

Does *Tribulus terrestris* L. affect hormonal responses following high-intensity resistance exercise?

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Abstract

Study aim: To investigate the effect of a 2-week supplementation with *Tribulus terrestris* L. (*TT*) on the responses of testosterone, cortisol, and thyroid hormones including triiodothyronine (T3) and thyroxine (T4) following an intensive session of resistance exercise (RE).

Materials and methods: Twenty-two healthy non-athlete men (23.8 ± 3.1 years) participated in this study were divided into two groups and were randomly assigned to receive either *TT* supplementation ($n = 11$) or a placebo ($n = 11$). They consumed two 250-mg capsules with *TT* or placebo (maltodextrin) per day and performed six REs with the intensity 80-90% of 1RM on the fifteen day of the experiment. Venous blood samples were drawn before supplementation, and before and after the RE session.

Results: The mean values of post-exercise testosterone and cortisol in both groups were significantly higher than pre-exercise and baseline ($p < 0.01$); however, there were no significant differences between the groups ($p > 0.05$). Likewise, despite a trend toward different levels of the responses of thyroid hormones with *TT* supplementation, there were no significant differences either in different time points or the groups ($p > 0.05$).

Conclusions: Short-term supplementation with *TT* appears to be ineffective in changing the hormonal responses measured in this study following a session of high-intensity RE.

Keywords: Herbal Plant – Strength Exercise – Testosterone – Cortisol – Thyroid hormones

Introduction

Over the last few decades, resistance exercise (RE) has become one the most rapidly growing exercises to improve physical fitness and body composition. Many people participating in RE programs aim to gain lean body mass and muscle strength, in which case the neuroendocrine system plays an important role. A single session of RE elicits significant acute hormonal responses that are more critical to tissue remodeling than long-term adaptations in resting hormonal levels [15]. These acute responses have been shown to increase the number of receptors and the possibility of hormone-receptor interactions within the muscle cells [11].

In men, high-intensity RE appears to acutely increase total testosterone and cortisol concentrations, as the most

frequent measured anabolic and catabolic hormones, respectively [15, 33]. In this regard, Kraemer et al. reported elevated serum total testosterone and cortisol immediately after heavy RE in resistance-trained men [14]. However, despite an impact on energy metabolism through increasing energy requirements and resting metabolic rate up to 24 h after exercise [12], acute effect of RE on circulating thyroid hormones have received less attention in the literature. In one of the few studies conducted in this area, significant reductions in triiodothyronine (T3) and thyroxine (T4) have been reported during one sever week of RE [21].

Many athletes, at all levels of competition, place great emphasis on the use of dietary supplements [8], although according to the International Olympic Committee (IOC) consensus statement, dietary supplements can play a small role in an athlete's sports nutrition plan [18]. Some dietary

supplements may be valuable in enhancing muscular adaptations to exercise, improving brain performance, decreasing delayed onset muscle soreness or pain, reducing pro-inflammatory mediators, improving recovery process, enhancing recovery from injury, reducing gastrointestinal problems, and decreasing respiratory tract infection illness load [2, 26]. The effect of using supplements, especially expected by athletes, is their influence on hormonal levels in response to exercise. Among these, *Tribulus terrestris* L. (*TT*) is a well-known androgenic herb in traditional medicine to improve sexual function in men through increasing endogenous testosterone production [13, 36]. However, evidence to date for the effectiveness of *TT* on promoting testosterone levels is limited and available literature collectively proposes that *TT* is ineffective for this purpose in humans [25]. In contrast, Milasius et al. demonstrated that *TT* supplementation for a period of 20 days significantly increased muscular power and blood testosterone among youth men [19]. Likewise, a recent animal study performed by Wu et al. [35] found that *TT* extracts (120 mg/kg) could enhance increases in testosterone plasma concentrations of rats undergoing high-intensity exercise [35]. *TT* has been shown to have favorable effects on exercise-induced muscle damage markers [16, 30], and anaerobic performance of trained athletes [16]. Despite conflicting results of the limited number of studies evaluating the efficacy of *TT* supplementation in athletes and healthy adults [22], there is insufficient information on the effect of this herbal plant on hormonal responses induced by high-intensity RE, such as cortisol and thyroid hormones. Therefore, the objective of the present study was to examine the effect of short-term consumption of *TT* supplement on the responses of testosterone, cortisol, T3, and T4 following a single session of intense whole-body RE. We hypothesized that supplementation would improve anabolic state in non-athlete young men after exercise.

