Consumption of nitrate containing vegetables and the risk of chronic kidney disease: Tehran Lipid and Glucose Study

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ABSTRACT

Background: There is growing evidence regarding the potential properties of nitrate-rich foods in development of chronic diseases. In this study, we investigated the association of nitrate-containing vegetables (NCVs) and the risk of chronic kidney disease (CKD).

Methods: We evaluated 1546 eligible adult participants of the Tehran Lipid and Glucose Study (TLGS), at baseline (2006–2008) and again after 3 years (2009–2011). Dietary intake was collected using the validated semi-quantitative food frequency questionnaire. Nitrate-containing vegetables and its categories including high-, medium-, and low-nitrate vegetables were defined. Estimated glomerular filtration rate (eGFR) and CKD were defined. Association between NCVs and CKD in the cross-sectional phase and the predictability of NCVs consumption in CKD occurrence were assessed using multivariable logistic regression models with adjustment for potential confounders.

Results: Mean dietary intake of energy-adjusted NCVs was 298.0 ± 177.3 g/day. Highest compared to the lowest tertile of NCVs was accompanied with a significantly lower mean eGFR (76.6 vs. 83.3, mL/min/1.73 m², \( p < 0.001 \)) and a higher prevalence of CKD (21.7 vs. 9.9%, \( p < 0.001 \)). At baseline, higher intake of high-NCVs was associated with a 48% higher chance of having CKD (OR = 1.48, 95% CI = 1.05–2.13). After 3 years of follow-up, there was no significant association between consumption of total NCVs and its categories with the occurrence of CKD.

Conclusion: Considering the lack of association between high-NCVs intakes and the risk of CKD in prospective analysis, additional research is recommended to clarify possible effect of nitrate intakes from vegetables on kidney function.

Introduction

Chronic kidney disease (CKD) is characterized by reduced estimated glomerular filtration rate (eGFR), which over time leads to kidney failure and cardiovascular disease (CVD). Individuals with CKD are at much higher risk of developing coronary artery disease, peripheral vascular disease, and cerebrovascular disease.

One of the principal pathophysiological mechanisms involved in the CKD is endothelial dysfunction. Activation of the renin–angiotensin system, oxidative stress, elevated asymmetric dimethyl arginine (ADMA), inflammation with increased circulating cytokines, and dyslipidemia are among pathways of endothelial dysfunction through which CKD and other CVD risk factors such as diabetes, hypertension, and obesity may lead to CVD.

Inorganic nitrate/nitrite, are naturally occurring compounds in foods, especially plant foods and vegetables, and are also used as food additives. Major sources of exogenous nitrate exposure are vegetables and drinking water, while exogenous nitrite sources are mainly processed meat and animal food products; more than 80–95% of dietary intake of nitrate is attributed to vegetables especially green leafy vegetables including lettuce and spinach, cabbage, rocket, red beetroot, and radish. Effect of dietary intakes of nitrate in health and disease is still a controversial issue. It has been investigated that nitrate and nitrite representing an important alternative source of nitric oxide (NO); various important biological functions of the nitrate–nitrite–NO pathway, recently highlight the potential role of nitrate and nitrite in development of chronic diseases. There is growing evidence suggests that NO pathway have protective effects against development of micro- and macro-vascular and renal disease. Studies show impressive NO-like cardiovascular effects of dietary nitrate such as lowering blood pressure and improving endothelial function, via sequential reduction of nitrate to nitrite and nitric oxide, and recently, in a
prospective study, we showed that higher intakes of nitrate containing vegetables decreased the occurrence of hypertension in adults.\textsuperscript{21}

Little is known regarding the association of nitrate and nitrite intakes or rich-nitrate-foods with kidney function and, to the best of our knowledge, this issue has not yet been investigated in the framework of a population-based, prospective examination. The main focus in this study, therefore, was to assess whether nitrate-containing vegetables associated with the risk of CKD.

