



Lack of Association Between Sodium Intake and Cytokine Levels

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Purpose: The complex pathogenesis of hypertension, potentially involving inflammatory pathways, remains elusive. This study aimed to evaluate the relationship between 24-hour urinary sodium excretion and inflammatory cytokines alongside C-reactive protein (CRP) in a nationwide Finnish sample.

Materials and methods: 265 participants from the FINRISK 2002 study were included in the analyses. Multivariable-adjusted associations of 24-hour urinary sodium with circulating CRP and 26 cytokines were examined.

Results: 24-hour urinary sodium was not significantly associated with any of the cytokines or CRP ($p \geq 0.02$ for all, significance at <0.001). Adjustments for age, sex, serum creatinine concentration, and alcohol intake did not alter these results.

Conclusion: This cross-sectional study revealed no associations between 24-hour urinary sodium and cytokine or CRP levels. This does not suggest reducing salt intake would be unbeneficial in hypertension. Additional research is required to clarify the mechanisms through which salt may induce hypertension. Assessing sodium intake in epidemiological studies is also challenging.

Keywords: inflammatory cytokines, C-reactive protein, urinary sodium concentration, salt intake

Introduction

Hypertension is among the leading causes of disease burden worldwide.¹ Despite the significant burden it imposes on global health, its complex pathogenesis, encompassing various mechanisms such as renal, vascular, neural, inflammatory, and immune pathways,² is not yet fully understood.

Increasing evidence has drawn focus to the possible role of pro- and anti-inflammatory cytokines in the pathogenesis of hypertension.³⁻⁷ Proinflammatory cytokines that may contribute to the development of hypertension include tumor necrosis factor (TNF)- α , interleukin (IL)-17, monocyte chemoattractant protein (MCP)-1 and IL-6.³⁻⁶ Past studies suggest that also C-reactive protein (CRP) is associated with hypertension.⁵ Conversely, certain other mediators, such as IL-10, have been identified as factors that may protect from developing hypertension.⁶

It has been suggested that salt may trigger the immune system, which plays a role in the development of sustained hypertension.⁸ The association of salt intake and proinflammatory cytokines has been investigated very scarcely previously. A Chinese study found a significant correlation between 24-hour urinary sodium excretion and IL-6 in 627 prehypertensive participants. In contrast, 24-hour urinary sodium excretion and TNF- α were not significantly correlated in that study.⁹ A recent Finnish randomized intervention study of 106 participants found that high salt intake did not alter the levels of 45 circulating cytokines.¹⁰

We endeavored to illuminate these questions by studying the associations of 24-hour urinary sodium excretion with cytokine and CRP levels in a population-based sample ($n=265$) comprising Finnish middle-aged adults.

Methods

Participants

In the present study, we analyzed data from the FINRISK 2002 study,¹¹ which included information on 24-hour urinary sodium excretion cytokine levels, and blood pressure (BP). A random sample of 13437 individuals aged 25–74 years was invited to participate, and 8799 (65.5%) of those invited took part in the study. A randomly chosen sub-sample of the participants was requested to collect a 24-h urine specimen, resulting in 909 individuals providing complete urine collections. Of those 909 participants, after exclusion of those with missing values on any of the covariates or cytokine data (n=552) and thereafter those on antihypertensive medication (n=92), a total of 265 participants were included in the analyses of the present study.

Health Examination

The participants completed a questionnaire on sociodemographic information, lifestyles, medications, and medical history at home. Physical examinations and blood sample taking were performed at a local study site by a trained staff in 2002. A nurse measured BP in sitting position three times on the right arm using a mercury sphygmomanometer and a 14×40 cm sized cuff after a 5-min rest. BP variables in the present study were defined as an average of the three BP measurements. Hypertension was defined as a systolic BP of 140 mmHg or more and/or a diastolic BP of 90 mmHg or more. The participants underwent measurements for height and weight, and body mass index was calculated as weight (kg) divided by height (m) squared. Alcohol intake was assessed through a questionnaire, capturing self-reported average weekly alcohol intake (g) over the past year.

