Estimation and Validation of Dietary Nitrate and Nitrite Intake in Iranian Population

Zahra BAHADORAN¹, Asghar GHALEMIE², *Parvin MIRMIRAN¹, Yadollah MEHRABI³, Fereidoun AZIZI⁴, Farzad HADAEGH⁵

1. Nutrition and Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Endocrine Physiology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Epidemiology, School of Public Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Prevention of Metabolic Disorders Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

*Corresponding Author: Email: mirmiran@endocrine.ac.ir

(Received 22 Aug 2017; accepted 18 Dec 2017)

Abstract

Background: The aim of this study was calibration of a nitrate (NO₃)/nitrite (NO₂) database for estimated its dietary intakes.

Methods: Overall, 250 healthy Tehranian adults were assessed in 2015 for dietary intakes of NO₃ and NO₂ and its serum and urine concentration. Food composition values for NO₃ and NO₂ were derived from a recent survey conducted on frequently consumed food items among Iranians. The correlation of dietary intakes of NO₃/NO₂ and its urinary and serum values was evaluated.

Results: Mean (±SD) intakes of dietary NO₃ and NO₂ were 505±160 and 7.7±2.2 mg/d, respectively. The correlation coefficient of intake and urinary NO₃ was 0.83 (95% CI=0.56-0.91) and 0.57 (95% CI=0.49-0.67) in men and women, respectively. A moderate agreement was also observed between NO₂ intake and its urinary levels (r=0.27, 95% CI=0.13-0.37, and 0.29, 95% CI=0.17-0.41, in men and women, respectively).

Conclusion: Using a national database of NO₃ and NO₂ content of food items along with a valid food frequency questionnaire could provide a valid estimation of dietary intakes of NO₃ in the target population.

Keywords: Nitrate; Nitrite; Diet; Food frequency questionnaire

Introduction

Inorganic nitrate (NO₃) and nitrite (NO₂) are compounds occurring in both natural and industrially processed foods; vegetables and drinking water are major sources of dietary NO₃ and vegetables especially green leafy vegetables, including lettuce and spinach, cabbage, rocket, red beet-root, and radish contribute approximately 80%-95% of the dietary intake of NO₃, whereas major dietary NO₂ intakes are usually from processed meat and animal food products (1, 2). Historically, there has been a long-term concern regarding adverse effects of dietary NO₃ and NO₂ due to its potential endogenous conversion to nitrosamines, and some acute and chronic toxicities.
such as methemoglobinemia, thyroid disorders and carcinogenesis (3-5). In contrast, recent research punctuates that dietary NO$_3$/NO$_2$ may induce several beneficial effects especially on cardiovascular system and metabolic pathways (6-8). There is also some evidence to support consideration inorganic NO$_3$/NO$_2$ as dietary nutrients (9). Despite growing agreement regarding an urgent need to clarify longitudinal risk-benefit assessment of NO$_3$ and NO$_2$ intakes on human health in the framework of population-based studies, there is a huge gap of knowledge in this regard (5, 10-12). Valid estimation of NO$_3$ and NO$_2$ exposure from diets seems to be a main challenging issue and important barrier to such investigations (13). Unlike other nutrients and food components, there is no valid applicable and comprehensive database such as the US Department of Agriculture (USDA) food composition table (FCT) for NO$_3$ and NO$_2$; substantial efforts have therefore been made during two past decades in several countries to develop such database (14-21).

Due to great variations in the NO$_3$ and NO$_2$ contents of foods, estimated dietary NO$_3$ and NO$_2$ intakes from FFQ needs to be confirmed by relevant serum or urine biomarkers (22). Serum and urinary NO$_3$ and NO$_2$ concentrations may be considered as reliable biomarkers of exogenous exposure to NO$_3$ and NO$_2$ and the majority of the urinary nitrate can be accounted for by dietary sources (23, 24); however, some confounders such as endogenous nitric oxide production, biological factors, physical activity, smoking, and some pathologic conditions may affect circulating levels of NO$_3$ and NO$_2$, an issue that need to be considered in the analysis (23).

To provide the opportunities for assessment of health related outcomes of dietary exposure to NO$_3$ and NO$_2$ in the framework of epidemiological studies and prospective cohorts, we recently developed a database for NO$_3$ and NO$_2$ contents of commonly consumed food items among Iranians (25).