Materials and methods

Participants

Following a notification call at the university, twenty-two non-athlete male college students were included from a total of thirty-one healthy volunteers. All participations were on the same level based on the answers to a structured questionnaire with 30 questions in five different sections including family history, medications, injuries, diseases, infections, and exercise history. Inclusion criteria were age range of 18 to 30 years, no history of injury or illness, no history of cardiovascular diseases and no engagement in regular physical activity within the six months prior to the study. Smoking and recent use of any type of supplements and medications were also considered as exclusion criteria. The present research protocol was

approved by the Institutional Review Board of the University (DT-15855). We conducted this study in accordance with the Declaration of Helsinki. Participation in the study was completely voluntary and all the participants received a clear explanation of experimental procedure. Therefore, they were aware of the potential benefits and risks of participating in the study.

Study design

This was a randomized double-blind, placebo-controlled study with repeated measures (i.e. three measurements including before supplementation, and before and after the exercise) design in which the participants were randomly divided into two groups of eleven persons including supplement and placebo. Neither the participants nor the experimenters knew to which condition each participant was assigned.

Procedures

Measurement of descriptive characteristics and familiarization with how to perform REs correctly were performed in the first session. The first blood samples were obtained during the second session to determine baseline values of the study variables. Subsequently, the maximum strength of muscle groups targeted in the RE protocol was assessed using the one-repetition maximum test (1RM) together with the Brzycki equation [4]. At the end of this session, bottles containing *TT* supplement or placebo capsules were randomly distributed among the participants such that all of the twenty-two bottles containing the capsules (eleven supplement and eleven placebo bottles) were placed in a non-transparent bag and the participants were asked to take one bottle. This procedure allowed us to categorize the participants into the supplement or placebo group in a random manner. All the bottles were coded in a double-blind manner by a third person who was not involved in the experiment to maintain blinding. Moreover, the supplement and placebo capsules were made in the same color and size to minimize the differences between them. During a two-week supplementation protocol, two 250-mg capsules of Chinese *TT* plant (fruit of the plant containing approx. 100 mg furostanol saponins, 27 mg total phenolic content, and 34 mg total flavonoids) that was a blend of *TT* powder and extract, or placebo (maltodextrin) were consumed every day in the morning and evening after the main meal.

Nutritional controls

The participants were instructed to maintain their routine daily physical activity throughout the experiment. They also recorded their food intake throughout the week (two working days and one weekend day) before supplementation, according to written and verbal instructions about the type and portion sizes of daily food

[29]. Likewise, food intake was also recorded 48 h prior the RE session.

Resistance exercise protocol

On the fourteenth day of supplementation period, the participants were instructed to eat the last meal at 10 pm and attended the gym to perform RE session at 10 am of the fifteenth day under fasted condition. Pre-test blood samples were taken before exercise after a resting period of 30 minutes. The participants performed low-intensity exercises and stretching before the main exercise protocol for 10 to 15 minutes. Circuit RE including bench press with Smith machine, leg press, lat pull down, leg extension and biceps curl barbell were then completed in three sets of 80, 85 and 90% of 1RM with 8 to 10 repetitions. A rest period of 45 and 120 seconds was considered between the sets and exercises, respectively [7].

Blood sampling and analyses

Venous blood samples (10 ml) were collected into the standard tubes in the same room at a temperature range of 22 to 24 °C. The samples were then stored at -20°C for subsequent analyses after separating serum by centrifugation at 3000 rpm for 10 minutes. Human ELISA kits (CUSABIO, China) were used to measure serum total testosterone and cortisol levels with a sensitivity of 0.05 ng/ml (0.17 nmol/L). Serum concentrations of total T3 and T4 were also measured by a spectrophotometer (ELISA reader, Metertech, M965, Taiwan) using human specific kits (Padyab Teb, Iran), with a sensitivity of 20 ng/dL and 1 µg/dL, respectively.

Statistical analyses

All data analysis was carried out by SPSS version 18.0 software (SPSS Inc., Chicago, IL) and $p < 0.05$ was considered to be statistically significant. Normal distribution of data was determined using Shapiro-Wilk test, where a p value more than 0.05 was considered normally distributed. Intra-group differences were analyzed by repeated measures analysis of variance (ANOVA) and post hoc Bonferroni tests where necessary. Between-group differences were also compared at the measured time points using

the independent-samples t -test, while Levene's test for equality of variances was met. Furthermore, an effect size statistic for each variable was reported using partial eta squared (η^2) and percent change was also calculated as: $[((\text{pre-test} - \text{post-test})/\text{pre-test}) \times 100]$.

Results

The characteristics of both studied groups, including the average age, height, weight, and body mass index (BMI) is presented in Table 1. There was only a significant difference between the groups in terms of BMI ($t = -3.63$, $p = 0.002$).

The groups had no significant differences in the approximate amount of energy intake, carbohydrate, protein, and fat over 48 h before the RE session (Table 2).

