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Factors Associated with Tocopherol Status in Obese Women: Effects of Diet Composition and Weight Loss

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Abstract

Objective: The objective of this study was to assess factors associated with plasma α-, β-, γ-, and δ-tocopherol in obese women and to examine change in tocopherol levels after a 1-year weight loss intervention across three dietary approaches. Factors examined were dietary factors (alcohol consumption, diet composition, and supplement use) and non-dietary factors (body mass index, physical activity, plasma cholesterol levels, waist circumference, and age).

Methods: Overweight/obese, nondiabetic women were randomly assigned to one of three diets: lower carbohydrate (45% energy), higher fat (35% energy), lower fat (20% energy), higher carbohydrate (65% energy), or walnut-rich (18% energy), higher fat (35% energy), lower carbohydrate (45% energy). Data and blood samples were obtained at baseline, 6- and 12-month clinic visits (n=245, 213, and 194 respectively).

Results: At baseline, age was directly related to plasma α-tocopherol and inversely related to γ- and δ-tocopherol ($P<0.05$ for each); body mass index was inversely associated with plasma α-tocopherol and positively associated with β-, γ- and δ-tocopherol ($P<0.05$ for each). Physical activity was directly associated with α-tocopherol at baseline ($P<0.05$) and inversely associated with β-tocopherol at 12 months ($P=0.03$). Dietary supplement use was positively associated with α-tocopherol at baseline ($P<0.05$) and 12 months ($P=0.007$), and negatively associated with 12-month γ-tocopherol ($P=0.02$). Plasma cholesterol was positively associated with 12-month α- ($P<0.001$), β- ($P=0.003$), and γ-tocopherol ($P=0.007$). The walnut-rich diet group had higher plasma γ-tocopherol concentration than other diet groups at 12 months ($P=0.002$).

Conclusions: Plasma tocopherol levels generally declined in association with weight loss in obese women, although age, adiposity, physical activity, plasma cholesterol, and dietary supplement use influenced these levels. Responses were similar to lower carbohydrate and lower fat diets, and walnut prescription minimized the reduction in plasma γ-tocopherol.

Keywords: Tocopherols; Weight loss; Diet composition; Walnuts

Abbreviations:

BMI: Body Mass Index; CV: Coefficient of Variation; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance; MET: Metabolic Equivalent; α-TTP: A-Tocopherol Transfer Protein

Introduction

The essentiality of vitamin E was identified in 1922 [1]. Vitamin E is the term used to collectively describe a group of lipid-soluble molecules (tocopherols and tocotrienols) that possess the chain-breaking, antioxidant activity of α-tocopherol. The phenolic hydroxyl group of vitamin E reacts with free radicals, protecting cell membrane fatty acids and plasma lipoproteins from lipid peroxidation [2,3]. Oxidative stress is defined as the imbalance in the body of the production of free radicals and the ability to counter these free radicals through antioxidants such as vitamin E [4]. Cardiovascular disease, cancer, cataracts, central neurodegenerative diseases, age-related macular degeneration, diabetes mellitus, and aging have all been associated with augmented levels of oxidative stress [5]. Vitamin E has been shown to slow the progression of aging and reduce the risks of these chronic diseases [5]. Thus, adequate levels of tocopherol are a goal for disease prevention. Both endogenous and exogenous factors, such as alcohol consumption [6], physical activity [7,8] and obesity [9], influence the production of reactive oxygen species and therefore may also affect the status of plasma tocopherols.

α-Tocopherol is the only molecule included in the estimation of vitamin E requirements [5]. However, most people consume more γ-tocopherol than any other congener due to its abundance in oils, seeds, and tree nuts [10,11]. In addition to reducing oxidative stress, plasma tocopherols are also influenced by factors such as plasma cholesterol (reflecting cholesterol-rich lipoproteins, which transport tocopherols in the circulation) and dietary supplement use.

Although previous investigators have identified numerous factors associated with α- and γ-tocopherol status, factors influencing β- and δ-tocopherol have been rarely examined. Based on the rat fetal resorption assay, the biological activity across these forms has been

defined as α-tocopherol, mg × 1.0; β-tocopherol, mg × 0.5; γ-tocopherol, mg × 0.1, and δ-tocopherol, mg × 0.03 [5]. α-Tocopherol generally predominates in plasma and other human tissues, typically with 4-10 times higher concentration than γ-tocopherol, with even smaller concentrations of β- and δ-tocopherol. All of these molecules are potent antioxidants, and it has been argued that all may contribute to reduced risk for chronic disease due to both antioxidant and nonantioxidant activities [10].

