3 weeks by either inhibition of ACE or blockade of AT_1R in male rats fed a high fructose, high salt diet such that MAP will be brought to a level identical to that of control glucose plus normal salt-fed Sprague Dawley rats will improve histopathological indices of cardiac fibrosis and mesangial hypercellularity as well as ameliorate left ventricular diastolic function, PWV, insulin sensitivity, GFR and albuminuria. We further hypothesize the high fructose plus high salt diet will result in little or no deleterious effects on cardiac, vascular or renal parameters in female rats.

2 Experimental design and methods

2.1 Animals

All rats were cared for in compliance with the principles of the National Research Council Committee *Guide for the Care and Use of Laboratory Animals*. The Wayne State University Institutional Animal Care and Use Committee approved the protocol and all amendments of the animal research plan. Experiments were performed on male and female Sprague Dawley rats (Envigo, Indianapolis, IN) that were 12 ± 1 week-old. Rats were permitted to acclimate for 48 h under controlled housing conditions (21–23°C; 06:00–18:00 light cycle; 18:00:06:00 dark cycle). They were permitted *ad libitum* access to standard rat chow containing 0.49% NaCl and water (Envigo Teklad, Indianapolis, IN) until initiated into the experimental protocol. Since our previous work demonstrated similar PWV and left ventricular diastolic function in glucose plus high salt or fructose plus normal salt diets compared with control glucose plus normal salt, we compared glucose normal salt rats (control group) with fructose high salt rats either with or without pharmacological treatment in accordance with the principle of reducing the number of animals used.

2.2 Protocols

The overall protocol is illustrated in Figure 1 (also see Supplementary Figure S1). After the two-day acclimation, rats underwent surgical placement of hemodynamic radiotransmitters (see below for all surgical procedure details) and permitted to recover, then baseline arterial pressures and heart rate were recorded for 6 days during which all rats were on *ad libitum* water plus 20% glucose plus 0.4% NaCl chow (TestDiet #1817915-209, Richmond, IN). Rats were

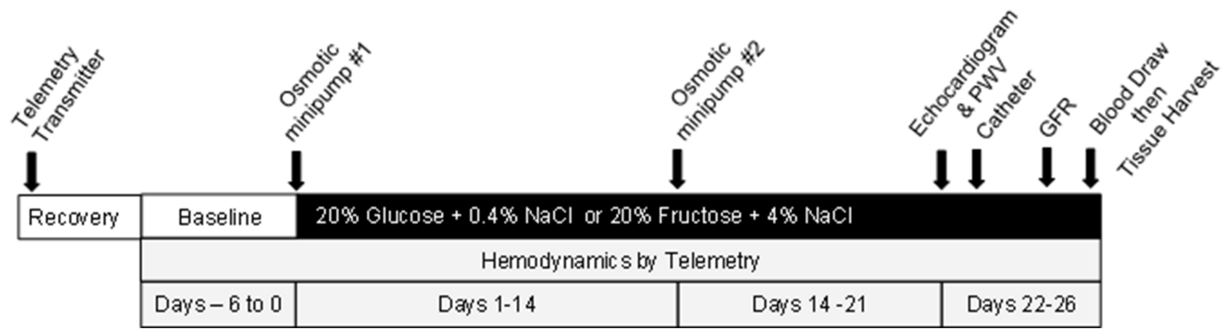

FIGURE 1
Schematic of methodology of this study.

then anesthetized and an osmotic minipump (Alzet 2002, Cupertino, CA) was inserted subcutaneously under isoflurane anesthesia to deliver either vehicle or the drug at a dose to bring MAP to a level as close to identical to that of the glucose-fed control group (37, 38). Due to different vehicle treatments required, two separate sets of rats were studied. Set one included the following: 20% glucose and 0.4% NaCl chow plus minipump to deliver isotonic saline vehicle (G + V), 20% fructose and 4% NaCl plus isotonic saline vehicle (F + V); and 20% fructose and 4% NaCl plus minipump delivering losartan 8 mg/kg/day (F + Los). Set two included 20% glucose and 0.4% NaCl chow plus isotonic saline: dimethylsulfoxide (DMSO) at a 1:1 proportion (G + V + D), 20% fructose and 4% NaCl plus saline: DMSO (F + V + D), and 20% fructose and 4% NaCl diet plus minipump to deliver enalapril 4 mg/kg/day (F + Enal) (TestDiet #1818296-209, Richmond, IN). The nutritional composition of the experimental diets are presented in Supplementary Tables S1, S2. Based on our previous study showing improvement in cardiovascular parameters with clonidine, we initially incorporated a third set of rats with clonidine 0.4 mg/kg/day (39) in the design but needed to eliminate this group due to complications resulting from diminished bowel motility (40). Vehicle or drug was administered over the course of (25–26 days). All chemicals and drugs were obtained from Millipore Sigma (Milwaukee, WI). Injection solutions were sterilized by passage through a Millex 0.22 mm filter (Millipore Sigma).

On day 21, each rat underwent echocardiography and evaluation of PWV under 2.5% isoflurane anesthesia. The next day, a vascular catheter was placed into the left carotid artery under ketamine 80 mg/kg and xylazine 10 mg/kg i.p. anesthesia for subsequent blood draws. Three days later, glomerular filtration rate (GFR) was assessed transdermally using FITC-sinistrin (MediBeacon, St. Louis, MO). One or two days later, blood was obtained from conscious rats for measurement of glucose and insulin. Immediately thereafter, they were anesthetized with sodium pentobarbital 40 mg/kg i.p. Urine was obtained directly from the bladder for subsequent assessment of albumin, and heart, aorta, and kidney were harvested and fixed in 10% formalin for histologic evaluation.

2.3 Surgical procedures

All rats received preemptive analgesia with Ethiqa 0.65 mg/kg s.c. (Fidelis, New Brunswick, NJ) prior to each surgical procedure.

2.3.1 Telemetry transmitter placement

Rats were anesthetized with ketamine/xyalazine as noted above. Briefly, the right inguinal area was prepped, the femoral artery was isolated and the gel-filled catheter of the hemodynamic transmitter (HD-S10, Data Sci. Intl., New Brighton, MN) was inserted and then advanced into the iliac and terminal aorta. The catheter was secured with 3-0 silk sutures (Ethicon, Johnson & Johnson, New Brunswick, NJ). The body of the transmitter was tunneled subcutaneously and stabilized with tissue adhesive (Vetbond, 3 M, Neuss, Germany). The incision was closed with 3-0 nylon sutures (Ethicon).

2.3.2 Osmotic minipump placement

Rats were anesthetized with 3% isoflurane in an induction chamber followed by 2% isoflurane by nose cone. They were placed in prone position on a heated platform. An osmotic minipump (Alzet 2002, Cupertino, CA) was inserted subcutaneously via a 1 cm incision in the dorsal area just to the left of the vertebrae and the incision was sutured. This osmotic pump was designed to deliver 0.5 mL/h for 14 days and the pump was primed to initiate infusion prior to insertion by incubating in saline at 37°C for 4 h prior to placement. On day 14, a second, identical osmotic minipump was inserted following the same procedures but placed to the right of the vertebral column. We changed to the two-pump protocol for all animals reported herein upon the recommendation of our veterinarian.

2.3.3 Vascular catheter placement

After ketamine/xylazine anesthesia, the left carotid artery was exposed, and the catheter was inserted, secured with 3-0 silk suture then tunneled subcutaneously and exteriorized at the base of the neck posteriorly as previously described (41).

2.3.4 Echocardiography

The same investigator (DK), blinded to the group assignment of the animals, performed all echocardiograms and PWV (Vevo 3100, Visual Sonics, Bothell, WA) by methods previous reported from Komnenov et al. (30). Rats were anesthetized with 3% in the induction chamber then maintained at 1.5% isoflurane by inhalation via nose cone. Rats were supine on a heated platform with all four limbs secured to the ECG electrodes by adhesive tape. Fur overlying the thorax was shaved and followed by applying a depilatory cream for 30 s (Nair, Pharmaceutical Innovations). Body temperature was monitored by rectal probe and maintained at 37°C. Echocardiographic recordings were performed using

standard techniques. Images were acquired via the MX-250S transducer to obtain a parasternal short axis view in M-mode at the level of the papillary muscles to ascertain systolic function and left ventricular dimensions. Left ventricular diastolic function was assessed using pulse wave Doppler recordings of transmitral flow velocities aligned in the apical four chamber view. Aortic PWV was determined using B-mod and pulse wave Doppler of aortic flow velocities at two positions along the aortic arch. The distance between the two positions was determined from the B-mode image. The distance was then divided by the transit time from position one to position two calculated using the ECG tracing as a reference.

2.3.5 Glomerular filtration rate

GFR was measured using bolus injection of fluorescein-isothiocyanate (FITC)-sinistrin as described by Schock-Kusch et al. (42) and previously used by our laboratory (19). Rats were briefly anesthetized with 3% isoflurane followed by 1.5% isoflurane. A small area of skin ~1.5 cm diameter was shaved and remaining fur was removed with a depilatory cream as described above. The LED-emitting optical transducer attached to an adhesive patch was positioned on the skin. Baseline measurements were recorded for about 3 min. Then, FITC-sinistrin, 5 mg/100 mg BW (Fresenius-Kabi, Lake Zurich, IL) was injected via catheter or tail vein and the animal was permitted to awaken. Recordings were taken for 2 h. FITC-sinistrin disappearance kinetics were analyzed over the first 90 min after injection via a three-compartment model. GFR was calculated using MB Studio software (MediBeacon, St. Louis, MO) as previously described (19, 42).

2.4 Terminal procedures

Eighteen to twenty-four hours after completion of GFR measurements, blood samples were obtained in conscious rats between 09:00–10:00. Glucose levels were measured on ~50 mL whole blood using a One-Touch Ultra glucose monitor (LifeScan, Inc., Malvern, PA) collected directly from the arterial catheter. For insulin assay, 1 mL blood was collected directly from the catheter into prechilled tubes containing 50 mL ethylenediamine tetra-acetic acid, immediately centrifuged at 3,000 rpm for 10 min at 4°C. Plasma was stored at −70°C until assay.

Rats were euthanized with sodium pentobarbital 120 mg/kg i.v. Urine was obtained directly by aspirating from the bladder. Hearts and kidneys were harvested, maintained in ice-cold saline, and weighed. Urine as well as samples of heart and kidney tissue were frozen and stored at −70°C. The remainder of heart and kidney tissue was placed into 10% formalin for 48 h and processed for paraffin embedding.

2.5 Histologic analyses

Ten micrometer sections of kidney were stained with periodic acid Schiff (PAS) according to the manufacturer's directions (Newcomer Supply, Middleton, WI). Morphometric analysis of glomerular mesangial cellularity was performed in accordance with the Oxford classification (43). Forty glomeruli were analyzed per rat. Briefly, mesangial cellularity was scored as follows: normal <4 mesangial cells/mesangial area, mild 4–5 mesangial cells/mesangial area, moderate 6–7 mesangial cells/mesangial area, and severe ≥8 mesangial cells/mesangial area. The score for a given glomerulus was taken as that of the most cellular area for that glomerulus. The region at the vascular pole was not included in the analyses.

Cardiac tissue was stained with picrosirius red per manufacturer's recommendations (Polysciences, Inc., Warrington, PA). Cardiac fibrosis was determined using ImageJ as the percentage of the area of picrosirius red staining vs. total tissue area of the left ventricle. All morphometric analyses were performed by a researcher blinded to the group assignment.

2.6 Hormonal and chemical assays

Plasma insulin was determined using rat insulin ELISA kit (Life Technologies-Invitrogen, Waltham, MA). Urine albumin was measured by albumin ELISA assay (Abnova, Walnut, CA). Creatinine was evaluated via standard colorimetric assay (Cayman, Ann Arbor, MI).

2.7 Statistical analyses

All data are presented as mean ± SE. The number of animals per group in the study ($n = 6$) was determined to achieve a power of 90% at an α-level of 0.05 to identify a difference of 100 mm/s in PWV with a standard deviation of 40 mm/s based on values from previous studies with this model (19, 30). Comparisons between baseline and post-treatment body weight in the same animal were performed by paired *t*-test. Comparisons among groups was accomplished by one-way ANOVA for multiple comparisons using Holm-Sidak *post hoc* analysis. Not all animals had sufficient urine in the bladder for collection. When *n* values deviate from the original assignment, the reason for missingness is provided and values imputed as the mean. A p-value <0.05 was considered significant.

Except for comparisons of body weight and organ weights at the beginning and the end of the study which were assessed by two-way ANOVA, the present study was not designed or powered to evaluate differences between sexes, but rather to assess the impact of RAS inhibition on fructose plus high salt on cardiovascular and renal parameters within a given sex.

3 Results

3.1 Metabolic and humoral parameters

Initial body weights of rats of the same age were significantly lower in female vs. male rats in each of the groups ($p < 0.01$) (Table 1). The difference between male and female rat weights was maintained throughout the study such that final body weights also differed significantly between male and female rats receiving the same treatment ($p < 0.01$). The final body weights of both male and female rats differed significantly from the initial body weight on each of the dietary regimens, but there was no difference between the weights of control glucose plus normal salt vs. fructose plus high salt-fed rats of either sex. Kidney and heart weights were similar among rats of the same sex and protocol, but kidney weights were significantly lower in each of the female vs. male rats within the same treatment group ($p < 0.01$). Non-fasting glucose did not differ among the groups. Plasma insulin was significantly lower in the male F + V group compared with the male G + V group. As well as the insulin was

TABLE 1 Baseline and final body weights, heart and kidney weights, blood glucose, insulin and glucose:insulin ratio in glucose + normal salt and fructose + high salt fed rats in losartan and enalapril treated groups of both sexes.

	n	Initial body weight (grams)	Final body weight (grams)	Both kidneys weight (grams)	Heart weight (grams)	Non-fasting glucose (mg/dL)	Insulin (μIU/mL)	Glucose: insulin ratio (nM/ nM × 10^6)
Males								
G + V	6	264.3 ± 9.1	308.3 ± 14.2†	1.953 ± 0.056	1.203 ± 0.127	165 ± 21	25.9 ± 2.9	60.9 ± 9.7
F + V	6	256.8 ± 4.4	302.2 ± 11.8†	2.013 ± 0.062	1.110 ± 0.057	136 ± 6	19.0 ± 0.9*	69.2 ± 8.1
F + Los	6	244.2 ± 7.9	302.0 ± 8.9†	2.232 ± 0.208	1.091 ± 0.030	140 ± 13	19.9 ± 2.5	55.4 ± 12.5
G + V + D	6	252.2 ± 10.6	319.5 ± 15.0†	1.960 ± 0.159	1.171 ± 0.048	132 ± 13	23.5 ± 1.4	49.5 ± 3.0
F + V + D	6	248.7 ± 8.9	305.8 ± 8.5†	2.118 ± 0.041	1.152 ± 0.029	124 ± 12	14.2 ± 1.6	72.5 ± 5.6*
F + Enal	6	254.5 ± 7.0	303.2 ± 5.3†	2.084 ± 0.164	1.102 ± 0.084	147 ± 12	29.8 ± 5.0	57.2 ± 4.5
Females								
G + V	6	234.3 ± 3.9	256.0 ± 5.2$^{\dagger\#}$	1.571 ± 0.059$^{\#}$	0.991 ± 0.041	138 ± 8	14.8 ± 2.5	85.1 ± 14.1
F + V	6	233.2 ± 6.4	250.2 ± 5.9$^{\dagger\#}$	1.689 ± 0.090$^{\#}$	0.954 ± 0.039	129 ± 7	20.6 ± 1.5	68.9 ± 3.0
F + Los	6	230.0 ± 5.1	254.8 ± 6.1$^{\dagger\#}$	1.594 ± 0.070$^{\#}$	0.953 ± 0.046	156 ± 13	16.9 ± 0.6	86.6 ± 9.6
G + V + D	6	240.0 ± 3.1	249.7 ± 5.6$^{\dagger\#}$	1.560 ± 0.059$^{\#}$	1.001 ± 0.030	142 ± 14	16.4 ± 1.9	81.6 ± 6.0
F + V + D	6	227.2 ± 2.0	241.8 ± 6.6$^{\dagger\#}$	1.564 ± 0.073$^{\#}$	1.009 ± 0.032	149 ± 16	23.7 ± 4.4	68.2 ± 12.6
F + Enal	6	230.0 ± 4.1	251.8 ± 3.8$^{\dagger\#}$	1.655 ± 0.077$^{\#}$	0.930 ± 0.038	159 ± 11	18.8 ± 3.3	88.2 ± 13.7

G + V, 20% glucose + 0.4% NaCl, saline vehicle treated; F + V, 20% fructose + 4% NaCl, saline vehicle; F + Los, 20% fructose + 4% NaCl, losartan 8 mg/kg/day; G + V + D, 20% glucose + 0.4% NaCl, saline:DMSO 1:1 vehicle; F + V + D, 20% fructose + 4% NaCl, saline:DMSO 1:1 vehicle; F + Enal, 20% fructose + 4% NaCl, enalapril 4 mg/kg/day. Values are mean ± SE. *$p < 0.05$ vs. G + V same sex; $^{\dagger}p < 0.05$ vs. baseline. $^{\S}p < 0.05$ vs. F + V + D same sex; $^{\#}p < 0.01$ vs. same dietary group, male rats.

TABLE 2 Baseline and post-treatment systolic and diastolic blood pressures and heart rates glucose + normal-salt and fructose + high salt fed rats rats in losartan and enalapril treated groups of both sexes.

	n	Baseline SBP (mmHg)	Post-treatment SBP (mmHg)	Baseline DBP (mmHg)	Post-treatment DBP (mmHg)	Baseline HR (bpm)	Post-treatment HR (bpm)
Males							
G + V	6	129.5 ± 2.3	124.4 ± 2.4	92.8 ± 1.8	94.4 ± 3.1	381 ± 8	371 ± 5
F + V	6	134.0 ± 3.2	138.9 ± 2.7*	91.0 ± 1.5	99.2 ± 2.3	393 ± 10	367 ± 3
F + Los	6	131.7 ± 1.8	125.1 ± 2.8§	89.9 ± 1.8	93.7 ± 3.0	400 ± 10	371 ± 5
G + V + D	6	131.2 ± 12.4	134.0 ± 3.2	90.5 ± 1.7	92.0 ± 1.8	394 ± 8	375 ± 4
F + V + D	6	129.7 ± 2.6	146.4 ± 4.9*†	93.4 ± 2.2	104.0 ± 3.7*†	387 ± 6	370 ± 3
F + Enal	6	136.4 ± 1.9	133.6 ± 1.6§	92.1 ± 1.2	89.8 ± 1.1§	383 ± 8	375 ± 8
Females							
G + V	6	128.1 ± 2.4	127.7 ± 3.8	88.7 ± 1.5	90.4 ± 2.4	406 ± 5	384 ± 10
F + V	6	130.4 ± 2.1	142.7 ± 5.4*†	89.7 ± 1.5	97.4 ± 2.9	390 ± 8	344 ± 7†
F + Los	6	134.8 ± 1.8	128.7 ± 1.8§	91.0 ± 2.0	84.1 ± 1.3§	411 ± 8	395 ± 5
G + V + D	6	129.5 ± 1.6	132.6 ± 2.4	89.3 ± 1.4	89.8 ± 1.6	390 ± 9	382 ± 5
F + V + D	6	129.9 ± 2.3	134.7 ± 2.9	90.7 ± 1.7	91.5 ± 2.2	386 ± 8	373 ± 2
F + Enal	6	133.3 ± 1.5	121.6 ± 2.6*§	91.9 ± 1.9	80.8 ± 2.2*§	396 ± 6	384 ± 4

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. Other abbreviations as in Table 1. Values a mean ± SE. *$p < 0.05$ vs. glucose-fed same group and sex; $^{\dagger}p < 0.05$ vs. baseline; $p < 0.05$ vs. fructose-fed vehicle-treated, same group and sex; $^{\S}p < 0.05$ vs. F + V + D and F + V same group and sex.

significantly higher in the male F + Enal (29.8 ± 5.0 mIU/mL) compared with F + V + D male rats (14.2 ± 1.6 mIU/mL) ($p < 0.05$). The insulin levels of female rats did not differ among the dietary groups. The plasma glucose:insulin ratio was significantly higher in the F + V + D group compared with the G + V + D group in male rats, but not differences were observed in the female rats (Table 1).

3.2 Systemic blood pressure and heart rate

Vehicle-treated fructose plus high salt-fed male rats displayed significantly higher systolic blood pressures than their respective glucose plus normal salt control group (Table 2). Systolic blood pressure in the female F + V group was significantly greater than its G + V

control, but the female F + V + D rats did not exhibit higher systolic pressure compared with the female G + V + D group. Figure 2 shows that the same pattern emerged for MAP in both male and female rats. MAP in the male F + V group was higher than the G + V group but did not achieve significance ($p < 0.065$). Diastolic blood pressure was elevated only in male F + V + D rats. As per experimental design, treatment with either losartan or enalapril restored systolic and MAP to levels no different from G + V or G + V + D groups in both male and female rats with the exception of female rats treated with enalapril (F + Enal) where both systolic and MAP were significantly lower than either G + V + D or F + V + D groups (Figure 2). Diastolic blood pressure was significantly lower in male F + Enal rats and female F + Los rats compared with their respective fructose-fed groups. Diastolic blood pressure in the female F + Enal group was lower than either female G + V + D or F + D + V rats. Heart rates did not differ among either male or female groups except for lower heart rate in F + V vs. G + V female rats (Table 2).

3.3 Cardiac echocardiography and histopathology

Table 3 depicts the results of echocardiography of LV at the end of the experiment. LV parameters are not significantly different among the groups in either male or female rats except for the cardiac output being significantly higher in the F + Enal group compared with the F + V + D (44.99 ± 3.63 mL/min) group in female rats. LV ejection fraction (Table 3); however, LV ejection fraction was similar across all treatments and in both sexes (Figures 3C,D,G,H). Losartan significantly decreased aortic PWV compared with G + V female rats (Figure 3E). Otherwise, PWV was not altered by 3 weeks of fructose plus high salt feeding across all groups. Losartan and enalapril exerted no effect on PWV in any of the other three groups (Figures 3A,B,F).

Picrosirius red staining as an index of collagen in LV tissue is shown in Figure 3. Fructose plus high salt diet markedly increased interstitial and perivascular fibrosis in vehicle-treated rats of both sexes and was significantly attenuated by AT_1R blockade (Figures 4A–D). ACE inhibition with enalapril significantly reduced LV collagen deposition in fructose-high salt-fed female rats (Figures 4C,D) but not male rats (Figures 4A,B).

3.4 Glomerular filtration rate and glomerular mesangial morphometry

GFR, assessed as the kinetics of the disappearance of FITC-sinistrin (Figures 5A–D), was significantly higher in male

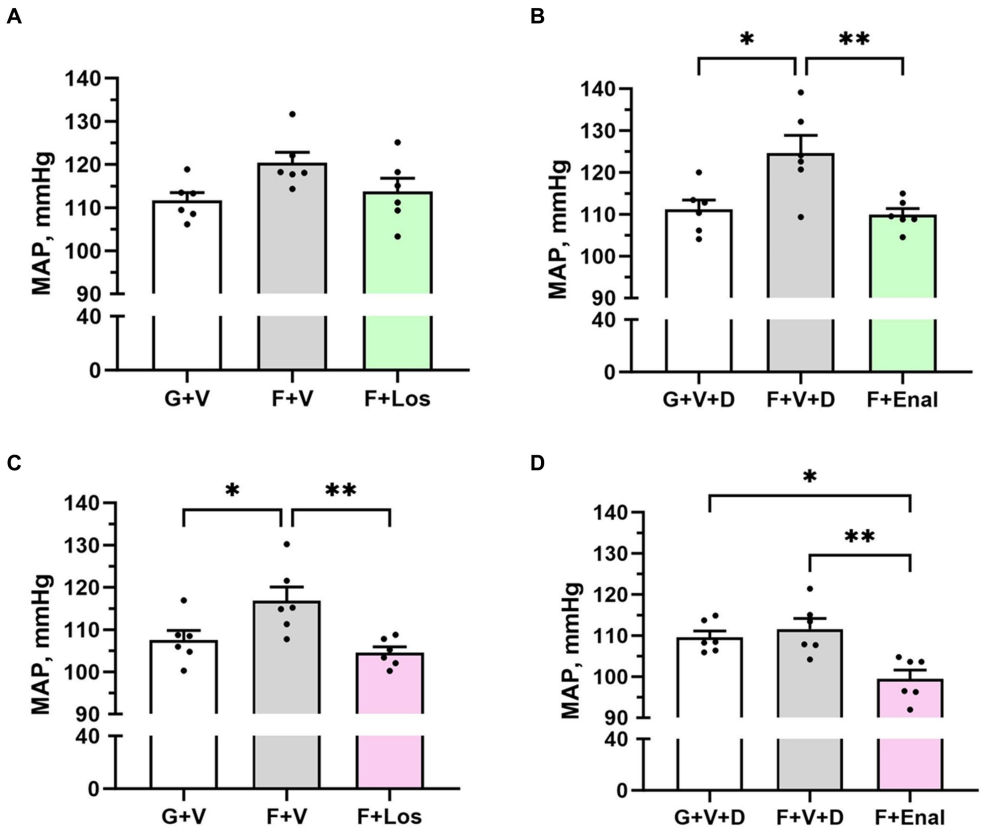

FIGURE 2
Mean arterial pressure (MAP) in male **(A,B)** and female **(C,D)** rats fed for 3 weeks on 20% glucose plus 0.4% NaCl (G) or 20% fructose plus 4% NaCl chow (F) and concurrently treated with either saline vehicle (V) or losartan (Los, 8 mg/kg/day s.c.): G + V, F + V, and F + Los; or saline:DMSO vehicle (V + D) or enalapril (Enal, 4 mg/kg/day s.c.): G + V + D, F + V + D, and F + Enal by osmotic minipump. Values are mean ± SE; $n = 6$ for each group. *$p < 0.05$ and **$p < 0.01$ by ANOVA.

TABLE 3 Echocardiographic parameters at the end of treatments in male and female glucose + normal salt and fructose + high salt-fed rats in losartan and enalapril treated groups of both sexes.

	Groups					
	G + V	F + V	F + Los	G + V + D	F + V + D	F + Enal
Males						
E/A	1.21 ± 0.12	1.15 ± 0.17	1.33 ± 0.06	1.18 ± 0.05	1.16 ± 0.06	1.11 ± 0.03
HR (BPM)	353 ± 13.5	361 ± 12.05	343 ± 10.17	342 ± 8.38	326 ± 9.99	357 ± 13.77
$LVID_S$ (mm)	3.86 ± 0.2	3.21 ± 0.2	3.47 ± 0.39	3.44 ± 0.32	3.79 ± 0.2	3.24 ± 0.2
$LVID_D$ (mm)	6.74 ± 0.3	5.95 ± 0.14	6.34 ± 0.24	6.70 ± 0.19	6.46 ± 0.17	6.29 ± 0.24
Fraction shortening (%)	42.9 ± 1.5	46.2 ± 2.54	45.7 ± 4.85	48.96 ± 3.47	41.46 ± 1.93	48.35 ± 2.76
Cardiac output (mL/min)	60.75 ± 5	48.9 ± 2.05	51.91 ± 4.07	61.95 ± 2.82	49.35 ± 2.41	57.17 ± 6.55
LV mass (mg)	632.6 ± 58	641.9 ± 28.6	632.2 ± 29.4	679.4 ± 35.2	639.5 ± 47.0	622.7 ± 70
$LVAW_S$ (mm)	2.72 ± 0.1	3.06 ± 0.1	2.90 ± 0.24	3.14 ± 0.1	2.97 ± 0.12	3.05 ± 0.1
$LVAW_D$ (mm)	1.77 ± 0.1	1.93 ± 0.1	1.98 ± 0.13	1.87 ± 0.12	1.88 ± 0.12	1.91 ± 0.08
$LVPW_S$ (mm)	2.66 ± 0.08	3.01 ± 0.17	2.67 ± 0.1	2.83 ± 0.2	2.57 ± 0.18	2.67 ± 0.31
$LVPW_D$ (mm)	1.64 ± 0.07	2.01 ± 0.12	1.69 ± 0.06	1.76 ± 0.09	1.73 ± 0.1	1.68 ± 0.12
Females						
E/A	1.26 ± 0.07	1.16 ± 0.08	1.40 ± 0.08	1.23 ± 0.1	1.27 ± 0.05	1.15 ± 0.09
HR (BPM)	347 ± 10.4	331 ± 9.63	334 ± 10.85	348 ± 9.48	337 ± 5.27	349 ± 5.82
$LVID_S$ (BPM)	3.28 ± 0.24	3.76 ± 0.31	3.27 ± 0.4	3.15 ± 0.26	3.60 ± 0.39	3.91 ± 0.22
$LVID_D$	6.28 ± 0.3	6.58 ± 0.25	5.98 ± 0.41	6.10 ± 0.19	6.13 ± 0.3	6.79 ± 0.22
Fraction shortening (%)	47.89 ± 2.1	43.24 ± 2.83	46.02 ± 3.57	48.54 ± 3.51	41.87 ± 3.52	42.56 ± 1.75
Cardiac output (mL/min)	54.38 ± 4.5	53.13 ± 2.33	45.3 ± 5.46	50.75 ± 2.83	44.99 ± 3.63	60.09 ± 2.93*
LV mass (mg)	514.3 ± 35	598.7 ± 38.1	553.2 ± 61.3	519.5 ± 29.5	555.5 ± 38.2	566.9 ± 45.1
$LVAW_S$ (mm)	2.80 ± 0.08	2.64 ± 0.09	2.69 ± 0.01	2.88 ± 0.07	2.69 ± 0.08	2.56 ± 0.1
$LVAW_D$ (mm)	1.70 ± 0.07	1.68 ± 0.04	1.76 ± 0.07	1.77 ± 0.05	1.71 ± 0.05	1.63 ± 0.04
$LVPW_S$ (mm)	2.57 ± 0.13	2.71 ± 0.09	2.70 ± 0.12	2.65 ± 0.1	2.58 ± 0.09	2.62 ± 0.13
$LVPW_D$ (mm)	1.51 ± 0.18	1.71 ± 0.12	1.78 ± 0.2	1.56 ± 0.08	1.76 ± 0.1	1.51 ± 0.08

E/A, ratio of early to late phase ventricular filling; HR, heart rate; CO, cardiac output; $LVID_S$, left ventricular systolic internal diameter; $LVID_D$, left ventricular internal diastolic internal diameter; LV mass, left ventricular mass; $LVAW_S$, left ventricular systolic anterior wall width; $LVAW_D$, left ventricular diastolic anterior wall width; $LVPW_S$, left ventricular systolic posterior wall width; $LVPW_D$, left ventricular diastolic posterior wall width. Values are mean ± SE; $n = 6$ for each group. $p < 0.05$ vs. F + V + D.

vehicle-treated fructose plus high salt rats compared with glucose plus normal salt rats (Figure 5B). Female rats in the F + Los group displayed significantly greater GFR than G + V rats (Figure 5C). Enalapril treatment in females did not achieve significance due to one rat with low GFR (Figure 5D).

Representative renal histology for male and female rats is shown in Figures 6A,C, respectively. Quantitative mesangial morphometry is depicted in Figures 6B,D. Fructose plus high salt diet resulted in a greater proportion of glomeruli exhibiting mesangial proliferation in both males and females but was more evident in male rats (Figures 6B,C). In male rats, both losartan and enalapril decreased mesangial hypercellularity similar to that in control glucose-fed rats (Figure 6B). In contrast, enalapril but not losartan decreased mesangial hypercellularity in female fructose plus high salt-fed rats (Figure 6D).

