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Review

Associations of metabolomic profiles with circulating vitamin E and urinary vitamin E metabolites in middle-aged individuals

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ABSTRACT

Vitamin E (α-tocopherol [α-TOH]) is transported in lipoprotein particles in blood, but little is known about the transportation of its oxidized metabolites. In the Netherlands Epidemiology of Obesity Study, we aimed to investigate the associations of 147 circulating metabolomic measures obtained through targeted nuclear magnetic resonance with serum α-TOH and its urinary enzymatic (α-CEHC) and oxidized (α-TLHQ) metabolites from 24-h urine quantified by liquid chromatography with tandem mass spectrometry. Multivariable linear regression analyses, in which multiple testing was taken into account, were performed to assess associations between metabolomic measures (determinants; standardized to mean = 0, SD = 1) and vitamin E metabolites (outcomes), adjusted for demographic factors. We analyzed 474 individuals (55% women, 45% men) with a mean (SD) age of 55.7 (6.0) y. Out of 147 metabolomic measures, 106 were associated ($P < 1.34 \times 10^{-3}$) with serum α-TOH (median β [interquartile range] = 0.416 [0.383–0.466]), predominantly lipoproteins associated with higher α-TOH. The associations of metabolomic measures with urinary α-CEHC have directions similar to those with α-TOH, but effect sizes were smaller and non-significant (median β [interquartile range] = 0.065 [0.047–0.084]). However, associations of metabolomic measures with urinary α-TLHQ were markedly different from those with both serum α-TOH and urinary α-CEHC, with negative and small-to-null relations to most very-low-density lipoproteins and amino acids. Therefore, our results highlight the differences in the lipoproteins involved in the transportation of circulating α-TOH and oxidized vitamin E metabolites. This indicates that circulating α-TOH may be representative of the enzymatic but not the antioxidative function of vitamin E.

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Introduction

Vitamin E (α-tocopherol [α-TOH]) is a fat-soluble and essential component in the -=nnnnnnnnnnnnnnn bjiiiiiiiiiiiiiiiiiiudiet. It is important in many physiological processes including fetal development and reproduction, neurodevelopment, and cognitive function; deficiency in vitamin E will induce defects in several developing organs, including primary manifestation in the central nervous system of cerebellar ataxia [1–3]. In addition, vitamin E has chain-breaking antioxidant activity by competitively reacting with lipid peroxyl radicals to ameliorate damage induced by lipid peroxidation [4].

Observational studies have shown associations between higher dietary intake or circulating levels of vitamin E and lower risk of diseases related to lipid peroxidation, such as cardiovascular and neurodegenerative diseases [5–9]. However, there is no evidence for a causal effect based on randomized clinical trials with vitamin E supplementation [10–13] or mendelian randomization [14,15]. A potential explanation lies in the bioactivity of vitamin E, which can be either catabolized via hepatic enzymatic pathways or oxidized in the periphery [16]. In the hepatic pathway, vitamin E is enzymatically converted to a spectrum of metabolites of carboxymethyl-hydroxychroman (CEHC), with successive shortening of the phytyl side chain, and then eliminated mainly via urine. Alternatively, α-tocopherol reacts with lipid peroxyl radicals, with the opening of the chromanol ring, and generates α-tocopherol quinone (α-TQ); thereafter, α-tocopherol quinone captures hydrogens, converting into α-tocopherol hydroquinone. After the β-oxidation and cyclization of the phytyl side chain, α-tocopheronic acid and α-tocopherol lactone are generated. Finally, α-tocopherol lactone is excreted as polar conjugates of α-tocopherol lactone hydroquinone (α-TLHQ) [17,18]. Therefore, circulating vitamin E levels might not represent the authentic antioxidative effect.

The intestinal absorption, hepatic metabolism, and cellular uptake of vitamin E largely follow similar processes as lipids. Briefly, vitamin E is emulsified by digestive enzymes to form micelles and subsequently absorbed by the intestine via passive diffusion or receptor-mediated transport, followed by circulation and distribution to target organs and tissues. Once internalized into the enterocytes, lipoproteins are the carriers of vitamin E vascular transportation, independent of the type of isomer. Vitamin E is absorbed and secreted in chylomicrons into the lymphatic system, then transformed into remnants acquired by the liver parenchymal cells via uptake mediated by low-density lipoprotein (LDL) receptors [19]. In the liver, different forms of vitamin E are sorted; the highly expressed α-TOH transport protein selectively and readily binds to α-TOH for secretion in very-low-density lipoproteins (VLDLs) and favors the discrimination of α-TOH among other isomers, protecting α-TOH from excessive degradation and excretion. The final acquisition of vitamin E by tissues is through chylomicron and VLDL catabolism, LDL uptake via LDL receptor, or lipoprotein transfer to membranes.

Apart from the major portion of circulating α-TOH carried by LDL particles, some of the VLDL-derived α-TOH can also be transferred to high-density lipoproteins (HDLs) during lipolysis. HDLs are important for the delivery of α-TOH to extrahepatic tissues, particularly to the central nervous system, and for facilitating the transport of α-TOH from the circulation back to the liver [20]. Therefore, vitamin E utility depends on mechanisms underlying lipoprotein metabolism and relies on lipoprotein-mediated production, processing, and uptake [21]. In addition to their role as vitamin E carriers, lipoproteins—in particular LDLs—are also susceptible to oxidative modifications that require antioxidant protection. However, it is unclear to what extent vitamin E is catabolized via hepatic enzymatic pathways or oxidized, and what lipoproteins are involved in the transportation of these two metabolism processes.

In the present study, we aimed to investigate the cross-sectional associations between circulating metabolomic profiles and circulating α-TOH and urinary enzymatic and oxidized α-TOH metabolites in middle-aged individuals in the Netherlands Epidemiology of Obesity (NEO) Study.

