



Universiteit
Leiden
The Netherlands

Oxidative stress in chronic diseases: causal inference from observational studies

Luo, J.

Citation

Luo, J. (2022, September 1). *Oxidative stress in chronic diseases: causal inference from observational studies*. Retrieved from <https://hdl.handle.net/1887/3454705>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3454705>

Note: To cite this publication please use the final published version (if applicable).

CHAPTER 5

Dietary-derived circulating antioxidants and risk of coronary heart disease: a Mendelian randomization study

Jiao Luo, Saskia le Cessie, Diana van Heemst, Raymond Noordam

J Am Coll Cardiol. 2021 Jan 5;77(1):45-54

Abstract

Objectives: To investigate possible causal associations between dietary-derived circulating antioxidants and primary CHD risk using two-sample Mendelian Randomization (MR).

Background: Previously, observational studies have identified associations between higher levels of dietary-derived antioxidants and lower risk of coronary heart disease (CHD), while randomized clinical trials showed no reduction in CHD risk following antioxidant supplementation.

Methods: Single-nucleotide polymorphisms (SNPs) for circulating antioxidants (vitamin E, C, retinol, β -carotene, and lycopene), assessed as absolute levels and metabolites, were retrieved from literature and were used as genetic instrumental variables. Summary statistics for gene-CHD associations were obtained from three databases (cases/controls): the CARDIoGRAMplusC4D consortium (60 801/123 504), UK Biobank (25 306/46 2011) and FinnGen study (7123/89 376), respectively. For each exposure, MR analyses were performed per outcome database, and subsequently meta-analyzed.

Results: Among the analytic sample of 768 121 individuals (93 230 cases), genetically predicted circulating antioxidants were not causally associated with CHD risk. For absolute antioxidants, the odds ratio (95% CI) for CHD ranged between 0.94 (0.63, 1.41) for retinol and 1.03 (0.97, 1.10) for β -carotene per unit increase in ln-transformed antioxidant values. For metabolites, the odds ratio ranged between 0.93 (0.82, 1.06) for γ -tocopherol and 1.01 (0.95, 1.08) for ascorbate per 10-fold increase in metabolite levels.

Conclusions: Evidence from our study did not support a protective effect of genetic predisposition to high dietary-derived antioxidant levels on CHD risk. Therefore, it is unlikely that taking antioxidants to increase blood antioxidants levels, will have a clinical benefit for the prevention of primary CHD.

Introduction

Coronary heart disease (CHD) is one of the foremost causes of mortality worldwide and is responsible for approximately 0.36 million of all deaths in the US and 1.78 million in Europe each year¹⁻³. Well-established risk factors for CHD include smoking, physical inactivity, inappropriate nutrition, overweight and obesity, high blood cholesterol and other lipids, high blood pressure, diabetes mellitus, and insufficient/long sleep², and interventions targeted to ameliorate these risk factors showed a significant reduction in CHD risk. Apart from conventional risk factors, oxidative stress has also been hypothesized as a vital component in the development and progression of CHD by promoting macromolecular damage and endothelium dysfunction⁴. Consequently, antioxidants, the scavengers of free radicals to diminish oxidative stress-induced damage, would be of interest as targets for primary CHD prevention⁵. Specifically, dietary-derived antioxidants, better known as vitamin E, C, and carotenoids, are the most easily accessible and modifiable approach for consideration.

Based on this hypothesis, a large amount of studies have been conducted to explore the association between antioxidants and primary CHD. In multiple observational studies, dietary intake, either as dietary components or supplements, or blood concentration of vitamin E, C, and carotenoids were associated with a lower risk of primary CHD⁶⁻¹². Similarly, adherence to a diet containing high amounts of antioxidants, irrespective of the type of antioxidants, was associated with a lower risk of cardiovascular diseases¹³. However, associations as these in observational studies are prone to biases including reverse causality and unmeasured confounding. While randomized clinical trials (RCTs) generally failed to demonstrate a causal benefit of antioxidants supplement on primary CHD¹⁴⁻²⁰, with the exception of lycopene supplement on cardiovascular risk factors²¹, there are notable limitations. For example, timing, dosage, duration, use of natural or synthetic antioxidants, as well as the uncertain time of onset and long-term progression of CHD pathogenesis might explain the observed null effect^{22,23}. Therefore, the conflicting results from observational studies and RCTs should be interpreted with caution, and the causality between dietary-derived antioxidants and CHD is still unclear.

Mendelian Randomization (MR) is an alternative approach to infer causality of life-long risk factors (exposure) on diseases (outcome) using genetic variants as instrumental variables²⁴. In the present study, we used MR analyses to assess the associations between genetically determined dietary-derived circulating antioxidants and their metabolites with primary CHD risk, in the absence of reverse causality and residual confounding factors.