Methods

Study population

This prospective study was conducted within the framework of the Tehran Lipid and Glucose Study (TLGS), which began in 1999–2001 on 15,005 participants, aged 3–75 years, residents of district No. 13 of Tehran in order to determine and prevent risk factors for non-communicable diseases.\textsuperscript{22} TLGS is an ongoing study and data are updated every 3 years.

The current study was conducted on adult men and women with complete data (demographics, anthropometrics, biochemicals, and dietary data), participated in the third (2006–2008) and fourth (2009–2011) TLGS examinations. After exclusion of the participants with under- or over-reported energy intake or specific diet including dietary recommendations for hypertension, hyperlipidemia or diabetes, 1538 subjects (663 men and 796 women) were eligible for cross-sectional analysis. For longitudinal analyses, after exclusion of participants with prevalent CKD (n = 239), the remaining non-CKD subjects were followed up to the fourth (2009–2011) TLGS examination; participants who left the study before follow-up examination were also excluded and final analyses was conducted on 1299 adults (652 men and 647 women).

This study was approved by the ethics committee of Research Institute for Endocrine Science, Shahid Beheshti University of Medical Sciences. Written informed consents were obtained from all participants.

Assessment of dietary intake and nitrate-containing vegetables

A trained dietitian using a 168-item validated semi-quantitative FFQ assessed dietary intakes of participants over the last year at baseline,\textsuperscript{23} following which the portion sizes of consumed foods were converted to grams. The validity of the food frequency questionnaire has previously been evaluated by comparing food groups and nutrient values determined from the questionnaire with values estimated from the average of twelve 24-h dietary recall surveys and the reliability has been assessed by comparing energy and nutrient intakes from two FFQ; Pearson correlation coefficients and intra-class correlation for energy and nutrients showed acceptable agreements between FFQ and twelve 24-h dietary recall surveys, and FFQ1 and FFQ2.\textsuperscript{24} However, since Iranian Food Composition Table is incomplete, and has limited data on nutrient content of raw foods and beverages, to analyze foods and beverages for their energy and nutrient content, we used the US Department of Agriculture Food Composition Table.\textsuperscript{25}

To obtain and exclude participants who had under or over reports of energy intakes, energy intake to basal metabolic rate ratio (EI/BMRest) was used; EI/BMRest is used extensively as a measure of the validity of energy intake in epidemiological studies.\textsuperscript{26} Since the participants were in overweight range (mean BMI was >26 kg/m\textsuperscript{2}), basal metabolic rate was calculated using Mifflin–St-Jeor Equation, because it provided the best estimation of age-specific energy intake of overweight and obese individuals.\textsuperscript{27} A cut-off limit of 1.55 for EI/BMRest, proposed by Goldberg et al.,\textsuperscript{28,29} was considered to identified under or over reports of energy intake.

In the current study, NCVs were calculated according to the Santamaria et al. study; moreover NCVs were categorized as low-nitrate (<50 mg/100 g fresh weight of vegetables), medium-nitrate (50–100 mg/100 g fresh weight of vegetables) and high-nitrate (>100 mg/100 g fresh weight of vegetables).\textsuperscript{30} Low-nitrate vegetables were included potato, broad bean, tomato, ketchup, cucumber, squash, eggplant, string bean, carrot, garlic, onion, pepper, mushroom, and watermelon; medium-nitrate vegetables included cabbage and turnip and high-nitrate vegetables include celery, lettuce, and spinach.

Demographic, anthropometric, and clinical measures

Demographics, anthropometrics, and biochemical measures were evaluated at baseline (2006–2008) and again after the follow-up examination (2009–2011). Trained interviewers collected information using pre-tested questionnaires. Weight was measured to the nearest 100 g using digital scales, while the subjects were minimally clothed, without shoes. Height was measured to the nearest 0.5 cm, in a standing position without shoes, using a tape meter. Body mass index was calculated as weight (kg) divided by square of the height (m\textsuperscript{2}). For blood pressure (BP) measurements, after a 15-min rest in the sitting position, two measurements of BP were taken, on the right arm, during a standardized mercury sphygmomanometer; the mean of the two measurements was considered as the participant’s BP.
To calculate physical activity levels the Kriska et al. questionnaire was used and it expressed as metabolic equivalent hours per week (METs h/week). To determine smoking status, subjects who smoked daily or occasionally were defined as current smoker and those who had never smoked or had quit smoking were defined as non-smokers. Information on medication usage for treatment of diabetes, hypertension, and lipid disorders was collected.

