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Dietary nitrate consumption and risk of coronary heart disease in women from the Nurses' Health Study

Jacklyn K. Jackson[1], **Geng Zong**[2], **Lesley K. MacDonald-Wicks**[1], **Amanda J. Patterson**[1], **Walter C. Willett**[2,3,4], **Eric B. Rimm**[2,3,4], **JoAnn E. Manson**[3,5], and **Mark A. McEvoy**[6]

[1]Priority Research Centre in Physical Activity and Nutrition, School of Health Sciences, Faculty of Health and Medicine, University of Newcastle, University Drive, Callaghan, New South Wales, 2308, Australia.

[2]Department of Nutrition, Harvard T.H. Chan School of Public Health, 677 Huntington Avenue, Boston, Massachusetts, 02115, United States.

[3]Department of Epidemiology, Harvard T.H. Chan School of Public Health, 677 Huntington Avenue, Boston, Massachusetts, 02115, United States.

[4]Department of Medicine Brigham and Women's Hospital and Harvard Medical SchoolChanning Division of Network Medicine, 75 Francis Street, Boston, Massachusetts, 02115 United States.

[5]Division of Preventive Medicine, Brigham and Women's Hospital and Harvard Medical School, 75 Francis Street, Boston, Massachusetts, 02115, United States.

[6]Centre for Clinical Epidemiology and Biostatistics, Hunter Medical Research Institute, University of Newcastle, Lot 1, Kookaburra Circuit, New Lambton Heights, New South Wales, 2305, Australia.

Abstract

The consumption of nitrate-rich vegetables can acutely lower blood pressure, and improve mediators shown to optimise vascular health. However, we do not yet understand the impact of long-term habitual dietary nitrate intake and its association with cardiovascular disease (CVD). Therefore the aim of this investigation was to examine the relationship between habitual dietary nitrate intakes and risk of coronary heart disease (CHD) in women from the Nurses' Health Study. We prospectively followed 62,535 women who were free from diabetes, CVD and cancer at baseline in 1986. Information on diet was updated every 4 years with validated food frequency questionnaires. The main outcome was CHD, defined by the occurrence of non-fatal myocardial infarction or fatal CHD. Cox proportional hazard regression models were used to estimate relative risks (RR) and 95% confidence intervals (CI). During 26 years of follow-up, 2257 cases of CHD

Corresponding author and contract details: Mark A. McEvoy, Centre for Clinical Epidemiology and Biostatistics, Hunter Medial Research Institute, University of Newcastle, Lot 1, Kookaburra Circuit, New Lambton Heights, New South Wales, 2305, Australia. Mark.McEvoy@newcaastle.edu.au Phone: +61 2 4042 0518.

Authorship

All authors contributed to the formulation of the research question and design of the study. J.K.J and G.Z carried out the analysis. J.K.J was principally responsible for the drafting of the written manuscript. All authors contributing to the writing and approving the final manuscript.

Conflict of Interest

None

were identified. When comparing the highest quintile of nitrate intake with the lowest quintile, in aged-adjusted analysis there was a protective association for CHD [RR=0.77, (95% CI: 0.68, 0.97); p=0.0002] which dissipated after further adjustment for smoking, physical activity, body mass index and race [RR= 0.91; (95% CI: 0.80, 1.04); P=0.27]. This magnitude of association was further attenuated once we adjusted for the Alternative Healthy Eating Index excluding vegetable and fruit consumption [RR: 1.04 (95% CI: 0.91, 1.20; P=0.34)]. Dietary nitrate intake was not related to risk of CHD after adjustment for other lifestyle and non-vegetable dietary factors in a large group of US women.

Keywords

Dietary Nitrate; Coronary Heart Disease; Myocardial Infarction; Women

Introduction:

Cardiovascular disease (CVD) is the leading cause of death worldwide, with more women than men dying each year due to CVD[1]. In the United States, CVD accounts for almost a third of female deaths, and an estimated 390,000 women develop new or recurrent myocardial infarction (MI) and/or coronary heart disease (CHD) annually[2, 3]. Despite these alarming trends, CVD is largely preventable with lifestyle modifications, including the consumption of a healthy diet[4].

