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ORIGINAL RESEARCH

Diet-Derived Antioxidants Do Not Decrease Risk of Ischemic Stroke: A Mendelian Randomization Study in 1 Million People

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BACKGROUND: Dietary intake and blood concentrations of vitamins E and C, lycopene, and carotenoids have been associated with a lower risk of incident (ischemic) stroke. However, causality cannot 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.

METHODS AND RESULTS: For each circulating antioxidant (vitamins E and 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 ($P<5\times10^{-8}$). We used summary statistics for single-nucleotide polymorphisms–stroke associations from 3 European-ancestry cohorts (cases/controls): MEGASTROKE (60 341/454 450), UK Biobank (2404/368 771), and the FinnGen study (8046/164 286). Mendelian randomization analyses were performed on each exposure per outcome cohort using inverse variance–weighted analyses and subsequently meta-analyzed. 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 (ORs) ranged between 0.94 (95% CI, 0.85–1.05) for vitamin C and 1.04 (95% CI, 0.99–1.08) for lycopene. For metabolites, ORs ranged between 1.01 (95% CI, 0.98–1.03) for retinol and 1.12 (95% CI, 0.88–1.42) 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.

Key Words: antioxidants ■ cardiovascular disease ■ diet ■ ischemic stroke ■ oxidative stress

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, 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.[8,9] Reactive oxygen species–induced damage can cause significant changes in the vascular system, ultimately influencing cerebral blood flow.[9] These

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CLINICAL PERSPECTIVE

What Is New?

- As more observational studies seem to report an association between antioxidant levels and reduced ischemic stroke occurrence, our study aimed to take a Mendelian randomization approach in order to investigate whether this association could be causal.

What Are the Clinical Implications?

- Our findings highlight that genetically determined antioxidant levels do not seem causally associated with lower ischemic stroke risk.
- Therefore, it seems unlikely that antioxidant supplementation is of clinical benefit to prevent ischemic stroke.

Nonstandard Abbreviations and Acronyms

IVW	inverse variance–weighted
MEGASTROKE	Multi-Ancestry Genome-Wide Association Study of 520 000 Subjects Identifies 32 Loci Associated With Stroke and Stroke Subtypes
MR	Mendelian randomization
MR-PRESSO	Mendelian Randomization Pleiotropy Residual Sum and Outlier

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 vitamins E and 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 vitamins E and C and carotenoids were associated with a 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 2-sample MR to assess the 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. The data from the UK Biobank are available upon acceptance of a research proposal by UK Biobank Recourses and payment of an access fee. The data from MEGASTROKE (Multi-Ancestry Genome-Wide Association Study of 520 000 Subjects Identifies 32 Loci Associated With Stroke and Stroke Subtypes) and FinnGen are freely available from their respective websites (MEGASTROKE: https://www.megastroke.org/, FinnGen: https://www.finngen.fi/en/). Additional methods can be found in Data S1. All participants provided written informed consent, and local research ethics committees and institutional review boards approved the individual studies.

Study Design

For our study, we conducted a 2-sample MR, which uses genetic instrumental variables as a proxy for the exposure.[21] Using this design, single-nucleotide polymorphism (SNP)-exposure and equivalent SNP–outcome associations were derived from genome-wide association studies. 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 3 principal assumptions. First, the genetic variants should be associated with the exposure. Second, 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 5 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 genome-wide association studies was performed to extract the leading SNPs as genetic instrumental variables. Whenever multiple genome-wide association studies were identified, only the largest study including replication was used.[23–29] All selected variants were associated with their corresponding exposure ($P<5\times10^{-8}$). 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 Table S1).[29] All selected variants passed standard quality control filters with an imputation score of ≥0.90, except for retinol-associated SNP rs117468033, which passed with a score of 0.65.

Outcome Data Sets

Summary statistics on the associations of the exposure-related SNPs with ischemic stroke were extracted from 3 large cohorts: the MEGASTROKE consortium, the UK Biobank, and the FinnGen study. Both the UK Biobank and the FinnGen study 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.

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 56 340) is a prospective general population cohort with 502 628 participants between the ages of 40 and 70 years recruited from the general population between 2006 and 2010[31]; 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 data sets. In our data set, we had data available on 2404 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 (version 2.3.2).

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 health care 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 were used. Within these data, there are 8046 reported cases of ischemic stroke and 164 286 controls.

Table 1. Summary Characteristics of MEGASTROKE, UK Biobank, and FinnGen

	MEGASTROKE	UK Biobank	FinnGen
Cases, n	67 162	2404	8046
Age (SD), y	69.1 (10.8)	61.5 (6.7)	…
Women, %	43	34	…
Controls	454 450	368 771	164 286
Age (SD), y	56.6 (15.6)	56.7 (8.0)	…
Women, %	51	54.5	…

MEGASTROKE indicates Multi-Ancestry Genome-Wide Association Study of 520 000 Subjects Identifies 32 Loci Associated With Stroke and Stroke Subtypes.

Statistical Analysis

All analyses were performed using R (version 3.6.1) statistical software (The R Foundation for Statistical Computing). MR analyses were performed using the R-based package "TwoSampleMR" (https://mrcieu.github.io/TwoSampleMR/).

