



Original article

Urinary oxidized, but not enzymatic vitamin E metabolites are inversely associated with measures of glucose homeostasis in middle-aged healthy individuals



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SUMMARY

Background & aims: Damage induced by lipid peroxidation has been associated with impaired glucose homeostasis. Vitamin E (α -tocopherol, α -TOH) competitively reacts with lipid peroxyl radicals to mitigate oxidative damage, and forms oxidized vitamin E metabolites. Accordingly, we aimed to investigate the associations between α -TOH metabolites (oxidized and enzymatic) in both circulation and urine and measures of glucose homeostasis in the general middle-aged population.

Methods: This cross-sectional study was embedded in the population-based Netherlands Epidemiology of Obesity (NEO) Study. α -TOH metabolites in blood (α -TOH and α -CEHC-SO₃) and urine [sulfate (SO₃) and glucuronide (GLU) of both α -TLHQ (oxidized) and α -CEHC (enzymatic)] were quantified by liquid chromatography coupled with tandem mass spectrometry (LC/MS–MS). Measures of glucose homeostasis (HOMA-B, HOMA-IR, Insulinogenic index and Matsuda index) were obtained from fasting and postprandial blood samples. Multivariable linear regression analyses were performed to assess the associations of α -TOH metabolites and measures of glucose homeostasis.

Results: We included 498 participants (45% men) with mean (SD) age of 55.8 (6.1) years who did not use glucose-lowering medication. While blood α -TOH was not associated with measures of glucose homeostasis, urinary oxidized metabolites (α -TLHQ-SO₃/GLU) were associated with HOMA-IR and Matsuda index. For example, a one-SD higher α -TLHQ-SO₃ was associated with 0.92 (95% CI: 0.87, 0.97) fold lower HOMA-IR and 1.06 (1.01, 1.11) fold higher Matsuda index, respectively. Similar results were obtained for the urinary α -TLHQ to α -CEHC ratio as a measure of oxidized-over-enzymatic conversion of α -TOH.

Conclusion: Higher urinary levels of oxidized α -TOH metabolites as well as higher oxidized-to-enzymatic α -TOH metabolite ratio, but not circulating α -TOH or enzymatic metabolites, were associated with lower insulin resistance. Rather than circulating α -TOH, estimates of the conversion of α -TOH might be informative in relation to health and disease.

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Abbreviations: α -TOH, alpha-tocopherol; α -CEHC, alpha-carboxymethylhydroxychroman; α -TLHQ, alpha-tocopheronolactone hydroquinone; LC-MS/MS, liquid chromatography-tandem mass spectrometry; BMI, body mass index.

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1. Introduction

Impaired glucose homeostasis is one of the first steps in the pathogenesis of type 2 diabetes mellitus (T2D). In addition to classical risk factors such as (central) obesity, lipid peroxidation,

which is an autocatalytic chain reaction induced by free radicals reacting with lipids, has been implicated to play an important role in the impairment of glucose homeostasis [1].

Vitamin E (α -tocopherol, α -TOH) has been unequivocally demonstrated as an effective lipophilic radical scavenger to prevent the chain propagation in lipid peroxidation [2]. In oxidative catabolism, α -TOH is initially oxidized to α -tocopheroxyl radical by one electron oxidation, and further reacts with lipid peroxides, opening the chromanol ring and consecutively forming α -tocopheryl quinone (α -TQ), α -tocopheryl hydroquinone (α -THQ) and α -tocopheronic acid. Subsequently, following by β -oxidation and cyclisation of the phytyl side chain, α -tocopherono lactone (α -TLHQ) will be generated (illustrated in our previous review [3]). Alternatively, in the hepatic enzymatic catabolism, α -TOH starts with ω -hydroxylation, and successively shortens the phytyl chain via multiple β -oxidation, generating α -carboxymethyl-hydroxychroman (α -CEHC). Thereafter, α -TLHQ and α -CEHC are both conjugated with glucuronic acid or sulfate to form water soluble polar metabolites and are excreted mainly via urine. The group of oxidized metabolites α -TLHQ therefore reflect the extent of lipid peroxidation and α -TOH bioactivity.

Despite multiple studies have investigated the associations between vitamin E and glucose homeostasis in both observational studies and clinical trials yet with inconsistent results [4–19], studies scarcely addressed the most important aspect of functional α -TOH, i.e., to what extent α -TOH acts as antioxidants. Interestingly, oxidized α -TOH metabolites have been linked to increased risk of multiple diseases with elevated levels of lipid peroxidation [20–22]. Theoretically, impaired glucose homeostasis related to excessive lipid peroxidation is likely to have an increased demand of antioxidants protection via α -TOH, and consequently lead to higher levels of urinary oxidized metabolites. Of note, the only one study specifically explored the association between oxidized metabolites and diabetics identified increased levels of α -TLHQ in diabetic children compared with age- and sex-matched healthy controls [23]. No data so far are available about the association of α -TOH oxidized metabolites with measures of glucose homeostasis in the general population.

We hypothesized that oxidized urinary vitamin E metabolites, but not circulating α -TOH or enzymatic metabolite, would positively associate with worse glucose homeostasis measures. In the present study we aimed to investigate the associations between α -TOH metabolites and measures of glucose homeostasis in a cross-sectional study of middle-aged healthy individuals embedded in the Netherlands Epidemiology of Obesity study (NEO).

