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Development of a reference database for assessing dietary nitrate in vegetables

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Abbreviations: 24-HDRs, 24 hour dietary recalls; FFQs, food frequency questionnaires; NO, nitric oxide; FW, fresh weight.

ABSTRACT

Scope: Nitrate from vegetables improves vascular health with short term intake. Whether this translates into improved long term health outcomes has yet to be investigated. To enable reliable analysis of nitrate intake from food records there is a strong need for a comprehensive nitrate content of vegetables database.

Methods and results: A systematic literature search (1980-2016) was performed using Medline, Agricola and CAB abstracts databases. The nitrate content of vegetables database contains 4237 records from 255 publications with data on 178 vegetables and 22 herbs and spices. The nitrate content of individual vegetables ranged from Chinese flat cabbage (median; range: 4240; 3004-6310 mg/kg FW) to corn (median; range: 12; 5-1091 mg/kg FW). The database was applied to estimate vegetable nitrate intake using 24-hour dietary recalls (24-HDRs) and food frequency questionnaires (FFQs). Significant correlations were observed between urinary nitrate excretion and 24-HDR ($r=0.4$, $P=0.013$); between 24-HDR and 12 month FFQs ($r=0.5$, $P<0.001$) as well as two 4 week FFQs administered 8 weeks apart ($r=0.86$, $P<0.001$).

Conclusion: This comprehensive nitrate database allows quantification of dietary nitrate from a large variety of vegetables. It can be applied to dietary records to explore the associations between nitrate intake and health outcomes in human studies.

INTRODUCTION

The understanding of the health effects of dietary nitrate has undergone a recent change. Once considered an environmental pollutant, potential carcinogen and inert end product of endogenous nitric oxide (NO) metabolism, nitrate (obtained primarily from green leafy vegetables and beetroot) is currently a candidate for the cardiovascular benefits of a vegetable-rich diet. The mechanism is via the sequential reduction of nitrate through an enterosalivary circulatory pathway to nitrite and NO, a molecule critical for cardiovascular health [1-3]. NO is a potent vasodilator that plays a key role in maintaining vascular homeostasis [4]. Decreased production and/or bioavailability of NO and impairment of endothelial function is a hallmark of a number of cardiovascular disorders including hypertension, atherosclerosis and ischaemic disease. Dietary nitrate, through the nitrate-nitrite-NO pathway, could preserve a healthy vasculature and enhance cardiovascular health. Indeed, over 30 randomised controlled clinical trials examining the benefits of dietary nitrate on blood pressure and vascular function have established cardioprotective benefits [5]. In contrast, there is a lingering health concern about nitrate intake and cancer [5]. This is due to the possible formation of carcinogenic N-nitroso compounds when ingested with an amine source. The International Agency for Research on Carcinogenicity (IARC) determined that "ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans" [6]. This reaction, is however, inhibited in the presence of antioxidant compounds such as polyphenols, vitamins C and E, which are also found in vegetables [7-9]. While estimates of the nitrate content of foods, including vegetables, have been generated for observational epidemiology studies in Spain [10] and the US [11, 12], there is a strong need for an accurate, comprehensive and up-to-date database to assess the role of dietary nitrate from vegetables with long term health benefits and risks in multi-national observational studies.

Dietary nitrate is predominantly found in vegetables accounting for ~80% of total nitrate intake [13]. Vegetables such as beetroot, lettuce, rocket and spinach are rich in nitrate while other vegetables, such as peas, potato and tomato, contain nitrate at lower concentrations [14]. In addition to the effects of different plant species other factors are recognised as having an effect on nitrate content. These include: climate; growing method (for example, open air vs undercover), cooking and preservation methods; month, season and year of cultivation; and the analytical procedure used to assess nitrate concentration [15-18]. There is currently no database in the public or commercial arena that has systematically collected information on the measured nitrate concentration of vegetables including details on factors known to affect nitrate concentration. Such a database will allow for improved estimation of nitrate intake. This is important to determine the relationship between nitrate intake from vegetables and long term health outcomes in different populations, the next critical step in dietary nitrate research. The standardisation and improved estimation of nitrate intake would lead to increased power to detect nitrate-health outcome relationships in both observational studies and randomised controlled trials. This approach is also vital for developing reference vegetable nitrate intakes that potentially could be included in dietary guidelines.