Urine Sample Collections

The 24-hour urine collection was mostly performed on Sundays. The participants were not informed about the purpose of the urine collection to prevent bias resulting from reduced salt intake before the collection. According to an external quality assurance program organized by Labquality (Helsinki, Finland), the bias of the sodium assay method was 0.2% (SD 1.85, n = 12). An ion-selective electrode was used in the analyses. Details of the urine collections have been described in more detail previously.¹²

Inflammatory Cytokines and CRP

The concentrations of 27 cytokines were measured from plasma samples using antibody-based magnetic bead kits (Bio-Plex Pro Human Cytokine 27-plex assay, Bio-Rad, Helsinki, Finland) for multiplexed protein quantification, following the manufacturer's instructions. The cytokines analyzed from the participants' blood samples were: RANTES (regulated on activation, normal T cell expressed and secreted), IL-1 β , IL1-RA (receptor antagonist), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70 (active form of interleukin-12 composed of p35 and p40 subunits), IL-13, IL-15, IL-17, BFGF (basic fibroblast growth factor), eotaxin, G-CSF (granulocyte colony-stimulating factor), GM-CSF (granulocyte-macrophage colony stimulating factor), IFN- γ (interferon gamma), PDGF-BB (platelet-derived growth factor), TNF- α , VEGF (vascular endothelial growth factor), CCL2 (C-C motif ligand 2), CCL3, CCL4, and CXCL10 (C-X-C motif chemokine ligand 10). Alongside the cytokine analyses, high-sensitivity CRP concentration was measured using an immunoturbidimetric method (Orion Diagnostica, Espoo, Finland). This was done on the Optima analyzer (Thermo Electron Corporation, Vantaa, Finland) at the laboratory of the Finnish Institute for Health and Welfare. The lowest detectable level was 0.2 mg/L, and any values falling below this threshold were recorded as 0.1.

Statistical Methods

For statistical analyses, we included only the cytokines for which less than 50% of the measurement data were missing. Consequently, IL-15 was excluded due to data limitations ([Supplementary Table S1](#)). To handle missing data, we imputed 5 complete datasets for cytokines using the Markov Chain Monte Carlo method. Our imputation models included age, sex, serum creatinine concentration, alcohol intake, and all cytokine concentration values. If a cytokine had values under

or above the detection limit, we imputed those missing values by randomly selecting them from a spread aligned with the cytokine's inherent distribution. We applied log transformations to cytokine concentrations.

We assessed the associations of 24-hour urinary sodium excretion and levels of cytokines and CRP with linear regression models. A separate model was run for each cytokine and CRP. All models were adjusted for age, sex, serum creatinine concentration, and alcohol intake. In these analyses, we used $p < 0.001$ as the level of significance to account for multiple testing and for the number of cytokines and CRP (0.05 divided by 27). All statistical analyses were performed using R, version 4.3.1 (R Core Team, Vienna, Austria).

Ethics

All participants gave written informed consent for their data to be used in scientific research purposes with anonymity. FINRISK 2002 was approved by the Ethical Committee on Epidemiology and Public Health of the Helsinki and Uusimaa Hospital District (decision number 87/2001).

Results

The Characteristics of the Study Population are Presented in Table 1

24-hour urinary sodium level did not associate with cytokine or CRP concentrations significantly ($p \geq 0.02$ for all, significance at <0.001) (Figure 1). These findings remained unaltered after adjustments for age, sex, serum creatinine concentration, and alcohol intake.

Had the threshold for statistical significance been < 0.05 , 24-hour urinary sodium excretion would have shown a significant association with IL-12p70 concentration ($p = 0.02$). However, the significance threshold determined in this work was 0.001 (0.05 divided by 27).

We reiterated the analyses in a population that also included those on antihypertensive medication, but the results remained practically unchanged (data not shown).