The primary objective of this study was to examine the factors associated with plasma α-, β-, γ-, and δ-tocopherol levels, including dietary factors (alcohol consumption, walnut prescription, supplement use) and non-dietary factors (body mass index [BMI], physical activity, plasma cholesterol levels, waist circumference, age), at baseline in overweight and obese women who were enrolled in a 1-year behavioral weight loss intervention. The secondary aim of this study was to examine the change in tocopherol levels at 6 and 12 months across three dietary approaches.

Methods

Study population

The study population and the intervention have been previously described [12]. Briefly, study participants (N=245) were nondiabetic overweight and obese women. Data and blood samples for assessing tocopherol status were available from the majority of the participants at baseline, 6- and 12-month clinic visits (n=245, 213, and 194 respectively). All participants supplied written informed consent, and the study protocol was approved and monitored by the UCSD institutional review board. Participants were randomized into one of three diet arms using a sequence stratified by menopausal status (older/younger than 55 years as a proxy) and insulin resistance status, calculated from the homeostasis model assessment-insulin resistance (HOMA-IR) index ([fasting glucose, mmol/L] × [insulin, ml U/L]/22.5) with HOMA-IR >3.0 considered indicative of insulin resistance [13]. The three diets consisted of: lower carbohydrate (45% energy), higher fat (35% energy); lower fat (20% energy), higher carbohydrate (65% energy); or walnut-rich (18% energy), higher fat (35% energy), lower carbohydrate (45% energy). Figure 1 shows the flow chart for participants for whom tocopherol data were analyzed in the present study.

Intervention

Details about the dietary guidance and intervention has been previously published and promoted a reduction in energy intake by 500 to 1000 kcal/day deficit (relative to expenditure) [12]. Participants randomized to the walnut-rich diet were instructed to consume an average of 42 g (1.5 oz.) walnuts per day within their reduced-energy diet. The physical activity goal for all participants was an average of at least 60 minutes/day of purposeful exercise at a moderate level of intensity [13].

Measurements

At baseline 6 and 12 month follow-up clinic visits, weight, height (baseline only), waist circumference, a fasting (≥ 6 hours) blood sample, and questionnaires were collected. The Global Physical Activity Questionnaire was utilized to collect self-reported physical activity data [14].

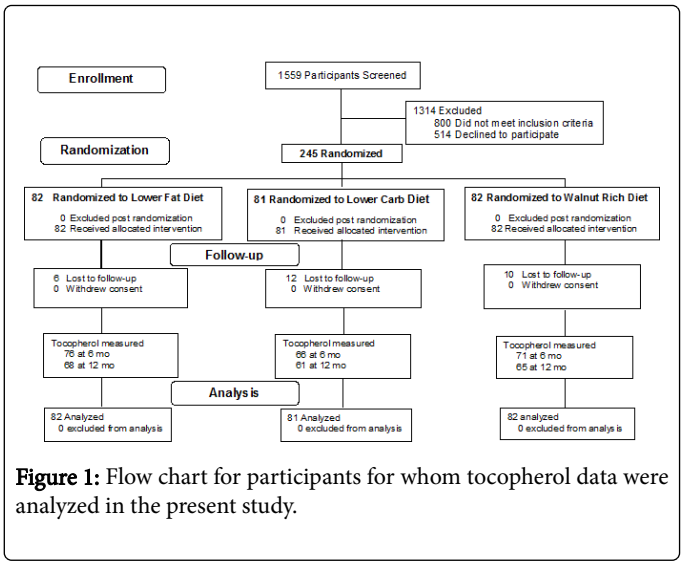

Figure 1: Flow chart for participants for whom tocopherol data were analyzed in the present study.

Laboratory measures

Laboratory measurements were conducted with plasma samples that had been frozen at -80C after blood collection and processing. Total cholesterol was determined enzymatically with the Kodak Ektachem Analyzer system (Johnson and Johnson Clinical Diagnostics, Rochester, NY, USA). Commercially prepared quality control samples, and laboratory participation in the College of American Pathologists Quality Assurance Program, were used to monitor accuracy and precision.

The detection and quantification of plasma tocopherols was accomplished by high performance liquid chromatography, using fluorescent detection at a wavelength of 295 nm excitation and 325 nm emission. Tocopherols were quantified by peak height using a standard curve prepared in bovine serum matrix from pure external compounds. Additionally, pooled in-house quality control samples were analyzed concurrently with batches of study samples, together with other commercially available reference samples, to monitor accuracy and precision. The mean intra-batch assay CV for α- and γ-tocopherols at 1.0 μmol/L was <2.3%, and for β- and δ-tocopherols at 0.125 μmol/L was <5.0%. The mean intra-batch assay CV for α- and γ-tocopherols at 10.0 μmol/L was <2.2%; and for β- and δ-tocopherols at 1.0 μmol/L was <4.0%. Also, the laboratory participates in the National Institute of Standards and Technology quality assurance program.