3.5 Urine albumin and creatinine ratio

Albuminuria tended to be higher in the fructose plus high salt-fed groups but achieved statistical significance only in the male F + V + D and female F + V groups compared with their respective glucose-fed counterparts. Urine albumin:creatinine ratio was higher in female F + V + D vs. G + V + D but did not achieve significance ($p < 0.075$). If both control groups and F + V and F + V + D groups are combined, rats of both sexes display significantly higher albumin:creatinine ratios with fructose plus high salt diet: 67.7 ± 12.3 vs. 121.6 ± 17.8 μg/mg [males (G + V and G + V + D) vs. (F + V and F + V + D), $p < 0.02$]; 52.7 ± 12.3 vs. 122.9 ± 12.7 μg/mg [females (G + V and G + V + D) vs. (F + V and F + V + D), $p < 0.01$]. Enalapril significantly diminished proteinuria in male rats fed a high fructose plus high salt diet (Figure 7).

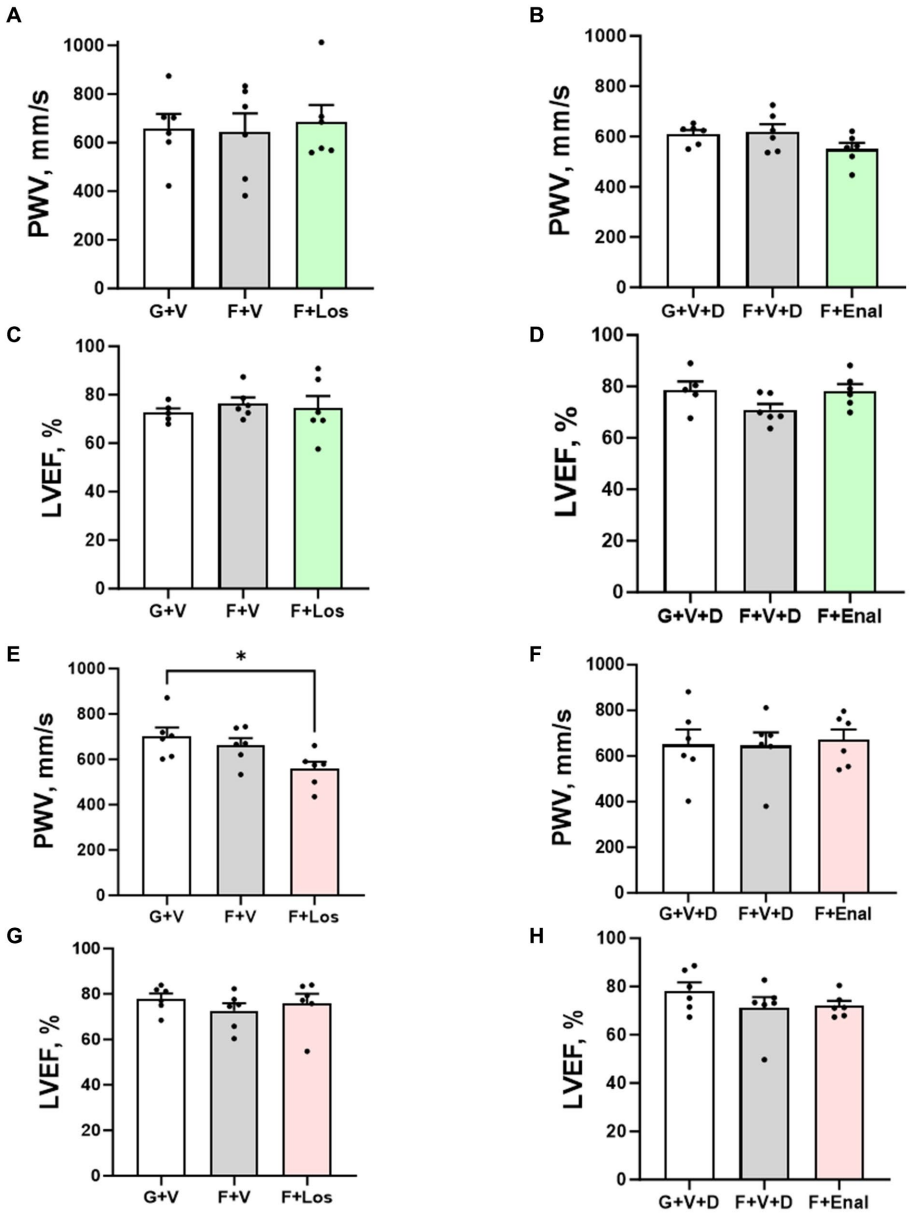

FIGURE 3
Aortic pulse wave velocity (PWV) and left ventricular ejection fraction (LVEF) in male **(A–D)** and female **(E–H)** rats fed for 3 weeks on 20% glucose plus 0.4% NaCl (G) or 20% fructose plus 4% NaCl chow (F) and concurrently treated with either saline vehicle (V) or losartan (Los, 8 mg/kg/day s.c.): G + V, F + V, and F + Los; or saline:DMSO vehicle (V + D) or enalapril (Enal, 4 mg/kg/day s.c.): G + V + D, F + V + D, and F + Enal by osmotic minipump. Values are mean ± SE; $n = 6$ for each group. *$p < 0.05$ by ANOVA.

4 Discussion

In this study, we hypothesized that salt-sensitive blood pressure and cardiorenal parameters induced by high fructose and high salt diet in Sprague Dawley rats would improve with RAS inhibition designed to restore arterial pressure to levels similar to control rats fed glucose and normal salt diet. The elevated mean arterial pressures in both male and female rats fed fructose and high salt are largely due to increases in systolic blood pressure except in fructose-fed male rats given the vehicle that contained DMSO where both systolic and diastolic pressures were elevated. The major findings of this study are that AT_1R antagonism but not ACE inhibition decreases aortic stiffness, whereas ACE inhibition augmented cardiac output in female fructose high salt-fed rats. Nonetheless, both enalapril and losartan substantially diminish cardiac fibrosis in both sexes. Higher GFRs occur only in male fructose plus high salt-fed rats receiving the saline-DMSO vehicle, the same group that displayed elevations in both systolic and diastolic blood pressures and elevated glucose:insulin ratio. This is consistent with extensive data showing renal hyperfiltration in pre-and early diabetic rats and humans (44). The mechanism underlying higher GFR in the fructose-high salt female rats with Ang II inhibition is less clear, but likely due to improved glomerular dynamics. Notably, mesangial hypercellularity is evident in both

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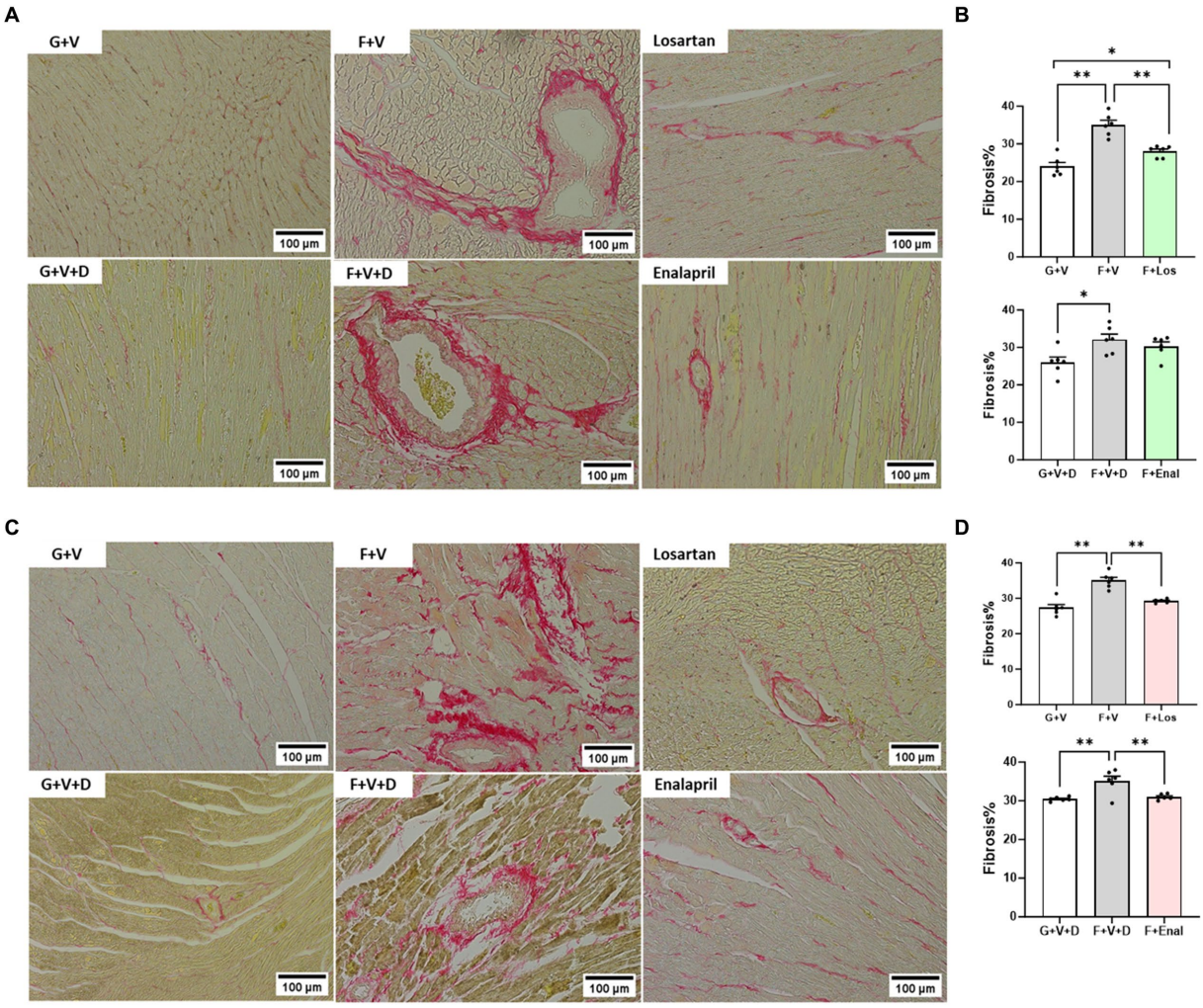

FIGURE 4
Picrosirius red staining for collagen in cardiac tissue in male **(A)** and female **(C)** rats fed for 3 weeks on 20% glucose plus 0.4% NaCl (G) or 20% fructose plus 4% NaCl chow (F) and concurrently treated with either saline vehicle (V) or losartan (Los, 8 mg/kg/day s.c.): G + V, F + V, and F + Los; or saline:DMSO vehicle (V + D) or enalapril (Enal, 4 mg/kg/day s.c.): G + V + D, F + V + D, and F + Enal by osmotic minipump. Quantitation of fibrosis as percentage of total LV area is shown for males **(B)** and females **(D)**. Values are mean ± SE; $n = 6$ for each group. *$p < 0.05$ and **$p < 0.01$ by ANOVA.

sexes on the fructose high salt diet but is most prominent in male rats regardless of the vehicle infused. Albuminuria tends to parallel the findings in mesangial hypercellularity with a profound decrease in male rats treated with enalapril.

4.1 Fructose and high salt: blood pressure and insulin resistance

Our findings are in alignment with previous studies showing that intake of a diet enriched in fructose and high salt results in higher blood pressures, an effect consistently observed in male rats (19, 20, 28, 45–47). The impact of such a diet on female rats is less consistent with some studies showing no change in blood pressure (30) and others demonstrating an increase in systolic blood pressure (48). Even within the present study, systolic and mean blood pressures are higher in one group of female rats fed fructose plus high salt diet and not in the other group. A role for varying effects of vehicle treatments cannot be discounted and provides a rationale for not combining the different vehicle-treated groups of either sex.

Additional factors may influence the impact of fructose on blood pressure. The approach used is important. Blood pressures assessed by plethysmography are higher (20, 48–50) compared with blood pressures obtained by direct arterial catheter or telemetry in freely moving rats within their home cages (19, 30, 45, 48). The influence of plethysmography on male rats appears to be greater compared with female rats (48). The present study was not designed to assess the role between sexes in the blood pressure response to fructose plus high salt diet; nevertheless, if such studies are undertaken consideration should be given to the potential confounding influences of methodology in evaluating differences in blood pressure between sexes.

The mode of delivery of dietary fructose may also be a factor in both blood pressure and insulin sensitivity. When fructose is incorporated into the chow, the proportion of calories provided by fructose can be rigorously controlled, thereby more closely mimicking the percentage of fructose calories ingested in the

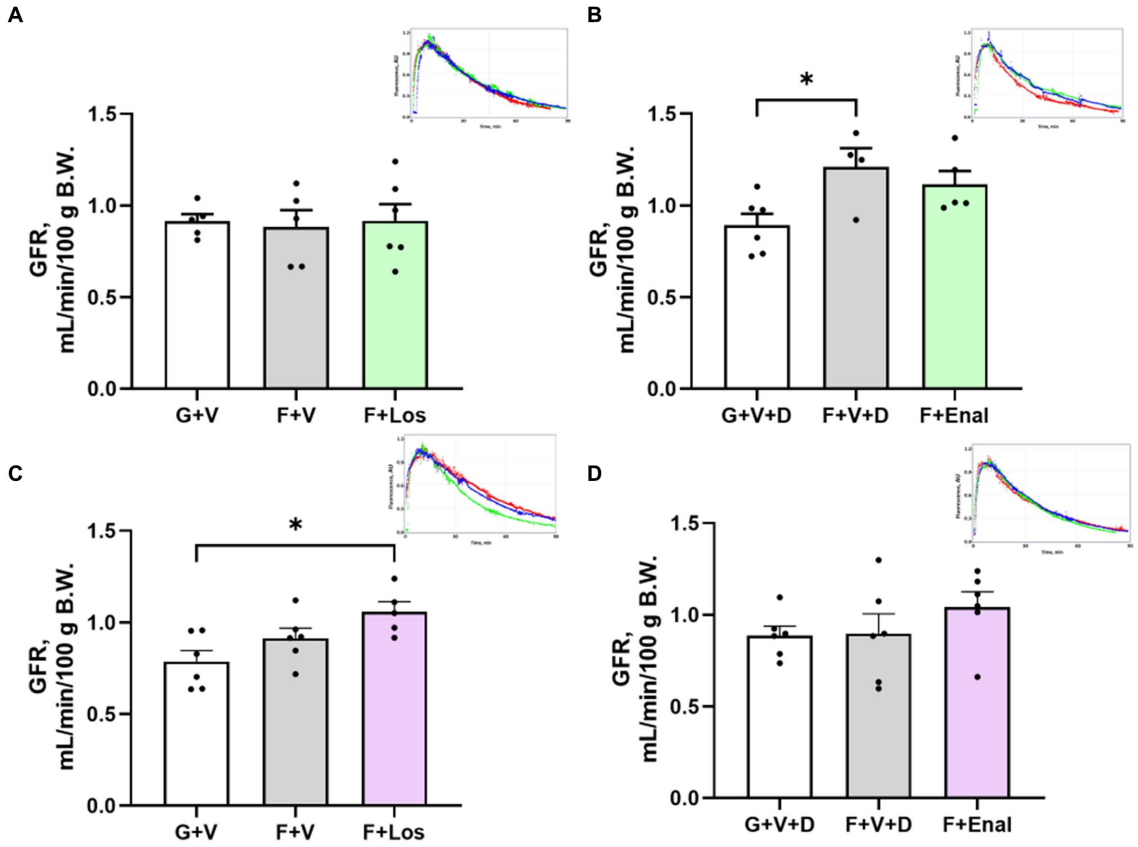

FIGURE 5
Glomerular filtration rate (GFR) in male **(A,B)** and female **(C,D)** rats fed for 3 weeks on 20% glucose plus 0.4% NaCl (G) or 20% fructose plus 4% NaCl chow (F) and concurrently treated with either saline vehicle (V) or losartan (Los, 8 mg/kg/day s.c.): G + V, F + V, and F + Los; or saline:DMSO vehicle (V + D) or enalapril (Enal, 4 mg/kg/day s.c.): G + V + D, F + V + D, and F + Enal by osmotic minipump. Insets are examples of single GFR determinations for glucose (blue), fructose (red) and drug treatment (green) in each set. Values are mean ± SE; $n = 5–6$ for each group. *$p < 0.05$ by ANOVA.

highest quintile of western population (51). When 20% (w/v) fructose is provided in the drinking water along with high salt diet in rats, the proportion of energy intake as fructose can vary from 40 to 58% of total daily calories (20). Since sodium absorption by the gut and reabsorption by renal tubular transporters is stimulated by fructose (52, 53), it is conceivable that the greater quantity of dietary fructose provided in drinking water when combined with high salt diet may exert a greater influence on blood pressure and other cardiorenal factors than when the fructose is present in chow.

Insulin resistance with fructose ingestion in rodents has been reported by other investigators (54) especially when fructose is combined with high salt diet (55) or for extended periods of time (56). In contrast, a difference in insulin sensitivity as measured by glucose:insulin ratio is evident only in the fructose-high salt male rats with vehicle plus DMSO compared with studies from our laboratory when fructose is provided in the drinking water (28, 29) or for longer periods of time in chow (47).

These factors may also apply to human consumption of fructose. Much of fructose in human diets is ingested as sugar-sweetened beverages but high fructose corn syrup is also present in a substantial number of other food products. There is evidence to suggest that dietary sugars ingested as beverages are disproportionately linked to higher blood pressure and metabolic syndrome compared with other sources fructose (57, 58).

4.2 Fructose and high salt: cardiovascular parameters

Several studies have shown that a fructose-rich diet blunts the suppression of renin secretion by high salt diet and results in higher plasma Ang II (20, 29, 46, 59). Although increased plasma concentrations of RAS components have not been universally observed (34), augmented expression of ACE and AT_1R in cardiac and aortic tissue has been observed (36, 60). Enhanced cardiac collagen deposition occurs in male rodents with prolonged fructose feeding (61, 62) or higher dietary fructose content (31). Cardiac fibrosis has also been reported in female rats fed high fructose diet (63). More extended periods of fructose plus high salt feeding result in diastolic dysfunction (47). The present histopathologic data demonstrate that cardiac fibrosis increases even after short term fructose plus high salt diet and prior to the ability to discern functional changes in left ventricular systolic or diastolic function in either male or female rats. Elevated blood pressure disrupts the equilibrium between collage types I and III synthesis and degradation resulting in collagen accumulation and myocardial fibrosis develop (64–66). Importantly, treatment with either an ACE inhibitor or AT_1R antagonist such that blood pressure is restored to a level comparable to that in their respective glucose plus normal salt counterparts successfully mitigates cardiac collagen deposition.

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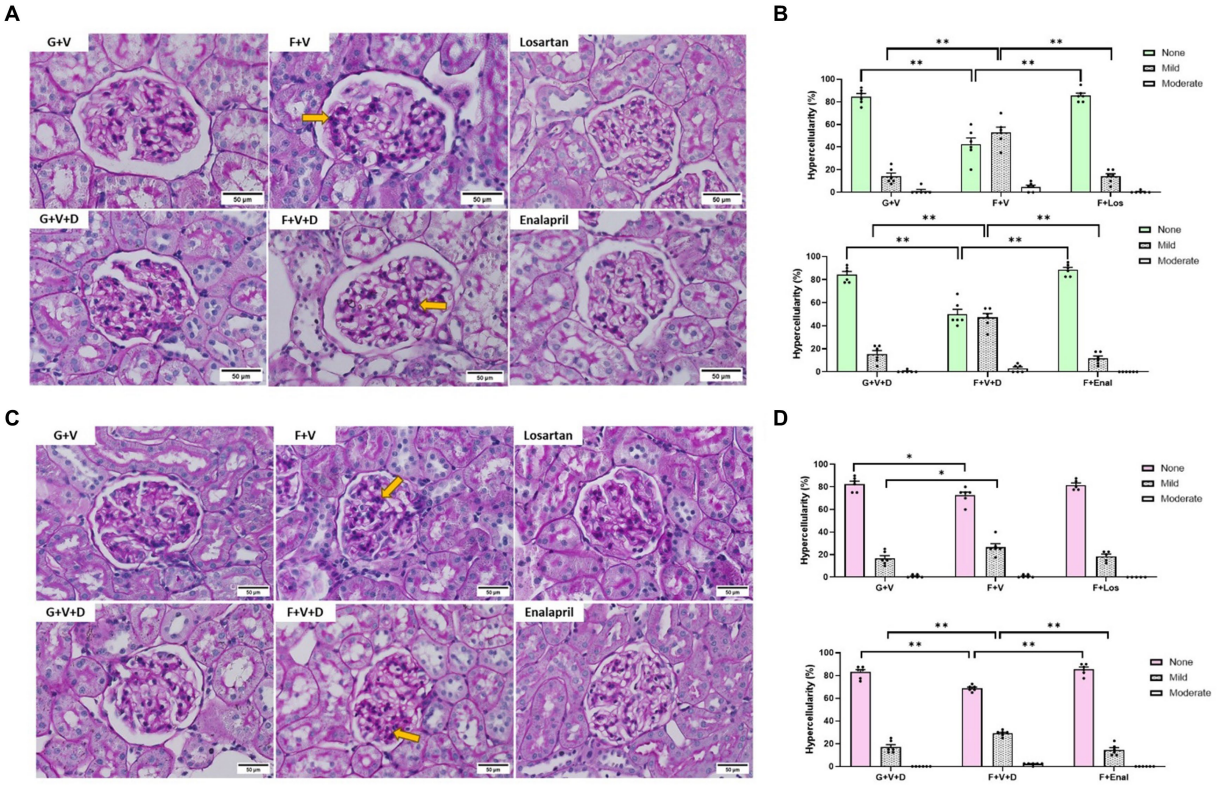

FIGURE 6
Periodic acid Schiff staining of representative glomeruli in male **(A)** and female **(C)** rats for 3 weeks on 20% glucose plus 0.4% NaCl (G) or 20% fructose plus 4% NaCl chow (F) and concurrently treated with either saline vehicle (V) or losartan (Los, 8 mg/kg/day s.c.): G + V, F + V, and F + Los; or saline:DMSO vehicle (V + D) or enalapril (Enal, 4 mg/kg/day s.c.): G + V + D, F + V + D, and F + Enal by osmotic minipump. Quantitative morphometry of mesangial hypercellularity is depicted for males **(B)** and females **(D)**. Yellow arrows indicate areas of increased mesangial hypercellularity. None, < 4; mild 4–5; moderate, 6–7; severe ≥8 mesangial cells/mesangial area. No severe glomeruli were observed. Values are mean ± SE, $n = 6$. *$p < 0.05$ and **$p < 0.01$ by ANOVA.

Dietary fructose-induced increases in aortic collagen have been reported as early as 1968 (67). PWV as an index of vascular stiffness is highly predictive of cardiovascular morbidity and mortality in humans (68, 69). Notably, aortic compliance in male but not female rats is diminished when given 20% fructose in their drinking water for 3 weeks (29, 30). A role for acute inhibition of sympathetic excitation (29) or enhancement of vagal activity (70) to improve vascular function with fructose-associated cardiometabolic alterations has been identified. Despite greater ACE and AT_1R mRNA expression (60) and protein content (36) in aortic tissue, acute inhibition of RAS fails to alter PWV, an index of aortic stiffness in fructose plus high salt fed rats (30). These results contrast with the improved vascular remodeling and stiffness with RAS inhibition in other models such as the spontaneously hypertensive rat (71), streptozotocin-induced diabetes (72), cystic renal disease (73) and iron-overload (74). Notably, aortic stiffness is not augmented within the 3-week timeframe in either sex with dietary fructose provided in rat chow rather than drinking water, nor is PWV altered with chronic RAS inhibition. It remains to be seen whether PWV may benefit from ACE or AT_1R blockade in rats fed this diet for longer periods of time when aortic stiffness is enhanced (47). Alternatively, other aberrations in factors such as NADPH oxidase, nitric oxide, and oxidative stress may contribute to disordered vascular compliance in this model independent of the RAS pathway (75).

4.3 Fructose and high salt: renal parameters

Albuminuria is an early index of renal injury. Elevated albumin excretion has been observed in male fructose-fed Dahl salt-sensitive rats (34) as well as Sprague Dawley rats fed fructose and high salt for 12 weeks (19). Albumin excretion shows greater variability in the current experiments since the study was not powered to evaluate albuminuria. When the groups are combined regardless of vehicle treatment, albuminuria is clearly augmented in the fructose plus high salt-fed groups of each sex. Moreover, RAS inhibition exerts a greater beneficial effect on albuminuria in male rats. The impact on albuminuria by fructose as well as RAS inhibition is consistent with the observed changes in mesangial hypercellularity which was more evident in the male rats. Mesangial cell proliferation occurs in response to activation of hexosamine flux which typically occurs under conditions of hyperglycemia (76, 77). Fructose may also enter the hexosamine pathway by action of glutamine: fructose-6-phosphate amidotransferase (GFAT) and may be augmented on a high fructose diet. Notably, Ang II activates the GFAT promoter in mesangial cells (78) and leads to oxidative stress (79). Importantly, the mesangial hypercellularity and albuminuria are evident at a time when GFR is not yet reduced. These findings are consistent with data showing glomerular hypertension and elevated single nephron GFR with a

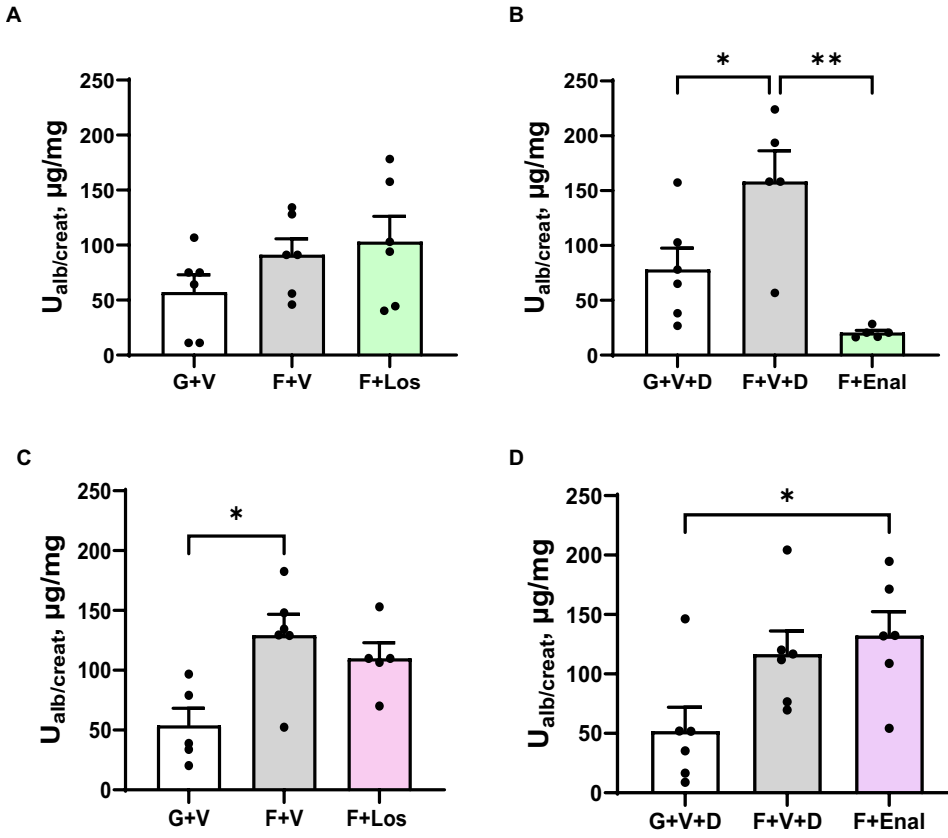

FIGURE 7
Urinary albumin to creatinine ratio (Ualb/create) in in male **(A,B)** and female **(C,D)** rats fed for 3 weeks on 20% glucose plus 0.4% NaCl (G) or 20% fructose plus 4% NaCl chow (F) and concurrently treated with either saline vehicle (V) or losartan (Los, 8 mg/kg/day s.c.): G + V, F + V, and F + Los; or saline:DMSO vehicle (V + D) or enalapril (Enal, 4 mg/kg/day s.c.): G + V + D, F + V + D, and F + Enal by osmotic minipump. Values are mean ± SE; $n = 6$ for each group. *$p < 0.05$ and **$p < 0.01$ by ANOVA.

high fructose diet (80). Indeed, more prolonged ingestion of a fructose plus high salt diet lowers GFR (19). Due to the association of fructose and high salt diet with elevated blood pressure, most studies on fructose effects in the kidney have focused on tubular sodium transport (53), but further investigations of a role in glomerular function and histopathology are also warranted.

4.4 Limitations

As noted above, there is a considerable difference in fructose calories when the fructose is delivered in the drinking water vs. rat chow such that the percentage of fructose calories provided with rat chow is specifically 20% of total caloric intake. If the data from Gordish et al. (20) is generalizable, when fructose is provided as 20% w/v in the drinking water the proportion of fructose calories is much higher. This may account for the lack of significant effects on insulin resistance, PWV, diastolic LV function and renal function observed in the fructose high salt-fed groups compared with our previous study where fructose was in the drinking water (30). A longer-term study of fructose and high salt exposure would be ideal; however, the mode, timing, and duration of delivery of RAS inhibitors will need to be carefully considered. The use of osmotic minipump delivery is superior giving the drugs in the drinking water especially as water intake may vary with fructose and salt intake making dosing irregular. On the other hand, a long-term study such as 12-weeks would require repeated minipump replacements. In addition, blood for evaluation of glucose and insulin was obtained from conscious animals to avoid changes imposed by the type or depth of anesthesia. This also limited the volume of blood that could be acquired without causing hypotension and influencing results and, in some cases, the catheters were occluded so that samples could not be obtained. A more rigorous measure of insulin sensitivity would have been the hyperinsulinemic euglycemic glucose clamp test; however, insulin is known to stimulate Ang II and to alter the actions of angiotensin peptides (81). Thus, the insulin infusion could have potentially altered parameters in our primary hypothesis, namely the RAS pathway so we chose the glucose:insulin ratio as an estimate of insulin sensitivity. Since urine for albumin and creatinine was obtained directly from the bladder at the time of harvesting, some samples were of insufficient volume to permit assay. Ideally, it would have been good to have had measure of renal blood flow or plasma flow but approaches in conscious rats similar to that used here for GFR are not yet available. It was necessary to use two different vehicles for enalapril and losartan, respectively. The study was powered to detect changes in PWV. Due to concerns regarding possible vehicle effects especially with the DMSO, this precluded

combining the groups not treated with RAS inhibition and thus diminished power for some of the other measurements.