It is clear that the best treatment for CVD is prevention, and diets containing greater quantities of vegetables appear effective at reducing the risk of CVD[5]. Previous findings from the Nurses' Health Study (NHS) indicate that green leafy vegetable consumption was significantly associated with a lower risk of CHD [RR: 0.78 (95% CI: 0.69, 0.88), P=0.0004][6]. Notably, green leafy vegetables (and root vegetables) are one of the richest sources of dietary nitrate, and account for approximately 60–80% of daily intake[7,8]. This may be physiologically important because dietary nitrate can be metabolised in the body via the enterosalivary nitrate-nitrite-nitric oxide (NO) pathway to produce cardio-protective NO[7,9].

NO is a major signalling molecule within the cardiovascular system, and has a primary role as a vascular vasodilator[10–12]. In addition, NO can facilitate many other anti-atherosclerotic functions by preventing blood clot formation, inflammation and by promoting the formation of new blood vessels[13, 14]. Therefore, reductions in NO bioavailability in humans have been implicated in the pathophysiology of CVD[15].

While hypertension has long been a major public health target for reducing CVD events, it is increasingly clear that additional CVD risk factors must be targeted concurrently[16, 17]. In a four week randomised controlled trial of 68 hypertensive patients, Kapil et al. found nitrate rich beetroot juice significantly reduced resting blood pressure [Systolic: −7.7 mmHg; Diastolic: −2.4 mmHg], and improved other mediators of vascular health as evidenced by improved endothelial function and reduced arterial stiffness, compared with a placebo group[18]. These findings are consistent with a recent systematic review and meta-analysis of randomised controlled trials, indicating an acute consumption of nitrate led to significantly lower blood pressure [systolic: −4.8 mmHg; diastolic: −1.7 mmHg], improved endothelial function, reduced arterial stiffness and reduced platelet aggregation[19]. These observed vascular improvements if sustained are of a clinically significant magnitude, estimated to reduce the incidence of deaths from CHD by approximately 9%, and CVD risk by 10%[20]. These findings suggest that dietary nitrate, as an effective NO donor in humans, may reduce major CVD risk factors and the development of atherosclerosis, MI and CHD[19].

Recent observational evidence indicates that higher long-term habitual nitrate intakes are associated with a lower risk of CVD mortality [21, 22]. However to the authors' knowledge no previous study has assessed the long-term impact of dietary nitrate intakes in relation to the risk of CHD specifically. Therefore, the aim of the present study was to examine whether higher usual dietary nitrate intakes in the NHS cohort are associated with reduced risk of CHD over 26-years of follow-up.

Subjects and Methods:

Study population

The NHS began in 1976, and included 121,700 US registered nurses[23]. Participants were female registered nurses aged 30–55 years old, from 11 US states. The NHS participants completed a baseline questionnaire that included assessment of lifestyle and medical history. Follow-up questionnaires have been sent biennially to participants to collect updated health, lifestyle and disease information; dietary data were first collected in 1980. A follow-up rate of approximately 90% was achieved in most follow-up cycles[24].

For the current investigation, the baseline year was 1986 as this included a more comprehensive semi-quantitative food frequency questionnaire (FFQ) than was used in 1980. We excluded participants with missing baseline age information (n=48), if responses were only to the 1986 questionnaire (n=700), if death occurred before baseline (n=5), and if there was a diagnosis with diabetes (n=2892), CVD (n=3351) and cancer (n=4464) at baseline. Participants with invalid (<600 or >3500 kcal/d) responses on the 1986 FFQ, or missing dietary nitrate data were also excluded (n=1525). Thus, the baseline population consisted of 62,535 women (Supplementary Figure 1). The study was approved by the Human Research Committee of the Brigham Women's Hospital. Informed consent was implied upon the return of a completed questionnaire.