Methods

Study population

The present study was embedded in the population-based prospective cohort NEO study, which aims to study the pathways that lead to obesity-related disorders. The NEO study was initiated in 2008, comprising 6671 participants ages 45 to 65 y. Detailed information on the study design and data collection has been given previously [22]. In brief, residents of the greater Leiden area with a self-reported body mass index (BMI) $\geq$ 27 kg/m^2 were eligible to participate. In addition, all inhabitants of the municipality of Leiderdorp were invited irrespective of their BMI. Participants were invited to the NEO study center of the Leiden University Medical Center for one baseline study visit. Blood samples were drawn after an overnight fast and separated into serum, and aliquots were stored at −80°C for later measurements. Participants were asked to collect their urine over 24 h and completed a general questionnaire at home with their demographic, lifestyle, and clinical data as well as specific questionnaires on diet and physical activity before their first visit. The urine sample was divided into aliquots and stored at −80°C for later analyses of urinary vitamin E metabolites. Additionally, participants were asked to bring all medications (prescribed medications, including blood-pressure-lowering medications, lipid-lowering medications, and glucose-lowering medications, as well as self-medications such as supplements) they were using for 1 mo preceding the study visit. The study was approved by the medical ethical committee of the Leiden University Medical Center, and all participants gave written informed consent.

A random subset of 35% baseline Leiderdorp participants (n = 599) was included in this cross-sectional analysis. We excluded individuals with urine collection less than 20 h and those for whom measurement of urinary vitamin E metabolites failed (n = 61). Given that the platform used for measurements contained a considerable number of lipoproteins, participants who were taking statins at the time of blood sampling (n = 38) were consequently excluded. We further excluded participants with implausible metabolite measurements (concentration $\leq$ 0, n = 4); missing or outlying urinary vitamin E metabolites (n = 7), serum vitamin E (n = 4), or metabolomic measures (n = 2); and missing data on confounding factors (n = 9). Therefore, the final number of participants in the present study was 474.

Metabolomic-measure profiling

The lipoprotein profiles were quantified using high-throughput ^{1}H-nuclear magnetic resistance metabolomics (Nightingale Health, Helsinki, Finland). This platform provides simultaneous quantification of 229 metabolites and ratios. After excluding the calculated ratios from the data set, we included 147 metabolites from 11 classes: lipoprotein subclasses (n = 98), lipoprotein particle sizes (n = 3), apolipoproteins (n = 2), fatty acids (n = 10), cholesterol (n = 9), glycerides and phospholipids (n = 9), amino acids (n = 8), ketone bodies (n = 2), inflammation (n = 1), glycolysis-related metabolites (n = 3), and fluid balance (n = 2). Lipoprotein subclasses were defined according to particle size as follows: chylomicrons and extremely large VLDLs with particle diameters $\geq$ 75 nm upward, five subclasses of VLDLs (XL-VLDLs: 64 nm, L-VLDLs: 53.6 nm, M-VLDLs: 44.5 nm, S-VLDLs: 36.8 nm, XS-VLDLs: 31.3 nm), intermediate-density lipoproteins (IDLs; 28.6 nm), three subclasses of LDLs (X-LDLs: 25.5 nm, M-LDLs: 23 nm, S-LDLs: 18.7 nm), and four subclasses of HDLs (XL-HDLs: 14.3 nm, L-HDLs: 12.1 nm, M-HDLs: 10.9 nm, S-HDLs: 8.7 nm). Detailed information, including quality-assurance measures and applications of the platform, has been given elsewhere [23].

Measurements of vitamin E and vitamin E metabolites

Circulating serum α-TOH was detected and quantified by Metabolon, Inc. (Durham, NC, USA), on a platform encompassing four methods of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS): LC-MS/MS negative, LC-MS/MS positive early, LC-MS/MS positive late, and LC-MS/MS polar. Given the relative quantification, the absolute amount of α-TOH per liter was not quantified, and therefore data are expressed not as a concentration but as peak height relative to an internal standard per sample, to allow for comparisons between individuals. More information about the quantifications has been given previously [24,25].

Urinary α-CEHC and oxidized α-TOH metabolites (α-TLHQ), presented as their sulfate or glucuronide conjugates, were measured by LC-MS/MS at University College London, UK, between March and May 2019 [26]. The final concentrations of α-TLHQ and α-CEHC were the sum of their corresponding sulfate and glucuronide isoforms.

Briefly, 100 μL thawed urine (from 24-h urine) was centrifuged for 10 min at 14 000g at room temperature and spiked with 10 μL of the internal standards

(100 μmol/L) lithocholic acid sulfate and androsterone D4-glucuronide, and 10 μL was subsequently injected into the LC-MS/MS system for detection. Metabolite separation was performed by a Waters Acquity UPLC BEH C8 column (1.7-μm particles, 50 mm × 2.1 mm; Waters Corp., Manchester, UK) plus a guard column containing an identical stationary phase. To minimize system contamination and carryover, eluents before and after the sample elution were discarded, and an additional blank sample (H_2O:MeCN) was run after each detection urine sample. Two separate peaks were observed for both α-TLHQ and α-CEHC conjugated with glucuronide, corresponding to major and minor isoforms. These isoforms have been previously described thoroughly by Pope et al. [27] and Sharma et al. [18,26]. The different elution times (minutes) for internal standards (lithocholic acid sulfate, 4.33; androsterone D4-glucuronide, 2.7) and each metabolite (2.39, 2.12, and 2.29 for α-TLHQ sulfate and glucuronide minor and major; 2.64, 2.50, and 2.56 for α-CEHC sulfate and glucuronide minor and major) guaranteed that all metabolites could be separated in a single chromatographic run. Metabolite analyses were then performed by MS using a Waters Acquity UPLC coupled to a triple-quadrupole Xevo TQ-S fitted with electrospray ionization in negative ion mode. Using multiple-reaction monitoring mode, specific parent and daughter ions were determined in scan mode and after collision-activated dissociation with argon. These ions were then used to quantify each α-TOH metabolite from transitions that corresponded to their molecular masses. Creatinine concentration is frequently used as a proxy of kidney function, and in cases of severe renal dysfunction the creatinine clearance rate will be "overestimated" because the active secretion of creatinine will account for a larger fraction of the total creatinine. Therefore, to correct for this dilution effect, urinary creatinine concentrations (mmol/L) were also measured by triple-quadrupole Micro Quattro mass spectrometry (MicroMass, Waters Corp.). Therefore, the final concentrations of α-TOH metabolites were in nmol/mmol of creatinine. A quality-control assessment was performed throughout the quantification to deal with the variations in sample quality and UPLC-MS/MS performance. Detailed information of the measurements has been given previously [28].

