Potential role of inflammation in relation to dietary sodium and β-carotene with non-alcoholic fatty liver disease: a mediation analysis

Yang Chen¹,², Min Wu³,⁵, Fuli Chen¹,⁵, Xiaoxiao Wen³, Liancheng Zhao⁴, Gang Li¹,⁶ and Long Zhou¹,⁶

© The Author(s) 2022

BACKGROUND: High sodium intake has been linked to the prevalence of non-alcoholic fatty liver disease (NAFLD), but underlying mechanism remains unclear. This study aims to explore the role of chronic inflammation in the association between sodium and NAFLD. We also observed whether β-carotene, which has a strong anti-inflammatory effect, lowers the odds of NAFLD.

METHODS: We performed mediation analyses to assess the mediating effects of C-reactive protein (CRP) and red cell distribution width (RDW) on the relationship between dietary sodium and NAFLD defined by the hepatic steatosis index (HSI) and the fatty liver index (FLI), respectively.

RESULTS: A total of 6725 participants were included in this study. Compared with the high sodium-low carotene group, participants in the high sodium-high carotene group had 16% and 26% lower odds for HSI and FLI-defined NAFLD, respectively. There were positive indirect effects of dietary sodium intake on the HSI-defined NAFLD (indirect effect: 0.0057, 95% CI: 0.0021–0.0091, P < 0.0001), as well as the FLI defined NAFLD (indirect effect: 0.0081, 95% CI: 0.0024–0.0162, P < 0.0001) when C-reactive protein (CRP) was considered as a mediator. The mediating effects were somewhat attenuated after further adjusting for dietary β-carotene intake. Similar results were found when RDW was considered as a mediator in the HSI-defined NAFLD analysis.

CONCLUSIONS: Higher sodium intake increases the odds of NAFLD by upregulating inflammation. Dietary β-carotene may attenuate this association by down regulating inflammation.

INTRODUCTION

High sodium intake has been confirmed as one of the most important risk factors for high blood pressure [1–3]. Excessive sodium intake also has been linked to higher risk of overweight/obesity as well as cardiovascular diseases [4–7]. Our previous study reported that dietary sodium intake was positively associated with non-alcoholic fatty liver disease (NAFLD) in a representative US general population [8]. NAFLD is a liver condition defined as the accumulation of intracellular fat in hepatocytes in the absence of excessive alcohol intake, and it is considered the hepatic expression of the metabolic syndrome. To date, the mechanisms underlying sodium intake in relation to NAFLD remain unclear. As components of metabolic syndrome, NAFLD, high blood pressure, and overweight/obesity share a common pathophysiology, that is, chronic inflammation [9–11]. As a systemic inflammation biomarker, C-reactive protein (CRP) has been linked to disease and mortality risk [9]. Recently, red cell distribution width (RDW) has also been suggested as a marker that reflect an underlying chronic inflammatory state [12–14]. We assume that high sodium intake increases the risk of NAFLD by upregulating inflammation. If this hypothesis is true, down regulating inflammation will improve hepatic steatosis caused by high sodium intake. β-carotene, a retinol precursor and antioxidant that is found in certain fruits and vegetables, has been found to have a strong anti-inflammatory effect [15–17]. Our second hypothesis, accordingly, is that high dietary β-carotene intake attenuates the effects of high sodium intake on NAFLD by down regulating inflammation. The present study aims to use mediation analyses to test the above two hypotheses by utilizing data from two cycles (cycle 2007–2008 and cycle 2009–2010) of the National Health and Nutrition Examination Survey (NHANES).

