Association of measures of body fat with serum alpha-tocopherol and its metabolites in middle-aged individuals

Meulmeester, F.L.; Luo, J.; Martens, L.G.; Ashrafi, N.; Mutsert, R. de; Mook-Kanamori, D.O.; ... ; Noordam, R.

Nutrition, Metabolism & Cardiovascular Diseases (2021) **31**, 2407–2415

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Association of measures of body fat with serum alpha-tocopherol and its metabolites in middle-aged individuals

Fleur L. Meulmeester [a,b], Jiao Luo [a,c], Leon G. Martens [a], Nadia Ashrafi [b], Renée de Mutsert [c], Dennis O. Mook-Kanamori [c,d], Hildo J. Lamb [e], Frits R. Rosendaal [c], Ko Willems van Dijk [f,g,h], Kevin Mills [b], Diana van Heemst [a], Raymond Noordam [a,*]

[a] *Department of Internal Medicine, Section of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands*
[b] *NIHR Great Ormond Street Biomedical Research Centre, Great Ormond Street Hospital and UCL Great Ormond Street Institute of Child Health, London, United Kingdom*
[c] *Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands*
[d] *Department of Public Health and Primary Care, Leiden University Medical Center, Leiden, the Netherlands*
[e] *Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands*
[f] *Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands*
[g] *Department of Internal Medicine, Division of Endocrinology, Leiden University Medical Center, Leiden, the Netherlands*
[h] *Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, the Netherlands*

Received 30 December 2020; received in revised form 4 May 2021; accepted 4 May 2021
Handling Editor: A. Siani
Available online 18 May 2021

KEYWORDS
Obesity;
Visceral adipose tissue;
Subcutaneous adipose tissue;
Vitamin E metabolites;
Alpha tocopherol;
Mass spectrometry;
Epidemiology

Abstract *Background and aims:* The accumulation of fat increases the formation of lipid peroxides, which are partly scavenged by alpha-tocopherol (α-TOH). Here, we aimed to investigate the associations between different measures of (abdominal) fat and levels of urinary α-TOH metabolites in middle-aged individuals.
Methods and results: In this cross-sectional analysis in the Netherlands Epidemiology of Obesity study (N = 511, 53% women; mean [SD] age of 55 [6.1] years), serum α-TOH and α-TOH metabolites from 24-h urine were measured as alpha-tocopheronolactone hydroquinone (α-TLHQ, oxidized) and alpha-carboxymethyl-hydroxychroman (α-CEHC, enzymatically converted) using liquid-chromatography-tandem mass spectrometry. Body mass index and total body fat were measured, and abdominal subcutaneous and visceral adipose tissue (aSAT and VAT) were assessed using magnetic resonance imaging. Using multivariable-adjusted linear regression analyses, we analysed the associations of BMI, TBF, aSAT and VAT with levels of urinary α-TOH metabolites, adjusted for confounders. We observed no evidence for associations between body fat measures and serum α-TOH. Higher BMI and TBF were associated with lower urinary levels of TLHQ (0.95 [95%CI: 0.90, 1.00] and 0.94 [0.88, 1.01] times per SD, respectively) and with lower TLHQ relative to CEHC (0.93 [0.90, 0.98] and 0.93 [0.87, 0.98] times per SD, respectively). We observed similar associations for VAT (TLHQ: 0.94 [0.89, 0.99] times per SD), but not for aSAT.
Conclusions: Opposite to our research hypothesis, higher abdominal adiposity was moderately associated with lower levels of oxidized α-TOH metabolites, which might reflect lower vitamin E antioxidative activity in individuals with higher abdominal fat instead.

* Corresponding author. Department of Internal Medicine, Section of Gerontology and Geriatrics, Leiden University Medical Center, PO Box 9600, 2300 RC, Leiden, the Netherlands.
E-mail address: r.noordam@lumc.nl (R. Noordam).

https://doi.org/10.1016/j.numecd.2021.05.001

Introduction

Obesity is characterized by excessive fat accumulation, and increases the risk of morbidity and mortality [1]. The accumulation of fat is an important contributor to the dysregulation of inter- and intracellular metabolic pathways, including lipid peroxidation [2,3]. In this process, oxidants such as free radicals cause peroxidative modification of lipids [4].

α-Tocopherol (α-TOH) is a lipid-soluble compound which is the most abundant and stable long-chain form of tocopherols *in vivo* [5]. α-TOH plays an essential role in the cell antioxidant defence system, where it acts as an effective scavenger of reactive oxygen species (ROS) in the autocatalytic chain reaction of free radical-mediated lipid peroxidation [4]. The metabolism of α-TOH can follow one of two pathways. Firstly, when α-TOH successfully inhibits chain propagation of lipid peroxidation, α-tocopheronolactone hydroquinone (α-TLHQ) is generated. Alternatively, α-TOH can be enzymatically converted in the liver to α-carboxymethyl-hydroxychroman (α-CEHC) [6,7]. Both α-TLHQ and α-CEHC are subsequently conjugated to either glucuronide or sulfate for secretion into urine. Specifically, α-TLHQ is depicted as a marker of antioxidant activity of α-TOH and a reflection of lipid peroxidation levels [8].

With respect to lipid peroxidation and its detrimental effects on health, multiple studies have studied the potential radical-scavenging role of α-TOH [5,7]. For example, studies have associated the level of α-TOH and metabolites to increased risk of disease, including Alzheimer's disease, cardiovascular diseases and fatty liver disease [6,7,9,10]. However, no data are available on the association between measures of body fat and α-TOH or α-TOH metabolites. One of the hypotheses is that the production of ROS is partially driven by pathological processes associated with excessive accumulation of adipose tissue [11–14]. This increased fat storage leads to an increased excretion of free fatty acids (FFAs) into the blood [15]. As a consequence, higher mitochondrial fatty acid oxidation in obese individuals [16–18], and in particular individuals with excessive visceral adiposity [15], as compared to normal weight individuals, is hypothesized to contribute to ROS generation through multiple pathways, including higher β-oxidation [19].