Statistical analysis

Statistical analysis was performed using the SAS software version 9.4 for Windows (SAS Institute Inc., Cary, North Carolina, USA). Levels of plasma α-, β-, γ-, and δ-tocopherol were compared at baseline across categories of covariates, using t-tests or analysis of variance. The cut points for BMI, waist circumference, alcohol consumption, and total plasma cholesterol were derived from the median level for each value (32.9 units, 105 cm, 2 drinks/week, and 204 mg/dL, respectively).

In accordance with the design of the randomized trial, change in each tocopherol within and between the diet interventions was examined using mixed models with diet, time, diet x time, and percent weight change as predictors. The change from baseline within each diet

group was controlled for weight, except in the model where weight was the outcome.

12 month tocopherol levels were examined in multivariate regression models controlled for the baseline level of the tocopherol, weight change, and additional independent predictors found to be significantly associated with baseline (cross-sectional) level in our bivariate analyses. Predictors that were deemed dependent upon each other included BMI, waist circumference, and weight loss. Physical activity was expressed in Metabolic Equivalent (MET)-hours/day. MET is defined as the ratio of a person's active metabolic rate to the metabolic rate at rest. The energy expenditure of sitting quietly (1 kcal/kg/hour) is equivalent to one MET. We hypothesized that the walnut-rich diet group would display a different tocopherol profile than subjects in the other diet arms, due to differential intake of tocopherols, and therefore, included walnut-rich diet assignment versus the other arms as one of the predictors. Beta coefficients are reported for each factor in the multivariate models to quantify associations. P values of 0.05 or less were considered significant.

Results

Baseline plasma tocopherol levels and covariates

In cross-sectional analysis at study entry (Table 1), α-tocopherol was positively associated with age (P<0.001), and γ- and δ-tocopherol were negatively associated with age (P<0.01 for each). However, β-tocopherol was not significantly associated with age. Tocopherols were also associated with BMI: α-tocopherol was inversely associated (P<0.05), whereas β-, γ-, and δ-tocopherol levels were directly associated with BMI (P<0.01). Waist circumference was not significantly associated with α-tocopherol, but was directly associated with β-, γ-, and δ-tocopherol (P<0.001 for each).

	N	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol
Age (years)					
<40	39	36.8 (1.1)*	0.45 (0.02)	7.5 (0.5)*	0.28 (0.03)*
40-49	60	39.3 (1.6)*	0.41 (0.02)	6.0 (0.5)*	0.17 (0.02)*
50-59	97	46.0 (1.9)*	0.43 (0.02)	6.0 (0.4)*	0.19 (0.02)*
60-72	49	50.4 (3.0)*	0.39 (0.02)	5.3 (0.5)*	0.16 (0.02)*
Body mass index (kg/m^2)[1]					
27-31.9	117	47.7 (2.3)*	0.40 (0.02)*	5.3 (0.4)*	0.17 (0.02)*
32-40	128	42.0 (1.2)*	0.44 (0.02)*	6.5 (0.3)*	0.21 (0.01)*
Waist (cm)[1]					
88.9-105	128	45.5 (1.6)	0.38 (0.01)*	5.3 (0.3)*	0.16 (0.01)*
106-127	117	41.8 (1.4)	0.46 (0.02)*	7.0 (0.3)*	0.23 (0.02)*
Current smoker					
No	236	43.8 (1.1)	0.42 (0.01)	6.2 (0.2)	0.20 (0.01)
Yes	7	40.1 (3.6)	0.32 (0.05)	4.8 (1.2)	0.16 (0.05)
Alcohol consumption					
None	83	44.6 (2.2)	0.41 (0.02)	5.9 (0.3)	0.18 (0.02)
1-2 drinks/week	86	43.1 (1.6)	0.42 (0.02)	6.2 (0.4)	0.21 (0.04)
3+ drinks/week	67	43.7 (1.9)	0.43 (0.02)	6.2 (0.4)	0.19 (0.02)
Total plasma cholesterol[1]					
124-204 mg/dL	124	40.7 (1.4)*	0.39 (0.01)*	5.8 (0.3)*	0.20 (0.01)
205-316 mg/dL	121	46.9 (1.6)*	0.45 (0.01)*	6.4 (0.3)*	0.19 (0.02)
Supplement use (Multivitamin and vitamin E)					
No	167	38.7 (0.7)*	0.44 (0.01)*	6.5 (0.3)*	0.21 (0.01)*
Yes	77	54.8 (2.7)*	0.38 (0.02)*	5.1 (0.4)*	0.16 (0.02)*
Physical activity category					
Low	114	38.8 (0.9)*	0.43 (0.02)	6.6 (0.3)	0.21 (0.02)
Moderate	68	46.0 (2.0)*	0.41 (0.02)	5.6 (0.4)	0.17 (0.02)
High	63	50.3 (3.0)*	0.42 (0.02)	5.7 (0.4)	0.19 (0.02)

Table 1: Tocopherol levels [mean (SEM) μmol/L] and covariates in obese women before randomization into a 3-arm weight loss intervention trial. *P<0.05. [1]Cutpoints for BMI, waist circumference, and total plasma cholesterol are derived from the median level for each value.

