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Original article

Weight loss program is associated with decrease α-tocopherol status in obese adults

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SUMMARY

Background & aims: Studies on changes in plasma α-tocopherol levels during body fat reduction in obese persons are not clear. The aim of the present study was to assess factors associated with α-tocopherol status in obese people and to examine changes in α-tocopherol status after a 6-week AntioxObesity weight loss program.

Methods: The study was conducted in 60 overweight or obese adults, aged 18–54 years old. Food intake data were collected using the 3-day record method and a semi-quantitative food-frequency questionnaire. Anthropometric measurements included: height (H), body weight, waist circumference (WC) and hip circumference (HC), body composition: fat mass (FM) and fat-free mass (FFM), subcutaneous fat (SF) and visceral fat (VF). Lipid profile, α-tocopherol concentration, glutathione peroxidase (GPx) activity, total antioxidant capacity (TAC) in plasma and superoxide dismutase (SOD) activity in erythrocytes were determined.

Results: Energy, fat, and carbohydrate intakes decreased significantly in all subjects ($P < 0.001$). Body weight, WC, body mass index (BMI), waist-to height ratio (WHtR), and FM, VF and SF decreased significantly during the 6 weeks in all subjects. Plasma α-tocopherol significantly decreased during the program ($P = 0.006$). No changes were observed for SOD activity, but GPx activity and TAC decreased significantly ($P = 0.001$; $P = 0,023$, respectively). Plasma α-tocopherol concentration after 6 weeks of the AntioxObesity program was strongly associated with baseline plasma α-tocopherol, changes in TC, VF and FM. Low α-tocopherol status (<20 μmol/L) was found in 78% of the women and 68% of the men, after 6 weeks of the AntioxObesity program. Men were characterized by a greater decrease in weight, BMI, WC, FM, VF, SF and TAC compared to women.

Conclusions: A 6-week weight loss program lowered α-tocopherol status in overweight and obese people. Low baseline α-tocopherol status and adiposity in obese adults negatively affected α-tocopherol status after 6 weeks weight loss program. These results, coupled with excessive weight and low α-tocopherol intake, led to the finding that there was an increased risk of oxidative stress diseases in adults on a reduced diet. Long-term dietary restriction program for obese patients should be monitored to avoid α-tocopherol deficiency, and take into account higher dietary α-tocopherol requirements for obese people.

Introduction

Obesity, due to its increasing prevalence in recent years, has become a public health problem, perceived as a global epidemic. Obesity is defined as a disease associated with chronic inflammation, manifesting in abnormal secretion of cytokines, proteins, and mediators of immune response with the activation of inflammatory signaling pathways [1–4]. Excessive body fat causes a series of metabolic (hormonal and immunological) disorders that lead to diabetes, hypertension, and hyperlipidemia (metabolic syndrome), which in turn can accelerate atherosclerosis and increase the risk of

Abbreviations: BMI, body mass index; FFM, fat-free mass; FM, fat mass; GPx, glutathione peroxidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SF, subcutaneous fat; SOD, superoxide dismutase; TAC, total antioxidant capacity; TC, total cholesterol; TAG, triacylglycerol; VF, visceral fat; WC, waist circumference; WHR, waist-hip ratio; WHtR, waist-to-height ratio.

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cardiovascular disease. Moreover, chronic inflammation enhances oxidative stress (an impaired balance between oxidants and antioxidants in the body) associated with hyperglycemia [5–7].

Excessive body fat, by exacerbating inflammation and oxidative processes, is linked to decreased plasma lipophilic micronutrients levels and antioxidant capacity [8–11]. α-Tocopherol, like other lipophilic compounds, such as vitamins A, D, and carotenoids is stored in adipose tissue, which affects its distribution to other tissues and their concentration in the blood. α-Tocopherol is one of eight forms of vitamin E. These are 4 tocopherols and 4 tocotrienols, which are a lipid-soluble and have antioxidant activity related to the protection of lipids from oxidation [12]. α-Tocopherol, is antioxidant with lipoperoxyl radical-scavenging activities and has potentially anti-inflammatory functions [13]. α-Tocopherol, especially the RRR-stereoisomer form, is the most biological active form of vitamin E. It is preferentially absorbed and incorporated into chylomicrons in the small intestine and via the thoracic lymph duct is delivered to the liver, where it is selectively incorporated into very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) by the hepatic α-tocopherol transfer protein and resecreted into the blood-stream. VLDL and LDL exchanges α-tocopherol with high-density lipoproteins (HDL), which seems to be the most effective α-tocopherol donor for cellular uptake. Plasma cholesterol, LDL and HDL are the major carriers of α-tocopherol in the circulation and plasma α-tocopherol concentration correlates with plasma lipids level [12,14]. Besides lipids protection from oxidation, it was found that α-tocopherol is involved in the expression of genes associated with glucose and lipid metabolism [14,15]. According to Blat et al. [16], adipose tissue contains 90% of total α-tocopherol, and α-tocopherol from adipocytes is released very slowly during α-tocopherol deficiency or under the specific conditions of hypermetabolism [17]. Several studies have confirmed inverse associations of obesity with plasma carotenoids [11,18], but there is still a lot of ambiguity about the association of obesity and weight loss with α-tocopherol status. Furthermore, it is known that variations in body composition are responsible for the differences in plasma lipid profile between men and women. Premenopausal women have a less proatherogenic plasma lipid profile than men, and accumulation of excess body fat appears to affect lipid kinetics differently in men and women [19].

Hence, the aim of the present study was to analyze the link between anthropometric and biochemical characteristics of obesity (particularly visceral and subcutaneous adipose tissue content, waist circumference, lipids profile and antioxidant capacity) and plasma α-tocopherol concentration after a 6-week weight loss program. We also hypothesized that fat mass reduction in overweight and obese women improves α-tocopherol status at a greater extent than in men.

Materials and methods

Ethics statement

This study was conducted according to the principles of the Declaration of Helsinki for experiments involving humans and was approved by the Bioethical Commission of the National Food and Nutrition Institute (No 1805/2011). All the participants provided written consent to participate in the study. Data obtained during the intervention was confidential and restricted to the participating investigators.

Participants and study design

The study was conducted in 2012–2014 at the Department of Human Nutrition, Warsaw University of Life Sciences (WULS-SGGW). The study involved 130 males and females, who fulfilled the following inclusion criteria: age 18–54; overweight or obese according to WHO definition: BMI ≥25 kg/m^2 and WC: men: <94 cm, women: <80 cm [20,21]; lack of hormone replacement therapy and hypolipemic drugs, consent to participate. Participants receiving pharmacological treatment or diagnosed with chronic diseases, allergies, food intolerances, and women in pregnancy, lactation, and menopausal were excluded from the study.

The participants were treated as part of the AntioxObesity weight loss program, involving the development, implementation, and evaluation of the efficacy of a therapeutic program for overweight and obese adults: comprehensive education on nutrition and physical activity. A total of 130 subjects were initially screened, 60 of whom completed the study (Fig. 1).