**Biochemical measures**

Fasting blood samples were taken after 12–14 h, from all study participants at baseline and follow-up phases. Serum creatinine levels were assayed using kinetic colorimetric Jaffe method. Fasting serum glucose (FSG) was measured by the enzymatic colorimetric method using glucose oxidase. The standard 2h serum glucose 2-h SG test was performed for all individuals who were not on anti-diabetic drugs. Analyses were performed using Pars Azmoon kits (Pars Azmoon Inc., Tehran, Iran) and a Selectra 2 auto-analyzer (Vital Scientific, Spankeren, Netherlands). Both inter- and intra-assay coefficients of variation of all assays were <5%.

**Definition of terms**

Chronic kidney disease was defined as estimated eGFR < 60 mL/min per 1.73 m². To calculate eGFR, the CKD-EPI creatinine equation, developed by Chronic Kidney Disease Epidemiology Collaboration, was used. As a single equation CKD-EPI has been expressed as follows:

\[
eGFR = 141 \times \min\left(\frac{\text{Scr}}{\kappa, 1}\right)^{1.209} \times \max\left(\frac{\text{Scr}}{\kappa, 1}\right)^{-0.218} \times 0.993^{\text{age}} \times 1.018 \begin{cases} \text{[if female]} & \times 1.159 \begin{cases} \text{[if black]} & \\
\end{cases} \\
\end{cases}
\]

In this equation, Scr is serum Cr in mg/dL; \(\kappa\) is 0.7 and 0.9 for men and women, respectively, \(\alpha\) is −0.329 and −0.411 for men and women, respectively; min indicates the minimum of \(\frac{\text{Scr}}{\kappa}\) or 1, and max indicates maximum of \(\frac{\text{Scr}}{\kappa}\) or 1.

Creatinine clearance rate (eCcr) using the Cockcroft–Gault formula as follows:

\[
eCcr = \frac{(140 – \text{age}) \times \text{body weight} \ (\text{kg}) \times (0.85 \text{ if female})}{72 \times \text{creatinine} \ (\text{mg/dL})}
\]

Diabetes was defined as FSG ≥ 126, 2-h SG ≥ 200 or use of anti-diabetic medications. Hypertension (HTN) was considered as systolic BP ≥ 140 or systolic BP ≥ 70 or current use of antihypertensive medications.

**Statistical analysis**

All analyses were conducted using SPSS software version 20.0 (SPSS Inc., Chicago, IL). Dietary intakes of NCVs were adjusted for total energy intake based on the residuals methods and categorized based on tertile ranges. General characteristics and dietary intakes of participants were assessed using the general linear model for continuous variables and Chi-square for non-continuous variables across tertiles of NCVs.

The association of total intakes of NCVs and its categories with creatinine clearance rate was assessed using linear regression analysis both at baseline and follow-up examinations. A univariate analysis was performed for common risk factors of CKD or potential confounding variables; variables with \(P < 0.2\) in the univariate analysis were selected for the final multivariable models; \(P < 0.05\) (\(P\) values for entry) determines which variables should be included in the multivariable model. Multivariable logistic regression models were used to estimate the odds (95% CI) of CKD, both at baseline and at the follow-up examination, in each tertile of NCV and its subgroups, with adjustment for confounding variables. Model 1, was adjusted for age and sex and BMI; in model 2, additional adjustment for smoking, education, physical activity, diabetes, and HTN was done. The final model was additionally adjusted for energy intake, total fiber, and potassium. The reference group was defined as the lowest tertile. \(P\) value < 0.05 was considered as a level of significance.

