Moderate walnut consumption improved lipid profile, steroid hormones and inflammation in trained elderly men: a pilot study with a randomized controlled trial

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ABSTRACT: The present study aimed to investigate the effect of walnut consumption on lipid profile, steroid hormones and inflammation in trained elderly men performing concurrent (resistance and endurance) training. Twenty healthy elderly males were divided into two matched groups, in a randomized controlled trial, that trained three sessions per week: concurrent training + dietary walnut consumption (15 g/day for six weeks, CTW: $n = 10$); concurrent training + control diet (CT: $n = 10$). Fasting blood samples were taken 48 hours before and after intervention for biochemical assessments. levels of high-density lipoprotein (HDL) increased only in CTW compared to baseline (19.8%, $p < 0.01$). Total cholesterol (TC), low-density lipoprotein (LDL) and triglyceride (TG) levels significantly decreased only for CTW (i.e., 13%, 18%, and 18.5% at $p < 0.01$ for all). Testosterone (T) increased after the training compared to pre-training for CTW and CT (10.3%, $p < 0.01$, 4.27% $p < 0.05$, respectively), but the increase was significantly higher in CTW ($p < 0.05$). Serum cortisol (C) was lower for CTW compared to CT ($p < 0.01$). C-reactive protein (CRP) decreased in CTW in comparison with CT. The present study revealed that 6-week moderate walnut supplementation (15 g/day) improved lipid profile, steroid hormones and systematic inflammation in aged men performing concurrent training. These findings could be attributable to the potential effect of polyunsaturated fatty acids (PUFA) contained in walnut (linoleic acid, n-6; linolenic acid, n-3).

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INTRODUCTION

Biological aging is associated with morphological and biochemical alterations that increase the risk of developing cardiovascular diseases, representing the major cause of morbidity and mortality in older adults [1]. Among these risk factors, blood glucose (BG) and lipid profile have been shown to have a greater impact on cardiovascular disease risk [2]. Moreover, aging is characterized by a reduction in systemic levels of testosterone (T) [3], a sex hormone with a profound influence on various tissues [4, 5]. Low T levels have various adverse health consequences, such as loss of muscle mass, increased fat mass, reduced aerobic capacity, and increased cardiovascular disease risk [3].

Health benefits of physical training are indisputable in combating age-related risks for disease and disability [6], and understanding the basis of such benefits is of high interest [7]. Concurrent training,

Abbreviations:
CT: concurrent training, CTW: concurrent training + walnut, HDL: high-density lipoprotein, BG: blood glucose, TC: total cholesterol, LDL: low-density lipoprotein, TG: triglycerides, T: testosterone, C: cortisol, CRP: C-reactive protein, PUFA: polyunsaturated fatty acids, LA: linoleic acid, ALA: α-linolenic acid, 6MWT: 6-minute walk test.

involving endurance and strength training performed within the same session, has been suggested as a more effective strategy in aging than either endurance or strength training performed alone because of its potential to simultaneously improve multiple components of fitness [8–10]. Nevertheless, studies investigating the biochemical responses during concurrent training in the elderly are relatively scarce [11]. It has been reported that high-density lipoprotein (HDL) was significantly improved after 12 weeks of concurrent training in the elderly [12]. Other research highlighted an improvement of T level in aging men [13]. However, concurrent training for 21 weeks had no effect on cortisol (C) level in older adults [13] and did not alter inflammation markers in middle-aged men [14].

Walnut is nutrient-dense food with a high content of unsaturated fat [15]. While most nuts are high in monounsaturated fatty acids, walnuts are predominantly composed of polyunsaturated fatty acids (PUFA) (47% of total weight), mainly linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3) [15]. In addition to a favourable lipid profile, walnut also contains other bioactive compounds such as fibre, phytosterols, L-arginine, polyphenols, melatonin, minerals and tocopherols [16]. Dietary intervention studies without physical training have demonstrated beneficial effects of walnut consumption on blood lipid profile [17] and coronary heart disease [18]. In this regard, it has been demonstrated that daily walnut supplementation of 43 g for 8 weeks significantly reduced non-HDL cholesterol and apolipoprotein-B in healthy men and postmenopausal women [15]. Other studies also showed that walnut supplementation improved endothelial function in hypercholesterolaemic [16] and type 2 diabetic older women [19]. It has also been shown that PUFA contained in walnut decreased glucocorticoid concentrations in guinea pigs [20], and played an important role in testicular steroidogenesis in male rats [21] but did not improve anti-inflammatory parameters in healthy older men [22].

