Oxidants and antioxidants as targets for cardiovascular disease prevention: evidence from observational and causal inference studies

Martens, L.G.

Citation

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DIET-DERIVED ANTIOXIDANTS DO NOT DECREASE RISK OF ISCHEMIC STROKE: A MENDELIAN RANDOMIZATION STUDY IN 1 MILLION PEOPLE

Leon G Martens
Jiao Luo
Ko Willems van Dijk
J Wouter Jukema
Raymond Noordam
Diana van Heemst

ABSTRACT

Background and aims
Dietary intake and blood concentrations of vitamin E, C, lycopene and carotenoids have been associated with a lower risk of incident (ischemic) stroke. However, causality cannot not be inferred from these associations. Here, we investigated causality by analyzing the associations between genetically-influenced antioxidant levels in blood and ischemic stroke using Mendelian Randomization (MR).

Methods
For each circulating antioxidant (vitamin E, C, lycopene, β-carotene and retinol), which were assessed as either absolute blood levels and/or high-throughput metabolite levels, independent genetic instrumental variables were selected from earlier genome wide association studies (p < 5x10^{-8}). We used summary statistics for Single-Nucleotide Polymorphisms (SNP)-stroke associations from three European-ancestry cohorts (cases/controls): MEGASTROKE (60,341/454,450), UK Biobank (2,404/368,771) and FinnGen study (8,046/164,286). MR analyses were performed on each exposure per outcome cohort using inverse-variance weighted analyses, and subsequently meta-analyzed.

Results
In a combined sample of 1,058,298 individuals (70,791 cases), none of the genetically-influenced absolute antioxidants or antioxidant metabolite concentrations were causally associated with a lower risk of ischemic stroke. For absolute antioxidants levels, the odds ratios (95% CI) ranged between 0.94 (95% CI: 0.85 to 1.05) for vitamin C and 1.04 (95% CI: 0.99 to 1.08) for lycopene. For metabolites, odds ratios ranged between 1.01 (95% CI: 0.98 to 1.03) for retinol and 1.12 (95% CI: 0.88 to 1.42) for vitamin E.

Conclusions
This study did not provide evidence for a causal association between dietary-derived antioxidant levels and ischemic stroke. Therefore, antioxidant supplements to increase circulating levels are unlikely to be of clinical benefit to prevent ischemic stroke.

INTRODUCTION

Stroke is the second leading cause of death and loss of disability-adjusted life years worldwide [1]. Ischemic stroke is caused by a disruption of cerebral blood flow causing a lack of oxygen in the affected area [2]. Several classical risk factors have been described as important in the pathogenesis of ischemic stroke, including smoking, obesity, diabetes mellitus, hypertension and dyslipidemia [3-7]. In addition to the traditional risk factors, oxidative stress has been hypothesized to be a vital trigger in the occurrence of stroke via excessive production of Reactive Oxygen Species (ROS) [8, 9]. ROS-induced damage can cause significant changes in the vascular system, ultimately influencing cerebral blood flow [9]. These detrimental effects, including increasing vasodilation, platelet aggregation, increased endothelial permeability and the formation of local lesions [9], could consequently lead to an increased risk of stroke [10].

Therefore, antioxidants, which are scavengers of free radicals and thereby diminish oxidative damage, can be hypothesized to decrease the risk of disease occurrence. Antioxidants, such as vitamin E, C and carotenoids, are of specific interest given that they are accessible and their intake is easily modifiable. Several studies have already been conducted examining the association between antioxidants and the occurrence of stroke [8, 10-14]. In these studies, dietary intake, either as dietary components or supplements, or blood concentration of vitamin E, C and carotenoids were associated with lower risk of first occurrence of ischemic stroke [8, 11-14]. Similarly, adherence to a diet rich in antioxidants, irrespective of the type of antioxidants, has been associated with a lower risk of incident stroke [15].

However, associations in these observational studies inevitably suffer from the possibility of reverse causation and residual confounding, and should be interpreted with caution. Therefore, the causality between dietary-derived antioxidants and stroke is still unclear. A randomized controlled trial on vitamin E and β-carotene supplementation and stroke risk found inconsistent results [16]. Whereas a similar study on vitamin E supplementation and cardiovascular events found no effect [17]. In addition to randomized clinical trials, Mendelian Randomization (MR), in which genetic variants of a certain exposure are used as instrumental variables, is an alternative approach to infer causality of life-long risk factors (exposure) on diseases (outcome) [18-20]. In the present study, we used two-sample MR to assess the causal associations between genetically-influenced dietary-derived circulating antioxidants and their metabolites with ischemic stroke.

METHODS

The data on which the results were based were primarily summary-level data. The data from the UK Biobank is available upon acceptance of a research proposal by UK Biobank Recourses and payment of an access fee. The data from MEGASTROKE and FinnGen are freely available from their respective websites (MEGASTROKE: https://www. https://www.megastroke.org/, FinnGen: https://www.finngen.fi/en/).

