2020

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10.1007/s00394-019-01902-z

This is a post-peer-review, pre-copyedit version of an article published in the European Journal of Nutrition. The final authenticated version is available online at: http://dx.doi.org/10.1007/s00394-019-01902-z

Blekkenhorst, L.C., Lewis, J.R., Bondonno, C.P., Sim, M., Devine, A., Zhu, K. (2019). Vegetable diversity in relation with subclinical atherosclerosis and 15-year atherosclerotic vascular disease deaths in older adult women. *European Journal of Nutrition*, *59*, 217–230 doi: 10.1007/s00394-019-01902-z

Vegetable diversity in relation with subclinical atherosclerosis and 15-year atherosclerotic vascular disease deaths in older adult women

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Disclaimers: None.

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Sources of support: The Perth Longitudinal Study of Ageing in Women (PLSAW) study was funded by Healthway the Western Australian Health Promotion Foundation and by project grants 254627, 303169 and 572604 from the National Health and Medical Research Council (NHMRC) of Australia. The analysis was supported by an NHMRC of Australia

project grant 1084922. NHMRC of Australia Fellowships supported the salaries of JRL and JMH. None of these funding agencies had any role in the conduct of the study; collection, management, analysis or interpretation of the data; or the preparation, review or approval of the manuscript.

Short running head: Vegetable diversity and atherosclerotic disease.

Abbreviations: ASVD, atherosclerotic vascular disease; CCA-IMT, common carotid artery intima-media thickness; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; FRS, Framingham risk scores; HDL, high-density lipoprotein; ICD, International Classification of Diseases; ICPC-Plus, International Classification of Primary Care-Plus; LDL, low-density lipoprotein; NRVs, Nutrient Reference Values; PLSAW, Perth Longitudinal Study of Aging in Women; WADLS, Western Australian Data Linkage System (WADLS).

Clinical trial registry: The Perth Longitudinal Study of Aging in Women (PLSAW) trial registration ID is ACTRN12617000640303. This study was retrospectively registered on the Australian New Zealand Clinical Trials Registry at www.anzctr.org.au.

ABSTRACT

Purpose: Increasing vegetable intake and diversity are recommended to maintain better health. Evidence for the health benefits of vegetable diversity, separate from total intake, is scarce. We aimed to investigate the associations of vegetable diversity with subclinical measures of atherosclerosis and atherosclerotic vascular disease (ASVD) mortality.

Methods: Vegetable diversity was assessed within a validated food frequency questionnaire using a single question, 'How many different vegetables do you usually consume each day (<1 to ≥6 per day)'. Cox proportional hazards modelling was used to examine the association between vegetable diversity and ASVD mortality in 1,226 women aged ≥70 years without clinical ASVD or diabetes mellitus at baseline (1998). In 2001, B-mode ultrasonography was used to measure common carotid artery intima-media thickness (CCA-IMT) (n=954) and carotid plaque severity (n=968).

Results: Over 15 years (15,947 person-years) of follow-up, 238 ASVD-related deaths were recorded. For each additional different vegetable consumed per day there was a 17% lower hazard for ASVD mortality (HR=0.83, 95%CI=0.78, 0.93, P=0.001); a 1.7% lower mean CCA-IMT (B ± SE: -0.013 ± 0.004, P<0.001); and a 1.8% lower maximum CCA-IMT (B ± SE: -0.017 ± 0.004, P<0.001). Further adjustment for total vegetable intake attenuated the association between vegetable diversity and ASVD mortality (P=0.114), but not CCA-IMT (P=0.024). No association was observed between vegetable diversity and carotid plaque severity (P>0.05).

Conclusions: Vegetable diversity may contribute to benefits in lowering risk of ASVD in older women. The reduction in risk is partly explained by increased total vegetable consumption.

Keywords: cardiovascular diseases, atherosclerosis, mortality, vegetables, diversity, older women

INTRODUCTION

Atherosclerotic vascular diseases (ASVD), such as ischaemic heart disease and cerebrovascular disease, are the leading causes of morbidity and mortality worldwide [1]. Higher intakes of vegetables are consistently associated with a lower risk of atherosclerotic-related diseases [2-5]. This evidence has contributed to dietary recommendations around the world to increase vegetable intake for the prevention of chronic diseases. Despite widespread and long-running health promotion campaigns, vegetable intake amongst the population is still suboptimal. Efforts to increase vegetable intake are urgently required.

Vegetables contain a wide range of vitamins, minerals, fibres and phytochemicals that contribute to vascular health [6]. In particular, meta-analyses have demonstrated that fibre [7], magnesium [8], potassium [9], and flavonoids [10] are associated with a reduced risk of vascular diseases. Furthermore, available data suggest vitamin K [11], vitamin C [12], nitrate [13,14], and organosulphur compounds [15] may also have vascular health benefits. These protective components are found in various concentrations in different vegetables. This is the basis for dietary recommendations to increase diversity of vegetables in the diet. However, few studies have investigated the direct relationship between vegetable diversity and health outcomes.

Overall diet diversity has been shown to be associated with better health outcomes [16-18]. However, greater diet diversity does not necessarily equate to greater diet quality [19]. For example, greater diversity of unhealthy foods will compromise diet quality. Increasing total amount and diversity of vegetable intake are both considered integral components to diet quality. Previously epidemiological studies have shown inverse associations between vegetable diversity and cancer incidence [20] and inflammation [21]. However, there is limited evidence for ASVD-related outcomes, especially in older female populations [22,23]. Therefore, this study aimed to investigate the association of vegetable diversity in the diet of older women, with risk of ASVD mortality over 15 years of follow-up. We also investigated the association of vegetable diversity with subclinical measures of atherosclerosis in a subgroup of this population.

SUBJECTS AND METHODS

Ethics

The Perth Longitudinal Study of Aging in Women (PLSAW) was approved by the Human Ethics Committee of The University of Western Australia. Written informed consent was obtained from each participant. Human ethics approval for the use of linked data were provided by the Human Research Ethics Committee of the Western Australian Department of Health (project #2009/24).

Study population

This study consisted of participants within a 15-year prospective population-based cohort study (PLSAW) and has been described elsewhere [24]. Participants (n=1,500) were women aged 70-85 years at baseline (1998) living in Perth, Western Australia. Missing dietary data and exclusion of implausible energy intakes (<2,100kJ [500kcal] or >14,700kJ [3,500kcal]) resulted in 1,468 (97.9%) women being included for the assessment of baseline prevalence of ASVD and diabetes mellitus. Women with prevalent ASVD (n=152), diabetes mellitus (n=69) or both (n=21) at baseline were excluded, resulting 1,226 (81.7%) of participants being included for the ASVD mortality analysis. Of these women, carotid plaque was assessed in 968 (79.0%) women and common carotid artery intima-media thickness (CCA-IMT) was assessed in 954 (77.8%) women in 2001 (**Figure 1**). Baseline prevalence of ASVD and diabetes mellitus were *a prior* exclusion criteria as clinical diagnosis may have resulted in dietary changes and thereby attenuating the outcomes of interest. Baseline prevalence of ASVD was determined from principal hospital discharge diagnosis codes from 1980-1998 using the Hospital Morbidity Data Collection, linked via the Western Australian Data Linkage System (WADLS). Diagnosis codes for ASVD have been described elsewhere [24]. Baseline prevalence of diabetes mellitus was determined from medication use and was coded (T89001-T90009) using the International Classification of Primary Care – Plus (ICPC-Plus) method [25].