Confounding factors

To determine BMI, body weight was measured without shoes, and 1 kg was subtracted to correct for clothing weight. Smoking status was categorized into current smoker, former smoker, or non-smoker. Physical activity levels (in MET-h/week [MET: metabolic equivalent of task]) were estimated based on the frequency and duration of leisure physical activity over the past 4 wk reported by participants on the Short Questionnaire to Assess Health-Enhancing Physical Activity [29]. A semiquantitative food frequency questionnaire was used to assess food and beverage intake. Total energy intake (in kJ) and the Dutch Healthy Diet Index (DHD-index) were subsequently estimated based on dietary intake [30]. Use of vitamin E supplements was collected via questionnaires and was defined as either vitamin E supplement use only or multivitamin supplement use (yes/no).

Statistical analysis

Descriptive characteristics of the study population are presented as mean (SD) or median (interquartile range) for normally distributed variables and skewed variables, respectively, and as frequency (percentage) for categorical variables.

Vitamin E metabolites and metabolomic measures were log10-transformed to approximate a normal distribution. Observed metabolite concentrations located beyond 4 SDs from the mean after log10-transformation were classified as outliers and further excluded. Since missing data on the metabolomic measures were most likely due to concentrations that were lower than the limit of detection, these missing values were imputed by giving them the value of half of the minimum observed value for each metabolite. In addition, we assessed the percentage of missing data for each metabolomic measure. To compare the effect estimates—that is, the coefficients for different metabolomic measures obtained from the regression models—we standardized the log10-transformed metabolomic measures and the serum and urinary vitamin E measures (mean = 0, SD = 1), so that the regression coefficient with its corresponding 95% confidence interval (CI) can be interpreted as the mean change in SD of the outcome with respect to a 1-SD change in the determinant (standardized concentrations of the metabolomic measures).

Multivariable-adjusted linear regression models were fitted, with metabolomic measures as determinants, confounding factors as covariates, and vitamin E metabolites as outcomes. Based on prior knowledge, confounding factors included age, sex, BMI, physical activity, smoking habits, Dutch Healthy Diet Index, and total energy intake. Scatterplots were used to visualize the difference in both direction and effect size of the estimated associations among metabolomic measures with different vitamin E metabolites, and we calculated Pearson correlations between the effect estimates derived from the regression results. Therefore, the correlation indicates the similarity of those associations.

Sensitivity analysis

Use of vitamin E supplements may have an influence on metabolomic measures and vitamin E conversion, and will potentially distort the associations. However, given the high heterogeneity of vitamin E supplement use, as either vitamin E only or multivitamin use, as well as limited information on frequency, dosage, and natural or modified vitamin E acetate, we additionally performed the regression analyses in participants who did not take vitamin E or multivitamin supplements (n = 350). Furthermore, to test the modification of effects by obesity status, we stratified participants into normal weight (BMI < 25 kg/m^2, n = 217) and overweight (since only 59 were obese, with BMI > 30 kg/m^2, we combined individuals with BMI ≥ 25 kg/m^2, n = 257), and all multivariable regression models in the main analyses were conducted in each strata.

Given that most of the metabolomic biomarkers, especially lipid subclasses, were highly correlated, conventional correction for multiple testing (e.g., Bonferroni) is too stringent. Therefore, the "effective number" (Meff) procedure was used to identify independent metabolomic traits [31]. The final significance threshold of the P value was then defined as $0.05/37 = 1.34 \times 10^{-3}$. All the analyses were undertaken using R (version 3.6.1) statistical software (R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of the study population

We included 474 participants in the present study after excluding those with missing data or outliers in serum or urinary vitamin E measures; characteristics of the study population are presented in Table 1. The mean age was 55.7 (6.0) years, with a median BMI of 25.3 (23.1–27.8) kg/m^2; 55% of the participants were women and 45% were men. 124 (26%) participants used vitamin E supplements, and 50 (10%) were current smokers. Summaries of metabolomic biomarkers are presented in Supplementary Table 1. The percentages of missing data for individual metabolomic measure were all below 30%; the percentages for 9 out of 147 were 20% or higher, and 7 of those 9 were extremely large VLDL characteristics (Supplementary Fig. 1).

Main analyses

In the multivariable-adjusted linear regression model, 106 out of 147 metabolomic measures were associated with serum α-TOH with $P < 1.34 \times 10^{-3}$ (median effect size = 0.416 [0.383–0.466]; Fig. 1 and Supplementary Table 1). Three of the 106 associations

Table 1
Characteristics of the study population (n = 474)

Characteristic	Value
Demography	
Age (y)	55.7 (6.0)
Sex (M/F)	213/261 (45%/55%)
BMI (kg/m^2)	25.3 (23.1–27.8)
Lifestyle factors	
Dutch Healthy Diet Index	59.7 (8.4)
Energy intake (kJ/d)	9106 (7326–11 078)
Physical activity (MET-h/wk)	28.9 (16.0–48.8)
Smoking	
Current	50 (10%)
Former	222 (47%)
Never	202 (43%)
Vitamin E supplement use (yes)*	124 (26%)
Vitamin E metabolites	
Blood (log10-transformed)	
α-tocopherol	8.5 (0.1)
Urinary	
α-TLHQ (nmol/mmol creatinine)	1864.9 (1347.1–2770.3)
α-CEHC(nmol/mmol creatinine)	271.0 (181.4–439.4)
α-TLHQ (log10-transformed)	3.3 (0.2)
α-CEHC (log10-transformed)	2.4 (0.3)

BMI, body mass index; CEHC, carboxymethyl-hydroxychroman; MET, metabolic equivalent of task; TLHQ, tocopherol lactone hydroquinone
Values are presented as mean (SD), n (percentage), or median (interquartile range)
*Defined as use of either vitamin E supplement or multivitamin supplement.