Smokers had nominally lower levels of each tocopherol than non-smokers, but the differences were not significant owing to the small number of smokers (n=7) in the cohort. Self-reported alcohol intake was not significantly associated with levels of any of the four tocopherols.

Concentrations of α-, β-, and γ-tocopherol were positively associated with plasma cholesterol levels (P<0.05 for each). α-Tocopherol was also strongly associated with physical activity level (P<0.001). Subjects who reported taking multivitamin and/or vitamin E supplements had significantly higher levels of α-tocopherol (54.8 vs 38.7 μmol/L), and lower levels of β-, γ-, and δ-tocopherol (P<0.03 for each) than non-users of supplements.

Changes in tocopherol levels and weight by diet arm

Along with weight, plasma α-and β-tocopherol decreased by 12 months in each diet arm (Table 2, P<0.05 for each). γ-Tocopherol decreased in the lower fat and lower carbohydrate diet arms (P<0.05) but was not significantly different from baseline in the walnut-rich diet arm. δ-Tocopherol decreased only in the lower fat diet arm, but not in the lower carbohydrate or walnut-rich diet arms (P<0.05).

	Lower Fat		Lower Carb		Walnut-Rich		P between groups, mixed model
	N	Mean (SEM)	N	Mean (SEM)	N	Mean (SEM)	
Baseline levels							
α-Tocopherol (mol/L)	82	45.4 (1.9)	81	43.3 (1.8)	82	42.5 (1.9)	0.27
β-Tocopherol (mol/L)	82	0.45 (0.02)	81	0.43 (0.02)	82	0.39 (0.01)	0.01
γ-Tocopherol (mol/L)	82	6.2 (0.4)	81	6.4 (0.4)	82	5.7 (0.3)	0.18
δ-Tocopherol (mol/L)	82	0.20 (0.02)	81	0.21 (0.02)	82	0.17 (0.01)	0.08
Weight (kg)	82	89.7 (1.2)	81	90.0 (1.4)	82	90.0 (1.3)	0.84
Changes from baseline							
α-Tocopherol (μmol/L)							
6 Months	76	-4.5 (2.1)*	66	-2.8(2.4)	71	-2.7 (2.3)	0.72
12 Months	68	-6.8 (2.3)*	61	-6.0 (1.9)*	65	-6.8 (2.1)*	0.09
β-Tocopherol (μmol/L)							
6 Months	76	-0.06 (0.02)	66	-0.04 (0.02)	71	-0.05 (0.02)	0.07
12 Months	68	-0.10 (0.02)*	61	-0.09 (0.02)*	65	-0.06 (0.02)*	0.38
γ-Tocopherol (μmol/L)							
6 Months	76	-1.7 (0.3)*	66	-1.0 (0.4)	71	-0.5 (0.4)	0.09
12 Months	68	-1.9 (0.4)*	61	-1.7 (0.4)*	65	-0.5 (0.4)	0.10
δ-Tocopherol (μmol/L)							
6 Months	76	-0.06 (0.02)*	66	-0.08 (0.02)*	71	-0.04 (0.02)	0.33
12 Months	68	-0.09 (0.02)*	61	-0.09 (0.02)	65	-0.04 (0.02)	0.12
Weight (kg)							
6 Months	76	-7.5 (0.6)*	69	-5.4 (0.6)*	72	-6.8 (0.7)*	0.04
12 Months	76	-8.2 (1.0)*	69	-5.6 (0.8)*	69	-7.5 (0.9)*	0.13

Table 2: Baseline tocopherol levels and weight, and changes from baseline in obese women randomized into a 3-arm weight loss intervention trial. *P<0.05 change from baseline within diet group, controlled for weight (except in model where weight was the outcome).

Multivariate analysis for tocopherol levels at 12 months

In each of the multivariate models (Table 3), the baseline tocopherol level was positively associated with the 12-month level (P=0.03 for α-tocopherol and P<0.05 for β-, γ-, and δ-tocopherol). Weight change was directly associated with γ- and δ-tocopherol (P<0.05), but not α- or β-tocopherol. Plasma cholesterol was directly associated with α-, β-, and γ- tocopherol (P<0.01 for each). The direct relationship between plasma α-tocopherol and supplement use in the bivariate analysis was also observed in the multivariate analysis (P=0.007), and similarly, the inverse relationship between supplement use and levels of γ-tocopherol that was observed in the bivariate analysis was also seen in the multivariate analysis (P=0.02). Compared to women who did not take vitamin E supplements, supplement users had higher 12-month levels of α-tocopherol (+4.081 μmol/L) and lower levels of γ-tocopherol (-0.805 μmol/L) in the multivariate analysis (Table 3). However, supplement use was not significantly associated with β- or δ-tocopherol levels in multivariate analysis.