5 Conclusion

In summary, these data confirm earlier findings that as little as 3 weeks of high fructose and high salt diet results in elevated blood pressure in both male and female rats. This short-term diet regimen does not change PWV, LV function or GFR in either sex. RAS blockade with either an ACE inhibitor or AT_1R antagonist is able to restore MAP to levels fructose high salt-fed rats similar to control glucose-fed rats. The only improvement in PWV occurs in female rats treated with losartan. Despite the lack of change in functional cardiorenal parameters, significant histopathologic changes occur in both cardiac and renal mesangial tissues and are ameliorated by RAS inhibition. Urinary albumin excretion is a marker of glomerular dysfunction prior to the development of frank decline in renal function and is also ameliorated by RAS inhibition in male rats.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Institutional Animal Care and Use Committee, Wayne State University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

SS: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Validation. RP: Formal analysis, Investigation, Writing – review & editing. BB: Investigation, Writing – review & editing. DK: Investigation, Writing – review & editing, Supervision. NR: Formal analysis, Investigation, Supervision, Writing – review & editing, Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Validation, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1436958/full#supplementary-material

References

1. Minja NW, Nakagaayi D, Aliku T, Zhang W, Ssinabulya I, Nabaale J, et al. Cardiovascular diseases in Africa in the twenty-first century: gaps and priorities going forward. *Front Cardiovasc Med*. (2022) 9:1008335. doi: 10.3389/fcvm.2022.1008335

2. Rangaswami J, Bhalla V, Blair JEA, Chang TI, Costa S, Lentine KL, et al. Cardiorenal syndrome: classification, pathophysiology, diagnosis, and treatment strategies: a scientific statement from the American Heart Association. *Circulation*. (2019) 139:e840–78. doi: 10.1161/CIR.0000000000000664

3. Vijayaraghavan K, McCullough PA, Singh B, Gupta M, Enas E, Mohan V, et al. Cardiometabolic-renal disease in South Asians: consensus recommendations from the Cardio Renal Society of America. *Cardiorenal Med*. (2019) 9:240–51. doi: 10.1159/000499341

4. Junho CVC, Trentin-Sonoda M, Panico K, Dos Santos RSN, Abrahao MV, Vernier ICS, et al. Cardiorenal syndrome: long road between kidney and heart. *Heart Fail Rev*. (2022) 27:2137–53. doi: 10.1007/s10741-022-10218-w

5. Bright R. Cases and observations inllustrative of renal disease, accompanied with the secretion of albumnous urine. *Med Chir Rev*. (1836) 25:23–35.

6. Zoccali C, Mallamaci F, Adamczak M, de Oliveira RB, Massy ZA, Sarafidis P, et al. Cardiovascular complications in chronic kidney disease: a review from the European Renal and Cardiovascular Medicine Working Group of the European Renal Association. *Cardiovasc Res*. (2023) 119:2017–32. doi: 10.1093/cvr/cvad083

7. Tuegel C, Bansal N. Heart failure in patients with kidney disease. *Heart*. (2017) 103:1848–53. doi: 10.1136/heartjnl-2016-310794

8. Webster AC, Nagler EV, Morton RL, Masson P. Chronic kidney disease. *Lancet*. (2017) 389:1238–52. doi: 10.1016/S0140-6736(16)32064-5

9. Damman K, Valente MA, Voors AA, O'Connor CM, van Veldhuisen DJ, Hillege HL. Renal impairment, worsening renal function, and outcome in patients with heart failure: an updated meta-analysis. *Eur Heart J*. (2014) 35:455–69. doi: 10.1093/eurheartj/eht386

10. McAlister FA, Ezekowitz J, Tarantini L, Squire I, Komajda M, Bayes-Genis A, et al. Renal dysfunction in patients with heart failure with preserved versus reduced ejection fraction: impact of the new chronic kidney disease-epidemiology collaboration group formula. *Circ Heart Fail*. (2012) 5:309–14. doi: 10.1161/CIRCHEARTFAILURE.111.966242

11. Lofman I, Szummer K, Evans M, Carrero JJ, Lund LH, Jernberg T. Incidence of, associations with and prognostic impact of worsening renal function in heart failure with different ejection fraction categories. *Am J Cardiol*. (2019) 124:1575–83. doi: 10.1016/j.amjcard.2019.07.065

12. Zoccali C, Mallamaci F, Halimi JM, Rossignol P, Sarafidis P, De Caterina R, et al. Chronic cardiovascular-kidney disorder: a new conceptual framework. *Nat Rev Nephrol*. (2024) 20:201–2. doi: 10.1038/s41581-023-00789-8

13. Vondenhoff S, Schunk SJ, Noels H. Increased cardiovascular risk in patients with chronic kidney disease. *Herz*. (2024) 49:95–104. doi: 10.1007/s00059-024-05235-4

14. Silveira Rossi JL, Barbalho SM, Reverete de Araujo R, Bechara MD, Sloan KP, Sloan LA. Metabolic syndrome and cardiovascular diseases: going beyond traditional risk factors. *Diabetes Metab Res Rev*. (2022) 38:e3502. doi: 10.1002/dmrr.3502

15. Hamad R, Penko J, Kazi DS, Coxson P, Guzman D, Wei PC, et al. Association of low socioeconomic status with premature coronary heart disease in US adults. *JAMA Cardiol*. (2020) 5:899–908. doi: 10.1001/jamacardio.2020.1458

16. Savira F, Magaye R, Liew D, Reid C, Kelly DJ, Kompa AR, et al. Cardiorenal syndrome: multi-organ dysfunction involving the heart, kidney and vasculature. *Br J Pharmacol*. (2020) 177:2906–22. doi: 10.1111/bph.15065

17. Whaley-Connell A, Sowers JR. Basic science: pathophysiology: the cardiorenal metabolic syndrome. *J Am Soc Hypertens*. (2014) 8:604–6. doi: 10.1016/j.jash.2014.07.003

18. Mozaffarian D, Fahimi S, Singh GM, Micha R, Khatibzadeh S, Engell RE, et al. Global sodium consumption and death from cardiovascular causes. *N Engl J Med*. (2014) 371:624–34. doi: 10.1056/NEJMoa1304127

19. Levanovich PE, Daugherty AM, Komnenov D, Rossi NF. Dietary fructose and high salt in young male Sprague Dawley rats induces salt-sensitive changes in renal function in later life. *Physiol Rep*. (2022) 10:e15456. doi: 10.14814/phy2.15456

20. Gordish KL, Kassem KM, Ortiz PA, Beierwaltes WH. Moderate (20%) fructose-enriched diet stimulates salt-sensitive hypertension with increased salt retention and decreased renal nitric oxide. *Physiol Rep*. (2017) 5:e13162. doi: 10.14814/phy2.13162

21. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on clinical practice guidelines. *Circulation*. (2018) 138:e426–83. doi: 10.1161/CIR.0000000000000597

22. Weinberger MH, Fineberg NS, Fineberg SE, Weinberger M. Salt sensitivity, pulse pressure, and death in normal and hypertensive humans. *Hypertension*. (2001) 37:429–32. doi: 10.1161/01.HYP.37.2.429

23. Mitchell GF. Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. *J Appl Physiol*. (1985) 105:1652–60. doi: 10.1152/japplphysiol.90549.2008

24. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J*. (2006) 27:2588–605. doi: 10.1093/eurheartj/ehl254

25. Choi HY, Park SK, Yun GY, Choi AR, Lee JE, Ha SK, et al. Glycated albumin is independently associated with arterial stiffness in non-diabetic chronic kidney disease patients. *Medicine*. (2016) 95:e3362. doi: 10.1097/MD.0000000000003362

26. Pascual-Morena C, Cavero-Redondo I, Martinez-Garcia I, Rodriguez-Gutierrez E, Luceron-Lucas-Torres M, Moreno-Herraiz N, et al. Exploring the influence of insulin resistance on arterial stiffness in healthy adults: from the metabolic and cardiovascular health insights of the EVasCu study. *Nutrients*. (2024) 16:791. doi: 10.3390/nu16060791

27. Hill MA, Yang Y, Zhang L, Sun Z, Jia G, Parrish AR, et al. Insulin resistance, cardiovascular stiffening and cardiovascular disease. *Metabolism*. (2021) 119:154766. doi: 10.1016/j.metabol.2021.154766

28. Soncrant T, Komnenov D, Beierwaltes WH, Chen H, Wu M, Rossi NF. Bilateral renal cryodenervation decreases arterial pressure and improves insulin sensitivity in fructose-fed Sprague-Dawley rats. *Am J Physiol Regul Integr Comp Physiol*. (2018) 315:R529–38. doi: 10.1152/ajpregu.00020.2018

29. Komnenov D, Levanovich PE, Perecki N, Chung CS, Rossi NF. Aortic stiffness and diastolic dysfunction in Sprague Dawley rats consuming short-term fructose plus high salt diet. *Integr Blood Press Control*. (2020) 13:111–24. doi: 10.2147/IBPC.S257205

30. Komnenov D, Rossi NF. Fructose-induced salt-sensitive blood pressure differentially affects sympathetically mediated aortic stiffness in male and female Sprague-Dawley rats. *Physiol Rep*. (2023) 11:e15687. doi: 10.14814/phy2.15687

31. Wang X, Xu Z, Chang R, Zeng C, Zhao Y. High-fructose diet induces cardiac dysfunction via macrophage recruitment in adult mice. *J Cardiovasc Pharmacol Ther*. (2023) 28:107424842311622. doi: 10.1177/10742484231162249

32. Hinderer S, Schenke-Layland K. Cardiac fibrosis—a short review of causes and therapeutic strategies. *Adv Drug Deliv Rev*. (2019) 146:77–82. doi: 10.1016/j.addr.2019.05.011

33. Manitius J, Baines AD, Roszkiewicz A. The effect of high fructose intake on renal morphology and renal function in rats. *J Physiol Pharmacol*. (1995) 46:179–83.

34. Xu L, Hu G, Qiu J, Fan Y, Ma Y, Miura T, et al. High fructose-induced hypertension and renal damage are exaggerated in dahl salt-sensitive rats via renal renin-angiotensin system activation. *J Am Heart Assoc*. (2021) 10:e016543. doi: 10.1161/JAHA.120.016543

35. Kim M, Do GY, Kim I. Activation of the renin-angiotensin system in high fructose-induced metabolic syndrome. *Korean J Physiol Pharmacol*. (2020) 24:319–28. doi: 10.4196/kjpp.2020.24.4.319

36. Bundalo MM, Zivkovic MD, Romic S, Tepavcevic SN, Koricanac GB, Djuric TM, et al. Fructose-rich diet induces gender-specific changes in expression of the renin-angiotensin system in rat heart and upregulates the ACE/AT_1R axis in the male rat aorta. *J Renin Angiotensin Aldosterone Syst*. (2016) 17:1470320316642915. doi: 10.1177/1470320316642915

37. Xie HH, Shen FM, Zhang XF, Jiang YY, Su DF. Blood pressure variability, baroreflex sensitivity and organ damage in spontaneously hypertensive rats treated with various antihypertensive drugs. *Eur J Pharmacol*. (2006) 543:77–82. doi: 10.1016/j.ejphar.2006.05.034

38. Caldiz CI, de Cingolani GE. Insulin resistance in adipocytes from spontaneously hypertensive rats: effect of long-term treatment with enalapril and losartan. *Metabolism*. (1999) 48:1041–6. doi: 10.1016/S0026-0495(99)90203-2

39. Zicha J, Rezacova L, Behuliak M, Vaneckova I. Blood pressure reduction induced by chronic intracerebroventricular or peroral clonidine administration in rats with salt-dependent or angiotensin II-dependent hypertension. *Physiol Res*. (2022) 71:763–70. doi: 10.33549/physiolres.935041

40. Umezawa T, Guo S, Jiao Y, Hisamitsu T. Effect of clonidine on colonic motility in rats. *Auton Neurosci*. (2003) 107:32–6. doi: 10.1016/S1566-0702(03)00105-X

41. Rossi NF, Chen H, Maliszewska-Scislo M. Paraventricular nucleus control of blood pressure in two-kidney, one-clip rats: effects of exercise training and resting blood pressure. *Am J Physiol Regul Integr Comp Physiol*. (2013) 305:R1390–400. doi: 10.1152/ajpregu.00546.2012

42. Schock-Kusch D, Geraci S, Ermeling E, Shulhevich Y, Sticht C, Hesser J, et al. Reliability of transcutaneous measurement of renal function in various strains of conscious mice. *PLoS One*. (2013) 8:e71519. doi: 10.1371/journal.pone.0071519

43. Roberts IS, Cook HT, Troyanov S, Alpers CE, Amore A, Barratt J, et al. The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility. *Kidney Int*. (2009) 76:546–56. doi: 10.1038/ki.2009.168

44. Vallon V, Thomson SC. The tubular hypothesis of nephron filtration and diabetic kidney disease. *Nat Rev Nephrol*. (2020) 16:317–36. doi: 10.1038/s41581-020-0256-y

45. Cabral PD, Hong NJ, Hye Khan MA, Ortiz PA, Beierwaltes WH, Imig JD, et al. Fructose stimulates Na/H exchange activity and sensitizes the proximal tubule to angiotensin II. *Hypertension*. (2014) 63:e68–73. doi: 10.1161/HYPERTENSIONAHA.113.02564

46. Zenner ZP, Gordish KL, Beierwaltes WH. Free radical scavenging reverses fructose-induced salt-sensitive hypertension. *Integr Blood Press Control*. (2018) 11:1–9. doi: 10.2147/IBPC.S147674

47. Levanovich PE, Chung CS, Komnenov D, Rossi NF. Fructose plus high-salt diet in early life results in salt-sensitive cardiovascular changes in mature male Sprague Dawley rats. *Nutrients*. (2021) 13:3129. doi: 10.3390/nu13093129

48. Brostek A, Hong NJ, Zhang R, Forester BR, Barmore LE, Kaydo L, et al. Independent effects of sex and stress on fructose-induced salt-sensitive hypertension. *Physiol Rep*. (2022) 10:e15489. doi: 10.14814/phy2.15489

49. D'Angelo G, Elmarakby AA, Pollock DM, Stepp DW. Fructose feeding increases insulin resistance but not blood pressure in Sprague-Dawley rats. *Hypertension*. (2005) 46:806–11. doi: 10.1161/01.HYP.0000182697.39687.34

50. Sikora M, Konopelski P, Pham K, Wyczalkowska-Tomasik A, Ufnal M. Repeated restraint stress produces acute and chronic changes in hemodynamic parameters in rats. *Stress*. (2016) 19:621–9. doi: 10.1080/10253890.2016.1244667

51. Johnson RK, Appel LJ, Brands M, Howard BV, Lefevre M, Lustig RH, et al. Dietary sugars intake and cardiovascular health: a scientific statement from the American Heart Association. *Circulation*. (2009) 120:1011–20. doi: 10.1161/CIRCULATIONAHA.109.192627

52. Eren OC, Ortiz A, Afsar B, Covic A, Kuwabara M, Lanaspa MA, et al. Multilayered interplay between fructose and salt in development of hypertension. *Hypertension*. (2019) 73:265–72. doi: 10.1161/HYPERTENSIONAHA.118.12150

53. Soleimani M, Barone S, Luo H, Zahedi K. Pathogenesis of hypertension in metabolic syndrome: the role of fructose and salt. *Int J Mol Sci*. (2023) 24:4294. doi: 10.3390/ijms24054294

54. Toop CR, Gentili S. Fructose beverage consumption induces a metabolic syndrome phenotype in the rat: a systematic review and meta-analysis. *Nutrients*. (2016) 8:577. doi: 10.3390/nu8090577

55. Bezerra RM, Ueno M, Silva MS, Tavares DQ, Carvalho CR, Saad MJ, et al. A high-fructose diet induces insulin resistance but not blood pressure changes in normotensive rats. *Braz J Med Biol Res*. (2001) 34:1155–60. doi: 10.1590/S0100-879X2001000900008

56. Pektas MB, Sadi G, Akar F. Long-term dietary fructose causes gender-different metabolic and vascular dysfunction in rats: modulatory effects of resveratrol. *Cell Physiol Biochem*. (2015) 37:1407–20. doi: 10.1159/000430405

57. Malik AH, Akram Y, Shetty S, Malik SS, Yanchou NV. Impact of sugar-sweetened beverages on blood pressure. *Am J Cardiol*. (2014) 113:1574–80. doi: 10.1016/j.amjcard.2014.01.437

58. Semnani-Azad Z, Khan TA, Blanco Mejia S, de Souza RJ, Leiter LA, Kendall CWC, et al. Association of major food sources of fructose-containing sugars with incident metabolic syndrome: a systematic review and meta-analysis. *JAMA Netw Open*. (2020) 3:e209993. doi: 10.1001/jamanetworkopen.2020.9993

59. Chen C, Ahn EH, Kang SS, Liu X, Alam A, Ye K. Gut dysbiosis contributes to amyloid pathology, associated with C/EBPbeta/AEP signaling activation in Alzheimer's disease mouse model. *Sci Adv*. (2020) 6:eaba0466. doi: 10.1126/sciadv.aba0466

60. Nyby MD, Abedi K, Smutko V, Eslami P, Tuck ML. Vascular angiotensin type 1 receptor expression is associated with vascular dysfunction, oxidative stress and inflammation in fructose-fed rats. *Hypertens Res*. (2007) 30:451–7. doi: 10.1291/hypres.30.451

61. Xie XW. Liquiritigenin attenuates cardiac injury induced by high fructose-feeding through fibrosis and inflammation suppression. *Biomed Pharmacother*. (2017) 86:694–704. doi: 10.1016/j.biopha.2016.12.066

62. Xing SS, Tan HW, Bi XP, Zhong M, Zhang Y, Zhang W. Felodipine reduces cardiac expression of IL-18 and perivascular fibrosis in fructose-fed rats. *Mol Med*. (2008) 14:395–402. doi: 10.2119/2008-00024.Xing

63. Soutelo J, Samaniego YA, Fornari MC, Reyes Toso C, Ponzo OJ. Cardiometabolic changes in different gonadal female states caused by mild hyperuricemia and exposure to a high-fructose diet. *Int J Endocrinol*. (2018) 2018:6021259. doi: 10.1155/2018/6021259

64. Shahbaz AU, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC, McGee JE, et al. Fibrosis in hypertensive heart disease: molecular pathways and cardioprotective strategies. *J Hypertens*. (2010) 28:S25–32. doi: 10.1097/01.hjh.0000388491.35836.d2

65. Diez J. Mechanisms of cardiac fibrosis in hypertension. *J Clin Hypertens*. (2007) 9:546–50. doi: 10.1111/j.1524-6175.2007.06626.x

66. Cuspidi C, Ciulla M, Zanchetti A. Hypertensive myocardial fibrosis. *Nephrol Dial Transplant*. (2006) 21:20–3. doi: 10.1093/ndt/gfi237

67. Cohen AM, Shoshan S. Effect of glucose, fructose, and starch on aorta collagen in the rat. Preliminary note. *J Atheroscler Res*. (1968) 8:371–5. doi: 10.1016/S0368-1319(68)80071-7

68. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, et al. Aortic pulse wave velocity improves cardiovascular event prediction an individual participant meta-analysis of prospective observational data from 17,635 subjects. *J Am Coll Cardiol*. (2014) 63:636–46. doi: 10.1016/j.jacc.2013.09.063

69. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness a systematic review and meta-analysis. *J Am Coll Cardiol*. (2010) 55:1318–27. doi: 10.1016/j.jacc.2009.10.061

70. Dos Santos F, Moraes-Silva IC, Moreira ED, Irigoyen MC. The role of the baroreflex and parasympathetic nervous system in fructose-induced cardiac and metabolic alterations. *Sci Rep*. (2018) 8:10970. doi: 10.1038/s41598-018-29336-3

71. Zeng W, Zhang X, Lu Y, Wen Y, Xie Q, Yang X, et al. Neferine ameliorates hypertensive vascular remodeling modulating multiple signaling pathways in spontaneously hypertensive rats. *Biomed Pharmacother*. (2023) 158:114203. doi: 10.1016/j.biopha.2022.114203

72. Balogh DB, Molnar A, Degi A, Toth A, Lenart L, Saeed A, et al. Cardioprotective and antifibrotic effects of low-dose renin-angiotensin-aldosterone system inhibitors in type 1 diabetic rat model. *Int J Mol Sci*. (2023) 24:17043. doi: 10.3390/ijms242317043

73. Ng K, Hildreth CM, Avolio AP, Phillips JK. Angiotensin-converting enzyme inhibitor limits pulse-wave velocity and aortic calcification in a rat model of cystic renal disease. *Am J Physiol Renal Physiol*. (2011) 301:F959–66. doi: 10.1152/ajprenal.00393.2011

74. Fidelis HG, Mageski JGA, Goes SCE, Botelho T, Marques VB, Avila RA, et al. Blockade of angiotensin AT_1 receptors prevents arterial remodelling and stiffening in iron-overloaded rats. *Br J Pharmacol*. (2020) 177:1119–30. doi: 10.1111/bph.14904

75. Ismaeel A, Brumberg RS, Kirk JS, Papoutsi E, Farmer PJ, Bohannon WT, et al. Oxidative stress and arterial dysfunction in peripheral artery disease. *Antioxidants*. (2018) 7:145. doi: 10.3390/antiox7100145

76. James LR, Le C, Scholey JW. Influence of glucosamine on glomerular mesangial cell turnover: implications for hyperglycemia and hexosamine pathway flux. *Am J Physiol Endocrinol Metab*. (2010) 298:E210–21. doi: 10.1152/ajpendo.00232.2009

77. Lagranha CJ, Doi SQ, Pithon-Curi TC, Curi R, Sellitti DF. Glutamine enhances glucose-induced mesangial cell proliferation. *Amino Acids*. (2008) 34:683–5. doi: 10.1007/s00726-007-0002-9

78. James LR, Ingram A, Ly H, Thai K, Cai L, Scholey JW. Angiotensin II activates the GFAT promoter in mesangial cells. *Am J Physiol Renal Physiol*. (2001) 281:F151–62. doi: 10.1152/ajprenal.2001.281.1.F151

79. Cheng DW, Jiang Y, Shalev A, Kowluru R, Crook ED, Singh LP. An analysis of high glucose and glucosamine-induced gene expression and oxidative stress in renal mesangial cells. *Arch Physiol Biochem*. (2006) 112:189–218. doi: 10.1080/13813450601093518

80. Sanchez-Lozada LG, Tapia E, Jimenez A, Bautista P, Cristobal M, Nepomuceno T, et al. Fructose-induced metabolic syndrome is associated with glomerular hypertension and renal microvascular damage in rats. *Am J Physiol Renal Physiol*. (2007) 292:F423–9. doi: 10.1152/ajprenal.00124.2006

81. Zamolodchikova TS, Tolpygo SM, Kotov AV. Insulin in the regulation of the renin-angiotensin system: a new perspective on the mechanism of insulin resistance and diabetic complications. *Front Endocrinol*. (2024) 15:1293221. doi: 10.3389/fendo.2024.1293221

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The influence of salt reduction with encapsulated oleoresins on the quality of mayonnaise, mustard, and ketchup

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Introduction: Mayonnaise, mustard, and ketchup are table sauces enjoyed worldwide, adding flavour and texture to many dishes. However, these products often contain high sodium content, which contributes to health issues such as high blood pressure and cardiovascular disease. To address these concerns, reducing salt content in the sauces has become a significant goal for both manufacturers and consumers.

Objectives: This study investigates the effects of three formulations of microencapsulated (ME) oleoresins (F1, F2, and F3), derived from aromatic plants and spices, on the mineral content, physical–chemical properties, colour, and sensory profiles of mayonnaise, mustard, and ketchup.

Results: The addition of ME ingredients resulted in significant reductions in salt content across all sauces, with reductions up to 50% in mayonnaise, 45% in mustard, and 52% in ketchup, aligning with EU sodium guidelines and allowing for a "reduced Na/NaCl content" nutrition claim. Potassium levels in mustard and ketchup were sufficient to support health claims related to blood pressure maintenance, while chloride content was reduced in ME formulations, better aligning with dietary reference values. Physical–chemical analysis revealed that ME ingredients had minimal impact on parameters like pH, lipid oxidation, and viscosity, although significant differences were observed in specific areas, such as the consistency of ketchup and chloride content in mustard and ketchup. The use of inulin, as a carrier agent, helped maintain the sauces rheological properties. Mustard showed the most similarity to the control in terms of physical–chemical parameters. Colour analysis indicated minimal changes in mayonnaise, moderate changes in mustard, and significant differences in ketchup, particularly with the ME-F3 formulation, where the light-yellow ME ingredients had a pronounced effect on the darker sauce. Despite these differences, the sensory analysis demonstrated that the overall sensory profiles of the ME formulations were similar the like control for all sauces. Mayonnaise showed the closest resemblance, while mustard had slightly lower scores in flavour and saltiness. Ketchup followed the same trend as mayonnaise, with no significant sensory differences compared to the control.

Conclusion: These findings suggest that ME ingredients can be effectively used in condiment reformulation to achieve significant salt reduction without compromising sensory qualities, while also supporting health-related claims. By incorporating ME-based salt reduction strategies and exploring low-sodium alternatives, consumers can continue to enjoy their favourite sauces while minimising sodium intake. Embracing these changes not only benefits personal health but also aligns with the industry's commitment to offering more nutritious options.

KEYWORDS

salt reduction, sauces and condiments, encapsulated oleoresins, quality parameters, sensory analysis

1 Introduction

The use of table sauces dates back to various cultures and regions worldwide. The Global Market for condiments, seasonings, and sauces will reach USD 181.0 billion by 2025 (1). Emulsion-based sauces (e.g., mayonnaise) and ready-made vegetable seasonings (mustard and ketchup) are mixtures of ingredients designed to enhance food flavour. These sauces are staples on kitchen tables and are especially prevalent in fast food, with mayonnaise being the most common, followed by ketchup and mustard.

Several studies have shown that these products contain very high levels of salt (2, 3). Regular consumption of foods and sauces high in sodium chloride can increase the risk of developing high blood pressure, heart disease, and stroke. This measure has prompted several governments and health organizations to advocate for salt reduction strategies to improve public health (4).

The WHO aims to reduce the average population's sodium intake by approximately 30% by 2025 (5). In the European Union, national salt reduction initiatives have set maximum salt content targets for 12 food products, including sauces, condiments, and spices (6). In addition to setting industry targets, other policies have been considered to support reformulation. These include introducing taxes on high-salt foods and implementing labelling and communication strategies. Aligning these strategies with the food industry can improve public health and ensure consumer welfare.

Reducing salt in food can be challenging, given salt's crucial role in flavour, texture, and preservation. Consequently, the food industry must continuously innovate to maintain traditional tastes and textures while reducing salt content. Numerous studies have developed new technologies and strategies, such as adjusting processing operations, selecting quality ingredients and using additives to achieve a 30–50% reduction in salt intake without compromising the product's qualitative, physicochemical characteristics and sensorial aspects.

Table salt provides a salty taste to foods, and reducing it can alter the flavour profile, often resulting in acidic or sweet effects. Therefore, it is essential to imitate or replace the properties of salt flavours with other aromas or flavours. Although complex, this approach can significantly impact sodium intake from processed products. Testing is necessary to select substitute ingredients that best match the desired taste profile.

One strategy involves modifying formulations using mineral compounds like lithium, potassium, or ammonium chloride. However, these compounds can produce undesirable flavours, such as sour, metallic, and bitter notes (7). Additionally, potassium chloride (KCl) can pose health risks for individuals with Type 1 diabetes, necessitating limited consumption of this type of salt (8).

Another approach is the addition of organic sodium salts, such as acetate, glutamate, or citrate, although their salinity is significantly lower than sodium chloride (9). Non-mineral compounds, including amino acids (arginine, lysine, choline chloride) and flavour enhancers (10), such as monosodium glutamate (MSG), yeast extracts, alapyridine, arginine-apyridine derivatives, aromatic plants, spices (11), and hydrolyzed vegetable proteins (HVP) (12), have also been applied to enhance the salty taste of foods.

Taste enhancers work by activating receptors in the mouth and throat, helping to compensate for salt reduction. They stimulate receptors linked to the umami taste, improving the overall balance of taste perception in foods. According to the European Union, it is safe to use these taste enhancers. However, MSG has garnered a controversial reputation due to potential adverse effects in individuals with a vitamin B6 deficiency (13).

A promising new salt reduction technique involves combining flavour compounds from various foods and recipes, which may become increasingly important in future strategies.

Healthier alternatives, such as natural flavours derived from oleoresins, are increasingly used as food ingredients due to their uniformity in flavour, and aroma, microbiological stability, and ease of storage and transport. The rising demand for salt reduction in foods has significantly increased the use of oleoresins. However, challenges such as colour changes, low water solubility, and susceptibility to oxidation necessitate encapsulation, where sensitive compounds are enclosed within another substance (carrier) and released under specific controlled conditions.

Spray drying is a common method for obtaining encapsulated oleoresin flavours and aromas in powder form, suitable for incorporation into various matrices. Using inulin as a carrier meets technological and regulatory requirements, as it is biodegradable and has been Generally Recognized As Safe (GRAS) in the USA since 2002 (14). Microencapsulated oleoresins (ME), derived from aromatic plants and spices (15), have been applied in various food and culinary products (16), including fish (17), and fish products (18, 19). These applications allow for a salt reduction of over 25%, masking undesirable flavour components while maintaining or improving the flavour profile, texture, stability, and shelf-life.

This study investigates the effects of three formulations of microencapsulated (ME) oleoresins (F1, F2, and F3), derived from aromatic plants and spices, on the mineral content, physical–chemical properties, colour, and sensory profiles of mayonnaise, mustard, and ketchup. To our knowledge, this is the first study to use oleoresin-based microencapsulated (ME) ingredients to achieve a 25–50% salt reduction in these sauces, specifically targeting products manufactured by Mendes Gonçalves SA, a Portuguese company. The goal is to replace salt with encapsulated oleoresins to enhance flavour while meeting the functional, technical, and quality requirements of the sauces, aligning with consumer needs and preferences.

2 Materials and methods

2.1 Sauce ingredients

The sauce ingredients were provided by Mendes Gonçalves'. The control and ME ingredients (F1, F1-A, F1-B, F2, and F3) low salt mayonnaise, mustard and ketchup sauce samples were formulated as shown in Table 1, and the preparation is described in sections 2.2.1–2.2.3. The final product was transferred into an airtight bottle and packaged. All sauce samples were stored at room temperature (T = 20.00°C ± 0.05) until physicochemical and sensory analysis were carried out.

2.2 Products preparation (recipes)

2.2.1 Mayonnaise sauce preparation

The ingredients listed in Table 1 were used to prepare 1 kg of mayonnaise sauce for each of the three batches. In a bowl, the starch, sugar, salt, and ME (F1, F2, and F3) were mixed until a homogeneous mixture was obtained. Using a food processor (Bimby, Model TM31, Germany), the colouring agent was first added to the water, followed by the powders, and the mixture was homogenised for 2 min. The egg yolk was then added at low speed, followed by the oil, and mixed for 6 min until the emulsion system was established. Finally, the vinegar was added and mixed for a further 5 min.

2.2.2 Mustard sauce preparation

For the preparation of 1 kg of mustard sauce samples for each of the three batches, the ingredients listed in Table 1 were used, and the process was divided into two steps. In the first step, the mustard base was prepared by combining the mustard seeds with water (47.29% w/w) in a bowl and soaking them for at least 2 h. After soaking, the mustard seeds were transferred to a food processor (Bimby, Model TM31, Germany), where vinegar was added, and the mixture was milled for 5 min. The mixture was then dissolved in hot water at 90°C for 10 min.

In the second step, the mustard sauce was prepared by adding water, starch, sugar, salt or ME (F1, F2, and F3), and gum to the mustard base and blending for 5 min. The final product was transferred to an airtight jar and packaged.

2.2.3 Ketchup sauce preparation

The ingredients listed in Table 1 were used to prepare 1 kg of ketchup sauce for each of the three batches produced. In a container, the powders—sugar, salt or ME (F1, F2, and F3), gum, and starch—were mixed until well combined. In a food processor (Bimby, Model TM31, Germany), water was added to the tomato concentrate, mixed with the powders, and heated at 90°C for 15 min. Vinegar was then added, and the entire mixture was stirred for an additional 5 min to combine all the ingredients.

TABLE 1 Sauces and condiments formulations based on 1 kg for each treatment (w/w %) with varying salt levels and ME.