Ascertainment of diet and dietary nitrate exposure

A validated semi-quantitative FFQ was used to collect dietary data in 1986 and subsequently every 4 years after. The FFQ asked participants to report their usual daily intakes (never to ≥6 times/d) of a standard portion size (e.g. ½ cup cooked vegetables, 1 cup raw vegetables) for 126 food items. The reproducibility and validity of the FFQ responses were previously evaluated in a subset of NHS participants (n=127) using 7-day food records. The validation study found the FFQ correlated reasonably well with dietary record values, the correlation coefficients for all vegetables was 0.46, and ranged from 0.25 for kale, mustard, or chard greens, to 0.73 for lettuce[25–27]. The validity of this questionnaire has been recently confirmed in a larger sample using both diet record and multiple biomarkers of diet[28]. Responses from the FFQ were converted into average daily intakes by combining frequency information with nitrate content information obtained from the updated US Department of Agriculture food composition tables[29].

Serum and urinary nitrate levels are affected by both dietary nitrate and endogenous nitric oxide production, additionally nitrate has a short half-life (~5–8 hours), therefore currently there is no reliable biomarker to assess long-term dietary nitrate intakes[30]. However, experimental trials have demonstrated high nitrate intakes lead to higher urinary nitrate concentrations[31]. Therefore, urinary nitrate levels have been used to evaluate the validity of capturing nitrate intakes assessed by FFQ. This validation study was conducted in 59 individuals who responded to a FFQ[32]. The correlation coefficient between dietary nitrate intake reported from the FFQ and urinary nitrate intake was 0.59, after adjustment for within-person variation (sex, gender and body mass)[32]. This relatively high correlation, corresponds with the findings of similar validation studies and indicates that the FFQ was reasonably accurate at capturing nitrate intakes[32].

Intakes of dietary factors were evaluated using updated cumulatively averaged intakes, by averaging all available information up to the start of a 2-year follow-up interval (e.g. the average of the 1986, 1990 and 1994 values were used for the 1994 to 1996 follow-up period, and so on), which better represents long-term habitual intakes and reduces random error[33].

The Alternative Healthy Eating Index (AHEI) is a diet quality score based on specific foods and nutrients, in which a higher score has consistently been associated with lower risk of chronic diseases in clinical and epidemiologic investigations[34]. Specifically, a higher AHEI score reflects higher intakes of vegetables, fruit, wholegrains, nuts, unsaturated fatty acids and lower intakes of sugar-sweetened beverages, red/processed meat and sodium, while moderate alcohol is positively ranked compared with no or heavy alcohol consumption[34]. All AHEI components were scored from 0 (worst) to 10 (best), and the total AHEI score ranged from 0 (non-adherence) to 110 (perfect adherence).

Ascertainment of coronary heart disease

The primary endpoint for this study was incidence of CHD, defined by occurrence of non-fatal MI or fatal CHD after the return of the 1986 FFQ and before June 1, 2012. Participants (or next of kin if deceased) reporting a primary endpoint were asked for permission to access their medical records to validate the reported event. Records were reviewed by physicians who were blinded to the participant’s risk factor status. Deaths were identified by reports from the next of kin, the US postal system, or by using certificates obtained from state vital statistics departments or the National Death Index. Fatal CHD was categorized as “definite” only if confirmed by hospital records or autopsy report, or if CHD was listed as the cause of death on the death certificate and there was evidence of previous coronary disease. If no medical records were available, persons in whom CHD was the underlying cause on the death certificate were categorized as “probable” cases.

Statistical Analysis:

Each participant contributed person-time of follow-up from the date of return of the baseline questionnaire to the date of CHD diagnosis, death, last return of a validated questionnaire, or end of analysis follow-up, whichever came first. Each participant contributed only one endpoint and the cohort at risk of each 2-year follow-up period included only those who remained free from CHD at the beginning of each follow-up period.

For analyses of nitrate intake, participants were divided into quintiles for cumulatively updated daily dietary nitrate consumption, with the lowest quintile representing the reference category. Median values of dietary nitrate intake for each quintile were used to test for a linear trend across the quintiles.

Cox proportional hazards models with time-varying covariates with age in months as the time scale were used for all analyses using SAS 9.4 statistical software (SAS Institute, Inc., Cary, North Carolina), to estimate relative risks (RRs) and corresponding 95% confidence intervals (CIs). Statistical significance was defined as $P<0.05$.