SUBJECTS AND METHODS

Study population

Data were derived from two cycles of NHANES (2007–2008 and 2009–2010). A detailed description of the NHANES study design and methods is available elsewhere [18]. In total, 20,686 individuals completed the NHANES surveys between 2007 and 2010 within two cycles (10,149 in NHANES 2007–2008 and 10,537 in NHANES 2009–2010). In this study, we included individuals aged ≥20 years who completed two 24-h recalls with

1 Department of Cardiology, Sichuan Provincial People’s Hospital, University of Electronic Science and Technology of China, Chengdu, China. 2 School of Medicine, Sichuan Provincial People’s Hospital, University of Electronic Science and Technology of China, Chengdu, China. 3 Department of Epidemiology, College of Public Health and Health Professions and College of Medicine, University of Florida, Gainesville, FL, USA. 4 Division of Prevention and Community Health, National Center for Cardiovascular Disease, Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. These authors contributed equally: Yang Chen, Min Wu, Fuli Chen.

Received: 21 February 2022 Revised: 29 August 2022 Accepted: 5 September 2022
Published online: 15 September 2022
Fig. 1 Flowchart of the study participants' selection. A total of 6725 and 3237 participants were selected from the two cycles of National Health and Nutrition Examination Survey (NHANES) for hepatic steatosis index (HSI) and fatty liver index (FLI) analyses.

Data collection

Data were collected by trained interviewers during interviews in the participants’ homes and through medical examination and subsequent laboratory assessments in the Mobile Examination Center (MEC). The questionnaire contains a number of questions on demographics, health conditions, and lifestyles such as smoking, drinking, house income, and passive sedentary behavior.

The ethnicity was divided into Hispanic, non-Hispanic White, non-Hispanic Black, and other races. As the house income variable in our analysis, family monthly poverty level index was calculated by dividing family income by the poverty guidelines, specific to family size, as well as the appropriate year and state. The index was grouped into three categories (≤1.10, 1.11–1.85, >1.85).

According to participants’ answers to the questionnaire, smoking status was categorized into current, former, and never smoker. Drinkers were defined as participants drinking alcohol at least 12 times in the previous year. Sedentary time, defined as total time spent sitting in a typical day not including time spent sleeping, was used as a passive sedentary behavior indicator in our analysis.

Dietary intake was documented and validated utilizing the 24-h dietary history interview. Participants received two 24-h dietary recall interviews. The first dietary recall interview was collected face-to-face in the MEC, and the second was collected by telephone 3 to 10 days later. Dietary intake was estimated by the mean of the two dietary recalls. Dietary sodium (in mg/d) and β-carotene (in μg/day) intakes were used as the main exposure variables. We calculated the dietary inflammatory index (DII) score according to the calculating methods provided by a previous study [20]. DII is a literature-derived dietary tool for measuring individual dietary inflammatory potential. The DII score was used as a covariates in the analysis. Body weight (in kg) was measured using a digital weight scale and standing height (cm) was measured using a stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference (WC) was measured to the nearest 0.1 cm at the end of the participant’s normal expiration using a tape by the standard method.

Laboratory tests

Blood specimens were processed, stored, and shipped to Fairview Medical Center Laboratory at the University of Minnesota, Minneapolis, Minnesota for analysis. Serum alanine aminotransferase (ALT) was measured with the kinetic rate methods in Beckman Coulter UniCel® DxC800 Synchron Clinical System. Serum aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) were measured by enzymatic rate methods in Beckman Coulter UniCel® DxC800 Synchron Clinical System. Hemoglobin A1c (HbA1c) was measured by high-performance liquid chromatography (HPLC). The analyzer dilutes the whole blood specimen with Hemolysis & Wash Solution, and then injects a small volume of the treated specimen onto the HPLC analytical column. Separation is achieved by utilizing differences in ionic interactions between the cation exchange group on the column resin surface and the hemoglobin components. The separated hemoglobin components pass through the photometer flow cell where the analyzer measures changes in absorbance at 415 nm. The analyzer integrates and reduces the raw data, and then calculates the relative percentages of each hemoglobin fraction. We defined diabetes as HbA1c ≥6.5% and/or current treatment with a hypoglycemic agent or insulin [21]. The laboratory method used for triglycerides (TG) measurement was enzymatic assay using Roche Hitachi Modular P Chemistry Analyzer. CRP was measured by latex-enhanced nephelometry on a Behring nephelometer. Particle-enhanced assays are based on the reaction between a soluble analyte and the corresponding antigen or antibody bound to polystyrene particles. For the quantification of CRP, particles consisting of a polystyrene core and a hydrophilic shell are used in order to link anti-CRP antibodies covalently. A dilute solution of test sample is mixed with latex particles coated with mouse monoclonal anti-CRP antibodies. CRP concentrations are calculated by using a calibration curve. Data reduction of the signals is performed by using a storable logit-log function for the calibration curve. These assays are performed on a Siemens/Behring nephelometer for quantitative CRP determination. Red cell distribution width represents the size distribution spread of the erythrocyte population derived from the red blood cell (RBC) histogram. It is the coefficient of variation, expressed in percent, of the RBC size distribution. RBC was directly measured on the Coulter® HMX. A detailed description of laboratory methods and materials used in the tests is available at https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/labmethods.aspx?BeginYear=2007.