Before 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 (ORs) 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 as a result of 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 among the genetic variants, and a value that differs from zero indicates that the IVW estimate is biased.[33] Although considered a relatively inefficient approach (eg, large CIs), 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, Mendelian Randomization Pleiotropy Residual Sum and Outlier (MR-PRESSO) 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 *Q* statistic was performed 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 data sets and subsequently meta-analyzed to derive the pooled estimates for each exposure on the risk of ischemic stroke. Heterogeneity of the estimates among 3 data sets was performed by I^2, and corresponding *P* values were obtained from the Cochran *Q* test. When no heterogeneity was found among the 3 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 that our study had enough power given the current parameters. Separate power calculations were performed for the different subtypes of stroke (cardioembolic, large artery atherosclerosis, and small-vessel) in MEGASTROKE, as data were 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 subanalyses as well.

RESULTS

By combining the 3 cohorts, a total sample of 1 058 298 individuals, of which 70 791 included 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 previously described,[36] which ranged from 1.7% to 30.1% for absolute antioxidant levels and from 3.3% to 18.6% for metabolite antioxidants (Table 2). 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 nonsignificant 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 data sets (Figures 1 and 2). All analyses were performed with outcome ischemic stroke, as well as with outcomes cardioembolic, large artery atherosclerosis, and small-vessel stroke separately (Figures S1 and S2). 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.

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 3 cohorts was not significantly associated with ischemic stroke. Most notably, the pooled ORs were 0.94 (95% CI, 0.85–1.05) per 1 μmol/L of ascorbate, 1.04 (95% CI, 0.99–1.08) per 1 μg/dL of lycopene, and 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 >3 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 *Q*

Table 2. GWAS on Genetically Determined Diet-Derived Antioxidants

Exposure data sets	Absolute antioxidants				Metabolite antioxidants			
	N	Genetic variants	Explained variance, %	Unit	N	Genetic variants	Explained variance, %	Units
α-Tocopherol	…	…	…	…	7276	11	3.3	Log10-transformed metabolites concentration
γ-Tocopherol	…	…	…	…	5822	13	15.0	Log10-transformed metabolites concentration
β-Carotene	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

GWAS indicates genome-wide association studies.

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 ORs 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 *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 β-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 3284 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 >1 million participants, our study population could be seen as relatively young for stroke occurrence (especially in the UK Biobank population who were younger than age 70 years at study inclusion). In a population-based cohort study, stroke incidence increased from 1.7 at age 55 to 59 years, to 19.9 at age 80 to 84 years.[39] Thus, the