In our multivariable models a variety of factors were considered, based on the criteria outlined by Jager et al[35]. For example, to prevent over adjustment, confounding variables must: (1) have an association with the disease, in that, it should be a risk factor for the disease; (2) it must be associated with the exposure, i.e., it must be unequally distributed between exposure groups; and (3) it must not be an effect of the exposure[35]. Vegetable and fruit are the two major sources of dietary nitrate (contributing to approximately 80–90% of total nitrate intakes), indicating that vegetable and fruit intakes principally influence nitrate intakes. For this reason the AHEI without the vegetable and fruit component score was used in our multivariable models, to prevent risk of over adjustment. Our models also considered a variety of potential covariates. Covariates were self-reported data updated biennially from baseline including: smoking status (never, past, or current 1–14, 14–25, or ≥25 cigarettes/d), physical activity level (Metabolic equivalents per week (MET's/wk))[36], race (White, Hispanic, Asian, African) and body mass index (BMI) (calculated as weight in kilograms divided by height in metres squared (kg/m^2)[37] (Table 3). In a sensitivity analysis, we also adjusted our multivariable models for hypertension, high cholesterol, diabetes and family history of MI (self-reported physician diagnosis of disease, collected biennially). Further, stratified analyses were conducted to explore effect modification based on smoking status (smoker vs non-smoker), activity levels above or below the median (<18 METs/wk vs ≥18 METs/wk), alcohol consumption (consumer vs non-consumer), and obese BMI ($BMI<30kg/m^2$ vs $BMI\geq30kg/m^2$).

Results

During 1,473,035 person-years of follow-up (n=62,535), 2267 incident cases of CHD were identified.

At baseline, highest consumers of dietary nitrate also consumed more total fruit and vegetables, and therefore were more likely to have higher AHEI scores. Highest consumers were also more likely to use multivitamin supplements, have greater intakes of antioxidants (vitamin C, vitamin E), exercise more, and were less likely to smoke (Table 1).

The overall mean (SD) intake of nitrate was 152 (75) mg/d. Lettuce (Iceberg and Romaine) was the primary dietary source of nitrate intakes, followed by other green leafy vegetables including spinach, celery and broccoli (Table 2). Nitrate intakes were significantly associated with the AHEI scores (r=0.54, p<0.01), but this association was weakened after excluding the vegetable and fruit components from the score (r=0.38, p<0.01), as vegetables and fruit are the primary sources of dietary nitrate.

Compared with quintile 1 (lowest dietary nitrate (median: 63.5mg/d)), the age-adjusted RR of CHD for quintile 5 (highest dietary nitrate (273 mg/d)) was 0.77 [(95% CI, 0.68, 0.88) (p for trend; 0.0002)]. This association was no longer significant once adjusted for race, smoking, BMI and activity levels (Model 1) [RR comparing extreme quintiles: 0.91 (0.80, 1.04), p for trend; 0.27]. Findings were further attenuated once the AHEI score, without vegetable and fruit components was added to the model (Model 2), and adjusted for CVD risk factors including hypertension, high cholesterol, diabetes and family history of MI (Model 3) (Table 3).

Stratified analysis identified a possible interaction with alcohol and BMI (p<0.2), but not smoking or activity levels (Supplementary Table 1).

Discussion

In this prospective cohort study of mid-life and older women with 26 years of follow-up, we observed that a higher long-term intake of dietary nitrate (equivalent to approximately 2 servings of green leafy vegetables/d) was not associated with a lower risk of CHD, once adjusted for lifestyle factors including smoking status, race, BMI, physical activity and overall diet quality (AHEI). These findings were unexpected given that previous analysis of the NHS cohort, discovered a significant 30% risk reduction for CHD in women consuming on average 1.5 serves of green leafy vegetables per day[(6)]. However it is important to recognise that green leafy vegetables contain a variety of beneficial components required for vascular health including, fibre, folate and potassium, and that other foods including potatoes and to a lesser extent processed meats, can also contribute to total dietary nitrate intakes[(38–40)].