**Results**

Mean age of participants was 38.0 ± 12.0 years at baseline and 57.0% of participants were women. Mean dietary intake of energy-adjusted NCVs was 298.0 ± 177.3 g/day. General characteristics of participants are presented in Table 1; those in the highest, compared to the lowest tertile of NCVs were more likely to be women (61.6% vs. 51.3%, \(p < 0.001\)) and had higher BMI (27.2 vs. 26.0 kg/m², \(p < 0.01\)). In the highest compared to the lowest tertile of NCVs, significantly lower mean eGFR (76.6 vs. 83.3, mL/min/1.73 m², \(p < 0.001\)), creatinine clearance rate (85.8 vs. 96.7 mL/min, \(p < 0.001\)), and a higher prevalence of CKD (21.7 vs. 9.9%, \(p < 0.001\)) was observed. Mean dietary intakes of the participants across tertiles of NCVs are presented in Table 2; those in the highest compared to the lowest tertile of NCVs significantly consumed more total fiber, legumes, fruits,
vegetables, nuts, dairy products, and potassium. There was no significant difference in dietary intakes of carbohydrate, fat, protein, meat, processed meat, and sodium across tertiles of NCVs.

An inverse significant association was observed between high-NCVs and creatinine clearance rate ($\beta = -0.091$, $p = 0.018$ and $\beta = 0.92$, $p = 0.016$, at baseline and follow-up examination, respectively); medium- and low-NCVs were not related to creatinine clearance rate.

Odds ratio (95% CI) of CKD across the tertiles of NCVs at baseline, are shown in Table 3. A significant positive association was found between NCVs and chance of having CKD, in the highest compared to the lowest ter- tile in the first (OR = 1.50, 95% CI = 1.02–2.21) and second (OR = 1.48, 95% CI = 1.00–2.14) model. However, after additional adjustment for total energy intake, fiber, and potassium, this association was disappeared (OR = 1.26, 95% CI = 0.81–1.94). Moreover, after adjustment of all potential confounding variables, higher intake of high-NCVs was associated with a higher risk of CKD by 48% (OR = 1.48, 95% CI = 1.05–2.13).

No significant association was observed between dietary intakes of medium- or low-NCVs with the chance of having CKD at baseline.

Odds ratio (95% CI) for incidence of CKD across the tertiles of NCVs are shown in Table 4. After 3 years of follow-up, there was no significant association between consumption of total NCVs and its categories with the occurrence of CKD.

**Discussion**

Our results have indicated that high consumption of high-nitrate containing vegetables positively associated with risk of CKD but there was no association between intake of total-, low-, and medium nitrate containing vegetables and CKD at baseline. A lower eGFR as well as creatinine clearance rate, and a higher prevalence of CKD were observed across increasing consumption of nitrate-containing vegetables. However, after exclusion of patients with CKD, consumption of nitrate containing vegetables had no association with 3-year risk of CKD.

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**Table 1.** Baseline characteristics of participants according to the tertiles of NCV at baseline.