Atherosclerotic vascular disease mortality assessment

The primary outcome of this study was any death relating to ASVD. Linked mortality data were used to retrieve coded multiple cause of death data over 15 years, as previously described [24]. Any ASVD coded death from the primary or contributing causes of death were considered an ASVD-related death. Atherosclerotic vascular disease deaths were defined using diagnosis codes from the ICD-9-CM [26] and the International Statistical Classification of Diseases and Related Health Problems, 10th Revision, Australian Modification (ICD-10-AM) [27]. Diagnosis codes included deaths attributed to ischaemic heart disease (ICD-9-CM codes 410-414 and ICD-10-AM codes I20-I25); heart failure (ICD-9-CM code 428 and ICD-10-AM code I50); cerebrovascular disease, excluding haemorrhage (ICD-9-CM codes 433-438

and ICD-10-AM codes I63-I69, G45.9); and peripheral arterial disease (ICD-9-CM codes 440-444 and ICD-10-AM codes I70-I74).

Common carotid artery intima-media thickness and carotid plaque severity

Ultrasounds were conducted by the same sonographer using an 8.0-mHz linear array transducer attached to an Acuson Sequoia 512 ultrasound machine (Mountain View, CA, USA) and a standard image acquisition protocol [28]. To account for asymmetrical wall thickening, images were taken from three different angles (anterolateral, lateral and posterolateral) of the far walls of the distal 2 cm of the left and right common carotid arteries. The same technician conducted off-line analyses on all end-diastolic images using a semi-automated edge-detection software program. The mean and maximum CCA-IMT (mm) from each of the six images (three on either side) were averaged to give an overall mean CCA-IMT and maximum CCA-IMT. A short-term precision study was undertaken in 20 non-trial subjects using the same sonographer and technician. Repeated IMT measurements were made between 0 and 31 days apart (mean 10.3 days). The coefficient of variation for the repeat measures was 5.98% (calculated using the root-mean-square method) [29]. Focal plaque was then determined by examining the entire carotid tree (common carotid artery, carotid bulb, internal and external carotid). Carotid plaque severity was categorised according to the degree of stenosis: none to less advanced (<25% stenosis) or more advanced (≥25% stenosis) [30].

Dietary intake assessment

A semi-quantitative food frequency questionnaire (FFQ) developed by the Cancer Council of Victoria [31] was used to assess dietary intake at baseline (1998), 5 years (2003), and 7 years (2005). The FFQ has been validated against two 7-day weighed food records and was found to have reasonable correlations for all nutrients, except retinol [32]. The poor agreement for retinol was due to the inclusion of liver in the two weighed food records. Liver was not an item on the FFQ. Although total vegetable intake has not been specifically validated for this FFQ, beta-carotene, fibre and vitamin C all had reasonably good agreement [32]. Furthermore, the average servings of vegetable intake in this cohort (mean 2.6 servings) is the same for the Australian population of the same sex and similar age (75 years and over) [33].

The questionnaire comprised of 74 food items (including 25 vegetable items) and used four photographs of commonly consumed foods (potato, vegetables, steak, and casserole) to estimate portion size. In total there were 24/25 vegetable items listed in the FFQ that were used to estimate total vegetable intake (g/d) (**Supplemental Table 1**). The estimation of total vegetable intake did not include 'Potatoes, roasted or fried, including hot chips' as hot chips are not recommended as part of a healthy diet. 'Potatoes cooked without fat' were included. Energy (kJ/d), alcohol (g/d), and nutrient intakes were calculated by the Cancer Council of Victoria using the NUTTAB95 food composition database [35] and other data where necessary. Participants were supervised whilst completing the FFQ. Food models, food

charts, measuring cups, and measuring spoons were provided to improve accuracy of reported food consumption. Vegetable diversity (number/d) was obtained within the FFQ using the question, 'How many different vegetables do you usually eat per day?'. Responses ranged from <1 different vegetable per day to ≥6 different vegetables per day and were coded from 0 to 6.

Nutrient-Rich Foods Index

We assessed diet quality using the Nutrient-Rich Foods Index [36]. This index was calculated using the Nutrient Reference Values (NRVs) for Australia and New Zealand based on adult females aged >70 y [37], as previously described [13].

Baseline demographic and clinical assessment

Body weight (kg) and height (m) were measured using digital scales and a wall-mounted stadiometer. Level of physical activity (kJ/d) was assessed using a questionnaire and calculated using a validated method, as previously described [24]. Smoking history was coded as non-smoker or prior/current smoker if they had consumed more than one cigarette per day for more than three months. Socioeconomic status (SES) was calculated using the Socioeconomic Indexes for Areas developed by the Australian Bureau of Statistics [38], as previously described [24]. A detailed list of medications was obtained from each participant. Medication use was coded using the ICPC-Plus method [25]. Antihypertensive, statin and low-dose aspirin medications were used to adjust for cardiovascular risk factors such as hypertension and dyslipidaemia. Blood pressure was measured in 1,190 (97.1%) participants. Participants were rested for 5 minutes before blood pressure was taken on the right arm using a mercury column manometer whilst participants were in an upright position [39]. Baseline serum creatinine was analysed in 1,106 (90.2%) participants using an isotope dilution mass spectrometry (IDMS) traceable Jaffe kinetic assay for creatinine on a Hitachi 917 analyser (Roche Diagnostics GmbH, Mannheim, Germany). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [40]. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were analysed in 895 (73.0%) participants using a Hitachi 917 auto analyser (Roche Diagnostics, Mannheim, Germany). Low-density lipoprotein (LDL) cholesterol was calculated in 888 (72.4%) participants using the Friedewald's method [41].

Framingham 10-year general cardiovascular risk scores

The Framingham risk scores (FRS) were calculated using body mass index data in 1,188 participants based on the publication by D'Agostino and others [42], as previously described [24]. Scores were confirmed using an online calculator developed by D'Agostino and Pencina [42].

Statistics

Statistical significance was set at a 2-sided Type 1 error rate of $P<0.05$. All data were analysed using IBM SPSS Statistics for Windows, version 21.0 (IBM) and STATA, version 15.1 (StataCorp LLC). Descriptive statistics of normally distributed continuous variables were expressed as mean ± standard deviation (SD); non-normally distributed continuous variables were expressed as median and interquartile range; and categorical variables as number and proportion (%). Differences in baseline characteristics across vegetable diversity categories were tested using one-way ANOVA for normally distributed continuous variables and the Kruskal-Wallis test for non-normally distributed variables. Chi-squared test for independence was used to test for differences in baseline characteristics among vegetable serving groups for categorical variables.