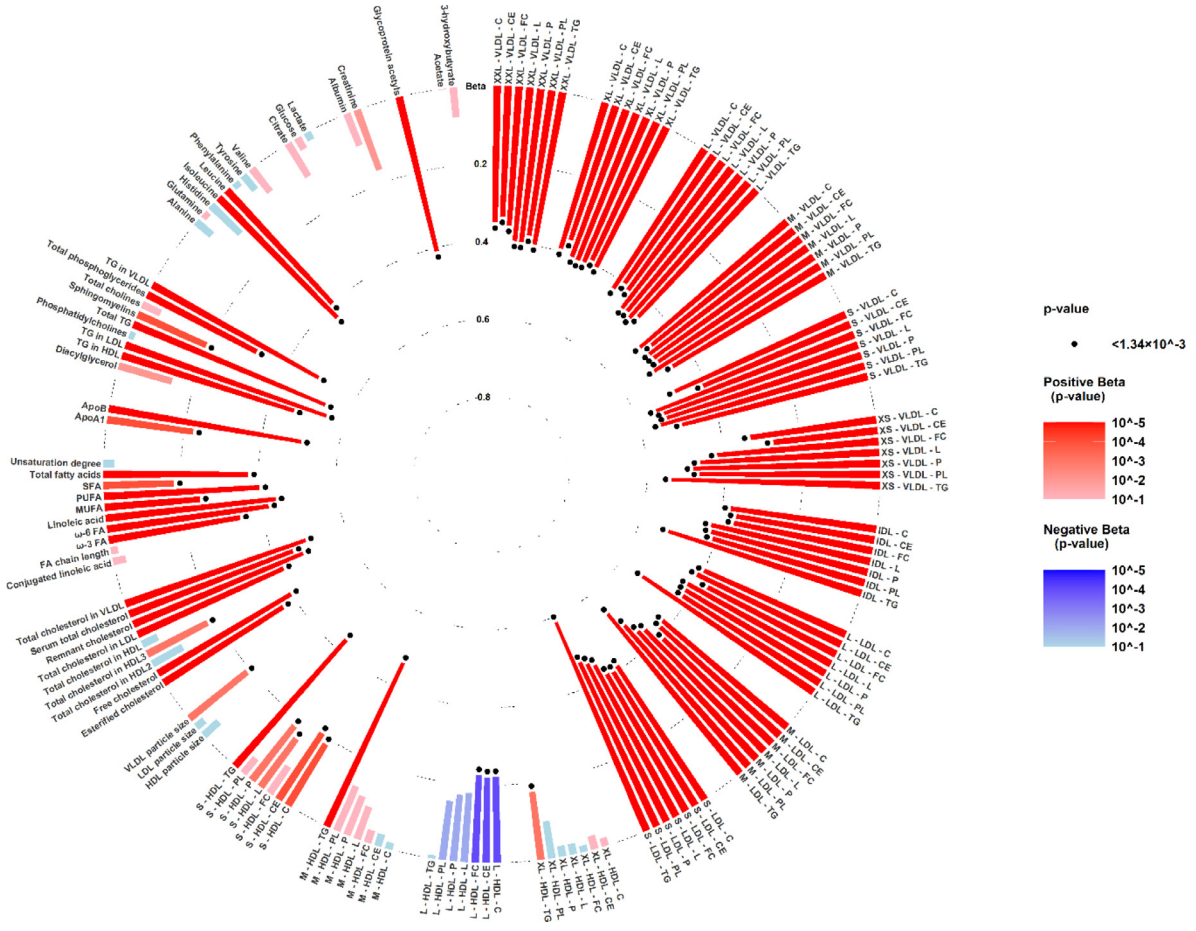

Fig. 1. Associations between 147 circulating metabolomic measures and circulating α-tocopherol. Associations were derived from a multivariable linear regression model in the study population (n = 474) adjusted for age, sex, body mass index, smoking status, Dutch Healthy Diet Index, energy intake, and physical activity. Effect estimates refers to the coefficients for different metabolomic measures obtained from the linear regression models. We standardized the log10-transformed metabolomic measures and the serum and urinary vitamin E measures (mean = 0, SD = 1), so that regression coefficients (with corresponding 95% confidence intervals) can be interpreted as the mean change in SD of the outcome with respect to a 1-SD change in the determinant (standardized concentrations of the metabolomic measures). Red and blue indicate positive and negative coefficients, respectively, and the gradients suggest different significant levels. A P value $< 1.34 \times 10^{-3}$ (0.05/37, where 37 is the number of independent metabolomic measures) is considered significant, indicated as a black dot above the bar. Full names and descriptive information for metabolomic measures are listed in Supplementary Table 1.

were negative: higher total cholesterol, cholesterol ester, and free cholesterol in large HDLs were associated with lower mean serum α-TOH—respectively effect estimates: −0.221 (95% CI, −0.330 to −0.112), −0.220 (95% CI, −0.329 to −0.112), and −0.225 (95% CI, −0.332 to −0.118) per 1-SD higher level of the metabolomic measure. In all other cases, higher levels of the metabolic measures were associated with higher α-TOH. These associations include VLDLs, IDLs, LDLs, and S-HDL, total cholesterol (not in HDL and HDL2) and its components, Apo-A and Apo-B, glycerides and phospholipids, and glycoprotein acetyls, with effect sizes ranging from 0.154 (95% CI, 0.063−0.244) SD for total lipids in small HDLs to 0.585 (95% CI, 0.509−0.660) SD for triacylglycerols in S-LDLs. Moreover, per-SD higher levels of leucine and isoleucine were associated with, respectively, 0.399-SD (95% CI, 0.290−0.507) and 0.434-SD (95% CI, 0.327−0.540) higher α-TOH.