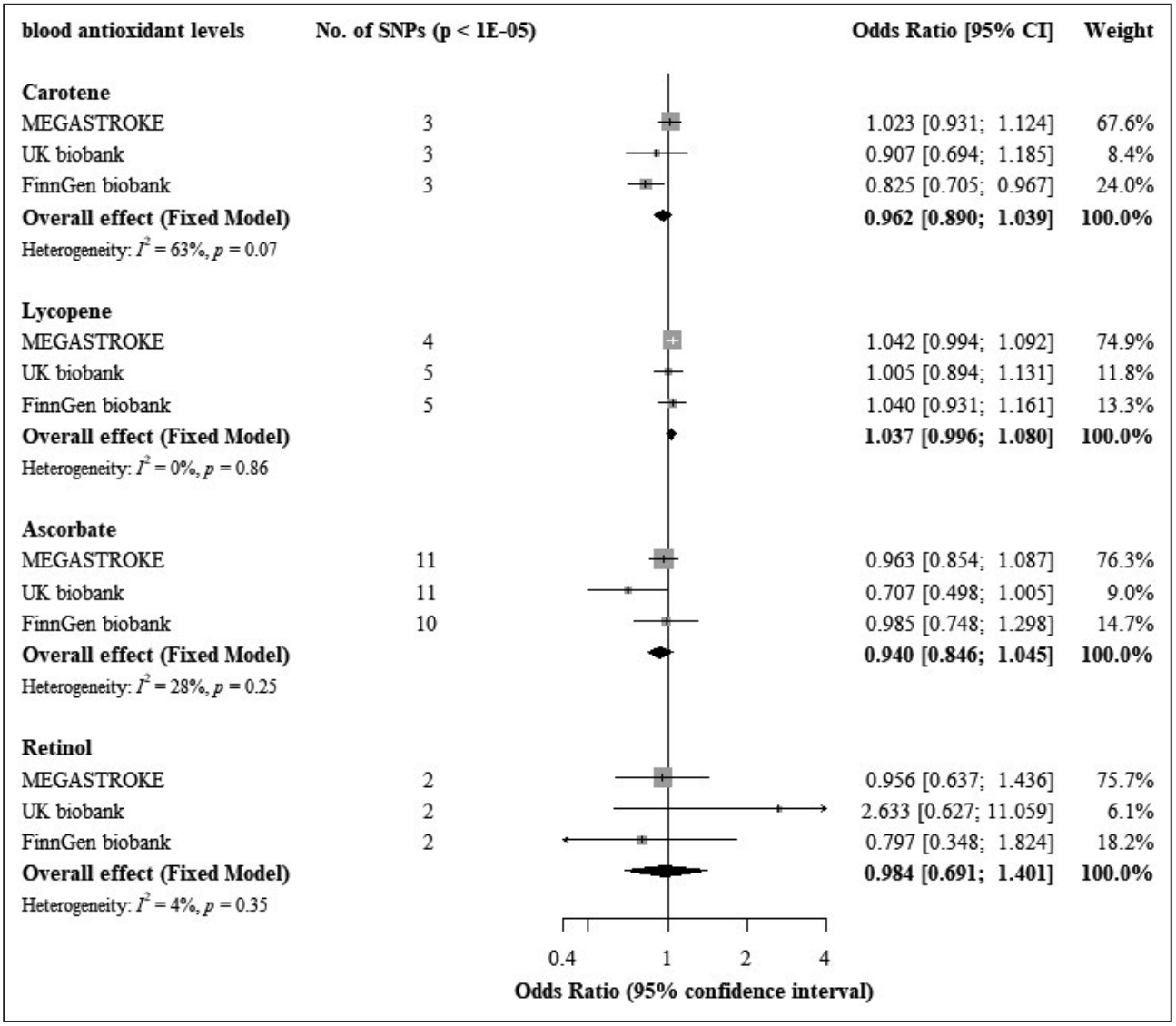

Figure 1. Causal association between absolute blood level antioxidants and ischemic stroke occurrence.
Estimated odds ratios represent the effect per unit increase in ln-transformed β-carotene and retinol, 1 μg/dL lycopene, and 1 μmol/L ascorbate on ischemic stroke. Results were obtained from an inverse variance–weighted analysis per outcome database and combined over the 3 databases using fixed-effect meta-analyses. MEGASTROKE indicates Multi-Ancestry Genome-Wide Association Study of 520 000 Subjects Identifies 32 Loci Associated With Stroke and Stroke Subtypes; SNP, single-nucleotide polymorphism.

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 among 3 independent cohorts with a large number of cases. As our null findings were generally consistent among our study cohorts, it is 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

