Antioxidant vitamin supplementation on muscle adaptations toresistance training: A double-blind, randomized controlled trial

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DMI and HPG shared senior authorship of this study. DMI carried out the formal analysis of the study and participated in its review and editing. HPG participated inthe conceptualization and design of the study, and its review, editing, and supervision. MMF participated in the conceptualization and design of the study, material preparation and data collection, carried out its original draft preparation, and par-ticipated in the review and editing. LAB, OBG, DVC, SSJ, MMD, and CRM participatedin the material preparation and data collection. All authors read and approved the final manuscript.
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A B S T R A C T

Objectives: The aim of this study was to examine whether antioxidant vitamin supplementation with vitaminC (VitC) and vitamin E (VitE) affects the hypertrophic and functional adaptations to resistance training in trained men.
Methods: This was a double-blind, randomized controlled trial in which participants were supplemented daily with VitC and VitE (n = 12) or placebo (n = 11) while completing a 10-wk resistance training programaccompanied by a dietary intervention (300 kcal surplus and adequate protein intake) designed to optimize hypertrophy. Body composition (dual-energy x-ray absorptiometry), handgrip strength, and one-repetition maximum (1-RM), maximal force (F0), velocity (V0), and power (Pmax) were measured in bench press (BP)and squat (SQ) tests conducted before and after the intervention. To detect between-group differences, mul-tiple-mixed analysis of variance, standardized differences, and qualitative differences were estimated. Rela- tive changes within each group were assessed using a paired Student's *t* test.
Results: In both groups, similar improvements were produced in BP 1-RM , SQ 1-RM SQ, and BP F0 ($P < 0.05$) after the resistance training program. A small effect size was observed for BP 1-RM ($d = 0.53$), BP F0 ($d = 0.48$),and SQ 1-RM ($d = 0.39$), but not for SQ F0 ($d = 0.03$). Dominant handgrip strength was significantly increased only in the placebo group ($P < 0.05$). According to body composition data, a significant increase was producedin upper body fat-free mass soft tissue (FFMST; $P < 0.05$) in the placebo group, whereas neither total nor segmental FFMST was increased in the vitamin group. Small intervention effect sizes were observed for upper body FFSMT ($d = 0.32$), non-dominant and dominant leg FFMST ($d = -0.39$; $d = -0.42$). Although a significant increase in total body fat was observed in both groups ($P < 0.05$) only the placebo group showed an increase in visceral adipose tissue ($P < 0.05$), showing a substantial intervention effect ($d = 0.85$).
Conclusions: The data indicated that, although VitC/VitE supplementation seemed to blunt upper bodystrength and hypertrophy adaptations to resistance training, it could also mitigate gains in visceral adipose tissue elicited by an energy surplus.

Keywords: Ascorbic acid; Vitamin C; Vitamin E; Dietary supplements; Muscle strength; Abdominal fat

Introduction

Oxidative stress is defined as an imbalance between the production of reactive oxygen and nitrogen species (RONS), and the capacityof the body's antioxidant defenses to eliminate them or repair the resulting damage [1]. RONS can cause oxidative damage to cellular components and have detrimental effects under both physiologic, such as during physical exercise [2], and disease conditions [3].

The antioxidant system is composed of enzyme and non- enzyme antioxidants. The latter can be classified as fat soluble when they are present in membranes, and lipoproteins, or water soluble, when they are found in extracellular and intracellular flu- ids [3,4]. Both can be ingested through the diet. Vitamin C (VitC),or ascorbic acid, a non-enzyme water-soluble antioxidant, is the first line of antioxidant defense in the human body [5]. VitC has multiple antioxidant actions because of its capacity to react with various RONS. Vitamin E (VitE) is a non-enzyme lipid-soluble anti- oxidant, with eight structural isomers of tocopherol and tocotrienol [6]. a-tocopherol, the most active form of VitE, is the most abundant fat-soluble antioxidant found in humans that protects against lipid peroxidation [3,7].

It has been well established that regular physical activity has considerable health benefits [5,8]. However, repeated skeletal muscle contractions generate RONS and, if intense and prolonged, exercise can cause oxidative damage to cells, both in untrainedand trained individuals [6,9]. Consequently, the intake of antioxidant supplements as a strategy to prevent or minimize the adverse effects of RONS generated during and after physical training is a common practice among athletes. However, current evidence for this way of supposedly reducing oxidative stress, accelerating recovery, and improving performance [10] is inconclusive [11,12].

On the contrary, there is growing evidence to suggest that RONSproduced during exercise play a key role in modulating cell-signaling pathways and many human redox-sensitive transcription factors [5]. RONS seem to mediate different processes such as mitochondrial biogenesis or the induction of endogenous antioxidant defense[13]. Consequently, high

doses of antioxidant supplements could interfere with certain adaptations to endurance training [14].