Figures 2 and 3 present the associations between metabolomic measures and, respectively, urinary α-CEHC and α-TLHQ metabolites. None of the analyses were statistically significant upon correction for multiple testing. Nevertheless, for α-CEHC the direction of the associations with metabolomic measures was similar to those for α-TOH with metabolomic measures; the effect sizes, however, were much smaller (median effect size = 0.065 [0.047−0.084]), as shown in Figure 2. For α-TLHQ, the strongest association was with diacylglycerol (0.126; 95% CI, 0.037−0.215; P = 0.006). The direction of the associations was substantially different from those for α-TOH with metabolomic measures, and only XS-VLDLs, IDLs, LDLs, XL-HDLs, and fatty acids showed associations in the same direction.

The effect estimates between metabolomic measures and circulating α-TOH were strongly correlated with the effect estimates of metabolomic measures with urinary α-CEHC (r = 0.86, $P < 0.001$). However, the correlations between the effect estimates of metabolomic measures with α-TOH and with urinary α-TLHQ, and between the effect estimates of metabolomic measures with α-CEHC and with urinary α-TLHQ, were very weak (respectively, r = 0.16, P = 0.05, and r = 0.22, P = 0.007; Fig. 4).

Sensitivity analyses

Excluding users of vitamin E supplements

We excluded 124 participants with either vitamin E or multivitamin supplementation, leaving 350 participants for further analyses. The associations between metabolomic measures and vitamin E metabolites in this group were generally consistent with the

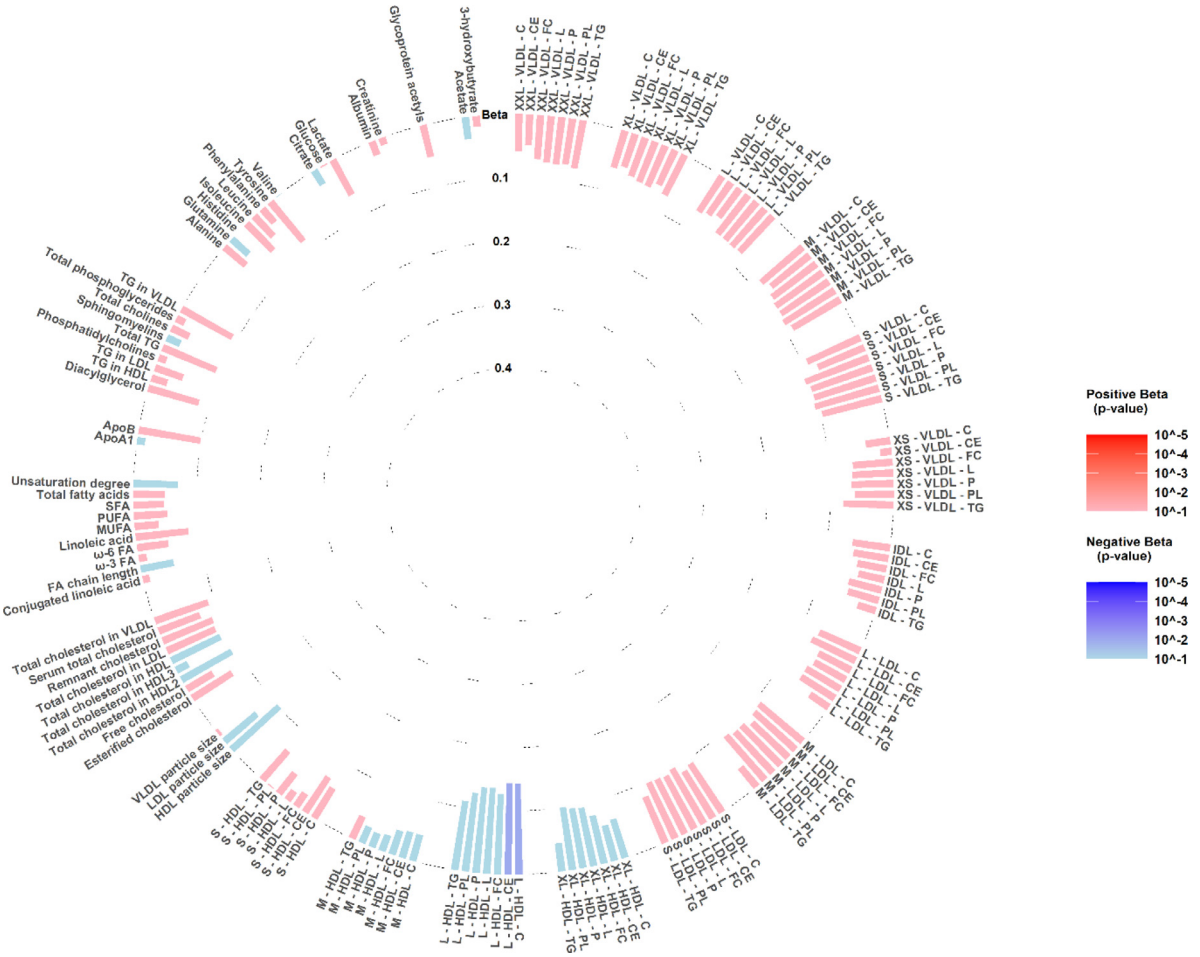

Fig. 2. Associations between 147 circulating metabolomic measures and urinary α-carboxymethyl-hydroxychroman. Associations were derived from a multivariable linear regression model in the study population (n = 474) adjusted for age, sex, body mass index, smoking status, Dutch Healthy Diet Index, energy intake, and physical activity. The figure legend is the same as for Figure 1, except that no significant associations were detected (all Ps > 1.34×10^{-3}).

analyses in the whole study population (Supplementary Fig. 2). However, several effect sizes became larger. Notably, 1-SD higher levels of total cholesterol and cholesterol esters in medium LDL and small LDL particles were associated, even after correction for multiple testing, with higher levels of α-CEHC, with respective effect sizes of 0.176 (95% CI, 0.071–0.280), 0.175 (95% CI, 0.070–0.280), 0.180 (95% CI, 0.075–0.285), and 0.179 (95% CI, 0.074–0.284) SD. Furthermore, 22 out of the 147 metabolomic measures (most notably 4 IDLs, 16 LDLs, total cholesterol, and total cholesterol in LDLs) were associated with α-TLHQ levels. Specifically, higher levels of LDL cholesterol subparticles—except for the amount of triacylglycerols in LDL particles—were associated with higher levels of α-TLHQ, with effect sizes ranging from 0.186 (95% CI, 0.079–0.293) SD for large LDL particles to 0.227 (95% CI, 0.121–0.333) SD for cholesterol esters in S-LDLs. In addition, 1-SD higher levels of total cholesterol and total cholesterol in LDLs were associated, respectively, with 0.186 (95% CI, 0.076–0.295) and 0.207 (95% CI, 0.102–0.313) SD higher α-TLHQ.

