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The Effect of Dietary Micro-Nutrients Intake on Abdominal Aortic Calcification: A Study Protocol for Systematic Review and Meta-Analysis

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Keywords:	Cardiovascular Disease, NUTRITION & DIETETICS, Drug Therapy, Nutritional support < GASTROENTEROLOGY, GERIATRIC MEDICINE



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Original protocol The Effect of Dietary Micro-Nutrients Intake on Abdominal Aortic Calcification: A Study **Protocol for Systematic Review and Meta-Analysis.** Etsay Weldekidan Tsegay^{1*}, Nigus Alemu Hailu², Meresa Berwo Mengesha³, Zenawi Hagos Gufue⁴ ^{1*}Department of Modern and Traditional Medicine Research, Tigray Health Research Institute, Mekelle, Ethiopia ²Department of Biomedical Sciences, College of Medicine and Health Sciences, Adigrat University, Adigrat, Ethiopia ³Department of Midwifery, College of Medicine and Health Sciences, Adigrat University, Adigrat, Ethiopia ⁴Department of Public Health, College of Medicine and Health Sciences, Adigrat University, Adigrat, Ethiopia **Corresponding Author** Etsay Weldekidan Tsegay (BSc, MSc) Department of Modern and Traditional Medicine Research, Tigray Health Research Institute, Mekelle, Ethiopia Email: etsay065@gmail.com.

30 Abstract

Introduction: Healthy dietary choices have an important role in preventing chronic diseases such as cardiovascular disease (CVD). Increasing evidence suggests micronutrient intake (essential minerals and vitamins) is associated with abdominal aortic calcification (AAC), which is an advanced marker of CVD. However, the existing reports seem inconsistent. Some studies reported micronutrients are associated with lower risk of AAC, while others have reported increasing risk. Therefore, this systematic review and meta-analysis sought to summarize the available evidence on the association of dietary micronutrient intake on AAC.

Methods and Analysis: A comprehensive systematic search of the PubMed/MEDLINE, EMBASE, Web of Science, and Google Scholar databases from their inception up to September 1, 2024, will be conducted. All clinical studies that report eligible exposure/s (dietary micronutrients intake) and outcome/s (presence/severity of AAC) will be included, and this systematic review and meta-analysis protocol will be reported following the revised PRISMA-P guidelines. The risk of bias for observational studies will be assessed using the Newcastle-Ottawa Scale (NOS) and Publication bias will be evaluated through visual inspection of funnel plots and the Egger's and Begg's regression tests. The Der Simonian and Laird random effects model meta-analysis will be calculated to provide pooled results and the weighted risk ratio with their 95% confidence intervals will be presented.

Ethics and dissemination: The results will be disseminated through publishing in a peer-reviewed
journal and public presentations at relevant local, national, and international conferences,
workshops, and symposiums. Ethical approval is not required as this is a systematic review of
publicly available data.

Prospero registration number: CRD42024575902

Keywords: Dietary micronutrients, abdominal aortic calcification, Cardiovascular disease.

1 2		
3 4	57	Strengths and limitations of this study
5 6	58	• This study will use meta-regression to identify sources of heterogeneity and identify
7 8	59	subgroups in which dietary micronutrients are more or less predictive of positive outcomes.
9 10	60	• To our knowledge, there has been no systematic review and meta-analysis that has
11 12	61	investigated the association between Dietary micronutrients and abdominal aortic
13	62	calcification (AAC).
14 15	63	• The main limitation of this review is the discrepancies in imaging modality, measurement
16 17	64	and reporting of AAC across studies but we attempted to overcome this by exploring these
18 19	65	aspects in pre-specified sub analyses.
20	66	• Further limitation is that this study has language restriction.
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86 Introduction

Abdominal aortic calcification (AAC) is an advanced marker of cardiovascular disease (CVD), occurring in one out of every three individuals between the ages of 45 and 54, and nine out of ten individuals aged 75 years and older (1). AAC is characterized by deposition of calcium within the arterial wall of abdominal aorta (2). It is independently associated with not only CVD risk (3), but also muscle strength decline (4), lower bone mineral density, fractures (5), falls (6), and dementia (7).

Despite AAC's association with debilitating medical conditions, the evidence regarding its
prevention and available treatment is very limited. Randomized trials and cohort studies have
examined the effect of intensive glycemic treatment, vitamin B, calcium supplementation,
phosphate binders, and etidronic acid on AAC, however the results shows no influence on AAC
occurrence or severity (8–15).

Dietary strategies, particularly micronutrients such as potassium, zinc, selenium, magnesium, Vitamin C and K have gained significant attention for their pivotal role in the prevention of CVDs (16). However, the relationship between dietary micronutrients intake and AAC remains uncertain. One cohort study indicated that a higher intake of dietary zinc was associated with an 8% lower risk of severe AAC after adjustment for age, gender, and ethnicity, though no association was found in the fully adjusted model (17). Similarly, higher consumption of dietary potassium (18), selenium (19), and copper (20) has been linked to decrease in AAC incidence (19). Furthermore, dietary copper intake has been associated with a decreased risk of severe AAC (21).

The relationship between dietary calcium intake and AAC is also complex. While higher dietary calcium intake has been associated with a lower prevalence of AAC at baseline, it has not been related to changes in prevalence of AAC over 2 to 5 years (12). This finding is inconsistent with a cross-sectional study on diabetic mellitus patients, which found no significant association between dietary calcium and AAC progression (22). However, in health adults, there is an independent correlation between increased dietary calcium intake and severe AAC (23).

1 A cross-sectional study in healthy participants found that increasing self-reported total magnesium 113 intake by 50 mg/day resulted in 12% decreased in presence of AAC (24), and another study

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reported that a 0.1 mmol/L increase in serum magnesium (Mg) being independently associated with a 1.1 point decrease in AAC score (25). Higher dietary Vitamin C intake has been linked to a reduced AAC score (16,26) and a lower risk of AAC in Adults (16). However, population based study have failed to demonstrate a significant correlation between dietary Vitamin C intake and risk of severe AAC (26). Furthermore, there is significant inverse relationship between higher folate (Vitamin B₉) intake and the risk of severe AAC (27), as well as a lower risk of AAC with higher dietary Vitamin A (28). Given the relationship between AAC and CVD, as well as the challenges it poses in terms of treatment, dietary micronutrients intake has drawn substantial attention for their potential role in preventing and ameliorating AAC (29). Although there are reasonable biological mechanisms that connect certain micronutrients to AAC, the current evidence is not conclusive. Additionally, the long-term effects of micronutrient intake on the presence/severity of AAC remain unclear. Therefore, there is a need to conduct a systematic review and meta-analysis to synthesize the robust evidence regarding the effect of micronutrient intake on the development, severity, or progression of AAC. Moreover, the association between specific dietary micronutrients intake and the presence and severity of AAC will be examined.

