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

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Diet-Derived Circulating Antioxidants and Risk of Coronary Heart Disease

A Mendelian Randomization Study

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ABSTRACT

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

OBJECTIVES The purpose of this study was to investigate possible causal associations between dietary-derived circulating antioxidants and primary CHD risk using 2-sample Mendelian randomization (MR).

METHODS Single-nucleotide polymorphisms for circulating antioxidants (vitamins E and C, retinol, β-carotene, and lycopene), assessed as absolute levels and metabolites, were retrieved from the published data and were used as genetic instrumental variables. Summary statistics for gene-CHD associations were obtained from 3 databases: the CARDIoGRAMplusC4D consortium (60,801 cases; 123,504 control subjects), UK Biobank (25,306 cases; 462,011 control subjects), and FinnGen study (7,123 cases; 89,376 control subjects). For each exposure, MR analyses were performed per outcome database and were subsequently meta-analyzed.

RESULTS Among an 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 for CHD ranged between 0.94 (95% confidence interval [CI]: 0.63 to 1.41) for retinol and 1.03 (95% CI: 0.97 to 1.10) for β-carotene per unit increase in ln-transformed antioxidant values. For metabolites, the odds ratio ranged between 0.93 (95% CI: 0.82 to 1.06) for γ-tocopherol and 1.01 (95% CI: 0.95 to 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. (J Am Coll Cardiol 2021;77:45-54) © 2021 by the American College of Cardiology Foundation.

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 United States and 1.78 million in Europe each year (1-3). 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 sleep (2), and interventions targeted to ameliorate these risk factors showed 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 dysfunction (4). Consequently, antioxidants, the scavengers of free radicals to diminish oxidative stress-induced damage, would be

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ABBREVIATIONS AND ACRONYMS

CAD = coronary artery disease

CHD = coronary heart disease

GWAS = genome-wide association studies

IVW = inverse-variance weighted

LD = linkage disequilibrium

MR = Mendelian randomization

OR = odds ratio

SNP = single-nucleotide polymorphisms

of interest as targets for primary CHD prevention (5). Specifically, dietary-derived antioxidants, better known as vitamins E and 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 vitamins E and C and carotenoids were associated with lower risk of primary CHD (6–12). Similarly, adherence to a diet containing high amounts of antioxidants, irrespective of the type of antioxidants, was associated with a lower risk of cardiovascular diseases (13). However, associations such as these in observational studies are prone to biases, including reverse causality and unmeasured confounding. Although randomized clinical trials (RCTs) generally failed to demonstrate a causal benefit of antioxidants supplement on primary CHD (14–20), with the exception of lycopene supplement on cardiovascular risk factors (21), 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.

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Mendelian randomization (MR) is an alternative approach to infer causality of lifelong risk factors (exposure) on diseases (outcome) using genetic variants as instrumental variables (24). 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.

