Association between Micronutrients Intake/Status and Carotid Intima Media Thickness: A Systematic Review

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ABSTRACT

Background Carotid intima media thickness (IMT) is a noninvasive marker of the extent and severity of subclinical atherosclerosis. Micronutrient intake may affect atherosclerosis and play a major role in the development of cardiovascular diseases (CVDs).

Objective The primary aim of this review was to synthesize the evidence regarding the association between carotid IMT and selected micronutrients.

Method The authors searched PubMed, Cochrane, and EMBASE databases from inception to June 2016 for selected micronutrients, CVD, carotid IMT, and antioxidants. Thirty-five original studies met the inclusion criteria and were reviewed following preferred reporting items for systematic review and meta-analysis guidelines.

Results Although not all studies found consistent results, the weight of the evidence suggests that high intakes and/or circulatory levels of magnesium, as well as vitamin D and the vitamin B group, may be associated with lower carotid IMT or reduced progression of carotid IMT. The majority of studies did not find any significant association between vitamin E and C and carotid IMT. Less evidence was available for associations of retinol, zinc, and iron with carotid IMT.

Conclusions In general, the current evidence concerning micronutrient intake and carotid IMT is largely inconclusive. Pragmatic clinical trials are required to determine whether dietary or supplemental intake of specific micronutrients alters carotid IMT, which is a surrogate measure of cardiovascular risk.

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CARDIOVASCULAR DISEASE (CVD) IS THE MAJOR cause of death worldwide. According to the World Health Organization, about 17.5 million people died from CVD during 2012, representing 31% of all deaths worldwide.[1,2] Intima media thickness (IMT) of large elastic artery walls, particularly the carotid artery in adults, is a well-established noninvasive measure of the extent and severity of subclinical atherosclerosis, the disease process that underlies the majority of cardiovascular events.[3] Importantly, carotid IMT is associated with risk of incident CVD events, including myocardial infarction and ischemic stroke, independent of established cardiovascular risk factors.[4,5]

There is a large body of evidence from epidemiologic studies describing the potential role of diet in CVD prevention.[6] Indeed, several studies have indicated that about 80% of coronary artery disease cases could be prevented by modifying lifestyle, specifically healthy eating habits, maintaining a healthy weight, and undertaking regular physical activity throughout the life span.[6] Furthermore, there is accumulating experimental, epidemiologic, and clinical data suggesting an association between micronutrient intake and reduced risk of CVD, specifically for those with antioxidant properties. Dietary antioxidants are recognized to have a protective effect against lipid peroxidation, which is associated with atherogenesis and CVD.[7] Specifically, prospective studies suggest that consumption of vitamins A, C, and E is associated with lower rates of CVD mortality.[8,9] Moreover, it has been reported that low circulatory levels of micronutrients such as magnesium and carotenoids may lead to adverse cardiovascular consequences, such as extensive and complex effects on atherosclerosis and atherogenesis.[10,11]

METHODS

Search Strategy

The authors identified published studies using PubMed, Cochrane, and EMBASE databases. Google Scholar was also used to search for further studies. The following terms were used individually or in combination based on medical subject heading terms: *IMT*, *micronutrient*, *CVD*, *carotenoid*, *retinol*, *vitamin E*, *vitamin C*, *vitamin D*, *vitamin B*, *magnesium (Mg)*, *zinc (Zn)*, *folate*, *iron*, and *antioxidant*. All studies were considered from inception to June 2016 with no language restriction but limited to human beings. Additional studies were identified using hand search of references from previous review articles. See Figure 1 for an example of the search strategy.

1. IMT OR ((*tunica intima* [MeSH terms] OR (*tunica* AND *intima*) OR *tunica intima* OR *intima*) AND *media* AND *thick* AND *ness*)) OR *CVD*
2. *micronutrients* [MeSH terms] OR (*carotenoids* [MeSH terms] OR *carotenoids* OR *carotenoid*)) OR (*vitamin a* [MeSH terms] OR *vitamin a* OR *retinol*)) OR (*vitamin e* [MeSH terms] OR *vitamin e*) OR (*ascorbic acid* [MeSH terms] OR (*ascorbic* AND *acid*) OR *ascorbic acid* OR *vitamin c*)) OR (*vitamin d* [MeSH terms] OR *vitamin d* OR *ergocalciferols* [MeSH trms] OR *ergocalciferols*)) OR (*vitamin b complex* [Pharmacological Action] OR *vitamin b complex* [MeSH Terms] OR "vitamin b complex" OR "vitamin b")) OR ("magnesium"[MeSH terms] OR *magnesium*)) OR (*selenium* [MeSH terms] OR *selenium*)) OR (*zinc* [MeSH terms] OR *zinc*)) OR (*folic acid* [MeSH terms] OR (*folic* AND *acid*) OR *folic acid* OR *folate*)) OR (*iron* [MeSH terms] OR *iron*)) OR (*antioxidants* [Pharmacological Action] OR *antioxidants* [MeSH terms] OR *antioxidants* OR *antioxidant*)
3. 1 and 2
4. *Cohort Studies* [MeSH terms] OR *Intervention Studies* [MeSH terms] OR *Clinical trial* [MeSH terms] OR *cohort** [MeSH terms] OR *intervention** [MeSH terms] OR *trial**
5. Filters: Human

Figure 1. Example of search strategy using PubMed for studies on the association between micronutrients and intima media thickness (IMT). Search strategy was modified for individual databases.