Few studies have prospectively investigated the relationship between dietary nitrate and cardiovascular related outcomes such as CHD and MI. To our knowledge, the only previous investigations of this relationship has been conducted in much smaller (n=1226–2229) Australian based cohorts, with shorter follow-up periods [(21, 22, 41)]. Blekkenhorst et al[(21)]. were the first to investigate prospectively the association of dietary nitrate (measured only at baseline) with Atherosclerotic Vascular Disease (ASVD) mortality including ischemic heart disease, heart failure, cerebrovascular disease (excluding haemorrhagic stroke) and peripheral arterial disease, in 1226 Australian women aged 70–85 years, followed up for 15 years. Finding from Blekkenhorst et al[(21)]. indicated that participants in the highest (>76.4 mg/d) compared with the lowest (<52.7 mg/d) tertile of total vegetable nitrate intake had a lower risk of ASVD mortality [HR: 0.79 (95% CI, 0.68, 0.93), p=0.004]. However, similar to our findings this association was attenuated following adjustment for a Nutrient-Rich Foods Index [HR: 0.85 (95% CI, 0.72, 1.01), p=0.072][(21)]. Within the same cohort Bondonno et al[(41)]. found that for every 1 standard deviation (31 mg/d) higher intake of total nitrate, there was an associated 18% lower risk of 14.5 year ischemic cerebrovascular disease events (p=0.017). More recently, Liu et al[(22)]. prospectively investigated the association of nitrate intake with CVD mortality in a sample of 2229 Australian men and women aged ≥49 years, followed up for 14 years. To which participants consuming the highest (>137.8 mg/d) compared with the lowest (<69.5 mg/d) quartile for vegetable nitrate intake were observed to be at a 37% lower hazards of CVD mortality [HR: 0.63 (95% CI: 0.41–0.95)][22].

In light of these previously published findings, it is perplexing to note that despite observing a similar trend to Blekkenhorst et al[21]. in the age-adjusted model, the fully adjusted model was not statistically significant. However, important differences exists between these studies which may account for this finding. First, different methods were used to estimate dietary nitrate intakes. The previous Australian based studies have drawn upon published nitrate databases to calculate intakes, as nitrate is not included as part of National food composition tables in Australia. This study on the other hand calculated nitrate intakes using the updated US Department of Agriculture food composition tables, which is most appropriate given that this study has been conducted in an American population. In light of these differences, it is not surprising that the nitrate intakes in the NHS have been estimated at levels markedly higher (mean nitrate intake: 152 mg/d) than those reported in the Australian based cohorts (Blekkenhorst et al[21]. mean nitrate: 80mg/d; Liu et al[22]. mean nitrate: 130mg/d). However, our mean nitrate intake estimations are similar to those previously reported in the NHS by Kang et al[42]. (mean nitrate intake: 142mg/d), in which higher dietary nitrate intakes were associated with a lower primary open angle glaucoma risk. In addition, experimental data indicates that dietary nitrate intakes of at least 130 mg/d are enough to exert cardiovascular benefits, including reductions in blood pressure and improved vascular function[19]. Second, the cardiovascular disease outcomes differed between studies. This study defined CHD as those with fatal and non-fatal CHD, while the Australian studies by Blekkenhorst et al[21]. and Liu et al[22]. focused on CVD mortality. This may account for the different findings, however it is important to highlight that the aim of this study was to determine if the development of CHD, as a culmination of atherosclerosis, and not just survival following the development of the disease (or a disease-related event) was associated with dietary nitrate intakes. This is important from the view point of prevention since dietary nitrate intakes targeted to prevent the development of CHD may be important.

Previously the AHEI score was shown to strongly predict the risk of CHD within women from the NHS[34]. Additionally, women in this cohort with a higher AHEI tended to be higher consumers of dietary nitrate (r_s=0.54, p<0.001), however this correlation was weakened when we considered the AHEI excluding vegetable and fruit component scores (r_s=0.38, p<0.001), as vegetables, followed by fruit are the primary dietary sources of nitrate, an association which is consistent with international cohorts[43]. Particular components including carotenoids, vitamin C, fibre, polyphenols, magnesium and potassium have been identified as important components of vegetables and fruit responsible for these beneficial effects[6]. Although long term dietary nitrate intakes may not yet have a clear independent effect, future studies must attempt to clearly identify if long term dietary nitrate could contribute to the apparent cardio-protective benefits of vegetable consumption, given that acute intakes of dietary nitrate have consistently shown beneficial effects for minimising CVD risk factors in small human clinical trials[19].