METHODS

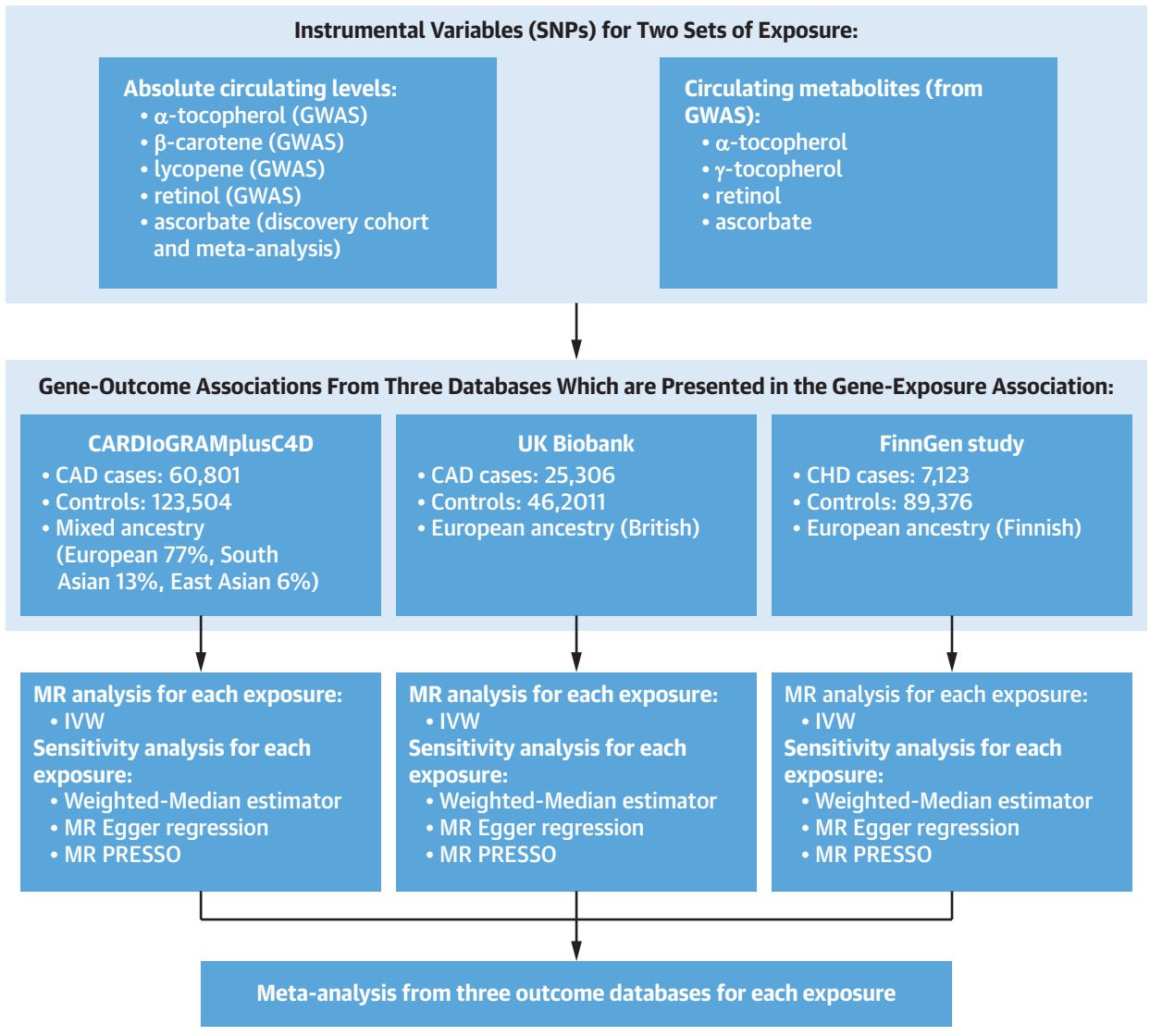
STUDY DESIGN. For the current study, we conducted 2-sample MR, which tests the association between genetic instrumental variable(s), as a proxy for the exposure, and outcome from 3 separate data sources and estimates the effect of an exposure on an outcome (25). MR is based on 3 principle assumptions, 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. In total, 5 main dietary-derived antioxidants were considered in the present study: vitamin E (α- 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 blood and their corresponding circulating metabolites that were quantified as relative concentrations in plasma or serum. For absolute antioxidant levels, α-tocopherol, β-carotene, lycopene, ascorbate, and retinol were identified, whereas for antioxidant metabolites, α-tocopherol, γ-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 (26–31). 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 (32). 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 7,781 European individuals (26). 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 disease (CAD) (35), 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 2,344 participants in the Nurses' Health Study (27). 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 (28).

FIGURE 1 Schematic Overview of the Present Study Design



SNPs for circulating antioxidants (vitamins E and C, retinol, β-carotene and lycopene), assessed as absolute levels and metabolites, were identified as genetic instrumental variables. Summary statistics for gene-CHD associations were obtained from 3 databases: CARDIoGRAMplusC4D consortium, UK Biobank, and FinnGen study, respectively. For each exposure, MR analyses (primary analysis using IVW and sensitivity analyses using weighted-median estimator, MR Egger regression, and MR PRESSO) were performed per outcome database, and subsequently meta-analyzed. 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 = Mendelian randomization; MR PRESSO = MR Pleiotropy RESidual Sum and Outlier; SNP = single-nucleotide polymorphism.

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 (29). As for ascorbate, 1 genetic variant ($p = 2.0 \times 10^{-7}$) was identified with over 15,000 participants (32). A summary of the demographic characteristics of the cohort that were used to generate genetic instrumental variables are presented in Supplemental Table 2.

Circulating antioxidant 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 = 7,276), 13 for γ-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.

Explained variance and instrument strength. Variance (R^2) in MR study refers to the proportion of total variation in the exposure that 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). 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 3 large databases, namely CARDIoGRAMplusC4D (Coronary Artery Disease Genome-Wide Replication and Meta-analysis plus the Coronary Artery Disease Genetics) consortium, UK Biobank, and the FinnGen study.