2. Methods

2.1. Study design and study population

This study was embedded in the population-based prospective Netherlands Epidemiology of Obesity (NEO) study, which is designed to investigate the pathways that are responsible for obesity-related disorders. The NEO study started in 2008 and includes 6671 individuals aged 45–65 years, with an oversampling of individuals with a self-reported body mass index (BMI) of 27 kg/m² or higher. Besides, all inhabitants aged between 45 and 65 years from the municipality of Leiderdorp were invited irrespective of their BMI. The study was approved by the medical ethical committee of the Leiden University Medical Center (LUMC), and all participants gave written informed consent. Detailed information on the study design and data collection has been described previously [24].

Participants were invited to come to the NEO study center of the LUMC for one baseline study visit after an overnight fast. Prior to

this study visit, participants collected their urine over 24 h and completed a general questionnaire at home in terms of their demographic, lifestyle, and clinical data in addition to specific questionnaires on diet and physical activity. Medication use within one month prior to the visit was asked to bring with participants and was recorded by research nurses. Fasting blood samples were drawn, and within five minutes later, a 400 mL, 600 kcal mixed meal (energy derived from protein, carbohydrate and fat were 16%, 50% and 34%, respectively) was consumed. Postprandial blood samples were then drawn at 30 and 150 min after the meal.

The present study cross-sectionally analyzed the baseline measurements. We included a random subset of 35% of the Leiderdorp participants from Western European ancestry with imaging and genomics information collected (N = 599). In total, 536 participants were eligible with urine collected for at least 20 h. We excluded participants for the main analyses with: 1) glucose-lowering medication use (n = 12) or no glucose homeostasis measures (n = 1); 2) sample failed to measure urinary metabolites (n = 1); 3) biologically implausible urinary metabolites measures due to sample problem (no TLHQ metabolites, n = 2); outliers (see 2.5. Statistical analysis) on blood (α -TOH) or urinary metabolites (n = 9); 3) missing data (n = 1) or outliers (n = 1) on HOMA-B or HOMA-IR; 4) missing data on potential confounders including physical activity, Dutch health diet index, education level and body fat percentage (n = 11, 1 overlap with vitamin E metabolites outliers). Consequently, a total of 498 individuals were used in the fasting analyses. In addition, we excluded individuals with [1] uncompleted mixed meal challenge [2]; missing data on Matsuda index or Insulinogenic Index, leaving a total of 448 participants in the postprandial analyses. Summary of the participant exclusions is presented in [Supplementary Fig. 1](#).

2.2. Alpha tocopherol metabolites measures

2.2.1. Alpha tocopherol (metabolite) measurements in blood

In fasting blood samples, α -TOH and α -CEHC sulfate conjugates (α -CEHC-SO₃) were detected and quantified by untargeted metabolomics provider Metabolon, Inc. (Durham, NC, USA) on a platform encompassing four liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) methods (LC-MS/MS negative, LC-MS/MS positive early, LC-MS/MS positive late and LC-MS/MS polar). More detailed descriptions have been described previously [25,26].

2.2.2. Alpha tocopherol metabolite measurements in urine

Urinary oxidized α -TOH metabolites (α -TLHQ) and enzymatic metabolites (α -CEHC), presented as their sulfate or glucuronide conjugates (α -TLHQ-SO₃, α -TLHQ-GLU, α -CEHC-SO₃, α -CEHC-GLU), were measured by LC-MS/MS at the University College London, UK.

Prior to the measurement, urine samples were thawed, and 100 μ l fresh urine was then centrifuged in Eppendorf tubes for 10 min at 13,000 rpm at room temperature and spiked with 10 μ l of the internal standards (100 μ mol/L), lithocholic acid sulfate (LA) and androsterone D4-glucuronide (AD4). Subsequently, samples were vortexed and transferred into screw-cap glass vials. 10 μ l was injected into the LC-MS/MS for detection.

The metabolites were separated using a Waters ACQUITY UPLC BEH C8 column (1.7 μ m particles, 50 mm \times 2.1 mm; Waters Corp, Manchester, UK) plus a guard column containing an identical stationary phase. The mobile phase was a gradient elution of solvent A (99.98% water; 0.01% (v/v) formic acid) and solvent B (99.98% acetonitrile/MeCN; 0.01% (v/v) formic acid), which were LC-MS grade or equivalent (Sigma–Aldrich Co. Ltd). The flow rate was set to 0.8 mL/min and the LC gradient was established by coordinating the solvents as follows: 95% solvent A plus 5% solvent B for

0–0.40 min; 80% solvent A plus 20% solvent B for 2 min; 0.1% solvent A plus 99.9% solvent B for 3.01–4 min; 95% solvent A plus 5% solvent B for 4.01–5 min. In order to minimize system contamination and carry over, the MS diverter valve was set up to discard the UPLC eluent before and after the sample elution, at 0–0.40 min and 4.01–5 min, respectively, as well as an additional run of blank sample (H₂O: MeCN) between each run of urine samples. Two peaks were observed for α -TLHQ and α -CEHC glucuronide conjugates, corresponding to major and minor isoforms. The different elution time (min) for internal standards (LA 4.33, AD4 2.7) and each metabolite (2.39, 2.12 and 2.29 for α -TLHQ sulfate, glucuronide minor and major, 2.64, 2.50, 2.56 for α -CEHC sulfate, glucuronide minor and major) guaranteed that all metabolites could be separated in a single chromatographic run.