Here, we described the performance of the database for estimation of NO$_3$ and NO$_2$ intake, in a representative Iranian adult population from the Tehran Lipid and Glucose Study, and evaluate the estimated values in relation to serum and urinary NO$_3$ and NO$_2$ levels.

Methods

Study population

The current calibration study was conducted in the subset of cohort members of the Tehran Lipid and Glucose Study (TLGS), an ongoing community-based prospective study being conducted to investigate and prevent non-communicable diseases, in a representative sample in the district 13 of Tehran, the capital city of Iran (26). Briefly, healthy non-smoker adults, aged 20-70 yr, free of type 2 diabetes, hypertension, coronary heart disease and renal dysfunction, were consecutively recruited from the TLGS, between Jul to Oct 2015 (26). Participants who had under- or over-report of total energy intakes (<800 kcal/d or >4200 kcal/d) or had specific diet (including dietary recommendations for HTN, hyperlipidemia or diabetes) were excluded and final analysis was conducted on 251 men and women.

Written informed consents were obtained from all participants, and the study protocol was approved by the Ethics Research Council of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Demographic and anthropometric measures

Trained interviewers collected information using standard questionnaires. Detailed measurements of variables in TLGS have been reported elsewhere (27). Weight was measured to the nearest 100 gr 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 (BMI) was calculated as weight (kg) divided by square of the height (m$^2$). Waist circumference (WC) was measured to the nearest 0.1 cm, midway between the lower border of the ribs and the iliac crest at the widest portion, over
light clothing, using a soft measuring tape, without any pressure to the body. For measurements of systolic (SBP) and diastolic blood pressure (DBP), after a 15-min rest in the sitting position, two measurements of blood pressure were taken on the right arm, during a standardized mercury sphygmomanometer; the mean of the two measurements was considered as the participant's blood pressure.

**Biochemical measures**

Blood and urine samples were taken after 12-14 h fasting from all study participants. Serum creatinine (Cr) levels were assayed by kinetic colorimetric Jaffe; the sensitivity of the assay was 0.2 mg/dL (range, 18–1330 μmol/L (0.2–15 mg/dL)) (28). Serum and urine NO3 and NO2 concentrations were measured by a rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite (29). This method has been previously validated in our laboratory and a review paper regarding serum nitric oxide metabolites (NOx) measurement has been published by our group (30, 31). Inter- and Intra-assay coefficients of variations of the assays were 5.2% and 4.4%, respectively; the sensitivity of the assay was 2.0 μmol/L and its recovery was 93 ± 1.5 %.

To assess intra-individual variance, caused by biological variation and random measurement errors in the urinary and serum values of NO3 and NO2, two blood and urine samples were collected over a 2-week period, in a sub-sample of the participants (n=41); repeated measurements of urine nitrate may be useful to account for within person variation for validation of NO3 intake assessed by food frequency questionnaire (FFQ) (22, 32).

**Dietary assessment**

A validated 168-item FFQ was used to assess typical food intakes over the previous year. Trained dietitians, with at least 5 yr of experience in the TLGS survey, asked participants to designate their intake frequency for each food item consumed during the past year on a daily, weekly, or monthly basis. Portion sizes of consumed foods reported in household measures were then converted to grams (33). The validity of the food frequency questionnaire has previously been evaluated by comparing food groups and nutrient values derived 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 (34).

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 (except NO3 and NO2), the US Department of Agriculture Food Composition Table was used (4).

**Estimation of NO3 and NO2 from diet**

Food composition values for NO3 and NO2 were derived from a recent survey conducted on frequently consumed food items among Iranians (25). Briefly, we determined the NO3 and NO2 contents of 87 food items including grains, legumes, fruits and vegetables, dairy products, meats and processed meats using validated spectrophotometric methods (25). Dietary NO3 and NO2 intake of the participants, were estimated by multiplying the reported frequency of consumption and portion size of each FFQ item by its NO3 and NO2 content, and summing values across all food items. In addition to calculating dietary NO3 and NO2 from all foods, plant- and animal-based NO3 and NO2 were separately calculated. Additional, we computed contribution of NO3 and NO2 that came from individual food groups including vegetables, fruits, grains, legumes, dairy, meats and processed meats.

**Estimation of NO3 and NO2 from drinking water**

NO3 and NO2 intakes from drinking water were derived by estimated daily water intakes of the participants, and measured values of NO3 and
NO\textsubscript{2} in tap water, using a spectrophotometric method (35).