It is worth noting however, in a stratified meta-analysis conducted by Jackson et al[19]., a high nitrate diet (rich in green leafy vegetables) was found to influence CVD risk factors including blood pressure [systolic: −2.4 mmHg; p=0.2; diastolic: −0.6 mmHg, p=0.5] and endothelial function [flow mediated dilatation: +0.5%, p=0.01) to a lesser extent than those observed with beetroot juice [systolic blood pressure: −5.7 mmHg; p<0.0001; diastolic blood pressure: −2.4 mmHg; p<0.0001; flow mediated dilatation: +0.8%, p<0.0001]. These finding indicate that other dietary components may be responsible for dampening the beneficial effects of dietary nitrate. This notion was recently supported by findings from Dewhurst-Trigg et al[44]. that in high to moderate nitrate containing vegetables including cabbage and broccoli, the cardiovascular benefits of nitrate were completely blocked in the co-presence of thiocyanate, which is thought to block the enterosalivary metabolism of dietary nitrate to NO via the nitrate-nitrite-NO pathway. Thus it is possible such mechanisms are at play in our cohort.

On the other hand, dietary nitrate is thought to produce greater bioactive effects when consumed within the context of a healthful diet (including fruits, vegetables, nuts and fish) as antioxidants, polyphenols and polyunsaturated fatty acids interact with nitrate to enhance the potency of its effects[45,46]. For example, vitamin C and polyphenols are abundant in a vegetable rich diet, and their combination with nitrate has been shown to favour the formation of NO via the nitrate-nitrite-NO pathway and even prolong the half-life of NO in the stomach[47, 48]. More recently, it was suggested that nitric oxides and unsaturated fatty acids (e.g. oleic, linoleic and arachidonic acid) from extra-virgin olive oil, nuts, fatty fish and lean meat can react in the stomach to produce nitroalkenes[49]. Nitroalkenes are potent cardio protective mediators, largely because they can stimulate enzymatic NO production, which is important for preventing hypertension, atherosclerosis, blood clotting and inflammation[50].

The strengths of our study include the large sample size, long follow-up period and presence of updated dietary and covariate data. However, a limitation of this study is its observational nature, and we therefore cannot exclude the possibility that findings are the result of residual confounding. However we have controlled for many known and potential risk factors related to the development of CHD in a prospective manner. Additionally, long-term prospective cohort studies are the strongest observational study design, as their prospective nature makes them less prone to biases, including reverse causation, recall bias, and selection bias, common to retrospective or cross-sectional studies[51].

In our study, participants who consumed greater quantities of dietary nitrate were also more likely to have a higher AHEI, be higher consumers of vegetables and have healthier lifestyles (e.g more physical activity, less likely to smoke cigarettes). Additionally, due to the complex nature of diet, self-reported dietary assessment methods including FFQs are prone to random and systematic error. This systematic error is likely to bias results towards the null, therefore it is possible that the association we reported is underestimating the true effect. However, FFQs are useful for assessing usual intakes over long periods of time, and the use of FFQs in this study has been validated against multiple diet records[32, 51]. In addition, the use of repeated measures of diets to calculate cumulative averages would minimise any potential random measurement error caused by within-person variation and accommodates for diet changes over time. Further, many other dietary variables assessed by FFQ have predicted higher or lower risks of CHD in this cohort[52].

Similar to other dietary constituents, external factors including farming practices, seasonal changes, storage conditions, transportation, processing and cooking practices are known to dramatically influence the nitrate content of fruits and vegetables, and therefore accurate and reliable estimation of dietary nitrate is difficult[46]. In particular, in the past there has been increasing pressures placed on farmers to alter cultivating practices in order to lower the nitrate content of vegetables (e.g. apply fewer nitrogen based fertilizers) due concerns of possible health risks[53]. This is despite the European Food Safety Authority concluding that the estimated exposure to nitrate from vegetables is unlikely to result in appreciable health risks[54]. However, it may be possible that such pressures have reduced dietary nitrate exposures from vegetables over time. On the other hand, it appears that the intakes of nitrate rich green leafy vegetables have increased over time, with US data indicating that fresh spinach consumption has increased from 0.3 kg/person in 1995 to 1.0 kg/person in 2005, likely driven by the increased availability of convenient, prewashed and pre-packaged spinach[55]. Thus it is possible that changes in food environments and availability, have driven higher dietary nitrate intakes over time.