After separation, the metabolites were then analyzed by MS using a Waters ACQUITY UPLC coupled to a triple-quadrupole Xevo TQ-S fitted with an electrospray ionization in negative ion mode. The gas temperatures persisted 600 °C for desolvation. In addition, nitrogen was used as the nebulizing gas with 7.0 Bar. α -CEHC and α -TLHQ are isobaric because of the same molecular mass (C₁₅H₂₂O₄). The cone voltages were set at 56 V and 54 V, and the collision voltages at 28 eV and 30 eV for sulfate conjugates and glucuronide conjugates, respectively. Running time for each sample is 5 min with a 20 μ L injection volume together with a partial loop with needle overfill mode. Using multiple reaction monitoring (MRM) mode, specific parent and daughter ions were determined in scan mode and following collision activated dissociation (CAD) with argon. These ions were then used to quantify each α -TOH metabolite from transitions previously established by Sharma et al. [23] (glucuronide conjugates 453.3 > 113.0 *m/z* and sulfate conjugates, 357.1 > 79.9 *m/z*) that corresponded to their theoretical molecular masses.

Urinary creatinine concentrations (mmol/L) were measured to correct dilution differences for each metabolite, by triple-quadrupole Micro Quattro mass spectrometry (MicroMass, Waters, UK) using deuterated creatinine as the internal standard. Therefore, the concentrations of α -TOH metabolites are expressed as nmol per mmol of creatinine. A quality control (QC) assessment was performed throughout the quantification both in creatinine and α -TOH metabolite assays to deal with the variations in sample quality and UPLC-MS/MS performance over time. Four QC samples were systematically interleaved every 50 urine samples to limit the amount of sample loss. The whole measurement protocol was developed and further modified by the detection group in London [23,27].

The final concentrations of glucuronide conjugates for α -TLHQ and α -CEHC were the sum of their corresponding major and minor isoforms. In addition to the measured single metabolite, total, glucuronide and sulfate conjugates ratios were further determined to reflect the α -TOH antioxidative capacity as well as lipid peroxidation levels taking α -TOH status into consideration, namely as the α -TLHQ-to- α -CEHC ratio, α -TLHQ-GLU-to- α -CEHC-GLU ratio, and α -TLHQ-SO₃-to- α -CEHC-SO₃ ratio.

2.3. Glucose homeostasis measures

For blood samples, plasma glucose concentrations were obtained by enzymatic and colorimetric methods (Roche Modular Analytics P800, Roche Diagnostics, Mannheim, Germany; CV < 5%), while serum insulin concentration were detected via an immunometric method (Siemens Immulite 2500, Siemens Healthcare Diagnostics, Breda, The Netherlands; CV < 5%). All these measurements were performed in the central clinical chemistry laboratory of the LUMC. Homeostatic model of insulin resistance (HOMA-IR), a marker of hepatic insulin resistance, homeostatic

model of and β -cell function (HOMA-B), a measure of β -cell to glucose-stimulated insulin secretion were then calculated by using the formula (fasting glucose \times fasting insulin)/22.5 and (20 \times fasting insulin)/(fasting glucose–3.5) respectively [28,29]. Matsuda index, which represents both hepatic and peripheral tissue sensitivity to insulin, was generated as $10000/\sqrt{(\text{fasting glucose} \times \text{fasting insulin})(\text{glucose}_{\text{mean}(0-150\text{min})} \times \text{insulin}_{\text{mean}(0-150\text{min})})}$ [30]. Moreover, Insulinogenic Index that also reflects β -cell function but specifically the first-phase insulin response to glucose challenge was calculated with the formula (insulin_{30min}–fasting insulin)/(glucose_{30min}–fasting glucose) [31].

2.4. Covariates

Education level was grouped into high (including higher vocational school, university, and postgraduate education) and low based on the Dutch Education system. Smoking habits were reported in three categories: current smoker, former smoker and never smoke. Familial diabetes history (yes/no) was collected through general questionnaire and defined as having a father, mother, or brother or sister with a diagnosis of diabetes mellitus. The frequency and duration of leisure physical activity over the past 4 weeks were reported by participants on the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH), which is expressed as metabolic equivalent (MET-hours per week). A semi-quantitative food frequency questionnaire was used to assess food and beverage intake, and total energy intake. Dutch Healthy Diet Index (DHD-index) were then calculated based on dietary intake [32]. Percentage body fat was measured by Bio Impedance Balance (TBF-310, Tanita International Division, UK). Total cholesterol levels (mmol/L) were obtained in fasting blood samples in our central clinical chemistry laboratory using standard assays. Lipid lowering medication defined as the use of statins fibrates and other lipid-lowering medication was recorded by medicine inventory by the research nurses. Information on the use of vitamin E supplements was collected through questionnaires as the combination of vitamin E supplements only or the use of multivitamin supplements.

2.5. Statistical analysis

2.5.1. Main analysis

Descriptive characteristics at the NEO baseline were presented as mean (standard deviation, SD) for normally distributed variables, median (interquartile range) for skewed variables, and frequency (proportions) for categorical variables. As the distribution of both blood and urinary vitamin E metabolites and the measures of glucose homeostasis (HOMA-B, HOMA-IR, Insulinogenic Index and Matsuda index) were skewed, these were natural log-transformed. Outliers (defined as located more than 4 SD from the mean) were removed after natural log transformation, comprising approximately 2% of the total observations.