AntioxObesity program

The main assumption of the AntioxObesity weight loss program was to investigate how the process of weight reduction influenced biochemical parameters (lipid profile) and oxidative stress biomarkers: the concentration of lipid antioxidants - α-tocopherol, vitamin A, carotenoids (β-carotene, lycopene, lutein), antioxidant enzyme activities, and total antioxidant capacity in blood serum without changes in the dietary intake of these compounds. Therefore, in the recommendations, particular attention was paid to the appropriate selection of fruits and vegetables, so that the intake of antioxidants was maintained at a similar level.

The AntioxObesity weight loss program was an intervention study in overweight and obese adults. The aim of the 6-week AntioxObesity program was weight/fat loss through a diet with a reduced energy value. The six-week weight loss program, divided into 3 stages, included (Table 1): assessment of nutritional value of diet based on 3 days of dietary self-reported records; anthropometric measurements: weight, height, waist and hip circumferences, body composition; biochemical analysis in plasma: α-tocopherol concentration, lipids profile, antioxidant potential, and SOD and GPx activity. All data from 1st and 3rd stage were used to determine the relationship between the 6-week AntioxObesity weight loss program on α-tocopherol status in conjunction with lipids profile, antioxidant enzyme activity, and plasma total antioxidant capacity in obese adults.

Compliance with dietary treatment

According to the main goals of dietary intervention, the dietary compliance criteria were: (1) Adequacy of proposed energy intake according to individual recommendations based on energy restriction (energy value reduced by ca. 500–700 kcal - 20–25% in relation to individuals baseline energy value) according to the individual's BMI; (2) Adequacy of carbohydrate intake; percentage of energy derived from carbohydrates between 50 and 55% ± 5%; (3) Adequacy of protein intake; percentage of energy from protein between 15 and 20% ± 5%; (4) Adequacy of fat intake - 25 and 30% ± 5% of total energy; (5) Adequacy of meal frequency, based on 3 main meals (breakfast, lunch, and dinner) and 2 snacks (mid-morning and mid-afternoon). Participants, who achieved 3 or more of the main goals of the 5 dietary intervention criteria, were considered as showing “global compliance”.

Assessment of dietary variables

Daily intakes of energy, macronutrients, and α-tocopherol were assessed on the basis of data from the 3-day dietary record method. The dietary record was conducted on the basis of widely accepted and applied rules [22]. Respondents were trained before

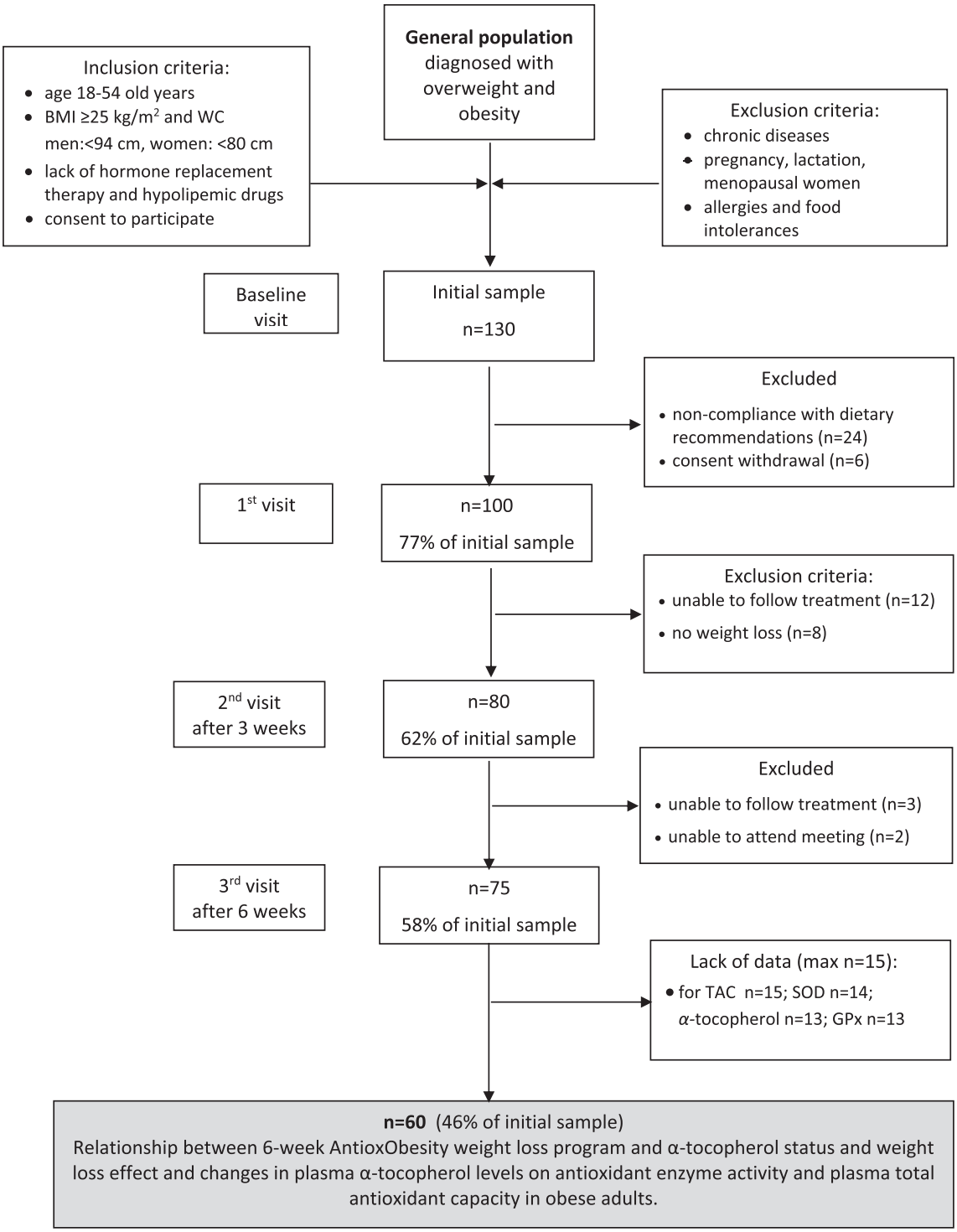

Fig. 1. Flowchart: study design and data collection.

participating in the survey on how to self-report all foods and beverages consumed daily to provide reliable estimates of dietary intake. In the Department of Human Nutrition, the diary was checked by a nutritionist in the participants' presence. The nutritionist asked for detailed information about the foods and drinks recorded, such as portion sizes and preparation methods using food models of products and dishes (the Polish Atlas of Food Products and Dish Portion Sizes). When necessary, the food diary was corrected by the nutritionist during the visit. After the review, food intake data were converted to food volume/weight (in mL or g). These data of the 3-days dietary records, three consecutive days (two weekdays and one weekend day) were entered into a nutritional software program (Diet 5.0) based on the latest available information on food composition tables from Poland [23], to evaluate average daily energy value, macronutrients, and α-tocopherol in the diets. In Polish nutrient database vitamin E content was evaluated as an α-tocopherol equivalent (mg α-TE), thus 0.8 conversion factor was used to estimate mg of α-tocopherol intake.

Table 1
The overall content of the AntioxObesity weight loss program.