The effect estimates of metabolomic measures with circulating α-TOH were strongly correlated with the effect estimates of metabolomic measures with urinary α-CEHC (r = 0.69, $P < 0.001$), whereas no correlation was found between the effect estimates of metabolomic measures with circulating α-TOH and with urinary α-TLHQ (r = −0.013, P = 0.88). A moderate correlation was observed for the estimates between metabolomic measures with circulating α-CEHC and with urinary α-TLHQ (r = 0.49, $P < 0.001$; Supplementary Fig. 3). However, this correlation was mainly due to the association with LDLs (r = 0.18, P = 0.053 after excluding the LDL subclass from the list of metabolic measures).

Stratification analyses by obesity

In the normal-weight subgroup analyses (n = 217), the associations of metabolomic measures and serum α-TOH were analogous to those obtained from the main analyses. However, the associations with HDLs were no longer significant, resulting in slightly fewer (95 out of 147) significant associations. The median significant effect size was 0.375 (95% CI 0.330–0.414). Similarly, the associations between metabolomic profiles with α-CEHC did not differ materially. Interestingly, diacylglycerol was significantly positively related to α-TLHQ. However, the associations between HDLs and α-TLHQ became stronger, whereas the association between extremely large VLDLs and XL-VLDLs and α-TLHQ turned positive and stronger compared to the estimates from the whole population, despite being insignificant.

In the overweight subgroup analyses (n = 257), the magnitude of the estimates from regression analyses were generally larger than those derived from the main analyses in all three vitamin E metabolites. Particularly, the associations of metabolomic profiles with α-TOH remained in the same directions, and 99 out of 147 associations were significant (median effect size = 0.497 [95% CI

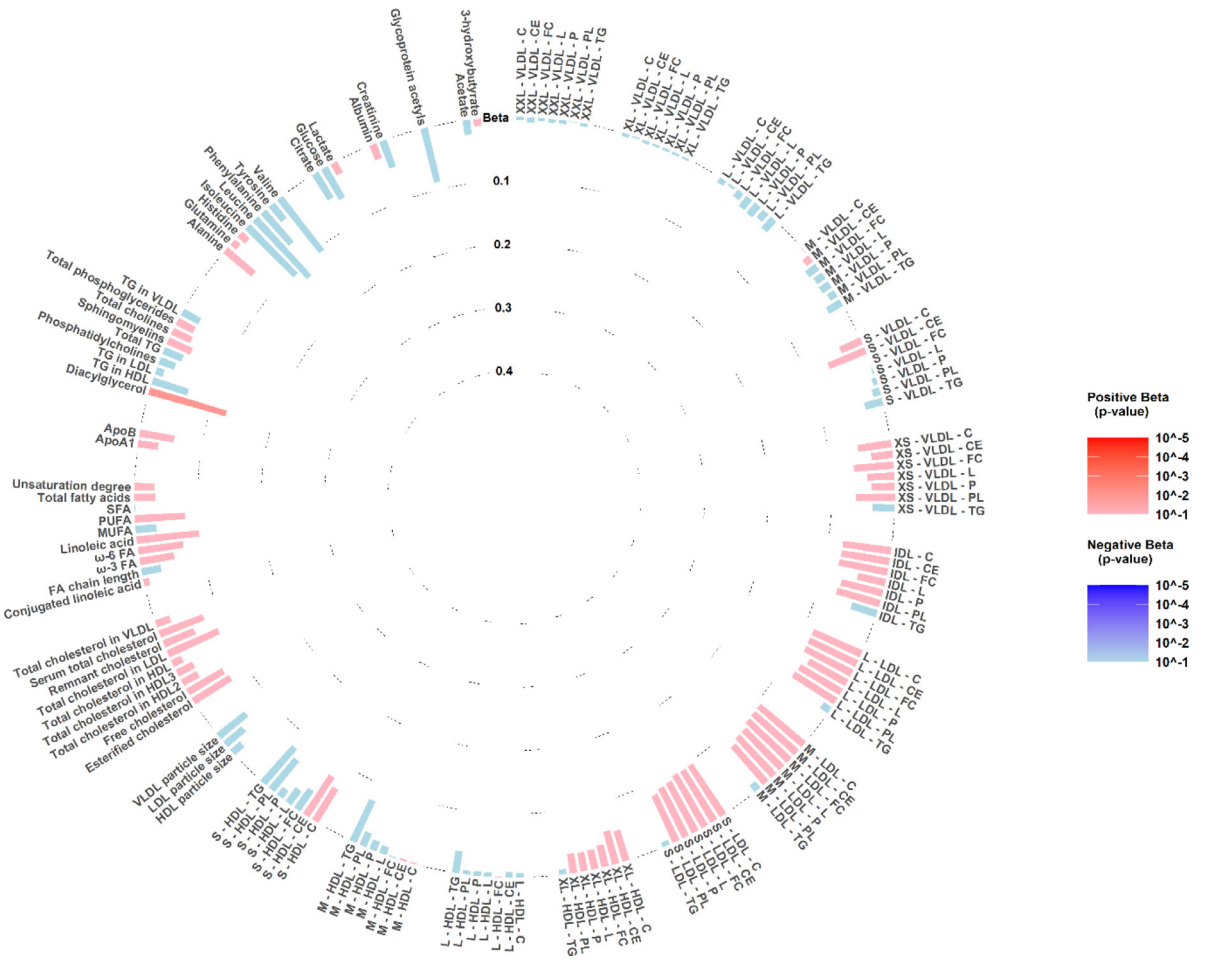

Fig. 3. Associations between β 147 circulating metabolomic measures and urinary α-tocopherol lactone hydroquinone. Associations were derived from a multivariable linear regression model in the study population (n = 474) adjusted for age, sex, body mass index, smoking status, Dutch Healthy Diet Index, energy intake, and physical activity. The figure legend is the same as for Figure 1, except that no significant associations were detected (all Ps $> 1.34 \times 10^{-3}$).

