



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

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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 (\pm SD) intakes of dietary NO₃ and NO₂ were 505 \pm 160 and 7.7 \pm 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 beetroot, 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 NO₃/NO₂ may induce several beneficial effects especially on cardiovascular system and metabolic pathways (6-8). There is also some evidence to support consideration inorganic NO₃/NO₂ as dietary nutrients (9). Despite growing agreement regarding an urgent need to clarify longitudinal risk-benefit assessment of NO₃ and NO₂ 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 NO₃ and NO₂ 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 NO₃ and NO₂; 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 NO₃ and NO₂ contents of foods, estimated dietary NO₃ and NO₂ intakes from FFQ needs to be confirmed by relevant serum or urine biomarkers (22). Serum and urinary NO₃ and NO₂ concentrations may be considered as reliable biomarkers of exogenous exposure to NO₃ and NO₂ 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 NO₃ and NO₂, 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 NO₃ and NO₂ in the framework of epidemiological studies and prospective cohorts, we recently developed a database for NO₃ and NO₂ contents of commonly consumed food items among Iranians (25).

Here, we described the performance of the database for estimation of NO₃ and NO₂, 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 NO₃ and NO₂ 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 (m²). 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 μ mol/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 (NO_x) 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 μ mol/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 con-

verted to grams (33). 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 (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. Additionally, 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

NO₂ in tap water, using a spectrophotometric method (35).

Statistical analysis

Dietary intakes of NO₃ and NO₂ were adjusted for total energy intake, according to residuals methods (36). Log-transformed of the variables with non-normal distribution (serum and urine NO₃ and NO₂) were used in the analyses. Mean \pm 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 NO₃ and NO₂ 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 NO₃, 1.95 and 1.85 urinary and serum NO₂.

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 $\mu\text{mol/l}$, $P < 0.05$). There was no significant difference in urinary and serum values of NO₃ and NO₂ between men and women.

Estimated daily consumption of NO₃ and NO₂, from total dietary intake, individual food groups, and drinking water are shown in Table 2. Mean (\pm SD) intakes of dietary NO₃ and NO₂ 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 \pm 12.3	41.8 \pm 12.5	41.5 \pm 12.4
Body mass index (kg/m ²)	27.0 \pm 3.8	27.1 \pm 4.8	27.1 \pm 4.5
Waist circumference (cm)	94.4 \pm 10.0	89.2 \pm 12.3	90.9 \pm 11.8
Systolic blood pressure (mm Hg)	109 \pm 9.5	105 \pm 11.5	107.0 \pm 11.1
Diastolic blood pressure (mm Hg)	75.7 \pm 5.7	72.1 \pm 7.1	73.2 \pm 6.9
Serum creatinine ($\mu\text{mol/l}$)	104 \pm 9.4	88.1 \pm 9.0	93.1 \pm 11.8
Urinary NO ₃ ($\mu\text{mol/l}$)	805 (342-1150)	718 (425-1096)	730 (396-1110)
Urinary NO ₂ ($\mu\text{mol/l}$)	132 (70.0-140)	130 (69.4-142)	131 (69.4-142)
Serum NO ₃ ($\mu\text{mol/l}$)	41.9 (21.6-74.5)	43.4 (23.1-65.4)	43.2 (22.6-67.8)
Serum NO ₂ ($\mu\text{mol/l}$)	15.3 (10.4-19.3)	15.0 (11.8-18.8)	15.1 (11.7-19.0)

Mean \pm SD (median and inter-quartile range)

Mean intakes (\pm SD) of NO₃ and NO₂ from drinking water were 36.6 ± 11.5 and 2.8 ± 0.9 mg/d. There was no significant difference in daily consumption of NO₃ and NO₂ from diet and drinking water, between men and women. A significant higher intake of plant-based NO₃ as well as NO₃ intakes from vegetables was observed in women ($P < 0.05$). In men, compared to women, a higher intake of animal-based NO₃ and NO₃ intake from grains was observed ($P < 0.05$).

Pearson correlation coefficients between total intakes and urinary and serum concentrations of

NO₃ and NO₂ are reported in Table 3. Crude correlation between intake and urinary levels of NO₃ 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 NO₃ 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-

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 NO₂ 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 weak 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	Men			Women		
	Crude R (P-value)	Partial R (P-value) ¹	Corrected R (95% CI) ²	Crude R (P-value)	Partial R (P-value) ¹	Corrected R (95% CI) ²
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)

¹Adjusted for age, body mass index and serum creatinine levels.