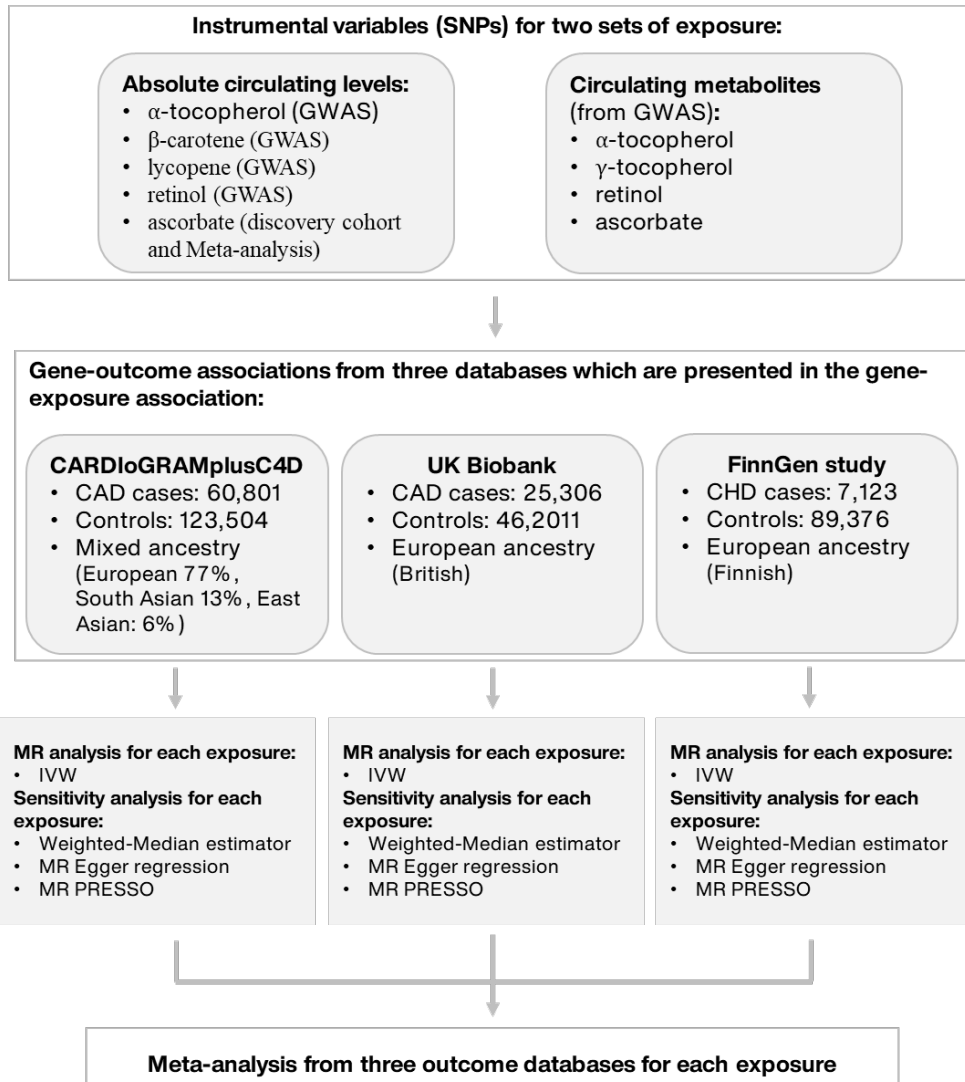


Figure 1 Schematic overview of the present study design

CAD: Coronary Artery Disease; CARDIoGRAMplusC4D: Coronary Artery Disease Genome-Wide Replication and Meta-analysis plus the Coronary Artery Disease Genetics; CHD: Coronary Heart Disease; GWAS, genome-wide association study; IVW: Inverse-Variance weighted; MR PRESSO: MR Pleiotropy RESidual Sum and Outlier.

Methods

Study design

For the current study, we conducted a two-sample MR, which tests the association between genetic instrumental variable(s), as a proxy for the exposure, and outcome from three separate data sources, and estimates the effect of an

exposure on an outcome²⁵. MR is based on 3 principle assumptions, notably that the genetic variant(s) should be 1) associated with the exposure, 2) associated with the outcome exclusively through the exposure, and 3) independent of any measured and unmeasured confounders. Data used in the present study are publicly available, and ethical approval and informed consent were obtained in each original study. A schematic overview of the present study design is presented in **Figure 1**.

Selection of genetic instrumental variables

Five main dietary-derived antioxidants were considered in the present study, which included vitamin E (α -tocopherol and γ -tocopherol), β -carotene, lycopene, vitamin C (L-ascorbic acid or ascorbate), and retinol. We considered both antioxidants that were measured as authentic absolute levels in the blood, and their corresponding circulating metabolites that were quantified as relative concentrations in plasma or serum, respectively. For absolute antioxidants levels, α -tocopherol, β -carotene, lycopene, ascorbate, and retinol were identified, while for antioxidants metabolites, α -tocopherol, γ -tocopherol, ascorbate, and retinol were used.

Genome-Wide Association Studies (GWAS) were searched to extract leading SNPs as genetical instrumental variables. When we identified multiple GWAS for a single trait, only the largest study with replication was used²⁶⁻³¹. Though GWAS was not available for absolute ascorbate levels, a study with a two-stage design which used a discovery cohort and five 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 and therefore was considered as qualified for genetic instrument extraction³². A summary table of instruments is presented in **Supplemental Table 1**.

Absolute circulating antioxidants

Three SNPs for α -tocopherol levels were identified in a GWAS with 7781 European individuals²⁶. However, those 3 loci were previously reported to be associated with lipid metabolism and/or regulation in GWAS on lipid levels^{33,34} or coronary artery disease³⁵, 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 β -carotene levels were identified in a GWAS within 2344 participants in the Nurses' Health Study²⁷. 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 adults²⁸. Two SNPs (LD < 0.001, $p < 5 \times 10^{-8}$) associated with circulating retinol levels were identified in a GWAS of 5006 Caucasian individuals from two cohorts²⁹. As for ascorbate, one genetic variant ($p = 2.0 \times 10^{-7}$) was identified with over 15 000 participants³². Summary of the demographic characteristics of the cohort that were used to generate genetic instrumental variables is presented in **Supplemental Table 2**.

Circulating antioxidants metabolites

Genetic variants for each metabolite at suggestive genome-wide significance level ($p < 1 \times 10^{-5}$) were extracted from published GWAS ^{30,31}, notably 11 instruments for α -tocopherol ($n = 7276$), 13 for γ -tocopherol ($n = 5822$) and 14 for ascorbate ($n = 2063$) derived from 7824 adult individuals from 2 European population studies, 24 for retinol ($n = 1957$) from 1960 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.