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3 4	138	Methods and analysis
5 6 7	139	Eligibility criteria
8	140	Population
9 10	141	This review will focus on observational human studies of dietary micronutrients intake and their
11 12	142	effect on severity /presence of AAC diagnosed using different modality.
13 14	143	Exposure
15	144	Micronutrients intake
16 17	145	Vitamins A, B-complex (B1, B2, B3, B5, B6, B7, B9, B12), C, D, E, and K and calcium, iron,
18 19	146	magnesium, zinc, selenium, iodine, copper, manganese, and chromium
20	147	Outcome of interest
21 22	148	Primary outcome
23 24	149	The primary outcome will be presence and severity of AAC. Presence of AAC identified from
25 26	150	either radiography, dual X-ray absorptiometry (DXA) machine, MRI, or CT scan and will be
20	151	presented as AAC present or absent. Severity AAC will be categorized as low (lowest reported
28 29 30	152	category), moderate (middle reported categories) and high (highest reported category).
31 32	153	Secondary outcome of interest will be the effect of specific dietary micronutrient intake in severity
33 34	154	and progression of AAC (rate of change in calcification volume, area, or score over time). This
35	155	review defines AAC as the presence of calcified deposits in the abdominal aorta identified from
36 37 38	156	radiography, dual X-ray absorptiometry (DXA), MRI, or CT scan.
39 40 41	157	Setting and Languages
42 43	158	This study will not restrict studies by settings, and only articles reported by English language will
44 45	159	be included.
40 47 48	160	Study design
49 50 51	161	Observational cohort studies (both retrospective and prospective cohort studies), case-control
52 53	162	studies, and cross-sectional studies that report eligible exposure(s) and outcome(s) will be included
54 55 56 57 58	163	in this review.

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1 2		
2 3 4	164	Inclusion criteria
5 6	165	• Observational studies in humans. These include cohort (both retrospective and prospective
7 8	166	cohort studies), case-control and cross-sectional studies that report eligible exposure(s)
9 10	167	and outcome(s)
10	168	• AAC assessed by any methodology.
12 13	169	• Report of any dietary micronutrient effect on abdominal aortic outcome
14 15 16	170	Exclusion criteria
17 18	171	We will exclude case series, case reports, commentaries, editor letters, and reviews that do not
19	172	contain any original data. We will exclude animal research from this review.
20 21 22 23	173	Search engines.
23	174	To discover relevant studies, comprehensive literature searches on dietary micronutrients intake
25 26	175	and AAC will be conducted in PubMed/MEDLINE, EMBASE, the Web of Science core
27 28	176	collection, Google Scholar, and CINAHL. Because no publication date limits were specified, the
29 30	177	search will run from inception to September 1, 2024. The information sources will be updated
31	178	prior to the submission of this evaluation. To ensure that all relevant studies are included in this
32 33	179	review, the information sources will be augmented by examining the reference lists of selected
34 35 36	180	studies and systematic reviews of comparable scope.
37 38	181	Search strategies
39 40	182	The search strategy will be developed using a combination of medical subject headings and
41 42	183	keywords related to both exposure and the outcome of interest. The literature search will be
43 44	184	conducted without any date constraints. The search strategies for PubMed/MEDLINE is provided
45	185	in Table 1.
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BMJ Open: first published as 10.1136/bmjopen-2024-096551 on 23 May 2025. Downloaded from http://bmjopen.bmj.com/ on June 7, 2025 at Agence Bibliographique de Table 1: Pilot search strategies in PubMed/MEDLINE database 192 Search arm Search term Data Search Number source restrictions articles retrieved PubMed/ English Dietary #1 Protected by copyright, including for uses related to text and Medline language 96 (dietary[Text Word])) OR (micronutrient*[Text Word])) OR (trace element[Text Word]))) Hits=1,055,480 2 #2 Micronutrients ((((((((((((((((((vitamin*[Text Word]) OR (mineral*[Text Word])) (calcium[Text Word])) OR OR (magnesium[Text Word])) OR (vitamin K[Text Word])) OR (phosphorus[Text Word])) OR (zinc[Text Word])) OR (copper[Text Word])) OR (selenium[Text Word])) OR (Sodium[Text Word])) OR (Chloride[Text Word])) OR (Sulfur[Text Word])) OR (Iron[Text Word])) OR (Manganese[Text Word])) OR (Iodine[Text Word])) OR (Fluoride[Text Word])))) data mining, AI training, and similar technologies Hits=2,716,784 #3 3 Abdominal aortic ((((abdominal aortic calcification) OR (abdominal calcification calcification)) OR (aortic calcification of abdomen))))) Hits=5,488 #1 AND #2 AND #3 4 The word "OR" was used to combine search terms within each arm and the word "AND" was 193 used to connect the three search arms (#1 AND #2 AND #3). 194 195 49 196 50 51 197 52 53 198 54 199 55 56 57 58 8 59 For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml 60

Protocol

The systematic review and meta-analysis will be reported in accordance with the revised Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P) recommendations (Figure 1). If protocol amendments are required after registration, the date, justification, and details of the changes for each part will be provided.

Data management

Covidence will import, screen, store, and analyze the results of the literature search. Covidence will automatically remove duplicates while also manually checking for study similarities (year of publication, author's name, volume, issue, etc.) via authors.

209 Selection of studies

EWT, ZHG, NAH and MBM will review the retrieved citations to determine study eligibility. In brief, the process for selecting studies for inclusion in the review and meta-analysis will be as follows: merge all identified records using EndNote, remove duplicate records of the same report, retrieve full text of potentially relevant reports, link together multiple reports of the same study (using the first or largest report as the primary record and subsequent reports to supplement other data), examine full-text reports for compliance with eligibility criteria, correspond with investigators, where appropriate, to clarify study eligibility and request missing data; make final decisions on study inclusion.

3 218 Extraction of data

A data extraction form will be developed as a standard data collection instrument. A pilot test of the form will be done among groups, and the form will be changed as needed based on group feedback. Data from included studies will be extracted independently by three reviewers (EWT, NAH and MBM). Reviewers will conduct a calibration exercise to ensure that their evaluation methodologies are consistent across reviewers. In the event of a disagreement between reviewers, the third investigator (ZHG) will be consulted.

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Data items

> The following data elements will be collected from the included studies: (i) study information: title, author name, year of publication, country of study, journal, sample size, study duration, study design, follow-up period, and limitations. (ii) population: characteristics of the participants, such as mean age, social economic status, race, or ethnicity, and whether there was a diagnosis of AAC. (iii) exposure: dietary micronutrient type and quantity. (iv) outcome: a composite of outcome occurrences, the commencement of the follow-up period, and the length of follow-up for outcome variables: (v) effect measures: reported effect measures for composite and distinct outcomes, if available, including p-values, standard deviation, and confidence intervals; and (vi) funding sources.