| Nitrate containing vegetables | Tertile 1 | Tertile 2 | Tertile 3 | p |
|------------------------------|-----------|-----------|-----------|---|
| Total NCV (g/day)            | <203      | 203-332   | ≥332      |   |
| Median                       | 146       | 261       | 428       |   |
| Age (years)                  | 35.3 ± 11.6 | 38.4 ± 12.1 | 40.4 ± 11.8 | 0.04 |
| Male (%)                     | 54.7      | 45.0      | 29.3      | 0.001 |
| Body mass index (kg/m²)      | 26.2 ± 0.2 | 26.4 ± 0.2 | 27.2 ± 0.2 | 0.018 |
| Physical activity (Met-h/week)| 34.1 ± 2.4 | 35.8 ± 2.4 | 40.4 ± 2.4 | 0.18 |
| Smoking (%)                  | 6.9       | 10.3      | 7.8       | 0.02 |
| Education level (%)          | 1.2       | 1.6       | 2.3       | 0.13 |
| Illiterate                   | 70.7      | 74.2      | 72.8      | 0.12 |
| Diploma                      | 28.1      | 24.2      | 24.9      | 0.26 |
| Systolic blood pressure (mm Hg) | 108 ± 0.5 | 107 ± 0.5 | 106 ± 0.5 | 0.10 |
| Diastolic blood pressure (mm Hg) | 71.7 ± 0.4 | 71.2 ± 0.4 | 70.9 ± 0.4 | 0.44 |
| Serum creatinine (mg/dL)     | 1.04 ± 0.01 | 1.05 ± 0.01 | 1.05 ± 0.01 | 0.48 |
| eGFR (ml/min/1.73 m²)        | 83.5 ± 0.8 | 80.0 ± 0.8 | 76.6 ± 0.8 | 0.001 |
| Creatinine clearance rate (ml/min) | 96.7 ± 1.2 | 90.9 ± 1.2 | 85.8 ± 1.1 | 0.001 |
| Chronic kidney disease (%)   | 9.9       | 15.1      | 21.7      | 0.001 |

Data are mean ± SE unless stated otherwise. Analysis of variance and chi-square test were used for continuous variables for non-continuous variables. NCV: Nitrate-containing vegetables.

**Table 2.** Mean dietary intakes of participants according to the tertiles of NCV.

| Nitrate containing vegetables | Dietary intake | Tertile 1 | Tertile 2 | Tertile 3 | p   |
|------------------------------|----------------|-----------|-----------|-----------|-----|
| Total NCV (g/day)            | Range          | <203      | 203-332   | ≥332      |     |
| Median                       | 146            | 261       | 428       |           |     |
| High-nitrate vegetables      | 8.8 ± 6.8      | 13.1 ± 10.6| 24.5 ± 18.8| <0.001 |
| Medium nitrate vegetables    | 4.9 ± 3.8      | 8.2 ± 4.2 | 14.9 ± 7.6 | <0.001 |
| Low-nitrate vegetables       | 141 ± 61       | 246 ± 64  | 444 ± 195 | <0.001 |
| Energy intake (kcal/day)     | 2399 ± 28      | 2258 ± 28 | 2301 ± 28 | 0.06  |
| Carbohydrate (%)             | 57.4 ± 0.3     | 56.8 ± 0.3| 58.2 ± 0.3| 0.87   |
| Fat (%)                      | 31.4 ± 0.3     | 32.5 ± 0.2| 31.3 ± 0.3| 0.22   |
| Protein (%)                  | 13.4 ± 0.1     | 13.5 ± 0.1| 13.8 ± 0.1| 0.07   |
| Total fiber (g/day)          | 35.7 ± 0.7     | 37.3 ± 0.7| 39.4 ± 0.7| 0.005  |
| Potassium (mg/day)           | 3190 ± 38      | 3694 ± 37 | 4372 ± 38 | <0.001 |

Data are shown as Mean ± SEM. To compare the dietary intakes of participants across tertiles of NCV general linear model was used with adjustment of sex, age, and energy intake. NCV: Nitrate-containing vegetables.
Table 3. Baseline association (Odds ratio and 95% CI) of chronic kidney disease according to tertiles of NCV categories.