Visit	Data collected
Baseline	30 min consultation about 6-week AntioxObesity weight loss program; initial measurements and recruitment
1st visit	Assessment of nutritional value of diet on the basis of data from 3-day records and a semi-quantitative food-frequency questionnaire
	Anthropometric measurements (weight, height, waist, and hip circumferences) and body composition (FM, FFM, SF, VF)
	Blood pressure measurement
	Blood collection - biochemical analysis: lipid profile, lipid micronutrients, antioxidative biomarkers
dietary intervention	Identifying goals of program, overview of nutritional values, and start reduced caloric diet by ca. 500–700 kcal/day
	Participants recommended to engage in normal physical activity, suitable to individual abilities and health
2nd visit (3 weeks)	Assessment of nutritional value of diet on the basis of data from the 3-day record method
	Anthropometric measurements
	Blood pressure measurement
	Dietitian consultation - nutrition recommendations adjusted to individual needs and health status
3rd visit (6 weeks)	Assessment of nutritional value of diet on the basis of data from 3-day records and a semi-quantitative food-frequency questionnaire
	Anthropometric measurements (weight, height, waist, and hip circumferences) and body composition (FM, FFM, SF, VF)
	Blood pressure measurement
	Blood collection - biochemical analysis: lipid profile, lipid micronutrients, antioxidative biomarkers

FM, fat mass; FFM, fat-free mass; SF, subcutaneous fat; VF, visceral fat.

Assessing adequacy of α-tocopherol intake, EAR - 12 mg/d and RDA - 15 mg/d levels were used [24].

Anthropometry, body composition

All anthropometric measurements, according to the standardized procedures [25], were taken with light clothing and without shoes twice and averages were calculated. Height (H) was measured with a portable stadiometer with the head in horizontal Frankfurt plane and recorded with a precision of 0.1 cm. Weight was taken in light indoor clothes without shoes using the same electronic digital scale to the nearest 0.1 kg. Waist circumference (WC) was measured using a stretch-resistant tape that provides constant 100 g tension, at the mid-way point between the iliac crest and the costal margin (lower rib) [21] on the anterior axillary line in a resting expiratory position. The hip circumference (HC) measurement was taken at the widest part of the buttocks, with the tape parallel to the floor [26]. The waist-hip ratio (WHR) and the waist-to-height ratio (WHtR) were calculated to determine fat tissue distribution. The WHR was calculated as the WC/HC and interpreted according to WHO criteria, considered 0.90 for men and 0.85 for women to assess central obesity [26]. The WHtR was calculated at the WC/H and value $\geq$ 0.5 was used as a central obesity measure [21]. Body mass index (BMI) was calculated as weight (kg)/height (m^2). BMI was categorized according to WHO [20,21]. BMI$\geq$25 kg/m^2 was used as overweight and BMI$\geq$30 kg/m^2 as obesity measures. Bioelectrical impedance analysis (BIA) (Maltron BioScan 920 ver.1.1) was used to assess fat mass (FM) and fat-free mass (FFM), including subcutaneous fat (SF) and visceral fat (VF). BIA was performed under standardized conditions according to the manufacturer's protocol. All measurements were performed with light clothing and with metal objects (e.g. jewelry, keys) removed. Whole body BIA measurements were performed by placing two adhesive single-use skin electrodes (purchased from Maltron International Ltd, UK) on the right hand and foot, respectively, on the patient when lying in supine position. The device applies a current of 400 mA at a constant frequency of 50 kHz.

The VF and SF measurements were performed in the standing position with four pairs of electrodes positioned on the trunk. The measurements were done at a frequency of 50 kHz, with an impedance range of 5–1100 Ω.

Biochemical analysis

Venous blood samples were taken at baseline and after the 6-week program after an overnight fast (12 h) in the morning (9–10 a.m.) with minimal stasis and maintained at 4 °C until plasma or serum was separated for biochemical analyses. Plasma and serum samples were collected after centrifugation (1000 × g for 10 min at 4 °C) and stored frozen (−80 °C) until analysis (no longer than 2 months, except erythrocytes and plasma for SOD and GPx activity, which were analyzed immediately, according to the manufacturer's instructions.

Lipid profile (total cholesterol, HDL cholesterol, and triglyceride levels) was determined through standard enzymatic analyses using commercial HYDREX kits (product numbers: total cholesterol - HXB104; HDL cholesterol - HXB106; triglycerides - 17628). LDL level was calculated using the Friedwald formula [27]. The results are expressed as mmol/L and compared with ESC/EAS guidelines [28].

Plasma α-tocopherol concentration was assessed using high-performance liquid chromatography (HPLC - Knauer) with a diode array detector, at scan wavelength of 280–300 nm. LiChroCART®250-4 RP-18 (4 × 250 mmol; 5 μm) with a precolumn (Merc, col. no. 841071, Darmstadt, Germany) was used. Acetonitryl/hexane/isopropanol (65:14:21; v/v/v) was applied as the eluent. The flow rate measured 0.8 ml/min. Samples were deproteinized with ethanol with ascorbic acid (1% in 0.1 M HCl) and 20 μl of internal standard (15 μg/ml, α-tocopherol acetate in ethanol). Extraction of α-tocopherol was performed twice with 1 ml hexane by vortex-mixing for 4 min and centrifuging at 3600 g for 10 min at 10 °C. The hexane extracts were collected, and the solvent was evaporated using CentriVap Concertrator (Labconco). The residue was dissolved in 200 μl of methanol, vortex-mixed for 30 s and transferred into a vial. The injection volume was 20 μL for all analyses. Plasma α-tocopherol concentrations were calculated by comparing with a corresponding calibration curve (standard of α-tocopherol from Sigma–Aldrich Inc. in ethanol) in a range of 1–10 μg/mL. Analytical recovery of α-tocopherol was 94%. The mean intra-day coefficients of variation (% CV) for α-tocopherol at 1.0 and 10 μg/mL were <3.3% and <3.2%, respectively. Similarly the corresponding values for the mean inter-day analysis were <3.0 and <3.5%, respectively. All reagents were of highest grade commercially available (HPLC purity) and were purchased from Merc (Darmstadt, Germany).

α-Tocopherol levels are expressed as μmol/L plasma, and lipid adjusted as α-tocopherol μmol on mmol of total cholesterol (TC), LDL-cholesterol, HDL-cholesterol, triglycerides (TAG), and total lipids (calculated as the sum of total cholesterol and triglycerides).

α-Tocopherol status was assessed according the following cut points: α-tocopherol plasma level >12 μmol/L, α-tocopherol: total TC ratio >2.22 μmol/mmol; α-tocopherol: total lipid ratio

Table 2
Characteristics of study population and changes in investigated parameters during the weight loss program.