In order to explore the α -TOH metabolism process, pairwise Pearson correlations were performed between circulatory and urinary metabolites. Multivariable linear regression analyses were performed to examine the association between α -TOH metabolites (determinant) and measures of glucose homeostasis (outcome). To facilitate interpretation and comparison between measures of glucose homeostasis, determinants were then z-transformed (mean = 0, SD = 1), so that the regression coefficient with its corresponding 95% confidence interval (CI) can be interpreted as the fold difference in the outcome with respect to a one-SD change in the determinant. For each outcome and determinant, four models were fitted. The basic regression model was adjusted for age and sex (Model 1). Model 2 was additionally adjusted for

potential confounders based on biological knowledge and previous studies including educational level (high or low), familial history of diabetes (yes or no), physical activity (MET hours per week), smoking habits (never smoke, current smoker or former smoker), Dutch Healthy Diet Index and total energy intake (kJ/day). Total cholesterol level (mmol/L) and lipid-lowering medication use (yes/no) were also included in fasting models for blood metabolites only, since the transportation and uptake of α -TOH largely depend on lipoproteins [33]. Moreover, obesity has been shown to directly have an influence on glucose homeostasis, especially insulin resistance [34], and meanwhile, obesity also increases lipid peroxidation levels [35] and might thus affect the conversion of α -TOH in the body. Therefore, we included total body fat (Model 3) as an indicator of fat content to explore the underlying mechanisms.

2.5.2. Sensitivity analyses

2.5.2.1. Urine sample collection missingness and other exclusions. To check whether the current study population is representative of the total study population, the study characteristics among individuals of urine sample collection missingness and other exclusion reasons were compared with the included participants separately. Chi-square test was used for categorical variables, while t test was used for normally distributed numeric variables and Mann-Whitely U test was used for non-normally distributed numeric variables.

2.5.2.2. Vitamin E supplement use. Vitamin E supplement use might have an influence on measures of glucose homeostasis as well as vitamin E conversion in the body. Vitamin E supplement use in the current study was defined as either the use of vitamin E supplements only or the use of multiple vitamin supplement. In order to fully rule out the supplement effect, we further performed sensitivity analysis in participants who did not use vitamin E supplements.

2.5.2.3. Censored normal regression. In the main analysis, we excluded all participants with glucose-lowering medication use. However, exclusion might underestimate the effect size of the determinants, reduce power, and could introduce (collider-stratification) bias in the analyses. A reliable approach to correct this dilution is to use censored normal regression model analysis which has been established previously [36]. Therefore, we additionally performed censored normal regression model analysis for the final adjusted model (Model 3) as in the main analysis.

All the analyses were undertaken using R (v3.6.1) statistical software (The R Foundation for Statistical Computing, Vienna, Austria). Pairwise correlation plots were performed using the software package “GGally”, and the censored normal linear regression models were performed using the software package “survival”.

3. Results

3.1. Characteristics of the study population

A total of 498 participants (45% men) were analyzed, of whom 448 were available for postprandial analyses. The baseline characteristics of the study population are presented in Table 1.

3.2. Main analysis

3.2.1. Pairwise correlation between blood and urinary metabolites

Correlations between circulatory and urinary α -TOH metabolites are shown in Fig. 1. In all participants, blood α -TOH was weakly associated with any urinary metabolite (r: 0.18–0.23), while blood

α -CEHC-SO₃ was moderately correlated with urinary metabolites (r: 0.31–0.49) with the highest correlation with urinary α -CEHC-SO₃ (r = 0.49). Urinary metabolites were highly correlated with each other with r ranging from 0.44 to 0.73.

3.2.2. Circulatory α -TOH and glucose homeostasis measures

In blood, we did not find evidence that α -TOH was associated with measures of glucose homeostasis after fully adjusting for potential confounders, as shown in Table 2.

3.2.3. Urinary metabolites and glucose homeostasis measures

The urinary oxidized metabolites α -TLHQ-SO₃ and α -TLHQ-GLU were associated with HOMA-IR and Matsuda index as visualized in Fig. 2 (summary statistics in Supplementary Table 2). For fasting measures (Figure 2A and B), in the age- and sex-adjusted basic

Table 1
Characteristics the study population.^a

	N = 498
Demography	
Age (years)	55.8 (6.1)
Sex (male)	225 (45.2%)
BMI (kg/m ²)	25.4 (23.1, 27.8)
Total body fat (%)	30.9 (8.3)
Education level (high)	253 (50.8%)
Family history of diabetes (yes)	136 (27.3%)
Lifestyle factors	
Dutch healthy diet index	59.8 (8.4)
Energy intake (KJ/day)	9106 (7304, 11053)
Physical activity a (MET-h/week)	29.4 (16.5, 48.8)
Smoking	
Current	53 (10.6%)
Former	235 (47.2%)
Never	210 (42.2%)
Vitamin E supplement use (yes) ^b	128 (25.7%)
Lipid-lowering medication use (yes)	28 (5.6%)
Vitamin E metabolites measurements	
Blood (log-transformed, no unit)^c	
α -tocopherol	19.7 (0.2)
α -CEHC-SO ₃ ^d	12.1 (0.6)
Urinary	
α -TLHQ-SO ₃ (nmol/mmol creatinine)	2.6 (1.6, 4.1)
α -TLHQ-GLU (nmol/mmol creatinine)	1822.5 (1339.3, 2744.8)
α -CEHC-SO ₃ (nmol/mmol creatinine)	165.4 (97.4, 298.3)
α -CEHC-GLU (nmol/mmol creatinine)	91.0 (62.3139.4)
α -TLHQ/ α -CEHC	7.1 (5.1, 9.9)
α -TLHQ-SO ₃ / α -CEHC-SO ₃	0.016 (0.011, 0.024)
α -TLHQ-GLU/ α -CEHC-GLU	20.8 (15.4, 28.5)
Urinary (log-transformed)	
α -TLHQ-SO ₃	1.0 (0.7)
α -TLHQ-GLU	7.6 (0.6)
α -CEHC-SO ₃	5.1 (0.8)
α -CEHC-GLU	4.5 (0.6)
α -TLHQ/ α -CEHC	1.9 (0.5)
α -TLHQ-SO ₃ / α -CEHC-SO ₃	-4.1 (0.7)
α -TLHQ-GLU/ α -CEHC-GLU	3.0 (0.4)
Glucose homeostasis	
Fasting levels	
HOMA-IR	1.9 (1.3, 2.9)
HOMA-B (%)	89.7 (63.8, 129.6)
Postprandial response^e	
Matsuda Index	5.6 (3.8, 7.9)
Insulinogenic index	7.2 (5.7, 9.4)