The effects of antioxidant vitamins on cellular adaptations to resistance training are less well understood [15]. Resistance training is a powerful stimulus for physiologic adaptations such as increased muscle strength and hypertrophy. Different mechanisms have been proposed to explain the role of RONS in regulating muscle hypertrophy [15] and force generation [16]. For instance, hydrogen peroxide appears to increase phosphorylation of the insulin-like growth factor (IGF)-I receptor and afterward, upregulates the pathway protein kinase B (Akt)-mammalian target rapamycin (mTOR)-ribosomal protein S6 kinase b-1 (P70S6K). Consequently, this pathway could be partially abolished by antioxidants [17]. Although some researchers have examined the effectsof VitC and VitE combined with resistance training [18], studies to date have varied widely in terms of training volumes and intensities, strength and hypertrophy assessment methods, trial duration, and participant age and training status.

Because of the widespread use of antioxidant vitamins [19] and the scarcity of evidence regarding their role in hypertrophic adaptations, this randomized controlled trial (RCT) sought to determine whether VitC/VitE supplementation could have an effect on resistance training induced functional and hypertrophic adaptations in trained individuals.

Methods

Participants

The participants recruited were 32 recreationally resistance-trained men aged 18 to 32 y. Participation was voluntary. A recreationally resistance-trained individuals was defined as a person who, for the past 8 mo, had undergone training involving at least one session per week [20]. Individuals were invited to participate if they were healthy and non-smokers. Exclusion criteria were the presence of cardiometabolic or musculoskeletal disorders and being under any form of supplementation. The use of any dietary supplements other than the experimental ones was not allowed during the intervention, and participants were requested to sus- pend the use of any supplements ≥2 wk before the intervention. For data analysis, 85% compliance with training and supplementation was required. Additionally, we made sure that participants reported good adherence to the planned diet designed to minimize nutrition-related biases.

Written consent was obtained before initiating the study and after the participants were made fully aware of the study's procedures, goals, and possible risks. The study protocol was conducted in line with the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of the Universidad Europea de Madrid.

This trial was registered at: https://clinicaltrials.gov/ (Identifier: NCT04828642). The protocol was published before trial commencement (2 April 2021).

Experimental design

This was a double-blind, RCT that took place in Madrid from April to June, 2021. Participants were randomly assigned to the supplementation groups VitC and VitE (VIT) or placebo (PLA). Dependent variables were assessed before and after the 10-wk intervention, which consisted of the intake of VitC plus VitE or PLA combined with a resistance training protocol. Participants avoided strenuous physical exercise outside the training protocol. If habitual, they could complete one non-resistance based training session per week. The week before the start of the study, they stopped doing any type of training.

The randomization, based on a computer-generated random allocation sequence, was performed by an external researcher and no personnel involved in the trial had access to it. During the full trial period, researchers assessing outcomes and participants were blinded to group allocation.

Training protocol

All participants followed a resistance training program 4 d/wk, consisting of two upper body training sessions and two lower body exercise sessions weekly, including exercises for all the major muscle groups and self-selected exercises for core and/or abdominal muscles. During the first 3 wk, sessions 4x10-12 repetition maximum (RM) with 2-min rest periods, and each repetition was executed at a velocity of 1:3 (1s concentric phase, 3s eccentric phase). In weeks 4 to 7, the load was 4x 8-10 RM with 2-min rest periods and a repetition execution velocity of 2:2s; and in weeks 8 to 10, the load was 4x 6-8 RM with 2- min rest periods and the repetition execution velocity was maximal:2s. Participants were instructed to record their loads in a training diary that was regularly checked by the researchers.

Supplementation with antioxidant vitamins

VitC capsules contained 1000 mg of ascorbic acid, VitE capsules contained 235 mg of DL-a- tocopherol acetate and placebo capsules contained maltodextrin (Vecos Nucoceutical). Placebo capsules were identical in shape, appearance, and taste to the vitamin capsules. Capsules were stored in two identical bins (VitC bin and VitE bin or their respective placebo) and labeled with a participant identification code. Labeling was performed by a researcher who was blind to participant codes, and allocation was conducted by another blinded researcher. Participants ingested one VitC and one VitE pill or two capsules of placebo every morning for 10 wk. Each week, participants were asked if they had consumed the supplement. At the end of the study, participants had to give back the remaining pills to ensure compliance.

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Diet control

Each participant completed a 4-d food diary before the start of the intervention. No differences were found between groups

in the intake of energy, proteins, carbohydrate, and fat, and the recommended dietary allowance of VitE and VitC was similarly covered by all participants [21] (Supplementary Table 1). Despite this, all study participants were instructed to follow a diet prescription adapted to their body weight. Energy surplus (300 kcal) was monitored in training days to maximize muscle gain [22], whereas diet was isocaloric during resting days. Protein intake was adjusted to recommendations of the International Society of Sports Nutrition [23] of an average of 2.01 g protein kg body weight d−1, and a supply of at least 0.25 to 0.4 g/kg in each meal. Carbohydrate intake was adjusted according to nutrition guidelines for resistance sports, consisting of 4 to 7 g/kg body weight d−1 [24]. The diet was distributed in three main meals and a post-training meal, which included protein (0.25-0.4 g/kg) and carbohydrate (1 g/kg) [22-24], or a snack on rest days. Foods containing high amounts of antioxidant compounds (more than two fruit juices, more than four cups of coffee or tea) and alcoholic beverages were avoided.