Here we present details on the construction of a nitrate database which is a systematic, comprehensive evaluation and collation of the currently available data on the nitrate content of vegetables, herbs and spices. For each record in the database, biological (vegetable type, strain or cultivar), environmental/ agricultural (growing method, month, season and year of sampling and country), processing (cooking method and preservation method) and measurement information (including variance, sample size and analytical method) is provided where available along with a complete literature citation. In order to highlight the potential utility of the nitrate database, as well as provide an example of the complexities of the variables that can affect nitrate content, the influence of growing method, season and cultivar on the nitrate content of lettuce was investigated. Finally, we used the database to assess the relationship between urinary nitrate excretion and estimated vegetable nitrate intake from 24 hour dietary recalls (24 HDR) in a group of men and women. We also assessed the correlations of vegetable nitrate intake between 24-HDR and 12 month food frequency questionnaires (FFQ) as well as two 4 week FFQs administered 8 weeks apart.

METHODS

Database development

Data collection

A systematic literature search was conducted according to the PRISMA 2009 Statement (**Figure 1**). Medline, Agricola and CAB (Commonwealth Agricultural Bureaux) abstracts were the major databases searched from January 1980 to January 2016. Key search terms (**Supplementary Table 1**), comprising subject headings and text words, included nitrate, nitrite, vegetables, food analysis, individual vegetable names and combinations of the listed terms. All searches were fully documented including search terms, search limits and the number of references found. Reports from government agencies were searched in Google Scholar and government agency websites. Additional studies were identified by searching reference lists of reviews and meta-analyses. References were imported into an Endnote® X7.0 (Thomson Reuters) library.

Data evaluation and selection

Titles and abstracts of the imported references were reviewed after the duplicates were removed. Full papers were collected for those meeting the following inclusion criteria: 1) they were published in English; 2) they were published between January 1980 – January 2016; and 3) they reported nitrate and/or nitrite concentration in vegetables. The full-text articles were subsequently assessed for eligibility by two study authors (LB and CB). Disagreement about study inclusion was resolved by consensus between two authors (LB and CB) and further discussion with a third (JH) if necessary. Articles were excluded if: 1) the nitrate and/or nitrite concentration was not provided or was unclear; 2) they reported an experimental study with no control or the data was reported under conditions of agricultural practice not conventionally used; 3) they were a nitrate and/or nitrite analysis method development paper with no validated reference method as a comparison; 4) the nitrate and/or nitrite concentrations were measured in vegetables prior to 1980; 5) the nitrate and/or nitrite concentrations were measured during growth of the vegetable prior to harvesting, in an inedible part of the vegetable, in an unknown vegetable or was not assessed in individual vegetables; and 6) the data in the article was compiled or part of a review (**Table 1**). Articles containing compiled data were used if the original source was not available or was not published in English.

Data compilation

Information on vegetables, sampling, measurement and publication were manually entered into a Microsoft Excel Spreadsheet. The database comprises four main sections:

1) Vegetable information. This section contains the vegetable name, Latin name, common alternate names, cultivar (if known), cooking method, preservation method and growing method. The codes used are detailed in **Supplementary Table 2**.
2) Sampling information. This section details month (if available), season (if available), year of sample (if available) and source of data (direct or compiled).
3) Measurement information. This section contains the nitrate and/or nitrite value as mg/kg (the relevant conversions were performed when necessary), type of value (mean, median), variance (SD, SE and/or range), sample size (if reported as a range the lowest number was used; if no sample size was reported or the sample size was unclear then a sample size of 1 was entered) and analytical method.
4) Publication information. This section includes year of publication, authors, title, country and type of publication.

Application of the database to estimate nitrate intake from vegetables

Participants

Participants were recruited by newspaper advertisement from the Perth general population for participation in a clinical trial investigating the effects of nitrate intake from vegetables on blood pressure. This trial was registered on the Australian New Zealand Clinical Trials Registry at www.anzctr.org.au (#ACTRN12615000194561) and ethics approval was obtained from the University of Western Australia Human Research Ethics Committee (RA/4/1/6872). For the purpose of assessing the nitrate database 41 participants consented to complete a FFQ, a 24 hour diet recall (24-HDR) and provide a spot urine sample. Of these participants, 30 also agreed to complete two identical 4 week FFQs 8 weeks apart. Screening was conducted at the University of Western Australia, School of Medicine and Pharmacology Unit, located at Royal Perth Hospital and consisted of a standard health and lifestyle questionnaire, blood pressure measurement, height and weight as well as routine laboratory analysis of a fasting blood sample. Exclusion criteria included: BMI <18.5 kg/m^2 or ≥35 kg/m^2; age <21 or >75 years; use of antihypertensive medication; diagnosed type 1 or 2 diabetes or fasting glucose >7.0mmol/L; being vegan or vegetarian; use of nitric oxide donors, organic nitrites and nitrates, sildenafil and/or related drugs; use of antibacterial mouthwash; use of antibiotics (with previous 2 months); current or recent (<12 months) smoking; history of symptomatic cardiovascular or peripheral vascular disease, or chronic kidney disease; recent history of a psychiatric illness or other major illness such as cancer; current or recent (within previous 6 months) significant weight loss or gain (>6% of body weight); alcohol intake >140 g per week for women or >210 g per week for men and/or binge drinking behavior; and women who were pregnant, lactating or wishing to become pregnant during the study.