Study Design

For our study, we conducted a two-sample MR, which uses genetic instrumental variables as a proxy for the exposure [21]. Using this design, SNP-exposure and equivalent SNP-outcome associations were derived from genome-wide association studies (GWAS). Subsequently, the SNP-outcome effect is divided by the SNP-exposure effect, and these are subsequently meta-analyzed to approximate the average genetically-influenced effect on the outcome. MR is based on several assumptions. First, the genetic variants should be associated with the exposure. Secondly, the association between these genetic variants and the outcome should be solely through the exposure. Finally, the genetic variants should be independent of any measured and unmeasured confounders.

Selection of genetic instrumental variables

To study the association between genetically-influenced levels of antioxidants and stroke, the genetic instruments of five diet-derived antioxidants were used, which included vitamin E (α-tocopherol and γ-tocopherol), β-carotene, lycopene, vitamin C (L-ascorbic acid or ascorbate) and retinol. For these antioxidants, we investigated either absolute blood levels, circulating metabolites which were quantified as relative concentrations in plasma or serum using high-throughput commercial platforms, or both. The metabolites were quantified as relative concentrations [22]. For each antioxidant, a search for GWAS was performed to extract the leading SNPs as genetic instrumental variables. Whenever multiple GWAS were identified, only the largest study including replication was used [23-29]. All selected variants were associated with their corresponding exposure ($p < 5 \times 10^{-8}$). A more detailed description and a summary table of the SNPs for each antioxidant used as instrumental variables can be found in the supplements and in our previous publication [22]. Different from our earlier work [22], we now used an updated genome-wide association study on vitamin C (all genetic instruments presented in **Supplementary Table 1**) [29]. All selected variants passed standard quality control filters with an imputation score of 0.90 or higher, except for retinol-associated SNP rs117468033, which passed with a score of 0.65.

Outcome datasets

Summary statistics on the associations of the exposure-related SNPs with ischemic stroke were extracted from three large cohorts; the MEGASTROKE consortium, the UK Biobank, and the FinnGen study. Both UK Biobank and FinnGen were not part of the main analyses of the MEGASTROKE consortium preventing inclusion of overlapping samples in the analyses. Available summary statistics can be found in **Table 1**.

Table 1. Summary characteristics MEGASTROKE, UK Biobank & FinnGen

	MEGASTROKE	UK Biobank	FinnGen
Cases	67 162	2 404	8 046
Age (SD), years	69.1 (10.8)	61.5 (6.7)	-
Women, %	43%	34%	-
Controls	454 450	368 771	164 286
Age (SD), years	56.6 (15.6)	56.7 (8.0)	-
Women, %	51%	54.5%	-

The MEGASTROKE consortium consisted of 60,341 cases and 454,450 controls collected from 29 studies. Of these participants, 86% were of European ancestry, 9% of East Asian ancestry, and the remaining participants were from African, South Asian, mixed Asian, and Latin American ancestry [30].

The UK Biobank cohort (project application number 56340) is a prospective general population cohort with 502,628 participants between the age of 40 and 70 years recruited from the general population between 2006 and 2010 [31], and more information can be found online (https://www.ukbiobank.ac.uk). The analyses were performed with participants of European ancestry, who were in the full released imputed genomics databases (UK10K + HRC). Follow-up information, including stroke occurrence, was retrieved through routinely available national datasets. In our dataset, we had data available on 2,404 cases of ischemic stroke, and 368,771 controls. We performed new genome-wide association analyses using logistic regression to assess the associations between genetic instruments and ischemic stroke, adjusted for age, sex and 10 principal components, and corrected for familial relationships using BOLT_LMM (v2.3.2).

The FinnGen study is an ongoing cohort study launched in 2017, which integrated the genetic data generated from biobank samples and health-related data from social and healthcare registers. Detailed information such as participating biobanks/cohorts, genotyping and data analysis are available at their website (https://www.finngen.fi/en/). For our current study, the freeze 4 data was used. Within this data, there are 8,046 reported cases of ischemic stroke, and 164,286 controls.

Statistical analysis

All the analyses were done using R (v3.6.1) statistical software (The R Foundation for Statistical Computing, Vienna, Austria). MR analyses were performed using the R-based

package "TwoSampleMR" (https://mrcieu.github.io/TwoSampleMR/).

Prior to the analysis, the "TwoSampleMR" package was used for harmonization of the exposure and outcome SNPs, in order to verify that the effect estimates are aligned in the same direction. Additionally, harmonization removes palindromic SNPs from the instrument pool.