CARDIoGRAMplusC4D assembled 60,801 cases and 123,504 control subjects 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 were 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). 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 (38), and CAD cases were retrieved from linkage with the 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 control subjects were identified. We performed logistic regression analyses to assess the associations between genetic instruments and CAD, adjusted for age, sex, and 10 principle components, and corrected for familial relationship using BOLT_LMM statistical package (version 2.3.2).

The FinnGen study is an ongoing nationwide cohort study launched in 2017, which combines genetic data from Finnish biobanks and health record data from Finnish health registries. Major CHD was defined as angina pectoris (I20), myocardial infarction (I21 to 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 7,123 cases of major CHD and 89,376 control subjects with complete instrument-CHD associations.

STATISTICAL ANALYSIS. All the analyses were undertaken using R version 3.6.1 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 variable (40). Results are expressed as odds ratios (ORs) on CHD risk for a corresponding unit change in absolute circulating levels of antioxidants on natural log-transformed levels (β-carotene and retinol), μg/dl (lycopene) or μmol/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 outcome. MR analyses were performed using the R-based package "TwoSampleMR."

Sensitivity analysis. To examine whether there was violation of the main MR assumptions due to directional pleiotropy, we performed MR-Egger regression analysis and weighted-median estimator (40–42). 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 biased (41). A weighted-median estimator analysis can provide a consistent valid estimate if at least one-half of the instrumental variables are valid (42). In addition, MR-PRESSO (MR Pleiotropy RESidual Sum and Outlier) was applied to detect and correct for horizontal pleiotropy through removing outliers (43), as implemented in the R-based package MRPRESSO. Furthermore, Cochran's Q statistic was used to test the heterogeneity among the estimated Wald ratios from different genetic variants (44). 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 3 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 3 studies and corresponding p value derived from Cochran's Q test. Given no heterogeneity was present across 3 databases, fixed-effect model meta-analyses were used to pool instrumental variable estimates across the 3 outcome databases for each exposure. All meta-analyses were performed in the R-based "meta" package.

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 (βgene-exposure), and with CHD (βgene-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 antioxidant levels were not associated with the risk of CHD in any of the 3 databases (**Figure 2**, Supplemental Table 5). Pooled OR for CHD per unit increase of antioxidants were 1.03 (95% confidence interval [CI]: 0.97 to 1.10) and 0.94 (95% CI: 0.63 to 1.41) for natural log-transformed β-carotene and retinol, 1.02 (95% CI: 0.99 to 1.06) for 1 μg/dl lycopene, and 1.00 (95% CI: 0.99 to 1.00) for 1 μmol/l ascorbate, respectively.

For β-carotene and lycopene with 3 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 the MR-PRESSO test for these 2 antioxidants in any of the databases.

In the sensitivity analysis for β-carotene with 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 ANTIOXIDANT METABOLITES AND CHD. Consistent with the findings from absolute circulating antioxidants, no association between genetically predicted circulating antioxidant 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 to 1.35) for α-tocopherol, 0.93 (95% CI: 0.82 to 1.06) for γ-tocopherol, 1.00 (95% CI: 0.98 to 1.02) for retinol, and 1.01 (95% CI: 0.95 to 1.08) for ascorbate.

Sensitivity analysis for metabolites on CHD risk are 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 = 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.