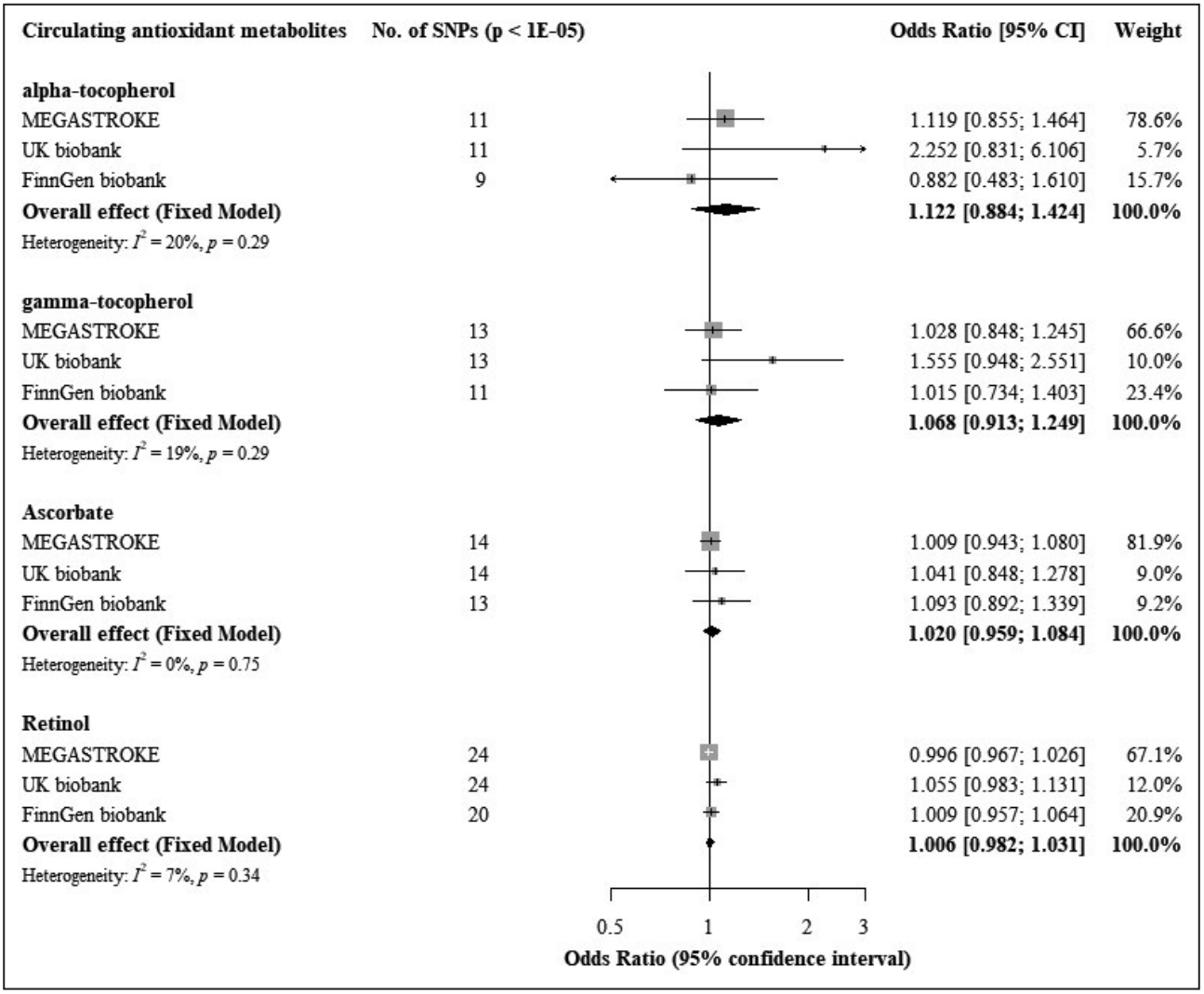

Figure 2. Causal association between circulating antioxidant metabolites and ischemic stroke occurrence.
Estimated odds ratios represent the effect per 10-fold increase in antioxidant metabolites' concentration on ischemic stroke. Results were obtained from an inverse variance–weighted analysis per outcome database and combined over the 3 databases using fixed-effect meta-analyses. MEGASTROKE indicates Multi-Ancestry Genome-Wide Association Study of 520 000 Subjects Identifies 32 Loci Associated With Stroke and Stroke Subtypes; SNP, single-nucleotide polymorphism.

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 3 cohorts comprising a total of 70 791 cases and performing a meta-analysis. Individually, the results from the 3 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 among 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, suggest 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 limits 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 vitamins E and C, lycopene, β-carotene, and retinol, and ischemic stroke. Therefore, antioxidant supplementation is unlikely to be of clinical benefit to prevent ischemic stroke.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Material

Data S1
Table S1
Figures S1–S2

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Supplemental Material

Data S1.

Supplemental Methods

SELECTION OF GENETIC INSTRUMENTAL VARIABLES.

In total, 5 main dietary-derived antioxidants were considered in the present study: vitamin E (a- and g-tocopherol), b-carotene, lycopene, vitamin C (L-ascorbic acid or ascorbate), and retinol. We considered both antioxidants that were measured as authentic absolute levels in blood and their corresponding circulating metabolites that were quantified as relative concentrations in plasma or serum. For absolute antioxidant levels, a-tocopherol, b-carotene, lycopene, ascorbate, and retinol were identified, whereas for antioxidant metabolites, a-tocopherol, g-tocopherol, ascorbate, and retinol were used. Genome-wide association studies (GWAS) were searched to extract leading single-nucleotide polymorphisms (SNPs) as genetic instrumental variables. When we identified multiple GWAS for a single trait, only the largest study with replication was used. Although GWAS data is not available for absolute ascorbate levels, a study with a 2-stage design, which used a discovery cohort and 5 replication cohorts and consequently meta-analysis, assessed the relationship between genetic variants located in vitamin C active transporter locus of SLC23A1 (solute carrier family 23 member 1) and circulating levels of ascorbic acid; it was therefore considered to be qualified for genetic instrument extraction. A summary table of instruments is presented in Table S1.

ABSOLUTE CIRCULATING ANTIOXIDANTS.

Three SNPs for a-tocopherol levels were identified in a GWAS with 7,781 European individuals. However, those 3 loci were previously reported to be associated with lipid metabolism and/or regulation in GWAS on lipid levels or coronary artery disease (CAD), and therefore were not considered for MR analysis due to likely pleiotropic bias. Three genetic variants (linkage disequilibrium [LD] <0.2 as indicated in the study; $p < 5 \times 10^{-8}$) associated with plasma b-carotene levels were identified in a GWAS within 2,344 participants in the Nurses' Health Study. Five variants (LD 0.001; $p < 5 \times 10^{-6}$) associated with circulating lycopene level were identified in a GWAS performed in 441 older Amish adults. Two SNPs (LD < 0.001; $p < 5 \times 10^{-8}$) associated with circulating retinol levels were identified in a GWAS of 5,006 Caucasian individuals from 2 cohorts.

Circulating antioxidant metabolites.

Genetic variants for each metabolite at suggestive genomewide significance level ($p < 1 \times 10^{-5}$) were extracted from published GWAS; notably, 11 instruments for a-tocopherol (n = 7,276), 13 for g-tocopherol (n = 5,822), and 14 for ascorbate (n = 2,063) were derived from 7,824 adult individuals from 2 European population studies, and 24 for retinol (n = 1,957) from 1,960 subjects of European descent. Linkage disequilibrium between all SNPs for the same exposure was assessed, and when LD was present (LD > 0.001), the variant with the smallest p value was selected. [22]

Table S1. Complete list of genetic instruments of diet-derived antioxidants.