Explained variance and instrument strength

Variance (R^2) in the MR study refers to the proportion of total variation in the exposure which is explained by the genetic instruments. R^2 for each trait were either derived from the original study or calculated based on the derived summary statistics in line with what has been described previously ³⁴, and ranged from 0.9% to 30.1% for absolute antioxidant levels, and from 3.3% to 18.6% for antioxidants metabolites, separately (**Supplemental Table 1**).

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.

Data source for instrument-outcome associations

Summary statistics for the associations of the identified exposure-related SNPs with primary CHD were extracted from three large databases, namely Coronary Artery Disease Genome-Wide Replication and Meta-analysis plus the Coronary Artery Disease Genetics (CARDIoGRAMplusC4D) consortium, UK Biobank, and FinnGen study.

CARDIoGRAMplusC4D assembled 60 801 cases and 123 504 controls for 48 studies, of which, 77% of the participants were of European ancestry, 19% were of south and east Asian ancestry, and a small proportion of Hispanic and African Americans. CAD cases were identified as an inclusive diagnosis of myocardial infarction, acute coronary syndrome, chronic stable angina, or coronary stenosis $> 50\%$ ^{36,37}. The summary statistics of the instruments-CAD associations were provided in the database.

The UK Biobank cohort 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 ³⁸ (<https://www.ukbiobank.ac.uk>). We restricted the analyses to the participants who reported their ancestry as European, and who were in the full released imputed genomics databases (UK10K + HRC). CAD diagnoses were coded according to the International Classification of Diseases (ICD) ³⁸, and CAD cases were retrieved from linkage with NHS database and were defined as angina pectoris (I20), myocardial infarction (I21 and I22), and acute and chronic ischemic heart disease (I24 and I25). In total, 25 306 cases and 46 2011 controls were identified. We performed logistic regression analyses to assess the associations between genetic instruments and CAD, adjusted for

age, sex, and 10 principal components and corrected for a familial relationship using BOLD_LMM (v2.3.2).

The FinnGen study is an ongoing nation-wide cohort study launched in 2017, which combines genetic data from Finnish biobanks and health record data from Finnish health registries (<https://www.finnngen.fi/en>). Major CHD was defined as angina pectoris (I20), myocardial infarction (I21-I23), ischemic heart diseases (I24 and I25), cardiac arrest (I46), and other unattended or cause unknown sudden death (R96 and R98). The analyses were based on the FinnGen data freeze 2, which consists of 7123 cases of major CHD and 89 376 controls with complete instruments-CHD associations.

Statistical analysis

All the analyses were undertaken using R (v3.6.1) statistical software (The R Foundation for Statistical Computing, Vienna, Austria).

Mendelian Randomization

The primary MR analysis was conducted by using Inverse-Variance weighted (IVW) regression analysis, which assumes the absence of invalid genetic instruments (e.g., no directional pleiotropy)³⁹. The mean effect estimate was obtained from each outcome database separately by a fixed-effect IVW meta-analysis of the Wald ratios (gene-outcome [log odds ratio] divided by gene-exposure associations) estimated for each instrumental variable⁴⁰. Results are expressed as odds ratios (OR) on CHD risk for a corresponding unit change in absolute circulating levels of antioxidants on natural log-transformed levels (β -carotene and retinol), $\mu\text{g/dL}$ (lycopene) or $\mu\text{mol/L}$ (ascorbate), or a 10-fold change in metabolites concentrations. When the MR assumptions are met, this odds ratio is an estimate of the causal effect of the exposure on the outcome. MR analyses were performed using the R-based package “TwoSampleMR” (<https://mrcieu.github.io/TwoSampleMR/>).

Sensitivity analysis

In order to examine whether there was a violation of the main MR assumptions due to directional pleiotropy, we performed MR-Egger regression analysis and Weighted-Median Estimator⁴⁰⁻⁴². In MR-Egger, the intercept estimates the average pleiotropic effect across the genetic variants; a value that differs from zero indicates that the IVW estimate is biased⁴¹. A Weighted-Median estimator analysis can provide a consistent valid estimate if at least half of the instrumental variables are valid⁴². In addition, MR-PRESSO (MR Pleiotropy RESidual Sum and Outlier) was applied to detect and correct for horizontal pleiotropy through removing outliers⁴³, as implemented in the R-based package MRPRESSO (<https://github.com/rondolab/MR-PRESSO>). Furthermore, Cochran’s Q statistic was used to test the heterogeneity among the estimated Wald ratios from different genetic variants⁴⁴. Additional sensitivity analyses were performed for β -carotene by using a stringent LD threshold of $r^2 < 0.001$, and for lycopene by restricting the analyses to only GWAS significant-level ($p < 5 \times 10^{-8}$) SNPs.

Meta-analysis of the estimates from three outcome databases

All exposure-specific MR analyses were performed in each outcome database of CARDIoGRAMplusC4D consortium, UK Biobank, and FinnGen study, separately, and then were meta-analyzed to generate the pooled estimates for each exposure on CHD risk. We calculated I^2 statistics to quantify heterogeneity between estimates from three studies and the corresponding p-value derived from Cochran's Q test. Given no heterogeneity was present across three databases, fixed-effect model meta-analyses were used to pool instrumental variable estimates across the three outcome databases for each exposure. All meta-analyses were performed in the R-based "meta" package (<https://cran.r-project.org/web/packages/meta/index.html>).

Results

Summary information of instruments identified for dietary-derived antioxidants and their metabolites are presented in **Supplemental Table 1**, and summary information of the cohorts contributing to the GWAS of absolute levels is given in **Supplemental Table 2**. Retinol and ascorbate are available both as absolute circulating antioxidants and metabolites. Detailed information on the genetic variants, their associations with antioxidants ($\beta_{\text{gene-exposure}}$), and with CHD ($\beta_{\text{gene-outcome}}$) across databases is given in **Supplemental Tables 3 and 4**. F-statistics for all genetic instruments used in the present study were above 10.