Based on these studies, we hypothesize obesity, and specifically the more metabolically active visceral adipose tissue (VAT), to be associated with higher lipid peroxidation in the body. Increased lipid peroxidation will in turn associate with higher levels of oxidized urinary α-TOH metabolites. In this study, we aim to examine on the association between (abdominal) measures of body fat and urinary α-TOH metabolite levels in middle-aged individuals.

Methods

Study design and study population

This study used data collected in the Netherlands Epidemiology of Obesity (NEO) study, a population-based, prospective cohort study designed to examine pathways that lead to obesity-related diseases [20]. The NEO study was initiated in 2008 and included 6671 Dutch participants aged between 45 and 65 years, with an oversampling of individuals with overweight or obesity. Detailed information about the study population and design has been described elsewhere [20]. To recruit participants, men and women living in the greater area of Leiden, The Netherlands, who met the age criterium and had a self-reported body mass index (BMI) of 27 kg/m^2 or higher were invited to participate. In addition, 45- to 65-year-old inhabitants from one municipality adjoining Leiden (Leiderdorp) were invited to participate in the NEO study, irrespective of their BMI. This resulted in a population of 1671 participants provided a reference group with a BMI distribution similar to that of the Dutch general population. Ethical approval was obtained from the Medical Ethical Committee of the Leiden University Medical Centre (LUMC) and written informed consent was obtained from all participants.

Participants were received at the NEO study site for several baseline measurements, including fasting blood sampling and sampling of the 24-h urine that was collected prior to the study visit. Additionally, participants were asked to fast for a minimum of 10 h and bring all medication they were using up until one month preceding the study visit. Moreover, participants completed a general questionnaire to report demographic and clinical information in addition to lifestyle-related questions.

A screening form was completed by all participants to inquire possible health risks or interference with MRI imaging (particularly metallic devices, claustrophobia, and a body circumference of >1.70 m). Of the participants who were eligible, approximately 40% were randomly selected to undergo an MRI assessment of abdominal subcutaneous fat and visceral fat.

For the present study, we only had resources available for a subpopulation of the total NEO population; to maximize statistical power and to not introduce selection bias caused by the oversampling of individuals with overweight and obesity, we selected participants from the Leiderdorp subpopulation with a BMI distribution assumed to be similar to the general population of whom we also had MRI-derived data available on abdominal fat (n = 599). Individuals with either missing data on exposure, outcome or covariates (n = 30) or had urine samples collected during a period of less than 20 h (n = 58) were excluded from the analyses, resulting in a total of 511 included participants (Supplementary Fig. 1).

Measures of body fat

To calculate the BMI of all participants, body weight was determined using a scale and height was measured with a vertically fixed, calibrated tape measure. Shoes were removed during all measurements and one kilogram was subtracted from the body weight to correct for the weight of clothing. BMI was then calculated by dividing the weight in kilograms by the height in meters squared. Percentage of body fat was assessed with a Tanita foot-to-

foot (FF) bioelectrical impedance analyser (BIA) system (TBF-310, Tanita International Division, UK). The validity of the values derived from BIA systems have been described before [21]. In addition, to test the reliability, repeated measurements were performed in a random sample of the participants (n = 72); the calculated intraclass correlation coefficient was 0.98.

Abdominal subcutaneous adipose tissue (aSAT) and visceral adipose tissue (VAT) were quantified by a turbo spin echo imaging protocol using magnetic resonance imaging (MRI), performed on a 1.5 T MR system (Philips Medical Systems, Best, the Netherlands). At the level of the 5th lumbar vertebra, three 10 mm thick transverse images were obtained during a breath-hold. Areas of aSAT and VAT were converted from the number of pixels to centimeters squared using in-house developed software (MASS, Medis, Leiden, the Netherlands) and the average of three slices was used in the analyses.

Measures of α-tocopherol in serum

Circulating α-TOH was detected and quantified in fasting serum samples by 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 information about the quantifications has been described previously [22,23]. Derived data represents the peak height relative to an internal standard per sample to allow for comparisons between individuals.

Measures of α-tocopherol conjugated metabolites in 24-h urine

The sulfated and glucuronidated conjugates of α-TLHQ and α-CEHC were synthesized by Pope et al. [24,25]. The internal standards (lithocholic acid sulfate [LA] and androsterone D4-glucuronide [AD4]) were purchased from Sigma–Aldrich Co. Ltd (Poole, Dorset, UK). Individual stock and working solutions of the internal standards were prepared in methanol and stored at −20 °C. All solvents used in this study were LC-MS/MS grade or equivalent and were likewise acquired from Sigma–Aldrich Co. Ltd.

Prior to the analysis, all NEO urine samples were thawed. Neat urine (100 μl) was then spun in Eppendorf tubes for 10 min at 13,000 rpm and spiked with 10 μl of the internal standards (100 μmol/L). Subsequently, the samples were vortexed and transferred into screw-cap glass vials. 10 μl was injected into the LC-MS/MS for analysis. This method includes both ultra-performance liquid chromatography (UPLC) for separation of the metabolites and tandem mass spectrometry for detection. The method was validated and modified from that developed by Sharma et al. [6].

