Short Communication

Sex-specific effects of dietary salt intake on circulating Alzheimer's disease-related biomarkers in aged rats

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Abstract

This study collected plasma samples from aged male and female Sprague Dawley rats (22–24 months old) with varying long-term dietary salt intake (low, 0.1% NaCl; normal, 0.4% NaCl; or clinically relevant high salt, 1% NaCl; for twelve weeks). Dementia-related biomarkers in the plasma, including amyloid-β peptide 1–42, tau protein, and glial fibrillary acidic protein, were measured using enzyme-linked immunosorbent assay kits. The primary outcome revealed sex differences in the impact of dietary salt on these biomarkers.

Keywords

Alzheimer's disease, amyloid-β, dietary salt intake, glial fibrillary acidic protein, sex differences, tau

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Introduction

The global average dietary salt intake is approximately twice the amount recommended by the World Health Organization (WHO).[1] Excessive salt consumption has been linked to cognitive impairment.[2,3] However, the studies supporting this finding have limitations. Human studies lack a reliable method to accurately measure individual salt intake,[4,5] and animal studies often use extremely high-salt diets—8 to 20 times more than the control—which may not be clinically relevant.[2,5] Furthermore, medical research has frequently neglected the distinct health concerns and physiological differences between men and women, resulting in knowledge gaps.

Alzheimer's disease (AD) develops gradually, progressing from an asymptomatic stage to a fully expressed clinical syndrome over many years.[6] Given the lack of effective therapies, timely referral of at-risk populations, such as older adults with high salt intake, is crucial for early diagnosis and prompt intervention.[7] This study collected plasma samples from aged male and female Sprague Dawley (SD) rats with varying long-term dietary salt intake. Dementia-related biomarkers in the plasma, including amyloid-β peptide 1–42 ($A\beta_{1-42}$), tau protein, and glial fibrillary acidic protein (GFAP), were measured. The primary outcome revealed differences in the impact of dietary salt on these biomarkers.

Methods

Animals and diets

Both male and female SD rats (22–24 months old) were purchased from Slac Laboratory Animal (Shanghai, China) and housed in standard cages in a temperature-controlled facility with a 12-h light/dark cycle. As previously described,[5] all animal experiments were approved by the Research Ethics Committee of Hangzhou Normal University (Approval reference# HSD20211205). The animal experimental procedures followed the National Institutes of Health Guide for the Care

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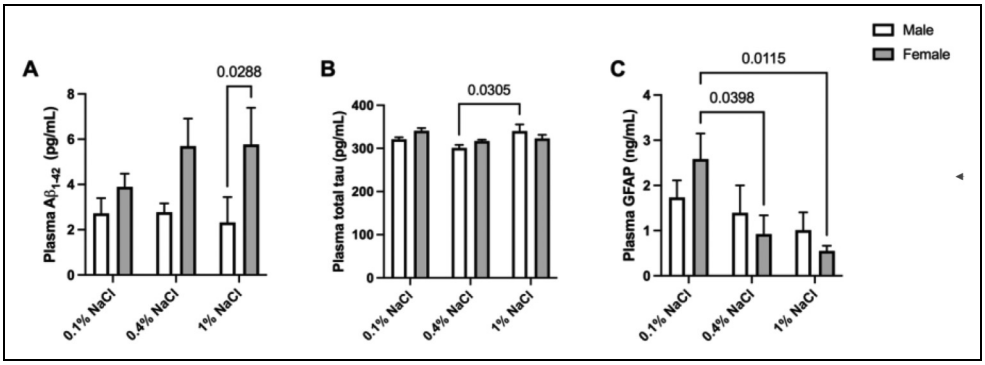

Figure 1. Sex differences in the impact of dietary salt on Alzheimer's disease-related plasma biomarkers in aged female and male Sprague-Dawley (SD) rats. Aged male and female SD rats (22–24 months old) were fed normal control chow (0.4% NaCl), low-salt chow (0.1% NaCl), or high-salt chow (1% NaCl) for 12 weeks. At the end of the study, blood samples were collected, and levels of dementia-related biomarkers were measured using enzyme-linked immunosorbent assay (ELISA) kits. These biomarkers included (A) amyloid-β peptide 1–42 ($A\beta_{1-42}$), (B) total tau protein, and (C) glial fibrillary acidic protein (GFAP). The diet groups are represented as follows: white block for 0.1% NaCl diet, gray block for 0.4% NaCl diet, and black block for 1% NaCl diet. Each group consisted of 4–6 rats. The results are presented as mean ± SEM and were analyzed using two-way ANOVA with Tukey's post hoc test.

and Use of Laboratory Animals. For a duration of 12 weeks, rats were provided with ad libitum access to tap water and chow with varying salt concentrations: normal control (0.4% NaCl), low-salt (0.1% NaCl), or high-salt (1% NaCl) from Xietong Biotechnology Co. (Hangzhou, China). Data collection and processing were performed randomly. The experimenters were blinded to the group allocations during measurements and assessments.