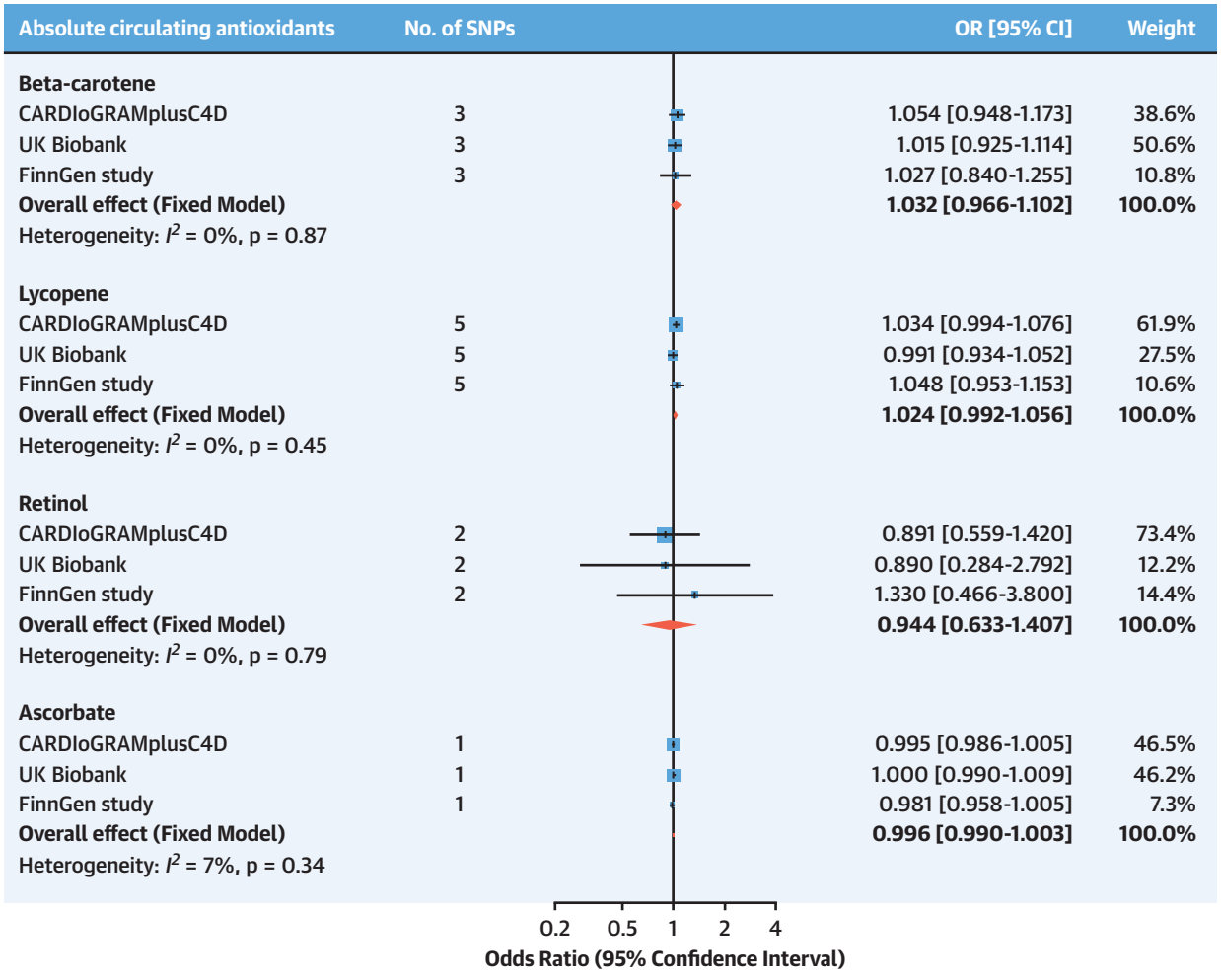
Heterogeneity was detected using Cochran's Q statistics for all metabolites in different databases, especially with outliers as identified using MR PRESSO. However, in the leave-one-out analyses, we found that the risk estimates of genetically predicted antioxidants' metabolites and risk of CHD did not change substantially after excluding 1 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 MR. 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 (**Central Illustration**).

Two previous studies using an MR approach found that a genetic predisposition to high vitamin E level was associated with increased risk of CAD (45,46).