The urinary α-TOH metabolites were separated using 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. 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; 0.01% (v/v) formic acid). 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. To minimise 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. The elution times for internal standards and α-TOH metabolites are presented in Supplementary Table 1. Two peaks were observed for α-TLHQ and α-CEHC conjugated with glucuronide, corresponding to major and minor isoforms. Likewise, these isoforms had been previously observed by Pope et al. [25] and Sharma et al. [6]. Only the major isomer was considered in this study.

After separation using LC, the α-TOH metabolites were analysed by tandem mass spectrometry using a Waters ACQUITY UPLC coupled to a triple-quadrupole Xevo TQ-S fitted with an electrospray ionization source, which ran in negative ion mode (ESI^-). The source and desolvation gas temperatures persisted at 150 °C and 600 °C, respectively. In addition, nitrogen was used as the nebulizing gas with 7.0 Bar. The cone voltages were set at 56 V and 54 V for sulfate conjugates and glucuronide conjugates, respectively, and the collision voltages were set at 28 eV and 30 eV, respectively. The total analysis time between each injection compromised 5 min.

Using multiple reaction monitoring (MRM) mode on the mass spectrometer, specific parent and daughter ions were determined in scan mode and following collision activated dissociation (CAD) with argon, respectively. These ions were then used to quantify each α-TOH metabolite transition, which are presented in Supplementary Table 2. These transitions had previously been established by Sharma et al. [6] and corresponded to the theoretical molecular masses of α-TLHQ and α-CECH for either sulfate and glucuronide conjugates. In this study, the analyses of TLHQ and CEHC comprise both sulfate and glucuronide conjugates, unless indicated otherwise. Accordingly, the ratio of α-TLHQ-to-α-CECH was determined to define levels urinary α-TOH metabolites. The ratio was calculated and then transformed into a logarithmic scale to approximate a normal distribution.

Method validation

To check the linearity, the response of the metabolite was compared to a calibration curve created with H_2O:MeCN solution with increasing concentrations of internal standards (AD4 and LA). Furthermore, the concentration of all four α-TOH metabolites in each patient urine sample was established by comparison of the ratio of the metabolite response to the response of the internal standard. The ratio of the areas for each metabolite over the corresponding internal standard was calculated separately using

TargetLynx software. The data acquired were then evaluated by linear regression for which GraphPad Prism version 7 was used to calculate the most suitable fit for the linear relationship. The correlation coefficients (r^2) were >0.97 for all metabolites.

To manage the variations in sample quality and UPLC-MS/MS performance over time, a quality control (QC) assessment was performed in the urinary creatinine and α-TOH metabolite assay. The QC samples (n = 4) were systematically interleaved after each 50 urine samples to limit the amount of sample loss that may arise due to an intermediate decrease in UPLC-MS/MS performance.

To correct for dilution differences between the samples from the participants, the urinary concentrations of creatinine were measured in the urine samples by triple-quadrupole Micro Quattro mass spectrometry (Micro-Mass, Waters, UK) using deuterated creatinine as the internal standard as being standard laboratory procedure. This method was developed in-house by the biological mass spectrometry unit. As a consequence, the concentrations of the α-TOH metabolite levels are therefore expressed per nmol of creatinine prior to the statistical analyses.

Covariates

Total energy intake (in kJ) and alcohol consumption (in g/day) were self-reported using a semi-quantitative 125-item food frequency questionnaire (FFQ) [26]. Tobacco smoking was documented in three categories, notably: (i) current smoker, (ii) former smoker, or (iii) never smoker. In addition, physical activity during leisure time (in MET-hours per week) was estimated using the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH) [27,28]. Correspondingly, participants reported on the frequency, duration and intensity of their physical activity during leisure times. Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric methods (Roche Modular Analytics P800, Roche Diagnostics, Mannheim, Germany; CV < 5%) and high-density lipoprotein (HDL)-cholesterol with the homogenous HDLc method (third generation) (Roche Modular Analytics P800, Roche Diagnostics; CV < 5%). Low-density lipoprotein (LDL) cholesterol concentration was estimated using Friedewald's formula. Lipid-lowering medication defined as the use of statins fibrates and other lipid-lowering medication was recorded by medicine inventory by the research nurses.

Participants were considered as diabetic based on self-reported diabetes, usage of medication or newly diagnosed diabetes with fasting plasma glucose (FPG) ≥7.0 mmol/L. In addition, menopausal status was assessed based on information on oophorectomy or hysterectomy and/or self-reported menopausal status. Accordingly, female participants were divided into three categories comprising pre-, peri-, and postmenopausal. Women who underwent a hysterectomy were categorized by age (premenopausal when <46 years, perimenopausal when 46–55 years, and postmenopausal when ≥55 years). The postmenopausal category also included women of the age 58 or older who did not report their menopausal status.

Statistical analysis

Characteristics of the study population were studied at baseline and expressed as mean (standard deviation [SD]), median (interquartile range [IQR]; for non-normally distributed data only), or proportion (%) in the whole study population and for men and women separately. All statistical analyses were performed using R (v3.6.1) statistical software (The R Foundation for Statistical Computing, Vienna, Austria).

We examined the associations between measures of overall adiposity (BMI and TBF) and serum α-TOH and urinary α-TOH metabolites using multivariable-adjusted linear regression analyses adjusted for age and sex (Model 1). In Model 2, we additionally adjusted for diabetes mellitus (yes/no), physical activity (MET-hours per week), total energy intake (kJ/day), smoking behaviour (never/former/current smoker) and alcohol intake (g/day), which we considered as potential confounding factors.