| Nitrate containing vegetables | Tertile 1 | Tertile 2 | Tertile 3 | p for trend |
|------------------------------|----------|----------|----------|-----------|
| Total nitrate containing vegetables | 1 | 1.31 (0.88–1.97) | 1.50 (1.02–2.21) | 0.04 |
| Model 2 | 1 | 1.26 (0.84–1.91) | 1.48 (1.00–2.14) | 0.05 |
| Model 3 | 1 | 1.18 (0.78–1.79) | 1.26 (0.81–1.94) | 0.34 |
| High-nitrate containing vegetables | 1 | 1.09 (0.77–1.56) | 1.43 (1.00–1.99) | 0.03 |
| Model 2 | 1 | 1.10 (0.77–1.58) | 1.44 (1.01–1.97) | 0.04 |
| Model 3 | 1 | 1.11 (0.76–1.60) | 1.48 (1.05–2.13) | 0.05 |
| Medium-nitrate containing vegetables | 1 | 1.12 (0.70–1.65) | 1.15 (0.78–1.67) | 0.56 |
| Model 2 | 1 | 1.10 (0.72–1.63) | 1.12 (0.77–1.65) | 0.64 |
| Model 3 | 1 | 0.80 (0.53–1.23) | 0.85 (0.57–1.26) | 0.58 |
| Low-nitrate containing vegetables | 1 | 1.24 (0.83–1.86) | 1.39 (0.91–2.22) | 0.04 |
| Model 2 | 1 | 1.24 (0.83–1.87) | 1.38 (0.90–2.20) | 0.08 |
| Model 3 | 1 | 1.15 (0.75–1.75) | 1.36 (0.88–2.12) | 0.11 |

Model 1: Adjusted for age and sex, body mass index. Model 2: Additional adjustment for smoking, education, physical activity, diabetes, and hypertension. Model 3: Additional adjustment for dietary intake of energy, fiber, and potassium. NCV: Nitrate-containing vegetables.

Table 4. The occurrence of chronic kidney disease according to tertiles of NCV categories after 3 years of follow-up.

| Nitrate containing vegetables | Tertile 1 | Tertile 2 | Tertile 3 | p for trend |
|------------------------------|----------|----------|----------|-----------|
| Total nitrate containing vegetables | 1 | 0.90 (0.46–1.79) | 1.12 (0.58–2.08) | 0.64 |
| Model 2 | 1 | 0.87 (0.44–1.71) | 1.03 (0.54–1.96) | 0.66 |
| Model 3 | 1 | 0.84 (0.42–1.70) | 0.93 (0.43–2.02) | 0.81 |
| High-nitrate containing vegetables | 1 | 0.95 (0.48–1.88) | 1.63 (0.89–2.99) | 0.09 |
| Model 2 | 1 | 0.79 (0.39–1.61) | 1.33 (0.71–2.49) | 0.11 |
| Model 3 | 1 | 0.76 (0.37–1.57) | 1.23 (0.66–2.53) | 0.23 |
| Medium-nitrate containing vegetables | 1 | 0.72 (0.39–1.34) | 0.74 (0.40–1.38) | 0.51 |
| Model 2 | 1 | 0.79 (0.42–1.48) | 0.77 (0.41–1.47) | 0.67 |
| Model 3 | 1 | 0.82 (0.43–1.56) | 0.78 (0.41–1.51) | 0.31 |
| Low-nitrate containing vegetables | 1 | 1.07 (0.55–2.08) | 1.15 (0.60–2.19) | 0.91 |
| Model 2 | 1 | 1.00 (0.51–1.98) | 1.07 (0.56–2.06) | 0.96 |
| Model 3 | 1 | 0.95 (0.44–2.00) | 0.96 (0.47–1.95) | 0.99 |

Model 1: Adjusted for age and sex, body mass index. Model 2: Additional adjustment for smoking, education, physical activity, diabetes, and hypertension. Model 3: Additional adjustment for dietary intake of energy, fiber, and potassium. NCV: Nitrate-containing vegetables.

To the best of our knowledge, this study was the first prospective population-based study that assessed the association of nitrate containing vegetables with risk of CKD over 3-year. Previous studies have indicated that consumption of fruit and vegetables by producing base and reducing dietary acid might slow eGFR reduction in patients with CKD. Lin et al. have shown that diet- and reducing dietary acid might slow eGFR reduction in patients with CKD.43 Fruits and vegetables are main source of fiber, potassium, and nitrate. Studies have reported that dietary potassium by reducing blood pressure and dietary fiber through decreasing inflammatory markers and blood pressure may protect against CKD.44,45 In this study, association between nitrate containing vegetables and 3-year risk of CKD was adjusted for dietary potassium and fiber to assess effect of dietary nitrate on incidence of CKD, however, no significant association was found. Vegetables especially leafy green vegetables are rich sources of nitrate and renoprotective effect of these foods may be related to their content of nitrate.46