Absolute circulating antioxidants and CHD

Overall, in the primary analyses using IVW, genetically determined absolute dietary-derived antioxidants levels were not associated with the risk of CHD in any of the three databases (**Figure 2 and Supplemental Table 5**). Pooled OR for CHD per unit increase of antioxidants were 1.03 (95% confidence interval, CI: 0.97, 1.10) and 0.94 (95%CI: 0.63, 1.41) for natural log-transformed β -carotene and retinol, 1.02 (95%CI: 0.99, 1.06) for 1 $\mu\text{g}/\text{dL}$ lycopene, and 1.00 (95%CI: 0.99, 1.00) for 1 $\mu\text{mol}/\text{L}$ ascorbate, respectively.

For β -carotene and lycopene with three or more genetic instruments, Weighted-Median estimator and MR-Egger regression were conducted. The estimates did not change substantially compared with IVW regression (**Supplemental Figure 1**). In addition, MR-Egger regression analysis suggested no evidence of overall pleiotropy, and there was no evidence of heterogeneity between individual genetic instrument estimation (**Supplemental Table 5**). In addition, no outlier SNP was identified in MR-PRESSO test for these two antioxidants in any of the databases.

In the sensitivity analysis for β -carotene with $\text{LD} < 0.001$ using 2 SNPs (rs6564851 and rs7501331), and for lycopene with only GWAS-level significant variant (rs7680948, $p < 5 \times 10^{-8}$), similar results were observed (**Supplemental Figure 2**).

Circulating antioxidants metabolites and CHD

Consistent with the findings from absolute circulating antioxidants, no association between genetically predicted circulating antioxidants metabolites concentration

and CHD risk was observed, as shown in **Figure 3**. The combined ORs for CHD per 10-fold increase in metabolites concentration were 1.00 (95% CI: 0.75, 1.35) for α -tocopherol, 0.93 (0.82, 1.06) for γ -tocopherol, 1.00 (0.98, 1.02) for retinol and 1.01 (0.95, 1.08) for ascorbate.

Sensitivity analyses for metabolites on CHD risk were provided in **Supplemental Table 6**. Estimates using the Weighted-Median estimator were consistently comparable to those from IVW regression. No horizontal pleiotropy was detected in MR-Egger regression, with the exception of retinol in UK Biobank database (intercept: -0.028, se: 0.010, p-value: 0.01). Although MR PRESSO detected outliers for α -tocopherol, retinol, and ascorbate in the UK Biobank database, the estimate did not change materially after correction.

Heterogeneity was detected using Cochran's Q statistics for all metabolites in different databases, especially with outliers as identified using MR PRESSO. However, in the leave-one-out analyses, we found that the risk estimates of genetically predicted antioxidants' metabolites and risk of CHD did not change substantially after excluding one SNP at each time, indicating that it is unlikely that potential driving SNPs could bias the causal association (data not shown).

Discussion

In the present study, we investigated the relationship between dietary-derived antioxidants and CHD risk using Mendelian Randomization. Instrumental variables were used as proxies for circulating antioxidants assessed both as absolute levels and metabolites, and comparable results were obtained. Our findings indicate that dietary-derived antioxidants are unlikely to be causal determinants of primary CHD risk.

Two previous studies using an MR approach found that a genetic predisposition to high a vitamin E level was associated with an increased risk of CAD^{45,46}. However, instruments selected in these studies play clear roles in lipid metabolism, which violated the InSIDE assumption in MR design and introduced bias in the effect estimates⁴⁷. To provide insights into the magnitude of the effects of genetic instruments on circulating antioxidant levels, we compared the effects from the genetic instruments and dietary supplementation (**Supplemental Tables 7 and 8**). The effects on circulating antioxidant levels achieved by genetic instruments, with the exception of ascorbate, are within the range of the effects observed by antioxidant supplementation in RCTs, for which we prioritized the trials on cardiovascular outcomes that have been included in previous meta-analyses¹⁴⁻²⁰. However, direct comparisons between these two effects should be interpreted with caution, given that the effect of genetic predisposition is assumed to be lifelong whereas the effect of supplementation only lasts for the duration of the trial. The exposure during the whole life course with a slightly minor effect could have a potential biological effect that exceeds the temporarily larger effect of supplements given the long period needed to develop coronary heart disease. The robust null results in our studies, however, suggest that lifelong exposure to somewhat higher antioxidant levels did not decrease the risk of CHD, in line with earlier findings from the trials and meta-analyses on trials.