Ingredient	Mayonnaise	Mustard	Ketchup
Water	58.54 ± 2.27	71.98 ± 1.48	34.29 ± 1.01
β carotene (A160a)	0.02 ± 0.00	–	–
Oil (vegetable)	26.37 ± 1.03	–	–
Tomato concentrate	–	–	28.92 ± 1.30
Sugar	2.74 ± 0.48	2.36 ± 0.16	23.01 ± 1.04
Vinegar	5.98 ± 0.74	10.72 ± 1.11	11.56 ± 1.01
Salt	0.35 ± 0.15	1.85 ± 0.20	1.52 ± 0.09
F1	0.35 ± 0.15	1.85 ± 0.20	1.52 ± 0.09
F1-A	0.17 ± 0.01	–	–
F1-B	0.35 ± 0.15	–	–
F2	0.35 ± 0.15	1.85 ± 0.20	1.52 ± 0.09
F3	0.35 ± 0.15	1.85 ± 0.20	1.52 ± 0.09
Gum	0.3 ± 0.11	0.10 ± 0.006	0.19 ± 0.04
Mustard	–	11.94 ± 0.99	–
Starch	5.5 ± 0.81	2.66 ± 0.30	2.90 ± 0.97
Egg	4.56 ± 0.37	–	–

The salt content added for formulations F1-A and F1-B was 0.53% (w/w).

TABLE 2 Physicochemical parameters of mayonnaise (M), mustard (Mu), ketchup (K), and control (Co), containing different added ingredients (F1, F2, and F3), respectively.

	pH	Acidity (%)	Density (Pa s)	Chloride (gKg^{-1})	TBA ($mgKg^{-1}$)	Viscosity (cP)	Consistency (cm/30 s)	Brix
M-Co	3.84 ± 0.00^{c}	0.72 ± 0.04^{a}	$1.00 \pm 0.01^{a,b}$	1.06 ± 0.04^{c}	0.02 ± 0.00^{a}	12653.33 ± 100.66^{c}	–	–
M-F1	3.82 ± 0.01^{b}	0.76 ± 0.02^{a}	0.99 ± 0.00^{a}	0.36 ± 0.00^{a}	0.02 ± 0.00^{a}	$14346.67 \pm 54.55^{a,b}$	–	–
M-F2	3.79 ± 0.01^{a}	0.78 ± 0.03^{a}	1.00 ± 0.00^{a}	0.38 ± 0.01^{a}	0.02 ± 0.00^{a}	14933.33 ± 612.32^{a}	–	–
M-F3	$3.83 \pm 0.00^{b,c}$	0.73 ± 0.04^{a}	1.00 ± 0.00^{a}	0.36 ± 0.00^{a}	0.02 ± 0.00^{a}	15186.67 ± 794.31^{a}	–	–
M-F1A	3.78 ± 0.00^{a}	0.73 ± 0.08^{a}	0.99 ± 0.00^{b}	0.55 ± 0.03^{b}	0.02 ± 0.00^{a}	$14213.13 \pm 477.21^{a,b}$	–	–
M-F1B	3.78 ± 0.00^{a}	0.75 ± 0.03^{a}	0.99 ± 0.00^{b}	0.51 ± 0.02^{b}	0.02 ± 0.00^{a}	$13387.67 \pm 371.66^{b,c}$	–	–
Mu-Co	3.78 ± 0.00^{b}	1.31 ± 0.02^{a}	1.07 ± 0.00^{a}	3.64 ± 002^{c}	–	18200.00 ± 0.00^{a}	–	–
Mu-F1	3.80 ± 0.01^{a}	1.31 ± 0.04^{a}	1.24 ± 0.31^{a}	1.87 ± 0.00^{a}	–	18617.67 ± 321.46^{a}	–	–
Mu-F2	3.82 ± 0.00^{c}	1.27 ± 0.02^{a}	1.06 ± 0.00^{a}	1.98 ± 0.00^{b}	–	17300.00 ± 476.97^{a}	–	–
Mu-F3	3.80 ± 0.00^{a}	1.32 ± 0.01^{a}	1.06 ± 0.00^{a}	1.86 ± 0.00^{b}	–	17916.67 ± 354.73^{a}	–	–
K-Co	3.71 ± 0.00^{a}	1.61 ± 0.01^{b}	1.17 ± 0.00^{a}	3.13 ± 0.00d	–	–	5.52 ± 0.36d	36.40 ± 0.44^{a}
K-F1	3.73 ± 0.00^{a}	1.55 ± 0.02^{a}	1.17 ± 0.00^{a}	1.66 ± 0.01^{b}	–	–	3.68 ± 0.11^{a}	38.43 ± 0.32^{c}
K-F2	3.72 ± 0.00^{a}	1.60 ± 0.01^{b}	$1.16 \pm 0.00^{a,b}$	1.64 ± 0.00^{a}	–	–	4.45 ± 0.07^{c}	$37.73 \pm 0.46^{b,c}$
K-F3	3.73 ± 0.00^{a}	1.57 ± 0.01^{a}	1.16 ± 0.00^{b}	1.71 ± 0.00^{c}	–	–	4.05 ± 0.07^{b}	$37.13 \pm 0.15^{a,b}$

Data are presented as the mean ± standard error of three experiments. *Samples with the same letters are not significantly different at 5%.

2.3 Physical, chemical, and sensory analysis

The sauces and condiments were analysed by physicochemical, textural and sensory analysis to evaluate the quality and consistency of the products and to monitor the production process to ensure that the products meet regulatory requirements and consumer expectations concerning taste, texture, and safety. Each physicochemical test was performed on control and ME ingredients (F1, F1-A, F1-B, F2, and F3) samples of low salt mayonnaise, mustard and ketchup sauces, after replacing the salt content of the control with 50% of each ME (mayonnaise, mustard, and ketchup) and 25% (mayonnaise), three bottles of 200 g for each batch these sauce products were analysed as replicates for each batch (Table 2).

2.3.1 Minerals

The salt content was measured by determining the potassium (K) and sodium (Na) levels using flame atomic absorption spectrophotometry (Spectr AA 55B spectrophotometer, Varian, Palo Alto, CA, United States) with a deuterium background correction, following the method described by (20). The concentrations were calculated using linear calibration curve generate from absorbance measurements of, at least, five different concentrations of standard solutions (KNO_3 and $NaNO_3$, dissolved in 0.5 M HNO_3).

The conversion of sodium to salt content was calculated based on the equivalence that 1 g of sodium is approximately equal to 2.5 g of salt (21).

2.3.2 Chloride

The chloride ion content of the mayonnaise, mustard, and ketchup analysis was determined by direct titration of a portion of the sample with 0.5 M AgNO3, according to Mohr's method (22), using a salt analyser (SALT-Matic 23, Crison, Spain).

2.3.3 Acetic acidity content and pH

Acetic acid content of the mayonnaise, mustard, and ketchup was carried out using a pH titrator (Easy Plus Easy, Mettler Toledo, United States). The pH of the sauces was determined using a pH meter (HI 2211 pH/ORP, Hanna Instruments, Romania).

2.3.4 Lipid oxidation

The oxidative state of mayonnaise samples was monitored using the 2-thiobarbituric acid (TBA) method by Abeyrathne et al. (23). Briefly, samples (5 g) were mixed with trichloroacetic acid (TCA) and ethylenediaminetetraacetic acid (EDTA), then shaken at 25°C for 2 min. The supernatant was filtered, centrifuged, and mixed with TBA solution. After incubating in boiling water for 40 min, the absorbance was measured at 530 nm. A standard curve was prepared using tetraethoxypropane (TEP) dilutions, and all determinations were performed in triplicate. The TBA index, expressed as mg of malondialdehyde (MDA) per 1,000 g sample, was calculated accordingly Equation 1 as follows:

$$TBA = \frac{72 \times c}{m \times v}(30 + mH) \qquad \text{Equation 1}$$

where *c*, is the concentration of MDA, expressed in μm, *v*, is the volume in mL, *H* is the sample humidity, in %, and *m*, is the mass of the test sample, in g. The result becomes the arithmetic mean of two parallel determinations, rounded to the nearest tenth.

2.3.5 Refractive index

The measurement of the refractive index to determine the ° Brix in ketchup condiments was performed using a refractometer (HI 96801, Hanna Instruments, Romania), at 20°C, after a calibration with distilled water.

2.3.6 Viscosity, density, and consistency

Viscosity was measured at 20°C using a rotary viscometer (Ametek Brookfield DV2T, United States) equipped with spindle 7 at 100 rpm and spindle 6 at 20 rpm for mayonnaise and mustard, respectively. Viscosity units are given in centipoise (cP). The density of the mayonnaises was determined by gravimetric method using an Erichsen pycnometer (50 mL, Mod. 209/IV, Germany) and units were expressed in pascal seconds (Pa-s). The Bostwick consistency of ketchup was assessed using a Bostwick consistometer (model LD-BC). The Bostwick consistometer measures sample flow in a graduated trough. A 50 mL compartment, separated by a spring-loaded gate, is filled and levelled. At 20°C, the gate is opened, and a stopwatch is started. After 30 s, the position of the sample is recorded with the consistency units expressed in centimetres per 30 s (cm/30s) (24).

2.3.7 Colour

The sauces colourimetry analysis was performed by measuring the colour parameters L* (luminosity between 0 - black and 100 - white), a* (reddish-green), b* (yellowish-blue) using a colourimeter Chroma meter CR-5 Konica Minolta colourimeter (Konica Minolta, Japan), using an illuminate D 65 and a 2° observation angle. The colour difference degree between the mayonnaise, mustard, and ketchup, containing different added ingredients, and control, ΔE_{ab}^* values was performed according to Macdougall (25) by means of the formula: $\Delta E_{ab}^* = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})}$ where ΔL^*, Δa^* Δb^* represent the difference between each parameter for the sauces and condiments, where each product without added ingredients was used as control. The ΔE_{ab}^* can be defined as the numerical comparison of a sample's colour to the control according to Colour difference ΔE–A Survey (26).

2.3.8 Sensorial analysis

Sensorial analysis was conducted in accordance with ISO 8586:2023 Sensory Analysis (27). A panel of untrained consumers in a blind test, comparing against a target, using a 9-point hedonic scale, where a score of 5 indicated "equal to target" and the scale ranged from "worst" to "best." The attributes measured and their descriptors were as follows: overall evaluation, taste, smell, appearance, texture and saltiness. Each product was assessed by at least seven different subjects and after microbiological tests, carried on samples using the company's internal protocols, and the results confirmed that the samples were within safe parameters, ensuring the safety of the tasters.

2.4 Statistics analysis

The statistics results were submitted to one-way ANOVA using multiple comparison tests (Tukey HSD) to identify differences between groups. Statistical analyses were tested at a 0.05 level of probability. The range, mean and relative standard deviation (RSD) of each parameter were calculated using the software, StatisticaTM 12.0 (28).

3 Results and discussion

3.1 Minerals analysis

The salt content (NaCl and KCl) in mayonnaise (M), mustard (Mu), and ketchup (K) (Figure 1) revealed significant differences ($p < 0.05$) with the addition of ingredients (F1, F2, and F3) compared to the control sauces (Co). This reduction in salt content was 50% for

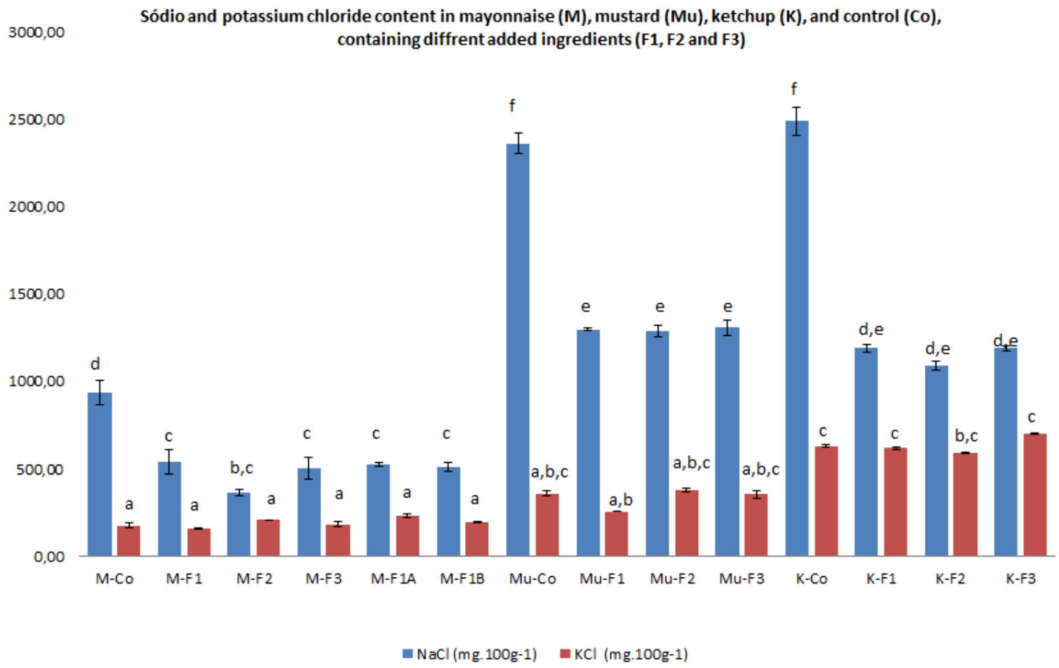

FIGURE 1
Sodium and potassium chloride content in mayonnaise (M), mustard (Mu), ketchup (K), and control (Co), containing different added ingredients (F1, F1A, F1B, F2, and F3), respectively. *Bars with the same letters are not significantly different at 5%.

the various sauces and 25% in mayonnaise when two specific ingredients (F1-A and F1-B) were used.

For mayonnaises prepared with ME ingredients, the NaCl content ranged from 360 to 540 mg per 100 g, corresponding to 145–217 mg of sodium per 100 g. In contrast, the control mayonnaise had nearly double the salt content at 937 mg per 100 g, equivalent to 375 mg of sodium per 100 g. However, many commercially available mayonnaise sauces contain much higher sodium levels, averaging 603.6 ± 54.38 mg per 100 g, as reported by (29) in mayonnaise sauces without salt reduction. Among the ME ingredients, F2 resulted in the lowest total NaCl content, although no significant differences ($p > 0.05$) were observed when compared to other ingredients (F1, F1-A, F1-B, and F3). These results fall within the EU Pledge recommendation for all emulsion-based sauces, which sets a limit of 750 mg of sodium per 100 g. The reformulation of mayonnaise sauce using ME as NaCl replacement resulted in a significant reduction of 42–62% in sodium NaCl content. Consequently, the reduction in sodium content can be applied for the nutrition claim of "reduced Na/NaCl content" (reduction ≥25% compared to the control) can be applied to all formulations developed using ME (30), aligning with dietary recommendations and public health concerns related to high sodium intake, such as hypertension and cardiovascular diseases.

For mayonnaise the KCl content in the ME formulations ranged from 159.62 to 232.89 mg per 100 g, while the control averaged 172.72 mg per 100 g. The processing did not result in significant differences ($p > 0.05$) among the ingredients, indicating that KCl is not essential in these formulations. Additionally, the increased potassium content does not support a health claim related to maintaining normal blood pressure, as the sauces contain less potassium than required (30).

The control mustard sample contained 2,363 mg per 100 g, corresponding to 945 mg of sodium per 100 g, exceeding the recommended threshold by 26%. This sodium level is slightly lower than the 1,100 mg per 100 g found in other commercial mustard brands (31, 32), but nearly double the amount detected in the reformulated mustard with ME oleoresins. In contrast, mustard samples with ME ingredients had salts content ranging from 1,289 to 1,307 mg per 100 g, equivalent to 516–523 mg of sodium per 100 g, meeting the EU Pledge recommendation for emulsion-based sauces. No significant differences ($p > 0.05$) were observed among the different ME ingredients (F1, F2, and F3). The nutrition claim can also be applied to all ME formulations (30), as each resulted in approximately a 45% reduction in sodium content.

The KCl content in ME mustard formulations ranged from 256.11 to 378.30 mg per 100 g, while the control averaged 357.45 mg per 100 g. These values also showed no significant differences ($p > 0.05$) across the ingredient formulations, indicating that the addition of KCl to the F2 formulation is unnecessary. In this case, the potassium content in the mustard sauces (≥ 350 mg per 100 g) supports a health claim related to maintaining normal blood pressure, as the sauces contain the required potassium levels (30). This may be attributed to the potassium content in the mustard seasonings that can ranged between 693 and 774 mg per 100 g (33), used during sauce processing.

In ketchup, the salt content ranged from 1,088 to 1,191 mg per 100 g, corresponding to 435–477 mg of sodium per 100 g. As expected, the control sample presented almost double this amount, with 2,489 mg of salt per 100 g, corresponding to 996 mg of sodium per 100 g. This level of sodium is similar to that found in other brands of ketchup (2), but almost double the amount detected in mustard reformulated with any of the three ME oleoresin formulations. As with mustard, no significant differences ($p > 0.05$) were observed between the different formulation (F1, F2, and F3). Salt and sodium levels in samples containing oleoresins met the EU Pledge recommendation for emulsion-based sauces, while the control exceeded this limit by 33%. Therefore, using any of the ME formulations, a reduction of around 52% in sodium content is achieved, allowing the previous nutritional claim to be applied (30).

For ketchup, the KCl content in ME formulations ranged from 588.37 to 698.89 mg per 100 g, while the control sample averaged 620.96 mg per 100 g. No significant differences ($p > 0.05$) were observed among the different ingredient formulations, indicating that adding KCl to the F2 formulation is unnecessary. With potassium content exceeding 350 mg per 100 g, these sauces already support a health claim related to maintaining normal blood pressure, as they provide more than the required potassium (30). The elevated potassium levels may be attributed to the potassium content in the tomatoes, which may vary between 403.02 ± 254.41 mg per g (34) used during sauce processing.

The chloride content in the control sauces (1.1–3.7 mg per 100 g) was statistically higher than in the formulations developed with ME oleoresins (0.6–2.0 mg per 100 g). This suggests that these formulations better align with the Dietary Reference Values (DRVs) for chloride, which range from 1.7 to 3.1 g per day for children and adults, respectively (35).

3.2 Chemical and physical changes

Regarding the physical–chemical parameters (Table 2), no significant differences ($p > 0.05$) were observed between the control and the samples with ME ingredients in the pH of the ketchup, the lipid oxidation (TBA) of the mayonnaise, and the viscosity of the mustard. However, significant differences ($p < 0.05$) were found between the control and ME samples for the pH of the mustard, the chloride content of the mustard and ketchup, and the consistency of all three sauces.

For mayonnaise, there were no significant differences ($p > 0.05$) in the pH between samples F1-A and F1-B. Additionally, no differences ($p > 0.05$) were observed in acetic acidity among all samples. A low pH and consistent acetic acid levels indicate that reducing salt and using encapsulated oleoresins did not compromise the safety and preservation of the sauces. Since acetic acid is the predominant acid, these products are expected to be toxic and destructive to bacterial pathogens, particularly under conditions of low pH and high titratable acidity (36). Regarding density, no differences ($p > 0.05$) were found between samples F1, F2, and F3, as well as between samples F1-A and F1-B.

Concerning viscosity, no significant differences ($p > 0.05$) were observed between samples F2 and F3. Unlike findings reported by other authors (37), reducing the salt content and altering the type of

TABLE 3 Colour parameters (L*, a*, b*) and total colour difference (ΔE_{ab}*) of mayonnaise (M), mustard (Mu), ketchup (K), and control (Co), containing different added ingredients (F1, F2, and F3), respectively.

	L*	a*	b*	$\Delta E_{a,b}$*
M-Co	90.00 ± 1.00^{d}	3.00 ± 0.00^{d}	$22.67 \pm 0.58^{a,b,c}$	–
M-F1	89.00 ± 1.00^{d}	4.00 ± 0.00^{d}	$23.33 \pm 0.58^{b,c}$	1.79 ± 0.42^{a}
M-F2	89.67 ± 0.58^{d}	4.00 ± 0.00^{d}	21.67 ± 0.58^{c}	1.53 ± 0.57^{a}
M-F3	89.00 ± 1.00^{d}	4.00 ± 0.00^{d}	$22.67 \pm 0.58^{a,b,c}$	1.61 ± 0.65^{a}
M-F1A	89.67 ± 0.58^{d}	4.00 ± 0.00^{d}	$22.00 \pm 0.00^{a,b,c}$	1.32 ± 0.21^{a}
M-F1B	89.00 ± 0.00^{d}	4.00 ± 0.00^{d}	$23.00 \pm 0.00^{b,c}$	1.45 ± 0.00^{a}
Mu-Co	69.33 ± 0.58^{c}	2.00 ± 0.00^{a}	49.67 ± 0.58^{d}	–
Mu-F1	67.67 ± 0.58^{c}	$2.67 \pm 0.58^{a,b}$	51.33 ± 0.58^{e}	$2.56 \pm 0.38^{a,b}$
Mu-F2	69.00 ± 0.00^{c}	$2.00 \pm 0.00^{a,b}$	51.67 ± 0.58^{d}	$2.27 \pm 0.50^{a,b}$
Mu-F3	68.33 ± 0.58^{c}	$3.00 \pm 0.00^{a,b}$	51.33 ± 0.58^{d}	$2.28 \pm 0.25^{a,b}$
K-Co	$22.00 \pm 0.00^{a,b}$	25.67 ± 0.55^{e}	20.33 ± 1.15^{a}	–
K-F1	20.33 ± 0.58^{a}	$24.00 \pm 0.00^{c,d}$	$21.67 \pm 0.58^{a,b}$	$2.75 \pm 0.60^{b,c}$
K-F2	$22.00 \pm 0.00^{a,b}$	$25.00 \pm 0.00^{d,e}$	$23.67 \pm 0.58^{b,c}$	$3.40 \pm 0.56^{b,c}$
K-F3	23.33 ± 1.53^{c}	23.00 ± 1.73^{c}	$22.67 \pm 2.08^{a,b,c}$	4.49 ± 0.98^{c}

Data are presented as the mean ± standard error of three experiments. *Samples with the same letters are not significantly different at 5%.

salt did not have a significant effect ($p > 0.05$) on viscosity. This may be attributed to the inulin used as a carrier agent for encapsulating the oleoresins, which has gelling properties that helped maintain the rheological properties of the mayonnaise (38).

For mustard, no significant differences ($p > 0.05$) were found for all physic-chemical parameters of all samples, with this sauce being the one that most resembles the control.

For ketchup, no significant differences ($p > 0.05$) were found in acidity between samples F1 and F3, not in density between the control and the F1 sample. However, significant differences ($p < 0.05$) in ketchup consistency were found between the control and the formulations containing oleoresins ME. The ketchup with F1 oleoresins ME exhibited lower consistency but had higher total soluble solids compared to the control. Typically, higher consistency is expected to correlate with higher total soluble solids, not lower (39). This discrepancy may be due to the inulin used as a carrier agent in the oleoresins ME, which dissolves in water and increases the overall concentration of soluble materials in the product.

These results indicate that ME ingredients have a selective impact on the physical and chemical properties of these sauces.

3.3 Colour parameters

Table 3 shows the total colour difference (ΔE_{ab}*) between samples with ME ingredients (F1, F1-A, F1-B, F2, and F3) compared to the control in the three types of sauces.

For mayonnaise, the lowest ΔE_{ab}* values were obtained, with no significant differences ($p > 0.05$) observed among all samples containing ME ingredients. Specifically, the ΔE_{ab}* values ranging from 1 to 2 for mayonnaise with ME (F1-A and F2-B) and a 25% salt reduction, as well as for mayonnaise with ME (F1 and F2) and a 50% salt reduction, were unnoticeable except to experienced consumers (26).

The colour difference for mustard is noticeable within the range of $2 < \Delta E_{ab}^{*} < 3.5$. In contrast, for ketchup, the highest ΔE_{ab}* value observed was 4.49 for ME-F3, indicating significant differences ($p < 0.05$) among the colour differences ($3.5 < \Delta Eab^{*} < 5$) of all ketchup samples containing ME ingredients. This suggests that the light-yellow colour of the ME ingredients has a more pronounced impact on darker sauces like ketchup, resulting in higher ΔE_{ab}* values.

3.4 Sensory analysis

The tasters rated the products positively overall, with an average score of around 5 on the hedonic scale of 1–9, that is, sensory analysis of all products revealed profiles similar to the control for all parameters (aroma, flavour, appearance, saltiness, texture and overall appearance) evaluated (Figures 2A–C). For mayonnaise, no significant differences were found between the formulations with ME oleoresins and the control, with this sauce being the one that most resembles the control.

While mustard presents slightly lower scores when compared to the control, mainly for flavour ($x = 4.1 \pm 1.4$) and salt ($x = 4.2 \pm 1.3$). Regarding ketchup sauce, the same trend was observed as for mayonnaise.

It was also found that there was no statistically significant difference between the samples.

By combining the results obtained, it was found that there was no preference for one sample over the other, so the panel members considered, based on the parameters evaluated, in the three new products, that they were not perceived by consumers, nor did they affect their acceptance.

These products are sauces used to enhance the flavour of foods in which salt is a relevant component. Several efforts have been made to reduce salt content of different foods, but no optimal solution has been found yet, and most of the studies highlight the perceived changes in taste due to the lack of salt or altered taste attributed to the substitute used (6, 9, 40). The fact that there were few differences in sensory

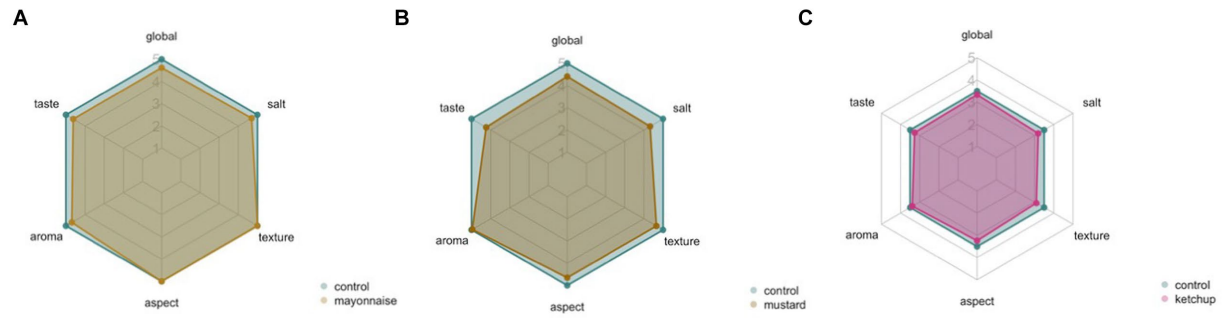

FIGURE 2
Sensory profile of mayonnaise **(A)**, mustard **(B)** and ketchup **(C)** sauces (after processing, $t = 0$). The results are expressed as mean values ± SD. A 5-point intensity scale was used, where 1 represents the worst and 5 is the best, with 5 indicating "equal to target." The evaluation parameters included: "global evaluation," "taste," "smell," "appearance," "texture," and "salt."

analysis when compared to the control, highlights the potential of the formulations to reduce the salt content of each product.

4 Conclusion

The incorporation of microencapsulated (ME) oleoresins in mayonnaise, mustard, and ketchup formulations successfully achieved significant salt reductions, ranging from 25 to 52%, while maintaining compliance with sodium guidelines set by the EU Pledge. The potassium content in mustard and ketchup was sufficient to support health claims related to maintaining normal blood pressure, highlighting the nutritional benefits of these reformulated sauces. Additionally, the reformulated sauces demonstrated lower chloride content, better aligning with dietary reference values and offering potential health benefits by reducing the risk of hypertension and cardiovascular diseases.

The study also revealed that the ME ingredients had minimal impact on the essential physical–chemical parameters of the sauces, such as pH, lipid oxidation, and viscosity, indicating that the basic integrity and texture of the products were preserved. However, some selective changes were observed, particularly in the consistency and chloride content of the sauces. Inulin, used as a carrier agent for the ME ingredients, played a crucial role in maintaining the rheological properties of the mayonnaise, further supporting the viability of these formulations.

Colour analysis showed that the ME ingredients had varying effects depending on the sauce. The colour difference was minimal in mayonnaise, moderate in mustard, and more pronounced in ketchup, particularly in darker formulations. Despite these variations, the sensory analysis confirmed that the overall sensory profiles of the reformulated sauces closely resembled the control samples, with mayonnaise showing the closest similarity. Mustard displayed slightly lower sensory scores, particularly in flavour and saltiness, though these differences were modest.

Overall, this study demonstrates that ME ingredients can be effectively used in condiment reformulation to achieve significant salt reduction while maintaining sensory quality, supporting health claims, and meeting regulatory standards. These findings underscore the potential of ME formulations to offer healthier condiment options without compromising consumer satisfaction.

Future research should focus on the long-term stability, microbial safety, and broader consumer acceptance of these reformulated products, while also assessing their impact on food safety and shelf-life for wider adoption.

The market for these sauces has a very interesting volume to invest in innovation and development. Thus, the formulation of innovative sauces can bring added value to the company that launches them, by presenting a tasty and "traditional" product, with a low salt content, meeting the current expectations and consumer's needs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

CS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. DC-B: Investigation, Resources, Writing – review & editing. AT: Formal analysis, Investigation, Writing – review & editing. AM: Investigation, Resources, Writing – review & editing. CV: Investigation, Writing – review & editing. DF: Investigation, Resources, Writing – review & editing. NK: Writing – review & editing.

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Conflict of interest

DC-B, AT, DF, and NK were employed by Mendes Gonçalves SA.

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References

1. Grand View Research Inc. (2024). Available at: https://www.grandviewresearch.com/press-release/global-sauces-dressings-condiments-market (Accessed June 5, 2024).

2. Shahar S, You YX, Zainuddin NS, Michael V, Ambak R, Haron H. Sodium content in sauces—a major contributor of sodium intake in Malaysia: a cross-sectional survey. *BMJ Open*. (2019) 9:e025068. doi: 10.1136/bmjopen-2018-025068

3. Action on Salt. Salt content of sauces. (2019). Available at: https://www.actiononsalt.org.uk/media/action-on-salt/news/surveys/2019/Salt-Content-of-Sauces-Report.pdf. (Accessed June 5, 2024).

4. World Health Organization, SHAKE, The salt habit, The SHAKE technical package for salt reduction. WHO. Geneva: WHO Library (2016).

5. World Health Organization. Using dietary intake modelling to achieve population salt reduction: A guide to developing a country-specific salt reduction model. Geneva: WHO Library (2018).

6. National Salt Initiatives Implementing the EU Framework for Salt Reduction Initiatives. (2009). Available at: https://health.ec.europa.eu/other-pages/basic-page/salt-campaign_en (Accessed June 13, 2023).

7. Valentová H, Panovská Z. Sensory evaluation | taste In: B Caballero, editor. Encyclopedia of Food Sciences and Nutrition. *2nd* ed. New York, NY: Academic Press (2003)

8. Coregliano-Ring L, Goia-Nishide K, Rangel ÉB. Hypokalemia in diabetes mellitus setting. *Medicina*. (2022) 16, 58:431. doi: 10.3390/medicina58030431

9. Olfa Ben Braïek and Slim Smaoui. Chemistry, safety, and challenges of the use of organic acids and their derivative salts in meat preservation. *J Food Quality*. (2021) 2021:1–20. doi: 10.1155/2021/6653190

10. Kemp SE, Beauchamp GK. Flavor modification by sodium chloride and monosodium glutamate. *Food Sci*. (1994) 59:682–6. doi: 10.1111/j.1365-2621.1994.tb05592.x

11. Rosa A, Pinna I, Piras A, Porcedda S, Masala C. Flavoring of sea salt with Mediterranean aromatic plants affects salty taste perception. *J Sci Food Agric*. (2022) 102:6005–13. doi: 10.1002/jsfa.11953

12. González-Pérez S, Arellano JB. 15 - vegetable protein isolates In: GO Phillips and PA Williams, editors. Woodhead publishing series in food science, technology and nutrition, handbook of hydrocolloids. *2nd* ed. London: Woodhead publishing (2009)

13. Conseil Européen de l'Information sur l'Alimentation. Sécurité alimentaire et qualité des aliments - Additifs alimentaires – Le glutamate monosodique. (2024). Available at: http://www.eufic.org/article/fr/Securite-alimentaire-qualite-aliments/additifs-alimentaires/artid/glutamate-monosodique/v (Accessed June 5, 2024).