Definition of NAFLD

NAFLD was defined using HSI and FLI, both of which have been validated externally and used in epidemiologic studies, in the absence of other causes of chronic liver disease [22–24]. HSI and FLI were calculated using the following two formulas:

1. HSI = 8 × (ALT/AST ratio) + BMI (+2, if female; +2, if diabetes). In this case, NAFLD was defined as HSI > 36.

2. FLI = [e0.955×log10(TG) + 0.193×BMI + 0.718×log10(GGT) + 0.053×WC - 15.74]/[1 + e0.955×log10(TG) + 0.139×BMI + 0.718×log10(GGT) + 0.053×WC - 15.74] × 100. In this case, NAFLD was defined as FLI ≥ 60.

Statistical analysis

Data were presented as mean ± standard deviation (SD) for continuous variables and as frequency (percentage) for categorical variables. Dietary sodium and β-carotene intake were dichotomized using their median values and were cross combined into four groups (Low sodium-
High carotene. Low sodium-Low carotene, High sodium-High carotene, and High sodium-Low carotene). We tested differences in baseline characteristics among the above four groups with one-way analysis of variance for continuous variables and Chi-square tests for categorical variables. The odds ratios (ORs) and 95% confidence intervals (CIs) for NAFLD of each group were estimated using unconditional binary logistic regression models. Potential covariates such as age, sex, ethnicity, education level, family monthly poverty level, passive sedentary hours, smoking status, drinking status, total energy intake, DII, and serum creatinine were included in multivariable analyses. The selection of covariates was based on clinical knowledge but not on statistical way (e.g., forward or backward). The restricted cubic spline model was used for the dose-response analysis. When we analyzing the dose–response relationship between sodium or β-carotene and NAFLD separately, we mutually adjusted for β-carotene and sodium in the model.

When performing mediation analysis, we followed the AGReMA statement, a guideline for reporting mediation analyses [25]. The variables of sodium intake, CRP, and RDW were standardized prior to analyses in order to compare effects across different variables by solving shifting or scaling issues. The mediation analyses include the following steps. First, linear regression was applied to examine the associations between sodium and CRP (or RDW), adjusting for age, sex, ethnicity, education level, family monthly poverty level, passive sedentary hours, smoking status, drinking status, total energy intake, DII, and serum creatinine (path α). Second, CRP (or RDW) was additionally controlled in the logistic regression model to estimate the direct effect of sodium intake on NAFLD, adjusting for the same covariates (path β). Third, the mediate function from the R mediation package with the causal analytical approach was applied to quantify the indirect effect (path α & path β) and the total effect (path γ) [26, 27]. To observe the effect of β-carotene on the mediating effects, we repeated the above analyses with adjustment for β-carotene. The mediation package is freely available for download via the Comprehensive R Archive Network (CRAN) at http://CRAN.R-project.org/package=mediation. A detailed description of the construction and statistic approaches for the mediation package is available at https://cran.r-project.org/web/packages/mediation/vignettes/mediation.pdf. The mediation analyses diagram for the association of dietary sodium and β-carotene intakes with NAFLD is shown in Fig. 2.

A two-tailed $P < 0.05$ was considered significant. Analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina) and R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria).