To investigate whether abdominal fat was specifically associated with serum α-TOH and urinary α-TOH metabolites, we assessed the association between VAT and aSAT and serum α-TOH and urinary α-TOH metabolites in the total study population. We adjusted the analyses for VAT and aSAT for the aforementioned considered confounding factors (Model 2). Additionally, VAT was adjusted for TBF and aSAT for VAT (Model 3).

The levels of serum α-TOH and urinary α-TOH metabolites were not normally distributed and therefore ln-transformed. To improve interpretability of the study results, we backtransformed all the beta coefficients from the linear regression analyses towards a ratio with 95% confidence interval. Furthermore, to be able to compare the different measures of (abdominal) body fat, we standardized these measures to a standard normal distribution (mean = 0; SD = 1). As a consequence, the results were presented as the fold difference in outcome variable, with corresponding 95% CI, per 1 SD of adiposity measure. As such, a ratio 1.1 per 1 SD can be interpreted as a 1.1 times higher outcome per 1 SD.

Additionally, we repeated all regression analyses stratified by sex given the large differences in body composition between men and women, and to test for possible effect modification by sex.

Results

Characteristics of study population

Characteristics of the study population are presented at baseline for the total study population as well as stratified by sex in Table 1. After excluding participants with missing data, our study population comprised 511 participants with a mean (SD) age of 55.9 (6.1) years, of whom 53% were women and median (IQR) BMI was 25.4 (23.1, 27.9) kg/m^2. Women had a higher median [interquartile range]

abdominal aSAT and more TBF than men (196 cm^2 [158, 238] aSAT in men, 251 cm^2 [191, 311] aSAT in women; 24% [21,28] TBF in men, 36% [32, 40] TBF in women), whereas men exhibited more VAT than women (101 cm^2 [72, 139] VAT in men, 57 cm^2 [37, 94] VAT in women). Furthermore, men had higher alcohol consumption than women. All other studied characteristics were similar between men and women.

Associations between body fat measures and serum α-tocopherol

BMI, TBF and aSAT were not associated with levels of α-TOH in serum (ratio: 1.00 [95% CI: 0.99, 1.01] per 1 SD BMI for model 1; ratio: 1.00 [95% CI: 0.98, 1.02] per 1 SD TBF for model 1; ratio: 1.00 [95% CI: 0.98, 1.01] per 1 SD aSAT for model 1), which remained similar after full adjustment of the considered confounding factors (Fig. 1). However, we observed associations of VAT with serum α-TOH, although the effect sizes were small. Furthermore, upon adjustment for covariates and TBF, higher VAT was not associated with higher α-TOH (1.01 times [95% CI: 0.99, 1.04] per 1 SD VAT).

Associations between body fat measures and urinary α-tocopherol metabolites

BMI was associated with lower levels of oxidized α-TOH metabolites (TLHQ) (0.95 times [95% CI: 0.90, 1.00] per 1 SD BMI for model 1) and TBF showed a similar direction of effect (0.94 times [95% CI: 0.88, 1.00] per 1 SD TBF for model 1). Both associations remained similar after full adjustment of the considered confounding factors (Fig. 2 and Supplementary Table 4). However, when stratified for sex, we observed associations between BMI and TBF,

Table 1 Baseline characteristics of the NEO participants stratified by sex.

	All (N = 511)	Male (N = 239)	Female (N = 272)
Demography			
Age (years)	56 (6)	56 (6)	56 (6)
Lifestyle factors			
Energy intake (kJ/day)	9129 (7304, 11,078)	10,406 (8875, 12,145)	7959 (6681, 9533)
Physical activity[a] (MET-h/week)	30 (17, 50)	30 (17, 50)	30 (17, 47)
Smoking			
Current	56 (10.9%)	28 (11.7%)	28 (10.3%)
Former	242 (47.4%)	121 (50.6%)	121 (44.5%)
Never	213 (41.7%)	90 (37.7%)	123 (45.2%)
Diabetes[b] (yes)	77 (15.1%)	47 (19.7%)	30 (11.0%)
Menopause (women only)			
Premenopause	–	–	45 (16.5%)
Perimenopause	–	–	67 (24.5%)
Postmenopause	–	–	160 (58.8%)
Vitamin E metabolites measurement			
Serum (natural log-transformed)			
α-tocopherol	19.7 (0.2)	19.7 (0.2)	19.7 (0.2)
Urinary			
α-TLHQ-GLU (nmol/mmol creatinine)	1825 (1334, 2723)	1519 (1194, 2256)	2081 (1465, 3035)
α-TLHQ- SO_3 (nmol/mmol creatinine)	2.6 (1.6, 4.1)	2 (1.4, 3.1)	3.3 (2.2, 5.4)
α-CEHC- GLU (nmol/mmol creatinine)	92 (62, 139)	83 (56, 119)	107 (69, 153)
α-CEHC- SO_3 (nmol/mmol creatinine)	166 (97, 298)	137 (76, 231)	200 (121, 346)
α-THLQ total (nmol/mmol creatinine)	1828 (1336, 2730)	1520 (1195, 2257)	2084 (1467, 3047)
α-CEHC total (nmol/mmol creatinine)	266 (180, 429)	223 (138, 356)	306 (214, 506)
α-TLHQ/α-CEHC	7.1 (5.1, 9.9)	7.6 (5.4, 10.5)	7.1 (4.8, 9.6)
Ln (α-TLHQ/α-CEHC)	2.0 (1.6, 2.3)	2.0 (1.7, 2.4)	2.0 (1.6, 2.3)
Body fat measurements			
BMI (kg/m^2)	25 (23, 28)	26 (24, 28)	25 (22, 27)
Total body fat percentage (%)	31 (24, 37)	24 (21, 28)	36 (32, 40)
aSAT (cm^2)	221 (172, 285)	196 (158, 238)	251 (191, 311)
VAT (cm^2)	82 (49, 116)	101 (72, 139)	57 (37, 94)
Serum lipid levels			
Total cholesterol (mmol/L)	5.7 (1.1)	5.6 (1.0)	5.9 (1.1)
HDL cholesterol (mmol/L)	1.6 (0.5)	1.3 (0.3)	1.8 (0.4)
LDL cholesterol (mmol/L)	3.6 (1.0)	3.6 (0.9)	3.6 (1.0)
Triglycerides (mmol/L)	1.2 (0.8)	1.4 (0.9)	1.0 (0.6)

Data are presented as mean (standard deviation) or median (interquartile range) for numerical variables, and number (proportions) for categorical variables.