To the best of the authors' knowledge, there is no scientific study that has attempted to investigate the effect of walnut supplementation on biological responses in healthy trained aged men. Thus, the aim of the present study was to examine the effects of walnut supplementation coupled with concurrent training on lipid profile, steroid hormones and systemic inflammation in physically active elderly men.

MATERIALS AND METHODS

Participants

Forty-six physically active elderly men (≥ 65 years) volunteered to participate in this study. Smoking and alcoholic participants or those

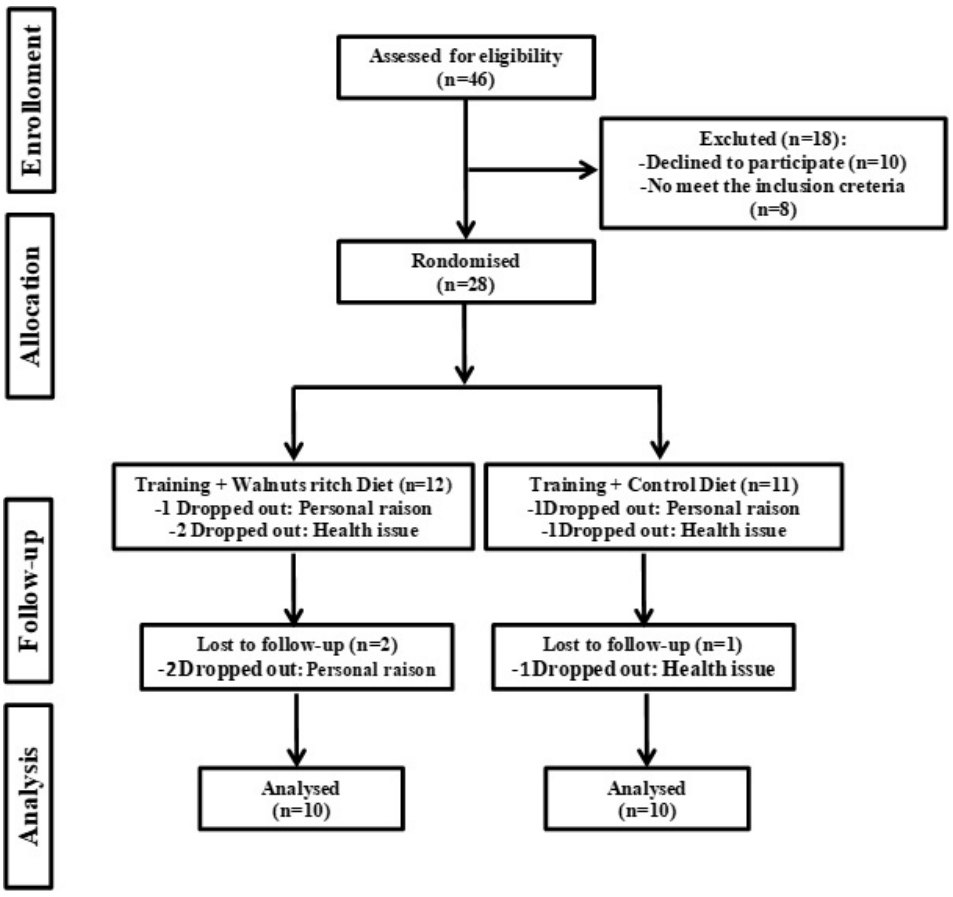

FIG. 1. Flowchart of study participants.

TABLE 1. Baseline of Anthropometric Measurements.

Variables	CT (M ± SD)	CTW (M ± SD)	P
Age (y)	66.9 ± 2.13	66.5 ± 2.68	0.71
Height (m)	1.71 ± 0.07	1.73 ± 0.06	0.62
Body mass (kg)	74.72 ± 7.95	73.09 ± 8.18	0.96
BMI (kg/m^2)	25.51 ± 2.48	24.5 ± 2.45	0.72
6MWT (m)	216 ± 15	213 ± 19.78	0.82

Note: CT: training group, CTW: training + walnut, Pre: Pre intervention, Post: Post intervention, M ± SD: Mean value ± standard deviation, BMI: body mass index, 6MWT: 6-minute walk test, $p > 0.05$ = no significant difference from CT.