**Statistical analysis**

Dietary intakes of NO\textsubscript{3} and NO\textsubscript{2} were adjusted for total energy intake, according to residuals methods (36). Log-transformed of the variables with non-normal distribution (serum and urine NO\textsubscript{3} and NO\textsubscript{2}) were used in the analyses. Mean ± standard deviation (SD), or median and inter-quartile range (IQR) was reported for the variables with normal and non-normal distribution, respectively. Pearson correlation and partial correlation test with adjustment of age, sex, body mass index and serum creatinine levels were used to estimate the correlation of dietary NO\textsubscript{3} and NO\textsubscript{2} intakes and their urinary and serum levels. The ratio of intra- to inter-individual variance (λ), calculated using the intra class correlation coefficient (ICC), was used for further adjustment of the correlation coefficients (22, 32). The λ was 1.98, 2.12 for urinary and serum NO\textsubscript{3}, 1.95 and 1.85 urinary and serum NO\textsubscript{2}.

All statistical analysis were conducted using SPSS (ver. 16.0, Chicago, IL, USA), and P<0.05 were considered significant.

**Results**

Mean age of the participants was 41.5±12.4 yr and 31.5% were men. General characteristics of the participants are shown in Table 1. A significant higher serum levels of creatinine was observed in men (104 vs. 88.1 µmol/l, P<0.05). There was no significant difference in urinary and serum values of NO\textsubscript{3} and NO\textsubscript{2} between men and women.

Estimated daily consumption of NO\textsubscript{3} and NO\textsubscript{2} from total dietary intake, individual food groups, and drinking water are shown in Table 2. Mean (±SD) intakes of dietary NO\textsubscript{3} and NO\textsubscript{2} were 505±160 and 7.7±2.2 mg/d, respectively.

**Table 1**: Characteristics of the study participants

| Variables                  | Men (n=79)       | Women (n=172)    | Total population (n=251) |
|----------------------------|------------------|------------------|--------------------------|
| Age (yr)                   | 40.8±12.3        | 41.8±12.5        | 41.5±12.4                |
| Body mass index (kg/m\textsuperscript{2}) | 27.0±3.8         | 27.1±4.8         | 27.1±4.5                 |
| Waist circumference (cm)   | 94.4±10.0        | 89.2±12.3        | 90.9±11.8                |
| Systolic blood pressure (mm Hg) | 109±9.5         | 105±11.5         | 107.0±11.1               |
| Diastolic blood pressure (mm Hg) | 75.7±5.7         | 72.1±7.1         | 73.2±6.9                 |
| Serum creatinine (µmol/l)  | 104±9.4          | 88.1±9.0         | 93.1±11.8                |
| Urinary NO\textsubscript{3} (µmol/l) | 805 (342-1150)  | 718 (425-1096)  | 730 (396-1110)           |
| Urinary NO\textsubscript{2} (µmol/l) | 132 (70.0-140)  | 130 (69.4-142)  | 131 (69.4-142)           |
| Serum NO\textsubscript{3} (µmol/l) | 41.9 (21.6-74.5) | 43.4 (23.1-65.4) | 43.2 (22.6-67.8)         |
| Serum NO\textsubscript{2} (µmol/l) | 15.3 (10.4-19.3) | 15.0 (11.8-18.8) | 15.1 (11.7-19.0)         |

Mean ±SD (median and inter-quartile range)

Mean intakes (±SD) of NO\textsubscript{3} and NO\textsubscript{2} from drinking water were 36.6±11.5 and 2.8±0.9 mg/d. There was no significant difference in daily consumption of NO\textsubscript{3} and NO\textsubscript{2} from diet and drinking water, between men and women. A significant higher intake of plant-based NO\textsubscript{3} as well as NO\textsubscript{3} intakes from vegetables was observed in women (P<0.05). In men, compared to women, a higher intake of animal-based NO\textsubscript{3} and NO\textsubscript{3} intake from grains was observed (P<0.05). Pearson correlation coefficients between total intakes and urinary and serum concentrations of NO\textsubscript{3} and NO\textsubscript{2} are reported in Table 3. Crude correlation between intake and urinary levels of NO\textsubscript{3} was 0.39 (P=0.013) and 0.19 (P=0.026) in men and women, respectively. Adjusting the relationship for age, body mass index and serum creatinine levels, resulted in a higher partial correlation coefficient (r=0.42, and r=0.29, P=0.001 in men and women, respectively). After correction for intra- and inter-individual variance, a stronger correlation between NO\textsubscript{3} intake and its urinary levels (r=0.83, 95% CI=0.56, 0.91, r=0.57, 95%CI=0.49-0.67, in men and women, respec-