Variables	All subjects (n = 60)			
	T_0	T_1	Δ T_1-T_0	P value*
Age	35.4 ± 8.9	–	–	–
Diet				
Energy (kcal/d)	2040 ± 608	1616 ± 705	−428	<0.001
Protein (g/d)	88.5 ± 22.5	86.6 ± 34.9	−1.9	0.271
Fat (g/d)	74.2 ± 32.5	52.0 ± 33.7	−22.1	<0.001
Carbohydrate (g/d)	266.4 ± 91.0	218.0 ± 96.0	−48.4	<0.001
α-tocopherol (mg/d)	7.6 ± 3.5	7.3 ± 4.3	−0.33	0.679
Anthropometry				
Weight (kg)	92.9 ± 17.0	88.8 ± 16.9	−3.9	<0.001
BMI (kg/m^2)	31.9 ± 4.5	30.1 ± 6.1	−1.8	<0.001
WC (cm)	93.9 ± 1.5	89.7 ± 11.3	−4.2	<0.001
WHR	0.86 ± 0.09	0.85 ± 0.09	−0.01	0.069
WHtR	0.55 ± 0.06	0.53 ± 0.06	−0.02	<0.001
Body composition				
FFM (%)	58.6 ± 9.0	60.0 ± 9.8	1.4	0.001
FM (%)	41.4 ± 9.0	40.0 ± 9.8	−1.4	0.001
VF (cm^2)	169.5 ± 4.7	145.5 ± 70.3	−26.6	0.002
SF (cm^2)	239.3 ± 0.3	224.0 ± 85.8	−19.2	0.025
Biochemical parameters				
TC (mmol/L)	5.00 ± 0.83	4.74 ± 0.73	−0.25	0.099
HDL (mmol/L)	1.33 ± 0.23	1.31 ± 0.23	−0.02	0.426
LDL (mmol/L)	3.08 ± 0.80	2.84 ± 0.70	−0.24	0.066
TAG (mmol/L)	1.31 ± 0.43	1.29 ± 0.40	−0.02	0.226
α-tocopherol (μmol/L)	18.2 ± 6.6	16.3 ± 6.9	−1.97	0.006
α-tocopherol:TC ratio (μmol/mmol)	3.86 ± 1.77	3.70 ± 1.94	−0.26	0.099
α-tocopherol:HDL ratio (μmol/mmol)	14.0 ± 5.29	12.97 ± 5.54	−1.65	0.006
α-tocopherol:LDL ratio (μmol/mmol)	6.66 ± 3.67	6.64 ± 4.39	−0.19	0.220
α-tocopherol:TAG ratio (μmol/mmol)	15.55 ± 7.62	13.66 ± 6.02	−2.28	0.015
α-tocopherol:lipids ratio (μmol/mmol)	3.05 ± 1.38	2.88 ± 1.40	−0.25	0.080
SOD (U/g hemoglobin)	161.2 ± 17.2	161.9 ± 19.7	0.75	0.868
GPx (U/L)	2639 ± 889	2182 ± 737	−448	0.001
TAC (μmol/L)	269.7 ± 21.1	263.9 ± 11.2	−5.72	0.023

Results expressed as means ± standard deviation. T_0, baseline; T_1, after 6 weeks; Δ T_1-T_0, changes; BMI, body mass index; WC, waist circumference; WHR, waist-hip ratio; WHtR, waist-to-height ratio; FFM, fat-free mass; FM, fat mass; VF, visceral fat; SF, subcutaneous fat; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TAG, triacylglycerol; SOD, superoxide dismutase; GPx, glutathione peroxidase; TAC, total antioxidant capacity.
*- Student's independent t test (significant difference $P \leq 0.05$).

>1.59 μmol/mmol as adequate concentration. α-Tocopherol plasma level >20 μmol/L was used as a strong antioxidant defense indicator and the cut point of decreased risk of cardiovascular disease [13,22,29,30]. In healthy individuals measurement of plasma α-tocopherol concentration is sufficient to establish actual α-tocopherol status, but for patients with metabolic disorders, when α-tocopherol concentration is low, lipid adjustment is necessary [9,30].

Superoxide dismutase (SOD) activity in erythrocytes was determined using the Randox reagent kit (RANSOD - SD-125, UK). *Glutathione peroxidase* (GPx) activity in blood was determined using the Randox reagent kit (RANSEL - RS-504, UK). Analyzes were performed according to the manufacture's protocol. Values are expressed as U/g hemoglobin and U/L plasma.

Total antioxidant capacity (TAC) of blood serum was determined using the colorimetric method based on the Trolox Equivalent Antioxidant Capacity (TEAC) assay, according to Re et al. [31].

Statistical analysis

The parameters analyzed in our study were presented as: means and standard deviation. Differences in all variables between baseline and after 6 weeks were compared by using an independent Student's t-test and Pearson's Chi^2. Repeated-measures ANOVA was used to assess the effects of gender and effects of time on dietary value, anthropometry, body composition and biochemical parameters.

Linear regression was used to investigate the relationship between changes in selected parameters and α-tocopherol status after 6 weeks the weight loss program. The final 3 multivariate models were specified. Based on the literature research, lipids profile, and adiposity measurements were included, as well models were adjusted on gender, age, α-tocopherol intake. The dependent variable was plasma α-tocopherol after 6 weeks and lipid-corrected plasma α-tocopherol after 6 weeks. The independent variables were baseline plasma α-tocopherol, changes between the end and beginning of the AntioxObesity program for BMI, WC, FM, VF, TC, HDL and for dietary fat. In addition, models were specified for each combination of gender.

For all analyses, P-values ≤ 0.05 were considered statistically significant. Statistical analyses were performed using STATISTICA software (version 12.0 PL; StatSoft Inc., Tulsa, OK, USA).

Results

General characteristics of the study population and changes in the determined parameters after 6 weeks weight loss program are presented in Table 2. Energy value, dietary fat, and carbohydrate intakes decreased significantly in all subjects ($P < 0.001$). Mean α-tocopherol intake was about 7.3–7.6 mg α-tocopherol/d and no significantly changes were observed after 6 weeks. Body weight, WC, BMI, WHtR, and FM decreased significantly after the 6 weeks in all subjects ($P < 0.05$), whereas the percentage of FFM significantly increased. VF and SF decreased significantly (about 27 and 19 cm^2) in all subjects. Plasma α-tocopherol ($P = 0.006$), α-tocopherol:HDL

Table 3
Effects of gender and time on anthropometric and biochemical parameters during the weight loss program.