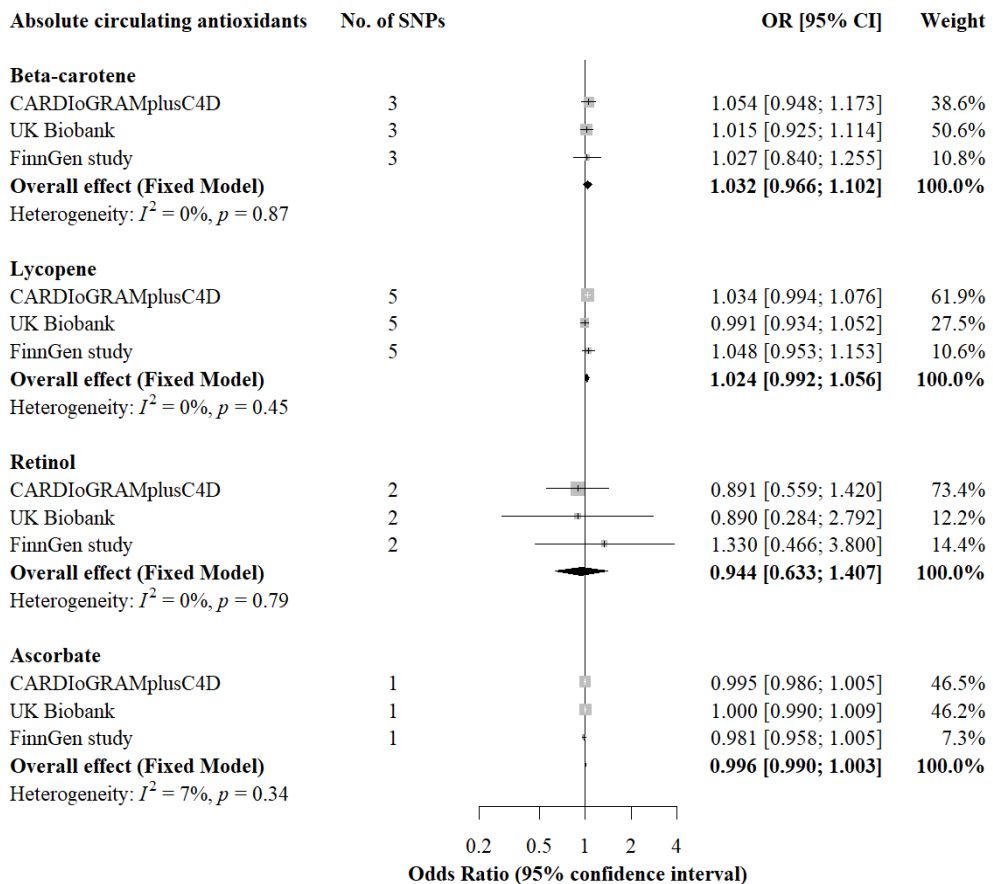


Figure 2 Causal association between absolute circulating antioxidants with Coronary Heart Disease

Estimated odds ratios for the effect of per unit increase in ln-transformed β -carotene and retinol values, 1 $\mu\text{g}/\text{dL}$ lycopene and 1 $\mu\text{mol}/\text{L}$ ascorbate on coronary heart disease, obtained from an Inverse-Variance Weighted (IVW) analysis, per outcome database separately and combined over the three databases using fixed-effect meta-analyses.

Study strengths and limitations

There are two main strengths in the present study. First, we used two separate sets of instrumental variables, for both absolute circulating antioxidants and their metabolites. Specifically, for retinol and ascorbate that is presented in both sets, similar results were generated, which is supportive of the robustness of our findings. Second, three large databases comprising 768 121 participants with 93 230 CHD cases for gene-outcome associations were meta-analyzed in the present study. The results from these 3 databases are generally consistent with no evidence of heterogeneity. Therefore, the precision for final MR estimates and the reliability of the results were significantly improved despite the limited number of strong genetic instruments.

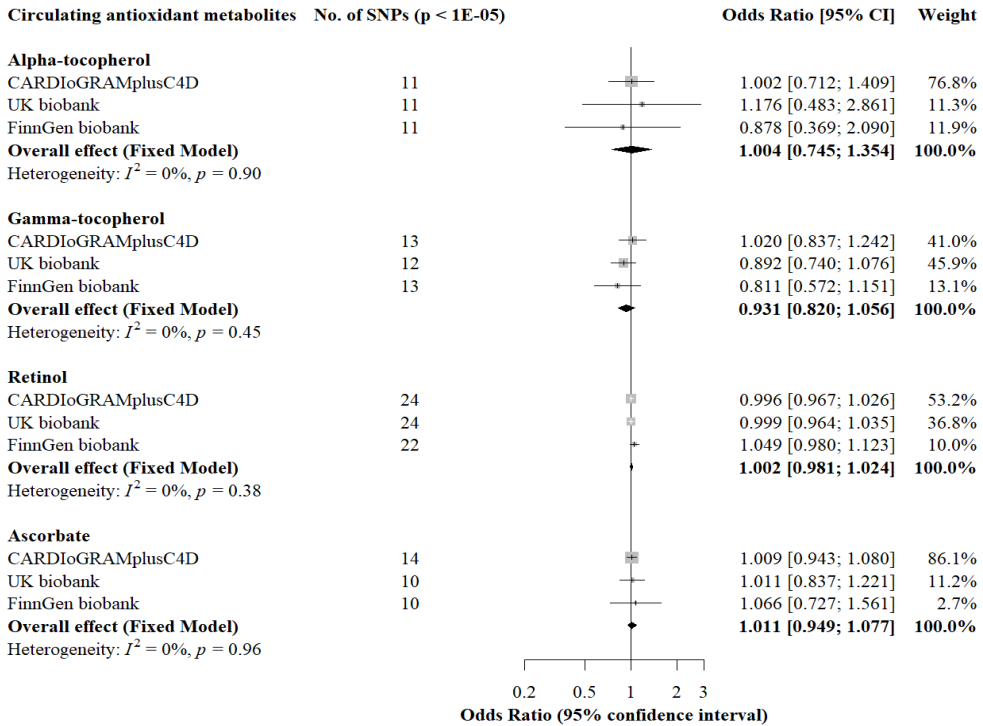


Figure 3 Causal association between circulating antioxidants metabolites with Coronary Heart Disease

Estimated odds ratios for the effect of per 10-fold increase in antioxidants metabolites' concentrations on coronary heart disease, obtained from an Inverse-Variance Weighted (IVW) analysis, per outcome database separately and combined over the three databases using fixed-effect meta-analyses.