Available at:  http://ijph.tums.ac.ir
tively), was observed. A moderate agreement was observed between NO₂ intake and its urinary levels (r=0.27 and 0.29, in men and women). There was no significant association between dietary NO₃ intake and its serum levels, neither in men (corrected r=0.07, 95% CI= -0.08-0.19) or women (corrected r=0.09, 95% CI= -0.03, 0.23). Dietary intakes and serum levels of NO₃ and NO₂ had a relatively week correlation in both men and women.

Table 2: Estimated daily consumption of nitrate and nitrite in the study population

| Variables     | Nitrate (mg/d)       | Nitrite (mg/d)       |
|---------------|----------------------|----------------------|
|               | Men (n=79)           | Women (n=172)        | Men (n=79) | Women (n=172) |
| Total         | 532±184              | 535±221              | 9.1±3.6   | 8.3±3.2       |
| Plan-based    | 498±166              | 515±216              | 5.5±2.0   | 5.4±2.2       |
| Animal-based  | 16.0±11.1            | 13.7±8.3             | 3.3±2.3   | 2.8±1.8       |
| Legumes       | 9.5±6.0              | 9.3±6.2              | 0.26±0.16 | 0.25±0.17     |
| Grains        | 190±86.3             | 137±71.1             | 2.6±1.6   | 1.9±1.0       |
| Vegetables    | 242±125              | 303±173              | 1.3±0.7   | 1.6±1.1       |
| Fruits        | 56.4±41.9            | 65.5±49.5            | 1.4±1.0   | 1.7±1.2       |
| Dairy products| 9.2±8.8              | 7.9±6.5              | 0.7±0.7   | 0.6±0.5       |
| Meats         | 5.9±6.0              | 5.2±4.6              | 2.1±2.0   | 1.8±1.6       |
| Processed meats| 0.9±1.1              | 0.6±0.8              | 0.5±0.4   | 0.5±0.3       |
| Drinking water| 40.2±11.9            | 34.9±11.0            | 3.1±0.93  | 2.7±0.86      |

Data are mean ±SD
* P<0.05 (independent t-test was used)

Table 3: Pearson correlation coefficients between total intakes and urinary and serum concentrations of nitrate and nitrite

| Variables     | Crude R (P-value) | Partial R (P-value) | Corrected R (95% CI) | Crude R (P-value) | Partial R (P-value) | Corrected R (95% CI) |
|---------------|-------------------|---------------------|----------------------|-------------------|---------------------|----------------------|
| Nitrate       |                   |                     |                      |                   |                     |                      |
| Urine         | 0.39 (0.001)      | 0.42 (0.002)        | 0.83 (0.56-0.91)     | 0.19 (0.026)      | 0.29 (0.001)        | 0.57 (0.49, 0.67)    |
| Serum         | 0.12 (0.35)       | 0.17 (0.42)         | 0.36 (0.24-0.46)     | 0.08 (0.31)       | 0.09 (0.17)         | 0.19 (0.07, 0.32)    |
| Nitrite       |                   |                     |                      |                   |                     |                      |
| Urine         | 0.13 (0.25)       | 0.14 (0.30)         | 0.27 (0.13-0.37)     | 0.16 (0.055)      | 0.15 (0.023)        | 0.29 (0.17, 0.41)    |
| Serum         | 0.02 (0.88)       | 0.04 (0.77)         | 0.07 (-0.08-0.19)    | 0.08 (0.31)       | 0.05 (0.48)         | 0.09 (-0.03, 0.23)   |

1 Adjusted for age, body mass index and serum creatinine levels.
2 Corrected for intra to inter-individual variance ratio (λ); λ was 1.98, 2.12 for urinary and serum NO₃, 1.95 and 1.85 urinary and serum NO₂