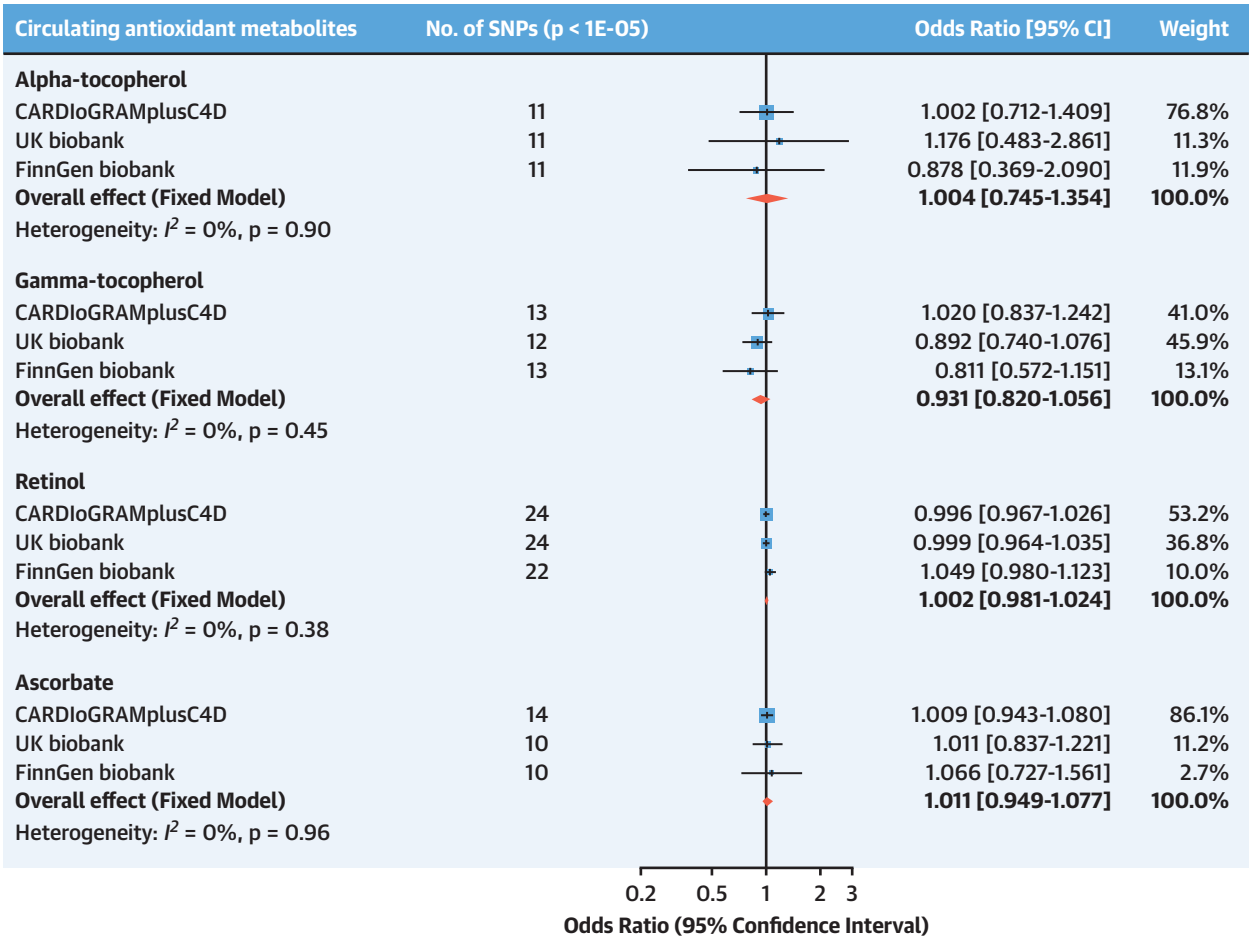
FIGURE 2 Causal Association Between Absolute Circulating Antioxidants With Coronary Heart Disease



Estimated ORs for the effect of per unit increase in ln-transformed β-carotene and retinol values, 1 μg/dl lycopene, and 1 μmol/l ascorbate on coronary heart disease, obtained from an IVW analysis, per outcome database separately and combined over the 3 databases using fixed-effect meta-analyses. CI = confidence interval; OR = odds ratio; other abbreviations as in Figure 1.

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 estimates (47). 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-analyses (14-20). However, direct comparisons between these 2 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 a 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 3 Causal Association Between Circulating Antioxidant Metabolites With Coronary Heart Disease