Risk of bias and quality assessment

The risk of bias for observational studies will be assessed using the Newcastle-Ottawa Scale (NOS). In addition, publication bias will be evaluated through visual inspection of funnel plots and the Egger's and Begg's regression tests. Summary estimates of confidence in the evidence will be assessed using the Grading of Recommendations Assessment Development and Evaluation (GRADE) of prognostic evidence. GRADE for evidence about prognosis starts with high quality evidence that can then be rated down. These criteria are based on; (1) five domains diminishing confidence (-1 for risk of bias, inconsistency, imprecision, indirectness, and publication bias) and (2) two situations increasing confidence (+1 or +2 for large-very large effect size and a +1 for a dose-response gradient). Two reviewers (NAH and MBM) will be assigned to evaluate the quality of each study, with a third reviewer (ZHG) consulted when conflicts emerge. The findings of the risk of bias assessment will be given in a table.

Statistical analysis and data synthesis

The analysis of outcome variables will be given according to either: (1) AAC present or absent (2) AAC classified as 'low' (referent—lowest reported group) against 'high' (all other groups) or (3) dose-response where AAC was assessed in three or more groups. We will use subgroup analysis to investigate clinical heterogeneity (general population, sex, race/ethnicity and age of cohort <60, 60-69, and \geq 70 years old) and methodological heterogeneity (risk of bias of studies, imaging modality such as radiograph, DXA, or CT and duration of follow-up for outcome measurement

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 $<5, 5-9, and \geq 10$ years). Meta-regression will be conducted using a random effects model in the above subgroup categories.

Between study heterogeneity will be calculated using I^2 statistic, with thresholds of 0-25%, 25% -49%, 50% -75%, and above 75% indicating low, moderate, high, and very high heterogeneity, respectively (30) where AAC events were reported individually pooled risk difference and risk ratios with 95% confidence intervals will be generated, followed by a summary estimate using Der Simonian-Laird random effects models. Furthermore, meta-analysis will be performed if the included studies are homogenous to calculate the effect of dietary micronutrients intake on AAC. This analysis will be performed using R version 3.6.1 software, and random effects will be used to determine the weights for the meta-analysis if necessary.

If various types of effect measures are utilized in the original studies, such as ORs, risk ratios, and hazard ratios, the meta-analysis will be further conducted separately for each type of effect measure. The study results will be reported in a sequential manner, starting with the primary outcomes, followed by secondary outcomes and important subgroup analysis, based on design, setup, and group classification methods will be conducted to investigate the possible causes of variability between studies and to explore the strength of the meta-analysis.

Ethics and dissemination

The results will be disseminated through publishing in a peer-reviewed journal and public presentations at relevant local, national, and international conferences, workshops, and symposiums. Ethical approval is not required as this is a systematic review of publicly available data.

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Declaration **Contributors:** All the authors have made substantial intellectual contributions to the development of the protocol: EWT, NAH, MBM, and ZHG contributed to the study concept and design. NAH and MBM formulated the search strategy. EWT led the writing of the manuscript and all authors conceived the conceptual ideas presented in the revised protocol critically. EWT and NAH drafted the risk of bias assessment section. ZHG developed the meta-bias and confidence in cumulative evidence sections. All authors read and approved the revised version and final supported versions. EWT has the primary responsibility for the final content. **Funding:** This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. **Competing interests:** The authors have declared that no competing interest exists. **Ethics approval:** The systematic review and meta-analysis does not require ethical approval. Patient consent for publication: Not required. Patient and public involvement statement: There is no patient or public involved in this systematic review/meta-analysis. Data Availability Statement: No datasets were generated or analyzed during the current study. However, all relevant data from this study will be made available upon study completion.





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BMJ Open

The Effect of Dietary Micro-Nutrients Intake on Abdominal Aortic Calcification: A Study Protocol for Systematic Review and Meta-Analysis

Journal:	BMJ Open
Manuscript ID	bmjopen-2024-096551.R1
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Primary Subject Heading :	Pharmacology and therapeutics
Secondary Subject Heading:	Nutrition and metabolism, Infectious diseases, Complementary medicine
Keywords:	Cardiovascular Disease, NUTRITION & DIETETICS, Drug Therapy, Nutritional support < GASTROENTEROLOGY, GERIATRIC MEDICINE



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2 3 4	1	Original protocol
5 6 7	2	The Effect of Dietary Micro-Nutrients Intake on Abdominal Aortic Calcification: A Study
7 8 9	3	Protocol for Systematic Review and Meta-Analysis
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12 13	5	Etsay Weldekidan Tsegay ^{1*} , Nigus Alemu Hailu ² , Meresa Berwo Mengesha ³ , Zenawi Hagos
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29 Abstract

Introduction: Healthy dietary choices have an important role in preventing chronic diseases such as cardiovascular disease (CVD). Increasing evidence suggests micronutrient intake (essential minerals and vitamins) is associated with abdominal aortic calcification (AAC), which is an advanced marker of CVD. However, the existing reports seem inconsistent. Some studies reported micronutrients are associated with lower risk of AAC, while others have reported increasing risk. Therefore, this systematic review and meta-analysis sought to summarize the available evidence on the association of dietary micronutrient intake on AAC.

Methods and Analysis: A comprehensive systematic search of the PubMed/MEDLINE, EMBASE, Web of Science, and Google Scholar databases from their inception up to September 1, 2024, will be conducted. All clinical studies that report eligible exposure/s (dietary micronutrients intake) and outcome/s (presence/severity of AAC) will be included, and this systematic review and meta-analysis protocol will be reported following the revised PRISMA-P guidelines. The risk of bias for observational studies will be assessed using the Newcastle-Ottawa Scale (NOS) and Publication bias will be evaluated through visual inspection of funnel plots and the Egger's and Begg's regression tests. The Der Simonian and Laird random effects model meta-analysis will be calculated to provide pooled results and the weighted risk ratio with their 95% confidence intervals will be presented.

Ethics and dissemination: The results will be disseminated through publishing in a peer-reviewed
 journal and public presentations at relevant local, national, and international conferences,
 workshops, and symposiums. Ethical approval is not required as this is a systematic review of
 publicly available data.

Prospero registration number: CRD42024575902

52 Keywords: Dietary micronutrients, abdominal aortic calcification, cardiovascular disease.

1 2 3	58	Strengths and limitations of this study
4 5	50	• This study will use meta regression to identify sources of beterogeneity and identify
6 7	59 60	subgroups in which dietary micronutrients are more or less predictive of positive outcomes
8 9	61	• This is the first systematic review and meta-analysis that has investigated the association
10 11	62	between dietary micronutrients and abdominal aortic calcification (AAC)
12	63	 The main limitation of this review is the discrepancies in imaging modality measurement
13 14	64	and reporting of AAC across studies but we attempted to overcome this by exploring these
15 16	65	aspects in pre-specified sub analyses
17 18	66	 Further limitation is that this study has language restriction
19 20	67	· · · · · · · · · · · · · · · · · · ·
20 21	68	
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87 Introduction

Abdominal aortic calcification (AAC) is an advanced marker of cardiovascular disease (CVD), occurring in one out of every three individuals between the ages of 45 and 54, and nine out of ten individuals aged 75 years and older ¹. AAC is characterized by deposition of calcium within the arterial wall of abdominal aorta ². It is independently associated with not only CVD risk ³, but also muscle strength decline ⁴, lower bone mineral density, fractures ⁵, falls ⁶, and dementia ⁷.