Discussion

In this study, conducted in the framework of a national population-based cohort, relatively high intakes of NO₃ and NO₂ (505±160 and 7.7±2.2 mg/d, respectively) were observed among Iranian population. A great association was observed between dietary NO₃ intakes with its urinary levels, especially in men, however the association was relatively weak for NO₃ intakes and its serum levels. Dietary intakes of NO₃ and NO₂ were higher than other reports from different population; the major contributors to nitrate intakes were vegetables including cucumber, green leafy vegetables, lettuce, and tomato, and grains including traditional breads and white rice. The major contributors to NO₂ intake were lamb and chicken meats, white rice, processed meats, and vegetables such as cucumber and tomato. Compared to other population, grains were the considerable source of plant-based NO₃ intake in our population, whereas green leafy vegetables such as spinach or other vegetables such as beetroot had a little contribution in our NO₃ intakes.
Median (inter quartile ranges) intakes of NO\textsubscript{3} and NO\textsubscript{2} were reported 309 (215-413) and 1.4 (1.1-1.8) mg/d in the Shanghai Women’s Health Study (37). Median NO\textsubscript{3} intakes in NIH-AAPR Diet and Health Study were 68.9 and 74.1 mg/d, and NO\textsubscript{2} intakes were 1.3 and 1.0 mg/d, in men and women, respectively (38). Moreover, our intake was approximately twofold of the acceptable daily intake (ADI) values, defined as 3.7 and 0.06 mg/kg body weight for NO\textsubscript{3} and NO\textsubscript{2}, respectively. In our population, the major contributors to NO\textsubscript{3} intakes were vegetables (51.2%) and grains (30.7%). Due to a relatively high NO\textsubscript{3} concentration in our traditional and industrial breads (50.0 mg 100 g\textsuperscript{-1}) (25), and high proportion of breads (320 g/d) in dietary pattern of Iranian population (39), NO\textsubscript{3} exposure from this food group was considerable. We previously reported that mean NO\textsubscript{3} content of lettuce, potato, radish, and cabbage samples was more than the maximum limits legislated by European countries; moreover, mean NO\textsubscript{2} contents of fruit samples were also relatively high (25). Dietary intakes of NO\textsubscript{3} from animal sources accounted for 34.4% of daily mean intake of NO\textsubscript{2} and the remainder of NO\textsubscript{2} intake was derived from plant sources. The major contributors to NO\textsubscript{2} intake were lamb and chicken meats, white rice, processed meats, and vegetables such as cucumber and tomato. High content of NO\textsubscript{3}/NO\textsubscript{2} in Iranian foods or high intake of NO\textsubscript{3}/NO\textsubscript{2}-containing foods may be contributed in high NO\textsubscript{3}/NO\textsubscript{2} diet in our population.

A critical overview of current literature shows a limited focus on valid estimation of NO\textsubscript{3} and NO\textsubscript{2} intakes (22, 40). Several models, including total diet study model, NO\textsubscript{3}/NO\textsubscript{2} database model, NO\textsubscript{3}/NO\textsubscript{2} core food model, the large database model, and a processed meat production model, have been suggested for assessment of dietary exposure of NO\textsubscript{3} and NO\textsubscript{2} in epidemiological studies (41). The core food approach, where the core foods are selected on the basis of their NO\textsubscript{3} and NO\textsubscript{2} content, seems the best model for estimating NO\textsubscript{3} and NO\textsubscript{2} intake from diet (41). However serum and urine NO\textsubscript{3} and NO\textsubscript{2} are affected by endogenous NO production and some factors such as physical activity, using plasma NO\textsubscript{3} and NO\textsubscript{2} in epidemiologic studies found to be feasible and the within-person variability is comparable to commonly used biomarkers (42); use of repeated measurements of urine NO\textsubscript{3} may be capture within person variation and may useful to evaluate the validity of NO\textsubscript{3} intake assessed by FFQ (43). There was a constant relationship between NO\textsubscript{3} intake and urinary excretion of NO\textsubscript{3} and NO\textsubscript{2} (β=0.6-0.8); increased NO\textsubscript{3} concentration in the water potentiated the relationship (40). Dietary NO\textsubscript{3} intake was significantly correlated with its urinary level (P=0.01; $r^2 = 0.07$) following consumption of a diet with high-NO\textsubscript{3} vegetables (44). A substantial correlation was reported between estimated intake of NO\textsubscript{3} from FFQ and urinary excretion levels ($r$=0.59, 95% CI=0.03-0.87), after correction for within person variation and adjustment for sex and body mass index (22); the authors concluded that FFQ assessment of NO\textsubscript{3} intake may provide valid information on usual NO\textsubscript{3} intake (22). Cross-checking of FFQ with other dietary assessment method such as 24h- dietary recall, also the performance of FFQ in assessing dietary NO\textsubscript{3} and NO\textsubscript{2} intake was comparable to that for many other nutrients; in a recent calibration study in the NIH-AAPR Diet and Health Study, energy-adjusted correlation coefficients between FFQ and 24h-recall based values of dietary nitrate and nitrite, for men and women, respectively were 0.59 and 0.57 for nitrate, and 0.59 and 0.58 for nitrite (38).