Body composition

Body composition was measured by dual-energy x-ray absorptiometry (DXA; Hologic QDR Discovery Wi, Bedford, MA, USA) [25] using Hologic APEX version 4.0.2. software. The instrument was calibrated using a lumbar spine phantom as recommended by the manufacturer. The test was the whole-body test in which participants were asked to maintain a supine position with slight abduction and external rotation of the hip on a stretcher for 8 min. A single trained DXA technician positioned the participants and performed the scans with the National Health and Nutrition Examination Study body composition correction function disabled [26].

Total and segmental soft tissue fat-free mass (FFMST) and fat mass (FM) were measured (kg) as trunk, upper left and right limbs, and lower left and right limbs. Total body FFMST (kg) was calculated as the sum of these measurements. Upper body FFMST (kg) was determined as the sum of FFMST values recorded for the arms and trunk, and lower body FFMST as the sum of those recorded for legs. For FM (kg), different body regions were measured: upper left and right limbs, trunk, and lower left and right limbs. Total body fat (kg) was determined as the sum of the values recorded for these regions. Visceral adipose tissue (VAT), android and gynoid fat (kg) were also measured. For the data analysis, measurements for the upper and lower left and right limbs were adjusted per the dominant or non-dominant limb.

Physical performance

Force, velocity, and power test

The variables maximal force (F0), velocity (V0), and power (Pmax) were assessed when executing an incremental test in bench press (BP) and squat (SQ) exercise according to a protocol described elsewhere [27,28], which considers the V0 decrease produced with increasing load. The test was performed in a Smith machine (Evolution Deluxe Smith Machine and Rack, Titanium Strength, Spain) until the individuals completed the 1-RM using a lineal encoder (Chronojump, Barcelona, Spain) at a frequency of 1000 Hz, and specific software for data analysis (Chronojump 1.8.1-95). This device has been previously validated to assess load displacement V0 in a resistance training machine [29]. Before the test, all participants performed 5 min of cardiovascular activity at moderate intensity as a general warm-up, followed by joint mobility exercises for the upper limbs and 4 to 5 min of passive rest before starting with the BP test. Once this test was completed, participants performed joint mobility exercises for the lower limbs followed by 4 to 5 min of passive rest and then executed the SQ test.

Handgrip strength

A calibrated handgrip dynamometer was used (Takei 5101, Tokyo, Japan) to determine isometric handgrip strength (HGS). Two measurements were made alternately per hand and the highest value used for analysis.

Statistical analysis

Sample size was calculated according to the data provided by Paulsen et al. [30]. Based on an SD of 0.7 and with an a error set at 0.05 and (1-ß) = 0.8, a mini- mum of 16 participants in each group was needed to detect a true difference of 0.7 kg in muscle mass, the primary outcome. For the secondary outcome, change in muscle strength (1-RM), based on an SD of 15 and with an a error set at 0.05 and (1-ß) = 0.8, we needed 16 volunteers in each group to detect a difference of 11%.

Descriptive statistics were calculated for each variable. The normality of the distribution of data was verified by the Shapiro Wilk test. Variables showing skewed distributions were log-transformed to obtain a normal distribution. Relative changes within each group were assessed using a paired Student's t test. To compare baseline variables the Student's t test, standardized differences (90% confidence level) and qualitative differences were estimated. To detect between- group differences, multiple 2 2 (group time) mixed-analysis of variance was performed and adjustments for multiple comparisons were made using the Bonferroni method by dividing the significance level of 0.050 by the number of com- parisons, additionally standardized differences (90% CL) and qualitative differences were estimated. The effect size of standardized differences was deter- mined by Cohen's d statistic, and the Hopkins' scale was used to determine the magnitude of the effect size, where 0 to 0.2 = trivial, 0.2 to 0.6 = small, 0.6 to 1.2 = moderate, 1.2 to 2 = large, and >2 = very large [31]. A practically worthwhile difference was assumed when the difference score was 0.2 of the between-subject SD. Qualitative assessment indicates the likelihood for the between-group differences to be substantial, referring to possible differences, to likely, to very likely, and to almost certain differences. The probability of a true difference between groups was qualitatively classified as almost certainly not: <0.5%; very unlikely: 0.5% to 5%; unlikely: 5% to 25%; possible: 25% to 75%; likely: 75% to 95%; very likely: 95% to 99.5%; and almost certain: >99.5%. A substantial effect was defined as >75% [32].

All statistical tests were carried out using the SPSS version 23 (IBM, Chicago, IL, USA) and Microsoft Excel software (Microsoft, Redmond, WA, USA).

Results

The final study population was therefore 23 individuals, 12 inthe VIT group (20.58 ± 1.78 y) and 11 in the PLA group (22.36 ± 4.18 y). Participant ow through the study is presented in the CONSORT (Consolidated Standards of Reporting Trial) diagram (Fig. 1).