Urine collection

Participants were instructed to collect a spot urine sample the night before their first visit, 60 to 120 minutes after consumption of their last meal of the day. The time of the sample collection was noted and the sample stored at 4ºC overnight.

24 hour diet recalls

At the first visit participants (n=41) were asked to recall in detail all foods and beverages consumed during the last 24 hours from the time they were interviewed. Emphasis on all vegetables consumed during this period was given. Participants were assisted in recalling portion size of foods and beverages consumed by using food models, photographs, charts, measuring cups and measuring spoons. Vegetable data was extracted from the 24-HDRs and then entered into FoodWorks (FoodWorks 7; Xyris Software) for the calculation of weight in grams. Individual vegetables (g) were then multiplied by the median nitrate content (mg/g) of that vegetable obtained from the nitrate content of vegetables database. The sum of nitrate values from individual vegetables was then calculated.

Food frequency questionnaires

At the first visit, participants (n=41) were also asked to complete a self-administered validated semi-quantitative FFQ developed by the Cancer Council Victoria, Australia [19]. This validated FFQ measures the usual frequency of food intake over a period of 12 months and comprises of a food list of 74 items with 10 frequency response options ranging from 'Never' to '3 or more times per day'. The questionnaire calculates portion size using three photographs of scaled portions for four different food types. The process of collection was identical and was supervised by a research assistant/registered nutritionist. Food models, photographs, charts, measuring cups and measuring spoons were provided for accuracy. The FFQ included questions about vegetable intake on 25 vegetables. Daily intake of these vegetables was estimated in g/day by the Cancer Council of Victoria. Each of the weights (g) of the 25 vegetables were multiplied by the median nitrate value (mg/g) of that vegetable. The sum of the nitrate values from individual vegetables was then calculated. A subset of these participants (n=30) also agreed to complete two identical modified versions of the Cancer Council of Victoria FFQ administered 8 weeks apart which assessed dietary intake over the previous 4 weeks instead of 12 months. These FFQs were administered during periods where participants were instructed to limit their intake of green leafy vegetables.

Urine nitrate, nitrite and creatinine

Nitrate and nitrite concentrations in urine were determined in frozen samples using gas chromatography-mass spectrometry (GC-MS) described previously [20]. Urinary creatinine measurements were performed by the Department of Clinical Biochemistry at Royal Perth Hospital using a kinetic colorimetric test (Roche, Indianapolis, IN) with an automated analyzer (Roche Hitachi 917).

Statistics

Descriptive statistics including number, mean (SD), median (IQR), minimum and maximum were used to summarise country of sample and individual vegetables. A large proportion of distributions of nitrate values in individual vegetables were not normally distributed therefore both mean and median values were used to describe the nitrate content (mg/kg FW) in Table 2 and 3.

The magnitude of the effect of factors known to potentially influence nitrate content was assessed in lettuce. Nitrate content of lettuce was log-transformed prior to all analyses due to its non-normality. Linear mixed models were used in unadjusted and publication ID (a unique number for each publication) adjusted models to investigate the differences of nitrate content amongst growing method (organic vs conventional and undercover vs open air), season and lettuce type. Publication ID was entered as a random factor treating each publication as a separate category rather than as a continuous covariate. This was used to adjust for variation in country, year and method of analysis. Bonferroni method was used to correct for multiple comparisons across categories.

Spearman's rho correlation coefficient was used to assess the linear relationship between urinary nitrate excretion and vegetable nitrate intake calculated from the 24-HDR (n=41). Agreement in estimated nitrate intake between the 24-HDR and the 12 month FFQ was assessed using Bland & Altman analysis (1986) (n=41). A paired t-test was used to evaluate the presence of fixed bias between methods. We also calculated the intraclass correlation coefficient (ICC) using a one way ANOVA with method of assessment (24-HDR or 12 month FFQ) and participant ID as fixed effects. The Pearson correlation coefficient (r) was used to assess the degree of linearity between the 2 methods. Spearman's rho correlation coefficient was used to assess the agreement between two 4 week FFQs administered 8 weeks apart (n=30). Statistical analyses were performed using SPSS v22 (SPSS Inc., Chicago, United States of America); SAS software (version 9.3; SAS Institute Inc); and STATA 14 (StataCorp, USA). Statistical significance was set at $p<0.05$ for all tests.