Study Selection

Citations from literature databases were imported into the referencing software Endnote X7.7.[12] Title and abstract screen were analyzed according to our defined inclusion criteria. Only studies with a clinical trial, case-control, cross-sectional, or prospective cohort design, with a control or referent group, were included. Case studies, case reports, animal studies, and ecologic studies in human beings were excluded. Review articles were collected for the purposes of reviewing the reference list and did not contribute to the final number of studies considered eligible unless they also contained original data. The study factor was intake or circulating micronutrient levels (including carotenoids, retinol, vitamin E, vitamin C, vitamin D, vitamin B, magnesium, zinc, folate, and iron) and the primary outcome was carotid IMT, and progression of preexisting atherosclerotic lesions was regarded as a secondary outcome.

Articles that meet the inclusion criteria were considered for full-text review, unless the article was not available after all attempts to retrieve it were exhausted. Full-text of articles that could not be assessed for relevance based on the title and abstract screen were also obtained to assess eligibility. Studies were not considered further when the title and abstract clearly indicated that the study did not meet the inclusion criteria described above. The primary reason for excluding studies was a case where the article did not contain original data relevant to our eligibility criteria.

Data Abstraction

The abstracts of all potential articles were reviewed independently by the authors initially to identify eligible publications for data abstraction. Relevant study attributes (qualitative and quantitative) were abstracted from the selected articles and reviewed for accuracy using the preferred reporting items for systematic reviews and meta-analyses checklist. Data extracted included title; authors; study design; participant characteristics; study factors (eg, dosage/concentration of micronutrients); analysis with adjustment for confounding; main outcome measure; and findings, including statistical significance. Studies were grouped by the study factor; that is, carotenoids, vitamin E, vitamin C, vitamin D, and minerals, for synthesis of findings.

Study Quality

Study quality was assessed by the two authors (B. Hosseini, MSc, and A. Saedisomeolia, PhD) using the Academy of Nutrition and Dietetics Quality Criteria Checklist.[13] The checklist includes the following criteria:

1. research question statement;
2. selection bias;
3. group comparability;
4. withdrawal handling;
5. blinding use;
6. description of intervention/therapeutic regimens/exposure factors;
7. outcome definition as well as validity and reliability of the measurements;
8. statistical analysis;
9. conclusions; and
10. sponsorship bias.

According to the checklist, when the majority of the answers to the above validity questions is "Yes" (including criteria 2, 3, 6, 7, and at least one additional "Yes"), the report should be designated with a plus symbol (+), whereas when the answers to the validity criteria questions 2, 3, 6, and 7 do not indicate that the study is potentially strong, the report should be designated with a neutral symbol (Ø). Finally, if six or more answers to the validity questions are "No," the report should be designated with a minus symbol (−). The methodologic quality rating of the included studies is detailed in Table 1.

RESULTS

Initially, 990 abstracts were identified. Based on the titles and abstracts, 125 articles were retrieved for full-text review. Finally, a total of 35 original articles qualified to be reviewed (see Figure 2). The summary of studies on the association between micronutrient and IMT is presented in Table 2.

Carotenoids and Carotid IMT

A total of eight studies, including one cohort,[14] five cross-sectional[11,15-18] and two case-control[19,20] studies investigated the relationship between carotenoids and carotid IMT. Overall, 480 participants with 18 months of follow-up were included from the cohort study, 16,734 participants from cross-sectional studies, and 339 cases and 340 controls from case-control studies. Cases were either subjects with aortic atherosclerosis[19] or those who exceeded the 90th percentile of carotid IMT.[20] The majority of studies measured the levels of major carotenoids,[15,16,19,20] whereas some studies assessed only serum levels of lycopene[11,17] or lutein and beta carotene.[14] One study assessed the dietary intakes of carotenoids using a 66-item food frequency questionnaire.[18] The quality rating of the studies was positive (Table 1). Among the identified studies, one study concluded carotid IMT was inversely associated with plasma carotenoids,[16] and another study[17] observed a negative correlation between serum lycopene and mean and maximal carotid IMT in men. However, some studies did not find any significant association between carotenoid levels and carotid IMT.[15,19,20] A cross-sectional study by Kritchevsky and colleagues[18] observed an inverse association between quintile of carotenoid consumption and prevalence of carotid artery plaques. The other studies found mixed results. One study[14] assessed the levels of several carotenoids; however, the authors observed a negative association between lutein only and carotid IMT. Another study[11] reported that plasma lycopene level was negatively correlated with carotid IMT in men; however, the correlation did not remain statistically significant after adjustments in women.

In summary, there are discrepant findings regarding the carotenoids and carotid IMT, a few studies showed a beneficial effect of carotenoids on carotid IMT. Results were similar for circulating biomarkers of intake.

Retinol and Carotid IMT

Retinol is found in a complex with retinol-binding protein 4 (RBP4) and transthyretin in the circulation and is associated with various cardiovascular risk factors.[21]

One study with a positive quality[21] reported that a low plasma level of vitamin A is probably associated with a higher carotid IMT. In a study of 96 participants by Bobbert and colleagues,[21] carotid IMT was positively correlated with RBP4, and retinol-binding protein/transthyretin ratio, although it had an inverse association with retinol, and retinol/RBP4 ratio.

To conclude, data concerning the effects of dietary intake of vitamin A, as well as plasma concentration of retinol on carotid IMT, are scarce, and as such, further investigations are warranted.

Tocopherols, Ascorbic Acid, and Carotid IMT

Seven studies, including one cross-sectional[22] study and six clinical trials,[23-28] determined the effects of vitamin C, E, or both on carotid IMT. Overall, 11,307 individuals were included from the cross-sectional study and 2,383 participants with the mean follow-up of 3.1 years were included from clinical trials. Of the clinical trials, three studies[23-25] investigated the effects of vitamin E supplementation (doses varied from 400 to 1,200 IU/day), whereas one study[26] determined the effect of daily vitamin C intake and another study[27] reported the effect of supplementation with both vitamins on carotid IMT. Two studies[23,27] had a positive quality, whereas five studies[22,24-26,28] were considered as being of neutral quality.