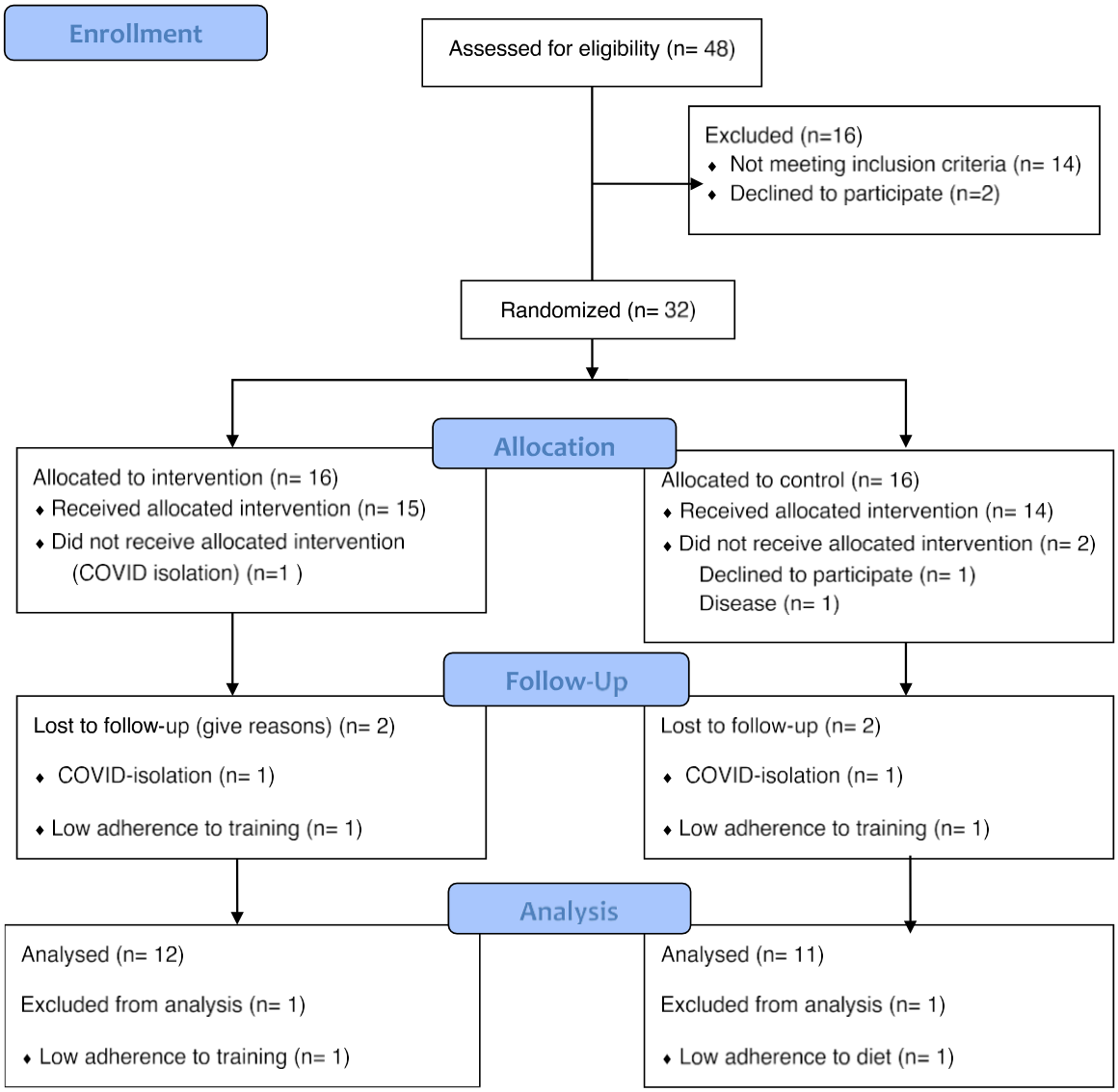

Fig. 1. CONSORT flow diagram.

Muscle strength

Self-reported training adherence was also similar between the two groups (VIT = 93.42 ± 5.50%; PLA = 94.36 6.12%; P = 0.608).

All the parameters of muscle strength evaluated were similar at baseline in both groups (Supplementary Table 2). However, a small between-group effect as assessed by Cohen's d was observed in isometric HGS in the dominant arm (d = 0.54) and a moderate effect in the non-dominant arm (d = 0.74). Effect sizes observed for 1-RM and F0 recorded in the BP and SQ tests were trivial, whereas small effects were observed for V0 and Pmax determined in both the SQ and BP tests (Supplementary Table 2).

Isometric HGS was only significantly improved postintervention in the dominant hand in the PLA group (P = 0.018), with no changes detected in the non-dominant hand in either group. In the BP exercise test, 1-RM and F0 were noted to increase significantly postintervention in both groups (VIT, P = 0.001; P = 0.002; PLA, P = 0.000; P = 0.002), whereas V0 and Pmax only improved in PLA (P = 0.005; P = 0.039). According to the SQ exercise test data, in both groups 1-RM increased (VIT, P = 0.004; PLA, P = 0.039), but nochanges were produced in F0, V0, or Pmax (Table 1).

When isometric HGS differences were compared between groups (time group), a small effect was found in the dominant hand (d = 0.25; % change VIT = 2.72%; PLA = 4.88%) and non-dominant hand (d = 0.29; VIT = 2.88%; PLA = 14.13%).