As previously discussed, nitrate intakes estimated using databases could have led to measurement error, thus limiting our findings. However, nitrate intakes captured from FFQ were previously found to be highly correlated with urinary nitrate, indicating that the average nitrate content of food does not vary substantially among individuals[32]. In fact, Ahluwalia et al. suggests dietary nitrate databases used in epidemiological research could be improved if “local” estimates of nitrate contents of vegetables were considered[56], however such specific databases do not currently exist. In addition, it is possible dietary nitrate intakes have been underestimated in our analysis, given that water nitrate intakes were not considered because of the limitation that the nitrate content of the water supply can vary dramatically by geographical region, and the NHS includes thousands of participants from across 11 US states[57]. Although nitrate is thought to be particularly high in well water sources in rural areas this represents very few participants, and even bottled water is yet to have specific legislation around specifying acceptable water nitrate levels. Thus any attempt to estimate water nitrate would be based on too many assumptions to be considered reliable. In addition, the omission of water nitrate from the analysis is consistent with the methodology of previously published literature [21, 22, 42].

Participants in the NHS were mostly non-Hispanic white women, and may not be representative of the general population. For example, mean fruit and vegetable intake in our population (5.7 servings/d) was much higher than national estimates (3.0 serving/d)[58], and despite the average caloric intake of our cohort matching US female caloric intakes, the average BMI of the NHS is approximately 25.1 kg/m^2, which is lower than that of the average US female population (BMI: 26.5 kg/m^2)[59]. Also, the overall prevalence of CHD in our sample was only 3.5%, which is significantly lower than the US average for middle-aged women (approximately 5.8%) [1]. Therefore results need to be interpreted within this context, and they provide support for the need to explore this relationship in other cohorts, including males and more diverse populations.

Conclusion

In this large prospective study of US women, we found that higher habitual intakes of dietary nitrate were not associated with a lower risk of developing CHD, after accounting for established lifestyle and non-vegetable/fruit dietary factors. Although nitrate has been shown to have a short term beneficial effect on CVD risk factors, we did not identify a clear independent long term effect of dietary nitrate for CHD prevention. For future investigations it will be important to further understand the role of dietary nitrate as a component of vegetables and overall diet quality. Dietary nitrate may represent an important component of vegetables, however continued research in diverse cohorts including males are required to understand whether findings are generalizable across populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1:

Age-standardized Baseline Characteristics in the NHS (1986) by Quintile of Nitrate Intake

Characteristic	Quintile 1 (N=12519)		Quintile 2 (N=12562)		Quintile 3 (N=12502)		Quintile 4 (N=12459)		Quintile 5 (N=12493)	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Age, years	51.0	7.3	51.5	7.1	52.2	7.1	52.8	7.0	53.5	6.9
BMI, kg/m^2	25.2	4.9	25.2	4.6	25.1	4.6	25.1	4.5	25.0	4.4
Physical Activity, METs/wk	10.2	16.1	12.2	18.3	14.1	20.2	15.7	21.3	19.1	24.9
AHEI *	43.0	9.5	47.2	9.3	50.2	9.4	53.9	9.6	58.5	9.8
Age-adjusted Characteristics	%		%		%		%		%	
White Caucasian	98		99		98		98		97	
Family history of MI	37		38		37		38		38	
Diagnosis of Hypertension †	22		22		23		22		24	
Diagnosis of Hypercholesterolemia †	10		11		11		11		12	
Current Smoker	27		21		20		19		18	
Multi-vitamin user	38		41		42		43		46	
Current Aspirin user	63		65		65		65		63	
Any Postmenopausal Hormone use	25		25		27		28		28	
Dietary Intake	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Caloric intake, kcal/d	1725	537	1792	526	1824	522	1768	502	1729	537
Alcohol intake, g/d	5.8	11.8	6.3	11.1	6.6	10.8	6.5	9.9	6.5	10.1
Median nitrate intake, mg/d ‡	63.5	15.7	102	9.2	134	9.5	171	12.9	273	105
Vitamin C intake, mg/d	271	334	307	342	329	348	368	378	444	428
Vitamin E intake, mg/d	34.2	74.1	37.3	75.7	38.8	75.5	43.5	81.3	50.2	89.7
Folate intake, mg/d	337	211	370	208	390	201	422	213	498	242
Crude fibre intake, g/d	3.9	1.2	4.6	1.2	5.1	1.3	5.6	1.4	6.7	1.8
Fruit and vegetable intake, serving/d	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Total Fruit and Vegetable Intake	3.5	1.7	4.8	1.8	5.6	2.0	6.4	2.2	8.1	2.9
Total Fruit Intake	1.9	1.3	2.3	1.4	2.6	1.4	2.7	1.5	3.0	1.7
Total Vegetable Intake	1.6	0.7	2.4	0.8	3.1	0.9	3.7	1.1	5.1	1.8
Total Green Leafy Vegetable Intake §	0.4	0.2	0.7	0.3	0.9	0.3	1.1	0.4	1.7	0.7