A cross-sectional study by Kritchevsky and colleagues[22] reported an inverse association between vitamin C consumption and carotid IMT in both sexes; however, dietary intake of vitamin E was inversely associated with IMT among women only. In addition, neither vitamin C nor vitamin E intakes were associated with carotid IMT in those who were younger than age 55 years. Moreover, the three long-term vitamin E trials[23-25] included in the review reported that vitamin E supplementation had no significant effect on carotid IMT progression. Another clinical trial[26] concluded progression of the carotid IMT over 3 years was significantly lower in the dietary intervention group, in which daily vitamin C consumption was significantly increased compared with the controls. A randomized trial[27] of both vitamin E and C showed that supplementation with vitamin C, E, or a combination of both did not lead to any change in carotid IMT progression in women, whereas the vitamin C and E intervention decreased carotid IMT progression significantly in men. The study suggested that the benefit of these vitamin supplements may be limited to men only, and possibly to men who are at higher risk of oxidative stress, such as smokers or those who have insufficient status of either dietary or endogenous antioxidants. In a longitudinal study,[28] the authors investigated the effect of self-selected supplemental vitamin C and E intake on carotid IMT, and reported that vitamin E supplementation significantly reduced carotid IMT progression among the placebo group. However, neither vitamin C nor vitamin E had any significant change on carotid IMT progression in the drug group.

Taken as a whole, data regarding the effect of either vitamin C or E intake on carotid IMT are inconclusive. A few studies show at least a nonsignificant association of these vitamins with carotid IMT and consistent lower risk of CVD with higher intake; however, most studies did not find any association. Moreover, it has been reported that the combination of vitamin E and C (one the antioxidant of lipid phase and other the antioxidant of water phase, respectively) can be more effective than their individual effects on the progression of carotid IMT in men, possibly related to higher background oxidative stress levels.

Vitamin D and Carotid IMT

One cross-sectional[29] and two case-control[30,31] studies investigated the association between vitamin D and carotid IMT. A total of 415 participants were included from the cross-sectional study, and a total of 539 cases and 424 controls were included from the case-control studies. Cases were either patients with type 2 diabetes[30] or individuals with human immunodeficiency virus.[31] One study had a neutral quality,[30] whereas the other studies[29,31] had a positive quality.

Both of the case-control studies concluded that 25-hydroxyvitamin D levels were negatively associated with carotid IMT, and those participants with hypovitaminosis D had a markedly higher carotid IMT.[30,31] In contrast, a Danish cross-sectional study of patients with type 2 diabetes[29] found no significant association between serum levels of vitamin D and carotid IMT after accounting for CVD risk factors.

Table 1. Quality assessment of studies on the association between micronutrients intakes/ status and carotid intima media thickness using the Academy of Nutrition and Dietetics Quality Criteria Checklist[12]

Author(s), y	Design	Q1[a]	Q2[b]	Q3[c]	Q4[d]	Q5[e]	Q6[f]	Q7[g]	Q8[h]	Q9[i]	Q10[j]	QA[k]
Bonithon-Kopp and colleagues,[15] 1997	Cross-sectional	Y[l]	Y	Y	UC[m]	N/A[n]	Y	Y	Y	Y	Y	Pos[o]
Iribarren and colleagues,[20] 1997	Case-control	Y	Y	Y	UC	N/A	Y	Y	Y	Y	Y	Pos
Kritchevsky and colleagues,[18] 1998	Cross-sectional	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Klipstein-Grobusch and colleagues,[19] 2000	Case-control	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Rissanen and colleagues,[11] 2000	Cross-sectional	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Dwyer and colleagues,[14] 2001	Cohort	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Rissanen and colleagues,[17] 2003	Cross-sectional	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Karppi and colleagues,[16] 2011	Cross-sectional	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Bobbert and colleagues,[21] 2010	Cross-sectional	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Kritchevsky and colleagues,[22] 1995	Cross-sectional	Y	Y	UC	UC	N/A	Y	Y	Y	Y	Y	Neu[p]
Azen and colleagues,[28] 1996	Clinical trial	Y	UC	Y	Y	UC	Y	Y	Y	Y	Y	Neu
Salonen and colleagues,[27] 2000	Clinical trial	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Pos
Lonn and colleagues,[24] 2001	Clinical trial	Y	UC	Y	Y	Y	Y	Y	Y	Y	Y	Neu
Hodis and colleagues,[23] 2002	Clinical trial	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Pos
Devaraj and colleagues,[25] 2007	Clinical trial	Y	Y	UC	UC	Y	Y	Y	Y	Y	Y	Neu
Ellingsen and colleagues,[26] 2009	Clinical trial	Y	UC	Y	Y	UC	Y	Y	Y	Y	Y	Neu
Targher, and colleagues,[30] 2006	Case-control	Y	UC	Y	UC	N/A	Y	Y	Y	Y	Y	Neu
Ross and colleagues,[31] 2011	Case-control	Y	Y	Y	UC	N/A	Y	Y	Y	Y	Y	Pos
Winckler and colleagues,[29] 2015	Cross-sectional	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Yang and colleagues,[32] 2010	Cross-sectional	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Ma and colleagues,[33] 1995	Cross-sectional	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Turgut and colleagues,[34] 2008	Clinical trial	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Pos
Moore and colleagues,[37] 1995	Case-control	Y	UC	UC	UC	N/A	Y	Y	Y	Y	Y	Neu
Kiechl and colleagues,[35] 1997	Cross-sectional	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Engberink and colleagues,[36] 2008	Cross-sectional	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Till and colleagues,[38] 2005	Clinical trial	Y	Y	Y	UC	Y	Y	Y	Y	Y	Y	Pos
Austen and colleagues,[42] 2006	Clinical trial	Y	Y	Y	UC	Y	Y	Y	Y	Y	Y	Pos
Tungkasereerak and colleagues,[41] 2006	Clinical trial	Y	Y	UC	N[q]	Y	Y	Y	Y	Y	Y	Neu
Held and colleagues,[43] 2008	Cross-sectional	Y	Y	Y	UC	N/A	Y	Y	Y	Y	Y	Pos