^a Data are presented as median (interquartile range) for numeric variables, and number (proportions) for categorical variables.

^b Vitamin E supplement use was defined as either vitamin E supplement use or multiple vitamin supplement use.

^c The concentrations were measured by Metabolon platform, and no units were presented.

^d Only complete cases in metabolon platform (n = 263).

^e Participants with completed meal challenge and no missing data on postprandial response were used (N = 448).

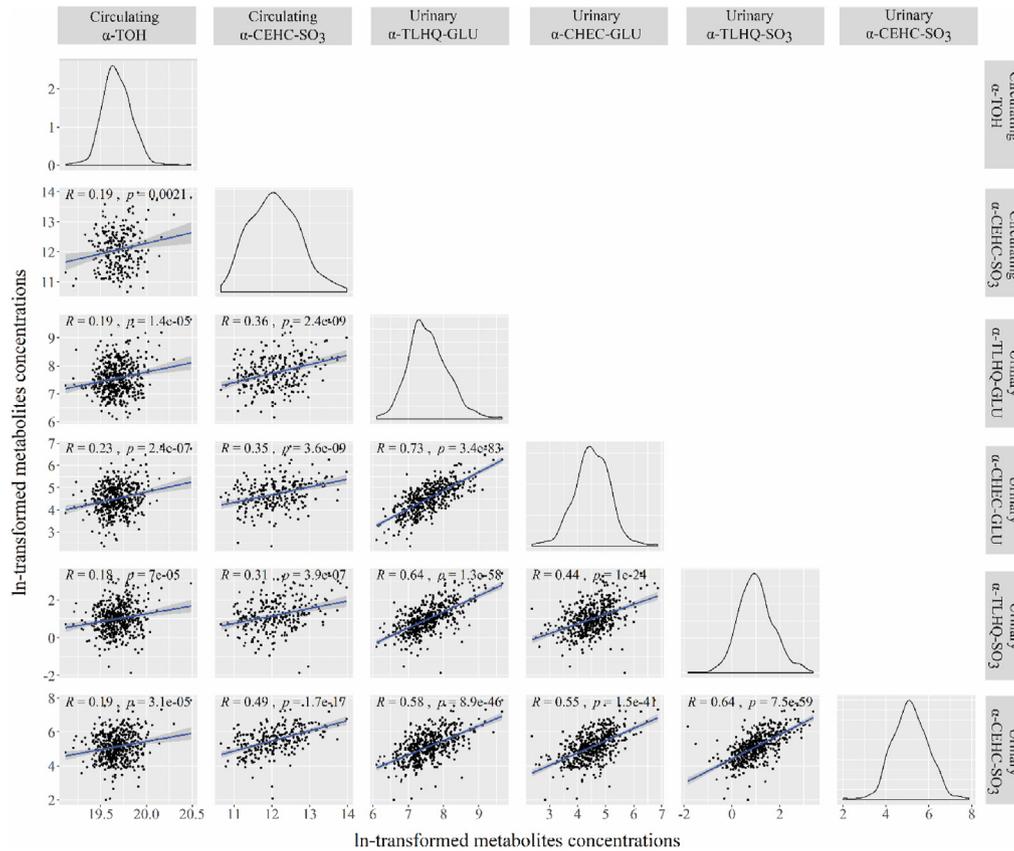


Fig. 1. Pairwise correlation. Pairwise correlation of α -tocopherol metabolites in blood and urine (N = 498). Pearson correlations were calculated after natural log-transformation of metabolites. For blood α -CEHC-SO₃, only the complete cases in the metabolon platform were used (n = 263).

model (Model 1), a one-SD higher α -TLHQ-SO₃ was associated with 0.93 [95% confidence interval (CI): 0.89, 0.98] fold lower HOMA-B and 0.89 (95% CI: 0.83, 0.94) fold lower HOMA-IR, respectively. However, when we additionally adjusted for other potential confounders including lifestyle factors and adiposity measures (Model 3), the association observed for HOMA-B diminished towards null and α -TLHQ-SO₃ was only associated with HOMA-IR (β : 0.92, 95% CI: 0.87, 0.97). Similarly, α -TLHQ-GLU followed the same pattern, and a one-SD higher α -TLHQ-GLU was associated with 0.93 (95% CI: 0.88, 0.98) fold lower HOMA-IR in the fully adjusted Model 3. This pattern was also observed in postprandial analyses, and α -TLHQ-SO₃ and α -TLHQ-GLU were only associated with Matsuda index (Fig. 2D), with a one-SD higher α -TLHQ-SO₃ corresponding to 1.06 (95% CI: 1.01, 1.11) fold higher Matsuda index, and a one-SD higher α -TLHQ-GLU corresponding to 1.08 (95% CI: 1.03, 1.13) fold higher

Matsuda index after adjustment for all potential confounders (Model 3). However, no association was found between enzymatic metabolites and any measures of glucose homeostasis.