14. Shoaib M, Shehzad A, Omar M, Rakha A, Raza H, Sharif HR, et al. Inulin: properties, health benefits and food applications. *Carbohydr Polym*. (2016) 147:444–54. doi: 10.1016/j.carbpol.2016.04.020

15. Serrano C, Sapata M, Oliveira M, Gerardo A, Viegas C. Encapsulation of oleoresins for salt reduction in food. *Acta Sci Pol Technol Aliment*. (2020) 19:57–71. doi: 10.17306/J.AFS.0772

16. Viegas C, Gerardo A, Mendes L, Ferreira R, Damas C, Sapata M, et al. Use of microencapsulation of aromatic plants and spices as a strategy for salt reduction for food and cooking. *J Culin Sci Technol*. (2022) 17:1–13. doi: 10.1080/15428052.2022.2088435

17. Rybicka I, Silva M, Gonçalves A, Oliveira H, Lourenço H, Marques A, et al. Development and quality assessment of smoked rainbow trout with reduced sodium content. *Int J Food Sci Technol*. (2023) 58:3658–68. doi: 10.1111/ijfs.16468

18. Estévez A, Camacho C, Correia T, Barbosa V, Marques A, Lourenço H, et al. Strategies to reduce sodium levels in European seabass sausages. *Food Chem Toxicol*. (2021) 153:112262. doi: 10.1016/j.fct.2021.112262

19. Oliveira H, Nabais A, Cristóvão M, Camacho C, Özkan B, Pires C, et al. Strategies to reduce sodium levels in canned fish products. 37th EFFoST International Conference 2023, Sustainable Food and Industry 4.0: Towards the 2030 Agenda, 6–8 November 2023 (2023). Available at: https://effostconference.com/wp-content/uploads/Abstract-Book-Posters-EFFoST-2023.pdf

20. Jorhem L. Determination of metals in foods by atomic absorption spectrometry after dry Ashing: NMKL1 collaborative study. *J AOAC Int*. (2000) 83:1204–11. doi: 10.1093/jaoac/83.5.1204

21. EU. Regulation (EU) no 1169/2011 of the European parliament and of the council on the provision of food information to consumers. *Off J Eur Union*. (2011) 304:18–63. Available at: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:en:PDF

22. Belcher R, Macdonald AMG, Parry E. On Mohr's method for the determination of chlorides. *Anal Chimica Acta*. (1957) 16:524–9. doi: 10.1016/S0003-2670(00)89979-1

23. Abeyrathne EDNS, Nam K, Ahn DU. Analytical methods for lipid oxidation and antioxidant capacity in food systems. *Antioxidants*. (2021) 10:1587. doi: 10.3390/antiox10101587

24. Mazaheri Tehrani M, Ghandi A. Modification of Bostwick method to determine tomato concentrate consistency. *J Food Eng*. (2007) 79:1483–6. doi: 10.1016/j.jfoodeng.2006.01.093

25. Macdougall DB. Colour in food. *1st* ed. England: Woodhead Lda (2002).

26. Colour Difference Delta E–A Survey. (2024) Available at: https://www.researchgate.net/publication/236023905_Colour_difference_Delta_E_-_A_survey (Accessed June 5, 2024).

27. ISO 8586:2023 Sensory Analysis. Selection and training of sensory assessors published (2023). Available at: https://www.iso.org/standard/76667.html

28. StatSoft. Inc. STATISTICA (data analysis software system) version 12. Tulsa, OA: StatSoft. Inc. (2024).

29. Rashed A, Noh M, Mohd MK, Rahman N, Tasirin A, Omar W, et al. The nutritional composition of mayonnaise and salad dressing in the Malaysian market. *Sains Malaysiana*. (2017) 46:139–47. doi: 10.17576/jsm-2017-4601-18

30. European Parliament and Council Regulation (EC). No 1924/2006 of the European Parliament and the council on nutrition and health claims made on foods. *Off J Eur Union*. (2006) 22:9–25. Available at: https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32006R1924

31. (2024) Available at: https://foodstruct.com/food/mustard (Accessed August 12 2024).

32. (2024) Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/172234/nutrients (Accessed August 12 2024).

33. (2024) Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/170929/nutrients (Accessed August 12 2024).

34. Ali MY, Sina AA, Khandker SS, Neesa L, Tanvir EM, Kabir A, et al. Nutritional composition and bioactive compounds in tomatoes and their impact on human health and disease: a review. *Food Secur*. (2020) 10:10045. doi: 10.3390/foods10010045

35. Turck D, Castenmiller J, de Henauw S, Hirsch-Ernst KI, Kearney J, Knutsen HK, et al. Dietary reference values for chloride. *EFSA*. (2019) 17:e05779. doi: 10.2903/j.efsa.2019.5779

36. Smittle RB. Microbiological safety of mayonnaise, salad dressings, and sauces produced in the United States: a review. *J Food Prot*. (2000) 63:1144–53. doi: 10.4315/0362-028x-63.8.1144. PMID: 10945595

37. Alvarez-Sabatel S, Martínez I, de Marañón J-C. Impact of oil and inulin content on the stability and rheological properties of mayonnaise-like emulsions processed by rotor-stator homogenisation or high pressure homogenisation (HPH). *Innov Food Sci Emerg Technol*. (2018) 48:195–203. doi: 10.1016/j.ifset.2018.06.014

38. Tavares E, Esmerino E, Santos-Junior V, Medeiros A, Bolini H. Dynamic aspects of salt reduction in tomato sauce by use of flavor enhancers and a bitter blocker. *Food Sci Technol Int*. (2020) 26:549–59. doi: 10.1177/1082013220913361

39. Torbica A, Belović M, Mastilović J, Kevrešan Ž, Pestorić M, Škrobot D, et al. Nutritional, rheological, and sensory evaluation of tomato ketchup with increased content of natural fibres made from fresh tomato pomace, food and bioproducts processing. *Food Bioproducts Proc*. (2016) 98:299–309. doi: 10.1016/j.fbp.2016.02.007

40. Jaenke R, Barzi F, McMahon E, Webster J, Brimblecombe J. Consumer acceptance of reformulated food products: a systematic review and meta-analysis of salt-reduced foods. *Crit Rev Food Sci Nutr*. (2017) 23;57:3357–72. doi: 10.1080/10408398.2015.11

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Spatiotemporal patterns of rheumatic heart disease burden attributable to high systolic blood pressure, high sodium diet, and lead exposure (1990 to 2019): a longitudinal observational study

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Background: Rheumatic heart disease (RHD) continues to be a significant global health concern, exhibiting unique regional disparities. Although there is a noted decline in the burden of RHD, the specific causatives for this decrease remain unclear. This study aims to identify and quantify the spatiotemporal patterns related to the RHD-attributable risk burden.

Methods: The data pertaining to deaths and disability-adjusted life years (DALYs) attributable to RHD risk were drawn from the Global Burden of Disease (GBD) study conducted from 1990 to 2019. These data, categorized by age, gender, and geographical location, highlighted risk factors including diets high in sodium, elevated systolic blood pressure (SBP), and lead exposure. To examine the long-term trends in RHD changes due to these specific risk factors, the average annual percentage change (AAPC) method was used.

Results: During the past 30 years, the highest decrease in RHD burden was attributed to high SBP. An AAPC of −2.73 [95% confidence interval (CI): −2.82 to −2.65] and − 2.45 (95% CI: −2.55 to −2.36) in deaths and DALYs was attributable to high SBP, while an AAPC of −3.99 (95% CI: −4.14 to −3.85) and − 3.74 (95% CI: −3.89 to −3.6) in deaths and DALYs was attributed to a diet high in sodium. Moreover, the trends in deaths and DALYs due to lead exposure also showed decreases with an AAPC of −2.94 (95% CI: −3 to −2.89) and − 3.46 (95% CI: −3.58 to −3.34) from 1990 to 2019. Oceania showed an upward trend of the RHD DALYs due to high SBP, with an AAPC of 0.23 (95% CI: 0.13 to 0.33). In general, countries in Oceania, East Asia, and South Asia had higher age-standard deaths and DALY rates of RHD due to diets high in sodium.

Conclusion: Our study has revealed that high SBP remains the prime risk factor contributing to the RHD burden. There are decreasing spatiotemporal patterns in RHD-related deaths and burdens. Gaining this knowledge is fundamental to making informed public health strategies and clinical decisions, especially concerning risk assessment, screening, and prevention initiatives.

KEYWORDS

age-period-cohort model, rheumatic heart disease, global burden, risk factors, socio-demographic index

Introduction

Rheumatic heart disease (RHD) is indeed a serious global health issue, resulting from acute rheumatic fever and leading to long-term heart complications. It poses a significant challenge, particularly in low- and middle-income regions such as South Asia and Sub-Saharan Africa (1, 2). According to a previous report, the DALYs for RHD were 10,673,882 in 2019 (3). As for mortality, the number of deaths due to RHD was approximately 0.31 million in 2019, underscoring its continued global impact (3).

Generally, there were many factors associated with the development of RHD, including overcrowding and poor housing, socioeconomic factors, improper health literacy, genetic background, and even inequity in healthcare (4, 5). Moreover, many metabolic, environmental, and behavioral factors, such as a diet high in sodium, high SBP, and lead exposure, are also associated with RHD (6). While RHD primarily results from an autoimmune reaction following streptococcal infections, emerging evidence suggests that elevated blood pressure, high sodium intake, and lead exposure can exacerbate cardiovascular damage and potentially influence the course of RHD. These factors May contribute to systemic inflammation, endothelial dysfunction, and overall cardiovascular burden, which can indirectly affect individuals with RHD. However, there has been no comprehensive estimation of the RHD-related burden attributable to these factors.

To the best of our understanding, the GBD is the sole dataset that provides a detailed view of the disease burden due to a particular set of risk factors. The database uses rigorous quality assurance procedures and presents evolving models across various scales over time. In addition, GBD 2019 furnishes the most recent data on each risk-outcome pair for cardiovascular diseases (CVDs) in 204 nations and territories, including their aggregated regions, spanning from 1990 to 2019. This provision facilitates the assessment of the burden attributable to risks for countries with low-income economies. Additionally, robust epidemiological evidence, such as a comprehensive spatiotemporal assessment of the RHD-related burden associated with these factors, would aid the development of effective prevention strategies that reduce the disease burden. Herein, we conducted this comparative study to estimate the burden and trends of RHD attributable to a diet high in sodium, elevated SBP, and lead exposure among different populations at global, regional, and national levels using the latest data from the GBD dataset, aiming to provide a comprehensive basis for the robust, evidence-based development of disease prevention strategies.

Methods

Data source

Data on the burden of RHD attributable to a diet high in sodium, high SBP, and lead exposure–across global, regional, and national levels, stratified by age and sex–were retrieved from the GBD 2019 datasets released by the Institute for Health Metrics and Evaluation (IHME). The GBD 2019 study used DisMod-MR 2.1, a Bayesian meta-regression tool, to estimate disease-related measures, including disability, across 369 diseases and injuries and 87 risk factors in 204 countries and territories over the past three decades (7). The population and data utilized in our study are derived from the GBD database, which aggregates data from a wide range of sources, including vital registration systems, health surveys, hospital records, and scientific literature. The GBD database covers nearly every country and region worldwide and systematically collects data to estimate disease burden over time. For our study, we analyzed data spanning from 1990 to 2019, focusing on the RHD burden attributable to high SBP, high sodium diet, and lead exposure. While the GBD study provides extensive global coverage, individual-level data or specific cohort details are not available as it uses aggregated data to generate estimates at the population level. This allows for a comprehensive analysis of disease burden trends over time across different regions and populations. In this study, the burden indicators of RHD included mortality numbers (X 1,000), DALY numbers (X 1,000), the age-standardized mortality rate (per 100,000), and the age-standardized DALY rate (per 100,000). Comprehensive methodology and statistical modeling for the GBD 2019 have been published elsewhere (7, 8). Briefly, the dietary intake of sodium was evaluated by calculating the average 24-h urinary sodium excretion, which was specified in grams per day. High dietary sodium was defined as an excretion exceeding 1–5 grams per day. High SBP was identified through the measurement of brachial SBP, reported in mmHg. The benchmark data used for mean SBP were derived from literary sources and household survey data, including reports such as STEPS and NHANES as per the GBD 2019. Regarding lead exposure, it has been distinguished in two separate ways, each corresponding to its unique pathway leading to attributable health losses. Acute lead exposure was measured in micrograms of lead per deciliter of blood (μg/dL), a condition known to be associated with IQ reduction in children. On the other hand, chronic lead exposure was measured in micrograms of lead per gram of bone (μg/g), a metric associated with elevated SBP and increased incidence of CVDs.

GBD study has been approved by the University of Washington Institutional Review Board. All CVDs were identified by the International Classification of Diseases and Injuries (ICD), 10th Revision.

The sociodemographic index (SDI) serves as a comprehensive assessment of health-related development within a given region or country. This composite indicator is calculated based on fertility rates, *per capita* income, and the level of educational attainment. The SDI values extend from 0 to 1, with low values indicating a low degree of health-related development and high values indicating a high degree of health development at both national and regional levels.

Based on the quintiles of the SDI, all countries are categorized into one of the five SDI zones, namely: low-SDI, low-middle-SDI,

middle-SDI, high-middle-SDI, and high-SDI regions. This classification provides a structured framework for comparative analysis of health-related development across different regions and nations worldwide.

Statistical analysis

The mortality and DALY rates per 100,000 population, inclusive of their 95% uncertainty intervals (UIs), attributed to RHD, are outlined by both age and gender. We examined the temporal trend shifts of RHD mortality and DALYs linked to a diet high in sodium, high SBP, and lead exposure through Joinpoint regression analysis (version 4.9.0.1 from the Statistical Research and Applications Branch, National Cancer Institute).

To depict the temporal trends of RHD mortality attributable to a diet high in sodium, high SBP, and lead exposure, we computed the average annual percent change (AAPC) and its corresponding 95% CI utilizing the Monte Carlo substitution method. We interpreted an increasing trend if the AAPC and its 95% CI exceeded zero. Conversely, an AAPC and its 95% CI of less than zero signified a decreasing trend. Any other values indicated a stable trend.

Results

Burden of RHD attributable to high SBP

Globally in 2019, there was a total number of 76.4 (51 to 114.7) deaths and 2446.4 (1672.9 to 3445.3) DALYs attributable to high SBP-related RHDs (Table 1). The corresponding rates were 1 death and 29.7 DALYs per 100,000 population (Table 1). The top three regions with high death rates were mainly in Oceania, South Asia, and Central Asia (Table 1), and the highest record of death rate and DALYs rate was observed in Vanuatu (4.9 per 100,000) and Indonesia (174.4 per 100,000) in 2019, respectively (Supplementary Tables S1, S2). From 1990 to 2019, the rates of RHD deaths and DALYs associated with high SBP showed decreasing trends (Figure 1). There was a spatially heterogeneous burden of RHD due to high SBP. Countries in high-income Asia Pacific, Eastern Europe, and Central Europe had the most significant decline, and Singapore, Hungary, and Poland were the top three countries that had fallen the furthest (Supplementary Tables S1, S2). Notably, Oceania was the only region to show an upward trend in RHD DALYs due to high SBP, with an AAPC of 0.23 (95%CI: 0.13 to 0.33), while the death rate showed a downward trend, with an AACP of −0.2 (95%CI: −0.28 to −0.11).

Among SDI quintiles, the global high SBP-related RHDs burden varied worldwide. In 2019, the highest death and DALY numbers were observed by the low-middle SDI quintile (Table 1). Furthermore, the highest death and DALY rates were observed in the low-middle SDI quintiles (Table 1). The high-SDI quintile experienced the lowest burden, while the high-middle SDI quintile had the highest downward trend for both death and DALY rates over the past three decades (Figure 1).

Significant gender differences existed in high SBP-related RHD burden. Females had much higher death rates than males, both globally and regionally (Figures 2A,B). In different age stratifications, males had much higher death numbers and rates than females before 40 years, while females showed higher death rates older than 40 years old in 2019 (Figures 3A,B). The most significant sex difference occurred in South Asia (Figures 2A,B). Some other notable patterns were observed in low-middle and low-SDI regions. During the past three decades, the trends of deaths and DALYs attributable to high SBP declined across all age groups (Figures 4A,B).

Both age-standardized rates of death and DALYs of RHD due to high SBP showed a moderate negative correlation with the SDI in 2019 (correlation coefficients were approximately −0.47 and − 0.41, respectively) at the national level (Figures 5A,B).

Burden of RHD attributable to a diet high in sodium

In 2019, globally, a total number of 11.9 (95%UI: 2.8 to 29.1) deaths and 390.8 (95%UI: 98.1 to 930.5) DALYs were caused by RHD due to a diet high in sodium, decreasing by 38 and 37% from 1990, respectively (Table 1). The countries with the highest absolute burden were China deaths: 5.5 (1.9 to 11.5) and DALYs: 163.9 (68.5 to 304.4; Supplementary Tables S3, S4). India, Pakistan, Brazil, the Democratic People's Republic of Korea, and Bangladesh were all on the list of the most burdened countries. The global age-standardized rates of deaths and DALYs were 0.1 (0 to 0.4, per 100,000) and 4.7 (1.2 to 11.2, per 100,000), respectively, with an AAPC of −3.99 (−4.14 to −3.85), and − 3.74 (−3.89 to −3.6) (Table 1). In general, countries in Oceania, East Asia, and South Asia had higher rates of deaths and DALYs (Table 1). Pakistan had the highest recorded death rate (0.5 per 100,000), and Solomon Islands had the highest DALY rate (14.2 per 100,000) (Supplementary Tables S3, S4). In terms of the temporal changes, high sodium intake attributable to RHD deaths and DALYs decreased annually across the world from 1990 to 2019, except for Belgium and Pakistan (Supplementary Tables S3, S4).

All SDI quintiles exhibited a downward trend in the absolute RHD burden due to high sodium intake during the study period (Table 1). Of which, The middle-SDI quintile led the largest reduction in RHD burden due to high sodium intake, with an AAPC of −5.21 (−5.5 to −4.92; Table 1) in deaths and an AAPC of −5.05 (−5.24 to −4.86; Table 2) in DALYs. Meanwhile, the rates of deaths and DALYs were higher in the low-middle SDI quintile in 2019 (Table 1).

Additionally, males had a higher burden of RHD than females worldwide (Figures 2C,D). A massive sex disparity was observed in Oceania, Central Asia, and East Asia, where death and DALY rates of men were almost three times those of women (Figures 2C,D). However, males had a lower burden of RHD than females in Central Sub-Saharan Africa, and Eastern Sub-Saharan Africa. In 2019, the age-standardized rates of deaths and DALYs from RHD related to high sodium intake increased with age (Figures 3C,D). Moreover, high sodium intake-related deaths and DALYs declined in each age group during the past 30 years, with the highest decrease noted in the 45–49 years age group (Figures 4A,B).

Both age-standardized rates of death and DALYs of RHD due to a diet high in sodium showed a strong negative correlation with the SDI in 2019 (correlation coefficients were approximately −0.46 and − 0.38, respectively) at the national level (Figures 5C,D).

Burden of RHD attributable to lead exposure

In 2019, globally, there were 7.7 (95% UI: 4.1 to 13.5) RHD deaths attributable to lead exposure, with a rate of 0.1 per 100,000 (95% UI: 0 to 0.2) (Table 1). Additionally, DALYs attributable to lead

TABLE 1 Global burden of rheumatic heart disease burden attributable to high systolic blood pressure, diet high in sodium, and lead exposure from 1990 to 2019.

Attributable factors	1990				2019			
	Death (X1000, persons, 95% UI)	ASRM (95% UI)	DALY (X1000, persons, 95% UI)	ASRD (95% UI)	Death (X1000, persons, 95% UI)	ASRM (95% UI)	DALY (X1000, persons, 95% UI)	ASRD (95% UI)
High systolic blood pressure								
Global	84 (56 to 127.7)	2.1 (1.4 to 3.3)	2677.4 (1784.6 to 3,904)	61.2 (41.1 to 89.8)	76.4 (51 to 114.7)	1 (0.6 to 1.4)	2446.4 (1672.9 to 3445.3)	29.7 (20.3 to 41.9)
High SDI	8.3 (5.3 to 13.8)	0.8 (0.5 to 1.3)	180.7 (123.7 to 276.8)	17.7 (12 to 26.5)	6 (3.3 to 11.7)	0.3 (0.2 to 0.5)	101.2 (65.3 to 174.3)	5.5 (3.7 to 8.9)
High-middle SDI	17.9 (12.2 to 27.2)	1.7 (1.2 to 2.6)	537.5 (375.1 to 781.3)	48 (33.7 to 71.5)	9.8 (6.4 to 16)	0.5 (0.3 to 0.8)	272.8 (186.3 to 408.3)	14 (9.7 to 21.1)
Middle SDI	26.2 (17 to 42.3)	2.7 (1.7 to 4.6)	824.5 (534.2 to 1225.5)	69.4 (45.8 to 109.7)	21.4 (14.3 to 33.7)	0.9 (0.6 to 1.5)	669 (460.2 to 987.4)	26.1 (17.6 to 38)
Low-middle SDI	23.4 (15.1 to 35.5)	3.8 (2.4 to 5.9)	828.4 (519.6 to 1,225)	111.4 (69.7 to 167.7)	28 (18.3 to 40.7)	2.1 (1.3 to 3.1)	965 (623.1 to 1347.3)	62.2 (41.1 to 87.6)
Low SDI	8.2 (4.9 to 13.3)	3.3 (2 to 5.6)	305.3 (186.2 to 481)	100.3 (61.2 to 161.1)	11.1 (7.3 to 16.4)	2.1 (1.3 to 3.2)	437.1 (283.9 to 621.2)	63.9 (42 to 91.4)
Andean Latin America	0.1 (0.1 to 0.2)	0.5 (0.3 to 0.8)	3.7 (2.1 to 6.4)	14.8 (8.9 to 25.8)	0.1 (0.1 to 0.2)	0.2 (0.1 to 0.4)	6.3 (3.8 to 10.5)	10.3 (6.3 to 17.1)
Australasia	0.2 (0.1 to 0.3)	0.7 (0.5 to 1.2)	3.9 (2.7 to 6)	16.8 (11.5 to 25.5)	0.1 (0.1 to 0.3)	0.3 (0.2 to 0.5)	2.8 (1.8 to 4.7)	6.2 (4.2 to 9.8)
Caribbean	0.3 (0.2 to 0.5)	1.1 (0.7 to 1.7)	13.3 (7.8 to 21)	44 (26.3 to 70.6)	0.3 (0.2 to 0.5)	0.7 (0.4 to 1.1)	16.2 (9.4 to 25.3)	32.2 (18.6 to 50.7)
Central Asia	1.2 (0.8 to 1.7)	2.2 (1.5 to 3.1)	48.6 (32.3 to 69.3)	85.9 (58.5 to 123)	1 (0.7 to 1.5)	1.2 (0.8 to 1.8)	41.7 (27.1 to 61.1)	44.7 (29.8 to 64.7)
Central Europe	3.1 (2.1 to 4.5)	2.1 (1.5 to 3)	91.5 (64.6 to 129.6)	62.4 (44.7 to 88.9)	0.8 (0.5 to 1.3)	0.4 (0.2 to 0.6)	19.2 (12.9 to 29.1)	10 (6.8 to 14.8)
Central Latin America	0.6 (0.4 to 1)	0.7 (0.5 to 1)	24.8 (15.7 to 38.2)	22.9 (15.4 to 36.5)	0.4 (0.3 to 0.6)	0.2 (0.1 to 0.3)	20.2 (13.5 to 31)	8 (5.4 to 12.5)
Central Sub-Saharan Africa	0.5 (0.3 to 0.8)	2 (1.1 to 3.4)	20.4 (11.9 to 31.1)	65.9 (40.9 to 103.9)	0.6 (0.3 to 1)	1.1 (0.6 to 2)	29.3 (16.9 to 47.9)	38.5 (22.5 to 63.6)
East Asia	27.8 (16.9 to 47)	3.7 (2.2 to 6.7)	763.5 (481.6 to 1180.3)	82.4 (51.6 to 134.5)	18.8 (11.7 to 30.1)	1 (0.6 to 1.7)	464.5 (304.1 to 706.9)	23.1 (15.2 to 35.3)
Eastern Europe	4.8 (3.3 to 7.2)	1.7 (1.2 to 2.5)	165.6 (117.3 to 239.5)	59.7 (42.4 to 87.4)	1.4 (1 to 2.2)	0.4 (0.3 to 0.7)	47.1 (31.8 to 70.2)	14.9 (10.3 to 21.8)
Eastern Sub-Saharan Africa	0.7 (0.4 to 1)	0.9 (0.5 to 1.4)	33 (19.7 to 49.2)	31.4 (19.8 to 47)	0.9 (0.6 to 1.4)	0.6 (0.4 to 1)	64.8 (38.3 to 100.5)	25.1 (16.2 to 37.7)
High-income Asia Pacific	1.1 (0.7 to 1.7)	0.6 (0.4 to 1)	22.4 (15.4 to 33.5)	11.4 (7.9 to 17.3)	1.1 (0.5 to 2.4)	0.2 (0.1 to 0.4)	14.6 (8.6 to 28.1)	3 (2 to 5.2)
High-income North America	2.4 (1.5 to 4)	0.7 (0.4 to 1.1)	52.1 (34.7 to 81.7)	15.1 (10.2 to 23.2)	1.5 (0.8 to 2.9)	0.2 (0.1 to 0.4)	30.8 (19.4 to 52.6)	5.1 (3.3 to 8.5)
North Africa and Middle East	1.9 (1.1 to 3.5)	1 (0.6 to 2.1)	72.3 (43.2 to 124.3)	32.9 (20 to 58.1)	1.9 (1.2 to 3.1)	0.4 (0.3 to 0.7)	84.9 (54.3 to 134.1)	15.3 (10 to 24.6)
Oceania	0.1 (0 to 0.2)	2.9 (1.4 to 5.2)	3.8 (1.8 to 6.7)	90 (44.7 to 159.4)	0.2 (0.1 to 0.4)	2.7 (1.4 to 4.7)	10.2 (4.9 to 17.7)	96.4 (47.8 to 163.6)
South Asia	28.6 (18 to 43.3)	4.9 (3.1 to 7.8)	1038.7 (647.8 to 1552.7)	142.3 (90.4 to 216.5)	38.4 (24.9 to 55)	2.7 (1.8 to 4.1)	1309.4 (851.4 to 1839.4)	81.5 (54 to 113.7)
Southeast Asia	2.4 (1.5 to 3.6)	0.9 (0.6 to 1.5)	90.8 (55 to 135.7)	27.1 (17.4 to 42.1)	1.8 (1.2 to 2.6)	0.3 (0.2 to 0.5)	77.4 (50.5 to 113.5)	11.3 (7.5 to 16.5)
Southern Latin America	0.5 (0.3 to 0.8)	1.2 (0.7 to 2.1)	12.5 (8.2 to 19.8)	27.1 (18 to 43)	0.4 (0.3 to 0.8)	0.5 (0.3 to 1)	10.5 (6.8 to 16.4)	13.3 (8.6 to 20.8)
Southern Sub-Saharan Africa	0.4 (0.2 to 0.5)	1.1 (0.7 to 1.6)	19.3 (11.4 to 28.5)	48.7 (30.3 to 70)	0.4 (0.2 to 0.5)	0.6 (0.4 to 0.9)	22.8 (13.7 to 34.2)	30.2 (19.2 to 44.4)
Tropical Latin America	0.7 (0.5 to 1.1)	0.7 (0.5 to 1.1)	36.4 (22.2 to 56.6)	29.6 (19.4 to 46.7)	0.7 (0.5 to 1.1)	0.3 (0.2 to 0.4)	42.8 (27.2 to 66.4)	17 (10.8 to 26.5)
Western Europe	5.7 (3.7 to 9.4)	1 (0.6 to 1.6)	116.9 (78.9 to 179.5)	20.9 (14.3 to 31.3)	4.2 (2.2 to 8.2)	0.4 (0.2 to 0.7)	61 (37.4 to 107.9)	6.6 (4.3 to 10.6)
Western Sub-Saharan Africa	1.2 (0.7 to 2.2)	1.5 (0.9 to 2.7)	43.8 (26.3 to 73.8)	41.5 (25.2 to 71.6)	1.3 (0.8 to 2.1)	0.7 (0.4 to 1.2)	69.9 (43.4 to 116.1)	25.9 (17 to 41.9)

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TABLE 1 (Continued)