No significant differences between groups were found in the BP or SQ measurements ($P > 0.025$). However, a small effect size was observed in BP 1-RM (d = 0.53; % change VIT = 12.40%; PLA = 18.30%), BP F0 (d = 0.48; VIT = 10.54%; PLA = 14.13%), BP V0 (d = 0.46; VIT = 3.78%; PLA = 8.24%), and in SQ 1-RM (d = 0.39;VIT = 9.85%; PLA = 8.29%), SQ V0 (d = 0.26; VIT = 0.02%;PLA = 5.79%), and SQ Pmax d = 0.42; VIT = 3.16%; PLA = 162.68%). Additionally, a substantial intervention effect on the BP 1-RM was revealed by the Hopkins' qualitative assessment method (Table 1).

Table 1
Changes in performance measures produced in response to the training/supplementation intervention

	Group	Pre, mean (SD)	Post, mean (SD)	*P* pre–post (intra-group)	% intra-group change from baseline	Between- group difference M (90% CL)	*P* (group × time)*	Between-group standardized differences (90% CL)†	Between-group qualitative assessment of difference ‡	
D handgrip (kg)	VIT	40.74 (4.01)	41.95 (4.06)	0.100	3.16 (5.81)	−1.42 (4.17)	0.611	0.25 (0.69)	55/31/14	Possible
	PLA	44.06 (7.45)	45.87 (6.79)	0.018	4.58 (5.80)					
ND handgrip (kg)	VIT	38.38 (4.01)	38.46 (3.24)	0.943	0.91 (11.05)	2.89 (7.20)	0.388	−0.29 (0.69)	12/29/59	Possible
	PLA	42.65 (6.54)	41.57 (5.65)	0.362	−1.98 (8.75)					
BP 1-RM (kg)	VIT	75.76 (16.30)	84.95 (18.63)	0.001	12.40 (9.16)	−5.90 (7.86)	0.203	0.53 (0.69)	79/17/4	Likely
	PLA	76.19 (17.67)	89.27 (18.53)	0.000	18.30 (12.63)					
BP F0 (n)	VIT	774.00 (148.47)	853.68 (166.80)	0.002	10.54 (8.81)	−3.59 (5.70)	0.249	0.48 (0.69)	75/19/5	Likely
	PLA	795.91 (175.29)	903.16 (178.82)	0.000	14.13 (6.85)					
BP V0 (m/s)	VIT	2.44 (0.22)	2.34 (0.26)	0.167	−3.78 (9.07)	4.46 (6.13)	0.271	−0.46 (0.69)	6/21/74	Possible
	PLA	2.33 (0.18)	2.13 (0.21)	0.005	−8.24 (7.90)					
BP Pmax (m/s/n)	VIT	474.00 (116.72)	497.98 (108.4)	0.764	5.80 (5.52)	1.40 (4.53)	0.743	−0.13 (0.70)	21/36/43	Possible
	PLA	464.65 (112.90)	485.18 (121.28)	0.039	4.39 (7.08)					
SQ 1-RM (Kg)	VIT	118.29 (22.62)	129.26 (21.22)	0.004	9.85 (8.40)	1.56 (6.84)	0.795	−0.39 (0.71)	8/24/68	Possible
	PLA	118.23 (19.75)	127.90 (23.52)	0.039	8.29 (10.62)					
SQ F0 (n)	VIT	2156.14 (352.33)	2214.98 (289.82)	0.308	3.42 (7.87)	0.60 (4.48)	0.938	0.03 (0.70)	34/37/28	Possible
	PLA	2168.50 (264.19)	2232.79 (321.12)	0.126	2.82 (5.20)					
SQ V0 (m/s)	VIT	2.48 (0.51)	2.43 (0.52)	0.713	0.02 (19.40)	−5.77 (14.66)	0.534	0.26 (0.72)	55/30/14	Possible
	PLA	2.65 (0.55)	2.73 (0.43)	0.634	5.79 (21.48)					
SQ Pmax (m/s/n)	VIT	1323.16 (334.82)	1329.61 (255.93)	0.764	3.16 (17.33)	−129.5 (201.0)	0.302	0.42 (0.74)	69/22/8	Possible
	PLA	1331.17 (527.97)	1510.71 (232.21)	0.283	132.68 (405.12)					

BP, bench press; CL, confidence level; D, dominant; F0, maximal force; Pmax, maximal power; V0, maximal velocity; ND, non-dominant; RM, repetition maximum; PLA, placebo group; SQ, squat; VIT, vitamin C+ vitamin E supplementation group
*Significant for between-group comparisons when $P < 0.002$ (i.e., 0.05/25 comparisons = 0.002).
†Threshold values for Cohen's ES were trivial (0.0–0.2), small (0.2–0.6), moderate (0.6–1.2), large (1.2–2), and very large (>2.0).
‡Numbers shown are the quantitative chances (%) that the effect is positive/trivial/negative. A substantial effect was defined as >75 %.