Higher oxidized-to-enzymatic metabolite ratios showed similar association with oxidized metabolites (Fig. 3 and summary statistics in Supplementary Table 3). For example, in the basic model (Model 1), a one-SD higher α -TLHQ-to- α -CEHC ratio was associated with 0.90 (95% CI: 0.85, 0.95) fold lower HOMA-IR (Figure 3B) and 1.11 (1.05, 1.17) fold higher Matsuda index (Fig. 3D). After adjustment for potential confounding factors (Model 3 A), the association attenuated, and a one-SD higher α -TLHQ-to- α -CEHC ratio was associated with 0.94 (95% CI: 0.89, 0.99) fold lower HOMA-IR and 1.07 (95% CI: 1.02, 1.12) fold higher Matsuda index. Similar associations were observed for sulfate conjugate ratio and glucuronide conjugate ratio.

Table 2
Associations between circulatory α -tocopherol and measures of glucose homeostasis in the general population.^a

	Fasting measures		Postprandial responses	
	HOMA-B	HOMA-IR	Insulinogenic index	Matsuda Index
Model 1	0.95 (0.91, 1.00)	0.97 (0.92, 1.03)	1.00 (0.96, 1.03)	0.99 (0.94, 1.04)
Model 2	0.98 (0.92, 1.04)	0.98 (0.91, 1.05)	1.01 (0.96, 1.05)	1.00 (0.94, 1.07)
Model 3	0.97 (0.92, 1.03)	0.97 (0.91, 1.03)	1.01 (0.96, 1.05)	1.01 (0.96, 1.06)

^a In total, 498 participants with available α -tocopherol were used for fasting HOMA-B and HOMA-IR analysis while 448 for postprandial Insulinogenic index and Matsuda Index analysis. Results are derived from multivariable linear regression coefficients with 95% confidence interval (CI) and were expressed as a one-standard deviation change in blood α -tocopherol with corresponding fold difference in log-transformed glucose homeostasis traits. Model 1: age and sex. Model 2: Model 1 + education level (high or low), familial history of diabetes (yes or no), physical activity (MET hours per week), smoking habits (never smoke, current smoker or former smoker), Dutch Healthy Diet index (DHD-index), energy intake (KJ/day), total cholesterol level (mmol/L) and lipid lowering medication (yes or no). Model 3: Model 2 + body fat percentage (%).

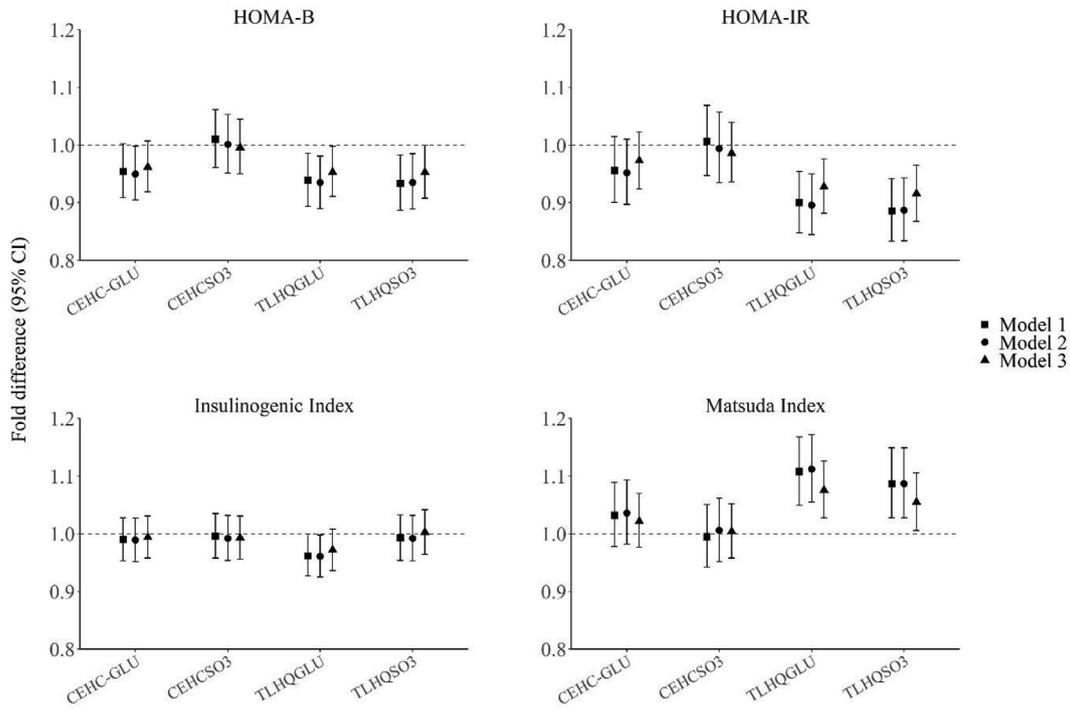


Fig. 2. Association between urinary metabolites and glucose homeostasis measurements. Association between urinary metabolites and (A) HOMA-B, (B) HOMA-IR, (C) Insulinogenic Index, and (D) Matsuda Index in general population. Results were based on general population (N = 498 for HOMA-B and HOMA-IR; N = 448 for Insulinogenic Index and Matsuda Index), and were derived from multivariable linear regression coefficient with corresponding 95% confidence interval (CI) and were expressed as fold difference in log-transformed outcomes with a one-standard deviation change in determinants. Model 1: adjust for age and sex. Model 2: Model 1 + educational level (high or low), familial history of diabetes (yes or no), physical activity (MET hours per week), smoking habits (never smoke, current smoker or former smoker), Dutch Healthy Diet Index and total energy intake (KJ/day). Model 3: Model 2 + total body fat percentage (%).