For our primary MR analysis, Inverse-Variance weighted (IVW) regression analyses were performed. This method assumes the absence of invalid genetic instruments such as SNPs affecting multiple exposures (pleiotropy) causing possible directional pleiotropy [19]. First, causal estimates were calculated per genetic instrument using the Wald ratio (SNP – outcome association divided by the SNP – exposure association) and subsequently meta-analyzed using the inverse-weighted meta-analyses weighted on the standard error of the SNP-outcome association (assuming no measurement error in the exposure) [32]. The calculated estimates were expressed as odds ratios (OR) on ischemic stroke per unit difference of the corresponding absolute circulating antioxidant levels (natural log-transformed levels for β-carotene and retinol, µg/dL for lycopene or µmol/L for ascorbate) or 10-fold change in antioxidant metabolite concentrations.

To ensure that the results obtained from the IVW analyses were not biased due to directional pleiotropy, we performed MR-Egger regression analysis and Weighted-Median Estimator when applicable [32]. In MR-Egger, the intercept depicts the estimated average pleiotropic effect across the genetic variants, and a value that differs from zero indicates that the IVW estimate is biased [33]. Although considered as a relatively inefficient approach (e.g., large confidence intervals), this method does not force the regression line to go through the intercept. The Weighted-Median estimator analysis can provide a consistent valid estimate if at least half of the instrumental variables are valid [20]. In addition, MR-PRESSO (MR Pleiotropy RESidual Sum and Outlier) was applied when possible to detect and correct for horizontal pleiotropy through removing outliers [34], as implemented in the R-based package MR-PRESSO (https://github.com/rondolab/MR-PRESSO)". The Cochran's Q statistic was performed in order to test the heterogeneity between the estimated Wald ratios from different genetic variants [35]. Additional sensitivity analyses were performed for the antioxidants β-carotene and lycopene.

The main MR analyses were performed in the individual datasets, and subsequently meta-analyzed to derive the pooled estimates for each exposure on the risk of ischemic stroke. Heterogeneity of the estimates across three datasets was performed by I^2, and corresponding p-value was obtained from the Cochran's Q test. When no heterogeneity was found amongst the three cohorts, a fixed-effect model meta-analysis was used to pool the instrumental variable estimates for each exposure. All meta-analyses were performed in the R-based "meta" package (https://cran.r-project.org/web/packages/meta/index.html).

A power calculation was performed for each genetically derived antioxidant separately (https://shiny.cnsgenomics.com/mRnd/). With power = 0.80, minimal effect

sizes (OR) differed from 0.98 to 0.93, which we deemed reasonable given the results of observational studies [11-14]. We therefore concluded our study had enough power given the current parameters. Separate power calculations were done for the different subtypes of stroke (cardioembolic, large artery atherosclerosis and small-vessel) in MEGASTROKE, as data was available. Minimal effect sizes needed ranged from 0.94 to 0.78. Although some of these were considered as an unreasonably achievable estimate, we decided to perform these sub-analyses as well.

RESULTS

By combining the three cohorts, a total sample of 1,058,298 individuals, of which 70,791 cases of ischemic stroke, and 987,507 controls, were analyzed to assess the association between diet-derived antioxidants and ischemic stroke. Variance explained (R^2) by the instruments for each trait were either derived from the original study or calculated based on the derived summary statistics and in line with the method described previously [36], and ranged from 1.7% to 30.1% for absolute antioxidant levels, and from 3.3% to 18.6% for metabolite antioxidants (**Table 2**). In order to minimize potential weak instrument bias, we considered an F-statistic of at least 10 as sufficient for performing an MR analysis, which is well-accepted in the field [37]. The in-between SNP heterogeneity was non-significant for all antioxidants in each cohort (p > 0.05). Additionally, we found no heterogeneity in the summary estimates from the MR analyses between the included datasets (**Figure 1 & Figure 2**). All analyses were performed with outcome ischemic stroke, as well as with outcomes cardioembolic, large artery atherosclerosis, and small-vessel stroke separately (**Supplementary Figure 1 & Figure 2**). As no consistent strong evidence favoring the hypothesis of higher levels resulting in a lower risk of stroke subtypes was found, only the main results are given here.

Table 2. GWAS on genetically determined diet derived antioxidants

Exposure Datasets	Absolute antioxidants				Metabolite antioxidants			
	N =	Genetic Variants	Explained Variance	Unit	N=	Genetic Variants	Explained Variance	Units
a-Tocopherol	-	-	-	-	7276	11	3.3%	log10-transformed metabolites concentration
γ-Tocopherol	-	-	-	-	5822	13	15.0%	log10-transformed metabolites concentration