RESULTS

Nitrate content of vegetables database

The Prisma flow chart showing the selection of included studies is shown in **Figure 1**. The database contains a total 4237 records, searched from January 1980 to January 2016, sourced from 255 references and includes data on 178 vegetables as well as 22 herbs and spices. The database contains information of the nitrate content of vegetables from 54 countries with a range of 1 - 553 entries per country and 1 - 51 vegetables per country (**Supplementary Table 3**). Information on cooking, preservation and growing method, month, season and year of sampling as well as analytical method and sample size were recorded where available.

The mean (SD), median (IQR), minimum and maximum nitrate content (mg/kg FW) as well as sample size and number of publications for individual vegetables (raw and fresh) with at least 3 publications are presented in **Table 2**. The median nitrate content (mg/kg FW) of individual vegetables with at least 3 publications from highest (Chinese flat cabbage: median; range: 4240; 3004-6310 mg/kg FW) to lowest (corn: median; range: 12; 5-1091 mg/kg FW) is shown in **Figure 2**. The median nitrate content (mg/kg FW) of individual vegetables grouped by family name is shown in **Figure 3**. The Latin and alternate names of the individual vegetables presented in Table 2 are detailed in **Supplementary Table 4**. The mean (SD), median (IQR), minimum and maximum nitrate content (mg/kg FW) as well as sample size and number of publications for herbs and spices with at least 3 publications is presented in **Table 3**.

The mean (SD), median (IQR), minimum and maximum nitrite content (mg/kg FW) as well as sample size and number of publications for individual vegetables (raw and fresh) as well as herbs and spices with at least 3 publications are presented in **Supplementary Tables 5 and 6** respectively.

Variables influencing the nitrate content of lettuce

The nitrate concentration of organically grown lettuce was significantly lower than conventionally grown lettuce (P=0.001,**Figure 4A**). This difference remained significant after adjustment for publication ID (P=0.009, **Figure 4B**). The nitrate concentration of lettuce grown undercover was significantly higher than lettuce grown open air (P<0.001, **Figure 4C**). This difference remained significant after adjustment for publication ID (P<0.001, **Figure 4D**).

The nitrate concentration of lettuce was significantly different among seasons in both unadjusted (**Figure 5A**) and publication ID adjusted (**Figure 5B**) models, with the exception of autumn vs. spring (both models) and summer vs. autumn (publication ID adjusted model) which was not significantly different from one another. The lowest nitrate concentration was observed in summer and the highest in winter.

The nitrate concentration varied widely between lettuce types in both unadjusted (**Figure 6A**) and publication ID adjusted (**Figure 6B**) models. The highest nitrate content was observed in Chinese lettuce and the lowest in Iceberg lettuce.

Application of the database to estimate nitrate intake from vegetables

The baseline characteristics of the participants (n=41) are presented in **Table 4**. The median (IQR) urinary nitrate was 81.9 (52.8 – 120.8) µmol/mmol creatinine. The mean (±SD) nitrate intake for the 12 month FFQ and 24-HDR amongst the 41 volunteers were 71.9 ± 33.9 mg/day and 89.3 ± 64.7 mg/day respectively. The mean (±SD) nitrate intake for the two 4 week FFQs, 30 volunteers on a low nitrate diet, administered 8 weeks apart were 45.6 ± 26.8 mg/day and 41.4 ± 40.36 mg/day. There was a positive correlation between urinary nitrate and 24-HDR (r=0.4, P=0.013), a positive correlation between 24-HDR and the 12 month FFQ (r=0.5, P<0.001; **Figure 7A**) and a positive correlation between the two 4 week FFQs administered 8 weeks apart (r=0.9, P<0.001). Bland Altman analysis and the paired t-test revealed a borderline significant fixed bias with the 12 month FFQ measuring 17.4 mg lower mean nitrate intake (mg/day) (95% CI: -35.9; 1.2, P=0.054) than the 24-HDR. The lower and upper limits of agreement for FFQ versus 24-HDR were -136.2 to +101.4 (**Figure 7B**). The ICC for reliability estimated from one-way ANOVA was ρ=0.38 (95% CI=0.12 to 0.65).