	α-Tocopherol (R^2=0.34)		β-Tocopherol (R^2=0.35)		γ-Tocopherol (R^2=0.44)		δ-Tocopherol (R^2=0.16)	
	Beta Coefficient	P value	Beta Coefficient	P value	Beta Coefficient	P value	Beta Coefficient	P value
Baseline level	0.102	0.03	0.497	<0.001	0.411	<0.001	0.17	<0.001
Weight change	0.036	0.71	0.002	0.3	0.059	0.009	0.002	0.04
Plasma cholesterol	0.132	<0.001	0.001	0.003	0.014	0.007	0	0.1
Supplement use	4.081	0.007	-0.037	0.1	-0.805	0.02	-0.011	0.42
Walnut-rich diet	-2.385	0.11	0.007	0.75	1.117	0.002	0.024	0.1
Age	0.144	0.07	-0.001	0.41	-0.021	0.26	0	0.77
Physical activity (MET h/d)	-0.18	0.1	-0.003	0.03	-0.04	0.11	-0.001	0.39

Table 3: Multivariate models[1] for tocopherol levels at 12 months among women randomized into a 3-arm weight loss intervention trial. [1]Each model was controlled for all predictors shown on table.

Women assigned to the walnut-rich diet arm had significantly higher levels of γ-tocopherol at 12 months (+1.117 μmol/L) than did subjects in the other diet groups (P=0.002). When controlled for cholesterol, weight, and other covariates, the walnut-rich diet group had a much smaller decrease in γ-tocopherol [mean(SEM) -0.5(0.4) vs-1.8(0.3) μmol/L, P=0.01]. Despite comparable weight loss at 12 months in each of the diet arms, subjects assigned to the walnut-rich diet did not have a significant decrease in γ-tocopherol, whereas subjects prescribed the other diets did.

Age was not significantly associated with tocopherol levels in any of the 12-month multivariate models. Physical activity level was inversely

associated with β-tocopherol as participants experienced a 0.003 μmol/L decrease for every MET-hour/day reported (Table 3).

Discussion

In addition to enhancing the current understanding of factors associated with α- and γ-tocopherol, this study also reports various factors associated with plasma β- and δ-tocopherol. Previous studies have identified numerous determinants of tocopherol levels but mainly reported those for α- and γ-tocopherol. Plasma tocopherol levels generally declined in association with weight loss in obese women, and responses were similar in response to lower carbohydrate and lower fat diets. Notably, walnut prescription minimized the reduction in plasma γ-tocopherol in association with weight reduction.

At baseline, plasma α-tocopherol was directly associated with age, which is consistent with results reported by previous investigators [15-17]. Succari et al. [16] suggested that lifestyle changes associated with aging could result in increased plasma tocopherol in older individuals. Others have argued that this association is caused by age-related increased plasma cholesterol levels [17], which is supported by our finding that tocopherols were no longer associated with age after adjusting for cholesterol level. Some previous studies observed that age did not influence serum [18] or hepatic tocopherol levels [19]. In this study, baseline plasma γ- and δ-tocopherol levels were found to be negatively associated with age. Similar results for γ-tocopherol in both men and women were reported in a previous study [20]. Campbell et al. [21] proposed that reduced food intake explains the reduction of tocopherol levels in association with age, which would support the inverse association of γ-tocopherol observed in our study as γ-tocopherol is the tocopherol found in the largest amount in the diet [10]. An association of δ-tocopherol with age has not been previously reported. The association between age and tocopherols may be due to transport disturbances [16], such as an age-related change in hepatic α-tocopherol transfer protein (α-TTP) expression. The widely accepted free radical theory of aging, conceived by Harman in 1956 [22], may also support the association of antioxidant levels, such as tocopherol, with age. This theory would mostly support an inverse association between antioxidant levels and age, as we observed for γ- and δ-tocopherol in unadjusted analyses.

BMI and waist circumference were generally found to be significantly associated with the plasma tocopherols (except for α-tocopherol and waist circumference). Previous studies have determined these anthropometric factors to be significantly associated with tocopherol levels as well [18, 20, 23]. Wallstrom et al. [24] found a positive association between α-tocopherol and BMI in men only, whereas Galan et al. [15] reported no association in either sex.