BMI, body mass index; CEHC, carboxymethyl-hydroxychroman; GLU, glucuronide; MET, metabolic equivalent; aSAT, abdominal subcutaneous adipose tissue; SO_3, sulfate; TLHQ, tocopheronolactone hydroquinone; VAT, visceral adipose tissue.

[a] Excluding traveling between home-work and daily activities at home/work.

[b] Based on self-reported DM or medication usage.

and TLHQ in women, but not in men. We observed no association between BMI and TBF with the enzymatically converted α-TOH metabolites (CEHC) (ratio: 1.02 [95% CI: 0.96, 1.08] per 1 SD BMI for model 1; ratio: 1.02 [95% CI: 0.94, 1.10] per 1 SD TBF for model 1). Separation of the urinary metabolites into either glucuronide or sulphate conjugates did not yield different associations between BMI and the metabolites, or between TBF and the metabolites (Supplementary Table 5). The observed effect estimates with TLHQ were somewhat larger in women than in men (Supplementary Table 6).

Higher VAT was marginally associated with lower levels of urinary TLHQ when adjusted for age and sex (0.94 times [95% CI: 0.89, 0.99] for model 1). This association persisted when adjusted for all considered confounding factors, but attenuated after adjustment for body fat percentage (Fig. 2 and Supplementary Table 4). We observed no associations between VAT and CEHC (ratio: 1.00 [95% CI: 0.92, 1.09] per 1 SD VAT for model 3). Similar results were observed after separation of the urinary metabolites into either glucuronide or sulphate conjugates (Supplementary Table 5). In addition, the results were similar in women. Men, however, showed no associations between VAT and α-TOH metabolites in any model (Supplementary Table 6).

With respect to aSAT, no associations were observed with levels of urinary α-TOH metabolites when adjusted for all considered confounding factors (TLHQ, ratio: 1.02 [95% CI: 0.96, 1.09] per 1 SD aSAT for model 3; CEHC, ratio: 1.03 [95% CI: 0.96, 1.12] per 1 SD aSAT; model 3) (Fig. 1, Supplementary Tables 3 and 4). Additionally, we observed no associations between aSAT and TLHQ or CEHC in men and women separately (Supplementary Table 5).

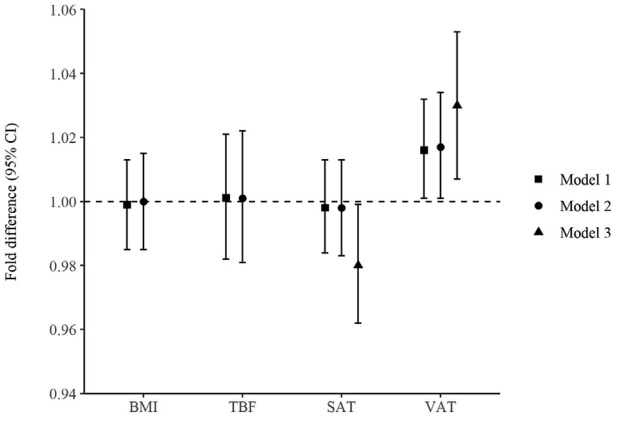

Figure 1 Associations between body fat measures and serum α-tocopherol in the overall study population. Results are derived from linear regression coefficients with 95% confidence interval (CI) and were expressed as a one-SD change in body fat measures with corresponding fold difference in log-transformed serum α-tocopherol concentration. Model 1: age and sex. Model 2: Model 1 + diabetes (yes or no), physical activity (MET hours/week), smoking habits (never smoke, current smoker or former smoker), energy intake (kJ/day), alcohol consumption (g/day), total cholesterol (mmol/L), and lipid lowering medication. Model 3: Model 2 + body fat percentage (%) for VAT, or + VAT for aSAT. BMI, body mass index; aSAT, abdominal subcutaneous adipose tissue; VAT, visceral adipose tissue.

Associations between body fat measures and the ratio of urinary oxidized-to-enzymatically converted α-tocopherol metabolites

In the analyses of the ratio of α-TOH metabolites, higher BMI, TBF and VAT were associated with a lower TLHQ-to-CEHC ratio (0.93 times [95% CI: 0.90, 0.98] per 1 SD BMI for model 1; 0.93 times [95% CI: 0.87, 0.98] per 1 SD TBF for model 1; 0.94 times [95% CI: 0.88, 1.00] per 1 SD VAT for model 3), which remained similar after full adjustment of the considered confounding factors (Fig. 1 and Supplementary Table 3). The effect estimates for VAT were somewhat larger in women than in men (Supplementary Table 5). However, aSAT was not associated with ratio TLHQ-to-CEHC after additional adjustment for VAT (ratio: 0.99 [95% CI: 0.93, 1.05] per 1 SD aSAT; model 3).