This study has some limitations to address. First, we are unable to test for a non-linear causal association between the antioxidant levels and CHD that has previously been suggested, especially for α -tocopherol and β -carotene⁶. Despite analytic methods having been developed, these require individual-level data of the exposure^{40,48}, and the published data we used are summary-level statistics, therefore we were not able to perform such analyses. Second, no sensitivity analysis could be performed for some absolute antioxidants (retinol and ascorbate) with limited genetic variants. Notwithstanding, these instruments are mapped in the genes which are crucial in the metabolism of antioxidants and are not associated with any other CHD risk factors in the GWAS catalog or PhenoScanner databases, suggesting no directional pleiotropy as also confirmed in the analyses. Third, only one SNP with a small R^2 of 0.9% for absolute ascorbate was used. However, with the considerable instrumental strength and large sample size and cases in which the analyses were conducted, we had more than sufficient statistical power to estimate a possible causal effect⁴⁹. In addition, results from ascorbate metabolites with larger R^2 (18.6%) gave very similar estimates which further reinforces the validity of the findings. Forth, protective effects of antioxidants might still exist in discriminatively selected subgroups who have

elevated oxidative stress levels, for example, vitamin E supplement provided cardiovascular-protective effects only in individuals with both diabetes and haptoglobin2-2 genotype. Besides, multiple treatments simultaneously might be more effective for multifactorial diseases since there could be a synergistic benefit from two agents with acceptable safety and efficacy, for example, traditional treatment plus antioxidants, to achieve better effects than antioxidants only⁵⁰. However, we could not explore these associations in the population with high risk or a known nutritional deficiency that might be more promising for antioxidant supplements or test the effect of antioxidants in combination with other treatments. Lastly, although no causal associations between circulating antioxidants and CHD risk were detected, we could not completely rule out the possibility that the effect size is too small to be identified even within our large sample size. Nevertheless, such a potential effect, if it exists at all by incorporating additional databases, will be extremely small and is unlikely to result in a clinically relevant reduction of CHD risk as obtained by other strategies, for instance, a 25% to 45% reduction of cardiovascular events of statins, physical activity or weight loss for primary CHD prevention⁵¹⁻⁵⁴.

Conclusion

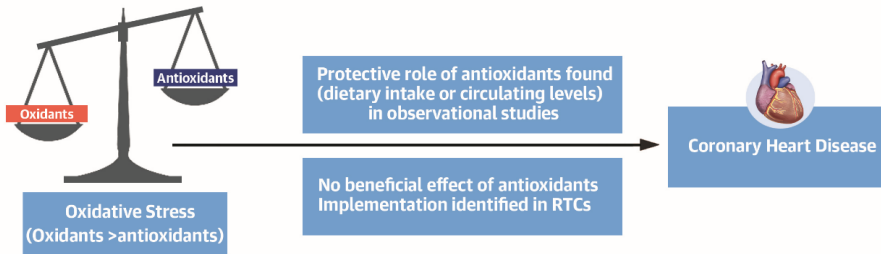
In conclusion, evidence from the present study did not support a beneficial role of circulating dietary-derived vitamin E, C, β -carotene, lycopene, or retinol on CHD risk in the general population. This signifies the absence of a substantial role of antioxidant supplements on CHD risk identified in RCTs and is in accordance with the recommendations from the U.S. Preventive Services Task Force¹⁹. Therefore, for healthy adults without nutritional deficiency, dietary-derived antioxidant supplement use that improves circulating antioxidant levels to prevent primary CHD is of limited clinical benefit.

Clinical Perspectives

Competency in Medical Knowledge: Dietary-derived circulating antioxidants (vitamin E, vitamin C, β -carotene, lycopene and retinol) are not causally associated with coronary heart disease in healthy adults without nutritional deficiency.

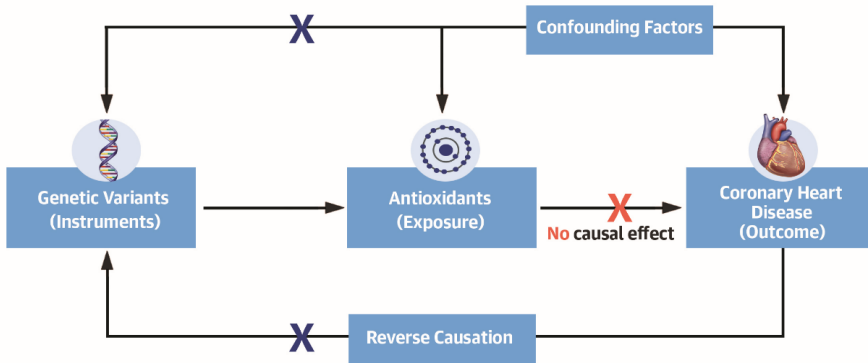
Translational Outlook: Future studies are needed to confirm the effect of dietary-derived antioxidants in discriminatingly selected subgroups who have elevated oxidative stress or known nutritional deficiency and to investigate the preventive and therapeutic effect of antioxidants in combination with other agents or traditional treatments that may generate synergistic benefit than antioxidants only.

Previous Controversial Evidence



5

Current Mendelian Randomization Study



Central Illustration Antioxidants and Coronary Heart Disease

Theoretically, antioxidants can act as scavengers of oxidants to mitigate oxidative stress-induced damage, thus preventing coronary heart disease. However, findings from our study demonstrated that there is no casual association between dietary-derived antioxidants with primary coronary heart disease risk.