0.423−0.561]). Likewise, the relationships of metabolomic measures with α-CEHC did not change substantially. However, with regards to α-TLHQ, the direction of the association with HDLs, notably large and medium HDLs, turned positive, though insignificant.

Discussion

In this cross-sectional study, we investigated the association between circulating metabolomic measures and urinary vitamin E metabolites. The direction of the estimates from metabolomic measures and circulating α-TOH and urinary α-CEHC were similar but with weaker effect sizes for α-CEHC than α-TOH, whereas α-TLHQ showed distinct associations. We found that 106 out of 147 metabolomic measures were associated with circulating α-TOH after correction for multiple testing, with no significant associations identified for associations with urinary α-CEHC and α-TLHQ. Sensitivity analyses in participants without vitamin E supplementation generally showed consistent results with the main analyses for each vitamin E metabolite, but with significant associations between IDLs, LDLs, total cholesterol, and cholesterol content in LDL particles and α-TLHQ.

Our study shows similar associations of metabolomic measures with urinary α-CEHC and with circulating α-TOH. The liver parenchymal cells acquire α-TOH through taking up chylomicron remnants, which contain a major proportion of absorbed α-TOH, and the highly expressed α-TOH transport protein facilitates the preferential enrichment of α-TOH in VLDL particles secreted by the liver. These processes are responsible for the regulation and release of α-TOH into circulation and consequently for its delivery to tissues. Therefore, vitamin E enzymatic activity in the liver is well regulated to maintain a certain level of α-TOH—that is, higher α-TOH gives higher α-CEHC [32]. However, the different associations observed between metabolomic measures and urinary α-TLHQ compared with blood α-TOH indicate that other processes that are not associated with α-TOH regulate the association for α-TLHQ. In addition, we observed that circulating α-TOH is associated with higher levels of most lipoprotein fractions and cholesterols. This observation may be somewhat counterintuitive given previous observations of higher vitamin E circulating levels with lower risk of atherosclerosis and cardiovascular diseases [6]. Nevertheless, individuals with hyperlipidemia have been found to have reduced uptake of the newly absorbed α-TOH into blood; the abnormal lipoprotein metabolism does not necessarily increase α-TOH delivery to the peripheral tissues, and this uptake reduction of α-TOH may be relevant to the pathogenesis of atherosclerosis [33]. In addition, our results are in line with previous research finding that α-TOH concentrations were correlated with serum lipid levels, and that the retention of plasma α-TOH was longer with higher serum total lipids [21]. This might be attributed to the notion that higher lipid concentrations can keep vitamin E from

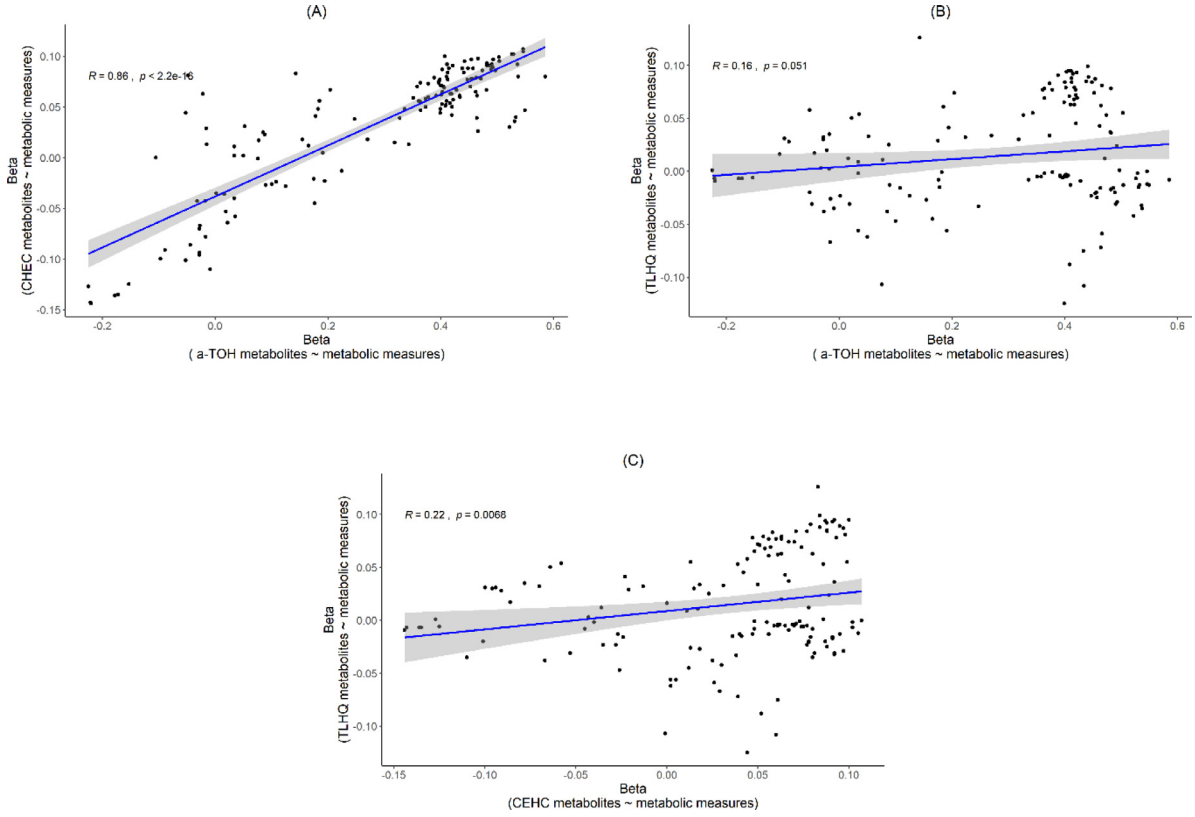

Fig. 4. Correlations of effect estimates between metabolomic measures with (A) circulating α-tocopherol and urinary α-carboxymethyl-hydroxychroman (α-CEHC), (B) circulating α-tocopherol and α-tocopherol lactone hydroquinone (α-TLHQ), and (C) urinary α-CEHC and α-TLHQ. Coefficients are derived from multiple linear regression of metabolomic measures (determinants) and vitamin E metabolites (outcomes). Correlation coefficients and *P* values are derived from Pearson correlation.