Variables	Women (n = 37)			Men (n = 23)			P value*		
	T_0	T_1	Δ T_1-T_0	T_0	T_1	Δ T_1-T_0	G	T	G x T
Age	34.1 ± 8.7	–	–	37.9 ± 9.1	–	–	0.1226	–	–
Diet									
Energy (kcal/d)	1884 ± 561	1460 ± 652	−424	2340 ± 590	1904 ± 665	−435	0.0046	0.0000	0.9449
Protein (g/d)	81.4 ± 19.3	77.9 ± 30.1	−3.5	101.7 ± 22.2	102.8 ± 38.1	1.1	0.0010	0.7534	0.5575
Fat (g/d)	66.8 ± 28.9	45.1 ± 25.2	−21.8	87.7 ± 34.8	64.9 ± 43.2	−22.8	0.0030	0.0004	0.9289
Carbohydrate (g/d)	251.2 ± 78.0	200.5 ± 95.8	−50.6	294.5 ± 107	250.2 ± 90.0	−44.3	0.0498	0.0000	0.7663
α-tocopherol (mg/d)	7.3 ± 3.5	6.8 ± 4.1	−0.50	8.1 ± 3.5	8.1 ± 4.7	−0.02	0.2724	0.6837	0.6980
Anthropometry									
Weight (kg)	86.3 ± 14.5	82.6 ± 14.3	−3.3	105.1 ± 14.8	100.6 ± 15.3	−4.9	0.0000	0.0000	0.0160
BMI (kg/m^2)	31.6 ± 4.6	30.4 ± 4.7	−1.2	32.6 ± 4.3	29.8 ± 8.3	−2.9	0.8653	0.0002	0.1111
WC (cm)	89.3 ± 8.9	85.2 ± 8.9	−3.8	102.5 ± 10.9	97.7 ± 10.9	−4.8	0.0000	0.0000	0.2997
WHR	0.82 ± 0.08	0.80 ± 0.06	−0.01	0.93 ± 0.08	0.92 ± 0.08	−0.01	0.0000	0.0415	0.5435
WHtR	0.54 ± 0.05	0.52 ± 0.05	−0.02	0.57 ± 0.07	0.55 ± 0.07	−0.03	0.0640	0.0000	0.5129
Body composition									
FFM (%)	55.3 ± 8.5	56.4 ± 9.2	1.1	64.8 ± 6.3	66.9 ± 6.7	2.0	0.0000	0.0014	0.2891
FM (%)	44.7 ± 8.5	43.6 ± 9.2	−1.1	35.2 ± 6.3	33.2 ± 6.7	−2.0	0.0000	0.0014	0.2892
VF (cm^2)	159.1 ± 54.0	139.3 ± 61.9	−23.6	188.8 ± 78.8	156.6 ± 83.9	−32.3	0.2033	0.0000	0.1585
SF (cm^2)	233.6 ± 73.4	225.4 ± 88.3	−14.3	249.9 ± 92.3	221.6 ± 83.1	−28.3	0.7838	0.0103	0.1535
Biochemical parameters									
TC (mmol/L)	5.06 ± 0.78	4.77 ± 0.78	−0.29	4.89 ± 0.90	4.67 ± 0.62	−0.15	0.4008	0.0538	0.5270
HDL (mmol/L)	1.30 ± 0.22	1.30 ± 0.22	0.00	1.38 ± 0.23	1.31 ± 0.23	−0.03	0.8531	0.9704	0.9556
LDL (mmol/L)	3.16 ± 0.78	2.87 ± 0.76	−0.30	2.91 ± 0.81	2.77 ± 0.58	−0.11	0.3421	0.0742	0.3855
TAG (mmol/L)	1.31 ± 0.41	1.31 ± 0.42	−0.02	1.31 ± 0.46	1.27 ± 0.35	−0.09	0.8647	0.3553	0.1964
α-tocopherol (μmol/L)	17.8 ± 6.4	16.1 ± 7.70	−1.76	19.0 ± 7.06	16.6 ± 5.77	−2.39	0.6429	0.0035	0.6410
α-tocopherol:TC ratio (μmol/mmol)	3.72 ± 1.65	3.63 ± 2.07	−0.03	4.11 ± 1.99	3.87 ± 1.67	−0.81	0.2851	0.0638	0.0821
α-tocopherol:HDL ratio (μmol/mmol)	14.03 ± 5.19	12.71 ± 5.81	−1.28	14.04 ± 5.59	13.59 ± 4.96	−2.52	0.3731	0.0030	0.3132
α-tocopherol:LDL ratio (μmol/mmol)	6.32 ± 3.41	6.57 ± 4.78	0.38	7.23 ± 4.14	6.79 ± 3.42	−1.54	0.3427	0.3088	0.0965
α-tocopherol:TAG ratio (μmol/mmol)	15.18 ± 7.41	13.25 ± 6.09	−1.83	16.20 ± 8.14	14.62 ± 5.98	−3.35	0.3303	0.0029	0.3597
α-tocopherol:lipids ratio (μmol/mmol)	2.94 ± 1.28	2.81 ± 1.47	−0.09	3.23 ± 1.56	3.03 ± 1.26	−0.64	0.2692	0.0245	0.0898
SOD (U/g hemoglobin)	161.3 ± 16.9	161.2 ± 18.58	−0.03	160.9 ± 18.1	163.3 ± 22.1	2.19	0.8376	0.7181	0.7107
GPx (U/L)	2625 ± 807	2118 ± 585	−507	2666 ± 1055	2307 ± 973	−341	0.5824	0.0000	0.4705
TAC (μmol/L)	264.7 ± 90.1	263.9 ± 87.2	−2.00	280.0 ± 32.0	267.1 ± 15.2	−13.2	0.0093	0.0167	0.0727

Results expressed as means ± standard deviation. T_0, baseline; T_1, after 6 weeks; Δ T_1-T_0, changes; G, gender; T, effects time; G x T, gender x effects time interaction effect; BMI, body mass index; WC, waist circumference; WHR, waist-hip ratio; WHtR, waist-to-height ratio; FFM, fat-free mass; FM fat mass; VF, visceral fat; SF, subcutaneous fat; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TAG, triacylglycerol; SOD, superoxide dismutase; GPx, glutathione peroxidase; TAC, total antioxidant capacity.
*- Repeated-measures ANOVA (significant difference $P \leq 0.05$).

Table 4
Percentage of participants who did not meet the reference values for lipids profile values and α-tocopherol status.

	All subjects (n = 60)			Women (n = 37)			Men (n = 23)		
	Baseline	After 6 weeks	P*	Baseline	After 6 weeks	P*	Baseline	After 6 weeks	P*
LDL (<3.0 mmol/L)	64.8	50.9	0.042	58.1	42.9	0.038	71.4	60.0	0.049
HDL (>1.0 mmol/L in men and >1.3 mmol/L in women)	69.1	68.9	0.983	57.1	58.1	0.939	90.0	92.9	0.062
TAG (<1.7 mmol/L)	85.5	88.9	0.611	85.7	87.1	0.870	85.0	92.9	0.384
α-tocopherol in plasma (>12 μmol/L)	19.6	23.2	0.105	21.6	27.0	0.088	15.6	15.6	1.000
α-tocopherol in plasma (>20 μmol/L)	60.7	75.0	0.049	59.5	78.4	0.019	63.2	68.4	0.073
α-tocopherol: TC ratio (>2.22 μmol/mmol)	5.6	11.4	0.038	8.6	12.9	0.047	0.0	7.7	0.219
α-tocopherol: lipids ratio (>1.59 μmol/mmol)	25.9	36.4	0.026	28.6	41.9	0.009	21.1	23.1	0.069

LDL, low-density lipoprotein; HDL, high-density lipoprotein; TAG, triacylglycerol; TC, total cholesterol.
*Chi^2 Pearson test (significant difference $P \leq 0.05$).

ratio (P = 0.006) and α-tocopherol:TAG ratio (P = 0.015) significantly decreased after 6 weeks the program in all subjects. No changes were observed for SOD activity, but GPx activity (P = 0.001) and TAC (P = 0.023) decreased significantly in all participants.