DISCUSSION

Here we present an up-to-date database of the nitrate content of vegetables. The key strength of this database is the systematic and comprehensive search of currently available literature that has provided important information on the nitrate content of a wide variety of vegetables as well as influencing factors: biological (vegetable type, strain or cultivar), environmental/ agricultural (growing method, month, season and year of sampling and country); processing (cooking method and preservation method) and measurement information (including analytical method). We have highlighted the potential complexities of some of these variables that can affect nitrate content by demonstrating that the nitrate content of lettuce differed significantly between organically and conventionally grown; between undercover and open air and across seasons. We have also shown that the database can be used to estimate vegetable nitrate intake as measured by 24-HDRs and FFQs with reasonable consistency.

The nitrate content of vegetables database an important tool in better understanding the relationship between nitrate intake from vegetables and health outcomes. It provides reliable values for vegetable nitrate concentrations that can be used in conjunction with other databases to calculate nutrient and non-nutrients from FFQs and/or dietary food records in randomised controlled trials and/or large epidemiological studies. This provides a unique opportunity for researchers to assess the benefits and possible risks of nitrate intake in different populations. The measured nitrate concentration from different countries will allow for values to be either generalized or specifically selected to determine nitrate intake. Additionally, the database will increase understanding of factors related to variation of nitrate concentration in vegetables.

Vegetable nitrate content depends on the biological properties of the plant. Nitrate accumulates differently in each part of the plant which can be listed in decreasing order of nitrate content as follows: petiole>leaf>stem>root>inflorescence>tuber>bulb>fruit>seed [13]. In our database, green leafy vegetables had the greatest concentration of nitrate with the highest nitrate values observed in Chinese flat cabbage and arugula (> 3000 mg/kg FW). Indeed, all vegetables with a nitrate concentration > 2000 mg/kg FW were green leafy vegetables. Among the stem vegetables, celery had the highest nitrate concentration (> 1000 mg/kg FW). Root vegetables that had the highest nitrate concentration were radish, rutabago, and beet (> 1400 mg/kg FW). Vegetables with a concentration of nitrate > 2000 mg/kg FW belonged to three families: Brassicaceae (Chinese flat cabbage, arugula, false pak-choi, mustard greens, potherb mustard, pak choi, Chinese broccoli and watercress), Amaranthaceae (Swiss chard and sea beet) and Valerianaceae (cornsalad). Nitrate content, however, varied considerably within each family. Nitrate concentration not only varied between vegetable types but also within species, cultivars and even genotype. For example, Chinese lettuce had 3 fold higher median nitrate content than iceberg lettuce. Where known, details of species and cultivar have been included in the database. Cilantro and basil had the highest nitrate concentration in the herbs and spices (> 1500 mg/kg FW) but given that only a small amount is generally consumed may not contribute significantly to nitrate intake.

In addition to the biological properties of the vegetable, the accumulation of nitrate is subject to environmental and/or agricultural factors. Fertilizer use, light intensity and temperature have been identified as the major factors that influence nitrate concentration in vegetables [17, 21, 22]. Application of fertilizers, in particular nitrogen fertilizers, facilitates the accumulation of nitrate in plant tissues. Thus conventionally grown vegetables tend to have a higher nitrate content compared to organically grown crops [23, 24]. In our database conventionally grown lettuce had a significantly higher nitrate concentration compared to organically grown lettuce. Light intensity and temperature play a crucial role in nitrate assimilation and variations in nitrate levels are observed in the same vegetable grown in different geographic and climatic conditions [22, 25]. Nitrate levels tend to be higher in samples from Northern Europe than those from Mediterranean countries. Additionally, higher nitrate levels are generally observed in winter than in summer and nitrate content in vegetables grown under glass are usually higher than in those grown outdoors in the same season and the same region [26]. These differences are recognised by the European Commission (EC Regulation No. 1881/2006) who set maximum limits for the nitrate content in lettuce and spinach according to harvest period and whether grown under cover or grown in open air. Indeed in our database lettuce grown in winter had the highest nitrate concentration as did lettuce grown undercover. Growing and preservation method, month, season and year of sampling and country of sampling are all included (where available) in the database, therefore researchers have the option of selecting the data relevant to the area and time frame being investigated.