Abbreviations: SD, Standard Deviation; BMI, Body Mass Index; METs, Metabolic Equivalents; AHEI, Alternative Healthy Eating Index; MI, Myocardial Infarction

*Alternative Healthy Eating Index, represents a total dietary score. Lowest=0, Highest=110;

†Self-reported diagnosis;

‡Values are the median dietary nitrate intakes for each quintile;

§Total green leafy vegetables includes: cabbage, spinach (cooked and raw), kale and lettuce (romaine and iceberg)

Table 2.

Top 10 food sources of dietary nitrate in the study population [*]

	Contribution of total dietary Nitrate intake (%) [†]		
Rank	**Food Source**	**Mean**	**SD**
1	Iceberg Lettuce	26.5	3.9
2	Romaine Lettuce	19.2	7.9
3	Cooked Spinach	6.5	0.3
4	Celery	6.1	0.7
5	Broccoli	5.7	0.5
6	Kale	3.5	1.7
7	Raw Spinach	3.0	0.6
8	Potato	3.0	1.0
9	Tomato Sauce	2.6	1.4
10	String Beans	1.9	0.2

Abbreviation: SD, Standard Deviation

[*]Average contribution to total dietary nitrate from 1986–2010 FFQ.

[†]Total % dietary nitrate contribution of the food listed: 78%

Table 3.

Risk of Coronary Heart Disease by Quintiles of Nitrate Intake in the NHS (1986–2012) *

Variable	Quintile 1 (<86 mg/d)	Quintile 2 (86–117 mg/d)	Quintile 3 (118–150 mg/d)	Quintile 4 (151–195 mg/d)	Quintile 5 (>195 mg/d)	P-value for trend
Number of cases	493	440	419	465	450	…
Person-years	293,249	294,919	295,097	295,112	294,658	…
RR (95% CI)						
Age-adjusted	1 [Reference]	0.88 (0.77,1.00)	0.78 (0.69,0.89)	0.83 (0.73,0.94)	0.77 (0.68,0.87)	0.0002
†**Model 1**	1 [Reference]	0.95 (0.84,1.09)	0.89 (0.79, 1.02)	0.97 (0.85,1.10)	0.91 (0.80,1.04)	0.27
‡**Model 2**	1 [Reference]	0.99 (0.87,1.13)	0.95 (0.83,1.08)	1.06 (0.93,1.21)	1.04 (0.91,1.20)	0.35
§**Model 3**	1 [Reference]	0.98 (0.98,1.12)	0.94 (0.83,1.08)	1.05 (0.92,1.19)	1.02 (0.89,1.17)	0.55

Abbreviations: ellipses, data no applicable; RR, relative risk; CI, Confidence Intervals

*Intake calculated using cumulative average (average of all available intake data from FFQ completed before each 2-year period at risk)

†Model 1: Multivariable analyses stratified by age in months and period at risk, and adjusted for smoking status, race, body mass index and physical activity.

‡Model 2: Model 1, adjusted for the Alternative Healthy Eating Index, excluding vegetables and fruit.

§Model 3: Model 2, adjusted for hypertension, high cholesterol, diabetes and family history of myocardial infarction.