Table 3 shows effects time or/and gender on investigated parameters. For dietary intake (energy, P = 0.0046; protein, P = 0.0010; fat P = 0.0030 and carbohydrate, P = 0.0498), anthropometrics measurements (weight, P = 0.0000; WC, P = 0.0000; WHR, P = 0.0000 and FM, P = 0.0000) and TAC (P = 0.0093) statistically significant effects of gender were stated. Men were characterized by a greater decrease in weight, BMI, WC, FM, VF, SF and TAC compared to women. A statistically significant effects of the time was found for dietary value (energy, P = 0.0000; fat, P = 0.0004 and carbohydrate, P = 0.0000), anthropometry and body composition (all variables) and biochemical parameters (α-tocopherol level, P = 0.0035; α-tocopherol:HDL ratio, P = 0.0030; α-tocopherol:TAG ratio, P = 0.002; α-tocopherol:lipids ratio, P = 0.0245; GPx, P = 0.0000) and TAC (P = 0.0167). Interaction between gender and effects of the time was found for the subjects' weight (P = 0.0160).

Table 4 shows the percentage of participants who did not meet the reference values for the lipids profile and α-tocopherol. The percentage of participants who did not meet the reference values for LDL decreased significantly in all subjects (P = 0.0420), women (P = 0.0038) and men (P = 0.0492) after 6 weeks of weight

Table 5
Multivariate models[a] between plasma α-tocopherol concentration after 6 weeks of the AntioxObesity program and selected predictors.

Predictors	α-tocopherol (μmol/L) after 6 weeks	
	β-coefficient	95% CI
All subjects (Overall model: r = 0.74; r^2 = 0.68; P < 0.0001)		
changes TC (mmol/L)	−0.781	−0.265; 0.109
changes HDL (mmol/L)	0.119	−0.069; 0.308
baseline α-tocopherol (μmol/L)	0.793*	0.612; 0.974
changes dietary fat (g/d)	0.084	−0.119; 0.286
changes VF (cm^2)	−0.195	−0.394; 0.004
changes FM (%)	0.208*	0.008; 0.408
changes BMI (kg/m^2)	0.037	−0.194; 0.268
changes WC (cm)	0.095	−0.103; 0.294
Women (Overall model: r = 0.81; r^2 = 0.74; P < 0.0001)		
changes TC (mmol/L)	−0.153	−0.366; 0.060
changes HDL (mmol/L)	0.080	−0.137; 0.296
baseline α-tocopherol (μmol/L)	0.808*	0.592; 1.024
changes dietary fat (g/d)	0.232	0.010; 0.454
changes VF (cm^2)	−0.153	−0.384; 0.078
changes FM (%)	0.201	−0.028; 0.431
changes BMI (kg/m^2)	0.090	−0.157; 0.337
changes WC (cm)	−0.081	−0.312; 0.150
Men (Overall model: r = 0.85; r^2 = 0.79; P = 0.0087)		
changes TC (mmol/L)	0.447	−0.694; 1; 589
changes HDL (mmol/L)	−0.428	−1.093; 0.237
baseline α-tocopherol (μmol/L)	1.004*	0.444; 1.563
changes dietary fat (g/d)	−0.373	−0.915; 0.168
changes VF (cm^2)	−0.603*	−1.199; −0.007
changes FM (%)	0.227	−0.433; 0.886
changes BMI (kg/m^2)	−0.064	−0.456; 0.328
changes WC (cm)	−0.110	−0.922; 0.702

Linear regression analysis adjusted for age, gender and α-tocopherol intake.
TC, total cholesterol; HDL, high-density lipoprotein; VF, visceral fat; FM, fat mass; BMI, body mass index; WC, waist circumference.
*, P ≤ 0.01.
[a], Each model was controlled for all predictors shown in table.

Table 6
Multivariate models[a] between plasma α-tocopherol: lipids ratio after 6 weeks of the AntioxObesity program and selected predictors.

Predictors	α-tocopherol: lipids ratio (μmol/mmol) after 6 weeks	
	β-coefficient	95% CI
All subjects (Overall model: r = 0.76; r^2 = 0.71; P < 0.0001)		
changes TC (mmol/L)	−0.188*	−0.369; −0.008
changes HDL (mmol/L)	0.119	−0.063; 0.300
baseline α-tocopherol (μmol/L)	0.821*	0.647; 0.996
changes dietary fat (g/d)	0.018	−0.178; 0.213
changes VF (cm^2)	−0.213*	−0.404; −0.021
changes FM (%)	0.128	−0.068; 0.317
changes BMI (kg/m^2)	0.108	−0.114; 0.330
changes WC (cm)	0.098	−0.093; 0.289
Women (Overall model: r = 0.83; r^2 = 0.77; P < 0.0001)		
changes TC (mmol/L)	−0.286*	−0.486; −0.086
changes HDL (mmol/L)	0.064	−0.139; 0.268
baseline α-tocopherol (μmol/L)	0.822*	0.619; 1.024
changes dietary fat (g/d)	0.205	−0.003; 0.414
changes VF (cm^2)	−0.199	−0.416; 0.018
changes FM (%)	0.110	−0.106; 0.325
changes BMI (kg/m^2)	0.167	−0.065; 0.0399
changes WC (cm)	−0.106;	−0.323; 0.112
Men (Overall model: r = 0.84; r^2 = 0.80; P = 0.0061)		
changes TC (mmol/L)	0.855	−0.203; 1.907
changes HDL (mmol/L)	−0.559	−1.173; 0.053
baseline α-tocopherol (μmol/L)	1.194*	0.682; 1.710
changes dietary fat (g/d)	−0.453	−0.954; 0.046
changes VF (cm^2)	−0.189	−0.744; 0.360
changes FM (%)	−0.143	−0.753; 0.465
changes BMI (kg/m^2)	−0.083	−0.441; 0.278
changes WC (cm)	−0.308	−1.064; 0.440

Linear regression analysis adjusted for age, gender and α-tocopherol intake.
TC, total cholesterol; HDL, high-density lipoprotein; VF, visceral fat; FM, fat mass; BMI, body mass index; WC, waist circumference.
*, P ≤ 0.01.
[a], Each model was controlled for all predictors shown in table.

reduction compared with baseline (Table 4). Moreover, we observed that lipid profile improved; for TC and LDL levels a decreasing tendency (P < 0.1) was found (Table 3). However, HDL levels were lower than references values in close to 60% of women and 90% of men, and TAG level did not meet references values in 85–93% of the respondents (Table 4).

Mean circulating levels of plasma α-tocopherol of about 16–19 μmol/L (Table 3) didn't indicate an α-tocopherol deficiency (<12 μmol/L), but we found that 22–27% women and 16% men (Table 4) had inadequate plasma α-tocopherol concentrations. Low α-tocopherol status (<20 μmol/L) was found at baseline in 60% of the women and 63% of the men, and it increased to 78% and 68% after 6 weeks, respectively, which indicates an increased risk of cardiovascular diseases and weak antioxidant defense.