The primary outcome of the study was any death relating to ASVD. Complete follow-up of death records were available for all participants that remained residents in Western Australia. The follow-up time for each participant was calculated in days from their baseline visit until their last day of follow-up, which was their date of death or 15-years of follow up. Cox proportional hazards modelling was used to assess the association between baseline vegetable diversity (number/d) and ASVD mortality using four models of adjustment: (i) unadjusted; (ii) age- and energy intake-adjusted; (iii) multivariable-adjusted; and (iv) multivariable-adjusted plus total vegetable intake (g/d). Covariates included in the multivariable-adjusted model were selected on an *a priori* basis and included age (years), BMI (kg/m^2), physical activity (kJ/d expended), alcohol intake (g/d), smoking history (yes/no), socioeconomic status (ordinal), the CAlcium Intake Fracture Outcome Study (CAIFOS) supplementation group of calcium vs. placebo, anti-hypertensive medication (yes/no), statin medication (yes/no), low-dose aspirin (yes/no), CKD-EPI eGFR ($ml/min/1.73m^2$), and energy intake (kJ/d). The randomisation of participants to the CAIFOS supplementation group of calcium vs. placebo from the first 5 years of the study was included as a covariate to take into account any confounding introduced. Vegetable diversity (number/d) was further explored by separating the variable into three groups (≤3 number/d, 4 number/d, and ≥5 number/d) and entered as a categorical variable in the three models of adjustment. The three groups were based on an approximately equal number of women in each of the three response categories. We tested for a trend using the median number of vegetable diversity within each group in separate Cox proportional hazards models. Cox proportional hazards assumptions were tested using the Schoenfeld's global test. No violations were detected ($P=0.359$).

Linear regression was used to investigate the association between CCA-IMT (mean and maximum) and vegetable diversity. The association of focal plaque severity (none/minimum versus moderate/high) and vegetable diversity was assessed using binary logistic regression.

Sensitivity analyses

Reverse causality bias was explored for ASVD mortality by excluding all deaths that occurred in the first 24 months of follow-up and the multivariable-adjusted analysis repeated. To adjust for the estimated 10-year risk of developing cardiovascular disease, the FRS was used in multivariable-adjusted models for ASVD mortality and CCA-IMT (mean and maximum). The multivariable-adjusted model consisted of all previously described covariates except for age, BMI, antihypertensive medication and smoking history as the FRS takes into account the participants' age, BMI, untreated systolic blood pressure and current smoking status.

The relationship between vegetable diversity (number/d) and total vegetable intake (g/d) was investigated using Spearman's rank-order correlation (rho). An interaction term between vegetable diversity (number/d) and total vegetable intake (g/d) was evaluated for ASVD mortality and CCA-IMT (mean and maximum) using the multivariable-adjusted model. The interaction was further explored by running the multivariable-adjusted analysis for vegetable diversity (number/d) stratified by low (<2 servings), moderate (2 to <3 servings) and high (≥3 servings) total vegetable intakes.

Since vegetable diversity may be a surrogate marker for a healthier diet, in separate multivariable-adjusted models for ASVD mortality and CCA-IMT (mean and maximum), we further adjusted for diet quality using the Nutrient-Rich Foods Index. We also considered the impact of individual dietary confounders by including intakes of fish, nuts, fruit [43], fibre, potassium, magnesium, vegetable-derived nitrate [13,14] and red meat on a variable-by-variable basis in multivariable-adjusted models for ASVD mortality and CCA-IMT (mean and maximum). We also considered the impact of fish, nuts, fruit and red meat entered together in a multivariable-adjusted model for ASVD mortality and CCA-IMT (mean and maximum). To account for possible change in vegetable diversity during the 15 years of follow-up, the average of vegetable diversity across baseline (1998), 5 years (2003), and 7 years (2005) was used in a multivariable-adjusted Cox proportional hazards model for ASVD mortality. To explore whether the relationship between vegetable diversity and any ASVD-related death, assessed using multiple cause of death data, was not due to vegetable diversity being associated with other causes of death, all-cause mortality and non-ASVD related mortality were investigated in multivariable-adjusted Cox proportional hazards models. Since vitamin D has been associated with subclinical atherosclerosis [44], we excluded participants that had received 1.2 g calcium carbonate plus 1000 IU of vitamin D (n=28) and repeated the multivariable-adjusted analyses for ASVD mortality and CCA-IMT (mean and maximum).

RESULTS

Characteristics of study population

The flow chart for participant selection is presented in **Figure 1**. Baseline participant demographics, medication use and biochemical analyses of all 1,226 study participants and by categories of vegetable diversity are presented in **Table 1**. These data did not identify any major differences in baseline cardiovascular risk. Most dietary intakes were significantly different across vegetable diversity categories (P<0.05) (**Table 2**). In particular, the high diversity group consumed more fish, nuts, fruit, fibre, potassium, and magnesium. The median (IQR) number of different vegetables consumed each day was 4.0 (3.0-5.0) and mean (SD) total vegetable intake was 196.5 (78.9) g/d (~2.6 servings/d).

Atherosclerotic vascular disease mortality

Over 15,947 person-years of follow-up (mean 13 years), there were 238 (19.4%) deaths relating to ASVD. Vegetable diversity (number/d) was inversely associated with ASVD mortality (P≤0.001). In the multivariable-adjusted model, for each additional different vegetable consumed per day there was a 17% lower hazard for ASVD mortality (**Table 3**). This relationship was attenuated after further adjustment for total vegetable intake (P=0.114) (Table 3). In categorical analyses (≤3 number/d, 4 number/d, and ≥5 number/d), there were significant trends between vegetable diversity and ASVD mortality in unadjusted (P_{trend}<0.001), age- and energy intake-adjusted (P_{trend}=0.001), and multivariable-adjusted (P_{trend}=0.001) models (Table 3; **Figure 2**). This relationship was attenuated after further adjustment for total vegetable intake (P=0.074) (Table 3).

When excluding participants who died in the first 24 months of the study (n=17), vegetable diversity (number/d) remained inversely associated with ASVD mortality (multivariable-adjusted HR=0.84, 95%CI 0.75, 0.94, P=0.003). Separate analysis that adjusted for the FRS did not change the interpretation of the association between vegetable diversity (number/d) and ASVD mortality (HR=0.81, 95%CI 0.73, 0.91, P<0.001).

There was a strong, positive correlation between vegetable diversity (number/d) and the total amount of vegetables (g/d) consumed (rho=0.77, P<0.001). There was also weak evidence for an interaction between vegetable diversity (number/d) and total vegetable intake (g/d) for ASVD mortality in the multivariable-adjusted model ($P_{interaction}$=0.081). ASVD mortality was therefore further explored by running the multivariable-adjusted analysis stratified by low (<2 servings), moderate (2 to <3 servings) and high (≥3 servings) total vegetable intakes. Benefits of vegetable diversity (number/d) were most evident among women with low total vegetable intakes (≤2 servings/d) (**Table 4**). However, given the lack of a statistically significant interaction in addition to overlapping confidence intervals in the stratified analysis, no definite differences can be confirmed.

Additional adjustment for diet quality, using the Nutrient-Rich Foods Index, did not change the interpretation of the association between vegetable diversity (number/d) and ASVD mortality (multivariable-adjusted HR=0.88, 95%CI 0.77, 0.99, P=0.038). Separate analyses that adjusted for individual dietary confounders (fish, nuts, fruit, fibre, potassium, magnesium, vegetable-derived nitrate and red meat) did not augment or attenuate the association between vegetable diversity (number/d) and ASVD mortality (**Table 5**).

To account for possible change in vegetable diversity during the 15 years of follow-up, the average was calculated across all three time points (baseline, 5 years, and 7 years) and entered in a multivariable-adjusted Cox proportional hazards model for ASVD mortality. This did not alter the interpretation of the association between baseline values and ASVD mortality (HR=0.80, 95%CI 0.70, 0.92, P=0.001). To explore whether the relationship between vegetable diversity and any ASVD-related death, assessed using multiple cause of death data, was not due to vegetable diversity being associated with other causes of death, non-ASVD related mortality was investigated in a multivariable-adjusted Cox proportional hazards model. Vegetable diversity (number/d) was inversely associated with all-cause mortality (n=473) (multivariable-adjusted HR=0.89, 95%CI 0.82, 0.92, P=0.002), but not associated with non-ASVD mortality (n=235) (multivariable-adjusted HR=0.94, 0.84, 1.05, P=0.285). Lastly, exclusion of participants that had received 1.2 g calcium carbonate plus 1000 IU of vitamin D (n=28) did not change the interpretation of the association between vegetable diversity (number/d) and ASVD mortality (multivariable-adjusted HR=0.84, 95%CI 0.75, 0.94, P=0.003).