**RESULTS**

A total of 6725 participants (3119 men and 3606 women) with a mean age of 53.2 ± 17.4 years were included in this study. The characteristics of the study population by the combination groups of dietary sodium and β-carotene intakes are presented in Table 1. Compared with participants with high sodium and low β-carotene intakes, those with high sodium and high β-carotene intakes were older and more likely to be white race, to have higher education level, to have higher family income, to have lower mean sodium intake, and CRP and RDW, adjusting for age, sex, ethnicity, education level, family monthly poverty level, smoking, drinking, sedentary time, total energy intake, DII, and serum creatinine. Besides, participants in the low sodium-high carotene group had the lowest odds for NAFLD compared with the high sodium-low carotene group.

Table 2 presents ORs and 95% CIs for the HSI and FLI defined NAFLD, respectively, by different combinations of dietary sodium and β-carotene intakes. Compared with the high sodium-low carotene group, participants in the high sodium-high carotene group had a lower odds for both the HSI and FLI defined NAFLD. The corresponding ORs and 95% CIs were 0.84 (0.73–0.98) for the HSI-defined NAFLD and 0.74 (0.60–0.92) for the FLI defined NAFLD after adjusting for age, sex, ethnicity, education level, family monthly poverty level, smoking, drinking, sedentary time, total energy intake, DII, and serum creatinine. Besides, participants in the low sodium-high carotene group had the lowest odds for NAFLD compared with the high sodium-low carotene group.

The dose-response relations of dietary sodium and β-carotene intakes with NAFLD defined by HSI and FLI were shown in Fig. 3. There was a significant positive dose–response relationship between dietary sodium intake and NAFLD after adjustment for covariates described above plus β-carotene ($P < 0.001$). In contrast, there was a significant inverse association between dietary β-carotene intake and NAFLD after adjustment for covariates including dietary sodium intake ($P < 0.05$).

Results from the mediation analysis for the association between dietary sodium intake and NAFLD with or without adjustment for dietary β-carotene intake were shown in Table 3. There were positive indirect effects of dietary sodium intake on the HSI-defined NAFLD (indirect effect: 0.0057, 95% CI: 0.0021–0.0091, $P < 0.0001$) as
well as the FLI defined NAFLD (indirect effect: 0.0081, 95% CI: 0.0024–0.0162, P < 0.0001) when CRP was considered as a mediator. As expected, the mediating effects were somewhat attenuated when we further adjusted for dietary β-carotene intake. Similar results were found when RDW was considered as a mediator in the HSI analysis. However, the mediating effect of RDW on the relationship between dietary sodium intake and FLI defined NAFLD did not reach the statistical significant level.

**DISCUSSION**

In this study on a representative sample of US adults, we found that dietary sodium intake was positively associated with NAFLD, while dietary β-carotene intake was inversely associated with NAFLD. Participants who had high sodium and high β-carotene intakes had a lower odds of NAFLD compared with those who had high sodium and low β-carotene intakes. The mediation analysis supports the association of dietary sodium with the NAFLD, being, at least partially, mediated by the effect of inflammation.

In recent years, several previous studies including our own study reported an independent relationship between dietary sodium intake and NAFLD. A study using data from the Korea National Health and Nutrition Examination Surveys (2008–2010) found significant associations between higher estimated 24-h urinary sodium excretion and NAFLD as assessed by both HSI (OR: 1.39, 95% CI: 1.26–1.55) and FLI (OR: 1.75, 95% CI: 1.39–2.20) [28]. Another study conducted in the Korea population also suggested that higher dietary sodium intake was associated with a greater prevalence of NAFLD in young and middle-aged general adults [29]. Results from the Prevention of Renal and Vascular End-Stage Disease (PREVEND) cohort study showed that higher dietary sodium intake was positively associated with NAFLD as defined by the FLI, with an OR per SD increment of 1.30 (95% CI: 1.21–1.41) [30]. Our previous study based on NHANES found that comparing with the lowest quartile of dietary sodium intake, the highest quartile had a non-significant difference compared with the group of high sodium-low carotene after Bonferroni adjustment.