Discussion

In this cross-sectional study, we aimed to address the associations between different measures of overall and abdominal body fat, and serum α-tocopherol and urinary α-tocopherol metabolite levels in 511 middle-aged individuals. The levels of urinary α-TOH metabolites were also expressed as the amount of TLHQ relative to the amount of CEHC as a measure of balance in the vitamin E metabolism. When adjusted for considered confounding factors, we observed weak associations between higher BMI and lower urinary TLHQ metabolites in the total study population. Additionally, higher BMI was moderately associated with lower oxidized-to-enzymatic α-TOH metabolite ratios. Results were similarly observed for TBF and VAT, but no clear association was observed between aSAT and the urinary α-TOH metabolites. These results were generally observed in women and not in men possibly due to the differences in body fat distribution between men and women. In general, these results were opposite to our original research hypothesis that adiposity would result in increased levels of lipid peroxides to be scavenged by vitamin E to become vitamin E metabolites.

The few studies that have investigated urinary α-TOH metabolites suggest that the levels of excreted oxidized metabolites of α-TOH are higher in cases of Alzheimer's disease and type 1 diabetes mellitus [6,9]. The discrepancies may be explained by several factors, including study design and population, sample size and used confounders. Importantly, they investigated a much younger study population than we used in the present study and had a different disease outcome, which possibly explains the discrepancy. In addition, Casati et al. analysed plasma tocopherols instead of urinary alpha-tocopherol metabolites [9]. In our study population, we observed that serum α-TOH was not associated with body fat measures. Therefore, the human body may not completely employ the circulating α-TOH as antioxidant, which could explain the discrepancy. Lastly, comparable studies included solely patients in their study population, resulting in a relatively small sample size [6,9].

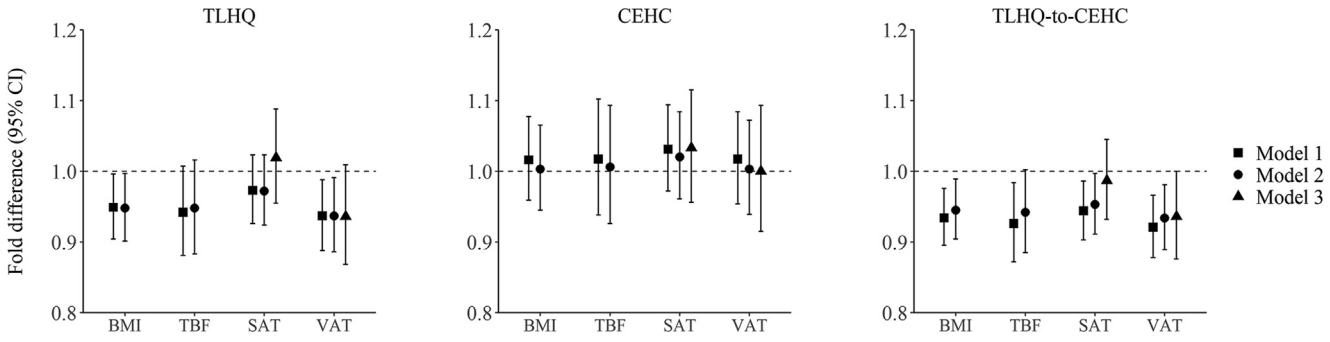

Figure 2 Associations between body fat measures and urinary α-tocopherol metabolites in the general population. Results are derived from linear regression coefficients with 95% confidence interval (CI) and were expressed as a one-SD change in body fat measures with corresponding fold difference in log-transformed urinary α-tocopherol metabolites or ratio. Model 1: age and sex. Model 2: Model 1 + diabetes (yes or no), physical activity (MET hours per week), smoking habits (never smoke, current smoker or former smoker), energy intake (kJ/day) and alcohol consumption (g/day). Model 3: Model 2 + body fat percentage (%) for VAT, or + VAT for aSAT. BMI, body mass index; CEHC, carboxymethyl-hydroxychroman; aSAT, abdominal subcutaneous adipose tissue; TBF, total body fat; TLHQ, tocopheronolactone hydroquinone; VAT, visceral adipose tissue.

In patients with the metabolic syndrome, it has previously been reported that the bioavailability of α-TOH was decreased and the elimination of α-TOH was delayed when compared to healthy adults [29]. Here, patients showed lower plasma and urinary levels of the enzymatically converted α-TOH metabolite, CEHC, independent of the amount of co-ingested dairy fat. Considering the metabolic syndrome and its associated higher rate of lipid peroxidation associated with obesity [16–18], it is very plausible that the over-produced oxidants outweigh the antioxidants such as α-TOH, resulting in a higher demand of antioxidants to balance the oxidative damage. To meet this demand, the conversion of α-TOH may shift to non-enzymatic oxidation, leading to a higher oxidized-to-enzymatic turnover of α-TOH. Due to the lower lipid peroxidation in our relatively healthy population, however, this metabolic shift may not occur, explaining the variation in enzymatic conversion of α-TOH. Furthermore, given that α-TOH is not the only antioxidant available in response to ROS production, the possibility remains that other defence mechanisms compensate for the compromised scavenging function of α-TOH in obesity [30,31].

With respect to abdominal fat, our results suggest that higher VAT is associated with a lower amount of excreted α-TOH metabolites beyond TBF. The observed association with only VAT and not aSAT suggests that excessive VAT increases the demand of α-TOH as an antioxidant. These observations agree with previous studies, which showed that FFAs are primarily released by VAT due to its high metabolic rate compared to aSAT [15]. Since FFAs are believed to induce the production of ROS [11–14,19,32], VAT may have a role in shifting the balance between oxidants and antioxidants towards to oxidants. In addition, excessive VAT releases approximately two to three times more interleukin 6 (IL-6), a pro-inflammatory cytokine, than aSAT [33], which is a leading source of oxidants [34,35]. Collectively, these findings advocate the hypothesis that VAT increases the demand of the antioxidant system function. However, the lower levels of excreted TLHQ (nonenzymatic conversion of a-TOH) requires additional studies to explain these findings with the other findings.