References

1. M. Nichols, N. Townsend, P. Scarborough, M. Rayner. Cardiovascular disease in Europe 2014: epidemiological update. *Eur Heart J* 2014; 35(42): 2950-9.
2. E. J. Benjamin, P. Muntner, A. Alonso, et al. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. *Circulation* 2019; 139(10): e56-e528.
3. E. S. Ford, U. A. Ajani, J. B. Croft, et al. Explaining the decrease in U.S. deaths from coronary disease, 1980-2000. *N Engl J Med* 2007; 356(23): 2388-98.
4. A. Daiber, F. D. Lisa, M. Oelze, et al. Crosstalk of mitochondria with NADPH oxidase via reactive oxygen and nitrogen species signalling and its role for vascular function. *Br J Pharmacol* 2017; 174(12): 1670-89.
5. K. Sugamura, J. F. Keaney. Reactive oxygen species in cardiovascular disease. *Free Radic Biol Med* 2011; 51(5): 978-92.
6. D. Aune, N. Keum, E. Giovannucci, et al. Dietary intake and blood concentrations of antioxidants and the risk of cardiovascular disease, total cancer, and all-cause mortality: a systematic review and dose-response meta-analysis of prospective studies. *Am J Clin Nutr* 2018; 108(5): 1069-91.
7. P. Knekt, J. Ritz, M. A. Pereira, et al. Antioxidant vitamins and coronary heart disease risk: a pooled analysis of 9 cohorts. *Am J Clin Nutr* 2004; 80(6): 1508-20.
8. G. Li, Y. Li, X. Chen, H. Sun, X. Hou, J. Shi. Circulating tocopherols and risk of coronary artery disease: A systematic review and meta-analysis. *Eur J Prev Cardiol* 2016; 23(7): 748-57.
9. B. Song, K. Liu, Y. Gao, et al. Lycopene and risk of cardiovascular diseases: A meta-analysis of observational studies. *Mol Nutr Food Res* 2017; 61(9).
10. J. Kim, J. Choi, S. Y. Kwon, et al. Association of Multivitamin and Mineral Supplementation and Risk of Cardiovascular Disease: A Systematic Review and Meta-Analysis. *Circ Cardiovasc Qual Outcomes* 2018; 11(7): e004224.
11. Z. Ye, H. Song. Antioxidant vitamins intake and the risk of coronary heart disease: meta-analysis of cohort studies. *Eur J Cardiovasc Prev Rehabil* 2008; 15(1): 26-34.
12. H. M. Cheng, G. Koutsidis, J. K. Lodge, A. W. Ashor, M. Siervo, J. Lara. Lycopene and tomato and risk of cardiovascular diseases: A systematic review and meta-analysis of epidemiological evidence. *Crit Rev Food Sci Nutr* 2019; 59(1): 141-58.
13. M. Parohan, J. Anjom-Shoae, M. Nasiri, et al. 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(6): 2175-89.
14. L. Al-Khudairy, N. Flowers, R. Wheelhouse, et al. Vitamin C supplementation for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev* 2017; (3).
15. L. Schwingshackl, H. Boeing, M. Stelmach-Mardas, et al. Dietary Supplements and Risk of Cause-Specific Death, Cardiovascular Disease, and Cancer: A Systematic Review and Meta-Analysis of Primary Prevention Trials. *Adv Nutr* 2017; 8(1): 27-39.
16. S. K. Myung, W. Ju, B. Cho, et al. Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systematic review and meta-analysis of randomised controlled trials. *BMJ* 2013; 346: f10.

17. S. P. Fortmann, B. U. Burda, C. A. Senger, J. S. Lin, E. P. Whitlock. Vitamin and mineral supplements in the primary prevention of cardiovascular disease and cancer: An updated systematic evidence review for the U.S. Preventive Services Task Force. *Ann Intern Med* 2013; 159(12): 824-34.
18. D. J. A. Jenkins, J. D. Spence, E. L. Giovannucci, et al. Supplemental Vitamins and Minerals for CVD Prevention and Treatment. *J Am Coll Cardiol* 2018; 71(22): 2570-84.
19. V. A. Moyer, on behalf of the U.S. Preventive Services Task Force. Vitamin, Mineral, and Multi-vitamin Supplements for the Primary Prevention of Cardiovascular Disease and Cancer: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med* 2014; 160(8): 558-64.
20. A. W. Ashor, R. Brown, P. D. Keenan, et al. Limited evidence for a beneficial effect of vitamin C supplementation on biomarkers of cardiovascular diseases: an umbrella review of systematic reviews and meta-analyses. *Nutr Res* 2019; 61: 1-12.
21. H. M. Cheng, G. Koutsidis, J. K. Lodge, A. Ashor, M. Siervo, J. Lara. Tomato and lycopene supplementation and cardiovascular risk factors: A systematic review and meta-analysis. *Atherosclerosis* 2017; 257: 100-8.
22. S. R. Steinhubl. Why Have Antioxidants Failed in Clinical Trials? *Am J Cardiol* 2008; 101(10, Supplement): S14-S9.
23. M. P. Murphy. Antioxidants as therapies: can we improve on nature? *Free Radic Biol Med* 2014; 66: 20-3.
24. D. A. Lawlor, R. M. Harbord, J. A. C. Sterne, et al. Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. *Stat Med* 2008; 27(8): 1133-63.
25. S. Burgess, R. A. Scott, N. J. Timpson, et al, Epic- InterAct Consortium. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol* 2015; 30(7): 543-52.
26. J. M. Major, K. Yu, W. Wheeler, et al. Genome-wide association study identifies common variants associated with circulating vitamin E levels. *Hum Mol Genet* 2011; 20(19): 3876-83.
27. S. J. Hendrickson, A. Hazra, C. Chen, et al. 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(6): 1379-89.
28. C. R. D'Adamo, A. D'Urso, K. A. Ryan, et al. A Common Variant in the SETD7 Gene Predicts Serum Lycopene Concentrations. *Nutrients* 2016; 8(2): 82.
29. A. M. Mondul, K. Yu, W. Wheeler, et al. Genome-wide association study of circulating retinol levels. *Hum Mol Genet* 2011; 20(23): 4724-31.
30. S. Y. Shin, E. B. Fauman, A. K. Petersen, et al. An atlas of genetic influences on human blood metabolites. *Nat Genet* 2014; 46(6): 543-50.
31. T. Long, M. Hicks, H. C. Yu, et al. Whole-genome sequencing identifies common-to-rare variants associated with human blood metabolites. *Nat Genet* 2017; 49(4): 568-78.
32. N. J. Timpson, N. G. Forouhi, M. J. Brion, et al. Genetic variation at the SLC23A1 locus is associated with circulating concentrations of L-ascorbic acid (vitamin C): evidence from 5 independent studies with >15,000 participants. *Am J Clin Nutr* 2010; 92(2): 375-82.
33. C. J. Willer, E. M. Schmidt, S. Sengupta, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet* 2013; 45(11): 1274-83.