**Table 1.** Characteristics of the study population by combinations of dietary sodium and β-carotene intakes (n = 6725).

| Characteristics                          | Low Na-High β-carotene | Low Na-Low β-carotene | High Na-High β-carotene | High Na-Low β-carotene | P-values |
|-----------------------------------------|------------------------|-----------------------|-------------------------|------------------------|----------|
| n                                       | 1513                   | 1847                  | 1851                    | 1514                   |          |
| Age, years                              | 59.0 ± 16.2            | 54.1 ± 17.9           | 52.2 ± 16.9*            | 47.4 ± 16.6            | <0.0001  |
| Men, n (%)                              | 430 (28.4)             | 631 (34.2)            | 1104 (59.6)             | 954 (63.0)             | <0.0001  |
| Ethnicity, n (%)                        | 403 (26.6)             | 573 (31.0)            | 394 (21.3)*             | 349 (23.1)             |          |
| Hispanic                                | 808 (53.4)             | 855 (46.3)            | 1092 (59.0)*            | 805 (53.2)             |          |
| White                                   | 254 (16.8)             | 370 (20.0)            | 258 (13.9)*             | 297 (19.6)             |          |
| Black                                   | 48 (3.2)               | 49 (2.7)              | 107 (5.8)*              | 63 (4.2)               |          |
| Others                                  | 377 (24.9)             | 669 (36.2)            | 346 (18.7)*             | 351 (23.2)             |          |
| Education, n (%)                        | 785 (51.9)             | 717 (38.8)            | 1176 (63.5)*            | 763 (50.4)             | <0.0001  |
| Less than high school                   | 718 (47.5)             | 819 (44.3)            | 1352 (73.0)             | 1030 (68.0)            | <0.0001  |
| High school                             | 223 (14.7)             | 282 (15.3)            | 237 (12.8)              | 204 (13.5)             | 0.1298   |
| More than high school                   | 829 (54.8)             | 1009 (54.6)           | 1352 (73.0)*            | 1030 (68.0)            | <0.0001  |
| Family monthly poverty levels           | 718 (47.5)             | 819 (44.3)            | 686 (37.1)              | 530 (35.0)             | <0.0001  |
| Smoking status, n (%)                   | 406 (26.8)             | 697 (37.7)            | 414 (22.4)*             | 473 (31.2)             |          |
| Current                                 | 143 (9.5)              | 356 (19.3)            | 224 (12.1)*             | 317 (20.9)             |          |
| Former                                  | 400 (26.4)             | 462 (25.0)            | 582 (31.4)*             | 385 (25.4)             |          |
| Never                                   | 970 (64.1)             | 1029 (55.7)           | 1045 (56.5)*            | 812 (53.6)             |          |
| Hypertension, n (%)                     | 718 (47.5)             | 819 (44.3)            | 686 (37.1)              | 530 (35.0)             | <0.0001  |
| Diabetes, n (%)                         | 223 (14.7)             | 282 (15.3)            | 237 (12.8)              | 204 (13.5)             | 0.1298   |
| Sedentary time, h/day                   | 5.1 ± 3.2              | 5.0 ± 3.2             | 5.8 ± 3.5*              | 5.4 ± 3.2              | <0.0001  |
| Dietary inflammatory index              | 0.5 ± 1.3              | 1.5 ± 1.1             | −0.9 ± 1.4*             | 0.1 ± 1.3              | <0.0001  |
| Total energy, kcal                      | 1527 ± 402             | 1479 ± 423            | 2433 ± 741              | 2428 ± 689             | <0.0001  |
| Body mass index, kg/m²                  | 28.5 ± 6.1             | 29.3 ± 6.6            | 29.0 ± 6.6*             | 29.9 ± 7.2             | <0.0001  |
| Log-transformed c-reactive protein, mg/dL | −1.7 ± 1.2             | −1.5 ± 1.2            | −1.8 ± 1.3*             | −1.7 ± 1.3             | <0.0001  |
| Log-transformed red cell distribution width, % | 2.6 ± 0.1              | 2.6 ± 0.1             | 2.5 ± 0.1               | 2.6 ± 0.1              | <0.0001  |