Common carotid artery intima-media thickness

At year three of the study, mean (SD) mean CCA-IMT was 0.778 (0.129) mm and mean (SD) maximum CCA-IMT was 0.922 (0.152) mm. In linear regression, for all models of adjustment, vegetable diversity (number/d) was inversely associated with mean and maximum CCA-IMT (**Table 6**). For each increase in different vegetable consumed per day there was an associated 0.013 mm (1.8%) lower mean CCA-IMT (P<0.001) and 0.017 mm (1.8%) lower maximum CCA-IMT (P<0.001) in multivariable-adjusted models. These relationships were independent of total vegetable intake (P<0.05 for both) (Table 6). Participants consuming ≥5 different vegetables per day had approximately 0.037 mm (4.8%) lower mean CCA-IMT and 0.047 mm (5.1%) lower maximum CCA-IMT compared to participants consuming ≤3 different vegetables per day. This relationship was attenuated after further adjustment for total vegetable intake (P>0.05 for both) (Table 6).

Separate analyses that adjusted for the FRS did not change the interpretation of the association between vegetable diversity (number/d) and mean CCA-IMT (unstandardized ß ± SE: -0.013 ± 0.003, P<0.001) and maximum CCA-IMT (unstandardized ß ± SE: -0.017 ± 0.004, P<0.001). In multivariable-adjusted models, there was no evidence of an

interaction between vegetable diversity (number/d) and total vegetable intake (g/d) for mean CCA-IMT ($P_{interaction}$=0.848) and maximum CCA-IMT ($P_{interaction}$=0.678).

Additional adjustment for diet quality, using the Nutrient-Rich Foods Index, did not change the interpretation of the association between vegetable diversity (number/d) and mean CCA-IMT (unstandardized ß ± SE: -0.012 ± 0.004, P=0.003) and maximum CCA-IMT (unstandardized ß ± SE: -0.016 ± 0.005, P=0.001). Separate analyses that adjusted for individual dietary confounders (fish, nuts, fruit, fibre, potassium, magnesium, vegetable-derived nitrate and red meat) did not augment or attenuate the association between vegetable diversity (number/d) and CCA-IMT (mean and maximum) (**Table 7**).

Exclusion of participants that had received 1.2 g calcium carbonate plus 1000 IU of vitamin D (n=28) did not change the interpretation of the association between vegetable diversity (number/d) and mean CCA-IMT (unstandardized ß=-0.013, SE=0.004, P<0.001) and maximum CCA-IMT (unstandardized ß=-0.017, SE=0.004, P<0.001).

Carotid plaque severity

Moderate to severe carotid stenosis (≥25% stenosis) was present in 120/968 (12.4%) of participants and none to minimal carotid stenosis (<25% stenosis) was present in 848/968 (87.6%). Vegetable diversity was not associated with carotid plaque severity (**Table 8**).

DISCUSSION

In this prospective cohort study of older adult women, we demonstrated that diversity of vegetable intake was inversely associated with ASVD mortality and CCA-IMT, a subclinical measure of atherosclerotic arterial wall thickening. These relationships were independent of lifestyle and cardiovascular risk factors as well as the intake of nutrient-rich foods associated with cardiovascular health. There was evidence that vegetable diversity was independent of total vegetable intake for CCA-IMT. However, this was not evident for ASVD mortality. There was some evidence to suggest that vegetable diversity might provide the greatest benefit for those consuming low vegetable intakes.

Diet diversity has been recommended for decades for its health benefits towards lowering risk of chronic diseases [19,45-48]. The rationale behind public health messages to increase diversity within the diet is that different foods contain different types and amounts of vitamins, minerals, fibres and phytochemicals that have been individually linked with lower chronic disease risk. Although many studies have linked diet diversity with chronic disease risk reduction, the benefits of diversity within the diet have not been fully elucidated. For example, some studies have linked higher diet diversity with excess energy intake and adverse health outcomes [49]. It is important to note, higher diet diversity does not necessarily equate to higher diet quality, which is likely a more important determinant for health outcomes [19]. Increasing amount and diversity of vegetables in the diet are both considered major components for increasing diet quality. Whether diversity of vegetable intake, separate from total amount and separate from fruit intake, is important for vascular health has yet to be established. Our study is one of only a few studies investigating the relationship of vegetable diversity, separate from fruit intake, with both subclinical and clinical vascular disease.

In a large prospective study of 71,141 women from the Nurses' Health Study and 42,135 men from the Health Professionals Follow-up Study, there was no evidence that quantity-adjusted fruit and vegetable diversity was associated with coronary heart disease (CHD) [50]. In another large prospective study (n=20,069 men and women), higher diversity of fruit and vegetables, independent of quantity, was not associated with CHD and stroke [51]. These results suggest quantity rather than diversity seem to be more important. However, it is hard to establish which one is more important, as quantity and diversity of fruit and vegetables are often highly correlated [51], and it is likely that both are important. More recently, studies have investigated vegetable diversity, separate from fruit intake. Conrad and colleagues found vegetable diversity and amount were both inversely associated with prevalent coronary heart disease, but not stroke or diabetes, in 38,981 adults from the National Health and Nutrition Examination Survey (NHANES) [52]. However, over a mean follow-up of 6.5 years greater vegetable amount, but not diversity, was inversely associated with all-cause, cardiovascular disease and coronary heart disease mortality [53].

To our knowledge, no studies have evaluated the relationship between intake of vegetable diversity and subclinical measures of atherosclerosis. Several studies have evaluated the relationship of vegetable diversity with inflammatory biomarkers, such as C-reactive protein (CRP) [21,22]. Inflammation has been proposed as a major contributor in atherosclerosis progression [54,55]. In a cross-sectional study of Puerto Rican adults (45-75 years), fruit and vegetable diversity was inversely associated with serum CRP concentrations [22]. This relationship remained after adjustment for confounding factors including total fruit and vegetable intake. In another cross-sectional study investigating the relationship of fruit and vegetable diversity with low-grade inflammation in 412 adolescents, greater vegetable diversity (≥13 categories per month), but not fruit diversity, was associated with a lower odds of having higher serum CRP concentrations (≤6 categories per month) [21]. This relationship was independent of total vegetable intake. These studies suggest higher diversity of vegetable intake, independent of total amount, may lower inflammation. Whether this translates to reducing the progression of atherosclerosis is yet to be established.

Although vegetable diversity has not been previously studied with subclinical measures of atherosclerosis, overall diet diversity has been investigated [56]. Hoebeeck et al [56] found higher diet diversity was inversely associated with femoral atherosclerosis in middle-aged (mean age: 46 years) men (n=1200) and women (n=1287). However, this relationship was not observed for carotid atherosclerosis, carotid plaque, carotid IMT, femoral plaque, or femoral IMT [56]. Other cardiovascular-related outcomes, such as type 2 diabetes have also been studied with overall diet diversity. Danquash et al [57] investigated the associations of between- and within- food group diversity with type 2 diabetes among Ghanaian migrants in Europe. The authors reported an inverse association between a constructed Food Variety Score (0-20) and type 2 diabetes adjusted for socio-demographic, lifestyle, and anthropometric factors. This Food Variety Score reflected the number of different food groups consumed on a weekly basis.