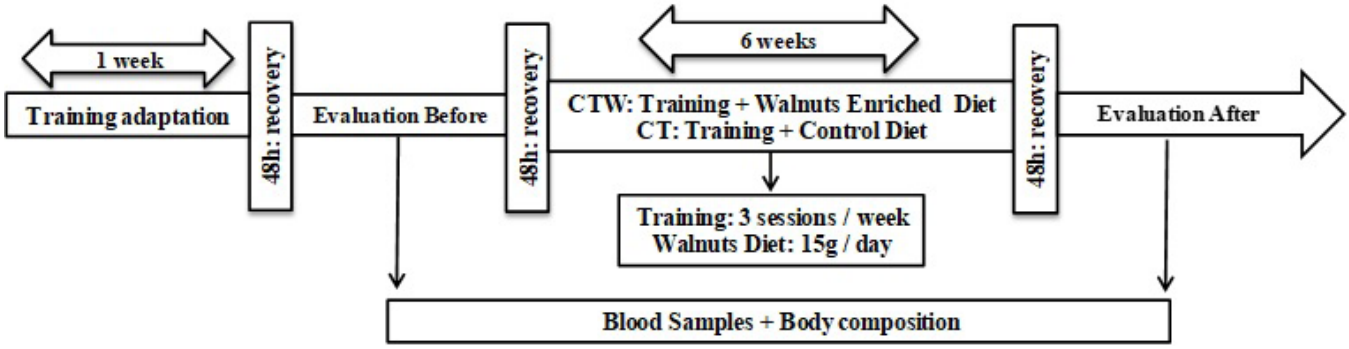

FIG. 2. Study Design. CT: training group + control diet, CTW: training + walnut diet, Pre: before intervention, Post: 48 hours after intervention, 6MWT: 6-minute walk test.

with a background in systematic physical training during the 2 months before the study were excluded. No participant was taking drugs that may influence the sleep/wake cycle. After receiving a thorough description of the protocol, its benefits and risks, each participant provided written informed consent and underwent a clinical examination which included a full medical check-up, anthropometric measurements, resting electrocardiogram (ECG) and 6-minute walk test (6MWT). Twenty-eight participants meeting the participation criteria were divided into two groups in a randomized controlled trial: concurrent training + walnut enriched diet (CTW, n = 15); concurrent training + control diet (CT, n = 13) (Figure 1). Eight participants were withdrawn from data analysis due to protocol violations: acute infection with the use of antibiotics > 6 days (n = 4), personal reason (n = 4) (i.e., obliged to leave the city due to family commitments) (Figure 1). Ultimately, twenty men completed pre- and post-intervention (CTW, n = 10; CT, n = 10) (Figure 1). The baseline anthropometric measurements and 6MWT of the participants are shown in Table 1.

Experimental design

The experimental protocol consisted of taking two blood samples 48 hours before and after concurrent strength and endurance training (three sessions/week) for six weeks associated with walnut supplementation for CTW (15 g/day) only (Figure 2). One week prior to and during the experimental period, participants were instructed to refrain from consuming any type of walnut, except for the walnut provided. To evaluate the short-term (6 weeks) effects of CT and CTW groups, assessments were made prior to the start and at the end of the 6-week training period. Walnut consumption for CTW was taken from the first day of training.

Before starting the protocol, participants were familiarized with training exercises for one week (Figure 2). They were also requested to avoid physical activity for 48 hours preceding each test.

Blood collection and biochemical analyses

The blood samples were conducted 48 hours before and after intervention (Figure 2). Fasting venous blood samples (10 ml) were drawn after supine rest in the morning at the Laboratory of Biochemistry. Samples were immediately centrifuged at 3000 rpm (× g) and 4°C for 15 min. Aliquots of the resulting plasma were stored at -80°C until analysed. All samples were analysed in the same assay run to eliminate inter-assay variance. All assays were performed with Bio-Rad control. BG was measured enzymatically using the hexokinase method. Total cholesterol (TC), HDL, LDL and triglycerides (TG) were measured enzymatically. C-reactive protein (CRP) was measured with the immune-turbidimetric method. T and C concentrations were assessed by chemiluminescent microparticle immunoassay (CMIA). For all the above assays, the COBAS 6000 automated system by

TABLE 2. Lipid composition of walnut consumption.