Discussion

The objective of this study was to investigate the association between 24-hour urinary sodium excretion and levels of various inflammatory cytokines and CRP. This research aimed to enhance our understanding of the possible inflammatory mediation in the relationship between salt intake and persistent hypertension. However, 24-hour urinary sodium excretion did not associate significantly with inflammatory cytokine and CRP concentrations.

In a recent Finnish dose-response study involving 106 participants, higher salt intake did not significantly impact the concentrations of 45 circulating cytokines. The study compared two groups: individuals following a habitual diet and those adhering to a Nordic healthy diet, further divided into reduced sodium and usual sodium intake groups. The study's

Table 1 Characteristics by Quintiles of 24-Hour Urinary Sodium Excretion

| Characteristics | Total | Q1 | Q2 | Q3 | Q4 | Q5 |
|--------------------------------------|-------------|------------|------------|------------|------------|-------------|
| N | 265 | 53 | 57 | 51 | 51 | 53 |
| Age (years) | 57.4±3.6 | 57.0±3.8 | 57.7±3.6 | 57.4±3.6 | 57.2±3.6 | 57.6±3.4 |
| Women | 134 (50.6%) | 43 (81.1%) | 40 (70.2%) | 27 (52.9%) | 15 (29.4%) | 9 (17.0%) |
| Body mass index (kg/m ²) | 27.0±4.1 | 27.0±4.2 | 27.0±4.6 | 26.3±3.8 | 27.5±3.9 | 27.5±4.0 |
| Systolic blood pressure (mmHg) | 139.5±18.9 | 139.5±17.4 | 141.6±22.4 | 135.3±17.9 | 142.3±18.3 | 138.5±17.5 |
| Diastolic blood pressure (mmHg) | 82.9±9.7 | 81.2±9.9 | 82.3±10.2 | 82.0±8.6 | 83.7±9.1 | 85.1±10.6 |
| 24-hour urinary sodium | | | | | | |
| Mean, mmol/day | 81.6±34.6 | 40.7±7.0 | 60.5±5.2 | 76.6±4.7 | 95.3±8.0 | 136.9±20.4 |
| Range, mmol/day | 28.0–182.0 | 28.0–52.0 | 54.0–68.0 | 69.0–84.0 | 86.0–109.0 | 110.0–182.0 |
| Dietary alcohol (ethanol) (g/week) | 83.0±120.9 | 82.4±151.5 | 71.2±118.3 | 76.4±106.6 | 87.3±128.2 | 98.6±94.5 |
| Serum creatinine (μmol/L) | 72.3±12.4 | 68.4±12.7 | 69.2±11.6 | 71.5±13.1 | 76.2±11.5 | 76.4±11.1 |

Notes: Values are means ± standard deviations for continuous data and numbers and percentages for categorical data. Q1–Q5, quintiles of 24-hour urinary sodium excretion.