Plasma biomarker assessment

Levels of plasma $A\beta_{1-42}$ (Cusabio, Wuhan, China), total tau (Baiyi Bio, Shanghai, China), and GFAP (Elabscience Biotechnology Co., Ltd, Wuhan, China) were measured using enzyme-linked immunosorbent assay (ELISA) kits. As previously described,[5] plasma was collected in EDTA anticoagulant tubes and centrifuged at 3000xg for 30 min at 4°C. For detailed procedures, refer to the manufacturers' manuals. Absorbance was measured at 450 nm using an automated microplate reader. Concentrations of $A\beta_{42}$ and tau were expressed in pg/mL, while GFAP levels were expressed in ng/mL. Four to six animals per group were used and all assays were performed in triplicate.

Statistical analysis

Statistical analyses were conducted using Prism 9 (GraphPad). Values were presented as mean ± SEM. Data analysis involved two-way ANOVA with Tukey's post-tests, setting the alpha level at 0.05 for all analyses.

Results

Aged female rats on a high-salt diet had higher levels of plasma $A\beta_{1-42}$ compared to aged male rats on the same diet ($p = 0.0288$; Figure 1(a)). There were no significant differences in plasma tau levels among aged female rats with varying dietary salt intake, but aged male rats on high-salt diets had higher plasma tau levels compared to those on normal diets ($p = 0.0305$; Figure 1(b)). While plasma GFAP tended to decline with increased salt intake in aged male rats, no significant differences were found (Figure 1(c)). Conversely, plasma GFAP was significantly higher in aged female rats on low-salt diets compared to those on normal ($p = 0.0398$; Figure 1(c)) and high-salt diets ($p = 0.0115$; Figure 1(c)).

Discussion

$A\beta_{1-42}$ and tau are classic proteins associated with AD.[8,9] GFAP, which is activated by inflammation, is also correlated with AD due to the relationship between chronic low-grade inflammation and dementia.[6] GFAP can serve as an early biomarker for AD, detectable over 10 years before symptom onset.[6] Among the AD-associated plasma biomarkers, $A\beta_{1-42}$ showed sex differences, with higher levels in aged female rats on high-salt diets. In contrast, plasma total tau was more sensitive in aged male rats, while GFAP was more sensitive in aged female rats.

Furthermore, excessive dietary salt intake is associated with cognitive impairment. Our findings indicate that a clinically relevant high-salt diet increases plasma total tau levels in aged male rats. It should be noted that besides total tau, tau phosphorylation is also linked to a high-salt diet.[10] However, plasma $A\beta_{1-42}$ and GFAP levels decreased in aged male rats on a high-salt diet, although not significantly. Interestingly, low-salt diets significantly increased plasma GFAP levels in aged female rats compared to both normal and high-salt diets, suggesting that low-salt diets may not benefit cognitive health in aged female rats.

There is controversy regarding the effects of high-salt diets on GFAP expression in the brain of mice. For example, one study found that eight months of high-salt diets did not affect GFAP+ astrocytes in the hippocampus and cortex of male wild-type and APP/PS1 mice (an AD model).[11] Another study found that ovariectomy female mice fed a high fat-sugar-salt diet developed gliosis (increased burden of astrocytes) and AD-brain pathology with cognitive impairment.[12]

Moreover, sex-specific effects of the association between GFAP and AD are also conflicted. Some studies found stronger association between Aβ burden and plasma p-tau epitopes in individuals with increased GFAP + astrocyte reactivity in men.[13] While another study showed higher GFAP levels in females than males and found a correlation between plasma GFAP and neurodegeneration only in females.[14]

Furthermore, one study found that plasma GFAP concentration was significantly increased in Aβ-positive groups compared with participants without Aβ pathology.[15] However, in contrast to plasma GFAP, cerebrospinal fluid GFAP concentration was significantly increased in non-AD patients compared to other groups.[15] These results indicate that although plasma GFAP might be a potential early predicting biomarker for AD, it still needs to be careful in explaining the results of plasma GFAP.

The strengths of this study are that our research is pioneered in investigating sex-specific effects of varying levels of dietary salt on AD-predicting plasma biomarkers in aged rats. Our study followed rigorous methodological standards, including randomized assignment and double-blind procedures. Nonetheless, the findings should be interpreted within the study's limitations. One limitation is that the sample size is small. Another limitation is the lack of insights into the potential mechanisms that may explain the observed sex-specific effects.

In conclusion, sex differences exist in AD-related plasma biomarkers in aged rats with varying dietary salt intake. This study provides valuable insights into the effects of sex and salt intake on early AD-predicting biomarkers, underscoring the importance of sex-specific dietary guidelines and early predictors for cognitive health.

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Statements and declarations

Author contributions

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability

All data related to this article are shown or available upon request from the corresponding authors.

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