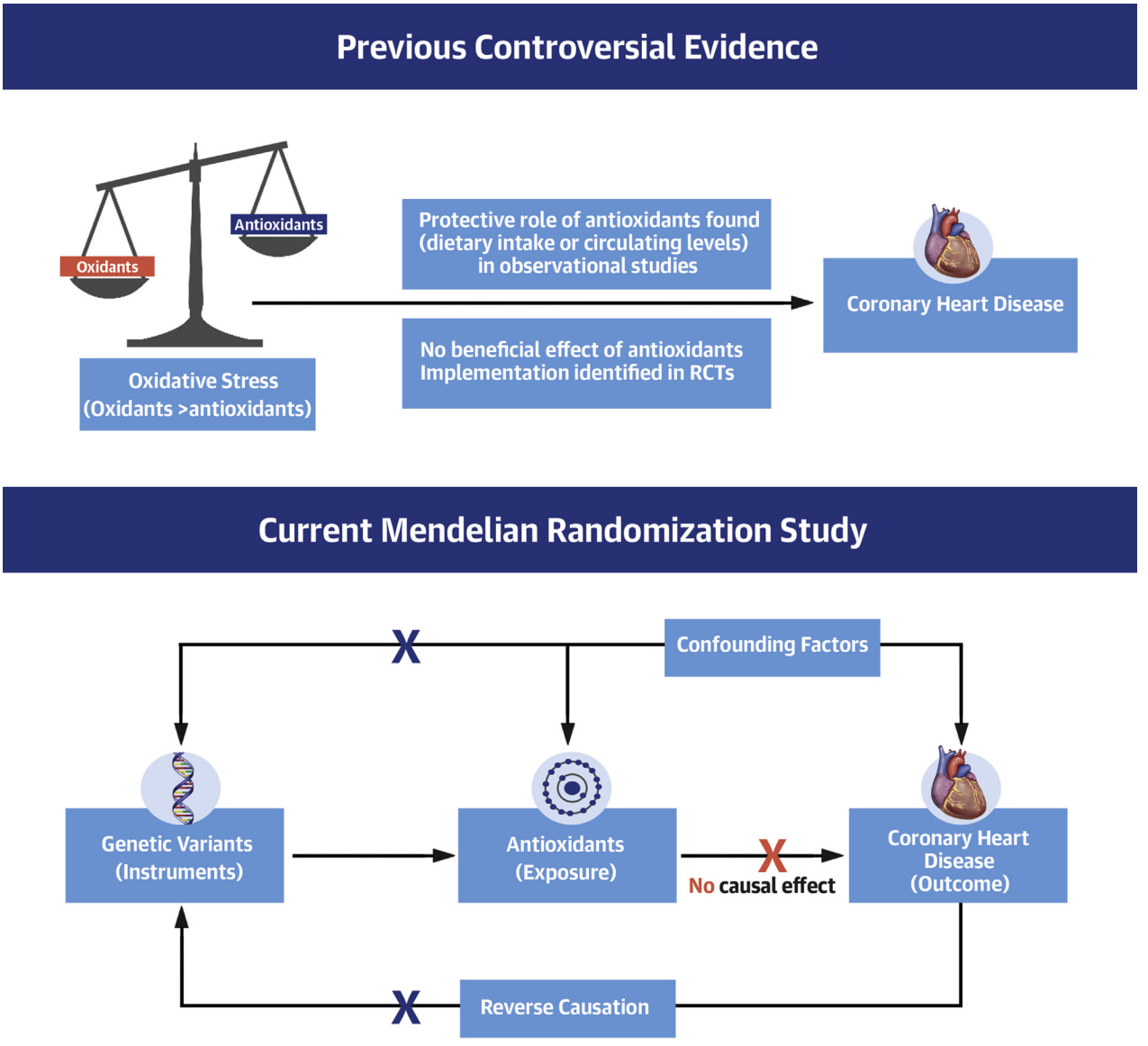


Estimated ORs for the effect of per 10-fold increase in antioxidants metabolites' concentrations on coronary heart disease, obtained from an IVW analysis, per outcome database separately and combined over the 3 databases using fixed-effect meta-analyses. Abbreviations as in Figures 1 and 2.

STUDY STRENGTHS. There are 2 main strengths in the present study. First, we used 2 separate sets of instrumental variables for both absolute circulating antioxidants and their metabolites. Specifically, for retinol and ascorbate that are presented in both sets, similar results were generated, which is supportive of the robustness of our findings. Second, 3 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.

STUDY LIMITATIONS. First, we are unable to test for a nonlinear causal association between the antioxidant levels and CHD that has previously been suggested, especially for α-tocopherol and β-carotene (6). Although analytic methods have been developed, these require individual-level data of the exposure (40,48); as the published data we used are summary-level statistics, we were therefore unable 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 that are crucial in the metabolism of antioxidants and are not associated with any other CHD risk factors in GWAS catalog or PhennoScanner

CENTRAL ILLUSTRATION Antioxidants and Coronary Heart Disease



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(Top) Antioxidants act as scavengers of oxidants to mitigate oxidative stress-induced damage, thus preventing coronary heart disease. Previously, observational studies found a protective role of antioxidants with lower risk of coronary heart disease, whereas randomized clinical trials (RCTs) generally failed to demonstrate a beneficial effect of antioxidant supplementation on coronary heart disease. **(Bottom)** Our 2-sample Mendelian randomization analyses, in the absence of residual confounding and reverse causation, do not support a causal role of circulating dietary-derived antioxidants in the prevention of coronary heart disease.

databases, suggesting no directional pleiotropy as also confirmed in the analyses. Third, only 1 SNP with an 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 effect (49). In addition, results from ascorbate metabolites with larger R^2 (18.6%) gave very similar estimates, which further reinforces the validity of the

findings. Fourth, protective effects of antioxidants might still exist in discriminatingly selected subgroups who have elevated oxidative stress levels; for example, vitamin E supplement provided cardiovascular-protective effects only in individuals with both diabetes and the haptoglobin2-2 genotype. Besides, multiple treatments simultaneously might be more effective for multifactorial diseases because there could be a synergistic benefit from 2 agents with acceptable safety and efficacy, for example, traditional treatment plus antioxidants to achieve better effects than antioxidants only (50). 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. Last, although no causal associations between circulating antioxidants and CHD risk was 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 prevention (51–54).