Attributable factors	1990				2019			
	Death (X1000, persons, 95% UI)	ASRM (95% UI)	DALY (X1000, persons, 95% UI)	ASRD (95% UI)	Death (X1000, persons, 95% UI)	ASRM (95% UI)	DALY (X1000, persons, 95% UI)	ASRD (95% UI)
Diet high in sodium								
Global	19.1 (6.4 to 42.3)	0.5 (0.2 to 1)	624.1 (213.1 to 1323.9)	14.2 (4.8 to 30.3)	11.9 (2.8 to 29.1)	0.1 (0 to 0.4)	390.8 (98.1 to 930.5)	4.7 (1.2 to 11.2)
High SDI	0.8 (0.2 to 2.2)	0.1 (0 to 0.2)	20.2 (5.3 to 49.6)	2 (0.5 to 4.9)	0.6 (0.1 to 2)	0 (0 to 0.1)	11.5 (2 to 32.2)	0.6 (0.1 to 1.7)
High-middle SDI	4.4 (1.5 to 9.1)	0.4 (0.1 to 0.8)	136 (50 to 271.7)	12.1 (4.4 to 24)	1.9 (0.6 to 4.1)	0.1 (0 to 0.2)	57.8 (19.9 to 120)	2.9 (1 to 6.1)
Middle SDI	9.1 (3.5 to 18.1)	0.9 (0.3 to 1.8)	296.2 (120.3 to 572.5)	24.4 (9.6 to 47.9)	4.6 (1.3 to 10)	0.2 (0.1 to 0.4)	145.9 (47.5 to 308)	5.6 (1.8 to 12)
Low-middle SDI	4 (1 to 9.7)	0.6 (0.2 to 1.6)	138.7 (33.6 to 335)	18.8 (4.7 to 45.8)	3.7 (0.6 to 9.9)	0.3 (0 to 0.7)	130.6 (21.8 to 345.2)	8.4 (1.4 to 22.2)
Low SDI	0.9 (0.1 to 2.7)	0.4 (0 to 1.1)	32.9 (3 to 103.2)	10.9 (1 to 34.1)	1.2 (0.1 to 3.5)	0.2 (0 to 0.7)	44.9 (4.4 to 136.7)	6.7 (0.7 to 19.8)
Andean Latin America	0 (0 to 0.1)	0.1 (0 to 0.3)	0.8 (0.1 to 2.3)	3 (0.2 to 9)	0 (0 to 0)	0 (0 to 0.1)	0.9 (0.1 to 2.7)	1.4 (0.1 to 4.3)
Australasia	0 (0 to 0)	0 (0 to 0.1)	0.2 (0 to 0.7)	0.9 (0.1 to 3.2)	0 (0 to 0)	0 (0 to 0.1)	0.2 (0 to 0.6)	0.4 (0.1 to 1.4)
Caribbean	0 (0 to 0.1)	0.1 (0 to 0.2)	0.8 (0.1 to 3)	2.6 (0.3 to 9.9)	0 (0 to 0.1)	0 (0 to 0.1)	0.8 (0.1 to 3.2)	1.7 (0.2 to 6.4)
Central Asia	0.2 (0 to 0.4)	0.3 (0.1 to 0.7)	6.7 (1.3 to 16.4)	12.1 (2.3 to 28.8)	0.1 (0 to 0.3)	0.1 (0 to 0.3)	3.9 (0.5 to 11)	4.2 (0.5 to 11.9)
Central Europe	0.7 (0.2 to 1.4)	0.5 (0.2 to 0.9)	19.8 (6.8 to 38.4)	13.6 (4.8 to 26.5)	0.2 (0.1 to 0.4)	0.1 (0 to 0.2)	4.3 (1.1 to 8.9)	2.2 (0.6 to 4.6)
Central Latin America	0.1 (0 to 0.2)	0.1 (0 to 0.2)	3.2 (0.4 to 9.1)	2.9 (0.3 to 8.1)	0 (0 to 0.1)	0 (0 to 0.1)	2.4 (0.3 to 6.7)	0.9 (0.1 to 2.6)
Central Sub-Saharan Africa	0 (0 to 0.1)	0.1 (0 to 0.4)	1.1 (0.1 to 4.6)	3.3 (0.4 to 14)	0 (0 to 0.1)	0.1 (0 to 0.2)	1.8 (0.2 to 8)	2.2 (0.2 to 9.8)
East Asia	12.7 (5.1 to 25)	1.5 (0.6 to 3)	398.5 (172.7 to 734.6)	40 (16.9 to 76.7)	5.7 (2 to 11.8)	0.3 (0.1 to 0.6)	168.3 (69.7 to 314.6)	8.1 (3.3 to 15.2)
Eastern Europe	0.5 (0.1 to 1.2)	0.2 (0 to 0.4)	16.3 (2 to 43)	5.9 (0.7 to 15.5)	0.1 (0 to 0.4)	0 (0 to 0.1)	4.2 (0.5 to 11.5)	1.3 (0.2 to 3.6)
Eastern Sub-Saharan Africa	0.2 (0 to 0.5)	0.2 (0 to 0.7)	7.2 (0.5 to 22.4)	7.5 (0.6 to 21.6)	0.1 (0 to 0.4)	0.1 (0 to 0.3)	6.6 (0.5 to 24.6)	3.1 (0.2 to 10.1)
High-income Asia Pacific	0.2 (0.1 to 0.5)	0.1 (0 to 0.3)	5.4 (1.6 to 11.3)	2.7 (0.8 to 5.7)	0.2 (0 to 0.6)	0 (0 to 0.1)	2.6 (0.4 to 7)	0.5 (0.1 to 1.4)
High-income North America	0.1 (0 to 0.5)	0 (0 to 0.1)	3.6 (0.4 to 12)	1.1 (0.1 to 3.6)	0.1 (0 to 0.5)	0 (0 to 0.1)	3.3 (0.4 to 9.8)	0.6 (0.1 to 1.6)
North Africa and Middle East	0.1 (0 to 0.3)	0 (0 to 0.1)	3 (0.6 to 11.5)	1.3 (0.3 to 5)	0.1 (0 to 0.3)	0 (0 to 0.1)	3.5 (0.7 to 12.9)	0.6 (0.1 to 2.3)
Oceania	0 (0 to 0)	0.6 (0.1 to 1.7)	0.4 (0.1 to 1.4)	12.8 (1.9 to 39.9)	0 (0 to 0.1)	0.4 (0 to 1.3)	0.9 (0.1 to 3.2)	10.8 (1.3 to 34.9)
South Asia	3.2 (0.3 to 9.4)	0.5 (0.1 to 1.6)	117.7 (12.1 to 341.1)	15.9 (1.6 to 46.9)	4.3 (0.5 to 12.4)	0.3 (0 to 0.9)	157.8 (17.5 to 440.4)	9.6 (1.1 to 27.1)
Southeast Asia	0.6 (0.1 to 1.2)	0.2 (0.1 to 0.5)	20.4 (4.8 to 44.9)	6.5 (1.6 to 13.9)	0.3 (0 to 0.8)	0.1 (0 to 0.1)	12.4 (1.7 to 31.5)	1.8 (0.2 to 4.6)
Southern Latin America	0.1 (0 to 0.2)	0.2 (0 to 0.5)	1.9 (0.2 to 5.3)	4.1 (0.4 to 11.5)	0 (0 to 0.2)	0.1 (0 to 0.2)	1.2 (0.1 to 3.6)	1.5 (0.1 to 4.4)
Southern Sub-Saharan Africa	0 (0 to 0.1)	0.1 (0 to 0.4)	2 (0.1 to 7.4)	4.8 (0.3 to 18)	0 (0 to 0.1)	0 (0 to 0.2)	1.9 (0.1 to 7.4)	2.5 (0.2 to 9.5)
Tropical Latin America	0.1 (0 to 0.2)	0.1 (0 to 0.2)	3.9 (0.3 to 11.8)	3.3 (0.3 to 9.4)	0.1 (0 to 0.2)	0 (0 to 0.1)	4.4 (0.4 to 13.3)	1.7 (0.2 to 5.3)
Western Europe	0.3 (0 to 1.1)	0.1 (0 to 0.2)	7.1 (1 to 23)	1.3 (0.2 to 4.2)	0.3 (0 to 1)	0 (0 to 0.1)	4.3 (0.6 to 13.7)	0.5 (0.1 to 1.6)
Western Sub-Saharan Africa	0.1 (0 to 0.4)	0.1 (0 to 0.5)	4.1 (0.3 to 16.3)	3.8 (0.3 to 14.8)	0.1 (0 to 0.4)	0.1 (0 to 0.2)	5.2 (0.4 to 21.5)	2 (0.2 to 7.7)

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TABLE 1 (Continued)

Attributable factors	1990				2019			
	Death (X1000, persons, 95% UI)	ASRM (95% UI)	DALY (X1000, persons, 95% UI)	ASRD (95% UI)	Death (X1000, persons, 95% UI)	ASRM (95% UI)	DALY (X1000, persons, 95% UI)	ASRD (95% UI)
Lead exposure								
Global	9.4 (5.1 to 16.1)	0.2 (0.1 to 0.4)	320.2 (168.9 to 543.6)	7.2 (3.9 to 12.2)	7.7 (4.1 to 13.5)	0.1 (0 to 0.2)	213.5 (106.8 to 372.7)	2.6 (1.3 to 4.6)
High SDI	0.2 (0.1 to 0.5)	0 (0 to 0)	5 (1.1 to 10.7)	0.5 (0.1 to 1.1)	0.2 (0 to 0.4)	0 (0 to 0)	2.6 (0.4 to 6.1)	0.1 (0 to 0.3)
High-middle SDI	1.1 (0.5 to 2.2)	0.1 (0.1 to 0.2)	33.4 (14.9 to 63)	3 (1.4 to 5.6)	0.6 (0.3 to 1.2)	0 (0 to 0.1)	13.1 (5.5 to 25.4)	0.7 (0.3 to 1.3)
Middle SDI	3.1 (1.6 to 5.5)	0.3 (0.2 to 0.6)	100.4 (49.8 to 172.9)	8.3 (4.3 to 14.9)	1.9 (0.9 to 3.4)	0.1 (0 to 0.2)	46.3 (21.8 to 84.7)	1.9 (0.9 to 3.4)
Low-middle SDI	3.7 (2.1 to 6.2)	0.6 (0.3 to 1)	134.4 (73.4 to 224.9)	17.9 (10.1 to 29.1)	3.6 (1.9 to 6)	0.3 (0.1 to 0.5)	103.4 (51 to 177)	7 (3.6 to 11.9)
Low SDI	1.3 (0.7 to 2.3)	0.5 (0.3 to 0.9)	46.9 (23.8 to 81.7)	15.3 (8 to 26.7)	1.5 (0.8 to 2.6)	0.3 (0.2 to 0.5)	48.1 (24 to 82.7)	7.8 (4.2 to 13.4)
Andean Latin America	0 (0 to 0)	0 (0 to 0.1)	0.5 (0.1 to 1)	1.7 (0.5 to 3.6)	0 (0 to 0)	0 (0 to 0)	0.4 (0.1 to 0.9)	0.6 (0.1 to 1.4)
Australasia	0 (0 to 0)	0 (0 to 0.1)	0.2 (0.1 to 0.4)	0.9 (0.3 to 1.7)	0 (0 to 0)	0 (0 to 0)	0.1 (0 to 0.3)	0.2 (0.1 to 0.5)
Caribbean	0 (0 to 0.1)	0.1 (0 to 0.2)	1.3 (0.5 to 2.5)	4.4 (1.8 to 8.4)	0 (0 to 0)	0 (0 to 0.1)	1 (0.3 to 2.1)	1.9 (0.6 to 4.1)
Central Asia	0 (0 to 0.1)	0.1 (0 to 0.1)	1.3 (0.2 to 3.4)	2.3 (0.3 to 6)	0 (0 to 0.1)	0 (0 to 0.1)	0.9 (0.1 to 2.4)	1 (0.1 to 2.7)
Central Europe	0.1 (0 to 0.2)	0.1 (0 to 0.1)	2.3 (0.4 to 5.3)	1.6 (0.2 to 3.7)	0 (0 to 0)	0 (0 to 0)	0.5 (0.1 to 1)	0.2 (0 to 0.5)
Central Latin America	0.1 (0 to 0.1)	0.1 (0 to 0.1)	2.5 (1.1 to 4.6)	2.3 (1.1 to 4.1)	0 (0 to 0.1)	0 (0 to 0)	1.2 (0.5 to 2.4)	0.5 (0.2 to 0.9)
Central Sub-Saharan Africa	0 (0 to 0)	0.1 (0 to 0.2)	0.9 (0.2 to 2.1)	3.1 (0.9 to 6.6)	0 (0 to 0.1)	0.1 (0 to 0.1)	1.2 (0.3 to 3)	1.8 (0.5 to 4.3)
East Asia	3.6 (1.9 to 6.5)	0.4 (0.2 to 0.9)	108.9 (56 to 189.9)	11.2 (5.8 to 19.9)	1.8 (0.8 to 3.3)	0.1 (0 to 0.2)	37.4 (18.1 to 67.2)	1.8 (0.9 to 3.3)
Eastern Europe	0 (0 to 0.2)	0 (0 to 0.1)	1.6 (0 to 5.6)	0.6 (0 to 2)	0 (0 to 0)	0 (0 to 0)	0.4 (0 to 1.4)	0.1 (0 to 0.5)
Eastern Sub-Saharan Africa	0.1 (0 to 0.1)	0.1 (0 to 0.2)	2.8 (1 to 5.6)	3 (1.2 to 5.5)	0.1 (0 to 0.1)	0 (0 to 0.1)	2.4 (0.7 to 5.9)	1.2 (0.5 to 2.5)
High-income Asia Pacific	0 (0 to 0)	0 (0 to 0)	0.4 (0 to 1)	0.2 (0 to 0.5)	0 (0 to 0.1)	0 (0 to 0)	0.3 (0 to 0.7)	0 (0 to 0.1)
High-income North America	0.1 (0 to 0.2)	0 (0 to 0.1)	2 (0.5 to 4.2)	0.6 (0.1 to 1.2)	0.1 (0 to 0.1)	0 (0 to 0)	0.9 (0.1 to 2.2)	0.1 (0 to 0.3)
North Africa and Middle East	0.2 (0.1 to 0.5)	0.1 (0.1 to 0.3)	8.7 (4 to 17.7)	3.9 (1.9 to 8)	0.2 (0.1 to 0.3)	0 (0 to 0.1)	6.7 (3 to 13)	1.3 (0.6 to 2.5)
Oceania	0 (0 to 0)	0.1 (0 to 0.3)	0.1 (0 to 0.4)	2.3 (0.1 to 8.7)	0 (0 to 0)	0.1 (0 to 0.2)	0.1 (0 to 0.6)	1.4 (0 to 5.6)
South Asia	4.7 (2.6 to 7.6)	0.8 (0.4 to 1.4)	172.4 (94.4 to 290.6)	23.4 (13.2 to 38.6)	5.1 (2.8 to 8.6)	0.4 (0.2 to 0.7)	151 (77.6 to 252.7)	9.9 (5.2 to 16.4)
Southeast Asia	0.1 (0 to 0.3)	0 (0 to 0.1)	5 (1.3 to 11.2)	1.4 (0.4 to 3)	0.1 (0 to 0.1)	0 (0 to 0)	2.4 (0.5 to 6)	0.4 (0.1 to 0.9)
Southern Latin America	0 (0 to 0)	0 (0 to 0.1)	0.3 (0 to 1)	0.8 (0 to 2.1)	0 (0 to 0)	0 (0 to 0)	0.2 (0 to 0.6)	0.2 (0 to 0.7)
Southern Sub-Saharan Africa	0 (0 to 0)	0 (0 to 0.1)	0.7 (0.2 to 1.8)	1.9 (0.5 to 4.4)	0 (0 to 0)	0 (0 to 0.1)	0.7 (0.1 to 1.8)	1 (0.2 to 2.3)
Tropical Latin America	0 (0 to 0.1)	0 (0 to 0.1)	2 (0.5 to 4.5)	1.6 (0.5 to 3.4)	0 (0 to 0.1)	0 (0 to 0)	1.1 (0.2 to 3)	0.5 (0.1 to 1.2)
Western Europe	0.2 (0 to 0.3)	0 (0 to 0.1)	3.1 (0.8 to 6.7)	0.6 (0.1 to 1.2)	0.1 (0 to 0.3)	0 (0 to 0)	1.8 (0.4 to 4.2)	0.2 (0 to 0.4)
Western Sub-Saharan Africa	0.1 (0 to 0.2)	0.1 (0 to 0.2)	3.1 (1 to 6.3)	2.9 (1 to 5.9)	0.1 (0 to 0.2)	0 (0 to 0.1)	2.9 (0.9 to 6.4)	1.2 (0.5 to 2.5)

SDI, sociodemographic index.

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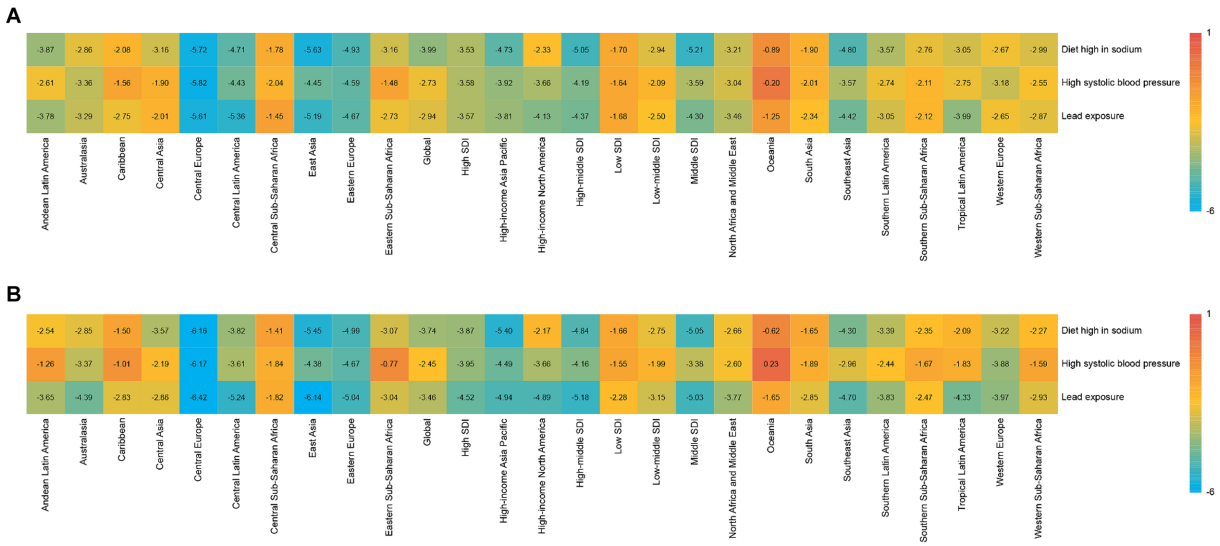

FIGURE 1
Average annual percentage change for rheumatic heart disease risk-attributable age-standardized mortality rate and age-standardized disability-adjusted life year (DALY) rate in 21 Global Burden of Disease regions classified by five sociodemographic index levels for both sexes in 1990–2019. SDI: sociodemographic index. **(A)** Mortality; **(B)** DALY.

exposure were 213.5 (95% UI: 106.8 to 372.7), corresponding to a rate of 2.6 per 100,000 (95% UI: 1.3 to 4.6) (Table 1). South Asia had the highest death and DALY rates in 2019. All 21 GBD regions showed downward trends of disease burden attributable to lead exposure from 1990 to 2019 (Figure 1). The three countries with the most deaths and DALYs attributable to lead exposure were India, China, and Pakistan (Supplementary Tables S5, S6). India, Pakistan, and Nepal were the countries with the highest rates of death (Supplementary Table S5), and India, Pakistan, and Afghanistan were those with the highest rates of DALYs (Supplementary Table S6). Most countries showed decreasing trends of disease burden due to lead exposure. Thailand and Singapore had the largest decreases in disease burden, while the highest increase was observed in Georgia (Supplementary Table S6).

At the SDI region level, the low-middle SDI region had the highest number of deaths and DALYs, but the highest rates of death and DALYs occurred in the low-SDI region, respectively. Of the 21 GBD regions, the highest number and rates of deaths and DALYs were in South Asia (Table 1). All SDI quintiles showed a downward trend of RHD deaths and DALYs attributable to lead exposure, where the high-middle SDI quintile had the largest changes (Figure 1).

The sex-specific death and DALY rates in locations were varied (Figures 2E,F). In most regions, males had a much higher burden than females, while this disparity reversed in South Asia. The global number of deaths in 2019 showed an unimodal distribution with age (Figures 3E,F). Age-specific mortality and DALY rates in males were higher than those in females before the age of 35–39, after which the trend reversed. Moreover, lead exposure-related deaths and DALYs declined in each age group except for the 90+ years age group during the past 30 years, with the highest decrease noted in the 25–29 years age group (Figures 4A,B).

Both age-standardized rates of death and DALYs of RHD due to lead exposure showed a moderate negative correlation with the SDI in 2019 (correlation coefficients were approximately −0.44 and −0.41, respectively) at the national level (Figures 5E,F).

Discussion

RHD is a significant global health concern, which caused approximately 0.31 million deaths worldwide in 2019 (9). In addition, the prevalence of RHD increased from 23.76 million in 1990 to 40.50 million in 2019, leading to predictions that cases of RHD could exceed 48 million by 2030 (10). Therefore, the disease represents an important global health issue requiring targeted prevention and control efforts. Herein, we conducted a comprehensive estimation of the burden of disease attributable to specific risk factors. Generally, we found that there appears to be a trend of decrease in global RHD-related numbers and rates of deaths and DALYs due to a diet high in sodium, high SBP, and lead exposure since 1990. The burden of RHD varied with different risk factors at regional and national levels, among which RHD disparities had different gradients of risk factors during this period. After that, tracking the disease burden caused by these risk factors helps formulate effective control measures to prevent RH.

Although the past 30 years have witnessed a decrease in high SBP-related RHD burden, high SBP still dominates among the risk-attributable factors in 2019. Hypertension May serve as a major risk factor for valvular heart disease, a known moderate risk factor for heart failure that is often associated with RHD (11). Higher SBP has been associated with an increase in the prevalence of CVDs. RHD, in particular, has been linked to an increased risk of fatal and non-fatal coronary heart disease and stroke (12). The burden of CVD attributable to hypertension is vast, which includes the impact on

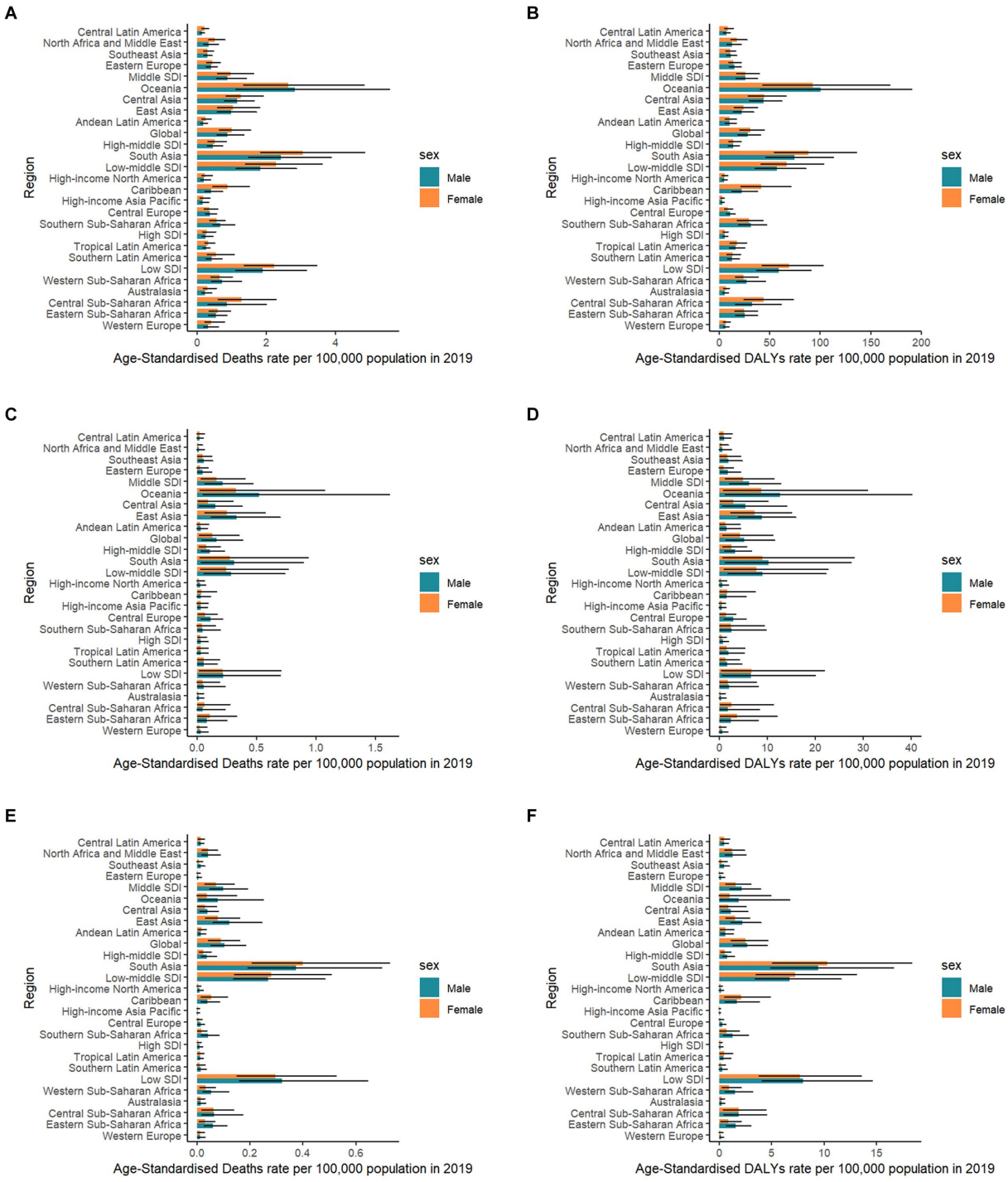

FIGURE 2
Rheumatic heart disease risk-attributable age-standardized mortality rate and age-standardized disability-adjusted life year (DALY) rate in 21 Global Burden of Disease regions classified by five sociodemographic index levels by sex in 2019. SDI: sociodemographic index. **(A)** Mortality due to high systolic blood pressure; **(B)** DALY due to high systolic blood pressure; **(C)** Mortality due to diet high in sodium; **(D)** DALY due to diet high in sodium; **(E)** Mortality due to lead exposure; and **(F)** DALY due to lead exposure.

RHD patients given the role of high blood pressure in heart disease and stroke (13). Over the past few decades (1990–2019), the global incidence of RHD has increased, particularly impacting poverty-stricken and marginalized populations. The correlation with elevated SBP adds to the overall disease burden (14). However, while high SBP is not a direct cause of RHD, it May escalate the severity or prognosis of RHD due to its impact on cardiovascular health. It is also worth mentioning that these sources offer different perspectives on the subject and should be read fully for a comprehensive understanding.

Evidence demonstrates that sodium intake shows a positive correlation with blood pressure, which could increase the risk for

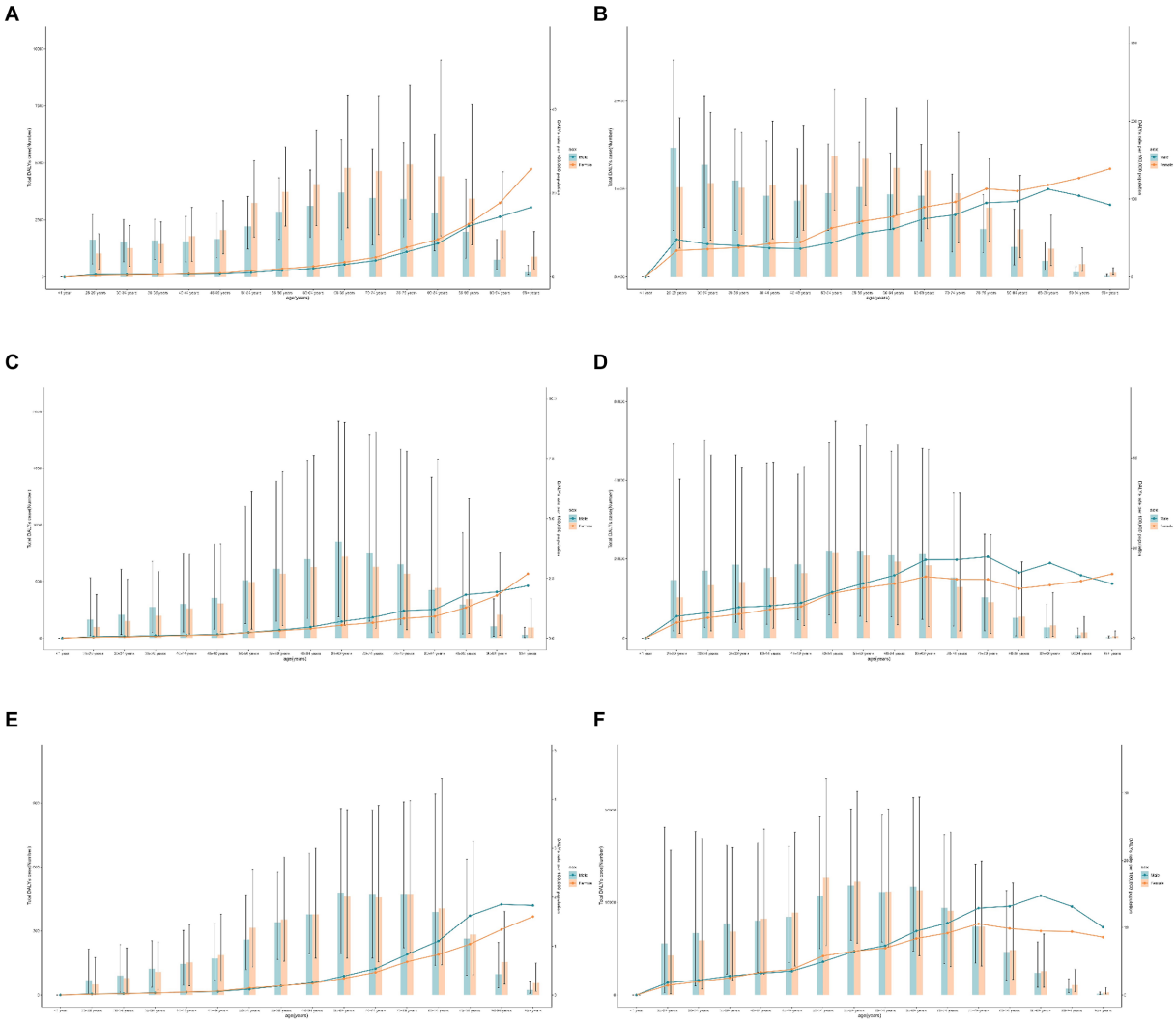

FIGURE 3
Global rheumatic heart disease risk-attributable age-standardized mortality rate and age-standardized disability-adjusted life year (DALY) rate by age and gender in 2019. **(A)** Mortality due to high systolic blood pressure; **(B)** DALY due to high systolic blood pressure; **(C)** mortality due to diet high in sodium; **(D)** DALY due to diet high in sodium; **(E)** Mortality due to lead exposure; and **(F)** DALY due to lead exposure.

CVDs, including RHD (15). Reducing salt intake could therefore form a key strategy to manage cardiovascular risks, including those related to RHD. As is always recommended, maintaining a balanced diet and limiting sodium intake can form part of a comprehensive strategy for overall cardiovascular health.

The escalating levels of lead exposure and its detrimental impact on human health emerged as a major public health concern during the 1990s. Lead exposure is known to contribute to the overall global burden of CVDs (16, 17). The overall estimations of GBD 2019 demonstrated that lead exposure accounted for 8.2, 7.2, and 5.65% of the global burden of hypertensive heart disease, ischemic heart disease, and stroke, respectively (16). In our study, we found that the DALYs attributable to lead exposure were 213,500, and its rate was 2.6 per 100,000, with the highest rate observed in developing countries.