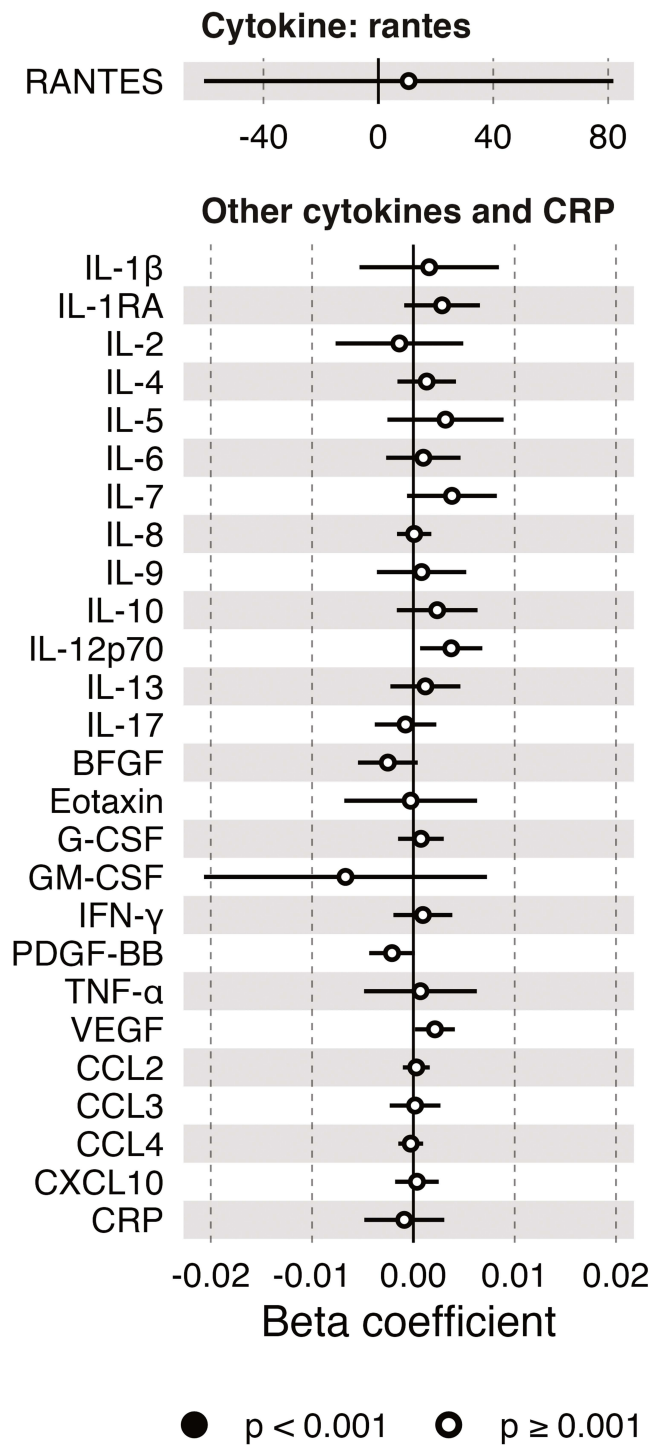


Figure 1 The associations of 24-hour urinary sodium excretion (mmol/day) with log-transformed cytokine and C-reactive protein (CRP) concentrations presented as beta coefficients and 95% confidence intervals. A separate model was run for each cytokine and CRP. All models were adjusted for age, sex, serum creatinine concentration, and alcohol intake. $p = 0.001$ was used as the level of significance to account for the number of cytokines. None of the depicted associations were statistically significant. RANTES, regulated on activation, normal T cell expressed and secreted.

Abbreviations: IL, interleukin; IL1-RA, interleukin-1 receptor antagonist; IL-12p70, active form of interleukin-12 composed of p35 and p40 subunits; BFGF, basic fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; IFN- γ , interferon gamma; PDGF-BB, platelet-derived growth factor; TNF- α , tumor necrosis factor α ; VEGF, vascular endothelial growth factor; CCL, C-C motif ligand; CXCL10; C-X-C motif chemokine ligand 10; CRP, C-reactive protein.

results suggested that excess salt intake may not trigger a cytokine-linked pathway in chronic low-grade inflammation,¹⁰ aligning with the findings of the present study.

The findings of a separate dose-response study conducted in Italy presented some disparities from the Finnish study. In the Italian study, researchers investigated the relationship between 24-hour urinary sodium excretion and inflammatory cytokine concentrations among patients with rheumatoid arthritis and systemic lupus erythematosus.¹³ Sodium reduction was negatively associated with IL-9 and transforming growth factor beta concentrations, suggesting that restricted sodium intake might attenuate the immune response in patients with autoimmune disease. However, the Italian study was conducted on only 29 patients, and the Finnish study was comprised of general adult population.