Despite AAC's association with debilitating medical conditions, the evidence regarding its
prevention and available treatment is very limited. Randomized trials and cohort studies have
examined the effect of intensive glycemic treatment, vitamin B, calcium supplementation,
phosphate binders, and etidronic acid on AAC, however the results shows no influence on AAC
occurrence or severity ⁸⁻¹⁵.

Dietary strategies, particularly micronutrients such as potassium, zinc, selenium, magnesium, Vitamin C and K have gained significant attention for their pivotal role in the prevention of CVDs ¹⁶. However, the relationship between dietary micronutrients intake and AAC remains uncertain ¹⁷. One cohort study indicated that a higher intake of dietary zinc was associated with an 8% lower risk of severe AAC after adjustment for age, gender, and ethnicity, though no association was found in the fully adjusted model ¹⁸. Similarly, higher consumption of dietary potassium ¹⁹, selenium ²⁰, and copper ²¹ has been linked to decrease in AAC incidence ²⁰. Furthermore, dietary copper intake has been associated with a decreased risk of severe AAC²².

The relationship between dietary calcium intake and AAC is also complex. While higher dietary calcium intake has been associated with a lower prevalence of AAC at baseline, it has not been related to changes in prevalence of AAC over 2 to 5 years ¹². This finding is inconsistent with a cross-sectional study on diabetic mellitus patients, which found no significant association between dietary calcium and AAC progression ²³. However, in health adults, there is an independent correlation between increased dietary calcium intake and severe AAC ²⁴.

A cross-sectional study in healthy participants found that increasing self-reported total magnesium
 intake by 50 mg/day resulted in 12% decreased in presence of AAC ²⁵, and another study reported
 that a 0.1 mmol/L increase in serum magnesium (Mg) being independently associated with a 1.1
 point decrease in AAC score ²⁶.

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Higher dietary Vitamin C intake has been linked to a reduced AAC score ^{16,27} and a lower risk of AAC in Adults ¹⁶. However, population based study have failed to demonstrate a significant correlation between dietary Vitamin C intake and risk of severe AAC ²⁷. Furthermore, there is significant inverse relationship between higher folate (Vitamin B₉) intake and the risk of severe AAC ²⁸, as well as a lower risk of AAC with higher dietary Vitamin A ²⁹. Given the relationship between AAC and CVD, as well as the challenges it poses in terms of treatment, dietary micronutrients intake has drawn substantial attention for their potential role in preventing and ameliorating AAC²⁹. Although there are reasonable biological mechanisms that connect certain micronutrients to AAC, the current evidence is not conclusive. Additionally, the long-term effects of micronutrient intake on the presence/severity of AAC remain unclear. Therefore, there is a need to conduct a systematic review and meta-analysis to synthesize the robust evidence regarding the effect of micronutrient intake on the development, severity, or progression of AAC. Moreover, the association between specific dietary micronutrients intake and the presence and severity of AAC will be examined.

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3 4	139	Methods and analysis
5 6 7	140	Eligibility criteria
8	141	Population
9 10	142	This review will focus on observational human studies of dietary micronutrients intake and their
11 12	143	effect on severity /presence of AAC diagnosed using different modality.
13 14	144	Exposure
15	145	Micronutrients intake
16 17	146	Vitamins A, B-complex (B1, B2, B3, B5, B6, B7, B9, B12), C, D, E, and K and calcium, iron,
18 19	147	magnesium, zinc, selenium, iodine, copper, manganese, and chromium
20	148	Outcome of interest
21 22	149	Primary outcome
23 24	150	The primary outcome will be presence and severity of AAC. Presence of AAC identified from
25 26	151	either radiography, dual X-ray absorptiometry (DXA) machine, MRI, or CT scan and will be
27	152	presented as AAC present or absent. Severity AAC will be categorized as low (lowest reported
28 29 30	153	category), moderate (middle reported categories) and high (highest reported category).
31 32	154	Secondary outcome of interest will be the effect of specific dietary micronutrient intake in severity
33 34	155	and progression of AAC (rate of change in calcification volume, area, or score over time). This
35	156	review defines AAC as the presence of calcified deposits in the abdominal aorta identified from
36 37 38	157	radiography, dual X-ray absorptiometry (DXA), MRI, or CT scan.
39 40	158	Setting and Languages
41 42	159	This study will not restrict studies by settings, and only articles reported by English language
43	160	(researchers lack the translation skills and have also resource limitation to pay for the translation
44 45	161	services) will be included.
46 47	162	Study design.
48 49	163	Observational cohort studies (both retrospective and prospective cohort studies), case-control
50 51 52	164	studies, and cross-sectional studies that report eligible exposure(s) and outcome(s) will be included
52 53 54	165	in this review.
55 56 57	166	
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3 4	167	Inclusion criteria
5 6	168	• Observational studies in humans. These include cohort (both retrospective and prospective
7 8	169	cohort studies), case-control and cross-sectional studies that report eligible exposure(s)
9 10	170	and outcome(s)
11	171	• AAC assessed by any methodology.
12 13 14	172 173	• Report of any dietary micronutrient effect on abdominal aortic outcome Exclusion criteria
15 16	174	We will exclude case series, case reports, commentaries, editor letters, and reviews that do not
17 18	175	contain any original data. We will exclude animal research from this review.
19	176	Search engines.
20 21	177	To discover relevant studies, comprehensive literature searches on dietary micronutrients intake
22 23	178	and AAC will be conducted in PubMed/MEDLINE, EMBASE, the Web of Science core
24 25	179	collection, Google Scholar, and CINAHL. Because no publication date limits were specified, the
26	180	search will run from inception to September 1, 2024. The information sources will be updated
27 28	181	prior to the submission of this evaluation. To ensure that all relevant studies are included in this
29 30 31 32 33 34 35	182	review, the information sources will be augmented by examining the reference lists of selected
	183	studies and systematic reviews of comparable scope.
	184	Search strategies
	185	The search strategy for PubMed will be developed using a combination of medical subject
36 37	186	headings (MeSH), keywords (text word), and "[Title/Abstract] related to both exposure and the
38	187	outcome of interest and combined using Boolean operators (AND, OR, NOT). The literature
39 40	188	search will be conducted without any date constraints. The search strategies for
41 42	189	PubMed/MEDLINEis provided in Table 1.
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Database	Search restriction	Search strategy	Number retrieve articles
PubMed/ MEDLIN E	Language/ English only	 #1 "Vascular Calcification"[MeSH Terms] OR "vascular calcification associated factor human"[Supplementary Concept] OR "abdom* aort* calcif*"[Title/Abstract] OR "aort* calcif*"[Title/Abstract] OR "vascular calcif*"[Title/Abstract] OR "arter* calcif*"[Title/Abstract] OR "acc"[Title/Abstract] OR "arter* calcif*"[Title/Abstract] OR "acc"[Title/Abstract] Hits=23,650 #2 "Micronutrients"[MeSH Terms] OR "Micronutrients"[Pharmacological Action] OR "Dietary Supplements"[MeSH Terms] OR "diet* micronutrient*"[Title/Abstract] OR "incronut* intake"[Title/Abstract] OR "nutrition* intake"[Title/Abstract] OR "vitamin* intake"[Title/Abstract] OR "nutrition* intake"[Title/Abstract] OR "vitamin* intake"[Title/Abstract] OR "diet* antioxidant*"[Title/Abstract] OR "trace element*"[Title/Abstract] OR "diet* antioxidant*"[Title/Abstract] OR "detect* acc"[Title/Abstract] OR "aca occurren*"[Title/Abstract] OR "calcifie* burden*[Title/Abstract] OR ("augment altern commun*[Journal] OR "acd appl ceram*[Journal] OR "aca"[All Fields] AND "calcif*"[Title/Abstract]) OR "vascular calcifie*"[Title/Abstract] OR ("augment altern commun*[Journal] OR "ava appl ceram*[Journal] OR "aca"[All Fields]) AND "mortalit*"[Title/Abstract]) OR "vascular calcifie*"[Title/Abstract] OR ("augment altern commun*[Journal] OR "ava appl ceram*[Journal] OR "aca"[All Fields]) AND "mortalit*"[Title/Abstract])) Hits=228,663 #1 AND #2 AND	827
		Filter on/ Language/ English only	778