Regardless of a good agreement of dietary NO\textsubscript{3} and its urinary levels, observed in our study, the correlation for serum values was week. This observation can be explained by the fact that ~ 90% of serum NO\textsubscript{3} and NO\textsubscript{2} in fasted state is derived from the L-arginine-NO pathway, and the halftime of NO\textsubscript{3} and NO\textsubscript{2} are only 5-8 h and 20-45 min, respectively (45). Plasma NO\textsubscript{3} and NO\textsubscript{2} have observed to be returned to its baseline level within 24 h, after a high-NO\textsubscript{3} diet (24). Serum NO\textsubscript{3} and NO\textsubscript{2} levels may therefore not accurately reflect dietary exposure and seems to be less useful for validation study.
To the best of our knowledge, this is the first estimation of dietary NO$_3$ and NO$_2$ intakes among Iranian population, based on a comprehensive NO$_3$ and NO$_2$ database (25) and dietary information derived from a validated FFQ (34), in the framework of a national-cohort on a representative population (27). Our findings should be interpreted considering some strengths and limitations. Use of a validated comprehensive FFQ to assess regular dietary intakes of the participants and estimation of NO$_3$/NO$_2$ based on measured values in frequently consumed food items among our population (25), compared to other previous studies relied on historic literature values, may fully reflect the accurate NO$_3$/NO$_2$ exposure from diet. Moreover, all food items identified as major contributors to dietary NO$_3$/NO$_2$ intakes were on our FFQ. Our estimation of NO$_3$ and NO$_2$ however did not capture cooking, peeling, pureeing, fermentation and other food processing methods may affect the NO$_3$ and NO$_2$ content. Moreover, data on storage time and condition, which can decrease NO$_3$ content of foods, were not available.

**Conclusion**

We report here a valid estimation of dietary exposure of NO$_3$ and NO$_2$ in an Iranian population, for the first time. Dietary NO$_3$ and NO$_2$ intake, estimated using TLGS FFQ, were greatly correlated with their urinary values, as the accurate surrogate of dietary intake. Using a national database of the NO$_3$ and NO$_2$ contents of food items along with a valid and reliable FFQ could provide a valid estimation of dietary intakes of NO$_3$ and NO$_2$ in the target population.

**Ethical considerations**

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

**Acknowledgements**

This study, as part of Ph.D. thesis of Ms. Zahra Bahadoran, was supported by the Research Institute for Endocrine Sciences of Shahid Beheshti University of Medical Sciences (grant No. 759). We thank the Tehran Lipid and Glucose Study participants and the field investigators of the Tehran Lipid and Glucose Study for their cooperation and assistance in physical examinations, biochemical evaluation and database management. The authors wish to acknowledge Ms. Niloofer Shiva for critical editing of English grammar and syntax of the manuscript.

**Conflict of interest**

The authors declare no conflict of interest.