CONCLUSIONS

Evidence from the present study did not support a beneficial role of circulating dietary-derived vitamins E or C, β-carotene, lycopene, or retinol on CHD risk in the general population. This signifies the absence of a substantial role of antioxidants supplements on CHD risk identified in RCTs and is in accordance with the recommendations from the U.S. Preventive Services Task Force (19). 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.

AUTHOR DISCLOSURES

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Diet-derived circulating antioxidants (vitamin E, vitamin C, β-carotene, lycopene, and retinol) are not causally associated with coronary heart disease in healthy adults.

TRANSLATIONAL OUTLOOK: Future studies should address the effect of diet-derived antioxidants alone and in combination with other interventions on cardiovascular outcomes in patients with nutritional deficiency or oxidative stress.

REFERENCES

1. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. Eur Heart J 2014;35:2950–9.

2. Benjamin EJ, Muntner P, Alonso A, et al. Heart disease and stroke statistics-2019 update: a report from the American Heart Association. Circulation 2019;139:e56–528.

3. Ford ES, Ajani UA, Croft JB, et al. Explaining the decrease in U.S. deaths from coronary disease, 1980-2000. N Engl J Med 2007;356:2388–98.

4. Daiber A, Di Lisa F, Oelze M, 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;174:1670–89.

5. Sugamura K, Keaney JF. Reactive oxygen species in cardiovascular disease. Free Radic Biol Med 2011;51:978–92.

6. Aune D, Keum N, Giovannucci E, 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;108:1069–91.

7. Knekt P, Ritz J, Pereira MA, et al. Antioxidant vitamins and coronary heart disease risk: a pooled analysis of 9 cohorts. Am J Clin Nutr 2004;80:1508–20.

8. Li G, Li Y, Chen X, Sun H, Hou X, Shi J. Circulating tocopherols and risk of coronary artery disease: A systematic review and meta-analysis. Eur J Prev Cardiol 2016;23:748–57.

9. Song B, Liu K, Gao Y, et al. Lycopene and risk of cardiovascular diseases: a meta-analysis of observational studies. Mol Nutr Food Res 2017;61.

10. Kim J, Choi J, Kwon SY, 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:e004224.

11. Ye Z, Song H. Antioxidant vitamins intake and the risk of coronary heart disease: meta-analysis of cohort studies. Eur J Cardiovasc Prev Rehabil 2008;15:26–34.

12. Cheng HM, Koutsidis G, Lodge JK, Ashor AW, Siervo M, Lara J. Lycopene and tomato and risk of cardiovascular diseases: a systematic review and meta-analysis of epidemiological evidence. Crit Rev Food Sci Nutr 2019;59:141–58.

13. Parohan M, Anjom-Shoae J, Nasiri M, Khodadost M, Khatibi SR, Sadeghi O. 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;58:2175–89.

14. Al-Khudairy L, Flowers N, Wheelhouse R, et al. Vitamin C supplementation for the primary prevention of cardiovascular disease. Cochrane Database Syst Rev 2017;3:CD011114.

15. Schwingshackl L, Boeing H, Stelmach-Mardas M, 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;8:27–39.

16. Myung S-K, Ju W, Cho B, 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. Fortmann SP, Burda BU, Senger CA, Lin JS, Whitlock EP. 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;159:824–34.

18. Jenkins DJA, Spence JD, Giovannucci EL, et al. Supplemental vitamins and minerals for CVD prevention and treatment. J Am Coll Cardiol 2018;71:2570–84.

19. Moyer VA, U.S. Preventive Services Task Force. Vitamin, mineral, and multivitamin supplements for the primary prevention of cardiovascular disease and cancer: U.S. Preventive Services Task Force Recommendation Statement. Ann Intern Med 2014;160:558–64.

20. Ashor AW, Brown R, Keenan PD, Willis ND, Siervo M, Mathers JC. 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. Cheng HM, Koutsidis G, Lodge JK, Ashor A, Siervo M, Lara J. Tomato and lycopene supplementation and cardiovascular risk factors: a systematic review and meta-analysis. Atherosclerosis 2017;257:100–8.