Figures 1-a, 1-b, and 1-c show testosterone and cortisol concentration levels, as well as testosterone/cortisol (T/C) ratio, in the groups at baseline, pre – and post-exercise. On the basis of repeated measures ANOVA, the interaction of time $\times$ groups were not significant for testosterone ($F = 2.36$, $p = 0.12$, $\eta^2 = 0.10$), cortisol ($F = 0.25$, $p = 0.71$, $\eta^2 = 0.01$), and T/C ratio ($F = 1.14$, $p = 0.31$, $\eta^2 = 0.05$).

Main effects were then compared across the time points in each group. Pairwise comparisons by Bonferroni test revealed that the average levels of testosterone after exercise in both groups was significantly higher than before

Table 1. Mean $\pm$ SD of physical characteristics of each group

	TT group (n = 11)	Control group (n = 11)	p value
Age [year]	23.7 $\pm$ 2.9	23.9 $\pm$ 3.5	0.9
Height [cm]	173 $\pm$ 5.2	171 $\pm$ 7.9	0.8
Weight [kg]	76 $\pm$ 5.5	78 $\pm$ 8.4	0.5
BMI [kg/m ²]	25.4 $\pm$ 0.9	26.0 $\pm$ 1.2	0.2

TT = *Tribulus terrestris* L.; BMI = body mass index.

Table 2. Mean $\pm$ SD of dietary intake of the groups

	TT group	Control group	p value
Energy intake [Cal]	2619.1 $\pm$ 303.6	2707.9 $\pm$ 445.3	0.59
Carbohydrate [g]	451.6 $\pm$ 53.2	469.7 $\pm$ 74	0.51
Protein [g]	119.6 $\pm$ 14.6	127.8 $\pm$ 21.3	0.3
Fat [g]	37.9 $\pm$ 4.3	39.6 $\pm$ 5.8	0.43

TT = *Tribulus terrestris* L.

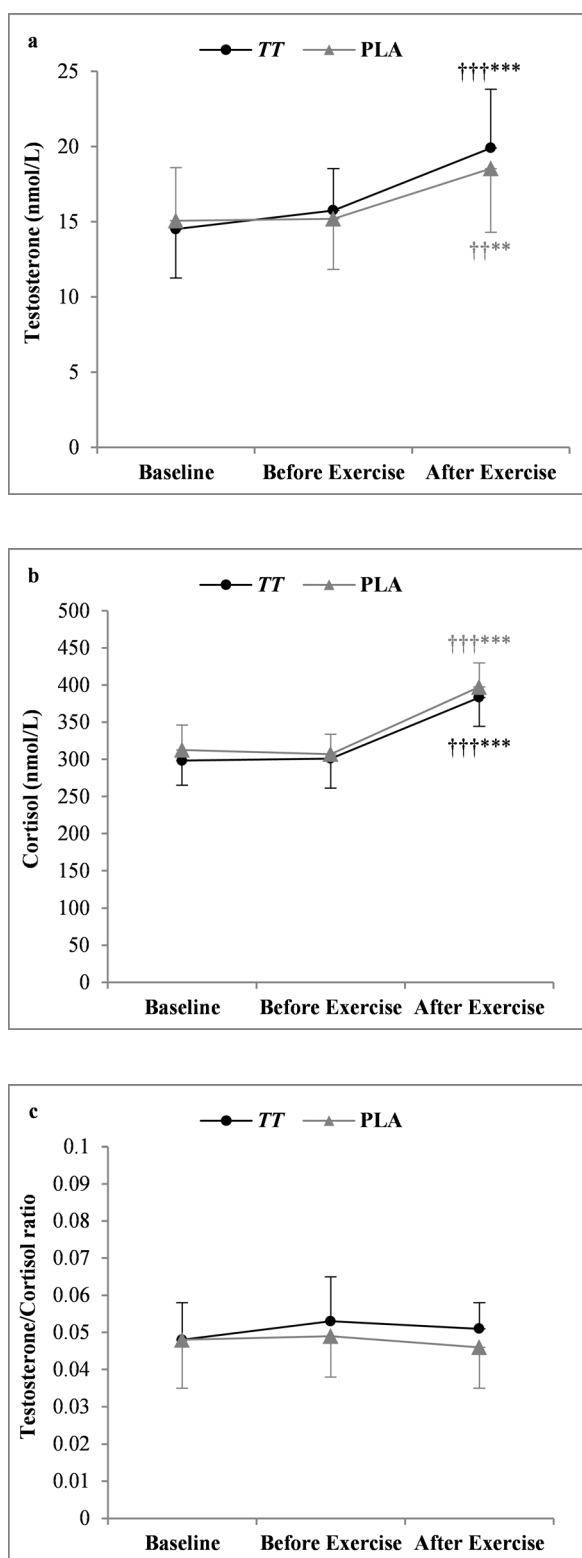


Figure 1. Serum levels of testosterone (a), cortisol (b), and testosterone/cortisol ratio (c) before a two-week supplementation (Baseline), and before and after a single session of high-intensity resistance exercise in *Tribulus terrestris* L. (TT, $n = 11$) and placebo (PLA, $n = 11$) groups. Data are shown as means (solid circles and triangles) $\pm$ standard deviation (error bars). *** $p < 0.001$ and ** $p < 0.01$ vs. baseline; ††† $p < 0.001$ and †† $p < 0.01$ vs. before exercise