Table 2. Continued

| Exposure Datasets	Absolute antioxidants				Metabolite antioxidants			
	N =	Genetic Variants	Explained Variance	Unit	N=	Genetic Variants	Explained Variance	Units
β-Caroten	2344	3	9.0%	µg/L in log-transformed scale	-	-	-	-
Lycopene	441	5	30.1%	µg/dL	-	-	-	-
Ascorbate	52018	11	1.87%	µmol/L	2063	13	18.6%	log10-transformed metabolites concentration
Retinol	5006	2	2.3%	µg/L in log-transformed scale	1957	24	4.8%	log10-transformed metabolites concentration
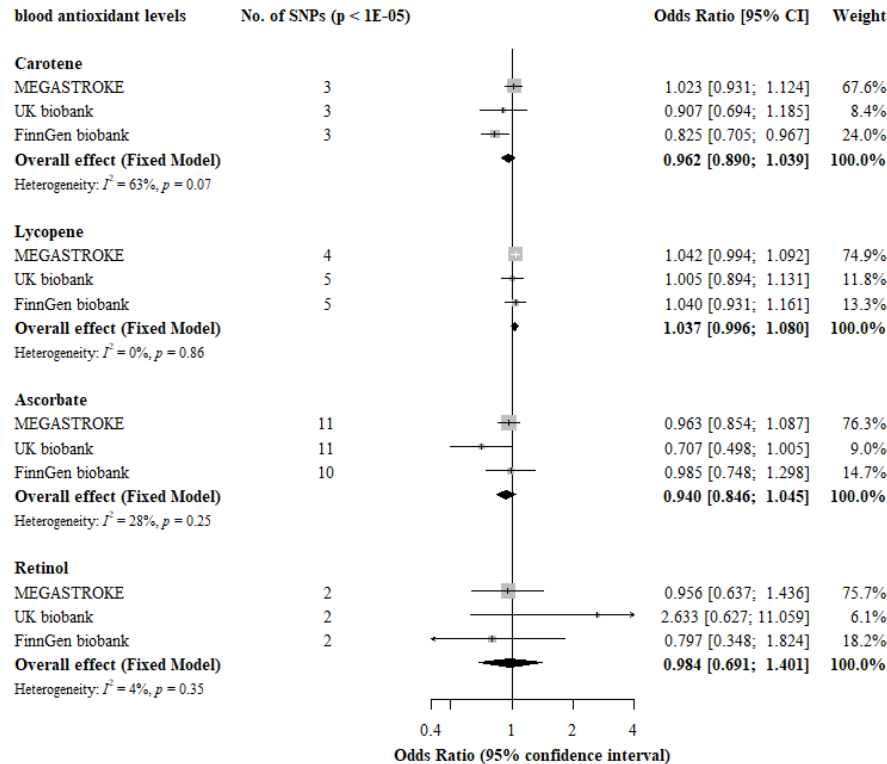
Figure 1. Causal association between absolute blood level antioxidants and ischemic stroke occurrence. Estimated ORs represent the effect per unit increase in ln-transformed β-Carotene and retinol, 1 µg/dL lycopene and 1 µmol/L ascorbate on ischemic stroke. Results were obtained from an IVW analysis per outcome database and combined over the three databases using fixed-effect meta-analyses.

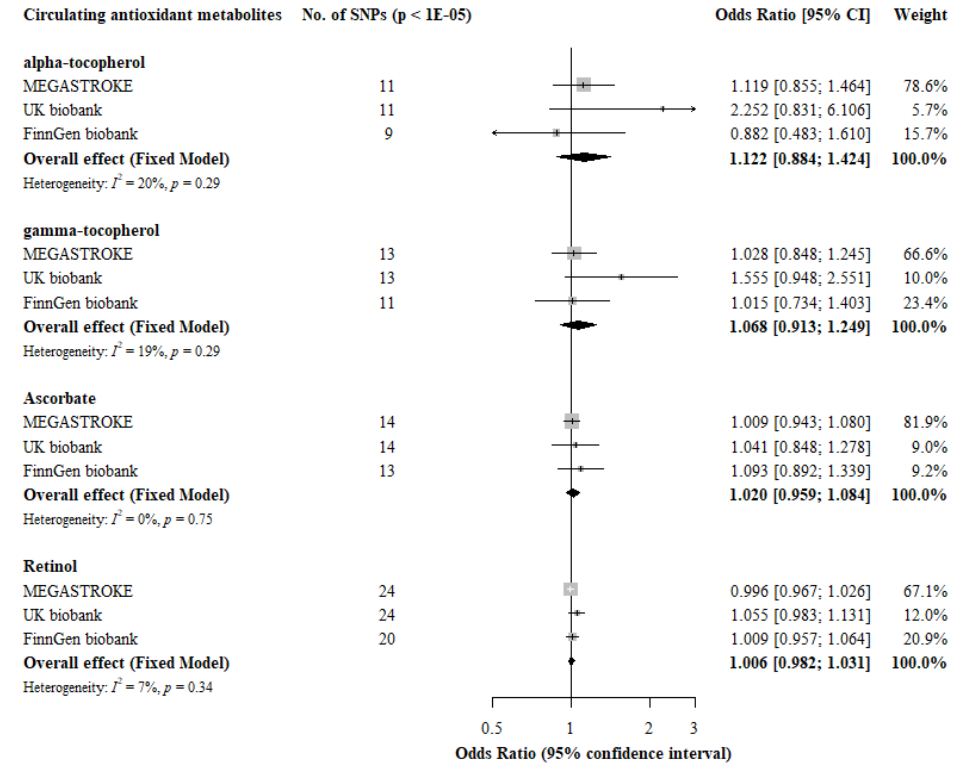CI = confidence interval; OR = odds ratio;

Figure 2. Causal association between circulating antioxidant metabolites and ischemic stroke occurrence. Estimated ORs represent the effect per 10-fold increase in antioxidant metabolites' concentration on ischemic stroke. Results were obtained from an IVW analysis per outcome database and combined over the three databases using fixed-effect meta-analyses.