reaching peripheral tissues, and the fact that the catabolism and uptake of lipoproteins decreases at high lipid concentrations. However, higher levels of large HDL particles were associated with lower levels of α-TOH. This may be because α-TOH content in HDL particles depends not only on tocopherol levels but also on HDL concentrations, and HDL α-TOH retention has been found to be related to high concentrations of HDL fractions [34]. Two branched-chain amino acids (leucine and isoleucine) are positively associated with higher α-TOH. Experimental studies have demonstrated that vitamin E is crucial for the maintenance of energy homeostasis, and its deficiency dysregulates energy metabolism and mitochondrial dysfunction, measured by extracellular oxygen consumption [35–38]. Ketogenic amino acids—particularly leucine, which can be used for ketone synthesis—were elevated in brains of zebrafish deficient in vitamin E, where there are probably elevated lipid peroxidation and metabolic disruptions [37,38]. Health states that are associated with increased oxidative stress are likely to have a greater antioxidant requirement, which would result in depletion of circulating levels of vitamin E and higher urinary concentrations of α-TLHQ. The negative although insignificant association of leucine with α-TLHQ contrasts with findings from previous experimental studies; understanding the underlying mechanisms will require additional efforts.

Results from sensitivity analyses after exclusion of supplement users were generally consistent with the main analyses, but with several significant associations of α-TLHQ. Notably, higher levels of substances that are susceptible to oxidative modifications, particularly IDL and LDL particles, were significantly associated with higher urinary α-TLHQ. Several fatty acids are also closely related to α-TLHQ, though not significantly. The inhibitory effect against lipid peroxidation of vitamin E decreases gradually from polyunsaturated fatty acids to cholesterol. In accordance, LDLs, as the lipoprotein particles most susceptible to oxidative modification, are associated with α-TLHQ. However, this does not include in LDL particles triacylglycerols, which are more resistant to lipid peroxidation. Therefore, oxidation rather than the level of α-TOH regulated the association of metabolomic measures and α-TLHQ. Despite the strong correlation (r = 0.48) between the effect estimates of metabolomic measures with α-CEHC and with α-TLHQ, this is predominantly driven by LDL particles.

In the stratification analyses by obesity, the associations of metabolomic measures with α-TOH and α-CEHC did not differ materially in general, but the effect sizes are larger in the overweight group, possibly due to the higher lipid levels in overweight individuals. Interestingly, the associations of lipoproteins, particularly VLDLs and HDLs, with α-TLHQ differed. Although none of the associations is significant, VLDLs are positively and HDLs negatively associated with α-TLHQ in the normal-weight group, whereas opposite directions were observed in the overweight group. Excess fat accumulation will certainly lead to elevated oxidative stress, and the supply of vitamin E by HDLs might be more important under conditions of oxidative stress due to the independence of regulatory mechanisms of cholesterol metabolism [39]. Previous efforts have demonstrated the exchange of α-TOH between lipoproteins, which may depend on the ratio of HDLs to LDLs [40]; the discrepancies of VLDL associations might imply a different transfer in people who are obese, provided distinct lipid profiles in them. However, the underlying mechanisms warrant further investigation.

One strength of the present study is that we simultaneously quantified the concentrations of circulating α-tocopherol and urinary

metabolites derived from two metabolic pathways, which facilitates the exploration of the circulating level versus the functional level. In addition, the measurement of urinary α-TLHQ was performed by LC-MS/MS, which deliberately avoids artifactual oxidation products of α-CEHC that might result from previous chromatography−mass spectrometry detection. The method we used measures the intact conjugate with minimal preparation and has been demonstrated with solid reliability and reproducibility [18,26].

Several limitations should also be noted. First, some associations increased after exclusion of users of vitamin E supplements, suggesting that the effect might be modified by use of supplements or that we might introduce collider stratification bias in the users of these supplements. However, the information on supplement use was very limited, as there were no data available on the dosages, frequencies, and natural or modified vitamin E acetate, resulting in a highly heterogenous group. Therefore, further exploration of the effect of use of vitamin E supplements on these associations was not feasible. Second, within our study population, we are not able to test these associations in different physiological situations that may affect the lipoproteins involved in transportation process, such as fasting or not [34,41,42], or in individuals with elevated lipid peroxidation that will reduce α-TOH bioavailability, such as lower bioavailability identified in people with metabolomic syndrome compared with healthy controls [43]. Third, we might still have insufficient power for some of these associations, particularly in the sensitivity analysis. However, we did not only perform statistical hypothesis testing, but apart from the point estimates obtained from our multivariable adjusted regression analyses illustrated in the main text, we also calculated confidence intervals for each estimate, as shown in Supplementary Table 1. CIs reflect the precision of the estimation in the sample. In addition, we had a specific focus on the similarity of the directionality of the associations between metabolomic measurements with circulating α-TOH and the associations between metabolomic measurements with circulating α-CEHC/α-TLHQ. Finally, the observational design could not rule out residual confounding.

Conclusion

Associations of metabolomic measures with circulating α-TOH and urinary oxidized vitamin E α-CEHC were very similar in direction, whereas associations of metabolomic measures with α-TLHQ were markedly different from the associations of metabolomic measures with both serum α-TOH and urinary α-CEHC. Our results highlight the differences in the lipoproteins involved in the transportation of enzymatic and oxidized vitamin E metabolites. This indicates that circulating α-TOH may be representative for the enzymatic but not the antioxidative function of vitamin E.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2021.111440.

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