Plasma α-tocopherol concentration after 6 weeks of the AntioxObesity program was strongly associated with baseline plasma α-tocopherol, changes in VF, and changes in FM (Table 5). Lipid-corrected α-tocopherol (Tables 6 and 7) after the 6-week program was associated with changes in TC, baseline plasma α-tocopherol, and changes in FM, VF for all subjects. The identified set of correlates explained 74%, 76%, and 74% of total variation in plasma α-tocopherol, the α-tocopherol: lipids ratio, and the α-tocopherol: TC ratio for all subjects. In women baseline plasma α-tocopherol concentration and changes in TC were statistically significant correlates in models, while a change in VF and in dietary fat were predictors in men (Tables 5–7).

Table 8 shows multivariable-adjusted linear regression models between plasma α-tocopherol and lipid-corrected α-tocopherol and selected predictors after the 6-week AntioxObesity program. Changes in TC, baseline plasma α-tocopherol, VF and FM were predictors of plasma α-tocopherol and lipid-corrected α-tocopherol in all subjects. In women changes in TC and baseline plasma α-tocopherol concentration correlated in models. While baseline plasma α-tocopherol concentration and changes in VF were statistically significant in men.

Discussion

The main findings of this substudy are: (1) the 6-week weight loss program brought about a decline in fat intake and α-tocopherol status, (2) baseline plasma α-tocopherol concentrations and changes in FM, VF proved to be good predictors of changes in α-tocopherol status, (3) gender no significantly influenced on α-tocopherol status after 6 weeks weight loss program, but in men more decrease of FM, VF and SF than in women, is associated with lower TAC.

In our study, average intake of α-tocopherol for about 90% of participants was inadequate at baseline and for 83% at the end of weight loss program. This is in line with the results by Agarwal et al. [32], who reported that compared to normal weight adults, obese adults had lower intakes of vitamin E and a higher prevalence of inadequacy. Most diets assuming a reduction of body mass are based on lowering the fat content in the diet, which decreases intakes of lipophilic micronutrients, like vitamin E. Some previous studies [33,34] reported that increasing fat intake (to 35–50% of calories) improved vitamin E status and did not negatively affect cardiovascular risk factors. Low-fat diets are associated with decreased vitamin E and n-3 fatty acid intakes [35]. In our study, after 6 weeks, the energy from fat decreased by about 3%, but the average intake of α-tocopherol remained at the same inadequate level. Results indicated that the selection of product groups is

Table 7
Multivariate models[a] between plasma α-tocopherol:TC ratio after 6 weeks of the AntioxObesity program and selected predictors.

Predictors	α-tocopherol: TC (μmol/mmol) after 6 weeks	
	β-coefficient	95% CI
All subjects (Overall model: r = 0.74; r^2 = 0.68; P < 0.0001)		
changes TC (mmol/L)	−0.240*	−0.427; −0.052
changes HDL (mmol/L)	0.158	−0.031; 0.347
baseline α-tocopherol (μmol/L)	0.769*	0.588; 0.950
changes dietary fat (g/d)	−0.005	−0.208; 0.199
changes VF (cm^2)	−0.240*	−0.439; −0.040
changes FM (%)	−0.166*	−0.034; 0.367
changes BMI (kg/m^2)	0.166	−0.066; 0.397
changes WC (cm)	0.133	−0.006; 0.332
Women (Overall model: r = 0.82; r^2 = 0.75; P < 0.0001)		
changes TC (mmol/L)	−0.320*	−0.531; −0.110
changes HDL (mmol/L)	0.105	−0.108; 0.319
baseline α-tocopherol (μmol/L)	0.782*	0.570; 0.995
changes dietary fat (g/d)	0.197	−0.022; 0.416
changes VF (cm^2)	−0.212	−0.439; 0.016
changes FM (%)	0.141	−0.085; 0.368
changes BMI (kg/m^2)	0.230	−0.013; 0.473
changes WC (cm)	−0.027	−0.255; 0.201
Men (Overall model: r = 0.96; r^2 = 0.88; P = 0.0141)		
changes TC (mmol/L)	0.594	−0.690; 1.178
changes HDL (mmol/L)	−0.453	−1.204; 0.294
baseline α-tocopherol (μmol/L)	1.154*	0.521; 1.783
changes dietary fat (g/d)	−0.667*	- 1.283;-0.058
changes VF (cm^2)	−0.507	−1.184; 0.164
changes FM (%)	−0.037	−0.783; 0.707
changes BMI (kg/m^2)	0.141	−0.302; 0.582
changes WC (cm)	−0.295	−1.210; 0.619

Linear regression analysis adjusted for age, gender and α-tocopherol intake.
TC, total cholesterol; HDL, high-density lipoprotein; VF, visceral fat; FM, fat mass; BMI, body mass index; WC, waist circumference.
*, P ≤ 0.01.
[a], Each model was controlled for all predictors shown in table.

important to create a future weight loss program, and special attention should be paid on choosing the adequate sources of fat and vitamin E (like oils, olives, dark-green leafy vegetables, whole grains, flax seeds, nuts, almonds) than limiting fat.

Weight loss after 6 weeks was associated with significantly lower plasma α-tocopherol concentration, which generally depended on its baseline concentration. Moreover, about 90% of men had HDL level lower than references value. After 6 weeks we observed the tendency in decreasing TC and LDL level. Most subjects (61–78%) had plasma α-tocopherol concentrations of less than 20 μmol/L, indicating an increased risk of cardiovascular disease and weak antioxidant defense. How Mah et al. [36] stated that the bioavailability of α-tocopherol is lower in patients with metabolic syndrome, which obesity is one of its component. Obese individuals have decreased intestinal absorption and hepatic secretion of α-tocopherol. It was explained by greater intestinal fat storage in adults with greater body fat. Lower α-tocopherol absorption, "trapping" of α-tocopherol within enterocytes and delayed secretion into lymphatic system, limit α-tocopherol availability in individuals with excessive body fat. In addition, inflammation and/or greater oxidation within the enterocyte or hepatocyte is associated with metabolic disorders, may limit α-tocopherol bioavailability [36,37]. On the other hand, lower α-tocopherol plasma status in obese people is explained by the accumulation of α-tocopherol in adipose tissue. α-Tocopherol, as well as carotenoids or vitamin D are stored in adipose tissue, but in comparison to other lipid soluble micronutrients, weight loss does not affect plasma α-tocopherol concentration [4]. The differential association of body mass with α-tocopherol and other lipid-soluble micronutrients argues against a generalized fat effect on oxidative stress and their metabolism. Schaefer et al. [38] found that only triglycerides are mobilized from a fat cell during up to 6 months of weight loss. Cholesterol and α-tocopherol released from the adipocyte are independent of factors controlling triglycerides afflux. Moreover, α-tocopherol stimulates the expression of PPARγ and lipid accumulation during adipocyte differentiation, and affects the endogenous synthesis of cholesterol, likely by modulating the cleavage of SREBPs [4]. Traber et al. [39] supposed that plasma α-tocopherol concentrations mainly are dependent on mechanisms control circulating lipids. In our program, weight loss and changes in diet (lower fat intake) caused a tendency (p = 0.066) to lowering LDL-cholesterol concentrations. Because under physiological fasting conditions α-tocopherol is mostly transported *via* LDL [40], it may explain the decrease in plasma α-tocopherol concentration during the 6 weeks.