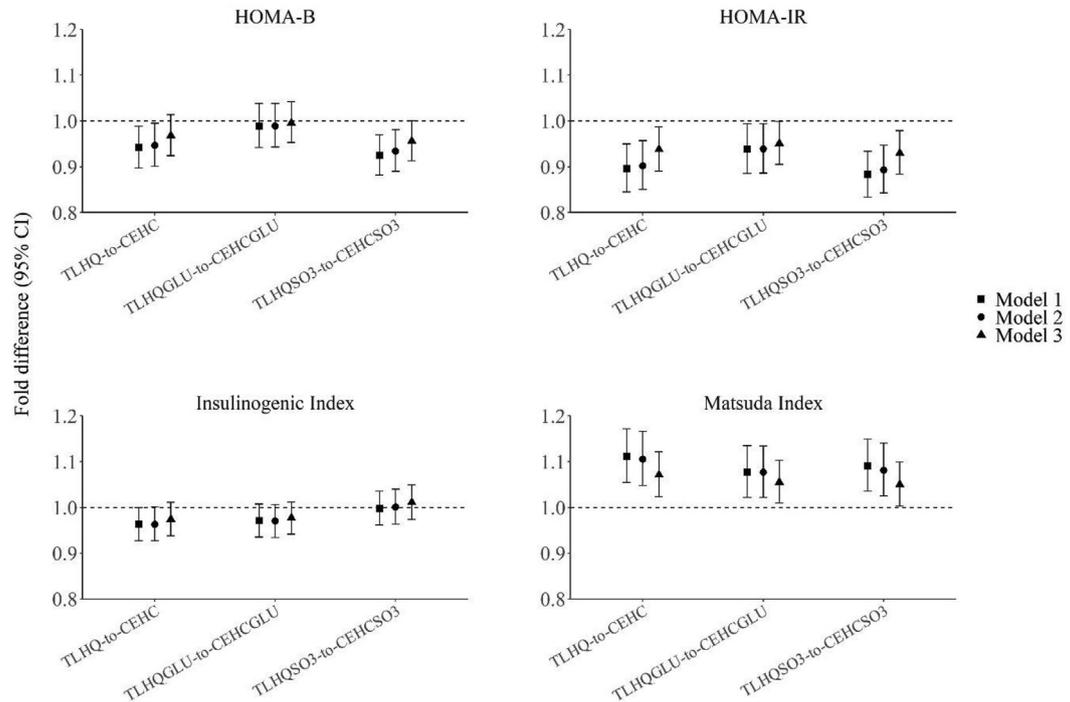


Fig. 3. Association between urinary enzymatic-to-oxidized metabolite ratios and glucose homeostasis measurements. Association between urinary metabolite ratios and (A) HOMA-B, (B) HOMA-IR, (C) Insulinogenic Index, and (D) Matsuda Index in general population. Results were based on general population (N = 498 for HOMA-B and HOMA-IR; N = 448 for Insulinogenic Index and Matsuda Index), and were derived from multivariable linear regression coefficient with corresponding 95% confidence interval and were expressed as fold difference in log-transformed outcomes with a one-standard deviation change in determinants. Model 1: adjust for age and sex. Model 2: Model 1 + educational level (high or low), familial history of diabetes (yes or no), physical activity (MET hours per week), smoking habits (never smoke, current smoker or former smoker), Dutch Healthy Diet Index and total energy intake (KJ/day). Model 3 A: Model 2 + total body fat percentage (%).

3.3. Sensitivity analysis

3.3.1. Urine sample collection missingness and other exclusions

Basic characteristics were compared between included participants (N = 498), individuals with less than 20 h or no urine collection (N = 63) and excluded participants due to other reasons (N = 38), as presented in [Supplementary Table 1](#). The participants excluded due to other reasons had higher percentages of family history of diabetes (27.3%, 30.2% versus 47.4%) and lipid-lowering medication use (5.6%, 11.1% versus 29.0%). Moreover, this group had slightly higher age and more male participants.

3.3.2. Vitamin E supplementation

Participants with vitamin E supplementation (n = 128) were further excluded in this sensitivity analysis, leaving 370 individuals for fasting and 337 for postprandial analyses, respectively. The correlation between α -TOH and other metabolites remained low. Blood α -CEHC-SO₃ was moderately correlated to urinary α -CEHC-SO₃ (r = 0.41) only ([Supplementary Fig. 2](#)). In addition, effect sizes did not change materially in the multivariable regressions after adjusting for confounders compared with the full population analyses ([Supplementary Table 4](#), [Figs. 3 and 4](#)).

3.3.3. Censored normal regression

In censored normal regression models with 12 individuals taking glucose-lowering medication included, 510 participants with complete data were included for fasting analysis, and 460 participants for postprandial analysis. We did not observe substantial differences in Model 3 ([Supplementary Table 5](#)) compared with the main analysis.