34. T. M. Teslovich, K. Musunuru, A. V. Smith, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* 2010; 466(7307): 707-13.
35. J. M. M. Howson, W. Zhao, D. R. Barnes, et al. Fifteen new risk loci for coronary artery disease highlight arterial-wall-specific mechanisms. *Nat Genet* 2017; 49(7): 1113-9.
36. M. Nikpay, A. Goel, H. H. Won, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet* 2015; 47(10): 1121-30.
37. P. Deloukas, S. Kanoni, C. Willenborg, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet* 2013; 45(1): 25-33.
38. C. Sudlow, J. Gallacher, N. Allen, 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(3): e1001779.
39. S. Burgess, A. Butterworth, S. G. Thompson. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* 2013; 37(7): 658-65.
40. J. R. Staley, S. Burgess. Semiparametric methods for estimation of a nonlinear exposure-outcome relationship using instrumental variables with application to Mendelian randomization. *Genet Epidemiol* 2017; 41(4): 341-52.
41. J. Bowden, G. Davey Smith, S. Burgess. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 2015; 44(2): 512-25.
42. J. Bowden, G. Davey Smith, P. C. Haycock, S. Burgess. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol* 2016; 40(4): 304-14.
43. M. Verbanck, C. Y. Chen, B. Neale, R. Do. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet* 2018; 50(5): 693-8.
44. M. Fd Greco, C. Minelli, N. A. Sheehan, J. R. Thompson. Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome. *Stat Med* 2015; 34(21): 2926-40.
45. T. Wang, L. Xu. Circulating Vitamin E Levels and Risk of Coronary Artery Disease and Myocardial Infarction: A Mendelian Randomization Study. *Nutrients* 2019; 11(9).
46. C. Fan, T. Huang, X. Kong, X. Zhang, Z. Zou, J. Xiao. Circulating vitamin E and cardiometabolic measures: a Mendelian randomization analysis. *J Clin Biochem Nutr* 2019; 65(2): 160-9.
47. P. C. Haycock, S. Burgess, K. H. Wade, J. Bowden, C. Relton, G. Davey Smith. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. *Am J Clin Nutr* 2016; 103(4): 965-78.
48. S. Burgess, N. M. Davies, S. G. Thompson, on behalf of EPIC-InterAct Consortium. Instrumental Variable Analysis with a Nonlinear Exposure–Outcome Relationship. *Epidemiology* 2014; 25(6): 877-85.
49. S. Burgess. Sample size and power calculations in Mendelian randomization with a single instrumental variable and a binary outcome. *Int J Epidemiol* 2014; 43(3): 922-9.
50. A. A. El-Aal, E. A. Abd El-Ghffar, A. A. Ghali, et al. The effect of vitamin C and/or E supplementations on type 2 diabetic adult males under metformin treatment: A single-blinded randomized controlled clinical trial. *Diabetes Metab Syndr* 2018; 12(4): 483-9.

51. F. Taylor, M. D. Huffman, A. F. Macedo, et al. Statins for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev* 2013; (1): Cd004816.
52. T. Varghese, W. M. Schultz, A. A. McCue, et al. Physical activity in the prevention of coronary heart disease: implications for the clinician. *Heart* 2016; 102(12): 904-9.
53. C. J. Lavie, R. V. Milani, H. O. Ventura. Obesity and cardiovascular disease: risk factor, paradox, and impact of weight loss. *J Am Coll Cardiol* 2009; 53(21): 1925-32.
54. J. W. Jukema, C. P. Cannon, A. J. de Craen, R. G. Westendorp, S. Trompet. The controversies of statin therapy: weighing the evidence. *J Am Coll Cardiol* 2012; 60(10): 875-81.

Supplementary materials

Supplemental Table 1 Summary of instruments for circulating dietary-derived antioxidants as both absolute levels and metabolites

Supplemental Table 2 Summary information of the cohorts contributing to the GWAS used for genetic instrumental variables extraction of absolute circulating antioxidants

Supplemental Table 3 Effect estimates of the associations between genetic instrumental variables for absolute circulating dietary derived antioxidants and risk of coronary heart diseases across three databases

Supplemental Table 4 Effect estimates of the associations between genetic instrumental variables for circulating dietary derived antioxidant metabolites and risk of coronary heart diseases across three databases

Supplemental Table 5 Associations between genetically predicted per unit increase in dietary-derived antioxidants and coronary heart disease in Mendelian Randomization analyses

Supplemental Table 6 Associations between genetically predicted per unit (\log_{10}) increase in dietary-derived antioxidants metabolites and coronary heart disease in Mendelian Randomization analyses

Supplemental Table 7 The effects of genetic instruments and dietary supplementation on circulating antioxidant concentrations

Supplemental Table 8 Circulating antioxidant concentration from antioxidants supplementation trials

Supplemental Figure 1 Estimated odds ratios for the effect of per unit increase in \ln -transformed β -carotene and 1 $\mu\text{g}/\text{dL}$ lycopene absolute levels on coronary heart disease, obtained from Weighted-Median estimator (upper panel) and MR Egger regression (lower panel), per outcome database separately and combined over the three databases using fixed-effect model meta-analysis.

Supplemental Figure 2 Sensitivity analysis for β -carotene by using a stringent LD threshold of < 0.001 , and for lycopene by restricting the analyses to only GWAS significant-level ($p < 5 \times 10^{-8}$) SNP only. Analyses were performed per outcome database separately and combined over the three databases using fixed-effect model meta-analysis

The Supplementary materials for this article can be found online at:
<https://www.jacc.org/doi/10.1016/j.jacc.2020.10.048>