Both anthropometric measurements were found to be negatively associated with α-tocopherol and positively associated with β-, γ-, and δ-tocopherol. The inverse association between α-tocopherol and BMI is consistent with the results of previous investigations which included both men and women [18,20,23]. In addition, low α-tocopherol levels were observed in individuals with increased abdominal adiposity [25]. Diminished plasma α-tocopherol levels in overweight and obese individuals may provide further insight as to why risk factors, such as abdominal adiposity [26] and obesity [27], are associated with increased risk for cardiovascular disease.

Because obesity is positively associated with oxidative stress [9,24,27,28], one could hypothesize that levels of antioxidants, such as tocopherols, would be reduced. Although this relationship was observed with α-tocopherol, the opposite was found for β-, γ-, and δ-tocopherol as their levels increased with increasing degree of obesity in the present study. The concentration of γ-tocopherol stored in adipose tissue has been found to exceed that found in plasma [29], which may support the direct association of γ-tocopherol with BMI and waist circumference. In addition, we observed a concomitant decrease in γ- and δ-tocopherol with weight loss at 12 months.

Lower plasma tocopherol levels in smokers, compared to non-smokers, have been previously reported [15,25,30]. Our cohort included few smokers, which limited our ability to detect differences by smoking status. We did not observe any significant association between alcohol consumption and plasma tocopherol levels despite the relatively large number of alcohol consumers in our cohort. Despite the production of reactive oxygen species from ethanol metabolism [6,31], other investigators also report no alcohol-related differences in plasma α- tocopherol levels [15,18].

Although exercise has been shown to increase oxidative stress [32] and lipid peroxidation [33], participants with a high level of physical activity had the highest plasma concentration of α-tocopherol at baseline in the present study. These results are consistent with those from other studies in which high levels of lipid-adjusted plasma α-tocopherol were found in participants with increased levels of physical activity [25]. Some oxidative stress may be beneficial for proper muscle homeostasis [34,35]. Additionally, Pingitore et al. [8] argued that antioxidant supplementation could affect the release of specific hormones and proteins that rely on the presence of certain reactive oxygen species. In multivariate analysis, β-tocopherol was the only tocopherol found to be associated with physical activity at 12 months. To our knowledge, this is the first study to examine physical activity as a possible determinant of plasma β- and δ-tocopherol concentrations.

Plasma cholesterol levels were found to be directly correlated with α-, β-, and γ- tocopherol at baseline and 12 months in this study, and these results are expected as tocopherols are transported in the circulation via cholesterol-rich lipoproteins [36]. Vitamin E deficiencies have been previously observed in those with hypocholesterolemia caused by fat malabsorption syndromes [37,38].

After a 12-month diet intervention, weight loss and changes in plasma tocopherol concentrations were observed in each dietary arm. All four tocopherols decreased significantly from the baseline levels in participants assigned to the lower fat diet arm. This would be expected because the lower fat diet group was advised to avoid foods containing nut and seed oils which are rich in tocopherols [11]. In the lower carbohydrate diet group, α-, β-, and γ-tocopherol concentrations also decreased from baseline after 12 months. There was a significant decrease in δ-tocopherol at 6 months; however, the decrease at 12 months was not significant. Johnstone et al. [39] also found a reduction in α- and γ-tocopherol levels in participants assigned to a lower carbohydrate diet (20% energy).

Walnuts contain an abundance of polyunsaturated fats, and they are also rich in γ-tocopherol [11]. Consequently, we observed the smallest decrease in γ-tocopherol within the walnut-rich diet group throughout the course of the 12-month weight-loss intervention. After controlling for covariates, the walnut-rich diet group was found to have higher γ-tocopherol concentrations at 12 months compared to the other diet arms. In earlier studies, an increase in γ-tocopherol concentration was observed in participants who were prescribed walnuts [40,41]. As seen in the other diet arms, significant reductions in α-and β-tocopherol were also observed in the walnut-rich diet arm.

At baseline, dietary supplement users had higher levels of α-tocopherol and lower levels of β-, γ-, and δ-tocopherol than nonusers. However, in the multivariate analysis, only α- and γ-tocopherol were found to be significantly associated with supplement use at 12 months. The same relationship was observed in both serum [24] and adipose tissue [42] in previous trials. The preferential secretion of α-tocopherol by hepatic α-TTP [36] may explain the observed relationship of α- and γ-tocopherol and supplement use. Vitamin E supplements generally contain large amounts of α-tocopherol (relative to dietary sources) which also likely competitively reduces intestinal uptake of other tocopherols.

This study has some limitations. Because our sample consisted entirely of women, these results may not be generalizable to men. Another limitation is the lack of detailed information about dietary intake; some variability in adherence to prescribed diet is likely. However, as we previously reported [43], changes in the red blood cell fatty acids, linoleic acid and alpha-linolenic acid, indicate good adherence in the walnut-rich diet group. Also, the weight loss demonstrated by study participants suggests that most were consuming a reduced-energy diet.