One of the strengths of this study is the direct assessment of abdominal fat with MRI in a relatively large study population. In addition, the extensive phenotyping of the NEO study allowed for adjustment of a wide range of possible confounding factors. One of the other strengths of this study is the application of liquid chromatography coupled to tandem mass spectrometry to detect α-TOH metabolites in 24-h urine, which is considered as an accurate and reliable method [36]. However, the present study also has a number of limitations. First, inherent to the observational and cross-sectional design, it is impossible to deduce either the direction or causality of the associations between different measures of body fat and urinary α-TOH metabolites from the presented results. Second, physical activity and consumption of food and alcohol were self-reported using questionnaires. Furthermore, we were not able to derive data on intake of vitamin E or other antioxidants through habitual nutritional intake from the FFQ data. Therefore, we were not able to correct for these factors, but adjusted for other factors related to health consciousness (most notably total energy intake and alcohol consumption) to capture as much confounding as possible in these circumstances. However, we previously published that vitamin E was uncorrelated to the vitamin E metabolites [37] meaning that vitamin E status does not yield differences in vitamin E conversion. Although being validated extensively [27,28,38], self-reported information on lifestyle, used for confounder adjustment, is susceptible to recall bias and/or measurement error. And last, the antioxidant system is extremely complex; it is well possible that the possible lower antioxidant activity in individuals with obesity are compensated by other antioxidative systems.

In conclusion, opposite to our research hypothesis, the results from the present study suggest that higher levels of overall and visceral fat were weakly associated with lower levels of oxidized α-TOH metabolites. This may reflect lower vitamin E antioxidative activity in individuals with high abdominal fat. These results were generally observed in

women and not in men possibly due to the differences in body fat distribution between men and women. Future longitudinal studies are required to study this observation in more detail.

Funding

This work was supported by the VELUX Stiftung [grant number 1156] to DvH and RN. JL was supported by the China Scholarship Counsel [No. 201808500155] and FM was supported by the Erasmus+Traineeship grant. Furthermore, this work was supported by the NIHR GOSH BRC to KM. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health, also the kind donations from the Szeban Peto Foundation. The NEO study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Centre, and by the Leiden University, Research Profile Area 'Vascular and Regenerative Medicine'.

Declaration of competing interest

Dennis O Mook-Kanamori is a part-time research consultant at Metabolon, Inc. All other authors declare no conflict of interest.

Acknowledgements

We greatly appreciate all participants of the Netherlands Epidemiology of Obesity study, and all participating general practitioners for inviting eligible individuals. We furthermore thank P.R. van Beelen and all research nurses for collecting the data, P.J. Noordijk and her team for sample handling and storage and I. de Jonge, MSc for all data management of the NEO study. We would like to thank the Peto Foundation for their kind donations and the support of the NIHR BRC at UCL Great Ormond Street Hospital.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2021.05.001.