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Table 1. Quality assessment of studies on the association between micronutrients intakes/ status and carotid intima media thickness using the Academy of Nutrition and Dietetics Quality Criteria Checklist[12] (*continued*)

Author(s), y	Design	Q1[a]	Q2[b]	Q3[c]	Q4[d]	Q5[e]	Q6[f]	Q7[g]	Q8[h]	Q9[i]	Q10[j]	QA[k]
Ntaios and colleagues,[39] 2010	Clinical trial	Y	Y	Y	UC	Y	Y	Y	Y	Y	Y	Pos
Kwok and colleagues,[40] 2012	Crossover trial	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Pos
Zureik and colleagues,[7] 2004	Clinical trial	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Pos
Kesse-Guyot and colleagues,[44] 2010	Cohort	Y	Y	Y	UC	N/A	Y	Y	Y	Y	Y	Pos
de Oliveira Otto and colleagues,[45] 2011	Cross-sectional	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Pos
Polidori and colleagues,[46] 2015	Cross-sectional	Y	Y	Y	UC	N/A	Y	Y	Y	Y	Y	Pos

[a]Q1=Question 1: Was the research question clearly stated?
[b]Q2=Question 2: Was the selection of study subjects/patients free from bias?
[c]Q3=Question 3: Were study groups comparable?
[d]Q4=Question 4: Was method of handling withdrawals described?
[e]Q5=Question 5: Was blinding used to prevent introduction of bias?
[f]Q6=Question 6: Were intervention/therapeutic regimens/exposure factor or procedure and any Comparison(s) described in detail? Were intervening factors described?
[g]Q7=Question 7: Were outcomes clearly defined and the measurements valid and reliable?
[h]Q8=Question 8: Was the statistical analysis appropriate for the study design and type of outcome indicators?
[i]Q9=Question 9: Are conclusions supported by results with biases and limitations taken into consideration?
[j]Q10=Question 10: Is bias due to study's funding or sponsorship unlikely?
[k]QA=quality assessment.
[l]Y=yes.
[m]UC=unclear.
[n]N/A=not applicable.
[o]Pos=positive.
[p]Neu=neutral.
[q]N=no.

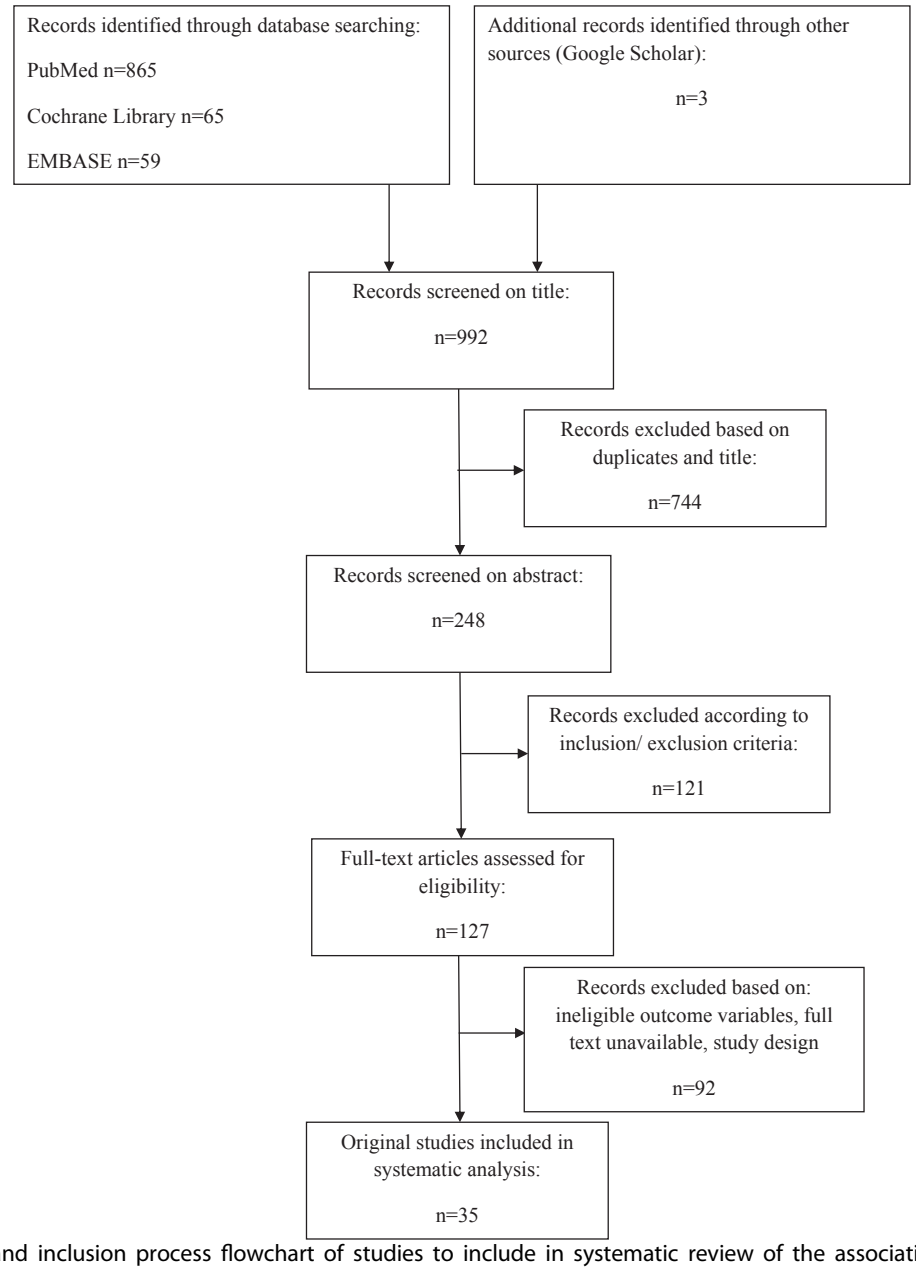

Figure 2. Search and inclusion process flowchart of studies to include in systematic review of the association between micronutrients and intima media thickness (IMT).