In summary, this study provides further insight regarding factors associated with plasma tocopherol status in overweight and obese women participating in a weight loss intervention. While many of the factors examined have been associated with oxidative stress, not all of them were significantly associated with tocopherol concentrations. Further, there were some differences in the direction of the associations across the tocopherols; e.g., age was directly associated with α-tocopherol but inversely associated with γ- and δ-tocopherol, and adiposity was negatively associated with α-tocopherol but positively associated with γ-, β- and δ-tocopherol. Plasma tocopherol levels generally declined in association with weight loss in these women, although age, adiposity, physical activity, plasma cholesterol, and dietary supplement use influenced these levels. Future studies should be directed towards understanding the mechanism of how these factors influence tocopherol absorption, kinetics, and metabolism.

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References

1. Evans HM, Bishop KS (1922) On the Existence of a Hitherto Unrecognized Dietary Factor Essential for Reproduction. Science 56: 650-651.
2. Traber MG, Atkinson J (2007) Vitamin E, antioxidant and nothing more. Free Radic Biol Med 43: 4-15.
3. Burton GW, Doba T, Gabe E, Hughes L, Lee FL, et al. (1985) Autoxidation of biological molecules. 4. Maximizing the antioxidant activity of phenols. Journal of the American Chemical Society 107: 7053-7065.
4. Sies H (2000) What is Oxidative Stress? Oxidative Stress and Vascular Disease, In: Keaney JF, Boston MA (eds): Springer US. 1-8.
5. Institute of Medicine, Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. 2000: Washington (DC), National academic press, US.
6. Lu Y, Cederbaum AI (2008) CYP2E1 and oxidative liver injury by alcohol. Free Radic Biol Med 44: 723-738.
7. Steinbacher P, Eckl P (2015) Impact of oxidative stress on exercising skeletal muscle. Biomolecules 5: 356-377.
8. Pingitore A, Lima GP, Mastorci F, Quinones A, Iervasi G, et al. (2015) Exercise and oxidative stress: potential effects of antioxidant dietary strategies in sports. Nutrition 31: 916-922.
9. Habib SA, Saad EA, Elsharkawy AA, Attia ZR (2015) Pro-inflammatory adipocytokines, oxidative stress, insulin, Zn and Cu: Interrelations with obesity in Egyptian non-diabetic obese children and adolescents. Adv Med Sci 60: 179-185.
10. Jiang Q, Christen S, Shigenaga MK, Ames BN (2001) gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention. Am J Clin Nutr 74: 714-722.
11. US Department of Agriculture, A.R.S, Nutrient Data Laboratory, USDA National Nutrient Database for Standard Reference, Release 28.
12. Le T, Flatt SW, Natarajan L, Pakiz B, Quintana EL (2016) Effects of Diet Composition and Insulin Resistance Status on Plasma Lipid Levels in a Weight Loss Intervention in Women. J Am Heart Assoc 5: e002771.
13. Bonora E (2000) Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 23: 57-63.
14. Cleland CL, Hunter RF, Kee F, Cupples ME, Sallis JF (2014) Validity of the global physical activity questionnaire (GPAQ) in assessing levels and change in moderate-vigorous physical activity and sedentary behaviour. BMC Public Health 14: 1255.
15. Galan P, Viteri FE, Bertrais S, Czernichow S, Faure H, et al. (2005) Serum concentrations of beta-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. Eur J Clin Nutr 59: 1181-1190.
16. Succari M, Garric B, Ponteziere C, Miocque M, Cals MJ, et al. (1991) Influence of sex and age on vitamin A and E status Age Ageing 20: 413-416.
17. Krasinski SD, Russell RM, Otradovec CL, Sadowski JA, Hartz SC, et al. (1989) Relationship of vitamin A and vitamin E intake to fasting plasma retinol, retinol-binding protein, retinyl esters, carotene, alpha-tocopherol, and cholesterol among elderly people and young adults: increased plasma retinyl esters among vitamin A-supplement users. Am J Clin Nutr 49: 112-120.
18. Gascon-Vila P, Garcia-Closas R, Serra-Majem L, Pastor MC, Ribas L, et al. (1997) Determinants of the nutritional status of vitamin E in a non-smoking Mediterranean population. Analysis of the effect of vitamin E intake, alcohol consumption and body mass index on the serum alpha-tocopherol concentration. Eur J Clin Nutr 51: 723-728.
19. Miyagi SJ, Brown IW, Chock JM, Collier AC (2009) Developmental changes in hepatic antioxidant capacity are age-and sex-dependent. J Pharmacol Sci 111: 440-445.
20. Ford ES, Schleicher RL, Mokdad AH, Ajani UA, Liu S (2006) Distribution of serum concentrations of alpha-tocopherol and gamma-tocopherol in the US population. Am J Clin Nutr 84: 375-383.
21. Campbell D, Bunker V, Thomas A, Clayton B (1989) Selenium and vitamin E status of healthy and institutionalized elderly subjects: analysis of plasma, erythrocytes and platelets. Br J Nutr 62: 221-227.
22. Harman D (1956) Aging: a theory based on free radical and radiation chemistry. J Gerontol 11: 298-300.
23. Mehmetoglu I, Yerlikaya FH, Kurban S (2011) Correlation between vitamin A, E, coenzyme Q(10) and degree of insulin resistance in obese and non-obese subjects. J Clin Biochem Nutr 49:159-163.
24. Wallstrom P, Wirfält E, Lahmann PH, Gullberg B, Janzon L, et al. (2001) Serum concentrations of beta-carotene and alpha-tocopherol are associated with diet, smoking, and general and central adiposity. Am J Clin Nutr 73: 777-785.