Values are presented as mean ± standard deviation or n (%) unless otherwise indicated. *Means there is significant difference compared with the group of high sodium-low carotene after Bonferroni adjustment.
Carotenoids form an important part of the human diet, consumption of which has been associated with many health benefits. β-carotene is one of the main constituents of carotenoids [31]. A community-based cross-sectional study conducted in a Chinese population observed a dose-dependent inverse association between β-carotene and NAFLD. The OR and 95% CI of NAFLD for the highest quartile was 0.32 (0.25–0.41) compared with the lowest quartile [32]. A case–control study enrolled 24

Table 2. Odds ratio (OR) and 95% confidence interval (CI) for NAFLD by the combinations of dietary sodium and β-carotene intakes.

|                | Low Na-High carotene | Low Na-Low carotene | High Na-High carotene | High Na-Low carotene |
|----------------|----------------------|---------------------|-----------------------|----------------------|
| HSI-defined NAFLD |                      |                     |                       |                      |
| n              | 1513                 | 1847                | 1851                  | 1514                 |
| No. of cases   | 814                  | 1061                | 1003                  | 922                  |
| Prevalence*, % | 53.8                 | 57.4                | 54.2                  | 60.9                 |
| ORs (95% CIs)  |                      |                     |                       |                      |
| Model 1b       | 0.65 (0.56–0.76)     | 0.78 (0.68–0.90)    | 0.74 (0.64–0.85)      | 1 (Reference)        |
| Model 2c       | 0.60 (0.51–0.71)     | 0.62 (0.53–0.73)    | 0.84 (0.73–0.98)      | 1 (Reference)        |
| FLI-defined NAFLD |                    |                     |                       |                      |
| n              | 727                  | 891                 | 892                   | 727                  |
| No. of cases   | 284                  | 391                 | 373                   | 376                  |
| Prevalence*, n (%) | 39.1                | 43.9                | 41.8                  | 51.7                 |
| ORs (95% CIs)  |                      |                     |                       |                      |
| Model 1b       | 0.54 (0.43–0.67)     | 0.71 (0.58–0.88)    | 0.64 (0.52–0.78)      | 1 (Reference)        |
| Model 2c       | 0.53 (0.41–0.68)     | 0.58 (0.46–0.73)    | 0.74 (0.60–0.92)      | 1 (Reference)        |

*P-value for difference in terms of prevalence of NAFLD equals to 0.0001.

bAdjusted for age and sex.
cAdjusted for age, sex, ethnicity, education level, family monthly poverty level, smoking, drinking, sedentary time, total energy intake, dietary inflammatory index, and serum creatinine.
dP-value for difference in terms of prevalence of NAFLD < 0.0001. The median values used in the HSI analysis were 3032 mg/day for sodium intake and 1217.5 µg/day for β-carotene intake. The median values used in the FLI analysis were 3049.5 mg/day for sodium intake and 1235.5 µg/day for β-carotene intake.

Fig. 3  Dose–response associations of dietary sodium and β-carotene intakes with no-alcoholic fatty liver disease (NAFLD). The restricted cubic spline model was basically adjusted for age, sex, ethnicity, education level, family monthly poverty level, smoking, drinking, sedentary time, total energy intake, dietary inflammatory index, and serum creatinine. A and C additionally adjusted for dietary β-carotene intake; B and D additionally adjusted for dietary sodium intake.