Accordingly, there is limited evidence regarding the associations of low vitamin D with higher carotid IMT.

Zinc and Carotid IMT

A cross-sectional analysis of baseline data from a prospective cohort study with a positive quality[32] indicated that dietary zinc intake was inversely associated with carotid IMT >80th percentile or ≥1 mm. Further studies are required.

Magnesium and Carotid IMT

A total of two studies, one cross-sectional study[33] and one clinical trial,[34] assessed the relationship between magnesium intake/status and carotid IMT. Overall, 15,248 participants were included from the cross-sectional study and 47 patients undergoing hemodialysis were included from the trial. Both studies were considered to have a positive quality.

The cross-sectional study[33] reported that serum magnesium was inversely correlated with carotid IMT in the women. Consistent with these findings are the results of a randomized trial[34] showing that carotid IMT was significantly improved in a magnesium citrate intervention group compared with the control group.

As such, studies indicate that high magnesium status might be involved in prevention of CVD and decreased IMT.

Table 2. Characteristics of studies on the association between carotid intima media thickness and micronutrients intakes/status

Author(s), y	Design	Sample size	Sample characteristics/ intervention	Results
Carotenoids and carotid IMT				
Bonithon-Kopp and colleagues,[15] 1997	Cross-sectional	1,187	Aged 59-7 1 y without any history of CAD[b] or stroke	Plasma carotenoids: ↔ IMT[a] ($P>0.05$)
Iribarren and colleagues,[20] 1997	Case-control	231 pairs	Matched pairs, cases with >90th percentile of IMT, controls with <75th percentile of IMT	Serum lycopene, alpha and beta carotene: ↔ OR[c] of elevated IMT ($P>0.05$)
Kritchevsky and colleagues,[18] 1998	Cross-sectional	12,773	Aged 45-64 y	↑ Quintile of carotenoid consumption: ↓ prevalence of carotid artery plaques
Klipstein-Grobusch and colleagues,[19] 2000	Case-control	108 cases vs 109 controls	≥55 y Male patients with aortic atherosclerosis vs healthy control	Serum lycopene: ↔ risk of aortic atherosclerosis (OR 0.55, 95% CI 0.25-1.22; P trend=0.13)
Rissanen and colleagues,[11] 2000	Cross-sectional	520	Middle-aged men and women (aged 45-69 y)	↓ Plasma lycopene: ↑ 17.8% IMT ($P<0.01$) in men Plasma lycopene: ↔ IMT ($P>0.05$) in women
Dwyer and colleagues,[14] 2001	Cohort	480	Aged 40-60 y with no history of heart attack, angina, revascularization, or stroke Follow-up: 18 mo	↑Plasma lutein: ↓ IMT progression ($P<0.01$) Plasma beta carotene: ↔ IMT progression ($P>0.05$)
Rissanen and colleagues,[17] 2003	Cross-sectional	1,082	Men aged 46-64 y	↑ Serum lycopene: ↓ mean and maximal IMT (Spearman correlation coefficients: $r=0.22$; $P< 0.001$, and $r=0.20$; $P<0.001$, respectively)
Karppi and colleagues,[16] 2011	Cross-sectional	1,212	Elderly men aged 61-80 y	↓ Plasma beta cryptoxanthin, lycopene, and alpha carotene: ↑ quartile of IMT (P for the differences were 0.043, 0.045, and 0.046, respectively)
Retinol and carotid IMT				
Bobbert and colleagues,[21] 2010	Cross-sectional	96	Age=55±1.3 y	↑Plasma retinol: ↓IMT ($r=-0.24$; $P<0.05$)
Tocopherols, ascorbic acid, and carotid IMT				
Kritchevsky and colleagues,[22] 1995	Cross-sectional	11,307	Aged 45-64 y	↑Vitamin C consumption: ↓mean IMT ($P=0.019$ for women and $P=0.035$ for men) ↑Vitamin E consumption: ↓mean IMT in women ($P=0.033$) ↑Vitamin C/ E consumption ↔ IMT in <55 y

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Table 2. Characteristics of studies on the association between carotid intima media thickness and micronutrients intakes/status (*continued*)