4. Discussion

In the present cross-sectional study, we aim to investigate the association between metabolites of vitamin E (α -TOH) and measures of glucose homeostasis in both fasting and postprandial state. When adjusted for potential confounders, we did not find evidence supporting an association between blood α -TOH with measures of glucose homeostasis. However, we observed that higher urinary oxidized metabolites of α -TOH, as well as oxidized-to-enzymatic metabolite α -TOH ratios, were associated with reduced insulin resistance. Additional adjustment for total body fat only minimally explained these observations. Sensitivity analysis including restriction to individuals not taking vitamin E or multivitamin supplements and censored normal regression model provided similar results emphasizing the robustness of the observations.

The lack of evidence supporting an association between plasma α -TOH with measures of glucose homeostasis observed in the present study is in accordance with some [8–12], but not all previous observational studies [6,7]. The discrepancies might be due to several factors such as study design, sample size as well as used confounders. In line with our study, in a more than 20 years follow-up study of middle-aged Swedish men, plasma α -TOH was not associated with future insulin response or T2D incidence [8]. Interestingly, in some clinical trials, α -TOH supplement only, which is mostly corresponding to an increase of blood α -TOH [37–39], was not beneficial for improvement of glucose levels, lipid levels or insulin sensitivity [17–19]. However, this may be explained by factors such as dosage, timing, duration and type of vitamin E.

With respect to oxidized metabolites, contrary to our hypotheses, we found associations between higher oxidized metabolites and better measures of glucose homeostasis. Previously, a study particularly identified that urinary α -TLHQ was higher in children with type 1 diabetes mellitus than in healthy controls [23]. Participants with metabolic syndrome also had decreased

concentration of urinary α -CEHC due to increased oxidative stress levels and inflammation in spite of rather similar plasma α -TOH concentration compared with healthy individuals, thereby indicating a higher antioxidative demands for α -TOH [40]. Moreover, metabolic syndrome patients had approximately 12% greater static oxidation reduction potential, i.e., oxidants, and 59% lower readily available antioxidant reserves compared with healthy adults [41]. An explanation for the inconsistency identified in our study may lie in the bioavailability and elimination of α -TOH. The bioavailability was shown to be reduced and the elimination delayed in metabolic syndrome patients who have increased lipid peroxidation, observed as lower plasma and urinary α -CEHC, independent of the co-ingested dairy fat amount [42]. In disease conditions where oxidants outweigh antioxidants, more antioxidants are required to diminish damage caused by oxidative stress. Together with the decreased hepatic turnover, the conversion of α -TOH may shift to a preference of non-enzymatic oxidation. However, in our relatively healthy population, lipid oxidation level is assumed to be relatively low and antioxidants outweigh oxidants with no delayed enzymatic conversion. In addition, other antioxidative systems still have the potential to neutralize oxidants, therefore, the scavenging function of α -TOH might be compensated. Furthermore, the excretion of these metabolites may also alter in different health conditions, and a higher/lower excretion via bile or feces may occur.

In the pairwise correlation analysis, the moderate correlation of α -CEHC-SO₃ between blood and urine is a validation of the measurement. However, very weak correlation was found between plasma α -TOH and oxidized metabolites indicating that the excretion of oxidized metabolites does not increase with the increase of circulating α -TOH. This raises the argument that despite of an increased level of circulating α -TOH, the body does not fully make use of this α -TOH as antioxidant. Taken together, the circulating level of α -TOH may not reflect antioxidative capacity and may not affect glucose homeostasis.

One of the strengths of the present study is the general population-based setting and the various number of confounding factors considered. Former studies of oxidized α -TOH metabolites and health outcomes have been conducted in relatively small patient cohorts, limiting the generalization of the results. Another strength is the measurement of oxidized metabolites. Previous chromatography-mass spectrometry (GC-MS) based method required long sample preparation of deconjugation, extraction and derivatization, and only detected free unconjugated metabolites [43,44]. These may result in artefactual oxidation products of α -CEHC during sample preparation, α -TOH acid and α -tocopheronolactone, better known as Simon metabolites [45,46]. The LC-MS/MS based method used in our study was developed and validated previously to have solid reliability and reproducibility [23,27], and the intact conjugate with minimal preparation ensures that the metabolites are unlikely due to artifact formation [23].

There are several limitations in this study. Firstly, a considerable number of individuals had no urine collection for at least 20 h, however, study characteristics were comparable with our study population. Secondly, since multiple urinary metabolites and study outcomes were used in the analyses, it could be argued that there might be multiple testing and chance of false-positive results. However, our exposures and outcomes were highly intercorrelated, and conventional corrections for multiple testing (e.g., Bonferroni) are too conservative. Lastly, because of the cross-sectional design, we are not able to rule out residual confounding or reverse causation.

In conclusion, the present study suggests that, in the middle-aged population, circulating α -TOH is not associated with glucose homeostasis measures. However, higher amounts of urinary

oxidized α -TOH metabolites and oxidized-to-enzymatic metabolite ratios are associated with lower insulin resistance. This finding supports the hypothesis that, rather than circulating α -TOH, its conversion might be more informative in relation to health and diseases. These findings also highlight the importance of disentangling the conversion preference of α -TOH in different health states in future studies.

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Author's contribution

The authors' responsibilities were as follows. RN, D.v.H: study conceptualization and funding acquisition; JL: conducted the statistical analysis and drafted the manuscript; S.le.C: data analysis; FM, NA, KM: performed the urinary metabolites detection; FR, R.de.M, DMK and K.v.D: were involved in the study design and administration and data interpretation; LM, FR, R.de.M, DMK, K.v.D: revised the manuscript for final submission; all authors: read and approved the final manuscript.

Conflicts of interest

Dr. Mook-Kanamori is a part time research consultant for Metabolon, Inc. The other authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clnu.2021.01.039>.

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