SNP	Trait type	Trait	Nearest Gene	Chromsome	effect allele	other allele	EAF	Exposure beta	se	p val
rs2232315	Absolute ciculating level	lycopene	G6PC2	chr2:169757432	A	G	0.03	0.740	0.15	1.00E-06
rs341075	Absolute ciculating level	lycopene	ART2P. AP005019.3	chr11:72257963	A	G	0.02	-0.870	0.17	6.00E-07
rs4635297	Absolute ciculating level	lycopene	BC039545	chr15:38327408	A	C	0.08	0.260	0.05	6.00E-07
rs6108801	Absolute ciculating level	lycopene	C20orf187	chr20:10989519	C	T	0.04	-0.480	0.09	4.00E-07
rs7680948	Absolute ciculating level	lycopene	SETD7	chr4:140447105	A	C	0.20	-0.190	0.03	5.00E-09
rs12934922	Absolute ciculating level	β-carotene	BCMO1	chr16:81301694	T	A	0.44	0.139	0.02	5.90E-10
rs4448930	Absolute ciculating level	β-carotene	BCMO1	chr16:81331002	C	G	0.15	0.066	0.03	3.80E-03
rs6564851	Absolute ciculating level	β-carotene	BCMO1	chr16:81264597	G	T	0.36	0.149	0.02	1.60E-24
rs7501331	Absolute ciculating level	β-carotene	BCMO1	chr16:81314496	T	C	0.24	-0.067	0.02	1.60E-05
rs10882272	Absolute ciculating level	retinol	RBP4	chr10:95348182	C	T	0.35	-0.030	0.01	7.00E-15
rs1667255	Absolute ciculating level	retinol	TTR	chr18:29187279	C	A	0.31	0.030	0.01	6.00E-14
rs6693447	Absolute ciculating level	ascorbate	RER1	chr1:2398751	T	G	0.551	0.039	0.006	6.25E-10
rs13028225	Absolute ciculating level	ascorbate	SLC23A3	chr2:219166533	T	C	0.857	0.102	0.009	2.38E-30
rs33972313	Absolute ciculating level	ascorbate	SLC23A1	chr5:139379813	C	T	0.968	0.36	0.018	4.61E-90
rs10051765	Absolute ciculating level	ascorbate	RGS14	chr5:177372991	C	T	0.342	0.039	0.007	3.64E-09

rs7740812	Absolute ciculating level	ascorbate	GSTA11P	chr6:52860989	G	A	0.594	0.038	0.006	1.88E-10
rs174547	Absolute ciculating level	ascorbate	FADS1	chr11:61803311	C	T	0.328	0.036	0.007	3.84E-08
rs117885456	Absolute ciculating level	ascorbate	SNRPF-DT	chr12:95855333	A	G	0.087	0.078	0.012	1.70E-11
rs2559850	Absolute ciculating level	ascorbate	CHPT1	chr12:101699681	A	G	0.598	0.058	0.006	6.30E-20
rs10136000	Absolute ciculating level	ascorbate	AKT1	chr14:104787244	A	G	0.283	0.04	0.007	1.33E-08
rs56738967	Absolute ciculating level	ascorbate	LOC105371356	chr16:79706644	C	G	0.321	0.041	0.007	7.62E-10
rs9895661	Absolute ciculating level	ascorbate	BCAS3	chr17:61379228	T	C	0.817	0.063	0.008	1.05E-14
rs10245705	Circulating metabolite's concentation	alpha-tocopherol	AC004520.1	chr7:26129672	T	C	0.02	-0.066	0.01	1.95E-07
rs7238006	Circulating metabolite's concentation	alpha-tocopherol	RP11-379L18.3	chr18:31927898	T	C	0.93	0.028	0.01	6.77E-07
rs11145330	Circulating metabolite's concentation	alpha-tocopherol	TJP2	chr9:71733603	A	C	0.89	0.032	0.01	1.95E-06
rs2074731	Circulating metabolite's concentation	alpha-tocopherol	SF3A1	chr22:30733914	A	C	0.17	-0.018	0.00	2.31E-06
rs1404410	Circulating metabolite's concentation	alpha-tocopherol	RNU6-102P	chr7:124248950	C	G	0.79	-0.024	0.01	4.57E-06
rs1532701	Circulating metabolite's concentation	alpha-tocopherol	SLC6A2	chr16:55698027	A	G	0.55	0.014	0.00	5.07E-06
rs261342	Circulating metabolite's concentation	alpha-tocopherol	LIPC	chr15:58731153	C	G	0.79	-0.017	0.00	5.41E-06
rs11992435	Circulating metabolite's concentation	alpha-tocopherol	RN7SL107P	chr8:81526083	A	G	0.95	0.033	0.01	6.38E-06
rs7930821	Circulating metabolite's concentation	alpha-tocopherol	ZDHHC13	chr11:19142695	T	C	0.02	0.067	0.01	7.53E-06
rs10163969	Circulating metabolite's concentation	alpha-tocopherol	NDUFV2	chr18:9067439	T	G	0.04	-0.035	0.01	9.39E-06