Nutrients 100 g	
Lipids (g)	65.44
Polyunsaturated fatty acids	48.42
Linoleic acid (18:2n_6) (g)	39.62
α-Linolenic acid (18:3n_3) (g)	8.8
Monounsaturated fatty acid	
Oleic acid (18:1n_9) (g)	11.1
Saturated fatty acid	
Palmitic acid (g)	4.15

Roche Diagnostics (Tokyo, Japan) and all relevant diagnostic reagents of the same company were used.

Dietary intervention

All participants received a detailed verbal explanation and written instructions on data collection procedure. Participants of the CTW group were asked to consume 15 g of walnut (*Juglans regia*) at 10h:00 am daily additionally to their habitual diets [23]. Documents were provided to assist participants with integrating walnut into their diets. Moreover, participants were reminded by phone to consume walnuts every day. No other specific dietary advice was provided and there were no restrictions on fat or calorie intake.

For the CT group, participants were asked to maintain their usual dietary habits during the period of intervention. Before starting the protocol, the fatty acid compositions of the walnut samples were determined and data are presented in Table 2.

Concurrent training programme

Both groups took part in a concurrent training programme during the same session that lasted 6 weeks. The strength exercises were performed first and were immediately followed by the endurance training since a previous study demonstrated that strength gains may be optimized with strength training prior to endurance in an intra-session exercise sequence [9]. CTW and CT groups performed the same exercise intensity and volume per session (see below). CTW trained on Mondays, Wednesdays, Fridays, and the participants of CT trained on Thursdays, Tuesdays, and Saturdays. All the training sessions were carefully supervised by at least 2 experienced personal trainers. The strength training programme included 6 exercises (leg press, leg extension, leg curl, seated row, bench press, and abdominal exercises). These exercises were chosen based on the recommendations of the American College of Sports Medicine (ACSM, 2009). During the first two weeks, participants performed three sets of 12–10 RM, progressing to 10–8 RM (weeks 3–4) to finish with three sets of 8–6 RM (weeks 5–6). During each set, the workload was adjusted when repetitions performed were either under or above the repetitions established [24]. The recovery between sets lasted ~120 s. The endurance training programme was based on treadmill running (Finnlo MaximumTR 8000). The endurance training session lasted 30 min with intensity individually monitored and maintained between 65 and 75% of the theoretical maximum heart rate [9]. 92% of the training programme was completed by all of the participants.

Statistical analyses

The statistical analyses were performed using the Statistica 10 software (StatSoft, Maisons-Alfort, France). Data were presented in the text, tables and figures as mean ± standard deviation (M ± SD). Normality of the distribution was checked and confirmed using the Shapiro-Wilk test. Unpaired Student's t-test was used to assess differences of anthropometric measurements and 6MWT between CTW and CT for baseline. The protocol-related effects were assessed using a two-way mixed analysis of variance (ANOVA) [(group (CT, CTW) × training (pre, post)] with repeated measures for training effect only. When ANOVA showed a significant effect, a Tukey post-hoc test was applied. Effect sizes were calculated as partial eta-squared η_p^2 to assess the practical significance of findings of the present study. Statistical significance was set at $p < 0.05$.

RESULTS

Blood glucose and lipid profile

The two-way ANOVA showed a significant effect of training for resting BG ($F_{(1,18)} = 17.1$, $p < 0.01$, $\eta_p^2 = 0.48$) with a significant interaction between the two conditions (training × group) ($F_{(1, 18)} = 5.6$, $p = 0.02$, $\eta_p^2 = .24$) for BG (Table 3). The post-hoc test showed that compared to baseline BG decreased post-intervention for CTW but not for the CT group.