Carotenoids form an important part of the human diet, consumption of which has been associated with many health benefits. β-carotene is one of the main constituents of carotenoids [31]. A community-based cross-sectional study conducted in a Chinese population observed a dose-dependent inverse association between β-carotene and NAFLD. The OR and 95% CI of NAFLD for the highest quartile was 0.32 (0.25–0.41) compared with the lowest quartile [32]. A case–control study enrolled 24
control subjects and 62 NAFLD patients, whose liver biopsies were collected and histological characteristics were assessed. Compared with individuals without hepatic steatosis, those with moderate steatosis had significantly decreased serum β-carotene (0.34 ± 0.03 vs. 0.21 ± 0.02 μmol/L). Moreover, β-carotene gradually decreased along disease progression from normal liver, simple steatosis to borderline steatohepatitis (P for trend ≤ 0.001) [33]. A prospective cohort study enrolled 2687 subjects who completed both NAFLD tests were classified into stable, improved and progressed groups according to changes in the degree of NAFLD between two visits. Analysis of covariance showed that ln-transformed serum β-carotene was positively associated with NAFLD improvement (P for trend < 0.05). After multivariable adjustment, mean difference in serum β-carotene was higher by 29.6% in the improved vs. progressed subjects [34].

The pro-inflammatory effect of sodium has been reported in several previous studies. In animal experiments, high sodium diet triggers TH17 (interleukin-17 (IL-17)-producing helper T cells) development and promotes tissue inflammation. TH17 cells are highly pro-inflammatory cells that are critical for clearing extracellular pathogens and for inducing multiple autoimmune diseases [35–37]. Results from a recent study showed that mice fed with a high-salt diet displayed obvious hepatic steatosis and inflammation. All these pathological changes persisted after salt withdrawal, displaying a memory phenomenon [38]. In human studies, high sodium intake was reported to be associated with changes in inflammatory markers including tumor necrosis factor-α (TNF-α) and adiponectin [39–41]. Contrary to the pro-inflammatory effect of sodium, β-carotene has a strong anti-inflammatory effect [42]. A previous study suggested that β-carotene decreases the severity of C–C motif chemokine ligand 4 (CCL4) induced hepatic inflammation and fibrosis in rats [43]. Another study reported that tomato powder (rich in β-carotene) inhibits hepatic steatosis and inflammation potentially through restoring sirtuin 1 (SIRT1) activity and adiponectin function [44].

To the best of our knowledge, this is the first study to explore the pro-inflammatory mechanism of higher sodium intake in relation to an increased odds of NAFLD in humans through a mediation analysis. Moreover, we also found that dietary β-carotene attenuates the mediating effects of inflammation on the association between dietary sodium intake and NAFLD. Our study has limitations: First, dietary sodium intake was measured from two 24-h dietary recalls instead of multiple 24-h urine collections. The latter is considered as the gold-standard for estimating sodium intake in humans. However, the lack of urinary sodium measurements in this study limits our efforts to use more objective sodium measurement indicators to verify the reliability of the results. Second, NAFLD was defined using HSI- and FLI-based predictive equations rather than hepatic biopsy. Although hepatic biopsy method has a better accuracy for the diagnosis of NAFLD, it is difficult to apply in large-scale population studies. Third, given the enormous complexity of the inflammatory response, CRP and RDW provide only limited information. Future research should thus focus on additional biomarkers, such as IL-1β, IL-6, TNF-α, and CD4+ T cell subsets. Lastly, the cross-sectional study design is subject to recall bias and this design cannot prove causality in itself. Findings from our study should be further confirmed in well-designed prospective cohort studies using more reliable sodium measurements in future.

**CONCLUSIONS**

This study suggests that higher sodium intake increases the odds of NAFLD by upregulating inflammation. Dietary β-carotene may attenuate this association by down regulating inflammation.
REFERENCES

1. 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.

2. Stamler J, Chan Q, Davivlus 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.

3. 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. BMJ. 2020;368:m315.

4. Zhou L, Stamler J, Chan Q, Van Horn L, Davivlus ML, Dyer AR, et al. Salt intake and prevalence of overweight/obesity in Japan, China, the United Kingdom, and the United States: the INTERMAP Study. Am J Clin Nutr. 2019;110:34–40.

5. Zhou L, Wen X, Peng Y, Zhao L, Yu Y. Salt added to food and body mass index: a bidirectional Mendelian randomisation study. Nutr Diet. 2021;78:315–23.