CI = confidence interval; OR = odds ratio;

Absolute antioxidant levels

For absolute blood antioxidants levels (**Figure 1**), we observed no evidence for associations with ischemic stroke, except for β-carotene when analyzed only within the FinnGen cohort. However, the pooled estimate for all three cohorts was not significantly associated with ischemic stroke. Most notably, the pooled odds ratios (95% confidence intervals) were 0.94 (95% CI: 0.85, 1.05) per 1 µmol/L ascorbate, 1.04 (95% CI: 0.99, 1.08) per 1 µg/dL lycopene, 0.96 (95% CI: 0.89, 1.04) and 0.98 (95% CI: 0.69, 1.40) per natural log-transformed β-carotene and retinol, respectively.

MR-Egger and weighted-median estimator regression were performed for antioxidants with more than three genetic instruments (notably β-carotene and lycopene). The estimates of both MR-Egger and weighted-median estimator were comparable with the IVW analyses. Furthermore, the MR-Egger intercept did not

deviate from zero (p-values>0.05). Additionally, MR-PRESSO did not detect any outliers, and Cochran's Q statistics detected no heterogeneity for the analyses of β-carotene or lycopene on ischemic stroke.

Circulating antioxidant metabolite levels
For circulating antioxidant metabolites (**Figure 2**), the pooled OR (95%CI) for ischemic stroke per 10-fold increase in metabolite concentration were 1.12 (95% CI: 0.88, 1.42) for α-tocopherol, 1.07 (95% CI: 0.91, 1.25) for γ-tocopherol, 1.02 (95% CI: 0.96, 1.08) for ascorbate and 1.01 (95% CI: 0.98, 1.03) for retinol using IVW.

Estimates derived from the MR-Egger and weighted-median estimator analyses were of similar direction and magnitude as the IVW analyses. Furthermore, no pleiotropic effect was identified by the intercept from MR-Egger or MR PRESSO, and no potential outlier was found via MR PRESSO. Cochran's Q statistics only detected heterogeneity in γ-tocopherol in the MEGASTROKE cohort.

DISCUSSION

We investigated whether we could find evidence supporting causality of the association between diet-derived antioxidants and ischemic stroke risk using MR. Circulating antioxidants, irrespective of how the levels were determined, were proxied by using genetic variants as instrumental variables. In an extreme sample size of 1,058,298 participants, 70,791 cases and 987,507 controls, we did not find evidence that genetically-influenced diet-derived antioxidant levels were associated with a lower risk of developing ischemic stroke. While B-carotene was associated with ischemic stroke in the FinnGen population, this association was not observed in the other included study populations and was therefore considered as a likely chance finding. These findings suggest that the previously observed association between antioxidants (either by dietary intake and/or serum levels) and ischemic stroke is not causal.

Previously, a meta-analysis of observational studies identified a 17% risk reduction of stroke in participants with high dietary vitamin E intake compared with those with low intake. However, the authors were cautious with interpreting this result given that only 3,284 events of ischemic stroke were reported, despite the study comprising over 220,000 participants, as well as the large heterogeneity of the individual contributing studies in the meta-analysis [38]. To date, there are no MR studies that have assessed the causal association of circulating antioxidants and ischemic stroke risk. In our previous study, we demonstrated that the effect of genetic variants on circulating antioxidant levels are generally comparable with those which would be achieved by dietary supplementation [22]. Given the robust and generally consistent null results in the present study that both absolute blood antioxidant levels and metabolites measured with high throughput

technology are not causally associated with ischemic stroke, it is not likely that dietary supplements that are to increase antioxidant concentrations in blood reduce the risk of ischemic stroke.