25. Ohrvall M, Tengblad S, Vessby B (1993) Lower tocopherol serum levels in subjects with abdominal adiposity. J Intern Med 234: 53-60.
26. Larsson B, Svardsudd K, Welin L, Wilhelmsen L, Bjorntorp P, et al. (1984) Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. Br Med J 288: 1401-1404.
27. Kopelman P (2007) Health risks associated with overweight and obesity. Obes Rev 1: 13-17.
28. Nasiri N, Moini A, Eftekhari-Yazdi P, Karimian L, Salman-Yazdi R (2015) Abdominal obesity can induce both systemic and follicular fluid oxidative stress independent from polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol 184: 112-116.
29. Burton GW, Traber MG, Acuff RV, Walters DN, Kayden H, et al. (1998) Human plasma and tissue alpha-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitamin E. Am J Clin Nutr 67: 669-684.
30. Bruno RS, Ramakrishnan R, Montine TJ, Bray TM, Traber MG (2005) α-Tocopherol disappearance is faster in cigarette smokers and is inversely related to their ascorbic acid status. Am J Clin Nutr 81: 95-103.
31. Hartman TJ, Baer DJ, Graham LB, Stone WL, Gunter EW, et al. (2005) Moderate alcohol consumption and levels of antioxidant vitamins and isoprostanes in postmenopausal women. Eur J Clin Nutr 59: 161-168.
32. Park SY, Kwak YS (2016) Impact of aerobic and anaerobic exercise training on oxidative stress and antioxidant defense in athletes. J Exerc Rehabil 12: 113-117.
33. Evans WJ (2000) Vitamin E, vitamin C, and exercise. Am J Clin Nutr 72: 647S-652S.
34. Musaro A, Fulle S, Fano G (2010) Oxidative stress and muscle homeostasis. Curr Opin Clin Nutr Metab Care 13: 236-242.
35. Niess AM, Simon P (2007) Response and adaptation of skeletal muscle to exercise-the role of reactive oxygen species. Front Biosci 12: 4826-4838.
36. Traber MG, Manor D (2012) Vitamin E. Adv Nutr 3: 330-331.
37. Pfeiffer RF (2014) Neurologic manifestations of malabsorption syndromes. Handb Clin Neurol 120: 621-632.
38. Ayuso BT, Martín MJ, Figueras P, Velilla MJ, Monzon MM (1994) Chronic polyneuropathy due to vitamin E deficiency. Neurologia 9: 300-302.
39. Johnstone AM, Lobley GE, Horgan GW, Bremner DM, Fyfe CL, et al (2011) Effects of a high-protein, low-carbohydrate v. high-protein, moderate-carbohydrate weight-loss diet on antioxidant status, endothelial markers and plasma indices of the cardiometabolic profile. Br J Nutr 106: 282-291.
40. Haddad EH, Gaban-Chong N, Oda K, Sabate J (2014) Effect of a walnut meal on postprandial oxidative stress and antioxidants in healthy individuals. Nutr J 13: 4.
41. Ros E, Nunez I, Perez-Heras A, Serra M, Gilabert R, et al. (2004) A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. Circulation 109: 1609-1614.
42. Handelman GJ, Epstein WL, Peerson J, Spiegelman D, Machlin LJ, et al. (1994) Human adipose alpha-tocopherol and gamma-tocopherol kinetics during and after 1 y of alpha-tocopherol supplementation. Am J Clin Nutr 59: 1025-1032.
43. Rock CL, Flatt SW, Pakiz B, Quintana EL, Heath DD, et al (2016) Effects of diet composition on weight loss, metabolic factors and biomarkers in a 1-year weight loss intervention in obese women examined by baseline insulin resistance status. Metabolism 65: 1605-1613.