Muscle hypertrophy

No significant differences in total or regional FFMST was observed at baseline between groups (Supplementary Table 2). However, a small between-group effect as assessed by Cohen's d was detected in FFMST in the dominant leg (d = 0.29) and non- dominant leg (d = 0.32; Supplementary Table 2).

After the 10-wk intervention, the variables FFMST for the dominant arm (P = 0.001), non-dominant arm (P = 0.028) and totalupper body muscle mass (P = 0.037) were higher only in the PLA group. In contrast, the VIT intervention did not lead to a significant increase in total or segmental muscle mass (Table 2).

Our intergroup comparisons (time group) revealed a small effect of the intervention on FFMST for the dominant arm (d = 0.56; VIT = 2.72%; PLA = 4.88%), trunk (d = 0.24; VIT = 1.29%; PLA = 2.09%), upper body (d = 0.32; VIT = 1.53%; PLA = 2.42%), non- dominant leg (d = —0.39; VIT = —0.09%; PLA = —1.6%), and dominant leg (d = —0.42; VIT = 1.12%; PLA = —0.78%). Additionally, through Hopkins' qualitative assessment, we detected a substantial intervention effect on upper body FFMST (Table 2).

Table 2
Changes in FFMST produced in response to the training/supplementation intervention

	Group	Pre, m (SD)	Post, mean (SD)	*P* pre–post (intra-group)	% intra-group change from baseline	Between- group difference M (90% CL)	*P* (group × time)*	Between-group standardized differences (90% CL)†	Between-group qualitative assessment of difference‡	
Body mass (kg)	VIT	73.07 (5.62)	73.89 (5.52)	0.108	1.16 (2.32)	−0.53 (1.83)	0.557	0.24 (0.70)	54/32/15	Possible
	PLA	74.39 (7.23)	75.67 (7.98)	0.069	1.69 (2.78)					
FFMST total (kg)	VIT	50.02 (3.65)	50.50 (2.96)	0.202	1.08 (2.56)	0.04 (1.86)	0.893	0.05 (0.69)	36/37/27	Possible
	PLA	50.96 (5.77)	51.53 (6.25)	0.203	1.04 (2.62)					
D- arm FFMST (kg)	VIT	3.43 (0.28)	3.52 (0.24)	0.071	2.72 (4.56)	−2.16 (3.01)	0.179	0.56 (0.69)	81/15/4	Likely
	PLA	3.43 (0.56)	3.59 (0.58)	0.001	4.88 (3.76)					
ND- arm FFMST (kg)	VIT	3.21 (0.26)	3.29 (0.24)	0.177	2.88 (6.22)	0.12 (3.69)	0.926	0.04 (0.69)	34/38/28	Possible
	PLA	3.23 (0.50)	3.33 (0.55)	0.028	2.76 (3.61)					
FFMST trunk (kg)	VIT	25.23 (2.14)	25.51 (2.61)	0.285	1.29 (3.51)	−0.80 (2.65)	0.550	0.24 (0.70)	54/32/14	Possible
	PLA	25.55 (2.70)	26.07 (2.74)	0.129	2.09 (3.88)					
Upper body FFMST (kg)	VIT	31.87 (2.38)	32.32 (1.93)	0.100	1.53 (2.88)	−0.89 (2.18)	0.430	0.32 (0.70)	62/28/11	Possible
	PLA	32.22 (3.68)	32.99 (3.84)	0.037	2.42 (3.18)					
D- Leg FFMST (kg)	VIT	9.07 (0.79)	9.15 (0.57)	0.519	1.12 (3.65)	1.90 (2.59)	0.376	−0.42 (0.69)	7/23/70	Possible
	PLA	9.34 (1.03)	9.28 (1.20)	0.552	−0.78 (3.56)					
ND- leg FFMST (kg)	VIT	9.08 (0.72)	9.06 (0.63)	0.968	−0.09 (3.70)	1.51 (2.28)	0.279	−0.39 (0.69)	8/24/68	Possible
	PLA	9.40 (1.13)	9.26 (1.27)	0.071	−1.60 (2.48)					
Total legs FFMST (kg)	VIT	18.15 (1.49)	18.21 (1.19)	0.455	0.19 (1.37)	0.41 (0.96)	0.305	−0.36 (0.69)	9/26/65	Possibly
	PLA	18.75 (2.15)	18.55 (2.46)	0.682	−0.22 (1.29)					

CL, confidence level; FFMST, fat-free mass soft tissue; D, dominant; ND, non-dominant; RM, repetition maximum; PLA, placebo group; VIT vitamin C + vitamin E supplementation group
*Significant for between-group comparisons when $P < 0.002$ (i.e., 0.05/25 comparisons = 0.002).
†Threshold values for Cohen's ES were trivial (0.0–0.2), small (0.2–0.6), moderate (0.6–1.2), large (1.2–2) and very large (>2).
‡Numbers shown are the quantitative chances (%) that the effect is positive/trivial/negative. A substantial effect was defined as >75%.

Body fat

A small intergroup effect size in total body fat (d = 0.21), leg fat (d = 0.38), and gynoid fat (d = 0.36) was observed at baseline (Supplementary Table 2).