**References**

1. Hord NG, Tang Y, Bryan NS (2009). Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am J Clin Nutr*, 90(1):1-10.
2. Bahadoran Z, Mirmiran P, Ghasemi A et al (2017). Vitamin C intake modify the impact of dietary nitrite on the incidence of type 2 diabetes: A 6-year follow-up in Tehran Lipid and Glucose Study. *Nitric Oxide*, 62:24-31.
3. Bahadoran Z, Mirmiran P, Ghasemi A et al (2015). Is dietary nitrate/nitrite exposure a risk factor for development of thyroid abnormality? A systematic review and meta-analysis. *Nitric Oxide*, 47:65-76.
4. Fewtrell L (2004). Drinking-water nitrate, methemoglobinemia, and global burden of disease: a discussion. *Environ Health Perspect*, 112(14):1371-1374.
5. Gilchrist M, Winyard PG, Benjamin N (2010). Dietary nitrate–good or bad? *Nitric Oxide*, 22(2):104-9.
6. Lundberg JO (2009). Cardiovascular prevention by dietary nitrate and nitrite. *Am J Physiol Heart Circ Physiol*, 296(5):H1221-3.
7. Lundberg JO, Carlstrom M, Larsen FJ, Weitzberg E (2011). Roles of dietary
inorganic nitrate in cardiovascular health and disease. *Cardiovasc Res*, 89(3):525-32.
8. Lundberg JO, Gladwin MT, Abluwalia A et al (2009). Nitrate and nitrite in biology, nutrition, and therapeutics. *Nat Chem Biol*, 5(12):865-9.
9. Bryan NS, Ivy JL (2015). Inorganic nitrite and nitrate: evidence to support consideration as dietary nutrients. *Nutr Res*, 35(8):643-54.
10. Milkowski A, Garg HK, Coughlin JR, Bryan NS (2010). Nutritional epidemiology in the context of nitric oxide biology: a risk-benefit evaluation for dietary nitrite and nitrate. *Nitric Oxide*, 22(2):110-9.
11. Sindelar JJ, Milkowski AL (2012). Human safety controversies surrounding nitrate and nitrite in the diet. *Nitric Oxide*, 26(4):259-66.
12. Habermeyer M, Roth A, Guth S et al (2015). Nitrate and nitrite in the diet: how to assess their benefit and risk for human health. *Mol Nutr Food Res*, 59(1):106-28.
13. Kochez A, Llosa Z (2011). *Nitrite and nitrate in human health and disease*. Springer Science & Business Media, pp.:36-42.
14. Ayaz A, Topcu A, Yurtagul M (2007). Survey of nitrate and nitrite levels of fresh vegetables in Turkey. *J Food Technol*, 5(2):177-9.
15. Chung SW, Tran JC, Tong KS et al (2011). Nitrate and nitrite levels in commonly consumed vegetables in Hong Kong. *Food Addit Contam Part B Surveill*, 4(1):34-41.
16. Chung S, Kim J, Kim M et al (2003). Survey of nitrate and nitrite contents of vegetables grown in Korea. *Food Addit Contam*, 20(7):621-8.
17. Suh J, Jin Pak O, Kang Y et al (2013). Risk Assessment on Nitrate and Nitrite in Vegetables Available in Korean Diet. *J Appl Biol Chem*, 56(4):205-11.
18. Santamaria P, Elia A, Serio F, Todaro E (1999). A survey of nitrate and oxalate content in fresh vegetables. *J Sci Food Agric*, 79(13):1882-8.
19. Keeton J, Osburn W, Hardin M, Longnecker M, Bryan N (2009). A national survey of the nitrite/nitrate concentrations in cured meat products and non-meat foods available at retail. https://www.pork.org/research/a-national-survey-of-the-nitritenitrate-concentrations-in-cured-meat-products-and-non-meat-foods-available-at-retail/
20. Sušin J, Kneel V, Gregorčič A (2006). A survey of nitrate and nitrite content of fruit and vegetables grown in Slovenia during 1996–2002. *Food Addit Contam*, 23(4):385-90.
21. Razgallah N, Chikh-Rouhou H, Boughattas I, M'hamdi M (2016). Nitrate contents in some vegetables in Tunisia. *Arch Agron Soil Sci*, 62(4):473-83.
22. Van den Brandt PA, Willett WC, Tannenbaum SR (1989). Assessment of dietary nitrate intake by a self-administered questionnaire and by overnight urinary measurement. *Int J Epidemiol*, 18(4):852-7.
23. Pannala AS, Mani AR, Spencer JP et al (2003). The effect of dietary nitrate on salivary, plasma, and urinary nitrate metabolism in humans. *Free Radic Biol Med*, 34(5):576-84.
24. Bondonno CP, Liu AH, Croft KD et al (2015). Short-term effects of a high nitrate diet on nitrate metabolism in healthy individuals. *Nutrients*, 7(3):1906-15.
25. Bahadoran Z, Mirmiran P, Jeddi S et al (2016). Nitrate and nitrite content of vegetables, fruits, grains, legumes, dairy products, meats and processed meats. *J Food Compost Anal*, 51:93-105.
26. Azizi F, Rahmani M, Emami H et al (2002). Cardiovascular risk factors in an Iranian urban population: Tehran lipid and glucose study (phase I). *Soz Praventivmed*, 47(6):408-26.
27. Azizi F, Ghanbarian A, Momenan AA et al (2009). Prevention of non-communicable disease in a population in nutrition transition: Tehran Lipid and Glucose Study phase II. *Trials*, 10:5.
28. Tohidi M, Hasheminia M, Mohebi R et al (2012). Incidence of Chronic Kidney Disease and Its Risk Factors, Results of Over 10 Year Follow Up in an Iranian Cohort. *PLoS One*, 7(9):e45304.
29. Miranda KM, Espey MG, Wink DA (2001). A Rapid, Simple Spectrophotometric Method for Simultaneous Detection of Nitrate and Nitrite. *Nitric Oxide*, 5(1):62-71.
30. Ghasemi A, Hedayaat M, Biabani H (2007). Protein precipitation methods evaluated for determination of serum nitric oxide end products by the Griess assay. *JMSR*, 2:29-32.
31. Ghasemi A, Zahediasl S (2012). Preanalytical and analytical considerations for measuring nitric oxide concentrations in plasma, blood cells, and tissue homogenates. *Nitric Oxide*, 26(2):77-82.