22. Steinhubl SR. Why Have Antioxidants failed in clinical trials? Am J Cardiol 2008;101:S14–9.

23. Murphy MP. Antioxidants as therapies: can we improve on nature? Free Radic Biol Med 2014;66:20–3.

24. Lawlor DA, Harbord RM, Sterne JAC, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med 2008;27:1133–63.

25. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG, Consortium E-I. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. Eur J Epidemiol 2015;30:543–52.

26. Major JM, Yu K, Wheeler W, et al. Genome-wide association study identifies common variants associated with circulating vitamin E levels. Hum Mol Genet 2011;20:3876–83.

27. Hendrickson SJ, Hazra A, Chen C, 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;96:1379–89.

28. D'Adamo CR, D'Urso A, Ryan KA, et al. A Common variant in the SETD7 Gene predicts serum lycopene concentrations. Nutrients 2016;8:82.

29. Mondul AM, Yu K, Wheeler W, et al. Genome-wide association study of circulating retinol levels. Hum Mol Genet 2011;20:4724–31.

30. Shin SY, Fauman EB, Petersen AK, et al. An atlas of genetic influences on human blood metabolites. Nat Genet 2014;46:543–50.

31. Long T, Hicks M, Yu HC, et al. Whole-genome sequencing identifies common-to-rare variants associated with human blood metabolites. Nat Genet 2017;49:568–78.

32. Timpson NJ, Forouhi NG, Brion MJ, 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;92:375–82.

33. Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. Nat Genet 2013;45:1274–83.

34. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 2010;466:707–13.

35. Howson JMM, Zhao W, Barnes DR, et al. Fifteen new risk loci for coronary artery disease highlight arterial-wall-specific mechanisms. Nat Genet 2017;49:1113–9.

36. Nikpay M, Goel A, Won HH, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. Nat Genet 2015;47:1121–30.

37. Deloukas P, Kanoni S, Willenborg C, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet 2013;45:25–33.

38. Sudlow C, Gallacher J, Allen N, 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:e1001779.

39. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol 2013;37:658–65.

40. Staley JR, Burgess S. Semiparametric methods for estimation of a nonlinear exposure-outcome relationship using instrumental variables with application to Mendelian randomization. Genet Epidemiol 2017;41:341–52.

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

42. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian Randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol 2016;40:304–14.

43. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet 2018;50:693–8.

44. Greco MF, Minelli C, Sheehan NA, Thompson JR. Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome. Stat Med 2015;34:2926–40.

45. Wang T, Xu L. Circulating vitamin E levels and risk of coronary artery disease and myocardial infarction: a Mendelian randomization study. Nutrients 2019;11:2153.

46. Fan C, Huang T, Kong X, Zhang X, Zou Z, Xiao J. Circulating vitamin E and cardiometabolic measures: a Mendelian randomization analysis. J Clin Biochem Nutr 2019;65:160–9.

47. Haycock PC, Burgess S, Wade KH, Bowden J, Relton C, Davey Smith G. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. Am J Clin Nutr 2016;103:965–78.

48. Burgess S, Davies NM, Thompson SG, for the EPIC-InterAct Consortium. Instrumental variable analysis with a nonlinear exposure–outcome relationship. Epidemiology 2014;25:877–85.

49. Burgess S. Sample size and power calculations in Mendelian randomization with a single instrumental variable and a binary outcome. Int J Epidemiol 2014;43:922–9.

50. El-Aal AA, El-Ghffar EAA, Ghali AA, Zughbur MR, Sirdah MM. 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;12:483–9.

51. Taylor F, Huffman MD, Macedo AF, et al. Statins for the primary prevention of cardiovascular disease. Cochrane Database Syst Rev 2013Cd004816.

52. Varghese T, Schultz WM, McCue AA, et al. Physical activity in the prevention of coronary heart disease: implications for the clinician. Heart 2016;102:904–9.

53. Lavie CJ, Milani RV, Ventura HO. Obesity and cardiovascular disease: risk factor, paradox, and impact of weight loss. J Am Coll Cardiol 2009;53:1925–32.

54. Jukema JW, Cannon CP, de Craen AJ, Westendorp RG, Trompet S. The controversies of statin therapy: weighing the evidence. J Am Coll Cardiol 2012;60:875–81.

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APPENDIX For supplemental tables, figures, and references, please see the online version of this paper.