In response to the intervention, both groups showed significant increases in total body fat (VIT, P = 0.035; PLA, P = 0.042) and leg fat (VIT, P = 0.018; PLA, P = 0.034). Only the VIT group displayed a significant increase in gynoid fat (P = 0.022), whereas only the PLA group showed a significant increase in VAT (P = 0.024; Table 3).

Our intergroup comparisons (time group) revealed a qualitativesubstantial effect in VAT (d = 0.85; VIT = 0.18%; PLA = 11.01%) (Table 3).

Table 3
Changes in body fat produced in response to the training/supplementation intervention

	Group	PRE M (SD)	POST M (SD)	P pre-post	% change	M (90% CL)	*P (between groups)*	Standardized differences (90% CL)	Qualitative assessment of difference/impact	
Total body fat (kg)	VIT	14.46 (2.40)	15.08 (2.34)	0.035	4.64 (6.66)	0.10 (4.69)	0.943	0.03 (0.69)	34/37/29	Possible
	PLA	14.98 (2.24)	15.62 (2.24)	0.042	4.54 (6.37)					
VAT (g)	VIT	376.59 (42.05)	275.16 (37.26)	0.917	0.18 (10.59)	-10.83 (8.75)	0.048	0.85 (0.69)	94/5/1	Likely
	PLA	270.88 (56.81)	295.15 (41.19)	0.024	11.01 (13.71)					
Arm fat (kg)	VIT	1.76 (0.37)	1.84 (0.32)	0.086	6.42 (10.46)	2.12 (6.37)	0.873	-0.06 (0.69)	26/37/37	Possible
	PLA	1.75 (0.32)	1.84 (0.40)	0.077	4.29 (6.69)					
Leg fat (kg)	VIT	5.82 (1.07)	6.13 (1.09)	0.018	5.71 (7.32)	-0.49 (5.39)	0.728	0.14 (0.70)	44/35/20	Possible
	PLA	6.28 (1.21)	6.65 (1.26)	0.034	6.21 (7.71)					
Android fat (kg)	VIT	1.09 (0.24)	1.14 (0.25)	0.222	5.79 (14.31)	0.99 (8.33)	0.907	-0.05 (0.69)	27/38/35	Possible
	PLA	1.07 (0.21)	1.11 (0.21)	0.065	4.80 (7.58)					
Gynoid fat (kg)	VIT	2.80 (0.53)	2.96 (0.54)	0.022	6.08 (8.20)	1.69 (5.54)	0.652	-0.18 (0.69)	18/34/48	Possibly
	PLA	3.02 (0.62)	3.14 (0.601)	0.094	4.39 (7.15)					

Confidence level (CL), fat-free mass soft tissue (FFMST), dominant (D), mean (M), non-dominant (ND), one-repetition maximum (1-RM), placebo group (PLA), standard deviation (SD), visceral adipose tissue (VAT), vitamin C + vitamin E supplementation group (VIT).

Discussion

The present study sought to investigate whether supplementation with VitC and VitE could affect the functional and hypertrophic adaptations produced in response to resistance training in healthy, trained individuals. To our knowledge, few investigations have examined the effects of VitC and VitE on resistance training adaptations, and only one study with trained participants has determined the effects of traditional heavy-load resistance training. As far as we know, this is the first study to assess antioxidant vitamin effects on resistance training adaptations focusing on muscle hypertrophy while participants followed a controlled diet. The results of the present RCT indicated that this supplementation might affect skeletal muscle hypertrophy and upper body strength improvement in response to 10 wk of resistance training.

Muscle strength

The conclusions of a meta-analysis by Dutra et al. [18] were thatVitC and VitE supplementation has no effect on muscle strength, as assessed through isokinetics. However, the participants of the studies included in this meta-analysis were elderly [33 35],untrained [36], or completed short isokinetic eccentric training programs (4 wk) [37,38]. Interestingly, Dutra et al. [36] found that VitC plus VitE supplementation in untrained young women did not improve muscle performance compared with women subjected to a control or PLA intervention. In another study whose participants were also untrained young woman, with training focused on maximizing strength and hypertrophy, no strength differences were found between groups [39].

Similar to our results, Paulsen et al. [30] carried out a 10-wk intervention consisting of VitC (1000 mg/d) and VitE (235 mg/d) intake combined with heavy-load resistance training four times perweek in recreationally resistance-trained men and women. Thisinvestigation showed that in both the experimental and PLA groups,1-RM in leg and upper body exercises improved. However, according to effect sizes, the PLA group seemed to show a greater increasein upper body strength including a significant difference for biceps curl and a similar increasing trend for maximal voluntary contraction of the knee extensors. Here, we also observed a trend toward a greater increase in BP F0 and 1-RM in the PLA group. Moreover, isometric HGS in the dominant hand was only significantly increasedin the PLA group. In the SQ test, although 1-RM tended to show a larger increase in the VIT group, this was not observed for F0.

The present results supporting those of Paulsen et al. [30] thus suggesting that supplementation with VitC and VitE could bluntupper body strength gains in response to a resistance training pro- gram in young, trained individuals. Although neither this study nor our study examined redox status, the mechanism underlying this finding could be that the presence of muscle-derived RONS is necessary for an optimal contractile force of skeletal muscle and this may have been modified by antioxidant supplementation [16].