There was a significant effect of training for resting TC ($F_{(1,18)}$= 43.3, p<0.01, $\eta_p^2 = 0.70$) with a significant interaction between the two conditions (training × group) ($F_{(1,18)}$= 11.9, p<0.01, $\eta_p^2 = 0.40$) (Table 3). The post-hoc test showed that TC decreased after intervention for CTW only. A significant effect of training was also observed for HDL ($F_{(1,18)} = 23.4$, $p < 0.01$, $\eta_p^2 = 0.56$) (Table 3). HDL increased after intervention for CTW but not for the CT group. A significant effect of training was also observed for LDL ($F_{1,18)} = 16.5$, $p < 0.01$, $\eta_p^2 = 0.47$) with a significant interaction between the two conditions (training × group) ($F_{1, 18)} = 6.8$ $p < .05$, $\eta_p^2 = .27$) was found (Table 3). LDL decreased after intervention for CTW only. Moreover, there was a significant effect of training on TG ($F_{(1,18)} = 11.01$, $p < 0.05$, $\eta_p^2 = 0.37$) with a significant interaction between the two conditions (training × group) ($F_{(1, 18)} = 13.50$, $p = 0.01$, $\eta_p^2 = 0.42$) (Table 3). TG decreased after intervention for CTW only.

Steroid hormones

A significant effect of training was observed for T ($F_{(1,18)} = 78$, $p < 0.001$, $\eta_p^2 = 0.81$) with a significant interaction between the two conditions (training × group) ($F_{(1, 18)} = 18.19$, $p < 0.01$, $\eta_p^2 = 0.50$). T increased after intervention for both groups (Figure 3).

TABLE 3. Effect of walnut supplementation associated with concurrent training on blood glucose, lipid profile and inflammation biomarker in aging men.

Variables	Groups	Times		Anova		
		Pre (M ± SD)	Post (M ± SD)	Training Effect $F_{(1,18)}$, p, ηp^2	Group Effect $F_{(1,18)}$, p, ηp^2	Interaction $F_{(1,18)}$, p, ηp^2
BG (mmol/l)	**CT**	5.14 ± 0.69	4.96 ± 0.99	17.1, < 0.01, 0.48	NS	5.6, = 0.02, 0.24
	GTW	5.41 ± 0.45	4.74 ± 0.38*			
TC (mmol/l)	**GT**	4.70 ± 0.41	4.50 ± 0.37	43.3, < 0.01, 0.7	NS	11.9, < 0.01, 0.4
	CTW	4.87 ± 0.44	4.23 ± .23*#			
HDL (mmol/l)	**CT**	1.13 ± 0.12	1.24 ± 0.20	23.4, < 0.01, 0.56	NS	NS
	CTW	1.11 ± 0.04	1.33 ± 0.03*			
LDL (mmol/l)	**CT**	2.71 ± 0.46	2.59 ± 0.63	16.5 < 0.01, 0.47	NS	6.8, = 0.01, 0.27
	CTW	2.80 ± 0.33	2.27 ± 0.78*			
TG (mmol/l)	**CT**	1.63 ± 0.18	1.51 ± 0.23	11.01, < 0.05, 0.37	NS	13.5, = 0.01, 0.42
	CTW	1.62 ± 0.17	1.32 ± 0.08 *			
CRP (mg/l)	**CT**	2.00 ± 0.29	2.1 ± 0.14	21.3, < 0.01, 0.5	NS	47.7, < 0.01, 0.70
	CTW	1.99 ± 0.30	1.43 ± 0.22 *#			

CT: training group, CTW: training + walnut, Pre: Before intervention, Post: 48 hours after intervention, M ± SD: Value ± standard deviation, BG: Blood glucose, HDL: High-density lipoprotein, TC: Total Cholesterol, LDL: Low-density lipoprotein, TG: Triglycerides, CRP: C-reactive protein, *: Significant difference with pre intervention ($p < 0.05$), #: Significant difference from CT ($p < 0.05$).

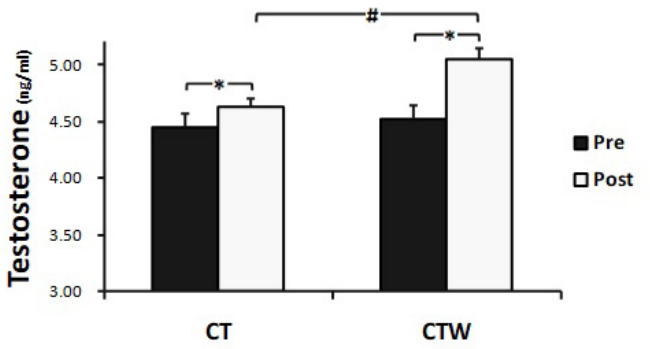

FIG. 3. Mean values and standard deviations of testosterone level for two groups (CT, CTW). CT: training group, CTW: training + walnut, Pre: Before intervention, Post: 48 hours after intervention, *: Significant difference with Pre intervention ($p < 0.05$), #: Significant difference from CT ($p < 0.05$).