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	201	Protocol
	202	The systematic review and meta-analysis will be reported in accordance with the revised Preferred
	203	Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P)
	204	recommendations (Figure 1). If protocol amendments are required after registration, the date,
	205	justification, and details of the changes for each part will be provided.
	206	Data management
	207	Covidence will import, screen, store, and analyze the results of the literature search. Covidence
	208	will automatically remove duplicates while also manually checking for study similarities (year of
	209	publication, author's name, volume, issue, etc.) via authors.
21 22	210	Selection of studies
22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37	211	EWT, ZHG, NAH and MBM will review the retrieved citations to determine study eligibility. In
	212	brief, the process for selecting studies for inclusion in the review and meta-analysis will be as
	213	follows: merge all identified records using EndNote, remove duplicate records of the same report,
	214	retrieve full text of potentially relevant reports, link together multiple reports of the same study
	215	(using the first or largest report as the primary record and subsequent reports to supplement other
	216	data), examine full-text reports for compliance with eligibility criteria, correspond with
	217	investigators, where appropriate, to clarify study eligibility and request missing data; make final
	218	decisions on study inclusion.
	219	Extraction of data
38 39	220	A data extraction excel form will be developed as a standard data collection instrument. For
40 41	221	standardization of the data extraction tool, a pilot test of the form will be done among five selected
42	222	articles, and the form will be changed as needed based on group feedback. Furthermore, reviewers
45 44	223	will conduct a calibration exercise to ensure that their evaluation methodologies are consistent
45 46	224	across reviewers. And data from included studies will be extracted independently by three
47 48	225	reviewers (EWT, NAH and MBM) In the event of a disagreement between reviewers, the third
49	226	investigator (ZHG) will be consulted.
50 51	227	Data items
52 53	228	The following data elements will be collected from the included studies: (i) study information:
54	229	title, author name, year of publication, country of study, journal, sample size, study duration, study
56 57	230	design, follow-up period, and limitations. (ii) Population: characteristics of the participants, such

as mean age, social economic status, race, or ethnicity, and whether there was a diagnosis of AAC. (iii) Exposure: dietary micronutrient type and quantity. (iv) Outcome: a composite of outcome occurrences, the commencement of the follow-up period, and the length of follow-up for outcome variables; (v) effect measures: reported effect measures for composite and distinct outcomes, if available, including p-values, standard deviation, and confidence intervals; and (vi) funding sources. **Risk of bias and quality assessment Risk of bias and Quality Assessment** The risk of bias for observational studies will be assessed using the Newcastle-Ottawa Scale (NOS). In addition, publication bias will be evaluated through visual inspection of funnel plots and the Egger's and Begg's regression tests. Summary estimates of confidence in the evidence will

be assessed using the Grading of Recommendations Assessment Development and Evaluation (GRADE) of prognostic evidence. GRADE for evidence about prognosis starts with high quality evidence that can then be rated down. These criteria are based on; (1) five domains diminishing confidence (-1 for risk of bias, inconsistency, imprecision, indirectness, and publication bias) and (2) two situations increasing confidence (+1 or +2 for large-very large effect size and a +1 for adose-response gradient). Two reviewers (NAH and MBM) will be assigned to evaluate the quality of each study, with a third reviewer (ZHG) consulted when conflicts emerge. The findings of the risk of bias assessment will be given in a table.

37
38 250 Statistical analysis and data synthesis

⁴⁰ 251 The analysis was conducted using the R statistical software version 4.4.3 ³⁰.

The analysis of outcome variables will be given according to either: (1) AAC present or absent (2)
AAC classified as 'low' (referent—lowest reported group) against 'high' (all other groups) or (3)
dose-response where AAC was assessed in three or more groups.

We will use subgroup analysis to investigate clinical heterogeneity (general population, sex, race/ethnicity and age of cohort <60, 60-69, and \geq 70 years old) and methodological heterogeneity (risk of bias of studies, imaging modality such as radiograph, DXA, or CT and duration of follow-up for outcome measurement <5, 5-9, and \geq 10 years). Meta-regression will be conducted using a random effects model in the above subgroup categories.

Between study heterogeneity will be calculated using I² statistic with thresholds of 0-25%, 25% – 49%, 50% - 75%, and above 75% indicating low, moderate, high, and very high heterogeneity, respectively ³¹ where AAC events were reported individually pooled risk difference and risk ratios with 95% confidence intervals will be generated, followed by a summary estimate using Der Simonian-Laird random effects models. Furthermore, meta-analysis will be performed if the included studies are homogenous to calculate the effect of dietary micronutrients intake on AAC. This analysis will be performed using R version 3.6.1 software, and random effects will be used to determine the weights for the meta-analysis if necessary.

If various types of effect measures are utilized in the original studies, such as ORs, risk ratios, and hazard ratios, the meta-analysis will be further conducted separately for each type of effect measure using R software. The study results will be reported in a sequential manner, starting with the primary outcomes, followed by secondary outcomes and important subgroup analysis, based on design, setup, and group classification methods will be conducted to investigate the possible causes of variability between studies and to explore the strength of the meta-analysis.

274 Sensitivity analysis

Sensitivity analysis will be carryout to assess the impact of the different dietary assessment
methods such as 24h recall, food frequency questioners, and dietary records.