Although rheumatic fever often affects children and adolescents (2), in 2019, the DALY rates of RHD attributable to the above risk exposures were positively associated with increasing age in both males and females, which indicates that the disease continues in youth into middle-aged and older adults. With the development of modern sophisticated biological valves and mechanical valves, the survival rate of patients with rheumatic heart valve disease is increasingly improving. However, children and adolescents are particularly vulnerable to long-term harm from lead, including increased risks of high blood pressure and heart disease (18). In addition, age-standardized mortality and DALYs from RHD attributable to lead exposure and hypertension were higher in men than in women before the age of 39, while the risk attributable to a high sodium diet was higher in women until the age of 80. This May be related to physical differences and lifestyle differences between men and women (19). Specifically, women often exhibit different patterns of risk factor exposure, disease progression, and health outcomes compared to men. Women with RHD May have different clinical presentations and

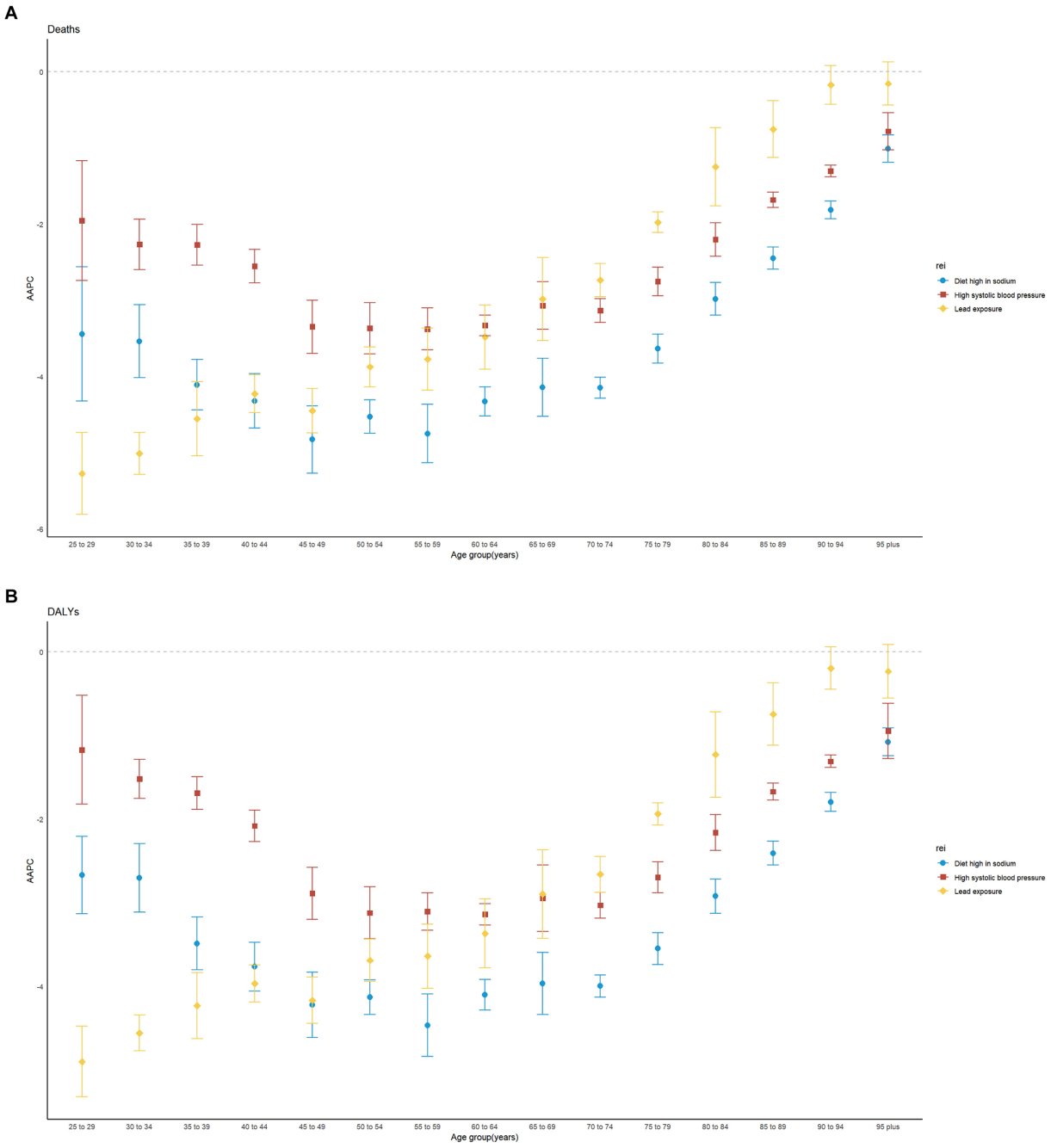

FIGURE 4
Average annual percentage change of age-standardized mortality rate and age-standardized disability-adjusted life year (DALY) rate for both sexes combined by age groups in 1990–2019 globally. AAPC: average annual percentage change. **(A)** Mortality; **(B)** DALY.

May be more susceptible to the adverse effects of conditions such as hypertension and dietary sodium intake, potentially due to physiological and hormonal factors. Additionally, gender differences in healthcare access and treatment-seeking behavior May further exacerbate these disparities, contributing to the observed patterns in RHD burden.

Since RHD accounts for a large proportion of DALYs (9) and imposes a relatively high burden (10), particularly in the low-middle and low-SDI countries, the preventive strategies against related risk factors, such as a diet high in sodium, high SBP, and lead exposure, seem to be cost-effective in reducing the disease burden. Measurements for behavioral risk factors such as adopting healthy diets, metabolic risk factors such as blood pressure control, as well as environmental risk factors such as reducing lead exposure, should also be emphasized.

The strengths of the current study include the comprehensive and up-to-date estimation of the situation and trends of the attributable burden to diet high in sodium, high SBP, and lead

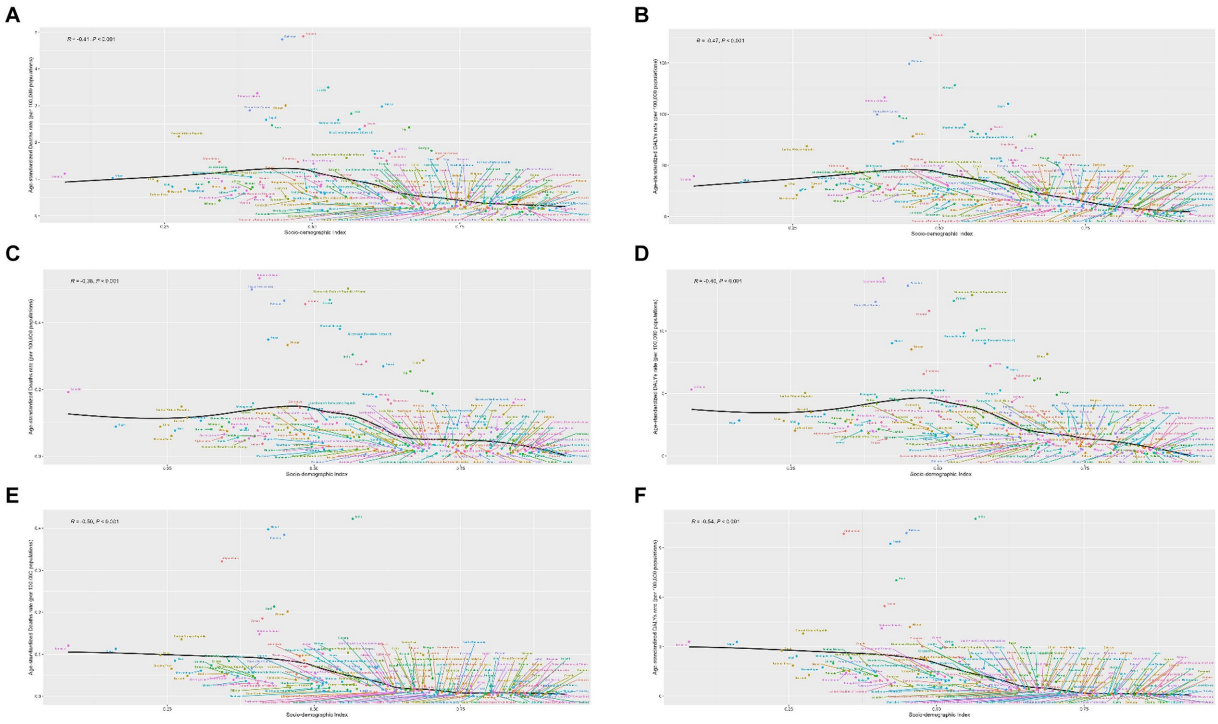

FIGURE 5
National age-standardized rates of mortality and disability-adjusted life year (DALY) of rheumatic heart disease risk due to available risk factors by socio-demographic index, 1990–2019. **(A)** Mortality due to high systolic blood pressure; **(B)** DALY due to high systolic blood pressure; **(C)** Mortality due to diet high in sodium; **(D)** DALY due to diet high in sodium; **(E)** mortality due to lead exposure; and **(F)** DALY due to lead exposure.

exposure and its attributable RHD, based on the data from GBD 2019 during 1990–2019. However, there were some major limitations of the present study. First, it resulted from GBD methodologies in data collection, thus, the availability and quality of primary data in regions with countries that have poor completeness rates of the data sources. In the absence of data, estimates rely heavily on the predictive validity of the modeling efforts applied to out-of-sample scenarios. Additionally, our data did not take into account the interplay among risk factors, which could possibly affect the reliability of the results. Third, we did not analyze the RHD burden attributable to other risk factors, including infectious agents, physical activity, and comorbidities because there are no data in the GBD database. This May overestimate the burden of RHD attributable to the included risk factors in this study. Fourth, the GBD study relies on observational data and statistical modeling to estimate disease burden and associated risk factors across time periods rather than following a specific cohort of individuals longitudinally. Moreover, there are potential biases due to the heterogeneity of the studies included in the GBD database. These studies vary in design, population characteristics, and data collection methods, which could introduce bias in the estimation of risk factors. Although the GBD study uses sophisticated modeling techniques to mitigate these biases, some residual bias May still exist and should be considered when interpreting the results. Finally, comprehensive and meticulous study is required to enhance the proof that supports understanding the mediating impacts of those factors on the burden of risk attributable to RHD.

Conclusion

Age-standardized global DALY and death rates of RHD attributed to a diet high in sodium, high SBP, and lead exposure decreased from 1990 to 2019, and both are reversely associated with SDI. Furthermore, we found that the major attributable age-standardized DALYs were from hypertension. Considering the substantial burden of RHD in certain regions, as well as the detrimental health impacts of associated risk factors, there is an immediate need for strategic attention and measures to regulate these factors and their consequent burden. Furthermore, the absence of dependable exposure information from every country worldwide emphasizes the demand for additional research to precisely ascertain the disease burden linked to these factors.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, JJ, upon reasonable request.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants or patients/participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

YaZ: Formal analysis, Software, Visualization, Writing – original draft, Writing – review & editing. JZ: Writing – original draft, Writing – review & editing. YL: Writing – original draft, Writing – review & editing. YuZ: Data curation, Writing – original draft, Writing – review & editing. LY: Writing – original draft, Writing – review & editing. KC: Conceptualization, Investigation, Software, Visualization, Writing – original draft, Writing – review & editing. JJ: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1419349/full#supplementary-material

References

1. Hu B, Feng J, Wang Y, Hou L, Fan Y. Transnational inequities in cardiovascular diseases from 1990 to 2019: exploration based on the global burden of disease study 2019. *Front Public Health*. (2024) 12:1322574. doi: 10.3389/fpubh.2024.1322574

2. Mao C, Sun X, Long D, Zhang M, Xu X, Gao X, et al. Epidemiological study of pediatric rheumatic heart disease: an analysis from the global burden of disease study 2019. *Int J Cardiol*. (2024) 400:131705. doi: 10.1016/j.ijcard.2023.131705

3. Ghamari SH, Abbasi-Kangevari M, Saeedi Moghaddam S, Aminorroaya A, Rezaei N, Shobeiri P, et al. Rheumatic heart disease is a neglected disease relative to its burden worldwide: findings from global burden of disease 2019. *J Am Heart Assoc*. (2022) 11:e025284. doi: 10.1161/JAHA.122.025284

4. Maaloul I, Bouzidi N, Kolsi R, Ameur SB, Abid L, Aloulou H, et al. Rheumatic cardiopathies and its risk factors: about 50 cases. *Ann. Cardiol. Angeiol. (Paris)*. (2024) 73:101676. doi: 10.1016/j.ancard.2023.101676

5. Nasir M, Taddesse K, Ahmed M, Argaw Z, Gebretensaye TG, Markos S. Health related quality of life and associated factors among adult patients with rheumatic heart disease patients in Ethiopia. *Int. J. Gen. Med.* (2023) 16:3403–12. doi: 10.2147/IJGM.S419118

6. Tong Z, Xie Y, Li K, Yuan R, Zhang L. The global burden and risk factors of cardiovascular diseases in adolescent and young adults, 1990-2019. *BMC Public Health*. (2024) 24:1017. doi: 10.1186/s12889-024-18445-6

7. GBD 2019 Risk Factors Collaborators. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the global burden of disease study 2019. *Lancet*. (2020) 396:1223–49. doi: 10.1016/S0140-6736(20)30752-2

8. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the global burden of disease study 2019. *Lancet*. (2020) 396:1204–22. doi: 10.1016/S0140-6736(20)30925-9

9. Ruan R, Liu X, Zhang Y, Tang M, He B, Zhang QW, et al. Global, regional, and National Advances toward the Management of Rheumatic Heart Disease Based on the global burden of disease study 2019. *J Am Heart Assoc*. (2023) 12:e028921. doi: 10.1161/JAHA.122.028921

10. Hu Y, Tong Z, Huang X, Qin JJ, Lin L, Lei F, et al. The projections of global and regional rheumatic heart disease burden from 2020 to 2030. *Front. Cardiovasc. Med.* (2022) 9:941917. doi: 10.3389/fcvm.2022.941917

11. Lee GB, Shin KE, Han K, Son HS, Jung JS, Kim YH, et al. Association between hypertension and incident infective endocarditis. *Hypertension*. (2022) 79:1466–74. doi: 10.1161/HYPERTENSIONAHA.122.19185

12. Kibone W, Bongomin F, Okot J, Nansubuga AL, Tentena LA, Nuwamanya EB, et al. High blood pressure prevalence, awareness, treatment, and blood pressure control among Ugandans with rheumatic and musculoskeletal disorders. *PLoS One*. (2023) 18:e0289546. doi: 10.1371/journal.pone.0289546

13. Cao X, Zhao Z, Kang Y, Tian Y, Song Y, Wang L, et al. The burden of cardiovascular disease attributable to high systolic blood pressure across China, 2005-18: a population-based study. *Lancet Public Health*. (2022) 7:e1027–40. doi: 10.1016/S2468-2667(22)00232-8

14. Ou Z, Yu D, Liang Y, Wu J, He H, Li Y, et al. Global burden of rheumatic heart disease: trends from 1990 to 2019. *Arthritis Res Ther*. (2022) 24:138. doi: 10.1186/s13075-022-02829-3

15. Wang K, Jin Y, Wang M, Liu J, Bu X, Mu J, et al. Global cardiovascular diseases burden attributable to high sodium intake from 1990 to 2019. *J Clin Hypertens (Greenwich)*. (2023) 25:868–79. doi: 10.1111/jch.14717

16. Rezaee M, Esfahani Z, Nejadghaderi SA, Abbasi-Kangevari M, Saeedi Moghaddam S, Ghanbari A, et al. Estimating the burden of diseases attributable to lead exposure in the North Africa and Middle East region, 1990-2019: a systematic analysis for the global burden of disease study 2019. *Environ Health*. (2022) 21:105. doi: 10.1186/s12940-022-00914-3

17. Zhou N, Huang Y, Li M, Zhou L, Jin H. Trends in global burden of diseases attributable to lead exposure in 204 countries and territories from 1990 to 2019. *Front Public Health*. (2022) 10:1036398. doi: 10.3389/fpubh.2022.1036398

18. Halabicky OM, Tellez-Rojo MM, Goodrich JM, Dolinoy DC, Mercado-Garcia A, Hu H, et al. Prenatal and childhood lead exposure is prospectively associated with biological markers of aging in adolescence. *Sci Total Environ*. (2024) 913:169757. doi: 10.1016/j.scitotenv.2023.169757

19. Lv Y, Cao X, Yu K, Pu J, Tang Z, Wei N, et al. Gender differences in all-cause and cardiovascular mortality among us adults: from Nhanes 2005-2018. *Front. Cardiovasc. Med.* (2024) 11:1283132. doi: 10.3389/fcvm.2024.1283132

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Insights into the salt levels in bread offers in Slovenia: trends and differences

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Objective: Bakery products are considered as one of main dietary sources of sodium/salt in Slovenia. Our main objective was to assess the salt content in bread in Slovenia, focusing into different bread categories and sales channels. The data collected in 2022 was compared with year 2012.

Methods: A follow-up study on salt content of bread sold in Slovenia was conducted. Bread samples were purchased in large retail shops and smaller bakeries across 11 statistical regions of Slovenia. Sodium content was determined by inductively coupled plasma mass spectrometry; salt content was calculated by multiplying sodium content with 2.54, assuming all sodium corresponds to sodium chloride.

Results: In 2022, 178 bread samples were purchased and analyzed. Weighted mean salt content in bread was 1.35 (95% CI 1.28–1.42) g/100 g in 2012, and 1.26 (95% CI 1.22–1.29) g/100 g in 2022, showing a 7% decrease. Notable differences in the salt content were observed between various bread subcategories and retail environments. In addition, a significant difference was observed between white wheat bread sold in large retail shops and smaller bakeries, where a higher salt content was observed.

Conclusion: While study results show small decrease in the salt content in bread in Slovenia in last decade, the salt reduction targets set by the WHO have not been met. Additional efforts are needed to stimulate bread reformulation with reducing salt content.

KEYWORDS

bread, sodium, salt, ICP-MS, Slovenia

1 Introduction

Sodium is one of the major cations in extracellular fluid. It enables proper signal transduction, muscle contraction and helps to regulate fluid balance in the body (1). While its role in the body is important, current intakes of sodium are much higher than needed or recommended, with the current global estimate of 3.95 g sodium/day (equivalent to 10.06 g salt/day), which is nearly twice the World Health Organization (WHO) recommended limit of 2 g sodium/day (equivalent to 5 g salt/day) (2). High sodium (salt) intake has been associated with the risk for developing gastric cancer (3, 4), and higher urinary calcium excretion, thereby increasing risk of osteoporosis and kidney stones (4). However, the most notable association, is with high blood pressure. Several systematic reviews and meta-analyses have shown that reducing sodium intake can lead to a reduction in systolic blood pressure (5,

6) and risk of cardiovascular disease (7). It has also been recognized as the most cost-effective strategy in reducing cardiovascular events and mortality rates (8, 9).

In 2013, the WHO published the Global action plan for prevention and control of noncommunicable diseases 2013–2020, which included a goal of 30% relative reduction in sodium/salt intake (10). To achieve this, comprehensive programs which engage different sectors (e.g., food manufacturers), as well as consumers, are needed. According to a systematic review by Trieu et al., majority of countries with salt reduction strategies use multifaceted approaches, implementing mainly food reformulation, consumer education, front-of-package labeling, interventions in public institution settings, and taxation (11). Most of these strategies have been recognized by the WHO as best-buy interventions for reducing sodium intake (12).

Reducing consumption of highly processed foods and food reformulation, are suggested as crucial for reducing sodium intake in the population (13, 14). This is understandable since processed foods represent about 75% of sodium intake in European and North American countries (1, 15). While specific food categories contributing most to sodium intake slightly vary between countries, cereals and cereal-based foods, and meat products are often among top contributors. This has been reported in countries such as United Kingdom and United States (16), and similarly in France (17) and New Zealand (18). Among cereal-based foods, bread and bakery products are suggested as main sources of salt in European countries (19). According to a recent systematic review of dietary sodium sources worldwide, they account for 25 to 40% of sodium contribution in many European countries and the United States (20). Bread and bakery products are therefore among main targets for food reformulation.

Sodium has an important role in bread, impacting both sensory characteristics and technological aspects, such as development of gluten structure, fermentation, mixing and baking (21, 22). Despite this, a gradual reduction of sodium content can be achieved (19, 23). An example of successful reduction was the United Kingdom, where sodium content in pre-packed bread was reduced by about 20% between 2001 and 2011 (24).

Since bread and bakery products were also recognized as the main contributors to sodium intake in Slovenia (25), efforts were made to reduce its content by setting voluntary sodium reduction targets (26). A recent study has shown limited improvement in sodium content in pre-packed foods, including pre-packed bread, between 2011 and 2020 (27). Similar results were also suggested for non-prepacked bread sold in retail shops in 2012 (28), however there has been no subsequent monitoring of these bread types since then.

Therefore, the main objective of this study was to assess the change in sodium/salt content of bread between 2012 and 2022. For year 2022 we additionally compare sodium/salt content in white wheat bread sold in small bakeries and large retail stores, and in different types (prepacked and unpacked), and different bread categories (white wheat, mixed and wheat toast). With consideration that the European Union regulations mandate food labeling of sodium in form of salt content, herein reported results are also presented for salt content, although all samples were analyzed for sodium content. Salt content was calculated assuming all sodium corresponds to sodium chloride.

2 Materials and methods

2.1 Sample collection

We conducted a longitudinal follow-up study investigating the salt content in bread available in Slovenian food supply, replicating a previous study published in 2012 (28). Bread samples were collected between November and December 2022 from large retail shops and smaller bakeries across Slovenia. In contrast to the 2012 study, which focused on bakeries and retail shops in the capital city metropolitan area, the 2022 sampling strategy incorporated suppliers in different statistical regions of Slovenia.

While an ideal bread sampling strategy would involve determining the number of breads based on accurate market share data for individual products within specific bread categories, such data was not readily available. Therefore, we utilized aggregate bread consumption estimates from household surveys of the Statistical Office of the Republic of Slovenia (SURS) conducted in 2010 and 2018, along with market share estimates for individual manufacturers, to determine the appropriate number of bread samples for each bread category. Similar to the sampling process employed in 2012, which prioritized bread types with higher consumption shares, as reported in the national survey results for household bread consumption in 2010, the 2022 sampling considered data from the bread type consumption survey as provided in the 2018 households' consumption report (29). Power analysis was performed with aim to determine the required sample size for detecting a statistically significant 10% reduction in salt content in retail white wheat bread between 2012 and 2022. This analysis utilized an effect size of 0.85, a significance level of 0.05, and a desired power of 0.8, with an allocation ratio of 2:1 (in comparison to 2012 sampling). The estimated sample size for white wheat bread in 2022 was $N = 28$. Sample size for other bread categories was recalculated with weighting-approach using the last available households' consumption report (29).

Regarding sampling in large retail shops, the sampling process stipulated that bread samples must originate from manufacturers and retailers selling bread across the country, to ensure comprehensive representation of the bread market. Retail shops affiliated with retail chains exceeding a 5% market share in the country's total food and other staple items sales, and bread manufacturers selling within these retail shops and maintaining at least 30 permanent employees and €2 million in annual revenues, were included in the sampling process. Additionally, breads with the highest turnover within the selected bread categories, as identified by shop assistants stationed at the bread-selling desks, were included in the sample. The 2022 sampling also encompassed breads listed on the government's price monitoring platform (30), which tracks bread prices to address concerns about rising food costs.

While the sampling conducted in the smaller bakeries in 2012 included only stores located in the metropolitan area (28), the sampling in 2022 was conducted in bakeries located in 11 official statistical regions of Slovenia, according to the Nomenclature of Territorial Units for Statistics (NUTS) - level 3 classification (31). This accounted for regions with approximately 97% of the country's population. Only one of the smallest regions (Lower Sava) was not included, due to logistic constraints. Bakery inclusion was based on their affiliation to the specific region and information obtained from Google Maps, which provided the names and locations of eligible bakeries. For each region, a separate sampling pool was established, and two bakeries were

randomly selected. We purchased one white and one wholegrain bread sample with the highest turnover, as recommended by the shop assistant working in the bakery store. Purchase of samples was done in neutral way, with researcher acting as an ordinary customer, asking for "bestselling white bread" and "bestselling wholegrain bread".

The combined estimated market share of all retail shops and bakery stores included in the bread selection for both years is estimated at >85%. Additionally, the bread manufacturers included in the study were estimated to cover at least 75% of all bread produced and sold in the country. The estimated market shares were obtained through publicly available sales data from retail shops and bread manufacturers for the sampling years.

While in 2012 altogether 45 bread samples were purchased (41 in large retail shops and 4 in small bakeries), the 2022 sampling included not only bread, but also toast bread and small bread rolls. The total sample in 2022 included 178 bread products, with 117 sampled in large retail shops and 61 in smaller bakeries. The decision for such increase in the sampling was done with consideration of the variability in the salt content observed in certain bread categories in the 2012 study. The data presenting the distribution of samples in selected bread categories per sampling year are provided in the Supplementary Table S1. The 2012 sampling included 14 white wheat bread samples, 9 half-white wheat breads, 6 dark breads, 16 mixed and 3 rye breads, while 2022 sample consist of 60 samples of white wheat breads, from which 28 were provided by small bakeries and 32 from large retail shops, 15 half-white wheat breads, 16 dark wheat breads, 29 mixed breads, 3 rye breads, 33 wholegrain breads (29 from smaller bakeries and 4 from large retail shops), 15 small white wheat bread rolls and 10 white wheat toast breads. Samples were categorized according to Slovenian Rules on quality of bakery products (32), which defines mixed wheat bread as bread made from different types of flour, with at least 51% of wheat flour. Rye bread is defined as bread made from rye flour, with up to 20% wheat flour and wholegrain wheat bread as bread containing at least 80% wholegrain wheat flour. The difference between white, half-white and dark wheat bread is mainly in the mineral (ash) content of the used wheat flour (33).

2.2 Sample preparation and sodium content determination

After purchase the bread samples were packaged in plastic bags, stored at room temperature (~23°C), and delivered to the laboratory.

The sodium content of bread samples in 2022 was determined in accredited laboratory Mérieux NutriSciences (Resana, Italy) using AOAC Official Method 2011.14 (34), utilizing quantification with inductively coupled plasma mass spectrometry (ICP-MS); results were expressed as mg/100 g. The sodium content in bread samples from 2012 was determined by potentiometric method, which quantifies sodium chloride content via chloride ions; the method uses silver nitrate solution as titrant (28). Salt content of bread samples was calculated by multiplying sodium content with 2.54, assuming all sodium corresponds to sodium chloride (NaCl).

2.3 Statistical analysis

Based on product type, each sample was assigned to a category as defined by national Rules on quality of bakery products (32). To draw conclusions on the mean salt content in Slovenia for overall category of bread, we used a weighting approach with consideration of national SURS data from 2010 and 2018 on household bread consumption. Specific weights for all sampling units (e.g., bread categories) were assigned to avoid bias due to the sampling technique. The weights used are presented in Supplementary Table S1.

For all bread products per sampling year and each bread subcategory, the mean, median, standard deviation, range, standard error, and confidence intervals were used to describe the salt content in grams per 100 grams of product. A one-way ANOVA analysis was conducted to determine the mean difference in salt content between white wheat breads purchased from small bakeries and large retail shops. Paired comparison t-tests were used to identify differences in salt content between analytical measurements and reported values in nutrition declarations (labels or online – where applicable) for pre-packed and un-packed bread products, and across different bread types. Additionally, linear mixed model analysis was used to determine the difference in the salt content of bread using the same bread categories in both sampling years and per different type of stores. Values were considered as statistically significant when p was <0.05. For statistical analyses and data representation IBM SPSS Version 27 (IBM SPSS, IBM Corp., Armonk, NY) and GraphPad Prism (GraphPad Software, version 8.2.0., San Diego, United States) was used.

3 Results

Results of monitoring bread salt content in Slovenia in 2022, and comparison with 2012 data (25, 28) are presented in Table 1.

In 2012 the lowest mean salt content was measured for dark wheat bread (1.32 g/100 g). While the salt content of white wheat bread (1.35 g/100 g) was lower compared to rye or half-white wheat bread, the standard deviation and range were higher than in other types of bread.

In 2022, the lowest mean salt content was also measured in dark wheat bread (1.15 g/100 g), while the highest content was observed in white toast bread (1.36 g/100 g) and wholegrain bread from smaller bakeries (1.37 g/100 g). Results also show high variability and range in certain categories such as white wheat bread (in smaller bakeries), wholegrain wheat bread and mixed breads.

The unweighted and weighted data on salt content in breads sold in Slovenia for years 2012 and 2022 are presented in Supplementary Table S2. The unweighted data show that our sampling sufficiently covered different bread categories, considering statistical data on bread type consumption (SURS, 2018). Weighting was used to provide insights about the salt content in "typical" bread consumed in Slovenia using SURS statistical data about the consumption of various types of bread. Such imaginary typical bread is constructed with fixed proportions of various bread types, according to their consumption share. Study results show a decrease of 7% in the salt content of such typical bread from year 2012 to 2022. In addition, a decrease of 12% in the salt content was observed specifically in white wheat bread from large retail shops in the same period.

Table 2 shows results of linear mixed effects model comparison analysis using the same categories of breads sampled in 2012 and 2022 in small bakeries and large retail shops. The results show significant decrease in the mean salt content between breads sampled

TABLE 1 Salt content of bread purchased in large retail shops and small bakeries across Slovenia in 2012 and 2022.

Type of bread	Purchase location	2012*				2022			
		N (%)	Mean (SD) salt content (g/100 g)	Median	Range	*N* (%)	Mean (SD) salt content (g/100 g)	Median	Range
White wheat bread	All	14 (31.1)	1.35 (0.25)	1.35	0.9	60 (33.7)	1.28 (0.20)	1.28	1.21
	Small bakeries	1	1.00			32	1.34 (0.21)	1.33	1.21
	Large retail shops	13	1.38 (0.24)	1.40	0.9	28	1.21 (0.16)	1.24	0.71
Half-white wheat bread	Large retail shops	9 (20.0)	1.37 (0.17)	1.40	0.6	12 (6.7)	1.24 (0.13)	1.21	0.51
Dark wheat bread	Large retail shops	6 (13.3)	1.32 (0.08)	1.30	0.2	16 (9.0)	1.15 (0.14)	1.12	0.53
Rye breads	Large retail shops	3 (6.7)	1.37 (0.06)	1.40	0.1	3 (1.7)	1.18 (0.13)	1.11	0.24
Mixed breads	All	13 (28.9)	1.36 (0.18)	1.40	0.7	29 (16.3)	1.22 (0.25)	1.15	0.97
	Small bakeries	3	1.50 (0.20)	1.50	0.4				
	Large retail shops	10	1.32 (0.16)	1.35	0.5				
Wholegrain**	All					33 (18.5)	1.36 (0.29)	1.33	1.44
	Small bakeries**					29	1.37 (0.30)	1.33	1.44
	Large retail shops					4	1.28 (0.24)	1.25	0.55
White wheat rolls	Large retail shops					15 (8.4)	1.29 (0.19)	1.34	0.66
White wheat toast bread	Large retail shops					10 (5.6)	1.36 (0.20)	1.39	0.55

SD, standard deviation. *Previous monitoring data was reported by Blaznik et al. (28) and Ribič et al. (25). **Wholegrain bread samples in small bakeries correspond to samples that were offered on the request for "bestselling wholegrain bread," however, considering that samples were purchased as non-prepacked and without written declaration, these could be also mixed breads, not meeting strict regulatory requirements for wholegrain bread.

TABLE 2 Results of linear mixed effect models comparing salt content of bread purchased in different type of stores in Slovenia in 2012 and 2022.

Type of store	Year of sampling	Mean	SE (Mean)	Difference	95% CI (Diff.)		*z*	*p*
Small bakeries	2012	1.375	0.09	−0.037	−0.23	0.15	−0.38	n.s
	2022	1.338	0.03					
Large retail shops	2012	1.351	0.03	−0.158	−0.23	−0.09	−4.51	<0.001
	2022	1.193	0.02					
Small bakeries	2012	1.375	0.09	−0.024	−0.21	0.16	−0.25	n.s
Large retail shops		1.338	0.03					
Small bakeries	2022	1.351	0.03	−0.144	−0.22	−0.07	−3.80	<0.001
Large retail shops		1.193	0.02					

CI, confidence interval; n.s, not significant; SE, standard error.

in year 2022 compared to 2012 in large retail shops. Additionally, although the similar trend was present in 2012, we evidenced significant difference in mean salt content in 2022 between the breads sold in small bakeries and those sold by large retail shops, where lower salt content was observed.

Using the one-way ANOVA and testing for between-subject effect related to type of retail environment, we found a significant difference ($p = 0.015$) between the salt content present in white wheat bread sold in small bakeries compared to white wheat bread sold in large retail stores, which commonly sell bread produced by large manufacturers. The white

wheat bread sold in small bakeries has a mean content of 1.34 (0.21) g salt/100 g, while the one sold in large retail shops has mean content of 1.21 (0.16) g salt/100 g. The salt content found in wholegrain breads purchased in small bakeries ($N = 29$) has the mean content of 1.37 (0.30) g salt/100 g, while wholegrain breads sold in large retail shops ($N = 4$) have 1.28 (0.24) g salt/100 g. Although the difference in means for wholegrain breads implies significant difference, the small sample size of samples purchased in large retail stores and the large variability within the sample obtained in small bakeries, did not provide the resource to further explore the observable difference. It should be mentioned that wholegrain bread samples in small bakeries correspond to samples that were offered by bakery staff when we asked for "bestselling wholegrain bread." These samples were non-prepacked and without any written declaration on the content of wholegrain ingredients and might not actually meet strict regulatory requirements for wholegrain bread (Figure 1).

For some bread samples purchased in larger retail shops we were able to access manufacturer data on the salt content, either from nutrition declaration on food labels or manufacturer website. Using pair-comparison t-test, we explored the difference between laboratory analyses and manufacturers' salt content data (Figure 2). We did not observe significant difference between the labeled and analytically determined salt content in prepacked breads, where regulation require mandatory declaration of the salt content. On the other hand, statistically significant difference was found in unpacked breads, where manufacturers' data on the salt content was taken from non-regulated websites.