Available at: http://ijph.tums.ac.ir
oxide metabolites in serum or plasma using the Griess method. Clin Lab, 58(7-8):615-24.
32. Beaton GH, Milner J, McGuire V et al (1983). Source of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. Carbohydrate sources, vitamins, and minerals. Am J Clin Nutr, 37(6):986-95.
33. Hosseini-Esfahani F, Jessri M, Mirmiran P et al (2010). Adherence to dietary recommendations and risk of metabolic syndrome: Tehran Lipid and Glucose Study. Metabolism, 59(12):1833-42.
34. Mirmiran P, Esfahani FH, Mehrabi Y et al (2010). Reliability and relative validity of an FFQ for nutrients in the Tehran lipid and glucose study. Public Health Nutr, 13(5):654-62.
35. García-Robledo E, Corzo A, Papaspyrou S (2014). A fast and direct spectrophotometric method for the sequential determination of nitrate and nitrite at low concentrations in small volumes. Mar Chem, 162:30-6.
36. Yilmaz B, Sahin K, Bilen H et al (2015). Carotenoids and non-alcoholic fatty liver disease. Hepatobiliary Surg Nutr, 4(3):161-71.
37. Aschebrook-Kilfoy B, Shu X-O, Gao Y-T et al (2013). Thyroid cancer risk and dietary nitrate and nitrite intake in the Shanghai Women’s Health Study. Int J Cancer, 132(4):897-904.
38. Inoue-Choi M, Virk-Baker MK, Aschebrook-Kilfoy B et al (2016). Development and calibration of a dietary nitrate and nitrite database in the NIH-AARP Diet and Health Study. Public Health Nutr, 19(11):1934-43.
39. Bahreynian M, Esmailzadeh A (2012). Quantity and quality of carbohydrate intake in Iran: a target for nutritional intervention. Arch Iran Med, 15(10):648-9.
40. Chilvers C, Inskip H, Caygill C et al (1984). A survey of dietary nitrate in well-water users. Int J Epidemiol, 13(3):324-31.
41. Pennington JAT (1998). Dietary exposure models for nitrates and nitrites. Food Control, 9(6):385-95.
42. Wang Y, Townsend MK, Eliassen AH, Wu T (2013). Stability and Reproducibility of the Measurement of Plasma Nitrate in Large Epidemiologic Studies. N Am J Med Sci (Boston), 6(2):82-6.
43. Bryan NS, Loscalzo J (2011). Nitrite and nitrate in human health and disease. Springer Science & Business Media, pp:132-146.
44. van Maanen JM, Pachen DM, Dallinga JW, Kleinjans JC (1998). Formation of nitrosamines during consumption of nitrate- and amine-rich foods, and the influence of the use of mouthwashes. Cancer Detect Prev, 22(3):204-12.
45. Yoon Y, Song J, Hong SH, Kim JQ (2000). Plasma nitric oxide concentrations and nitric oxide synthase gene polymorphisms in coronary artery disease. Clin Chem, 46(10):1626-30.