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277 Ethics and dissemination

The results will be disseminated through publishing in a peer-reviewed journal and public presentations at relevant local, national, and international conferences, workshops, and symposiums. Ethical approval is not required as this is a systematic review of publicly available data.

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42 43	420	Figure 1. Preferred Reporting Items for Systematic Review and Meta – Analysis
44 45	421 422	Protocols (PRISMA-P) 2020 statement ³²
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45	450	Contributors: All the authors have made substantial intellectual contributions to the dev

velopment of the protocol: EWT, NAH, MBM, and ZHG contributed to the study concept and design. NAH and MBM formulated the search strategy. EWT led the writing of the manuscript and all authors conceived the conceptual ideas presented in the revised protocol critically. EWT and NAH drafted the risk of bias assessment section. ZHG developed the meta-bias and confidence in cumulative evidence sections. All authors read and approved the revised version and final supported versions.

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	462	Patient consent for publication: Not required.
	463	Patient and public involvement statement: There is no patient or public involved in this
17	464	systematic review/meta-analysis.
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20 21	466	However, all relevant data from this study will be made available upon study completion.
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The Effect of Dietary Micro-Nutrients Intake on Abdominal Aortic Calcification: A Study Protocol for Systematic Review and Meta-Analysis

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1 2		
2 3 4	1	Original protocol
5 6	2	The Effect of Dietary Micro-Nutrients Intake on Abdominal Aortic Calcification: A Study
7 8	3	Protocol for Systematic Review and Meta-Analysis
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11 12	5	Etsay Weldekidan Tsegay ^{1*} Nigus Alemu Hailu ² Meresa Berwo Mengesha ³ Zenawi Hagos
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29 Abstract

> Introduction: Healthy dietary choices have an important role in preventing chronic diseases such as cardiovascular disease (CVD). Increasing evidence suggests micronutrient intake (essential minerals and vitamins) is associated with abdominal aortic calcification (AAC), which is an advanced marker of CVD. However, the existing reports seem inconsistent. Some studies reported micronutrients are associated with lower risk of AAC, while others have reported increasing risk. Therefore, this systematic review and meta-analysis sought to summarize the available evidence on the association of dietary micronutrient intake on AAC.

Methods and Analysis: A comprehensive systematic search of the PubMed/MEDLINE, EMBASE, Web of Science, and Google Scholar databases from their inception up to September 1, 2024, will be conducted. All clinical studies that report eligible exposure/s (dietary micronutrients intake) and outcome/s (presence/severity of AAC) will be included, and this systematic review and meta-analysis protocol will be reported following the revised PRISMA-P guidelines. The risk of bias for observational studies will be assessed using the Newcastle-Ottawa Scale (NOS) and Publication bias will be evaluated through visual inspection of funnel plots and the Egger's and Begg's regression tests. The Der Simonian and Laird random effects model meta-analysis will be calculated to provide pooled results and the weighted risk ratio with their 95% confidence intervals will be presented.

47 Ethics and dissemination: The results will be disseminated through publishing in a peer-reviewed
 48 journal and public presentations at relevant local, national, and international conferences,
 49 workshops, and symposiums. Ethical approval is not required as this is a systematic review of
 50 publicly available data.

Prospero registration number: CRD42024575902

52 Keywords: Dietary micronutrients, abdominal aortic calcification, cardiovascular disease.

1 2 3	50	Strongths and limitations of this study
4 5	20	Strengths and minitations of this study
6 7	59	• Sensitivity analysis will be carryout to assess the impact of the different dietary assessment
8	60	methods such as 24h recall, food frequency questioners, and dietary records.
9 10	61	• The main limitation of this review is the discrepancies in imaging modality, measurement
11 12	62	and reporting of AAC across studies but we attempted to overcome this by exploring these
13	63	aspects in pre-specified sub analyses.
14 15	64	• Further limitation is that this study has language restriction
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87 Introduction

Abdominal aortic calcification (AAC) is an advanced marker of cardiovascular disease (CVD), occurring in one out of every three individuals between the ages of 45 and 54, and nine out of ten individuals aged 75 years and older ¹. AAC is characterized by deposition of calcium within the arterial wall of abdominal aorta ². It is independently associated with not only CVD risk ³, but also muscle strength decline ⁴, lower bone mineral density, fractures ⁵, falls ⁶, and dementia ⁷.

Despite AAC's association with debilitating medical conditions, the evidence regarding its
prevention and available treatment is very limited. Randomized trials and cohort studies have
examined the effect of intensive glycemic treatment, vitamin B, calcium supplementation,
phosphate binders, and etidronic acid on AAC, however the results shows no influence on AAC
occurrence or severity ⁸⁻¹⁵.

Dietary strategies, particularly micronutrients such as potassium, zinc, selenium, magnesium, Vitamin C and K have gained significant attention for their pivotal role in the prevention of CVDs ¹⁶. However, the relationship between dietary micronutrients intake and AAC remains uncertain ¹⁷. One cohort study indicated that a higher intake of dietary zinc was associated with an 8% lower risk of severe AAC after adjustment for age, gender, and ethnicity, though no association was found in the fully adjusted model ¹⁸. Similarly, higher consumption of dietary potassium ¹⁹, selenium ²⁰, and copper ²¹ has been linked to decrease in AAC incidence ²⁰. Furthermore, dietary copper intake has been associated with a decreased risk of severe AAC²².

The relationship between dietary calcium intake and AAC is also complex. While higher dietary calcium intake has been associated with a lower prevalence of AAC at baseline, it has not been related to changes in prevalence of AAC over 2 to 5 years ¹². This finding is inconsistent with a cross-sectional study on diabetic mellitus patients, which found no significant association between dietary calcium and AAC progression ²³. However, in health adults, there is an independent correlation between increased dietary calcium intake and severe AAC ²⁴.

A cross-sectional study in healthy participants found that increasing self-reported total magnesium
intake by 50 mg/day resulted in 12% decreased in presence of AAC ²⁵, and another study reported
that a 0.1 mmol/L increase in serum magnesium (Mg) being independently associated with a 1.1
point decrease in AAC score ²⁶.

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Higher dietary Vitamin C intake has been linked to a reduced AAC score ^{16,27} and a lower risk of AAC in Adults ¹⁶. However, population based study have failed to demonstrate a significant correlation between dietary Vitamin C intake and risk of severe AAC ²⁷. Furthermore, there is significant inverse relationship between higher folate (Vitamin B₉) intake and the risk of severe AAC ²⁸, as well as a lower risk of AAC with higher dietary Vitamin A ²⁹. Given the relationship between AAC and CVD, as well as the challenges it poses in terms of treatment, dietary micronutrients intake has drawn substantial attention for their potential role in preventing and ameliorating AAC²⁹. Although there are reasonable biological mechanisms that connect certain micronutrients to AAC, the current evidence is not conclusive. Additionally, the long-term effects of micronutrient intake on the presence/severity of AAC remain unclear. Therefore, there is a need to conduct a systematic review and meta-analysis to synthesize the robust evidence regarding the effect of micronutrient intake on the development, severity, or progression of AAC. Moreover, the association between specific dietary micronutrients intake and the presence and severity of AAC will be examined.