References

[1] Manna P, Jain SK. Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: causes and therapeutic strategies. Metab Syndr Relat Disord 2015;13:423–44.
[2] Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752–61.
[3] Murdolo G, Piroddi M, Luchetti F, Tortoioli C, Canonico B, Zerbinati C, et al. Oxidative stress and lipid peroxidation by-products at the crossroad between adipose organ dysregulation and obesity-linked insulin resistance. Biochimie 2013;95:585–94.
[4] Niki E. Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence. Free Radic Biol Med 2014;66:3–12.
[5] Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. Free Radic Biol Med 2007;43:4–15.
[6] Sharma G, Muller DP, O'Riordan SM, Bryan S, Dattani MT, Hindmarsh PC, et al. Urinary conjugated α-tocopheronolactone–a biomarker of oxidative stress in children with type 1 diabetes. Free Radic Biol Med 2013;55:54–62.
[7] Torquato P, Bartolini D, Giusepponi D, Piroddi M, Sebastiani B, Saluti G, et al. Increased plasma levels of the lipoperoxyl radical-derived vitamin E metabolite α-tocopheryl quinone are an early indicator of lipotoxicity in fatty liver subjects. Free Radic Biol Med 2019;131:115–25.
[8] Luo J, Mills K, le Cessie S, Noordam R, van Heemst D. Ageing, age-related diseases and oxidative stress: what to do next? Ageing Res Rev 2020;57:100982.
[9] Casati M, Boccardi V, Ferri E, Bertagnoli L, Bastiani P, Ciccone S, et al. Vitamin E and Alzheimer's disease: the mediating role of cellular aging. Aging Clin Exp Res 2020;32:459–64.
[10] Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F. The role of vitamin e in human health and some diseases. Sultan Qaboos Univ Med J 2014;14:e157–65.
[11] Dale CE, Fatemifar G, Palmer TM, White J, Prieto-Merino D, Zabaneh D, et al. Causal associations of adiposity and body fat distribution with coronary heart disease, stroke subtypes, and type 2 diabetes mellitus: a mendelian randomization analysis. Circulation 2017;135:2373–88.
[12] Dymkowska D, Szczepanowska J, Wieckowski MR, Wojtczak L. Short-term and long-term effects of fatty acids in rat hepatoma AS-30D cells: the way to apoptosis. Biochim Biophys Acta 2006;1763:152–63.
[13] Fauconnier J, Andersson DC, Zhang SJ, Lanner JT, Wibom R, Katz A, et al. Effects of palmitate on Ca(2+) handling in adult control and ob/ob cardiomyocytes: impact of mitochondrial reactive oxygen species. Diabetes 2007;56:1136–42.
[14] Rachek LI, Musiyenko SI, LeDoux SP, Wilson GL. Palmitate induced mitochondrial deoxyribonucleic acid damage and apoptosis in l6 rat skeletal muscle cells. Endocrinology 2007;148:293–9.
[15] Spalding KL, Bernard S, Näslund E, Salehpour M, Possnert G, Appelsved L, et al. Impact of fat mass and distribution on lipid turnover in human adipose tissue. Nat Commun 2017;8:15253.
[16] Fukushima A, Lopaschuk GD. Cardiac fatty acid oxidation in heart failure associated with obesity and diabetes. Biochim Biophys Acta 2016;1861:1525–34.
[17] Lopaschuk GD. Fatty acid oxidation and its relation with insulin resistance and associated disorders. Ann Nutr Metab 2016;68(Suppl 3):15–20.
[18] Yesilbursa D, Serdar Z, Serdar A, Sarac M, Coskun S, Jale C. Lipid peroxides in obese patients and effects of weight loss with orlistat on lipid peroxides levels. Int J Obes 2005;29:142–5.
[19] Ly LD, Xu S, Choi SK, Ha CM, Thoudam T, Cha SK, et al. Oxidative stress and calcium dysregulation by palmitate in type 2 diabetes. Exp Mol Med 2017;49:e291.
[20] de Mutsert R, den Heijer M, Rabelink TJ, Smit JW, Romijn JA, Jukema JW, et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. Eur J Epidemiol 2013;28:513–23.
[21] Ling CH, de Craen AJ, Slagboom PE, Gunn DA, Stokkel MP, Westendorp RG, et al. Accuracy of direct segmental multi-frequency bioimpedance analysis in the assessment of total body and segmental body composition in middle-aged adult population. Clin Nutr 2011;30:610–5.
[22] Cirulli ET, Guo L, Leon Swisher C, Shah N, Huang L, Napier LA, et al. Profound perturbation of the metabolome in obesity is associated with health risk. Cell Metabol 2019;29:488–500.e2.
[23] Long T, Hicks M, Yu HC, Biggs WH, Kirkness EF, Menni C, et al. Whole-genome sequencing identifies common-to-rare variants associated with human blood metabolites. Nat Genet 2017;49:568–78.
[24] Pope SA. The analysis and identification of urinary metabolites of vitamin E in man using mass spectrometry and chemical synthesis. University College London; 2001.
[25] Pope SA, Burtin GE, Clayton PT, Madge DJ, Muller DP. Synthesis and analysis of conjugates of the major vitamin E metabolite, alpha-CEHC. Free Radic Biol Med 2002;33:807–17.
[26] Siebelink E, Geelen A, de Vries JH. Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults. Br J Nutr 2011;106:274–81.

[27] de Hollander EL, Zwart L, de Vries SI, Wendel-Vos W. The SQUASH was a more valid tool than the OBiN for categorizing adults according to the Dutch physical activity and the combined guideline. J Clin Epidemiol 2012;65:73–81.
[28] Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. J Clin Epidemiol 2003;56:1163–9.
[29] Mah E, Sapper TN, Chitchumroonchokchai C, Failla ML, Schill KE, Clinton SK, et al. α-Tocopherol bioavailability is lower in adults with metabolic syndrome regardless of dairy fat co-ingestion: a randomized, double-blind, crossover trial. Am J Clin Nutr 2015;102:1070–80.
[30] Adachi T, Toishi T, Wu H, Kamiya T, Hara H. Expression of extracellular superoxide dismutase during adipose differentiation in 3T3-L1 cells. Redox Rep 2009;14:34–40.
[31] Rindler PM, Plafker SM, Szweda LI, Kinter M. High dietary fat selectively increases catalase expression within cardiac mitochondria. J Biol Chem 2013;288:1979–90.
[32] Schönfeld P, Wojtczak L. Fatty acids as modulators of the cellular production of reactive oxygen species. Free Radic Biol Med 2008;45:231–41.
[33] Curti ML, Jacob P, Borges MC, Rogero MM, Ferreira SR. Studies of gene variants related to inflammation, oxidative stress, dyslipidemia, and obesity: implications for a nutrigenetic approach. J Obes 2011;2011:497401.
[34] Appari M, Channon KM, McNeill E. Metabolic regulation of adipose tissue macrophage function in obesity and diabetes. Antioxidants Redox Signal 2018;29:297–312.
[35] Kim SY, Jeong JM, Kim SJ, Seo W, Kim MH, Choi WM, et al. Pro-inflammatory hepatic macrophages generate ROS through NADPH oxidase 2 via endocytosis of monomeric TLR4-MD2 complex. Nat Commun 2017;8:2247.
[36] Roberts 2nd LJ, Oates JA, Linton MF, Fazio S, Meador BP, Gross MD, et al. The relationship between dose of vitamin E and suppression of oxidative stress in humans. Free Radic Biol Med 2007;43:1388–93.
[37] Luo J, Meulmeester FL, Martens LG, Ashrafi N, de Mutsert R, Mook-Kanamori DO, et al. Urinary oxidized, but not enzymatic vitamin E metabolites are inversely associated with measures of glucose homeostasis in middle-aged healthy individuals. Clin Nutr 2021 Feb 3. S0261-5614(21)00066-2.
[38] Verkleij-Hagoort AC, de Vries JH, Stegers MP, Lindemans J, Ursem NT, Steegers-Theunissen RP. Validation of the assessment of folate and vitamin B12 intake in women of reproductive age: the method of triads. Eur J Clin Nutr 2007;61:610–5.