Processing factors also have an effect on the nitrate concentration of vegetables. Nitrates are water soluble therefore washing and cooking has been shown to decrease the nitrate content of the vegetable [27-29]. Boiling vegetables can reduce the nitrate content up to 75% [27-29], however information regarding the effect of other cooking methods (microwaving, steaming, frying etc.) is limited. Pickling vegetables can reduce the nitrate content by approximately 45%. In the current database vegetables that have not been analysed as raw and fresh, cooking and preservation methods have been included. Other processing factors influencing the nitrate content of vegetables include storage time and conditions as well as peeling of the vegetables. Storage, particularly at room temperature, can reduce the nitrate content of fresh vegetables possibly due to the action of endogenous nitrate reductases or bacterial contamination [30]. Peeling of certain vegetables, for example potatoes, can significantly reduce the nitrate content as the highest concentration is found in and just below the skin [30, 31]. Information regarding peeling and storage were not found in the references obtained and thus were not included in this database.

There are several analytical methods for the determination of nitrate in vegetables [32]. The best method for nitrate determination in vegetable samples is considered to be the automated colorimetric method with reduction with cadmium. Other methods include nitrate-selective electrodes, spectrophotometric methods, ion chromatographic methods, polarimetric methods and UV screening methods. All these methods are represented in the database; however information regarding the degree of accuracy using these methods limited.

There is currently no reliable biomarker to assess nitrate intake. Urinary nitrate levels are affected by the L-arginine-NO synthase pathway as well as dietary nitrate intake, with approximately 70% of dietary nitrate consumed excreted in urine within 24 hours of consumption [33] . Urinary nitrate may provide an indication of short term intake but may not be good indicator of long-term nitrate intake because of variability in dietary nitrate intake across days. The 24-HDR captured vegetable nitrate intake in the previous 24 hours and the low positive correlation with urinary nitrate is consistent with levels being affected by different endogenous metabolic pathways. While 24-HDRs are recognised as being a more accurate method of assessing dietary intake for most nutrients, large cohort studies usually rely on FFQs as they are cost-effective, quick and easy to administer and are also more likely to provide better estimates of nutrient intakes over the long term. In this current study we have demonstrated a moderate positive correlation between nitrate intakes calculated from 24 HDRs and 12 month FFQs. This correlation is consistent with other nutrient intake method-comparison studies [34]. We also observed a strong positive correlation between two 4 week FFQs administered 8 weeks apart demonstrating good reproducibility.

CONCLUSION

In conclusion, there is convincing evidence that intake of vegetables, specifically green leafy vegetables, are associated with positive health outcomes. Clinical trials show that nitrate could be a major cardioprotective component of green leafy vegetables. In light of the new evidence of a potential positive association between dietary nitrate and health outcomes and the lingering concern about nitrate and risk of cancer, this comprehensive and up-to-date database of the nitrate content of vegetables presented here will be a timely and invaluable tool for researchers. It allows quantification of dietary nitrate from a comprehensive list of vegetables which can be used to examine the association between nitrate intake and health outcomes in observational studies in which nitrate content is assessed on the basis of recorded food intake and dietary intake recall surveys. These studies are the next critical step in dietary nitrate research and, together with results from randomised controlled clinical trials, could ultimately lead to the development of reference vegetable nitrate intakes that could be included in dietary guidelines.

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Authors' contributions: LCB, JMH and CPB designed research; LCB, SS and CPB conducted research; LCB, RJW and CPB analysed data; LCB and CPB wrote paper; CPB had primary responsibility for final content; LCB, RLP, NCW, KDC, JRL, AD, SS, RJW, JMH and CPB critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Table 1: Summary of full-text articles excluded (n=442)

Reasons for exclusion	Number of articles
Nitrate and/or nitrite concentration was not provided or was unclear	114
Experimental study with no control or a control not used in normal agricultural practice	204
Nitrate and/or nitrite analysis method development paper with no validated reference method as a comparison	22
Nitrate and/or nitrite concentration measured prior to 1980	4
Nitrate and/or nitrite concentration measured during growth of the vegetables prior to harvesting, in an inedible part of the vegetable, in an unknown vegetable or was not assessed in individual vegetables	57
Compiled data or part of a review	41

Table 2: Nitrate content of vegetables (in fresh weight); minimum sample size and number of publications from which the values were derived.[1]