There are several strengths to our study. Participants were consuming an average 2.6 servings of vegetables per day, which is the same for Australian women 75 years and over [33]. Such findings suggest that the results of our study may be applicable to a large proportion of Australian women. Dietary intake, including intake of vegetable diversity, was assessed at different time points throughout the 15-year follow-up period. Combining these data, which was completed as a sensitivity analysis, reduces bias associated with measurement error and misclassification. In addition, given the age of the participants and the extensive follow-up period, a high rate of deaths relating to ASVD was present, increasing the power to detect an association. Furthermore, follow-up of all death records for women that remained in Western Australia were obtained. This was likely most women given the age of participants and minimises the likelihood of loss to follow-up bias.

Several limitations need to be considered when interpreting the findings from this study. When categorising participants into the vegetable diversity groups, this resulted in differences in other food and nutrient intakes, including total energy

intake. We attempted to address this by adjusting for total energy intake and other confounding factors in multivariable-adjusted models. Although total energy intake was significantly higher among women consuming the highest diversity of vegetables, BMI was similar. This implies that individuals consuming higher vegetable diversity and hence higher energy intake should have been more physically active. However, physical activity levels were similar across the vegetable diversity groups. This suggests the physical activity questionnaire used in this study did not completely capture the entire amount of activity expended by participants, or that self-reported activity levels contributed to possible recall bias and measurement error [59]. In contrast, given the age of the participants, the women consuming high vegetable diversity could have lower muscle mass due to lower physical activity levels. There is also a possibility that recall bias could have occurred when assessing dietary intakes. Dietary intakes in this study were self-reported. Although participants were supervised whilst completing the FFQ on all occasions, recall bias and measurement error are possible leading to a biased estimate for the effect of vegetable diversity [59]. However, it is likely that any measurement error was non-differential, which would lead to an underestimate of the true effect of vegetable diversity. The last point to consider is that given the observational nature of the study, a causal relationship cannot be established and only the possibility of a causal relationship can be proposed. Results also cannot be generalised to older male and younger populations.

Conclusions

In this prospective cohort study, we found higher vegetable diversity was inversely associated with subclinical and clinical ASVD. There was evidence that vegetable diversity was independent of total vegetable intake for CCA-IMT. However, this was not evident for ASVD mortality. The reduction in ASVD risk was partly explained by higher total vegetable intake. There was also some evidence to suggest that vegetable diversity may provide the greatest benefits for those consuming low vegetable intakes. These findings should be interpreted with caution until they have been explored and replicated in other populations and in larger cohorts.

Acknowledgements: The authors wish to thank the staff at the Western Australian Data Linkage Branch; Hospital Morbidity Data Collection; Registry of Births, Deaths and Marriages; Victorian Department of Justice and Regulation; and the National Coronial Information System for their work on providing the data for this study.

Conflict of Interest (COI) Statement: All authors have no potential conflicts of interest.

Authors' contributions: LCB, JRL, MS, AD, RLP, JMH: study concept and design; JRL, AD, KZ, WHL, PLT, RLP: acquisition of data; LCB, RJW, JMH: statistical analysis and interpretation of data; LCB: drafting of the manuscript; all authors: critical revision of the manuscript for important intellectual content.

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Table 1. Baseline characteristics of all study participants and by categories of vegetable diversity[1].

	All participants	Vegetable diversity categories			
Participant demographics	**n = 1,226**	**≤3 number/d n=415**	**4 number/d n=379**	**≥5 number/d n=432**	**P value**
Age, years	75.1 ± 2.7	75.2 ± 2.7	75.0 ± 2.6	75.0 ± 2.6	0.286
BMI, kg/m^2	27.0 ± 4.6	27.0 ± 4.6	27.1 ± 4.6	26.9 ± 4.5	0.898
Body weight, kg	68.1 ± 12.1	67.9 ± 12.0	68.4 ± 12.3	68.1 ± 11.9	0.822
Physical activity, kJ/d, median (IQR)	460.5 (101.7-860.8)	419.8 (0.0-860.5)	454.9 (70.6-850.9)	493.4 (204.8-880.2)	0.091
Alcohol intake, g/d, median (IQR)	2.1 (0.3-10.4)	1.5 (0.3-9.7)	2.0 (0.4-9.9)	3.0 (0.3-11.6)	0.163
Smoking history[2], n (%)	441 (36.0)	146 (35.4)	138 (36.6)	157 (36.6)	0.923
Socioeconomic status[3]					0.865
Top 10% most highly disadvantaged, n (%)	41 (3.3)	14 (3.4)	10 (2.7)	17 (4.0)	
Highly disadvantaged, n (%)	146 (11.9)	59 (14.3)	38 (10.1)	49 (11.5)	
Moderate-highly disadvantaged, n (%)	194 (15.8)	68 (16.5)	62 (16.5)	64 (15.0)	
Low-moderately disadvantaged, n (%)	185 (15.1)	62 (15.0)	57 (15.2)	66 (15.5)	
Low disadvantage, n (%)	255 (20.8)	83 (20.1)	83 (22.1)	89 (20.9)	
Top 10% least disadvantaged, n (%)	394 (32.1)	127 (30.8)	126 (33.5)	141 (33.1)	
Treatment with calcium supplements, n (%)	641 (52.3)	213 (51.3)	201 (53.2)	227 (52.5)	0.868
Framingham risk score (%)[4]	20.6 ± 9.1	20.5 ± 9.2	20.6 ± 8.8	20.7 ± 9.2	0.946
Blood pressure[5]					
Systolic blood pressure, mmHg	137.7 ± 18.2	137.0 ± 18.2	138.6 ± 17.4	137.6 ± 18.9	0.499
Diastolic blood pressure, mmHg	73.4 ± 11.0	73.2 ± 11.0	74.0 ± 11.0	73.1 ± 11.1	0.509
Mean arterial pressure, mmHg	94.9 ± 11.9	94.5 ± 11.9	95.5 ± 11.7	94.6 ± 11.9	0.437
Medication use					
Anti-hypertensive medication, n (%)	493 (40.2)	168 (40.5)	141 (37.2)	184 (42.6)	0.293
Statin medication, n (%)	184 (15.0)	66 (15.9)	53 (14.0)	65 (15.0)	0.751
Low-dose aspirin, n (%)	193 (15.7)	74 (17.8)	60 (15.8)	59 (13.7)	0.249

Biochemical analyses					
CKD-EPI eGFR[6], ml/min/1.73m^2	67.6 ± 13.0	67.7 ± 12.6	66.8 ± 13.1	68.1 ± 13.2	0.361
Total cholesterol[7], mmol/L	5.9 ± 1.1	6.0 ± 1.1	5.9 ± 1.0	5.8 ± 1.1	0.129
HDL[7] cholesterol, mmol/L	1.5 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	1.4 ± 0.4	0.283
LDL[8] cholesterol, mmol/L	3.8 ± 1.0	3.8 ± 1.1	3.7 ± 0.9	3.7 ± 1.0	0.210
Triglycerides[7], mmol/L, median (IQR)	1.4 (1.0-1.9)	1.4 (1.0-1.9)	1.3 (1.1-1.8)	1.4 (1.0-1.9)	0.597

[1]Vegetable diversity (number/d) was assessed by how many different vegetables consumed per day. P values are a comparison between groups using ANOVA, Kruskal-Wallis test and Chi-square test where appropriate. Values are presented as mean ± SD unless otherwise stated.