Author(s), y	Design	Sample size	Sample characteristics/ intervention	Results
Azen and colleagues,[28] 1996	Clinical trial	146	Nonsmoking 40- to 59-y-old men with previous coronary artery bypass graft surgery randomized to colestipol/niacin plus diet or placebo plus diet 22 Subjects had an on-trial average supplementary vitamin E intake of ≥100 IU/d (high users) and 29 subjects had an average on-trial supplementary vitamin C intake of ≥250 mg/d (high users)	↑Vitamin E supplementation: ↓ IMT progression (P=0.03) within the placebo group Vitamin E/C supplementation: ↔ IMT progression within the drug group
Salonen and colleagues,[27] 2000	Clinical trial	520	Aged 45-69 y with serum cholesterol ≥5.0 mmol/L randomized to either 136 IU vitamin E, 250 mg slow-release vitamin C, a combination of these, or placebo twice daily for 3 y	Vitamin E/C or C+E supplementation: ↔ IMT progression in women Vitamin C+E supplementation: ↓ IMT progression in men (0.011 mm [year 1]), compared with either all other men (vitamin E group: 0.018 mm [year 1], vitamin C group: 0.017 mm [year 1]; P=0.009)
Lonn and colleagues,[24] 2001	Clinical trial	732	Aged ≥55 y with vascular disease or diabetes and at least 1 other risk factor and without heart failure or a low left ventricular ejection fraction randomized to ramipril 2.5 mg/d or 10 mg/d and vitamin E (400 IU/d) or their matching placebos for 4.5 y	Vitamin E supplementation: ↔ IMT progression
Hodis and colleagues,[23] 2002	Clinical trial	332	Aged ≥40 y with an low-density lipoprotein cholesterol level ≥3.37 mmol/L (130 mg/dL) and no clinical signs or symptoms of CVD[d] randomized to vitamin E (400 n IU/d) or placebo for 3 y	Vitamin E supplementation: ↔ IMT progression
Devaraj and colleagues,[25] 2007	Clinical trial	90	Patients with CAD randomized to vitamin E (1,200 IU/d) or placebo for 2 y	Vitamin E supplementation: ↔ IMT progression
Ellingsen and colleagues,[26] 2009	Clinical trial	563	Men aged 70±5 y randomized to 1 of 4 groups (including dietary intervention (↑vitamin C consumption), n-3 supplementation, both, or neither) for 3 y	↑Vitamin C consumption: ↓ IMT progression (P=0.006)

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Table 2. Characteristics of studies on the association between carotid intima media thickness and micronutrients intakes/status (*continued*)

Author(s), y	Design	Sample size	Sample characteristics/ intervention	Results
Vitamin D and carotid IMT				
Targher and colleagues,[30] 2006	Case-control	390 Pairs	Patients with type 2 diabetes vs age- and sex-matched healthy controls	Hypovitaminosis D vs normal vitamin D levels: ↑ carotid IMT (1.10±0.15 vs 0.87±0.14 mm; $P<0.001$)
Ross and colleagues,[31] 2011	Case-control	149 Cases vs 34 controls	Patients with human immunodeficiency virus infection on antiretroviral therapy vs healthy controls	↓ 25-hydroxy vitamin D levels: ↑OR of carotid IMT (OR 10.62, 95% CI 1.37-82.34; $P<0.01$)
Winckler and colleagues,[29] 2015	Cross-sectional	415	Aged ≥30 y with type 2 diabetes and 25<BMI<40	Vitamin D serum levels: ↔ IMT
Zinc and carotid IMT				
Yang and colleagues,[32] 2010	Cross-sectional	4,564	Aged 40-89 y without clinical manifestation of CVD	↑Dietary zinc intake: ↓ IMT
Magnesium and carotid IMT				
Ma and colleagues,[33] 1995	Cross-sectional	15,248	Aged 45-64 y, blacks and whites	↑serum Mg[e] levels: ↓ IMT in women ↑serum Mg levels: ↔ IMT in men
Turgut and colleagues,[34] 2008	Clinical trial	47	Patients on hemodialytic randomized to receive either phosphate binder + Mg citrate orally at a dosage of 610 mg every other day or only calcium acetate therapy as a phosphate binder for 2 mo	Mg supplementation vs placebo: ↓ Bilateral IMT (−0.27±0.1 vs 0.05±0.1 mm; $P<0.001$ for left IMT, −0.17±0.1 vs 0.03±0.1 mm; $P<0.002$ for right IMT)
Iron and carotid IMT				
Moore and colleagues,[37] 1995	Case-control	—	Cases with carotid intima media thickening vs controls	↑ Serum ferritin concentration: ↔ IMT
Kiechl and colleagues,[35] 1997	Cross-sectional	826	Aged 40-79 y	↑ Iron stores: ↑carotid atherosclerosis, ↑ progression of pre-existing atherosclerotic lesions
Engberink and colleagues,[36] 2008	Cross-sectional	819	Aged 50-70 y	↑NTBI[f]: ↔ IMT

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Table 2. Characteristics of studies on the association between carotid intima media thickness and micronutrients intakes/status (*continued*)

Author(s), y	Design	Sample size	Sample characteristics/ intervention	Results
Vitamin B group and carotid IMT				
Till and colleagues,[38] 2005	Clinical trial	50	Patients (aged 60±8 y) with IMT ≥1 mm randomized to receive 2.5 mg folic acid, 25 mg vitamin B-6, and 0.5 mg vitamin B-12 or placebo daily for 1 y	B group supplementation vs placebo: ↓ IMT (−0.08±0.17 mm vs 0.07±0.25 mm; P=0.02)
Austen and colleagues,[42] 2006	Clinical trial	10	Cyclosporin A-treated renal transplant recipients randomized to receive folate supplementation (5 mg/d) or placebo for 3 mo	Folate supplementation: ↔ IMT
Tungkasereerak and colleagues,[41] 2006	Clinical trial	54	Chronic hemodialysis patients with hyperhomocysteinemia randomized to receive oral 15 mg folic acid, 50 mg vitamin B-6, and 1 mg vitamin B-12 daily (treatment group) or oral 5 mg folic acid alone (control group) for 6 mo	B group supplementation: ↔ IMT
Held and colleagues,[43] 2008	Cross-sectional	923	Patients with vascular disease or diabetes	↑Plasma folate concentration: ↓ IMT (P<0.05)
Ntaios and colleagues,[39] 2010	Clinical trial	103	Patients with at least 1 cardiovascular risk factor randomized to receive either a daily dose of 5 mg folic acid or placebo for 18 mo	Folic acid supplementation vs placebo: ↓ IMT (−0.028±0.015 mm vs 0.020±0.005 mm; P<0.05)
Kwok and colleagues,[40] 2012	Crossover trial	50	Healthy vegetarians (vegetarian diet for at least 6 y) randomized to receive vitamin B-12 (500 μg/d) or placebo were for 12 wk with 10 wk of placebo-washout before crossover, and then open label vitamin B-12 for additional 24 wk	Vitamin B-12 supplementation vs placebo: ↓ IMT (−0.02±0.00 mm vs 0.01±0.00 mm; P<0.05)
Combination of micronutrients and carotid IMT				
Zureik and colleagues,[7] 2004	Clinical trial	1,162	Aged >50 y free of symptomatic chronic diseases and apparently healthy randomized to daily receive either a combination of antioxidants (120 mg vitamin C, 30 mg vitamin E, 6 mg beta carotene, 100 μg selenium, and 20 mg zinc) or placebo for 7.2±0.3 y	Antioxidants supplementation: ↔ IMT