Nitric oxide (NO) generally is produces from L-arginine. However, recent investigations have indicated that NO also generated by nitrate–nitrite–NO pathway from nitrate supplementation or dietary nitrate. After ingestion of vegetables, nitrate absorbs and reduces to nitrite by commensal bacteria in mouth cavity. Then nitrite swallowed and further reduces to NO by enzymatic and non-enzymatic pathway in blood and tissue and elevates NO availability.17

In this study, no association between nitrate-containing vegetables and 3-year incidence of CKD was observed. Animal study have indicated that NO deficiency contribute to pathogenesis of CKD through HTN development and glomerular ischemia. In contrast to CKD lead to low NO production that may have adverse effect on CKD progression15,49–51; hence, clinical trials and experimental studies have shown that administration of nitrite or nitrate supplement by reduction of blood pressure and improvement of ischemia perfusion injury ameliorate CKD progression.15,48 Kanematsu et al. have report that administration of nitrate sodium prevents kidney damage in rats. They suggested that this effect was related to oxidative stress reduce through NO and other nitrogen bioactive species but not reduce blood pressure.16 Carlstrom et al. have shown that consumption of low and high dose of nitrate supplementation in salt-induced hypertensive rats had renoprotective effect in compared with control rats. Moreover, changes in eGFR were not significant between two groups.17

Considering potential endogenous conversion of inorganic nitrate/nitrite to NO, the role of nitrate–nitrite–NO pathway has been highlighted in several health and disease states in recent years. Renoprotective effects of nitrate/nitrite has been attributed to the hypothesis that...
stimulation of a nitrate–nitrite–NO pathway, with inorganic nitrate/nitrite, could limit arteriolar responsiveness to angiotensin II and markedly attenuate angiotensin II-induced hypertension by inhibiting superoxide production via nitric oxide synthase-independent NO generation.57

In a clinical trial, Carpentier et al. have indicated that after nitrate supplementation (450 mg/day) in young men, there were no significant differences on GFR between control and intervention groups over a week. They showed that dietary nitrate had no effect on kidney function.58 However, no prospective study has been investigated association between dietary nitrate and risk of CKD yet. We cannot fully explain lack of association between consumption of nitrate containing vegetables and risk of CKD, a possible explanation could be health condition of our participants. In the current study, participants were healthy at baseline and it is likely consumption of nitrate containing vegetables is not effective on development of CKD in healthy subjects. Another explanation is related to unmeasured residual confounding factors which may be responsible for no observed association.

This study had some strengths and limitations. The main strength of the present study was its population-based design. Other strength was applied of a validated FFQ that was completed by trained dietitians and was not self-reported which reduce measurement bias. In counter to lack of data on the nitrate content of the vegetables as well as dietary, nitrate/nitrite was the main limitation of this study. Moreover, some inherent limitations of observational studies including selection bias, information bias in measuring exposure or outcome, and non-differential misclassification, should be considered in interpretation of the findings.

In conclusion, our findings in cross-sectional setting provided documents for an inverse association of high-NCVs consumption with renal function; higher intakes of high-NCVs was related to a lower rate of creatinine clearance, eGFR and increased odds of CKD, independent of potential confounding variables. However, these findings did not confirm in the prospective setting. Since cross-sectional association could not support causality, these findings therefore could not imply on adverse effect of high-nitrate containing vegetables on renal function. Additional research is recommended to clarify the possible effect of nitrate intakes from vegetables on kidney function.

Considering lack of epidemiological studies in relation to long-term effects of dietary nitrate/nitrite intakes on renal function and the risk of kidney diseases, further cohort studies are required to clarify the possible association. Use of nitrate/nitrite supplementation in experimental studies may also help to clarify potential risk-benefit of nitrate–nitrite–nitric oxide pathway on renal function.

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Disclosure statement

The authors declare no conflict of interest.

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