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3 4	139	Methods and analysis
5 6 7	140	Eligibility criteria
8	141	Population
9 10	142	Adults with or at risk of abdominal aortic calcification will be the population interest.
11 12	143	Exposure
12	144	Micronutrients intake
15	145	The intervention is intake of micronutrients such as Vitamins A, B-complex (B1, B2, B3, B5, B6,
16 17	146	B7, B9, B12), C, D, E, and K and calcium, iron, magnesium, zinc, selenium, iodine, copper,
18 19	147	manganese, and chromium. To measure the intake and status of the listed micronutrients, a
20	148	combination of dietary assessment, biochemical tests, and functional evaluations is typically used.
21 22	149	Comparator
23 24	150	For this systematic review and meta-analysis protocol, the reference group comprises individuals
25 26	151	with lower intake or absence of the dietary micro-nutrients.
27	152	Outcome of interest
28 29	153	Primary outcome
30 31	154	The primary outcome will be presence and severity of AAC. Presence of AAC identified from
32	155	either radiography, dual X-ray absorptiometry (DXA) machine, MRI, or CT scan and will be
34	156	presented as AAC present or absent. Severity AAC will be categorized as low (lowest reported
35 36 37	157	category), moderate (middle reported categories) and high (highest reported category).
38 39	158	Secondary outcome of interest will be the effect of specific dietary micronutrient intake in severity
40 41	159	and progression of AAC (rate of change in calcification volume, area, or score over time). This
42	160	review defines AAC as the presence of calcified deposits in the abdominal aorta identified from
43 44 45	161	radiography, dual X-ray absorptiometry (DXA), MRI, or CT scan.
46 47	162	Setting and Languages
48 49	163	This study will not restrict studies by settings, and only articles reported by English language will
50	164	be included.
51 52	165	Study design.
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3 4	166	Observational cohort studies (both retrospective and prospective cohort studies), case-control
5 6	167	studies, and cross-sectional studies that report eligible exposure(s) and outcome(s) will be included
7 8 9	168	in this review.
10 11 12	169	Inclusion criteria
13 14	170	• Observational studies in humans. These include cohort (both retrospective and prospective
15 16	171	cohort studies), case-control and cross-sectional studies that report eligible exposure(s)
17	172	and outcome(s)
18 19	173	• AAC assessed by any methodology.
20 21 22	174 175	• Report of any dietary micronutrient effect on abdominal aortic outcome Exclusion criteria
23 24	176	We will exclude case series, case reports, commentaries, editor letters, and reviews that do not
25 26	177	contain any original data. We will exclude animal research from this review.
20	178	Search engines.
28 29	179	To discover relevant studies, comprehensive literature searches on dietary micronutrients intake
30 31	180	and AAC will be conducted in PubMed/MEDLINE, EMBASE, the Web of Science core
32	181	collection, Google Scholar, and CINAHL. Because no publication date limits were specified, the
33 34	182	search will run from inception to September 1, 2024. The information sources will be updated
35 36	183	prior to the submission of this evaluation. To ensure that all relevant studies are included in this
37 38	184	review, the information sources will be augmented by examining the reference lists of selected
39	185	studies and systematic reviews of comparable scope.
40 41	186	Search strategies
42 43	187	The search strategy for PubMed will be developed using a combination of medical subject
44	188	headings (MeSH), keywords (text word), and "[Title/Abstract] related to both exposure and the
45 46	189	outcome of interest and combined using Boolean operators (AND, OR, NOT). The literature
47 48	190	search will be conducted without any date constraints. The search strategies for
49 50	191	PubMed/MEDLINEis provided in Table 1. In addition to the MeSH terms, this systematic review
51	192	and meta-analysis protocol will incorporate the PICO framework (Population, Intervention,
52 53 54 55 56	193	Comparator, and Outcome).

We will apply the PICO framework in our review protocol as follows: Population: This targets

Individuals at risk of or diagnosed with abdominal aortic calcification (AAC); Intervention: The intervention is Dietary intake of micro-nutrients mainly vitamins and minerals. Comparator: We will compare Individuals with lower intake or absence of the dietary micro-nutrients. Outcome: Primary outcomes of interest will include Presence/severity/progression of abdominal aortic calcification, measured via imaging (e.g., CT scans, lateral spine radiographs, or echocardiography), while other reported outcome events can be considered secondary outcomes. to peer terien only

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Database	Search restriction	Search strategy	Num of retri artic
PubMed/ MEDLIN E	Language/ English only	 #1 "Vascular Calcification"[MeSH Terms] OR "vascular calcification associated factor human"[Supplementary Concept] OR "abdom* aort* calcif*"[Title/Abstract] OR "aort* calcif*"[Title/Abstract] OR "arter* calcif*"[Title/Abstract] OR "acc"[Title/Abstract] OR "arter* calcif*"[Title/Abstract] OR "acc"[Title/Abstract] Hits=23,650 #2 "Micronutrients"[MeSH Terms] OR "Micronutrients"[Pharmacological Action] OR "Dietary Supplements"[MeSH Terms] OR "diet* micronutrient*"[Title/Abstract] OR "vitamin* intake"[Title/Abstract] OR "nincronut* intake"[Title/Abstract] OR "nincronut* intake"[Title/Abstract] OR "nincronut* intake"[Title/Abstract] OR "mineral* intake"[Title/Abstract] OR "trace element*"[Title/Abstract] OR "diet* antioxidant*"[Title/Abstract] OR "trace element*"[Title/Abstract] OR "diet* antioxidant*"[Title/Abstract] Wits= 864,097 #3 "Disease Progression"[MeSH Terms] OR ("Disease Progression"[MeSH Terms] OR (("presen*"[All Fields] AND "of aac"]Title/Abstract] OR "calcif*"[Title/Abstract] OR "calcif*" burden"[Title/Abstract] OR ("sever*"[All Fields] AND "of aac"[Title/Abstract] OR "calcif*"[Title/Abstract] OR "calcif*"[Title/Abstract] OR "calcif*"[Title/Abstract] OR "calcif*"[Title/Abstract] OR "calcif*"] wide acevelop*"[Title/Abstract] OR ("augment altern commun"]Journal] OR "calcif*"[Citle/Abstract] OR ("augment altern commun"]Journal] OR "av appl ceram"[Journal] OR "ace"[All Fields] AND "calcif*"[Title/Abstract]) OR "vascular calcif*"[Title/Abstract] OR (("augment altern commun"]Journal] OR "av appl ceram"[Journal] OR "ace"[All Fields]) AND "mortalit*"[Title/Abstract]))) Hits=228,663 #1 AND #2 AND #3 Hits=827 	827
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4 5 6 7 8 9 10 11 12 13 14 15	226	Protocol
	227	The systematic review and meta-analysis will be reported in accordance with the revised Preferred
	228	Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P)
	229	recommendations (Figure 1). If protocol amendments are required after registration, the date,
	230	justification, and details of the changes for each part will be provided.
	231	Data management
16	232	Covidence will import, screen, store, and analyze the results of the literature search. Covidence
18	233	will automatically remove duplicates while also manually checking for study similarities (year of
19 20	234	publication, author's name, volume, issue, etc.) via authors.
21 22	235	Selection of studies
23	236	EWT, ZHG, NAH and MBM will review the retrieved citations to determine study eligibility. In
24 25	237	brief, the process for selecting studies for inclusion in the review and meta-analysis will be as
26 27	238	follows: merge all identified records using EndNote, remove duplicate records of the same report,
28 29	239	retrieve full text of potentially relevant reports, link together multiple reports of the same study
30	240	(using the first or largest report as the primary record and subsequent reports to supplement other
31 32	241	data), examine full-text reports for compliance with eligibility criteria, correspond with
33 34	242	investigators, where appropriate, to clarify study eligibility and request missing data; make final
35 36	243	decisions on study inclusion.
37	244	Extraction of data
38 39	245	A data extraction excel form will be developed as a standard data collection instrument. For
40 41	246	standardization of the data extraction tool, a pilot test of the form will be done among five selected
42	247	articles, and the form will be changed as needed based on group feedback. Furthermore, reviewers
45 44	248	will conduct a calibration exercise to ensure that their evaluation methodologies are consistent
45 46	249	across reviewers. And data from included studies will be extracted independently by three
47 48	250	reviewers (EWT, NAH and MBM) In the event of a disagreement between reviewers, the third
49	251	investigator (ZHG) will be consulted.
50 51	252	Data items
52 53	253	The following data elements will be collected from the included studies: (i) study information:

title, author name, year of publication, country of study, journal, sample size, study duration, study
 design, follow-up period, and limitations. (ii) Population: characteristics of the participants, such

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as mean age, social economic status, race, or ethnicity, and whether there was a diagnosis of AAC. (iii) Exposure: dietary micronutrient type and quantity. (iv) Outcome: a composite of outcome occurrences, the commencement of the follow-up period, and the length of follow-up for outcome variables; (v) effect measures: reported effect measures for composite and distinct outcomes, if available, including p-values, standard deviation, and confidence intervals; and (vi) funding sources. **Risk of bias and Quality Assessment** The risk of bias for observational studies will be assessed using the Newcastle-Ottawa Scale

(NOS). In addition, publication bias will be evaluated through visual inspection of funnel plots and the Egger's and Begg's regression tests. Summary estimates of confidence in the evidence will be assessed using the Grading of Recommendations Assessment Development and Evaluation (GRADE) of prognostic evidence. GRADE for evidence about prognosis starts with high quality evidence that can then be rated down. These criteria are based on; (1) five domains diminishing confidence (-1 for risk of bias, inconsistency, imprecision, indirectness, and publication bias) and (2) two situations increasing confidence (+1 or +2 for large-very large effect size and a +1 for a dose-response gradient). Two reviewers (NAH and MBM) will be assigned to evaluate the quality of each study, with a third reviewer (ZHG) consulted when conflicts emerge. The findings of the risk of bias assessment will be given in a table.

- Statistical analysis and data synthesis
- The analysis will be conducted using the R statistical software version 4.4.3 30 .

The analysis of outcome variables will be given according to either: (1) AAC present or absent (2)

AAC classified as 'low' (referent—lowest reported group) against 'high' (all other groups) or (3)

dose-response where AAC was assessed in three or more groups.

We will use subgroup analysis to investigate clinical heterogeneity (general population, sex, race/ethnicity and age of cohort <60, 60-69, and \geq 70 years old) and methodological heterogeneity (risk of bias of studies, imaging modality such as radiograph, DXA, or CT and duration of follow-up for outcome measurement <5, 5-9, and \geq 10 years). Meta-regression will be conducted using a random effects model in the above subgroup categories.

Between study heterogeneity will be calculated using I² statistic with thresholds of 0-25%, 25% – 49%, 50% - 75%, and above 75% indicating low, moderate, high, and very high heterogeneity,

respectively ³¹ where AAC events were reported individually pooled risk difference and risk ratios with 95% confidence intervals will be generated, followed by a summary estimate using Der Simonian-Laird random effects models. Furthermore, meta-analysis will be performed if the included studies are homogenous to calculate the effect of dietary micronutrients intake on AAC.

Random effects will be used to determine the weights for the meta-analysis if necessary. A qualitative synthesis will be performed if there is significant heterogeneity ($I^2 \ge 50\%$ or P < 0.1) or in situations where the data are incomplete or unsuitable for meta-analysis. Moreover, when appropriate, additional analytical strategies will be evaluated to strengthen the robustness and depth of the analysis, such as subgroup analysis, sensitivity analysis, or meta-regression. Furthermore, to minimize the risk of bias and ensure the reliability of the findings, studies with certain designs (e.g., high risk of bias or methodological limitations) will be excluded from the analysis.

If various types of effect measures are utilized in the original studies, such as ORs, risk ratios, and hazard ratios, the meta-analysis will be further conducted separately for each type of effect measure using R software. The study results will be reported in a sequential manner, starting with the primary outcomes, followed by secondary outcomes and important subgroup analysis, based on design, setup, and group classification methods will be conducted to investigate the possible causes of variability between studies and to explore the strength of the meta-analysis.

³⁶₃₇ 304 Meta-bias (es)

Outcome reporting biases will be assessed by comparing outcomes documented in research protocols with those reported in the actual study reports. Additionally, sensitivity analysis will used to evaluate the impact of selective reporting on the results of meta-analyses, if deemed necessary. Funnel plots will also be employed to investigate potential publication bias. Furthermore, sensitivity analysis will be carryout to assess the impact of the different dietary assessment methods such as 24h recall, food frequency questioners, and dietary records.

49 311 Ethics and dissemination

The results will be disseminated through publishing in a peer-reviewed journal and public presentations at relevant local, national, and international conferences, workshops, and symposiums. Ethical approval is not required as this is a systematic review of publicly available data.

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3 4	423	1. Figure legends
5 6 7 8	424 425 426	Figure 1. Preferred Reporting Items for Systematic Review and Meta–Analysis Protocols (PRISMA-P) 2020 statement ³²
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2 3	452	Declaration
4 5 7 8 9 10	453	Contributors: All the authors have made substantial intellectual contributions to the development
	454	of the protocol: EWT, NAH, MBM, and ZHG contributed to the study concept and design. NAH
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	456	conceived the conceptual ideas presented in the revised protocol critically. EWT and NAH drafted
11 12	457	the risk of bias assessment section. ZHG developed the meta-bias and confidence in cumulative
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31 32 33 34	468	However, all relevant data from this study will be made available upon study completion.
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