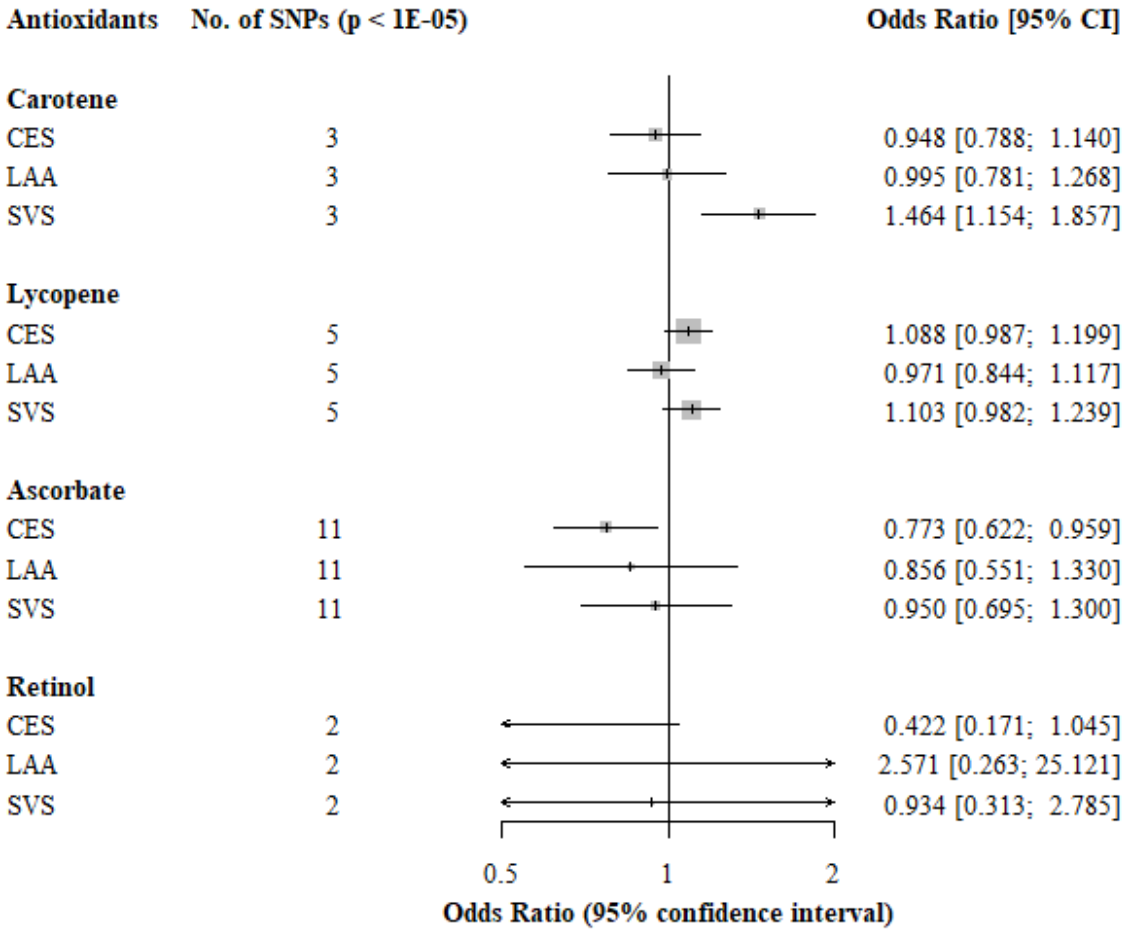
rs10935814	Circulating metabolite's concentation	alpha-tocopherol	SELENOT	chr3:150352873	A	G	0.10	-0.037	0.01	9.44E-06
rs6713914	Circulating metabolite's concentation	ascorbate	AC007131.2	chr2:59523041	C	T	0.44	-0.059	0.01	3.22E-07
rs577596	Circulating metabolite's concentation	ascorbate	AL008721.1	chr22:25931606	A	G	0.38	-0.057	0.01	6.68E-07
rs11167905	Circulating metabolite's concentation	ascorbate	PRELID2	chr5:144906846	C	T	0.14	-0.080	0.02	9.83E-07
rs6834631	Circulating metabolite's concentation	ascorbate	RP11-170N16.1	chr4:122315665	G	T	0.04	-0.131	0.03	1.03E-06
rs7112460	Circulating metabolite's concentation	ascorbate	HYLS1	chr11:125751859	T	C	0.93	0.108	0.02	1.14E-06
rs6826474	Circulating metabolite's concentation	ascorbate	MANBA	chr4:96650120	T	C	0.96	-0.138	0.03	1.56E-06
rs8105491	Circulating metabolite's concentation	ascorbate	CTC-260E6.6	chr19:20403988	T	G	0.85	-0.070	0.01	2.30E-06
rs808686	Circulating metabolite's concentation	ascorbate	AL121761.1	chr20:19717135	A	G	0.49	0.060	0.01	3.01E-06
rs13069990	Circulating metabolite's concentation	ascorbate	LSAMP	chr3:115620801	T	C	0.62	-0.051	0.01	4.44E-06
rs2070006	Circulating metabolite's concentation	ascorbate	FGA	chr4:155513866	C	T	0.37	-0.051	0.01	4.76E-06
rs13103690	Circulating metabolite's concentation	ascorbate	SLC2A9	chr4:9972778	G	T	0.47	0.047	0.01	5.20E-06
rs9606290	Circulating metabolite's concentation	ascorbate	MYO18B	chr22:20218237	A	G	0.26	0.159	0.04	6.32E-06
rs9419004	Circulating metabolite's concentation	ascorbate	ADGRA1	chr10:134914522	C	G	0.28	-0.254	0.06	6.53E-06
rs8057559	Circulating metabolite's concentation	ascorbate	RP11-140I24.2	chr16:73317172	T	C	0.96	0.140	0.03	9.10E-06
rs1060467	Circulating metabolite's concentation	gamma-tocopherol	CYP4F11	chr19:16024538	A	G	0.59	0.023	0.00	2.61E-07
rs5994305	Circulating metabolite's concentation	gamma-tocopherol	SEC14L2	chr22:30820707	A	G	0.83	0.031	0.01	7.15E-07