6. Strazzullo P, D’Elia L, Kandala NB, Cappuccio FP. Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. BMJ. 2009;339:b4567.

7. Aburto NJ, Ziolkowska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: systematic review and meta-analyses. BMJ. 2013;346:f1326.

8. Zhou L, Yang Y, Feng Y, Zhao X, Fan Y, Rong J, et al. Association between dietary sodium intake and non-alcoholic fatty liver disease in the US population. Public Health Nutr. 2021;24:993–1000.

9. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med. 2019;25:1822–32.

10. Polimeno L, Del BM, Baratta F, Perri L, Albanese F, Pastori D, et al. Oxidative stress: new insights on the association of non-alcoholic fatty liver disease and atherosclerosis. World J Hepatol. 2015;7:1325–36.

11. Stefan N, Haring HU, Cusi K. Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. Lancet Diabetes Endocrinol. 2019;7:313–24.

12. Dogan S, Celikbilek M, Zararsiz G, Derici K, Sivgin S, Guven K, et al. Red blood cell distribution width as a non-invasive marker for the assessment of inflammation in non-alcoholic steatohepatitis. Hepatogastroenterology. 2015;62:393–8.

13. Kim J, Im JS, Choi CH, Park CH, Lee JI, Son KH, et al. The Association between Red Blood Cell Distribution Width and Sarcopenia in U.S. Adults. Sci Rep. 2020;12:11484.

14. Ferreira JP, Lamiral Z, Bakris G, Mehta C, White WB, Zannad F. Red cell distribution width in patients with diabetes and myocardial infarction: an analysis from the EXAMINE trial. Diabetes Obes Metab. 2021;23:1580–7.

15. Rodriguez-Rodríguez E, López-Sobaler AM, Navia B, Andrés P, Jiménez-Ortega AI, Ortega RM. β-carotene concentration and its association with inflammatory biomarkers in Spanish school children. Ann Nutr Metab. 2017;71:80–7.

16. Schultz H, Ying G, Dunaij LF, Dunaij DF. Rising plasma beta-carotene is associated with diminishing C-reactive protein in patients consuming a dark green leafy vegetable-rich, low inflammatory foods everyday (LIFE) diet. Am J Lifestyle Med. 2019;15:634–43.

17. Cheng J, Balbuena E, Miller B, Erogul A. The role of β-carotene in colonic inflammation and intestinal barrier integrity. Front Nutr. 2021;8:734800.

18. Zipp G, Chiappa M, Porter KS, Oschega Y, Lewis BG, Dostal J. National health and nutrition examination survey: plan and operations, 1999-2010. Vital Health Stat. 2013;1:37.

19. Zhou L, Feng Y, Yang Y, Zhao X, Fan Y, Rong J, et al. Diet behaviours and hypertension in US adults: the National Health and Nutrition Examination Survey 2013–2014. J Hypertens. 2019;37:1230–8.

20. Liu N, Ma F, Feng Y, Ma X. The association between the Dietary Inflammatory Index and Thyroid Function in U.S. adult males. Nutrients. 2021;13:3330.

21. Zhou L, Li X, Li S, Wen X, Peng Y, Zhao L. Relationship between dietary choline intake and diabetes mellitus in the National Health and Nutrition Examination Survey 2007–2010. J Diabetes. 2021;13:554–61.

22. Lee JH, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. Dig Liver Dis. 2010;42:503–8.

23. Bedogni G, Bellantoni S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol. 2006;6:33.

AUTHOR CONTRIBUTIONS

L Zhou and GL contributed to the study design and implementation, and is the guarantor of this work. L Zhou, YC, MW, and FC contributed to study conception and data interpretation. L Zhou contributed to data acquisition. L Zhou, YC, MW, and FC contributed to data analysis and drafted the manuscript. XW, L Zhao, and GL contributed to revision of this manuscript. All authors have approved the final version to be published.
COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
Correspondence and requests for materials should be addressed to Gang Li or Long Zhou.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022