[2]n=1,218.

[3]n=1,215.

[4]n=1,188.

[5]n=1,190.

[6]n=1,106.

[7]n=895.

[8]n=888.

CKD-EPI, chronic kidney disease EPIdemiology; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2. Dietary intakes of all study participants and by categories of vegetable diversity[1].

	All participants	Vegetable diversity categories			
Dietary intakes	**n = 1,226**	**≤3 number/d n=415**	**4 number/d n=379**	**≥5 number/d n=432**	**P value**
Vegetable diversity, number/d, median (IQR)	4.0 (3.0-5.0)	3.0 (2.0-3.0)	4.0 (4.0-4.0)	5.0 (5.0-6.0)	<0.001
Vegetables, g/d	196.5 ± 78.9	129.4 ± 46.9	195.7 ± 48.2	261.5 ± 70.3	<0.001
Energy, kJ/d	7,146.5 ± 2,091.9	6,790.4 ± 2,086.0	7,201.5 ± 2,053.9	7,440.2 ± 2,084.9	<0.001
Total fat, g/d	64.7 ± 23.3	63.3 ± 23.4	64.9 ± 22.4	65.7 ± 24.1	0.313
Saturated fat, g/d	25.8 ± 11.2	26.2 ± 11.4	25.8 ± 10.7	25.4 ± 11.4	0.606
Monounsaturated fat, g/d	22.5 ± 8.7	21.6 ± 8.4	22.6 ± 8.4	23.3 ± 9.1	0.018
Polyunsaturated fat, g/d	10.6 ± 4.8	10.2 ± 4.9	10.7 ± 4.6	11.1 ± 4.8	0.018
Omega 3 fatty acids, g/d, median (IQR)	1.2 (0.9-1.7)	1.1 (0.9-1.5)	1.2 (0.9-1.7)	1.3 (1.0-1.7)	<0.001
Dietary cholesterol, mg/d	238.6 ± 99.6	232.8 ± 100.2	240.6 ± 94.0	242.6 ± 103.7	0.328
Protein, g/d	79.5 ± 26.4	73.7 ± 26.6	80.1 ± 25.7	84.6 ± 25.8	<0.001
Carbohydrate, g/d	191.1 ± 58.1	179.6 ± 57.0	193.3 ± 57.1	200.2 ± 58.3	<0.001
Sugar, g/d	92.1 ± 31.9	85.9 ± 31.4	93.1 ± 31.4	97.2 ± 31.9	<0.001
Fibre, g/d	22.8 ± 7.8	19.4 ± 6.9	23.2 ± 7.2	25.7 ± 7.8	<0.001
Potassium, mg/d	2,948.9 ± 844.9	2,571.2 ± 772.0	2,986.5 ± 787.2	3,278.9 ± 815.6	<0.001
Magnesium, mg/d	298.9 ± 93.0	267.8 ± 89.4	303.5 ± 89.5	324.9 ± 90.9	<0.001
Fruit, g/d	256.1 ± 131.5	212.7 ± 121.4	266.4 ± 124.3	288.7 ± 136.0	<0.001
Nuts, g/d, median (IQR)	0.6 (0.2-2.7)	0.3 (0.0-1.8)	0.6 (0.2-1.9)	1.0 (0.2-4.3)	<0.001
Fish, g/d, median (IQR)	19.3 (9.3-35.7)	16.2 (7.6-30.4)	20.6 (9.8-35.3)	22.8 (11.5-44.0)	<0.001
Red meat, g/d, median (IQR)	42.2 (23.7-69.5)	37.8 (19.8-63.4)	43.9 (25.7-72.0)	44.9 (26.0-73.2)	0.009
Processed meat, g/d, median (IQR)	10.4 (4.9-20.6)	10.1 (4.4-19.3)	11.2 (5.1-23.8)	9.8 (4.6-20.7)	0.123
Nutrient-Rich Foods Index	75.2 ± 24.4	73.3 ± 22.8	75.8 ± 24.0	76.5 ± 26.1	0.146

[1]Vegetable diversity (number/d) was assessed by how many different vegetables consumed per day. P values are a comparison between groups using ANOVA and Kruskal-Wallis test where appropriate. Values are presented as mean ± SD unless otherwise stated.

Table 3. The association between vegetable diversity (number/d) and ASVD mortality[1]

	All participants		Vegetable diversity categories			
	HR (95%CI)		**≤3 number/d**	**4 number/d**	**≥5 number/d**	**P for**
	n=1,226	**P value**	**n=415**	**n=379**	**n=432**	**trend[2]**
Median vegetable diversity, number/d			3.0	4.0	5.0	
Atherosclerotic vascular disease						
Deaths, n (%)	238 (19.4)		110 (26.5)	56 (14.8)	72 (16.7)	
Unadjusted	0.82 (0.74, 0.91)	<0.001	1.00 (Referent)	0.50 (0.36, 0.69)	0.57 (0.43, 0.77)	<0.001
Age- and energy intake-adjusted	0.84 (0.76, 0.93)	0.001	1.00 (Referent)	0.53 (0.38, 0.73)	0.61 (0.45, 0.82)	0.001
Multivariable-adjusted[3]	0.83 (0.75, 0.93)	0.001	1.00 (Referent)	0.50 (0.35, 0.71)	0.60 (0.43, 0.83)	0.001
Multivariable-adjusted plus total vegetable intake	0.87 (0.73, 1.03)	0.114	1.00 (Referent)	0.53 (0.36, 0.78)	0.68 (0.42, 1.09)	0.074

[1]Results are presented as HR (95% CI) per different vegetable consumed per day (number/d) using Cox proportional hazards modelling.

[2]P values are a trend test using the median values of each vegetable diversity category in Cox proportional hazards models.

[3]Multivariable-adjusted model included age, BMI, physical activity, alcohol intake, smoking history, socioeconomic status, the CAIFOS supplementation group of calcium vs. placebo, anti-hypertensive medication, statin medication, low-dose aspirin, CKD-EPI eGFR and energy intake.

ASVD, atherosclerotic vascular disease; CAIFOS, Calcium Intake Fracture Outcome Study; CKD-EPI, chronic kidney disease EPIdemiology; eGFR, estimated glomerular filtration rate.

Table 4. The association between vegetable diversity (number/d) and ASVD mortality stratified by low (<2 servings), moderate (2 to <3 servings), and high (≥3 servings) total vegetable intakes[1]

Vegetable serving categories	Deaths, n (%)	Hazard Ratio (95%CI)	P value
≤2 servings/d	83/355 (23.4)	0.77 (0.61, 0.99)	0.042
2 to <3 servings/d	98/486 (20.2)	0.84 (0.65, 1.09)	0.196
≥3 servings/d	57/385 (14.8)	1.23 (0.85, 1.78)	0.265

[1]Results are presented as HR (95% CI) per different vegetable consumed per day (number/d) using Cox proportional hazards modelling adjusted for age, BMI, physical activity, alcohol intake, smoking history, socioeconomic status, the CAIFOS supplementation group of calcium vs. placebo, anti-hypertensive medication, statin medication, low-dose aspirin, CKD-EPI eGFR and energy intake.