rs261301	Circulating metabolite's concentation	gamma-tocopherol	ALDH1A2	chr15:58686939	T	C	0.13	0.032	0.01	2.06E-06
rs1013104	Circulating metabolite's concentation	gamma-tocopherol	RP11-471L13.2	chr17:12187236	T	C	0.44	-0.021	0.00	3.83E-06
rs7038957	Circulating metabolite's concentation	gamma-tocopherol	RNA5SP291	chr9:106539439	T	C	0.83	-0.029	0.01	3.86E-06
rs7350776	Circulating metabolite's concentation	gamma-tocopherol	RYR3	chr15:33689075	C	G	0.70	0.024	0.01	3.86E-06
rs10077932	Circulating metabolite's concentation	gamma-tocopherol	LINC02142	chr5:2450679	T	C	0.14	-0.040	0.01	4.08E-06
rs10520845	Circulating metabolite's concentation	gamma-tocopherol	MYO10	chr5:16879933	A	C	0.02	0.191	0.04	5.27E-06
rs13336771	Circulating metabolite's concentation	gamma-tocopherol	LONP2	chr16:48318728	A	C	0.17	0.062	0.01	7.39E-06
rs10492212	Circulating metabolite's concentation	gamma-tocopherol	EEF1B2P4	chr12:107296945	T	C	0.16	-0.027	0.01	8.66E-06
rs2794327	Circulating metabolite's concentation	gamma-tocopherol	MEGF6	chr1:3512341	T	C	0.67	-0.036	0.01	8.78E-06
rs6821770	Circulating metabolite's concentation	gamma-tocopherol	SORCS2	chr4:7732805	A	G	0.14	0.038	0.01	8.92E-06
rs10466757	Circulating metabolite's concentation	gamma-tocopherol	BCAT1	chr12:24984990	A	T	0.16	0.063	0.01	9.56E-06
rs58411567	Circulating metabolite's concentation	retinol	DNAH10	chr12:124387574	A	G	0.25	-0.208	0.05	3.02E-07
rs1176744	Circulating metabolite's concentation	retinol	HTR3B	chr11:113803028	C	A	0.31	-0.207	0.05	3.52E-07
rs945817	Circulating metabolite's concentation	retinol	RNU6-248P	chr6:75454012	A	G	0.83	-0.275	0.05	6.46E-07
rs1842947	Circulating metabolite's concentation	retinol	OR3A3	chr17:3316976	G	A	0.49	-0.192	0.04	8.34E-07
rs2417325	Circulating metabolite's concentation	retinol	GRIN2B	chr12:14171015	T	C	0.09	0.329	0.08	1.29E-06
rs149478645	Circulating metabolite's concentation	retinol	CTTNBP2	chr7:117400313	G	A	0.97	-0.507	0.14	1.30E-06

rs7926028	Circulating metabolite's concentation	retinol	RP11-347H15.6	chr11:50213631	T	G	0.47	-0.135	0.04	2.75E-06
rs17005512	Circulating metabolite's concentation	retinol	RP11-576N17.4	chr4:83113756	C	G	0.17	-0.218	0.06	2.77E-06
rs3898702	Circulating metabolite's concentation	retinol	LMCD1-AS1	chr3:8011098	T	C	0.21	-0.217	0.05	3.02E-06
rs9586119	Circulating metabolite's concentation	retinol	TET1P1	chr13:88613487	C	T	0.11	0.355	0.08	3.34E-06
rs149113848	Circulating metabolite's concentation	retinol	AC084193.1	chr2:81021743	G	C	0.99	-0.964	0.27	3.47E-06
rs75308833	Circulating metabolite's concentation	retinol	CTD-2197M16.1	chr5:34602282	T	C	0.97	-0.494	0.15	3.51E-06
rs10019071	Circulating metabolite's concentation	retinol	RN7SKP13	chr4:181442264	A	G	0.01	0.657	0.16	3.64E-06
rs12955464	Circulating metabolite's concentation	retinol	RP11-419J16.1	chr18:10141045	G	C	0.87	-0.234	0.06	3.71E-06
rs6550239	Circulating metabolite's concentation	retinol	RAB5A	chr3:20001318	A	G	0.73	-0.183	0.05	4.40E-06
rs139726207	Circulating metabolite's concentation	retinol	SERPINE2	chr2:223878661	G	A	0.91	0.370	0.11	4.46E-06
rs112293959	Circulating metabolite's concentation	retinol	ZFAND6	chr15:80358561	G	A	0.99	-0.429	0.13	5.70E-06
rs1153379	Circulating metabolite's concentation	retinol	AP000472.3	chr21:16678993	A	G	0.95	-0.323	0.08	6.10E-06
rs114515641	Circulating metabolite's concentation	retinol	IGSF11	chr3:118837344	G	T	0.03	0.414	0.13	7.12E-06
rs3890033	Circulating metabolite's concentation	retinol	TNK2	chr3:195591527	C	T	0.50	0.144	0.04	8.56E-06
rs79262371	Circulating metabolite's concentation	retinol	UBE3AP2	chr21:28344552	C	T	0.02	0.569	0.19	8.76E-06
rs2147337	Circulating metabolite's concentation	retinol	SIRPG	chr20:1612282	G	T	0.66	0.158	0.04	9.00E-06
rs9546522	Circulating metabolite's concentation	retinol	DCLK1	chr13:36719430	A	G	0.37	0.134	0.05	9.12E-06