In addition, it is interesting to note that no effect of weight loss program on SOD activity was found, but significantly lower GPx activity. Similarly, an important reduction in GPx activity in obese patients compared with the control group was confirmed by Monzo-Beltran et al. [41] in a one-year follow-up study, but other authors [42–44] supposed that levels of plasma antioxidants could

Table 8
Multivariable-adjusted linear regression models between plasma α-tocopherol concentration and lipid-corrected α-tocopherol after 6 weeks of the AntioxObesity program and selected predictors (P ≤ 0.01, for all presented predictors).

Predictors	α-tocopherol (μmol/L)		α-tocopherol: lipids ratio (μmol/mmol)		α-tocopherol: TC ratio (μmol/mmol)	
	β-coefficient	95% CI	β-coefficient	95% CI	β-coefficient	95% CI
All subjects	Overall model: r = 0.63; r^2 = 0.60; P < 0.0000		Overall model: r = 0.73; r^2 = 0.70; P < 0.0001		Overall model: r = 0.85; r^2 = 0.69; P < 0.0001	
changes TC (mmol/L)	–	–	–	–	−0.319	−0.493; −0.144
baseline α-tocopherol (μmol/L)	0.706	0.520; 0.892	0.758	0.573; 0.943	0.766	0.594; 0.939
changes VF (cm^2)	–	–	−0.182	−0.355; −0.008	–	–
changes FM (%)	0.208	0.030; 0.385	0.218	0.046; 0.391	−0.221	−0.394; −0.048
Women	Overall model: r = 0.66; r^2 = 0.63; P < 0.0001		Overall model: r = 0.73; r^2 = 0.71; P < 0.0001		Overall model: r = 0.84; r^2 = 0.69; P < 0.0001	
changes TC (mmol/L)	–	–	−0.314	−0.517; −0.111	−0.434	−0.643; −0.225
baseline α-tocopherol (μmol/L)	0.789	0.569; 1.008	0.778	0.575; 0.981	0.760	0.551; 0.969
Men	Overall model: r = 0.57; r^2 = 0.48; P = 0.0052		Overall model: r = 0.80; r^2 = 0.74; P = 0.0021		Overall model: r = 0.81; r^2 = 0.75; P = 0.0011	
baseline α-tocopherol (μmol/L)	0.704	0.338; 1.070	0.935	0.561; 1.309	0.945	0.575; 1.314
changes VF (cm^2)	–	–	–	–	−0.411	−0.787; −0.034

Linear regression analysis adjusted for age, gender and α-tocopherol intake.
TC, total cholesterol; VF, visceral fat; FM, fat mass.

be reduced and antioxidative enzyme activity should be increased, because obesity is positively associated with oxidative stress. The decrease of α-tocopherol caused a decrease of TAC, which indicated decreased antioxidative defense during fat loss. TAC value after 6 weeks more decrease in men than in women. Our findings indicated the need of further studies to explanation these differences.

In relation to adiposity indicators, we obtained interesting results for plasma α-tocopherol concentrations, since the decrease in α-tocopherol level was associated with a decrease in adiposity. Obesity, especially VF, is a confirmed risk factor for metabolic disorders [45–47]. Central adiposity and VF was found to be a stronger predictor of plasma α-tocopherol concentration. Our results are in line with several prior studies [10,11,48] that reported that α-tocopherol serum levels were positively related to BMI and central obesity (WC, WHtR, WHR). They explained the role of α-tocopherol as a gene modulator, especially involved in glucose and lipid metabolism. Waniek et al. [49] observed a strong association of the α-tocopherol/cholesterol ratio with VF. VF is significantly reduced during weight loss and a greater VF loss was associated with a higher baseline level of VF and higher baseline BMI.

VF is preferentially lost in the early part of weight loss [50]. This may explain our findings regarding decreasing plasma α-tocopherol, TAC in association with VF and fat loss, especially in men. Adipose tissue is an endocrine organ and especially VF releases more inflammatory markers [50], which could be responsible for increased oxidative stress leading to reduced α-tocopherol levels [36].

In summary, this study provides further insight regarding factors associated with plasma α-tocopherol status in overweight and obese adults during 6 weeks weight loss. Nutritional intervention, coupled with nutrition motivational interviewing, should be regarded as an effective dietary plan for weight loss, but special attention should be paid to α-tocopherol status at the beginning of fat reduction. Baseline α-tocopherol status is a strong predictor of a decrease in plasma α-tocopherol and weakened antioxidative defense during weight loss. Traber et al. [13] and Mah et al. [36] pointed out the lack of correlation between dietary α-tocopherol intakes and circulating α-tocopherol. In healthy adults, intakes of 12–15 mg α-tocopherol daily are sufficient to provide adequate vitamin E status [13], but requires further research in adults with metabolic disorders. Future studies should consider gender and obesity-related physiologic effects on circulating α-tocopherol levels. It is known that serum α-tocopherol is gender specific [36] due to hormonal differences and gender-dependent differences in the activation of the CYP enzymes involved in vitamin E metabolism [40], but our results didn't confirm this effect. But we found that strong predictors of α-tocopherol status in women were baseline α-tocopherol and changes of TC, while in men - baseline α-tocopherol and changes of VF, which requires explanation in further studies.

Several limitations of the present study should be mentioned. The main limitation of this study was the lack of inflammatory markers and oxidative damage, such as C-reactive protein, interleukins, tumor necrosis factor (TNFα), and isoprostanes. In future studies on α-tocopherol status assessment, larger and longer trials are needed to investigate the effect of gender and anthropometric parameters on α-tocopherol metabolism. In our study, we assessed only α-tocopherol, which generally predominates in plasma, but other forms of vitamin E, especially γ-tocopherol have antioxidant properties and it is the predominant form in food [51,52]. Moreover additional and better than plasma α-tocopherol plasma concentration biomarkers of vitamin E status, like adipose tissue α-tocopherol concentration or urinary α-tocopherol catabolites: α-carboxyethyl hydroxychromanol (α-CEHC) and α-carboxymethylbutyl hydroxychromanol (α-CMBHC) and biliary and fecal excretion of α-tocopherol and its catabolites should be assessed [37].

Although the study had small subject numbers, the findings lay the ground for further studies, and the analyzed relationships can be used as tools in a study of adiposity and the cardiometabolic profile in adults. Moreover, the present study proved that low baseline α-tocopherol status and adiposity in obese adults negatively affected α-tocopherol status after 6 weeks weight loss program. These results, coupled with excessive weight and low α-tocopherol intake, led to the finding that there was an increased risk of oxidative stress diseases in adults on a reduced diet. Long-term dietary restriction program for obese patients should be monitored to avoid α-tocopherol deficiency, and take into account higher dietary α-tocopherol requirements for obese people.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

The paper was written by all authors.

Statement of authorship

JH, AS conducted research; AS, MG, JF biochemical analysis; JH, MG, JF, AS analysis and interpretation data; MG, JF drafting of manuscript; JH did a critical review of the manuscript; JH, MG had primary responsibility for final content. All authors read and approved the final manuscript.

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