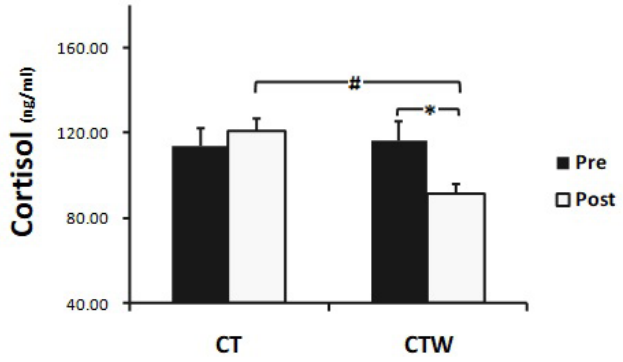

FIG. 4. Mean values and standard deviations of cortisol level for two groups (CT, CTW). CT: training group, CTW: training + walnut, Pre: Before intervention, Post: 48 hours after intervention, *: Significant difference with Pre intervention ($p < 0.05$), #: Significant difference from CT ($p < 0.05$).

Moreover, T was higher for CTW as compared to CT after intervention (Figure 3). A significant training effect on C was observed ($F_{(1,18)} = 7.1$, $p < 0.01$, $\eta_p^2 = 0.28$) with a significant interaction between the two conditions (training × group) ($F_{(1,18)} = 22.1$, $p < 0.01$, $\eta_p^2 = 0.55$). C decreased after intervention for CTW only (Figure 4).

Inflammation biomarker

A significant effect of training was observed for CRP ($F_{(1,18)} = 21.3$, $p < 0.01$, $\eta_p^2 = 0.5$) with a significant interaction between the two conditions (training × group) ($F_{(1,18)} = 47.7$, $p < 0.01$, $\eta_p^2 = 0.7$) (Table 3). CRP decreased after intervention for CTW only. Moreover, the decrease of CRP after intervention was significantly higher for CTW compared to CT (Table 3).

DISCUSSION

The present study clearly revealed that daily supplementation with 15 g of walnuts associated with concurrent training for six weeks significantly increased T and HDL levels. Moreover, BG, TC, LDL, C and CRP decreased only for CTW compared to CT.

The present findings showed that BG was significantly decreased after intervention (12.38%) for CTW but did not change for the CT group. Accordingly, it has been reported that walnut supplementation decreased BG and risk of insulin resistance in participants with type 2 diabetes [25, 26]. The lack of decrease in BG for the CT group is in accordance with a previous study indicating that fasting BG did not change after 21 weeks of combined or isolated strength and endurance training in middle-aged and older women [27].

TC, TG and LDL were decreased by 13%, 21%, 18%, respectively and HDL increased by 14.4% for CTW. These findings highlighted the beneficial effect of walnut supplementation associated with concurrent training in aged men. Nevertheless, for the sake of comparisons, studies on the effect of walnut supplementation associated with physical training in aging are limited. Regarding the effect of CT alone, a review by Tambalis et al. [11] suggested that some combined training protocols have been effective in lowering LDL and increasing HDL, while others have not. In this context, it has been reported that LDL was significantly reduced following concurrent training in healthy young men [28]. For isolated training, it has been shown that strength training improved lipid profile in obese older women [29]. In the present study, the reduction in TC and LDL was not significant in the CT group. The discrepancy with former studies may be explained by the short duration of the training protocol (6 weeks vs 12 weeks) and the cohort age (aging vs young) [11]. The present results highlighted that walnut supplementation associated with combined training improved lipid profile in aged men despite the relatively low dose of walnut (i.e., 15 g/day). This suggests potentially interesting benefits of walnut supplementation when associated with physical training. Concerning the effect of walnut supplementation alone on lipid profile, several randomized controlled trials have found a reduction in TC and LDL with different doses of walnut for young healthy people [15, 30, 31]. Indeed, it has been shown that daily walnut (43 g) consumption for 8 weeks significantly reduced non-HDL cholesterol [15]. Another study showed that LA and ALA contained in the walnut diet (44 g/day for 4 weeks) lowered non-HDL levels in healthy young women [30]. It has also been demonstrated that including walnut as part of a regular diet for 6 months improved plasma lipid profile [31].