(*continued on next page*)

Table 2. Characteristics of studies on the association between carotid intima media thickness and micronutrients intakes/status (*continued*)

Author(s), y	Design	Sample size	Sample characteristics/ intervention	Results
Kesse-Guyot and colleagues,[44] 2010	Cohort	1,026	Middle-aged participants followed up for 7.5 y	Dietary micronutrients: ↔ IMT ↑intakes of folate, vitamin C, beta carotene: ↓ IMT
de Oliveira Otto and colleagues,[45] 2011	Cross-sectional	5,181	Aged 45-84 y and free of diabetes and cardiovascular disease	↑Intakes of Mg: ↓ IMT Intakes of iron, beta carotene, vitamin C, E: ↔ IMT
Polidori and colleagues,[46] 2015	Cross-sectional	192	Aged ≥65 y with no or very mild cognitive impairment	↑Plasma levels of vitamin C and E: ↓ IMT

[a]IMT= intima media thickness.
[b]CAD=coronary artery disease.
[c]OR=odds ratio.
[d]CVD=cardiovascular disease.
[e]Mg=magnesium.
[f]NTBI=nontransferrin bound.

Iron and Carotid IMT

Two cross-sectional studies[35,36] as well as one case-control study[37] addressed the association between iron status and carotid IMT. A total of 1,645 participants were included from the cross-sectional studies. Cases were patients with carotid intima-media thickening. One study had a quality rating of neutral,[37] whereas the other two studies[35,36] had a positive quality. Of the identified studies, two studies[36,37] did not observe any significant association between iron stores and IMT. Kiechl and colleagues[35] reported iron stores were inversely correlated with carotid atherosclerosis, and also the progression of preexisting atherosclerotic lesions.

Taken together, the majority of studies suggest that iron and its metabolites are not associated with carotid IMT, although further studies are likely warranted.

Vitamin B Group and Carotid IMT

Six studies, including five clinical trials[38-42] and one cross-sectional study,[43] investigated the relationship between vitamin B group intake and carotid IMT. A total of 267 participants were included from the clinical trial and 923 individuals were included from the cross-sectional study. Two trials[39,42] investigated the effects of folate supplementation and one study[40] examined the effects of vitamin B-12 on carotid IMT. The other randomized clinical trials[38,41] determined the effects of a combination of B-6, folate, and B-12 supplementation. The quality rating of one study was neutral,[41] whereas the other studies[38-40,42,43] had a positive quality.

The majority of studies showed a beneficial effect of vitamin B group intervention either alone or in combination on carotid IMT,[38-40] whereas two studies[41,42] did not report any significant change following vitamin B group supplementation. Moreover, Held and colleagues[43] reported plasma folate concentration was negatively correlated with carotid IMT.

In summary, although there are discrepant findings regarding the vitamin B group and carotid IMT, the majority of studies show associations in the same direction, consistent with a beneficial effect of vitamin B intake in carotid IMT. More research is required to ascertain the effects of these vitamins on carotid IMT.

Combinations of Micronutrients and IMT

Although the majority of studies have investigated the effects of a specific micronutrient on progression of carotid IMT, four studies, including one clinical trial,[7] one cohort study,[44] and two cross-sectional studies,[45,46] have determined the association between several micronutrients intake/status and IMT. Overall, 1,162 participants were included from clinical trials, 1,062 individuals with the follow-up of 7.5 years from the cohort study, and 5,373 participates were included from cross-sectional studies. One cross-sectional study[46] assessed the plasma levels of selected micronutrients, whereas another one investigated the relationship between micronutrients intakes and carotid IMT.[45] The quality rating of all studies was positive.

Of the included studies, two studies[7,44] did not report any association between micronutrients and carotid IMT, whereas both of the cross-sectional studies[45,46] reported an inverse

association between some micronutrients intake/status and carotid IMT.

Accordingly, data regarding the effects of combinations of several micronutrients on IMT are scarce, and the conducted studies have not attained conclusive results.

DISCUSSION

This systematic review assessed 35 original studies on the association between selected micronutrients and carotid IMT in human beings, and highlighted that although not all studies showed consistent results, the majority found an inverse association between both intakes and circulatory levels of magnesium, as well as vitamin D and vitamin B group with carotid IMT. Less evidence was available for associations of retinol, zinc, and iron with carotid IMT. No significant association was reported regarding vitamin C and E by most of the studies.