Despite the use of data from more than 1 million participants, our study population could be seen as relatively young for stroke occurrence (especially in the UK Biobank population who were below age 70 years at study inclusion). In a population-based cohort study, stroke incidence increased from 1.7 at age 55-59, to 19.9 at age 80-84 [39]. Thus, the current population might show different results when studied several years from now. The low number of cases observed in the UK Biobank population is likely explained by the lower mean age of the individuals included in the analysis, and the UK Biobank is considered a healthier population than the general UK population. Furthermore, MEGASTROKE contained cohorts with an oversampling of stroke cases. As our primary research hypothesis was focused on ischemic stroke, we did not investigate different stroke subtypes as primary outcomes. This decision was motivated by the up to 6 times lower number of cases per subtype. Therefore, analyses of these stroke subtypes were considered as underpowered to draw firm conclusions from, as could also be seen from our power calculation. However, importantly, our findings on ischemic stroke were robust across different analysis methods and were generally consistent across three independent cohorts with a large number of cases. As our null findings were generally consistent across our study cohorts, it is very unlikely that changes in antioxidant levels will yield any clinically relevant reduction in ischemic stroke risk. These findings were also in line with our earlier work in which we found no associations between these antioxidants in relation to coronary heart disease [22]. Together with our present findings, this would suggest that antioxidants do not affect the risk of developing atherogenic cardiovascular disease. However, this is contrary to the data from experimental settings in which oxidative stress does play an important role in the onset of atherosclerosis [40, 41]. This has given rise to the hypothesis that circulating antioxidant levels might not be representative of antioxidant capacity, and that increasing antioxidant levels in blood (either by nutritional intake or supplements) do not necessarily result in additional antioxidative effects. This hypothesis was supported by a number of our earlier studies in which we investigated the associations of vitamin E and its enzymatic and oxidative metabolites with lifestyle factors and subclinical disease outcomes [42, 43]. In brief, we showed in these studies that vitamin E concentrations were not correlated with the urinary enzymatic and oxidative vitamin E metabolite levels, and lifestyle factors and subclinical disease outcomes showed different associations with vitamin E concentrations than with their oxidized metabolites.

The present study has several strengths. First, a large sample size was studied, by combining three cohorts comprising a total of 70,791 cases and performing a meta-analysis. Individually, the results from the three cohorts are consistent with each other and with the final meta-analysis. We did not detect any heterogeneity between-SNPs

for every antioxidant in each cohort, or across each cohort. Additionally, by performing MR-egger and weighted-median estimator analyses, the final MR estimates should be seen as a reliable result despite the sometimes low explained variance of certain genetic instruments. Second, we used separate sets of instrumental variables by looking at both absolute blood levels as well as metabolite levels of antioxidants. The similar findings, especially in regards to ascorbate and retinol which are analyzed with both their absolute blood concentration and the relative metabolite levels, suggests general robustness of our findings. However, some limitations should also be considered with respect to the interpretation of the results. First, participants included in our study are predominantly of European descent, which will limit the extrapolation to other populations. Second, sensitivity analyses for some instrumental variables with limited number of genetic variants as instrumental variables could not be performed. Third, since we were only able to study a selected number of antioxidants, we cannot fully exclude the hypothesis that other antioxidants could have protective effects on ischemic stroke.

In summary, our study did not provide evidence supporting a causal association between diet-derived levels of antioxidants vitamin E, C, lycopene, β-carotene and retinol, and ischemic stroke. Therefore, antioxidant supplementation is unlikely to be of clinical benefit to prevent ischemic stroke.

Disclosures
None

Conflict of interest statement
The authors declare to have no conflict of interest.

Supplemental Material
Data S1. Supplemental Methods, Table S1, Figures S1-S2.

REFERENCES

1. Wang, H., *Global age-sex-specific fertility, mortality, healthy life expectancy (HALE), and population estimates in 204 countries and territories, 1950-2019: a comprehensive demographic analysis for the Global Burden of Disease Study 2019.* Lancet, 2020. **396**: p. 1160-1203.

2. Boehme, A.K., C. Esenwa, and M.S. Elkind, *Stroke Risk Factors, Genetics, and Prevention.* Circ Res, 2017. **120**: p. 472-495.

3. Banerjee, C., Y.P. Moon, M.C. Paik, T. Rundek, C. Mora-McLaughlin, J.R. Vieira, R.L. Sacco, and M.S. Elkind, *Duration of diabetes and risk of ischemic stroke: the Northern Manhattan Study.* Stroke, 2012. **43**: p. 1212-7.

4. Bhat, V.M., J.W. Cole, J.D. Sorkin, M.A. Wozniak, A.M. Malarcher, W.H. Giles, B.J. Stern, and S.J. Kittner, *Dose-response relationship between cigarette smoking and risk of ischemic stroke in young women.* Stroke, 2008. **39**: p. 2439-43.

5. Horenstein, R.B., D.E. Smith, and L. Mosca, *Cholesterol predicts stroke mortality in the Women's Pooling Project.* Stroke, 2002. **33**: p. 1863-8.

6. Lewington, S., R. Clarke, N. Qizilbash, R. Peto, R. Collins, and C. Prospective Studies, *Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies.* Lancet, 2002. **360**: p. 1903-13.

7. Suk, S.H., R.L. Sacco, B. Boden-Albala, J.F. Cheun, J.G. Pittman, M.S. Elkind, M.C. Paik, and S. Northern Manhattan Stroke, *Abdominal obesity and risk of ischemic stroke: the Northern Manhattan Stroke Study.* Stroke, 2003. **34**: p. 1586-92.