ASVD, atherosclerotic vascular disease; CKD-EPI, chronic kidney disease EPIdemiology; eGFR, estimated glomerular filtration rate.

Table 5. Multivariable-adjusted HRs and 95% CIs for ASVD mortality for vegetable diversity (number/d) with individual additional adjustments for potential dietary confounders[1]

	All participant n=1,226	**P value**
Vegetable diversity		
Multivariable-adjusted[2] plus fish, g/d	0.83 (0.75, 0.93)	0.002
Multivariable-adjusted plus nuts, g/d	0.84 (0.75, 0.94)	0.002
Multivariable-adjusted plus fruit, g/d	0.83 (0.74, 0.93)	0.002
Multivariable-adjusted plus fibre, g/d	0.86 (0.76, 0.97)	0.014
Multivariable-adjusted plus potassium, mg/d	0.88 (0.78, 1.00)	0.048
Multivariable-adjusted plus magnesium, mg/d	0.87 (0.77, 0.98)	0.019
Multivariable-adjusted plus vegetable-derived nitrate, mg/d	0.83 (0.72, 0.95)	0.006
Multivariable-adjusted plus red meat, g/d	0.84 (0.75, 0.94)	0.002
Multivariable-adjusted plus fruit, nuts, fish and red meat, g/d	0.84 (0.75, 0.94)	0.003

[1]Results are presented as HR (95% CI) per different vegetable consumed per day (number/d) using multivariable-adjusted Cox proportional hazards modelling with individual additional adjustments for potential dietary confounders for vegetable diversity (number/d) and ASVD mortality.

[2]Multivariable-adjusted model included age, BMI, physical activity, alcohol intake, smoking history, socioeconomic status, the CAIFOS supplementation group of calcium vs. placebo, anti-hypertensive medication, statin medication, low-dose aspirin, CKD-EPI eGFR and energy intake.

ASVD, atherosclerotic vascular disease; CAIFOS, Calcium Intake Fracture Outcome Study; CKD-EPI, chronic kidney disease EPIdemiology; eGFR, estimated glomerular filtration rate.

Table 6. The association between vegetable diversity (number/d) and CCA-IMT

	All participants[1]		Vegetable diversity categories[2]			
	B ± SE		**≤3 number/d**	**4 number/d**	**≥5 number/d**	
	n=954	**P value**	**n=302**	**n=300**	**n=352**	**P value**
Mean CCA-IMT, mm						
Unadjusted	-0.014 ± 0.003	<0.001	0.797 ± 0.007	0.780 ± 0.007	0.760 ± 0.007	0.001
Age- and energy intake-adjusted	-0.013 ± 0.003	<0.001	0.795 ± 0.007	0.780 ± 0.007	0.762 ± 0.007	0.004
Multivariable-adjusted[3]	-0.013 ± 0.004	<0.001	0.795 ± 0.010	0.775 ± 0.010	0.758 ± 0.010	0.003
Multivariable-adjusted plus total vegetable intake	-0.012 ± 0.005	0.023	0.792 ± 0.011	0.775 ± 0.010	0.761 ± 0.011	0.129
Maximum CCA-IMT, mm						
Unadjusted	-0.018 ± 0.004	<0.001	0.948 ± 0.009	0.921 ± 0.009	0.900 ± 0.008	<0.001
Age- and energy intake-adjusted	-0.017 ± 0.004	<0.001	0.946 ± 0.009	0.921 ± 0.009	0.901 ± 0.008	0.001
Multivariable-adjusted	-0.017 ± 0.004	<0.001	0.945 ± 0.012	0.916 ± 0.012	0.898 ± 0.012	0.001
Multivariable-adjusted plus total vegetable intake	-0.014 ± 0.006	0.024	0.938 ± 0.013	0.916 ± 0.012	0.904 ± 0.013	0.136

[1]Results analysed by linear regression and are presented as unstandardised B and SE per different vegetable consumed per day (number/d).

[2]Results analysed by linear regression and are presented as estimated mean and SE.

[3]Multivariable-adjusted model included age, BMI, physical activity, alcohol intake, smoking history, socioeconomic status, the CAIFOS supplementation group of calcium vs. placebo, anti-hypertensive medication, statin medication, low-dose aspirin, CKD-EPI eGFR and energy intake.

CCA-IMT, common carotid artery intima-media thickness; CAIFOS, Calcium Intake Fracture Outcome Study; CKD-EPI, chronic kidney disease EPIdemiology; eGFR, estimated glomerular filtration rate.

Table 7. Multivariable-adjusted linear regression analysis with individual additional adjustments for potential dietary confounders[1]

	Mean CCA-IMT, mm n=954		Maximum CCA-IMT, mm n=954	
	B ± SE	**P value**	**B ± SE**	**P value**
Vegetable diversity				
Multivariable-adjusted[2] plus fish, g/d	-0.012 ± 0.004	0.001	-0.016 ± 0.004	<0.001
Multivariable-adjusted plus nuts, g/d	-0.013 ± 0.004	<0.001	-0.016 ± 0.004	<0.001
Multivariable-adjusted plus fruit, g/d	-0.012 ± 0.004	0.001	-0.015 ± 0.004	<0.001
Multivariable-adjusted plus fibre, g/d	-0.013 ± 0.004	0.001	-0.017 ± 0.005	<0.001
Multivariable-adjusted plus potassium, mg/d	-0.010 ± 0.004	0.018	-0.012 ± 0.005	0.011
Multivariable-adjusted plus magnesium, mg/d	-0.012 ± 0.004	0.002	-0.015 ± 0.004	0.001
Multivariable-adjusted plus vegetable-derived nitrate, mg/d	-0.011 ± 0.005	0.045	-0.014 ± 0.006	0.030
Multivariable-adjusted plus red meat, g/d	-0.013 ± 0.004	<0.001	-0.017 ± 0.004	<0.001
Multivariable-adjusted plus fruit, nuts, fish and red meat, g/d	-0.012 ± 0.004	0.002	-0.015 ± 0.004	0.001

[1]Results are presented as unstandardized B and SE per different vegetable consumed per day (number/d) using multivariable-adjusted linear regression with individual additional adjustments for potential dietary confounders for vegetable diversity (number/d) and CCA-IMT.

[2]Multivariable-adjusted model included age, BMI, physical activity, alcohol intake, smoking history, socioeconomic status, the CAIFOS supplementation group of calcium vs. placebo, anti-hypertensive medication, statin medication, low-dose aspirin, CKD-EPI eGFR and energy intake.

CCA-IMT, common carotid artery intima-media thickness; CAIFOS, Calcium Intake Fracture Outcome Study; CKD-EPI, chronic kidney disease EPIdemiology; eGFR, estimated glomerular filtration rate.

Table 8. The association between vegetable diversity (number/d) and carotid plaque severity[1]

	All participants		Vegetable diversity categories			
	OR (95% CI)		**≤3 number/d**	**4 number/d**	**≥5 number/d**	**P for trend[2]**
	n=968	**P value**	**n=306**	**n=303**	**n=359**	
Median vegetable diversity, number/d			3.0	4.0	5.0	
Carotid plaque severity						
Unadjusted	0.93 (0.79, 1.08)	0.335	1.00 (Referent)	0.85 (0.53, 1.37)	0.83 (0.53, 1.32)	0.439
Age- and energy intake-adjusted	0.93 (0.79, 1.09)	0.360	1.00 (Referent)	0.85 (0.53, 1.37)	0.84 (0.53, 1.33)	0.459
Multivariable-adjusted[3]	0.93 (0.78, 1.09)	0.360	1.00 (Referent)	0.89 (0.53, 1.50)	0.81 (0.49, 1.35)	0.419
Multivariable-adjusted plus total vegetable intake	0.81 (0.63, 1.05)	0.107	1.00 (Referent)	0.77 (0.43, 1.37)	0.61 (0.30, 1.24)	0.172

[1]Results analysed by logistic regression and are presented as ORs (95%CI) per different vegetable consumed per day (number/d).