Several mechanisms have been suggested through which micronutrients can play a role in carotid IMT, and thereby, CVD events. Carotenoids present in plasma have been shown to act as antioxidants[19] because they can quench singlet oxygen, a potential cause of lipid peroxidation, which is considered a potential initiator of CVD.[19,47] The highest physical quenching rate constant of all carotenoids with singlet oxygen has been attributed to lycopene, the open-chain isomer of beta carotene.[11] High carotenoids concentrations in blood or adipose tissue are linked with reduced risk of CVD.[17] Likewise, findings from several studies have indicated that low circulating levels of carotenoids may play a role in atherogenesis and progression of carotid atherosclerosis.[19,22] In line with these findings, a negative correlation between carotenoids and IMT has been described in some of the reviewed studies; however, most of the studies did not show any significant association between carotenoids and IMT. Some studies observed stronger association for lycopene, which may be attributable to its higher antioxidant capabilities.

It has been suggested that RBP4 may exacerbate insulin resistance, because elevated RBP4 levels have been reported in patients with type 2 diabetes. Bobbert and colleagues[21] suggested that circulating RBP4 can be regarded as a marker for CVD, especially in insulin resistance or type 2 diabetes, and the authors also found that plasma retinol is negatively correlated with mean carotid IMT. One study showed a negative association between vitamin C and E and carotid IMT. It is well documented that they are two of the most important dietary antioxidants.[27] Several studies indicate that supplementation with vitamin E, vitamin C, or both may result in a decreased risk of specific chronic diseases such as ischemic heart disease.[48] Specifically, in exerting its antioxidant properties, vitamin E is oxidized into the nonactive α-tocopheroxyl radical, which can be reduced back to α-tocopherol by vitamin C.[26] Vitamin E may also exhibit other antiatherogenic properties because it plays a role in inhibition of smooth muscle cell proliferation, platelet aggregation, monocyte adhesion, oxidized low-density lipoprotein cholesterol uptake, and cytokine production.[23] Accordingly these vitamins may have a role in the regression of IMT; however, findings from the included studies were inconclusive.

Another investigated micronutrient in this review was vitamin D. It has been suggested that vitamin D may favorably affect cardiovascular health through multiple mechanisms, including downregulation of the renin–angiotensin system, enhancement in insulin secretion and insulin sensitivity[49] protection against angiogenesis, and modulation of inflammatory processes.[50] Specifically, hypovitaminosis D is linked with increased circulating concentrations of matrix metalloproteinase-9, which controls vascular wall remodeling, and supplementation with vitamin D reduces serum levels of matrix metalloproteinase-9. Moreover, vitamin D deficiency may lead to a decrease in apolipoprotein A-1, and vitamin D supplementation appears to improve the elastic properties of the arterial wall.[51,52] Some studies found an inverse association between vitamin D and carotid IMT; however, this was not supported by all the conducted studies.

Several potential mechanisms underlying the cardiovascular benefits of zinc have been suggested.[53] Atherogenesis constitutes of the oxidation of low-density lipoprotein cholesterol and its uptake into macrophages, as well as apoptosis of endothelial cells.[54] Low-density lipoprotein cholesterol oxidation can be inhibited by zinc via macrophages and endothelial cells. Moreover, the activity of caspase enzymes involved in apoptotic pathways is inhibited by zinc.[53] There is very limited evidence linking zinc intake with subclinical atherosclerosis, and only one cross-sectional study found an inverse correlation between dietary zinc intake and IMT. Moreover, there is accumulating experimental and clinical evidence suggesting that hypomagnesemia may play a significant role in the progression of CVDs in the general population.[55] The fact that arterial blood pressure can be regulated by magnesium may have a favorable influence on the development of atherosclerotic lesions.[55] Moreover, some evidence suggests that magnesium is a potent inhibitor of the calcification process and that experimental magnesium deficiency seems to induce vascular calcification,[34] which suggests that it may be also involved in IMT. Furthermore, few experimental studies demonstrate that orally administrated magnesium suppresses the progression of atherosclerotic lesions.[56,57] Several studies reported an inverse association between magnesium intake as well as circulatory level and carotid IMT potentially via pathways involving competition with calcium as a divalent element.

Emerging evidence suggests that iron status could influence the risk of CVD. Specifically, elevated iron stores are regarded as a risk factor for developing CVD. In terms of the association between iron and carotid IMT, one study[35] suggests that iron may contribute to the progression of atherosclerosis and may be responsible for the sex differences observed in CVD between men and women.[58] It has been reported that iron could promote free radical formation via Fenton reaction, which may lead to injury of the arterial walls and atherosclerosis.[36] This hypothesis is in the same line with epidemiologic evidence that reported an association between body iron stores and myocardial infarction and endothelial function.[59] As previously proposed, frequent blood donation, resulting in lowered body iron stores, might give some protection against accelerated atherosclerosis.[60] However, two studies did not find any significant association between iron status and carotid IMT.[36,37]

There is strong evidence suggesting that hyperhomocysteinemia is linked with risk of atherosclerotic vascular disease.[61] B vitamins and also other nutrients can

decrease homocysteine concentrations.[62] Data regarding the effects of B vitamins are inconclusive. The majority of studies concluded that vitamin B group intake was inversely associated with carotid IMT.

It should be also noted that the studies included in our systematic review were heterogeneous in nature, with diverse study populations, sample sizes, and study durations. Moreover, the findings are based mostly on observational studies, and data are prone to reverse bias as well as subject recalls and interviewer bias. Another limitation of this review is the consideration of published studies only. Because studies with positive findings are more likely to be published, publication bias cannot be excluded. The main strength of this systematic review is the extensive, systematic literature search. Clear inclusion criteria and an explicit approach to collecting data were also used.

CONCLUSIONS

The net evidence from published studies is consistent with an inverse association of dietary antioxidants with IMT, in particular for magnesium, vitamin D, and the vitamin B group. Rigorous pragmatic randomized clinical trials are needed before recommendations can be made specifically related to the consumption of antioxidant supplements in patients at risk of CVD.

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