8. Rodrigo, R., R. Fernandez-Gajardo, R. Gutierrez, J.M. Matamala, R. Carrasco, A. Miranda-Merchak, and W. Feuerhake, *Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities.* CNS Neurol Disord Drug Targets, 2013. **12**: p. 698-714.

9. Allen, C.L. and U. Bayraktutan, *Oxidative stress and its role in the pathogenesis of ischaemic stroke.* Int J Stroke, 2009. **4**: p. 461-70.

10. Shirley, R., E.N. Ord, and L.M. Work, *Oxidative Stress and the Use of Antioxidants in Stroke.* Antioxidants (Basel), 2014. **3**: p. 472-501.

11. Yochum, L.A., A.R. Folsom, and L.H. Kushi, *Intake of antioxidant vitamins and risk of death from stroke in postmenopausal women.* Am J Clin Nutr, 2000. **72**: p. 476-83.

12. Liu, S., J.E. Manson, M.J. Stampfer, K.M. Rexrode, F.B. Hu, E.B. Rimm, and W.C. Willett, *Whole grain consumption and risk of ischemic stroke in women: A prospective study.* JAMA, 2000. **284**: p. 1534-40.

13. Sanchez-Moreno, C., A. Jimenez-Escrig, and A. Martin, *Stroke: roles of B vitamins, homocysteine and antioxidants.* Nutr Res Rev, 2009. **22**: p. 49-67.

14. Gillman, M.W., L.A. Cupples, D. Gagnon, B.M. Posner, R.C. Ellison, W.P. Castelli, and P.A. Wolf, *Protective effect of fruits and vegetables on development of stroke in men.* JAMA, 1995. **273**: p. 1113-7.

15. Parohan, M., J. Anjom-Shoae, M. Nasiri, M. Khodadost, S.R. Khatibi, and O. Sadeghi, *Dietary total antioxidant capacity and mortality from all causes, cardiovascular disease and cancer: a systematic review and dose-response meta-analysis of prospective cohort studies.* Eur J Nutr, 2019. **58**: p. 2175-2189.

16. Leppala, J.M., J. Virtamo, R. Fogelholm, D. Albanes, P.R. Taylor, and O.P. Heinonen, *Vitamin E and beta carotene supplementation in high risk for stroke: a subgroup analysis of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study.* Arch Neurol, 2000. **57**: p. 1503-9.

17. Yusuf, S., G. Dagenais, J. Pogue, J. Bosch, and P. Sleight, *Vitamin E supplementation and cardiovascular events in high-risk patients.* N Engl J Med, 2000. **342**: p. 154-60.

18. Lawlor, D.A., R.M. Harbord, J.A. Sterne, N. Timpson, and G. Davey Smith, *Mendelian randomization: using genes as instruments for making causal inferences in epidemiology.* Stat Med, 2008. **27**: p. 1133-63.

19. Burgess, S., A. Butterworth, and S.G. Thompson, *Mendelian randomization analysis with multiple genetic variants using summarized data.* Genet Epidemiol, 2013. **37**: p. 658-65.

20. Bowden, J., G. Davey Smith, P.C. Haycock, and S. Burgess, *Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator.* Genet Epidemiol, 2016. **40**: p. 304-14.

21. Burgess, S., R.A. Scott, N.J. Timpson, G. Davey Smith, S.G. Thompson, and E.-I. Consortium, *Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors.* Eur J Epidemiol, 2015. **30**: p. 543-52.

22. Luo, J., S. le Cessie, D. van Heemst, and R. Noordam, *Diet-Derived Circulating Antioxidants and Risk of Coronary Heart Disease: A Mendelian Randomization Study.* J Am Coll Cardiol, 2021. **77**: p. 45-54.

23. Long, T., M. Hicks, H.C. Yu, W.H. Biggs, E.F. Kirkness, C. Menni, J. Zierer, K.S. Small, M. Mangino, H. Messier, et al., *Whole-genome sequencing identifies common-to-rare variants associated with human blood metabolites.* Nat Genet, 2017. **49**: p. 568-578.

24. D'Adamo, C.R., A. D'Urso, K.A. Ryan, L.M. Yerges-Armstrong, R.D. Semba, N.I. Steinle, B.D. Mitchell, A.R. Shuldiner, and P.F. McArdle, *A Common Variant in the SETD7 Gene Predicts Serum Lycopene Concentrations.* Nutrients, 2016. **8**: p. 82.

25. Shin, S.Y., E.B. Fauman, A.K. Petersen, J. Krumsiek, R. Santos, J. Huang, M. Arnold, I. Erte, V. Forgetta, T.P. Yang, et al., *An atlas of genetic influences on human blood metabolites.* Nat Genet, 2014. **46**: p. 543-550.