²Corrected 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 NO₃ and NO₂ were reported 309 (215-413) and 1.4 (1.1-1.8) mg/d in the Shanghai Women's Health Study (37). Median NO₃ intakes in NIH-AAPR Diet and Health Study were 68.9 and 74.1 mg/d, and NO₂ intakes were 1.3 and 1.0 mg/d, in men and women, respectively (38). Moreover, our intakes was approximately twofold of the acceptable daily intake (ADI) values, defined as 3.7 and 0.06 mg/kg body weight for NO₃ and NO₂, respectively. In our population, the major contributors to NO₃ intakes were vegetables (51.2%) and grains (30.7%). Due to a relatively high NO₃ concentration in our traditional and industrial breads (50.0 mg 100 g⁻¹) (25), and high proportion of breads (320 g/d) in dietary pattern of Iranian population (39), NO₃ exposure from this food group was considerable. We previously reported that mean NO₃ content of lettuce, potato, radish, and cabbage samples was more than the maximum limits legislated by European countries; moreover, mean NO₂ contents of fruit samples were also relatively high (25). Dietary intakes of NO₂ from animal sources accounted for 34.4% of daily mean intake of NO₂ and the remainder of NO₂ intake was derived from plant sources. The major contributors to NO₂ intake were lamb and chicken meats, white rice, processed meats, and vegetables such as cucumber and tomato. High content of NO₃/NO₂ in Iranian foods or high intake of NO₃/NO₂-containing foods may be contributed in high NO₃/NO₂ diet in our population.

A critical overview of current literature shows a limited focus on valid estimation of NO₃ and NO₂ intakes (22, 40). Several models, including total diet study model, NO₃/NO₂ database model, NO₃/NO₂ core food model, the large database model, and a processed meat production model, have been suggested for assessment of dietary exposure of NO₃ and NO₂ in epidemiological studies (41). The core food approach, where the core foods are selected on the basis of their NO₃ and NO₂ content, seems the best model for estimating NO₃ and NO₂ intake from diet (41).

However serum and urine NO₃ and NO₂ are affected by endogenous NO production and some

factors such as physical activity, using plasma NO₃ and NO₂ 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 NO₃ may be capture within person variation and may useful to evaluate the validity of NO₃ intake assessed by FFQ (43). There was a constant relationship between NO₃ intake and urinary excretion of NO₃ and NO₂ ($\beta=0.6-0.8$); increased NO₃ concentration in the water potentiated the relationship (40). Dietary NO₃ intake was significantly correlated with its urinary level ($P=0.01$; $r^2 = 0.07$) following consumption of a diet with high-NO₃ vegetables (44). A substantial correlation was reported between estimated intake of NO₃ 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 NO₃ intake may provide valid information on usual NO₃ intake (22). Cross-checking of FFQ with other dietary assessment method such as 24h- dietary recall, also the performance of FFQ in assessing dietary NO₃ and NO₂ 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-recal 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 NO₃ 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 NO₃ and NO₂ in fasted state is derived from the L-arginine-NO pathway, and the half-lives of NO₃ and NO₂ are only 5-8 h and 20-45 min, respectively (45). Plasma NO₃ and NO₂ have observed to be returned to its baseline level within 24 h, after a high-NO₃ diet (24). Serum NO₃ and NO₂ 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 NO₃ and NO₂ intakes among Iranian population, based on a comprehensive NO₃ and NO₂ 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 NO₃/NO₂ 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 NO₃/NO₂ exposure from diet. Moreover, all food items identified as major contributors to dietary NO₃/NO₂ intakes were on our FFQ.

Our estimation of NO₃ and NO₂ however did not capture cooking, peeling, pureeing, fermentation and other food processing methods may affect the NO₃ and NO₂ content. Moreover, data on storage time and condition, which can decrease NO₃ content of foods, were not available.

Conclusion

We report here a valid estimation of dietary exposure of NO₃ and NO₂ in an Iranian population, for the first time. Dietary NO₃ and NO₂ 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 NO₃ and NO₂ contents of food items along with a valid and reliable FFQ could provide a valid estimation of dietary intakes of NO₃ and NO₂ 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.

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Conflict of interest

The authors declare no conflict of interest.

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