The present results clearly demonstrate an improvement in T (12%, 4.2% respectively for CTW and CT) with a significantly greater increase for CTW. This result showed the beneficial effect of CTW in T metabolism. Concerning the effect of physical training alone, the present findings agree with previous investigations reporting increased basal T in older men following CT [13]. This fact is important for medical practitioners because exercise has been proposed as an initial treatment for low T [32]. Concerning the isolated walnut effect on T level, several randomized controlled trials have shown that walnut or walnut oil improved steroid hormones levels in human and animal models [21, 33, 34]. Indeed, it has been shown that walnut oil had stimulating effects on the male reproductive system and could increase plasma T levels by influencing the pituitary-testicular axis [21]. Otherwise, it has been demonstrated that both ALA and LA in walnut can affect reproductive processes through different mechanisms [35]. They provide precursors for the synthesis of prostaglandins and regulate the expression patterns of key enzymes evolved in the metabolism of steroids and prostaglandins [35].

In the present study, serum C decreased by 21% in the CTW group. As previously shown by others in elderly men [10] and in young recreational endurance runners [36], CT alone did not affect serum C levels in the present study. These findings may suggest that participants were in an increased state of anabolism due to a potential reduction in protein breakdown [24]. Indeed, it has been demonstrated that C is primarily related to catabolic processes, the degradation of proteins from skeletal muscle being just one of them [24]. The decreased C levels in the present study are in accordance with a previous study conducted in guinea pigs after walnut supplementation [20]. In the same line, fatty acid analyses showed that the used walnuts contained a large amount of PUFA such as ALA (40 g/100 g) and LA (8.8 g/100 g). This finding may explain the greater improvement in T metabolism and the decreases in serum C levels in the CTW group. Indeed, PUFA enriched by ALA decreased glucocorticoid concentrations and increased T metabolism in guinea pigs [20].

This investigation clearly demonstrated that CRP decreased by 28% comparatively with baseline for CTW only. The effect of CT confirmed previous studies indicating no significant changes in CRP after combined resistance and endurance training in healthy middle-aged men [14, 37]. However, some studies demonstrated a decrease in CRP after 10 weeks of moderate resistance training in sedentary men [38]. This divergence between results may be related to methodological issues between studies, such as training protocols that used different volumes and/or intensities, differences in the characteristics of participants (e.g., aged vs. young), and differences in baseline CRP levels [38]. Accordingly, it has been shown that CRP levels decreased by 45% in hypercholesterolaemic participants consuming an LA-rich diet [39]. The decrease of CRP levels in CTW could also be related to the levels of melatonin contained in walnut, since melatonin is known as an anti-inflammatory hormone in aging and age-related diseases [40].

A limitation of the present study is the lack of a control group. Nevertheless, even if no group of non-training participants was included, it is of interest to mention that the reference group was a matched participant cohort that did train but did not consume walnut at all. This allowed us to perform interesting comparisons and obtain significant results despite the small sample size of participants. In addition, specific blood markers of lipid profile (i.e., apolipoprotein-B) were not assessed. This warrants further research including replication experiments.

CONCLUSIONS

The present study showed that concurrent training associated with walnut supplementation (15 g/day) improved T and HDL levels for CTW to a larger extent than CT. Moreover, BG, TC, LDL, C and CRP

concentrations significantly decreased only for CTW. It seems that the improved lipid profile could be due to the PUFA contained in walnut. In future studies, we will focus on walnut and blood levels of PUFA and melatonin for better understanding of the mechanism by which walnut intake could improve lipid profile and inflammation in the elderly. It is concluded that moderate walnut consumption could amplify the beneficial effect of concurrent training in the elderly.

Ethical standards

The authors declare that the experiments comply with the current laws of the country in which they were performed. The study was registered at the Pan African Clinical Trial Registry (PACTR202004543859924) and performed between March 2017 and May 2017 at the Laboratory of Biochemistry, CHU Habib Bourguiba, Sfax, Tunisia, LR19ES13. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the local institutional review board of protection of persons (CPP SUD 0033/2017).

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Conflict of interest declaration

The authors declare that they have no conflict of interest.

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