In addition to IL-9,¹⁴ TNF- α and IL-6 are recognized as cytokines that may be linked to hypertension development, whereas the anti-inflammatory cytokine IL-10 is considered a potential protective factor against hypertension.^{3,5,6} An *in vivo* study involving six human participants in a spaceflight simulation demonstrated that reduced dietary salt intake led to decreased IL-6 levels and increased IL-10 levels, while no significant changes in TNF- α levels were observed.¹⁵ Conversely, recent findings suggest that high salt intake in rodents suppresses the production of pro-inflammatory cytokines such as TNF- α and IL-6.¹⁶

The variance in the present study's findings compared to other studies may, in part, be attributed to publication bias, which tends to favor published research with more positive outcomes. Additionally, the challenges of capturing associations between 24-hour urinary sodium excretion and levels of inflammatory cytokines and CRP could be particularly pronounced in diverse global settings. The complexity of these relationships underscores the need for nuanced, and perhaps region-specific, investigations to comprehensively understand the interplay between sodium intake, inflammatory markers, and hypertension.

The strengths of the present study include the use of 24-hour urinary sodium sampling, which is the gold standard for assessing sodium intake in population studies. Another strength of this study is the population-based sample. Our study has several limitations. One limitation was the scarcity of data on medications beyond antihypertensive drugs. Many other drugs, such as glucocorticoids and non-steroidal anti-inflammatory drugs, have the potential to alter cytokine production.^{17,18} Additionally, due to lack of adequate follow-up data the present study utilized only a cross-sectional design. An additional limitation is the absence of measurements for certain factors related to BP regulation and immune response, such as renin-angiotensin system activation, nitric oxide, plasma asymmetric dimethylarginine levels, and other immune activation markers. Including these factors could provide further insights into the physiological impacts of sodium intake. It is possible that individuals in better overall health may show a higher level of enthusiasm for participating in public health studies, particularly those involving demanding tests, such as urinary sampling. Longitudinal analyses are needed in the future to evaluate long-term effects of salt intake on inflammatory cytokines.

Beyond the above limitations, additional considerations are important when interpreting the immunological aspects of our findings. The present study explored the relationship between urinary sodium excretion and inflammatory factors in a cohort of normal subjects. The rationale behind investigating this relationship in subjects without inflammatory diseases or sodium excretory issues was to establish a baseline understanding of these interactions in a healthy population. This foundational knowledge is crucial for future studies that may explore similar relationships in diseased states.

It is well-documented that high salt intake can induce immune responses,¹⁹ yet the impact of normal salt intake on inflammatory markers remains less understood. This study sought to fill this gap by examining urinary sodium excretion across different quintiles without explicitly measuring daily salt intake. The apparent negative results observed in this study can serve as valuable control data for future research involving subjects with high salt intake or induced immune responses. Future studies should incorporate precise dietary assessments to strengthen the findings. The negative results observed in this study could also be attributed to other nutritional factors that counterbalanced the effects of increased salt intake. Factors such as potassium, magnesium, and essential fatty acids, which have antihypertensive properties and are precursors to vasoactive eicosanoids,^{20,21} may have played a role. Additionally, genetic polymorphisms and lifestyle factors such as exercise may have contributed to the observed resistance to increased salt intake.

The lack of a high salt intake assessment and the absence of a disease state with enhanced immune responses are notable limitations. Future research should aim to compare these relationships in populations with known inflammatory

conditions or those subjected to high salt diets. Such studies could provide more definitive insights into the role of sodium in modulating immune responses.

In conclusion, our cross-sectional analyses revealed no significant associations between 24-hour urinary sodium excretion and levels of inflammatory cytokines and CRP. The findings of our study do not suggest that reducing salt intake would be unbeneficial in hypertension management. Instead, they underscore the need for further studies to understand the complex pathogenesis of hypertension. Furthermore, they emphasize the epidemiological challenges involved in assessing sodium intake in population studies.

Data Sharing Statement

The FINRISK 2002 data are available from the THL Biobank based on a research application, as explained on the website of the THL Biobank (<https://thl.fi/en/research-and-development/thl-biobank/for-researchers/sample-collections/the-national-finrisk-study-1992-2012>).

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Disclosure

Dr Teemu Niiranen reports personal fees from Servier Finland, personal fees from Astra Zeneca, outside the submitted work. The authors declare that they have no other competing interests.

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