Vegetable	Mean (SD) mg/kg	Median (IQR) mg/kg	Minimum mg/kg	Maximum mg/kg	Minimum sample size	No of publications
Amaranth	1121 (1514)	498 (30-1962)	1	4340	53	7
Arugula	3475 (2448)	3441 (1805-4608)	18	8778	2086	17
Asparagus	84 (68)	83 (18-141)	11	209	329	11
Bean sprouts	467 (1267)	45 (23-99)	11	4410	121	7
Bean, garden	374 (247)	340 (161-493)	51	1145	675	16
Beet	1581 (1171)	1429 (907-1919)	4	7470	1807	34
Bitter melon	390 (199)	380 (231-538)	122	710	37	5
Black salsify	2321 (3903)	92 (43-NA[2])	43	6827	21	3
Broccoli	403 (393)	280 (206-438)	8	1890	507	20
Brussels sprouts	206 (430)	32 (23-65)	7	1400	210	11
Cabbage	59 (678)	427 (218-800)	2	6315	3552	66
Carrot	196 (187)	145 (70-269)	1	1454	4238	59
Cauliflower	291 (405)	171 (87-320)	1	2468	728	29
Celeriac	443 (318)	308 (201-695)	86	1179	486	7
Celery	1871 (3121)	1188 (589-2098)	30	20851	908	30
Chicory	63 (61)	50 (9-NA[2])	9	129	7	3

Chicory, leaves	1041 (812)	615 (532-1721)	485	2529	75	5
Chinese amaranth	2308 (1665)	2000 (1014-3757)	218	4800	44	5
Chinese broccoli	2173 (844)	2220 (1350-2948)	1100	3150	27	3
Chinese cabbage	1619 (2669)	1044 (860-1426)	0	25065	1161	26
Chinese flat cabbage	4518 (1671)	4240 (3004-NA[2])	3004	6310	12	3
Corn	280 (541)	12 (6-822)	5	1091	42	3
Cornsalad	2602 (1499)	2985 (1170-3781)	132	4695	890	8
Cucumber	257 (252)	185 (72-344)	1	1021	1862	38
Eggplant	358 (310)	289 (135-450)	30	1300	435	19
Endive	1054 (671)	907 (551-1416)	35	2660	531	21
Endive, Belgium	429 (692)	106 (49-1133)	40	1465	1064	4
False pak-choi	3186 (785)	2994 (2600-3728)	2100	4505	72	4
Fennel	1305 (841)	1033 (683-1825)	313	3261	156	10
Fenugreek	1369 (2130)	757 (54-1773)	2	6743	39	6
Garden cress	1857 (1272)	2064 (704-2580)	74	4319	42	5
Kale	1748 (1096)	1689 (623-2699)	122	3482	443	11
Kohlrabi	1270 (833)	1130 (734-1769)	130	2919	364	13
Leek	406 (507)	232 (130-492)	0	3110	1173	28
Lettuce						
Total	1851 (1122)	1685 (974-2693)	1	10490	22668	102

Lettuce, Butterhead	1332 (977)	1070 (457-2057)	168	3652	3809	16
Lettuce, Chinese	3947 (3802)	3008 (1300-7064)	1300	10490	111	4
Lettuce, Curly	2039 (1028)	1900 (1185-2720)	630	5085	403	8
Lettuce, Iceberg	943 (505)	945 (681-1078)	31	4130	3099	28
Lettuce, Leaf	1154 (628)	1339 (467-1675)	222	1940	185	9
Lettuce, Lollo Rossa	2153 (929)	2170 (1505-2720)	3	4670	224	8
Lettuce, Oak Leaf	2133 (887)	2132 (1518-2592)	640	6750	591	9
Lettuce, Romaine	1292 (748)	1171 (891-1479)	105	3585	635	15
Malabar spinach	1018 (291)	1045 (728-1280)	641	1340	18	4
Mushroom	30 (28)	22 (8-49)	1	117	233	11
Mustard greens	2596 (959)	2766 (1624-3397)	1100	3787	51	6
New Zealand spinach	1218 (463)	1316 (735-1602)	608	1630	21	3
Okra	137 (116)	110 (40-252)	9	306	20	6
Onion	146 (377)	77 (31-114)	1	3204	795	32
Onion, green	375 (308)	314 (157-410)	95	1326	26	11
Onion, spring	570 (237)	532 (410-833)	210	840	44	5
Pak choi	2489 (1381)	2500 (1536-3695)	130	4500	84	6
Parsnip	152 (125)	83 (59-280)	37	340	31	5
Pea	97 (184)	26 (8-111)	5	660	491	11
Pepper, Bell	839 (2039)	95 (43-201)	2	11240	1004	27