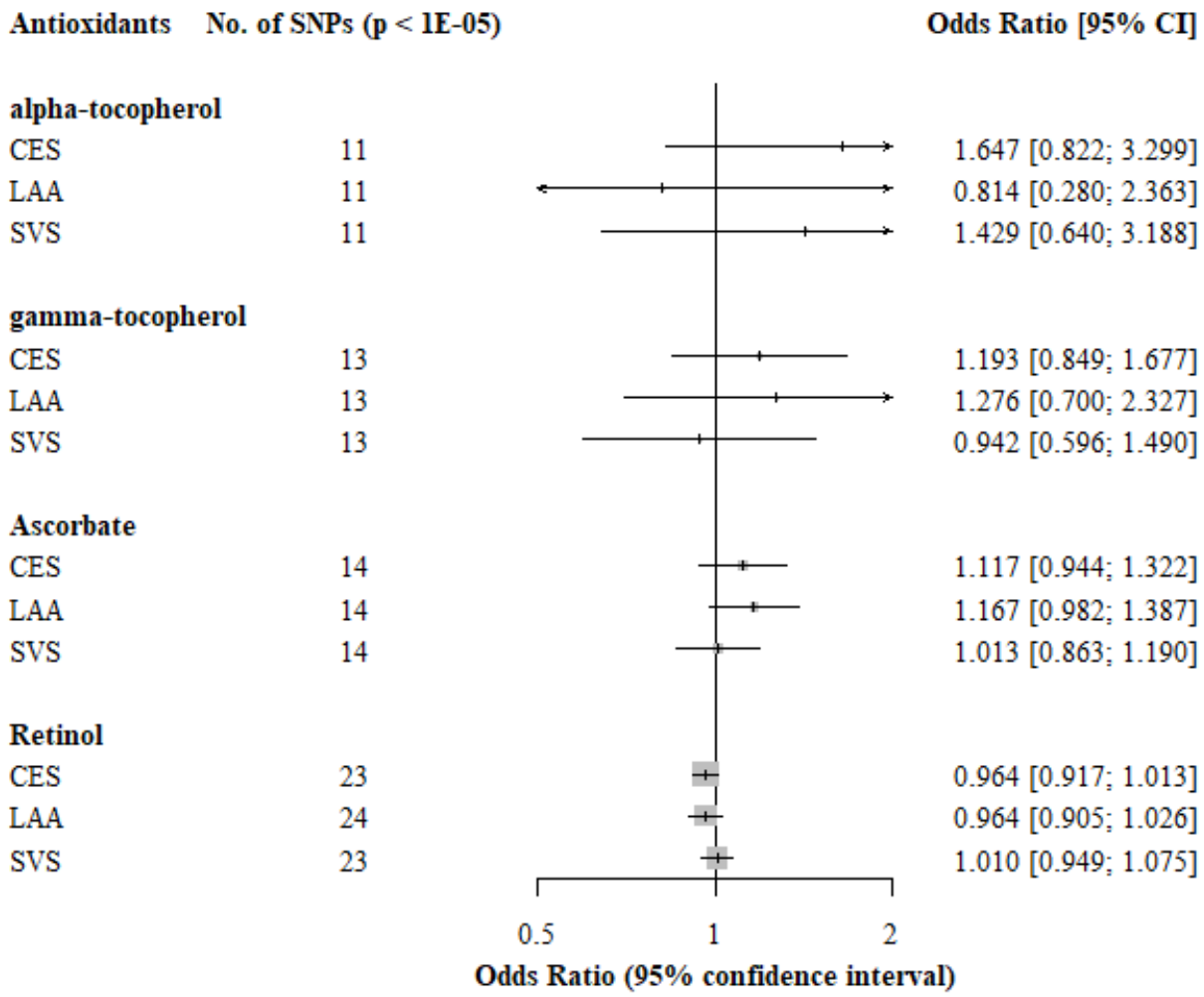
rs2367816	Circulating metabolite's concentation	retinol	HNRNPA1P61	chr2:33873296	G	A	0.26	0.228	0.05	9.46E-06
rs4737112	Circulating metabolite's concentation	retinol	CYP4F44P	chr8:43393138	G	T	0.10	-0.226	0.08	9.75E-06
rs4135385	Circulating metabolite's concentation	retinol	CTNNB1	chr3:41279440	G	A	0.76	0.212	0.05	9.80E-06
rs118025446	Circulating metabolite's concentation	retinol	CEP162	chr6:84904002	A	G	0.04	-0.481	0.12	9.84E-06

Figure S1. Causal association between circulating antioxidant levels and cardioembolic stroke (CES), large artery atherosclerosis (LAA), and small vessel stroke (SVS).



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.

Figure S2. Causal association between circulating antioxidant metabolite levels and cardioembolic stroke (CES), large artery atherosclerosis (LAA), and small vessel stroke (SVS).



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.