[2]P values are a trend test using the median values of each vegetable diversity category in logistic regression models.

[3]Multivariable-adjusted model included age, BMI, physical activity, alcohol intake, smoking history, socioeconomic status, the CAIFOS supplementation group of calcium vs. placebo, anti-hypertensive medication, statin medication, low-dose aspirin, CKD-EPI eGFR and energy intake.

CAIFOS, Calcium Intake Fracture Outcome Study; CKD-EPI, chronic kidney disease EPIdemiology; eGFR, estimated glomerular filtration rate.

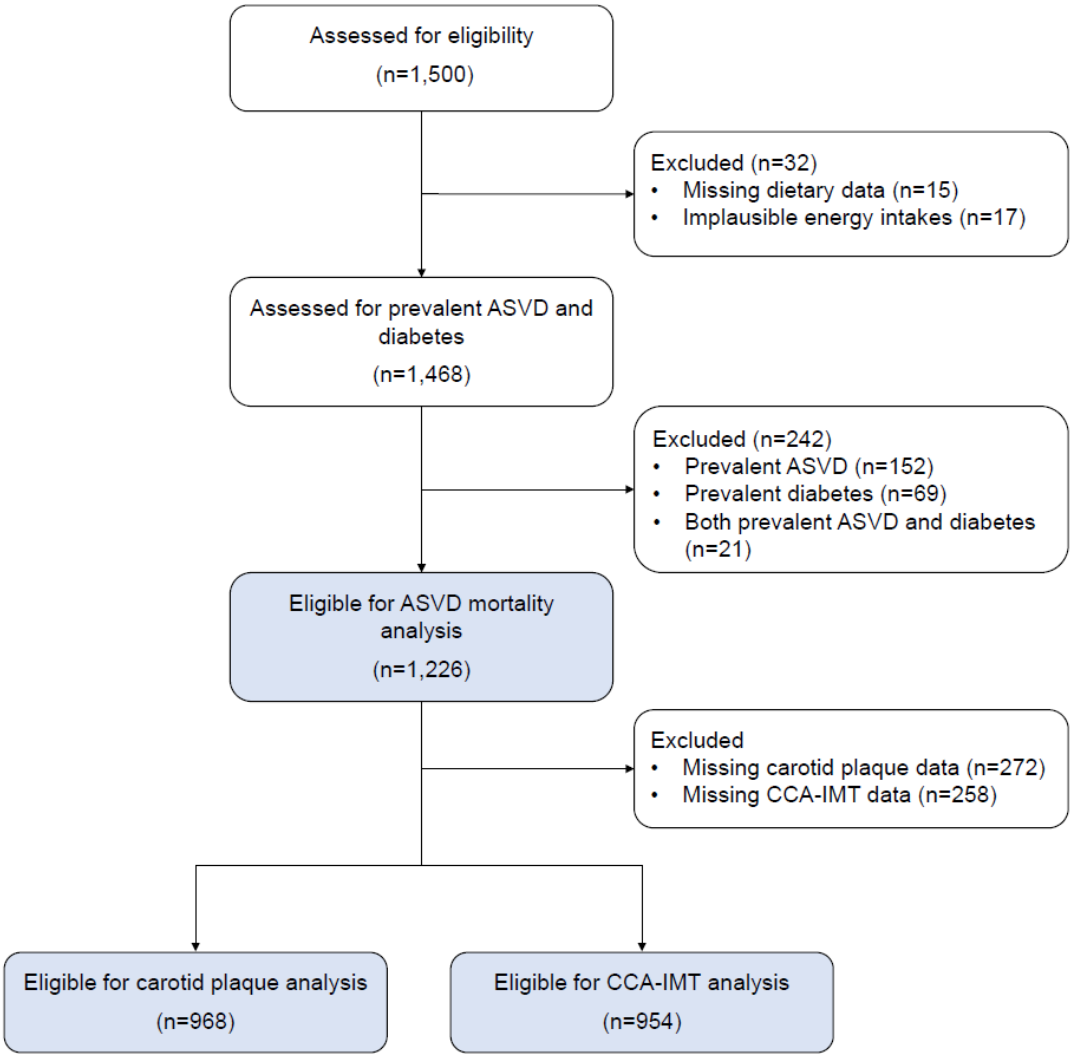

Figure 1. Participant flow chart. ASVD, atherosclerotic vascular disease; CCA-IMT, common carotid artery intima-media thickness.

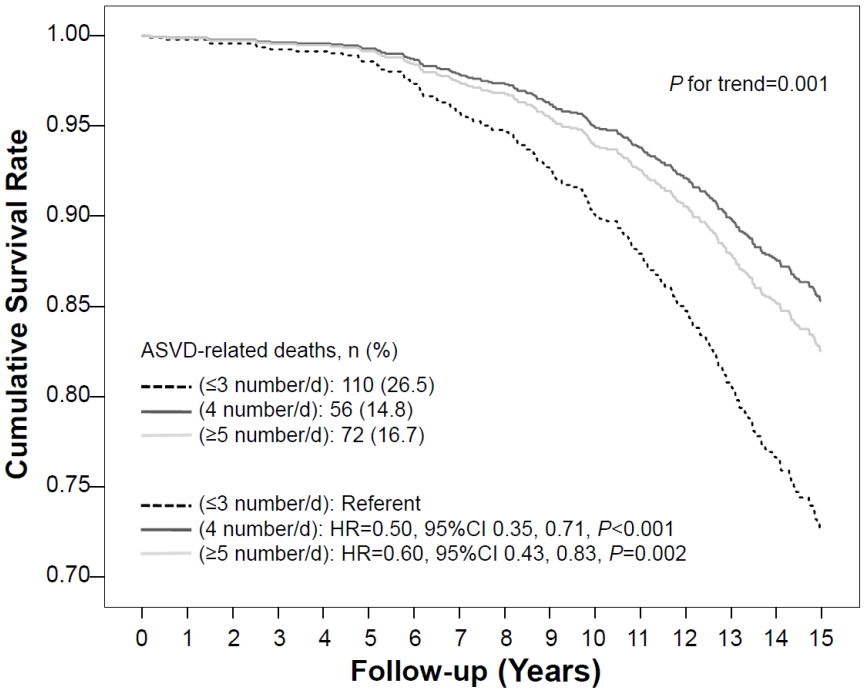

Figure 2. Multivariable-adjusted cumulative survival curves for atherosclerotic vascular disease mortality according to vegetable diversity categories. Multivariable-adjusted model included age, BMI, physical activity, alcohol intake, smoking history, socioeconomic status, the CAIFOS supplementation group of calcium vs. placebo, anti-hypertensive medication, statin medication, low-dose aspirin, CKD-EPI eGFR and energy intake. CKD-EPI, chronic kidney disease EPIdemiology; eGFR, estimated glomerular filtration rate.