Potato	161 (119)	114 (93-194)	10	1091	5980	60
Potherb mustard	2426 (972)	2678 (1790-3345)	828	3500	77	5
Pumpkin	415 (521)	252 (130-472)	18	2470	159	11
Radicchio	191 (233)	64 (15-380)	9	667	73	5
Radish	1662 (979)	1530 (1100-2190)	0	4653	1786	35
Rhubarb	1098 (1598)	201 (150-NA[2])	150	2943	72	3
Rutabaga	1696 (1436)	1513 (390-2879)	118	4071	16	3
Sea beet	1817 (1012)	2183 (674-NA[2])	674	2595	8	3
Spinach	1926 (1207)	1802 (1140-2535)	1	9379	9547	89
Squash	587 (533)	504 (168-845)	25	2003	345	17
Sweet potato	129 (224)	54 (18-110)	10	717	60	8
Swiss chard	2077 (989)	2275 (1627-2766)	23	3622	1459	13
Taro	391 (252)	329 (188-655)	164	740	34	3
Tomato	388 (902)	60 (25-193)	0	4700	1678	54
Turnip	1707 (2616)	660 (378-1993)	38	11159	494	20
Water spinach	1449 (1013)	1150 (679-2436)	280	3180	59	5
Watercress	2322 (1618)	1748 (1223-3806)	1	4827	101	12
Winter melon	439 (164)	463 (253-594)	230	635	70	3
Yam	303 (188)	359 (106-445)	35	460	16	4

[1] References available in online supplementary material

[2]NA, not available. 75th percentile could not be calculated, only 3 entries available.

Table 3: Nitrate content of herbs and spices (in fresh weight); minimum sample size and number of publications from which the values were derived.[1]

Herb or spice name	Mean (SD) mg/kg	Median (IQR) mg/kg	Minimum mg/kg	Maximum mg/kg	Minimum sample size	No of publications
Basil	1864 (1623)	1596 (726-2893)	56	4695	74	5
Chive	473 (245)	360 (278-266)	270	819	136	3
Cilantro	1931 (1507)	1687 (522-3400)	240	4273	77	10
Dill	1719 (1387)	1121 (572-3178)	54	3911	102	11
Garlic	319 (454)	121 (69-229)	14	1513	116	11
Garlic chive	1618 (1934)	1020 (570-1900)	140	5828	66	4
Ginger root	1300 (900)	1300 (507-2030)	70	2659	39	4
Mint	779 (684)	504 (186-1343)	75	1892	24	7
Parsley	818 (941)	418 (211-1130)	26	4848	584	29

[1] References available in online supplementary material

Table 4: Characteristics of the participants (n=41; males n=24, females n=17)

Characteristic	**Range**	**Mean**	**SD**
Age (years)	39-74	62	9.5
Height (m)	1.5-1.9	1.7	0.1
Weight (kg)	50-112	78	13
Body mass index (kg/m^2)	20-35	27	4
Systolic blood pressure (mm Hg)	121-148	133	8
Diastolic blood pressure (mm Hg)	58-89	77	8
Heart rate (bpm)	44-79	62	7
Total cholesterol (mmol/L)	3.7-8.9	5.5	1.2
HDL cholesterol (mmol/L)	0.8-2.9	1.4	0.4
LDL cholesterol (mmol/L)	1.9-6.4	3.5	1.1
Triglycerides (mmol/L)	0.5-3.2	1.3	0.6
Glucose (mmol/L)	4.6-6.7	5.3	0.5

Figure legends

Fig. 1. Prisma flow chart.

Fig. 2. Median nitrate content (mg/kg FW) of individual vegetables with ≥3 publications presented from highest to lowest.

Fig. 3. Median nitrate content (mg/kg FW) of individual vegetables grouped by family name.

Fig 4: Nitrate content (geometric mean ± 95% CI) of lettuce by growing method. Organic versus conventionally grown lettuce (A) unadjusted and (B) publication ID adjusted. Undercover versus open air grown lettuce (C) unadjusted and (D) publication ID adjusted.

Fig. 5. Nitrate content (geometric mean ± 95% CI) of lettuce by season. (A) unadjusted and (B) publication ID adjusted.

Fig. 6. Nitrate content (geometric mean ± 95% CI) of lettuce by type. (A) unadjusted and (B) publication ID adjusted.

Fig. 7. **(A)** Scatter plot of mean nitrate intake (mg/day) measured by FFQ and 24-HDR showing Pearson's correlation coefficient, **(B)** Bland-Altman plot showing mean difference (bias) and limits of agreement (95% CI's) in mean nitrate intake (mg/day) estimated with the FFQ and the 24-HDR. Lines are (——) bias, (– – –) 95% CI for the bias, limits of agreement (**– – –**) and (– · – ·) 95% CI for the limits of agreement.