26. Hendrickson, S.J., A. Hazra, C. Chen, A.H. Eliassen, P. Kraft, B.A. Rosner, and W.C. Willett, *beta-Carotene 15,15'-monooxygenase 1 single nucleotide polymorphisms in relation to plasma carotenoid and retinol concentrations in women of European descent.* Am J Clin Nutr, 2012. **96**: p. 1379-89.

27. Mondul, A.M., K. Yu, W. Wheeler, H. Zhang, S.J. Weinstein, J.M. Major, M.C. Cornelis, S. Mannisto, A. Hazra, A.W. Hsing, et al., *Genome-wide association study of circulating retinol levels.* Hum Mol Genet, 2011. **20**: p. 4724-31.

28. Major, J.M., K. Yu, W. Wheeler, H. Zhang, M.C. Cornelis, M.E. Wright, M. Yeager, K. Snyder, S.J. Weinstein, A. Mondul, et al., *Genome-wide association study identifies common variants associated with circulating vitamin E levels.* Hum Mol Genet, 2011. **20**: p. 3876-83.

29. Zheng, J.S., J. Luan, E. Sofianopoulou, F. Imamura, I.D. Stewart, F.R. Day, M. Pietzner, E. Wheeler, L.A. Lotta, T.E. Gundersen, et al., *Plasma Vitamin C and Type 2 Diabetes: Genome-Wide Association Study and Mendelian Randomization Analysis in European Populations.* Diabetes Care, 2021. **44**: p. 98-106.

30. Malik, R., G. Chauhan, M. Traylor, M. Sargurupremraj, Y. Okada, A. Mishra, L. Rutten-Jacobs, A.K. Giese, S.W. van der Laan, S. Gretarsdottir, et al., *Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes.* Nat Genet, 2018. **50**: p. 524-537.

31. Sudlow, C., J. Gallacher, N. Allen, V. Beral, P. Burton, J. Danesh, P. Downey, P. Elliott, J. Green, M. Landray, et al., *UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age.* PLoS Med, 2015. **12**: p. e1001779.

32. Burgess, S. and S.G. Thompson, *Interpreting findings from Mendelian randomization using the MR-Egger method.* Eur J Epidemiol, 2017. **32**: p. 377-389.

33. Bowden, J., G. Davey Smith, and S. Burgess, *Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression.* Int J Epidemiol, 2015. **44**: p. 512-25.

34. Verbanck, M., C.Y. Chen, B. Neale, and R. Do, *Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases.* Nat Genet, 2018. **50**: p. 693-698.

35. Greco, M.F., C. Minelli, N.A. Sheehan, and J.R. Thompson, *Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome.* Stat Med, 2015. **34**: p. 2926-40.

36. Teslovich, T.M., K. Musunuru, A.V. Smith, A.C. Edmondson, I.M. Stylianou, M. Koseki, J.P. Pirruccello, S. Ripatti, D.I. Chasman, C.J. Willer, et al., *Biological, clinical and population relevance of 95 loci for blood lipids.* Nature, 2010. **466**: p. 707-13.

37. Pierce, B.L., H. Ahsan, and T.J. Vanderweele, *Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants.* Int J Epidemiol, 2011. **40**: p. 740-52.

38. Cheng, P., L. Wang, S. Ning, Z. Liu, H. Lin, S. Chen, and J. Zhu, *Vitamin E intake and risk of stroke: a meta-analysis.* Br J Nutr, 2018. **120**: p. 1181-1188.

39. Hollander, M., P.J. Koudstaal, M.L. Bots, D.E. Grobbee, A. Hofman, and M.M. Breteler, *Incidence, risk, and case fatality of first ever stroke in the elderly population. The Rotterdam Study.* J Neurol Neurosurg Psychiatry, 2003. **74**: p. 317-21.

40. Kattoor, A.J., N.V.K. Pothineni, D. Palagiri, and J.L. Mehta, *Oxidative Stress in Atherosclerosis.* Curr Atheroscler Rep, 2017. **19**: p. 42.

41. Forstermann, U., N. Xia, and H. Li, *Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis.* Circ Res, 2017. **120**: p. 713-735.

42. Martens, L.G., J. Luo, F.L. Meulmeester, N. Ashrafi, E.W. van Eekelen, R. de Mutsert, D.O. Mook-Kanamori, F.R. Rosendaal, K.W. van Dijk, K. Mills, et al., *Associations between Lifestyle Factors and Vitamin E Metabolites in the General Population.* Antioxidants (Basel), 2020. **9**.

43.	Luo, J., F.L. Meulmeester, L.G. Martens, N. Ashrafi, R. de Mutsert, D.O. Mook-Kanamori, F.R. Rosendaal, K. Willems van Dijk, S. le Cessie, K. Mills, et al., *Urinary oxidized, but not enzymatic vitamin E metabolites are inversely associated with measures of glucose homeostasis in middle-aged healthy individuals.* Clin Nutr, 2021. **40**: p. 4192-4200.