Muscle hypertrophy

In the meta-analysis by Dutra et al. [18], the effect of antioxidant vitamin supplementation on hypertrophy adaptations in response to resistance training was also assessed. However, no valid conclusions could be drawn because of the different methods of the studies examined. Although one of the studies conducted in older adults found a greater increase in fat-free mass gain [34], results could not be replicated in a subsequent study with the same protocol and a larger sample size [33]. In contrast, Bjørnsen et al. [35] reported a greater increase in rectus femoris thickness in their PLA group compared with a VitC/VitE supplementation group. Notwithstanding, these three studies were performed on elderly individuals and thus are not comparable to ours, as the aging process is characterized by enhanced oxidative stress [40] and a lower hypertrophy capacity. One of the studies in untrained women found no between-group differences in rectus femoris thickness. The only study performed in trained adults detected no significant differences in muscle mass after chronic antioxidant supplementation. In contrast, a similar study in untrained women showed that total fat-free mass was only increased in individualsin the PLA group after a 10-wk resistance training protocol [39].

In the present study, participants in the PLA group only experienced a significant increase in upper body FFMST, which was greater than in the VIT group. Notwithstanding, in neither group was there a significant increase in lower body FFMST. Lower body strength tended to be greater in the VIT group, yet the percentage change produced in both groups was small. Consequently, we suggest a tendency of VitC/VitE supplementation to blunt any upper body FFMST increase produced in response to a resistance training and nutrition program targeting hypertrophy.

Remarkably, Paulsen et al. [30] reported that VitC/VitE supple- mentation interfered with exercise-induced signaling in muscle cells after a resistance training session, observing reduced phosphorylation of P70S6K and mitogen-activated protein kinases p38 and extracellular signal-regulated kinase (ERK)1/2. In line with these results, Makanae et al. [41] observed that oral VitC attenuated overload-induced skeletal muscle hypertrophy in rats and this effectwas related to diminished of ERK1/2 and P70S6K phosphorylation attributed to the antioxidant. Furthermore, different RONS havebeen reported as essential signals for regulation of the mTOR- P70S6K pathway [15,17,42]. As mentioned previously, Handaya ningsih et al. [17] observed that endogenous and exogenous hydro- gen peroxide enhanced the activation of the IGF-I receptor and subsequently, upregulated Akt-mTOR-P70S6K. On the contrary, antioxidant administration reduced the IGF-I receptor phosphorylation, and consequently the signaling pathway Akt-mTOR-P70S6K was downregulated. Additionally, Ito et al. [42] observed that peroxynitrite and nitric oxide actives the transient receptor potential cat-ion channel subfamily member 1 (TRPV1), causing an increase of intracellular Ca^{2+} concentration and triggering mTOR activation.

Body fat

During the present intervention, participants ingested an energy surplus (300 kcal) to maximize skeletal muscle hypertrophy according to protocols described elsewhere [22]. Consequently, in both groups, body fat mass was significantly increased. However, only the PLA group underwent a VAT increase. Previous studies have either not investigated changes in body fat [36,38] or have shown that no changes in body fat occur during the intervention [20,34,35]. According to Bobeuf et al. [34], only VitC/VitE intake and/or resistance training can prevent increases in total andabdominal body fat in elderly individuals. On the contrary, Dutraet al. [39] observed a reduction in body fat mass in their PLA group but not in their vitamin and control groups, although VAT was not analyzed. However, none of these studies was designed to inducean energy surplus so we cannot compare their findings with the body fat changes detected in our participants.

VitC supplementation has been shown to reduce VAT in obese mice fed a high-fat diet, an effect thought to be mediated partly by the upregulated expression of proliferator-activated receptor a (*PPAR*-a) target genes responsible for fatty acid b-oxidation [43]. More longitudinal studies are needed to confirm the possible effects of VitC/VitE supplementation in preventing VAT increasesin response to surplus energy.

Limitations and strengths

A major strength of this study was its robust experimental design based on a double-blind RCT including placebo and a con- trolled diet and training program. Other studies have only analyzed participant diets before or after a training intervention by recommending products to be avoided (other supplementation, coffee, alcohol, sources rich in antioxidants) and following a routine diet [20,34,35]. In the present investigation, each participant was given a personalized diet to follow based on nutritional recommendations targeting muscle hypertrophy. However, adherence to these nutritional targets differed between participants. Body composition was measured by a practical criterion reference method, DXA [44], as this technique has been used in most of the studies withthe same purpose [33,34,39] and has shown a satisfactory level of precision for the measurement of VAT in population with a body mass index >18.5 kg/m^2 [45]. As a limitation, we should mention that protein synthesis and oxidative stress markers were not analyzed. Such data could help elucidate the biological mechanisms underpinning our results. Also, participants were not supervised when training, although to check training adherence, each week they reported weights lifted in the BP and SQ tests. Another limitation was the small number of participants due to a high withdrawal rate during a high-commitment intervention lasting 10 wk.

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