4 Discussion

White wheat bread is most commonly consumed bread type in Slovenia, and therefore also most critical for food reformulation, because lowering of its salt content would have highest public-health effects. Interestingly we observed a significant difference between salt content in white wheat bread sold in large retail shops and small bakeries, with the latter having higher mean salt content (1.21 vs. 1.34 g/100 g, respectively). The reasons for such difference could be related to consumer preferences in the neighborhoods of specific small bakeries, differences in the manufacturing codes of practices (i.e., small bakeries are not included to Chamber of Commerce and Industry of Slovenia, which organize voluntary salt reduction program in Slovenia for major bread manufacturers), or technological variations which stem from specific characteristics of the used ingredients. These may also in part explain the high salt content range for white wheat bread sold in smaller bakeries (1.21 g/100 g), which is a consequence of very different amounts of salt used in the manufacturing process. A study on artisanal and industrial bread in Italy reported a higher overall salt content of common white wheat bread (1.5 (0.4) g/100 g) than our findings, however, unlike our results the salt content in industrially produced bread in Italy was higher than that of artisanal bread (35). Furthermore, an analysis of salt content in bread sold in the capital of neighboring Croatia indicated even higher salt levels, with the average content in white wheat bread reaching 2.00 (0.03) g/100 g in larger industrial bakeries and 2.23 (0.07) g/100 g in smaller bakeries (36). Similar results were also observed in Spain and Poland, where the average salt content in white wheat bread was determined at 2.09 (0.3) g/100 g and ~ 2.29 g/100 g, respectively (37, 38). Interestingly, the average salt content in wholegrain wheat bread was slightly higher than in white, half-white or mixed wheat bread. This is in line with results from a study done in Poland (38). Nonetheless, the variability in salt content of this bread type was high and could be a consequence of the use of different ingredients (e.g., using higher amount of salt to improve taste and aroma of wholegrain bread) and technologies, or variations in sodium content of different wheat flour types (39). It should be also

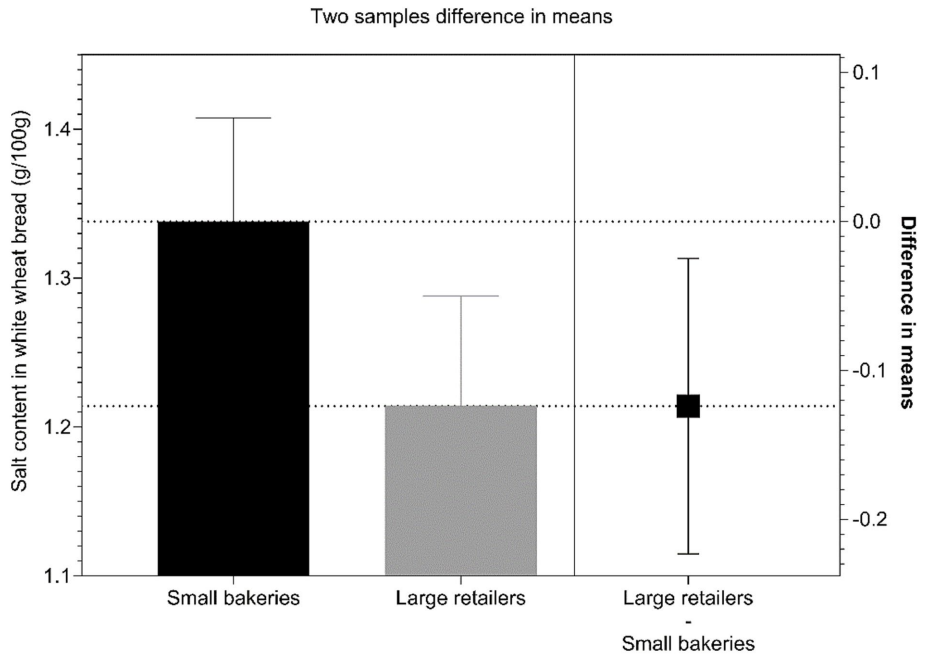

FIGURE 1
Analysis of differences in mean salt content (g/100 g) in white wheat breads purchased in small bakeries and large retail shops in 2022.

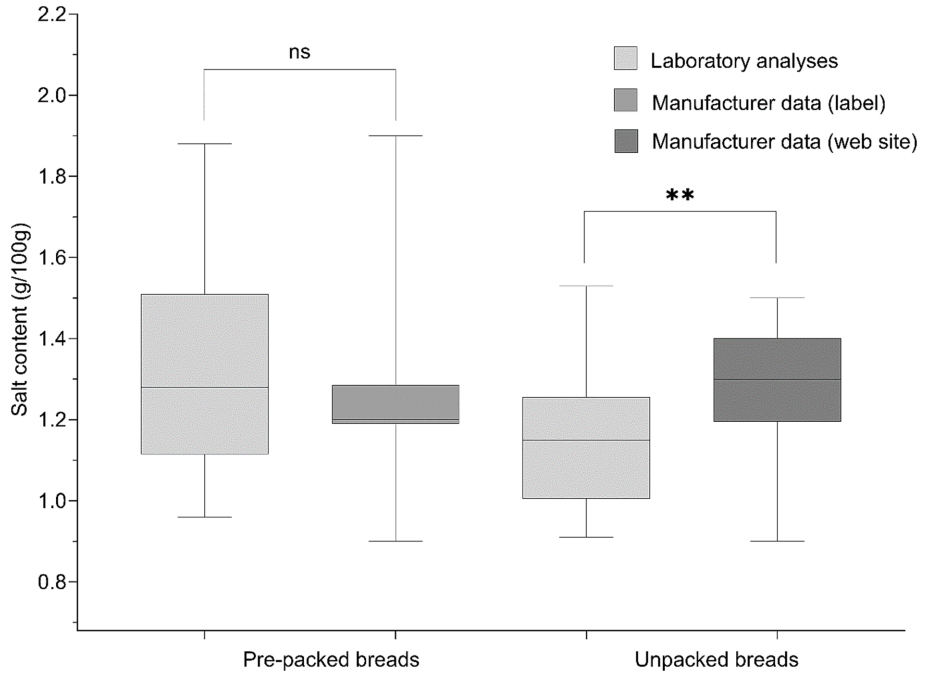

FIGURE 2
Box-and-whisker plots comparing salt content using laboratory analyses and manufacturer data nutrition declarations on food labels or websites of manufacturers/retailers for unpacked and prepacked breads (**represent significance at $p < 0.01$; ns, not significant difference).

noted that our sample of wholegrain breads in large retail shops was rather small, because of very limited offers of wholegrain breads in Slovenia. Analytically measured salt content in our samples of dark wheat bread and rye bread showed a lower salt content than other types (1.15 g/100 g and 1.18 g/100 g, respectively). This is almost half of salt content of dark wheat bread from bakeries in Zagreb (Croatia) where it was determined to be between 2.51 and 2.56 g/100 g (36). For rye bread, our results indicate lower mean content than that of both wholemeal rye bread and mixed wheat-rye bread from Poland (38).

We also observed significant differences between analytically measured salt content in un-packed breads, compared to manufacturers' salt content data published on websites, where nutrition declaration data can be provided voluntarily also for non-prepacked foods. We found that manufacturers provided data on higher salt content that was actually found in bread samples, which might be explained with the fact that on-line data is not regularly updated, even if foods are being reformulated. On the other side, we did not find such differences on sample of prepacked foods, where manufacturers data on the salt content was taken from mandatory nutrition declaration of food label. While in our study food labels were quite reliable, this cannot be projected to other jurisdictions, with different manufacturing practices and regulations. For example, Bernardo et al. compared sodium content determined by laboratory analysis and in nutrition declaration on bread rolls in Brazil and highlighted notable differences between −5 and 23% for rolls made from proprietary recipes, and between 16.1 and 46.9% in processed dough bread rolls (40). The observed difference between analytically measured values and nutrition declaration values could be due to different methods used to determine them. According to EU legislation, the labeled values should, according to the individual case, be average values and based on either the manufacturer's analysis of the food; a calculation from the known or actual average values of the ingredients used; or a calculation from generally established and accepted data (41).

Between 2010 and 2020, Slovenia implemented a national action plan to reduce salt intake in the Slovenian population (26). The plan included voluntary targets for various food categories such as bread and bakery products, meat products, salty snacks and breakfast cereals, which were based on the targets set in the United Kingdom. However, previous monitoring of bread in 2012 showed that the targets were not met, and a similar conclusion can be drawn for our results for year 2022. Furthermore, in 2021 the WHO introduced global sodium benchmarks for various food categories. Specifically, a target value of 330 mg sodium/100 g was set for leavened bread made from any type of cereal flour, which corresponds to approximately 0.84 g salt/100 g (42). Unfortunately, none of the analyzed breads in 2022 met this target, which highlights the need for further actions on reformulation and lowering the salt content.

Salt (sodium chloride) is an important ingredient in bread and bakery products as it stabilizes the gluten structure, modulates the fermentation rate, improves the color of the crust and texture and reduces water activity, which prolongs shelf life (43). Gradually reducing the salt content in bread to a set target is considered as the most common strategy. Studies on acceptability of reduced-sodium bread have reported that sodium reductions of up to 30–50% are acceptable to consumers (44–46). However, simply reducing the amount of salt used could have certain disadvantages such as the requirement of other producers' compliance to lower salt content or the possibility of consumers to simply purchase bread that does not have reduced salt content (47), which also indicates the importance of

consumer education for the success of reducing salt content and intake. Furthermore, reducing salt content beyond a certain point and without other additives/substitutes or production modifications, can lead to uncontrolled fermentation, changes in sensory profile, pale color of the crust due to an insufficient Maillard reaction, and a shorter shelf life, as salt also plays an important role in food safety. Since it reduces water activity and thus limits microbial growth, lowering the salt content requires careful adjustments with other strategies (48). In addition, reducing salt content can also lead to production difficulties due to increased dough stickiness. The mitigate such effects, replacing a proportion of sodium chloride with other salts is possible. Among them, potassium chloride seems to be promising, as potassium helps to maintain a similar dough rheology to doughs with sodium chloride (49). According to results of several studies, replacing ~25–50% of sodium chloride with potassium chloride is possible without decreasing product quality or increasing production difficulties. Other strategies for lowering the salt content of bread have also been explored, such as using taste enhancers (e.g., yeast extracts, monosodium glutamate, etc), creating taste contrasts by spatially distributing different salt concentrations (e.g., using two bread doughs with different concentrations), using coarse-grained salt, or modifying flavor by adding other herbs and spices (23, 47).

We used weighting approach to provide insights about changes in the salt content in the general category of bread in Slovenia. With consideration of statistical data on the consumption of various bread types, weighting approach was used to construct salt content for typical bread in Slovenia. This approach indicated a 7% decrease in salt content in bread category between the years 2012 and 2022 (Year 2012: 1.35 g/100 g; year 2022: 1.26 (95% CI 1.22–1.29) g/100 g). Considering very long observation period, we could label such progress as very conservative. This is particularly evident, when such a progress is compared to some other countries. For example, Brinsden et al. reported that, on average the salt content in packed bread in the United Kingdom has fallen by 20%, from 1.23 ± 0.19 g salt/100 g in 2001, to 0.98 ± 0.13 g salt/100 g in 2011 (24). In a recent report on the percentage of food categories meeting the salt reduction targets (average and maximum) for 2017, it was reported that 95% of in-home and 54% of out-of-home bread and roll products were at or below the maximum target of 0.45 g sodium/100 g (or approx. 1.14 g salt/100 g) (50).

While salt content of bread is relatively low, bread represents a staple food and is one of the main sources of salt in most European countries (19). In a nationally representative study of dietary habits and intake in Slovenian population, it was estimated that adult men consume an average of 177 grams of bread per day, while women consume 119 grams per day (51). Considering our results on the mean salt content of typical bread sold in Slovenia, this consumption corresponds to an estimated daily salt intake of about 2.2 grams for men and 1.5 grams for women. Compared to the very recently published data on salt consumption of Slovenian adults based on urinary sodium concentration (52), these values would represent about 19% of the daily salt intake for men and 17% for women. Furthermore, compared to the recommended daily salt intake of less than 5 grams, salt intake from bread accounts for about 30 to 46% of the recommended intake, respectively.

Our study indicates that activities to reduce the salt content in bread in Slovenia should be strengthened in future. This is even more important for smaller bakeries which are not included in voluntary salt reduction program, organized within Chamber of Commerce and Industry of Slovenia and which aims to gradually reduce the salt content of bread and bakery products. This program's first target was 5% reduction of the salt content by the end of 2022. However, no new targets have been set since then and therefore the results of our study will provide an important basis for future reformulation strategies and reduction targets in Slovenia. Additionally, it would be interesting to examine the salt reduction strategies large producers and smaller bakeries themselves use and their opinions on it, as it could provide better insight and starting point for the next reformulation strategy.

While a reduction in the salt content of bread could potentially have an impact on the overall salt intake of the Slovenian population, it would also be interesting to investigate how this affects iodine intake. In countries such as the Netherlands, it was shown that bread makes an important contribution to adequate iodine intake (53), as the salt used in the production of bread is iodized. In addition, an investigation of the changes in sodium and potassium intake in the case of a (partial) substitution of salt with KCl in bread would be informative, as would an economic evaluation of such a strategy. Furthermore, with consideration of wide sampling approach, our study enables further longitudinal observation of changes of the composition of bread in Slovenian food supply, both in large retailers and small bakeries.

4.1 Strengths and limitations

Our study included the main categories of bread available in both large retail shops and smaller bakeries in the 11 statistical regions of Slovenia. The salt content of bread in Slovenia has not been evaluated before to this extent. The sodium (salt) content of bread samples was analyzed by chemical analysis with ICP-MS, which has the potential to yield accurate results for foods with lower sodium concentrations. This approach also provides valuable insight, especially considering that nutritional information for unpacked breads is usually not displayed to the consumers at the point of purchase. Another strength of the study was that all samples were purchased in the real-life retail environment and not provided by the manufacturers. This approach assured us that we analyzed samples of bread, which are indeed available to consumers.

However, we should also mention some study limitations. Although we used a weighing approach to mitigate bias due to sampling, it cannot be completely ruled out as we asked the bakery worker to choose bestselling white and wholegrain bread. The sample size for certain bread categories (e.g., wholegrain bread) was relatively small and with high variability in the salt content; therefore, we could not explore the differences between different sale points in depth. At this point, we should mention that the small sample size is due to the limited availability of wholegrain bread in large retail stores in Slovenia. We should also mention that the bread samples were purchased as they were offered and may deviate from the quality standards for bread set by law in Slovenia (e.g., wholegrain bread with lower percentage of wholegrain flour than defined in the Rules on quality of bakery products (32)). This limitation is particularly relevant for the sample of breads from small bakeries, where some of the wholegrain breads may actually be categorized as mixed breads,

but we did not have access to ingredient lists to verify this. We should also mention that different laboratory analytical methods were used for determination of salt content in years 2012 and 2022, and therefore comparisons between our results from 2012 and 2022 should be interpreted with caution. This also applies to comparisons with the results of other countries, as many used the potentiometric or Volhard method to determine the salt content, which can be less accurate as the ICP-MS method used in our study for year 2022. Additionally, we calculated the salt content on the assumption that all sodium corresponds to sodium chloride. However, sodium may also have been added in the form of other sodium salts (e.g., sodium carbonates), which could lead to a slight overestimation of the salt content.

5 Conclusion

Study results showed small decrease in the salt content in bread category in Slovenia in the last decade. The decrease was somewhat more notable in the subcategory of white wheat bread sold in large retail shops. However, the targets for sodium/salt reduction set by the WHO have not been met. A large variability between bread samples has also been observed. This study provides an important insight into salt content reduction progress and emphasizes the importance of strengthening activities on the reformulation of bread. This is even more important for smaller bakeries, which are currently not efficiently included into voluntary bread reformulation program.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

SK: Conceptualization, Writing – original draft, Writing – review & editing. HH: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. UB: Conceptualization, Supervision, Writing – review & editing. MH: Investigation, Writing – review & editing. EH: Investigation, Writing – review & editing. AK: Investigation, Writing – review & editing. IP: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1473362/full#supplementary-material

References

1. Turck D, Castenmiller J, de Henauw S, Hirsch-Ernst KI, Kearney J, Knutsen HK, et al. Dietary reference values for sodium. *EFSA J*. (2019) 17:e05778. doi: 10.2903/j.efsa.2019.5778

2. Powles J, Fahimi S, Micha R, Khatibzadeh S, Shi P, Ezzati M, et al. Global, regional and national sodium intakes in 1990 and 2010: a systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. *BMJ Open*. (2013) 3:e003733. doi: 10.1136/bmjopen-2013-003733

3. D'Elia L, Rossi G, Ippolito R, Cappuccio FP, Strazzullo P. Habitual salt intake and risk of gastric cancer: a meta-analysis of prospective studies. *Clin Nutr*. (2012) 31:489–98. doi: 10.1016/j.clnu.2012.01.003

4. Cappuccio FP. Cardiovascular and other effects of salt consumption. *Kidney Int Suppl*. (2013) 3:312–5. doi: 10.1038/kisup.2013.65

5. He FJ, Li J, MacGregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. *BMJ*. (2013) 346:f1325–5. doi: 10.1136/bmj.f1325

6. Huang L, Trieu K, Yoshimura S, Neal B, Woodward M, Campbell NRC, et al. Effect of dose and duration of reduction in dietary sodium on blood pressure levels: systematic review and meta-analysis of randomised trials. *BMJ*. (2020) 368:m315. doi: 10.1136/bmj.m315

7. Ma Y, He FJ, Sun Q, Yuan C, Kieneker LM, Curhan GC, et al. 24-hour urinary sodium and potassium excretion and cardiovascular risk. *N Engl J Med*. (2022) 386:252–63. doi: 10.1056/NEJMoa2109794

8. Webb M, Fahimi S, Singh GM, Khatibzadeh S, Micha R, Powles J, et al. Cost effectiveness of a government supported policy strategy to decrease sodium intake:

global analysis across 183 nations. *BMJ*. (2017) 356:i6699. doi: 10.1136/bmj.i6699

9. Bibbins-Domingo K, Chertow GM, Coxson PG, Moran A, Lightwood JM, Pletcher MJ, et al. Projected effect of dietary salt reductions on future cardiovascular disease. *N Engl J Med*. (2010) 362:590–9. doi: 10.1056/NEJMoa0907355

10. World Health Organization. Global action plan for the prevention and control of noncommunicable diseases, 2013–2020. Geneva: World Health Organization, pp. 1–103. (2013).

11. Trieu K, Neal B, Hawkes C, Dunford E, Campbell N, Rodriguez-Fernandez R, et al. Salt reduction initiatives around the world – a systematic review of Progress towards the global target. *PLoS One*. (2015) 10:e0130247. doi: 10.1371/journal.pone.0130247

12. World Health Organization. Best buys and other recommended interventions for the prevention and control of noncommunicable diseases. Geneva: World Health Organization (2017). 28 p.

13. World Health Organization. Accelerating salt reduction in Europe: A country support package to reduce population salt intake in the WHO European region. Copenhagen: World Health Organization (2020). 49 p.

14. Hendriksen MAH, Verkaik-Kloosterman J, Noort MW, van Raaij JMA. Nutritional impact of sodium reduction strategies on sodium intake from processed foods. *Eur J Clin Nutr*. (2015) 69:805–10. doi: 10.1038/ejcn.2015.15

15. Brown IJ, Tzoulaki I, Candeias V, Elliott P. Salt intakes around the world: implications for public health. *Int J Epidemiol*. (2009) 38:791–813. doi: 10.1093/ije/dyp139

16. Anderson CAM, Appel LJ, Okuda N, Brown IJ, Chan Q, Zhao L, et al. Dietary sources of sodium in China, Japan, the United Kingdom, and the United States, women and men aged 40 to 59 years: the INTERMAP study. *J Am Diet Assoc*. (2010) 110:736–45. doi: 10.1016/j.jada.2010.02.007

17. Meneton P, Lafay L, Tard A, Dufour A, Ireland J, Ménard J, et al. Dietary sources and correlates of sodium and potassium intakes in the French general population. *Eur J Clin Nutr*. (2009) 63:1169–75. doi: 10.1038/ejcn.2009.57

18. Eyles HC, Cleghorn CL. Dietary sources of sodium across the diverse New Zealand adult population. *Prev Med*. (2022) 29:101927. doi: 10.1016/j.pmedr.2022.101927

19. Kloss L, Meyer JD, Graeve L, Vetter W. Sodium intake and its reduction by food reformulation in the European Union - a review. *NFS J*. (2015) 1:9–19. doi: 10.1016/j.nfs.2015.03.001

20. Bhat S, Marklund M, Henry ME, Appel LJ, Croft KD, Neal B, et al. A systematic review of the sources of dietary salt around the world. *Adv Nutr*. (2020) 11:677–86. doi: 10.1093/advances/nmz134

21. Belz MCE, Ryan LAM, Arendt EK. The impact of salt reduction in bread: a review. *Crit Rev Food Sci Nutr*. (2012) 52:514–24. doi: 10.1080/10408398.2010.502265

22. Pashaei M, Zare L, Khalili Sadrabad E, Hosseini Sharif Abad A, Mollakhalili-Meybodi N, Abedi A-S. The impacts of salt reduction strategies on technological characteristics of wheat bread: a review. *J Food Sci Technol*. (2022) 59:4141–51. doi: 10.1007/s13197-021-05263-6

23. Silow C, Axel C, Zannini E, Arendt EK. Current status of salt reduction in bread and bakery products – a review. *J Cereal Sci*. (2016) 72:135–45. doi: 10.1016/j.jcs.2016.10.010

24. Brinsden HC, He FJ, Jenner KH, MacGregor GA. Surveys of the salt content in UK bread: progress made and further reductions possible. *BMJ Open*. (2013) 3:e002936. doi: 10.1136/bmjopen-2013-002936

25. Ribic CH, Zakotnik JM, Seljak BK, Policnik R, Blaznik U, Mis NF, et al. Estimation of sodium availability in food in Slovenia: results from household food purchase data from 2000 to 2009. *Zdr Varst*. (2014) 53:209–19. doi: 10.2478/sjph-2014-0021

26. Ministrstvo za zdravje. Nacionalni akcijski načrt zazmanjševanje uživanja soli v prehrani prebivalcev Slovenije za obdobje 2010–2020 [national action plan for reducing salt intake in the diet of the population of Slovenia for the period 2010–2020]. Ljubljana: Ministrstvo za zdravje (2010). 45 p.

27. Krušič S, Hristov H, Hribar M, Lavriša Ž, Žmitek K, Pravst I. Changes in the sodium content in branded foods in the Slovenian food supply (2011–2020). *Nutrients*. (2023) 15:4304. doi: 10.3390/nu15194304

28. Blaznik U, Škrjanc B, Hlastan RC. Končno poročilo o rezultatih analiz vsebnosti soli v kruhu, mesnih izdelkih in obrokih v letu 2012 [final report on the results of analyses of salt content in bread, meat products and meals in the year 2012]. Ljubljana: Inštitut za varovanje zdravja RS (2013). 8 p.

29. Statistični urad Republike Slovenije. *Količina doma porabljenih živil in pijač na člana gospodinjstva, letno povprečje [Average annual quantity of food and beverages consumed per household member]*. SiStat (2022). Available at: https://pxweb.stat.si/SiStatData/pxweb/en/Data/-/0878705S.px/.

30. Ministrstvo za Kmetijstvo Gozdarstvo in Prehrano. *Primerjaj cene [Compare prices]*. (2022). Available at: https://www.primerjaj-cene.si/sl/hrana/.

31. Eurostat. Statistical regions in the European Union and partner countries. NUTS and statistical regions 2021. Luxembourg: Publications Office of the European Union, pp. 1–188. (2022).

32. Pravilnik o Kakovosti Pekovskih Izdelkov [Rules on the Quality of Bakery Products]. *Ljubljana: Ministrstvo za kmetijstvo, gozdarstvo in prehrano*. p. 9. (2015).

33. Pravilnik o Kakovosti Izdelkov iz Žit [Rules on the Quality of Cereal Products]. *Ljubljana: Ministrstvo za kmetijstvo in okolje*. p. 19. (2014).

34. AOAC. Determination of calcium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium, and zinc in fortified food products by microwave digestion and inductively coupled plasma-optical emission spectrometry: single-laboratory validation and ring trial. *AOAC Int*. (2011) 95:177–85. doi: 10.5740/jaoacint.CS2011_14

35. Carcea M, Narducci V, Turfani V, Aguzzi A. A survey of sodium chloride content in Italian artisanal and industrial bread. *Food Secur*. (2018) 7:181. doi: 10.3390/foods7110181

36. Delaš Aždajić M, Delaš I, Aždajić S, Štimac Grbić D, Vahčić N. A cross sectional study of salt content in bakery bread in Zagreb, Croatia. *Arh Hig Rada*. (2019) 70:219–23. doi: 10.2478/aiht-2019-70-3277

37. Pérez Farinós N, Santos Sanz S, Mª Ángeles DR, Yusta Boyo MJ, Robledo T, Castrodeza JJ, et al. Salt content in bread in Spain, 2014. *Nutr Hosp*. (2017) 35:650–4. doi: 10.20960/nh.1339

38. Winiarska-Mieczan A, Kwiatkowska K, Kwiecień M, Baranowska-Wójcik E, Wójcik G, Krusiński R. Analysis of the intake of sodium with cereal products by the population of Poland. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. (2019) 36:884–92. doi: 10.1080/19440049.2019.1605209

39. Ertl K, Goessler W. Grains, whole flour, white flour, and some final goods: an elemental comparison. *Eur Food Res Technol*. (2018) 244:2065–75. doi: 10.1007/s00217-018-3117-1

40. Bernardo GL, Inoue TB, Höfelmann DA, Feldman CH, Veiros MB. Sodium and salt content of Portuguese rolls produced in a city of southern Brazil: a comparison of laboratory analysis, food labelling and nutrition standards. *Public Health Nutr*. (2021) 24:2337–44. doi: 10.1017/S1368980020000786

41. European Commission. *Guidence document for competent authorities for the control of complience with EU legislation on: regulation (EU) no 1169/2011 of the European Parliament and of the council of 25 October 2011 on the provision of food information to consumers, amending regulations (EC) no 1924/2006 and (EC) no 1925/2006 of the European Parliament and of the council, and repealing commission directive 87/250/EEC, council directive 90/496/EEC, commission directive 1999/10/EC, directive 2000/13/EC of the European Parliament and of the council, commission directives 2002/67/EC and 2008/5/EC and commission regulation (EC) no 608/2004 and council directive 90/496/EEC of 24 September 1990 on nutrition labelling of foodstuffs and directive 2002/46/EC of the European Parliament and of the council of 10 June 2002 on the approximation of the laws of the member states relating to food supplements with regard to the setting of tolerances for nutrient values declared on a label*, pp. 1–15. (2012).

42. World Health Organization. WHO global sodium benchmarks for different food categories. Geneva: World Health Organization (2021). 32 p.

43. Ferrari GT, Proserpio C, Stragliotto LK, Boff JM, Pagliarini E, Oliveira VR. Salt reduction in bakery products: a critical review on the worldwide scenario, its impacts and different strategies. *Trends Food Sci Technol*. (2022) 129:440–8. doi: 10.1016/j.tifs.2022.10.013

44. La Croix KW, Fiala SC, Colonna AE, Durham CA, Morrissey MT, Drum DK, et al. Consumer detection and acceptability of reduced-sodium bread. *Public Health Nutr*. (2015) 18:1412–8. doi: 10.1017/S1368980014001748

45. Lobo CP, Ferreira TA. Hedonic thresholds and ideal sodium content reduction of bread loaves. *Food Res Int*. (2021) 140:110090–8. doi: 10.1016/j.foodres.2020.110090

46. Bolhuis DP, Temme EHM, Koeman FT, Noort MWJ, Kremer S, Janssen AM. A salt reduction of 50% in bread does not decrease bread consumption or increase sodium intake by the choice of sandwich fillings. *J Nutr*. (2011) 141:2249–55. doi: 10.3945/jn.111.141366

47. Dunteman A, Yang Y, McKenzie E, Lee Y, Lee SY. Sodium reduction technologies applied to bread products and their impact on sensory properties: a review. *Int J Food Sci Technol*. (2021) 56:4396–407. doi: 10.1111/ijfs.15231

48. Lorén N, Niimi J, Höglund E, Albin R, Rytter E, Bjerre K, et al. Sodium reduction in foods: challenges and strategies for technical solutions. *J Food Sci*. (2023) 88:885–900. doi: 10.1111/1750-3841.16433

49. Avramenko NA, Tyler RT, Scanlon MG, Hucl P, Nickerson MT. The chemistry of bread making: the role of salt to ensure optimal functionality of its constituents. *Food Rev Int*. (2018) 34:204–25. doi: 10.1080/87559129.2016.1261296

50. Niblett P, Coyle N, Little E, Fitzpatrick J, Nicholas J, Knowles B, et al. Salt targets 2017: Second progress report. A report on the food industry's progress towards meeting the 2017 salt targets. London: Public Health England (2020). 62 p p.

51. Gregorič M, Hristov H, Blaznik U, Koroušić Seljak B, Delfar N, Pravst I. Dietary intakes of Slovenian adults and elderly: design and results of the National Dietary Study SI.Menu 2017/18. *Nutrients*. (2022) 14:3618. doi: 10.3390/nu14173618

52. Kugler S, Blaznik U, Rehberger M, Zaletel M, Korošec A, Somrak M, et al. Twenty-four hour urinary sodium and potassium excretion in adult population of Slovenia: results of the manjsoli.Si/2022 study. *Public Health Nutr*. (2024) 27:e163. doi: 10.1017/S1368980024001605

53. Verkaik-Kloosterman J, Buurma-Rethans EJM, Dekkers ALM, Van Rossum CTM. Decreased, but still sufficient, iodine intake of children and adults in the Netherlands. *Br J Nutr*. (2017) 117:1020–31. doi: 10.1017/S0007114517000733

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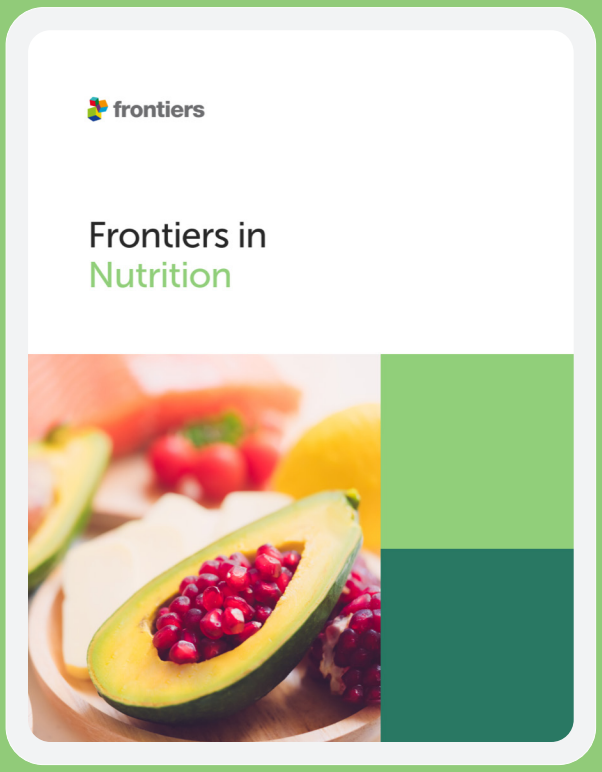

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