exercise (TT = 28%, $p = 0.001$; placebo = 23%, $p = 0.003$) and baseline values (TT = 40%, $p < 0.001$; placebo = 25%, $p = 0.003$). However, post-exercise levels of testosterone did not differ between the supplement and placebo groups ($t = -0.91$, $p = 0.37$). Although significant elevations ($p < 0.001$) in serum cortisol levels were observed in both groups compared with before exercise (TT = 29%; placebo = 30%) and baseline (TT = 29%; placebo = 28%), there were no significant differences between the groups in terms of post-exercise alterations ($t = 0.78$, $p = 0.44$). Likewise, there were no significant differences between the groups concerning post-exercise values of T/C ratio ($t = 1.31$, $p = 0.20$).

The changes in serum concentrations of T3 and T4 are presented in Figures 2-a and 2-b, respectively. No statistically significant interaction of time $\times$ groups was noted

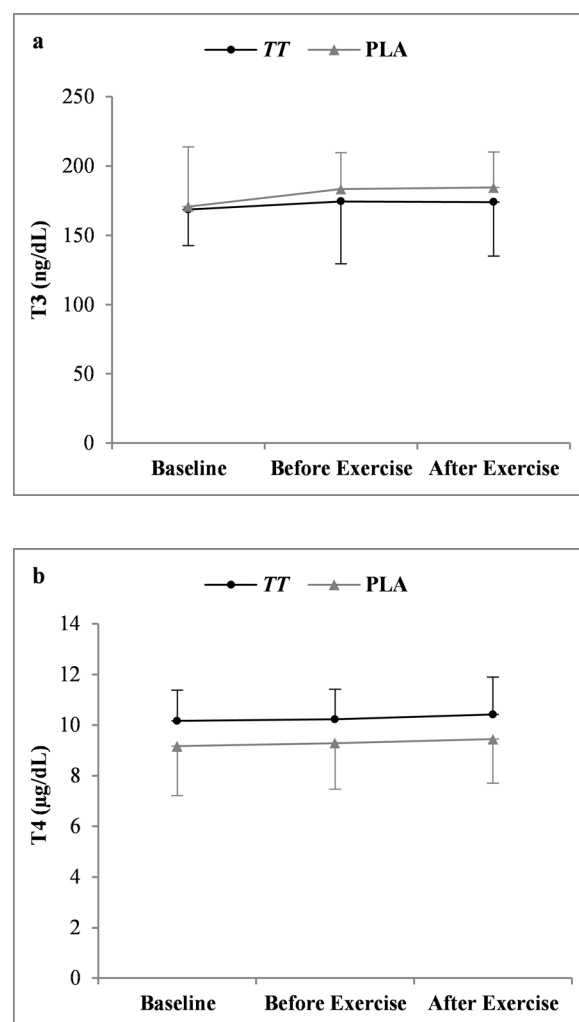


Figure 2. Serum levels of triiodothyronine (T3, a) and thyroxine (T4, b) before a two-week supplementation (Baseline), and before and after a single session of high-intensity resistance exercise in *Tribulus terrestris* L. (TT, $n = 11$) and placebo (PLA, $n = 11$) groups. Data are shown as means (solid circles and triangles) $\pm$ standard deviation (error bars)

for T3 ($F = 0.22$, $p = 0.66$, $\eta^2 = 0.01$) and T4 ($F = 0.006$, $p = 0.9$, $\eta^2 < 0.001$). Moreover, there were no significant main effects for the thyroid hormones ($p > 0.05$). Likewise, post-exercise alterations of T3 ($t = -0.74$, $p = 0.46$) and T4 ($t = 0.99$, $p = 0.33$) did not differ between the groups.

Discussion

The objective of this research was to examine the effect of short-term supplementation with *TT* on serum concentrations of thyroid hormones, testosterone, and cortisol in response to a single session of intense circuit RE. In the present study, non-athlete participants were recruited to exclude the effects of exercise experience or previous consumption of supplements from the results. To the best of our knowledge, few studies have investigated the effect of this herbal supplement on these responses in human. We here observed that, the mean concentration of testosterone and cortisol, from pre- to post-test values, was increased by around 28% and 29% in the *TT* group, respectively, and by around 23% and 30% in the placebo group, respectively. Nevertheless, there was no significant difference between the groups ($p > 0.05$). In addition, the changes of T/C ratio at different time points did not reach statistically significant level between the groups ($p > 0.05$).

In the present study, we measured the variables at 3 different time points including i) before supplementation (baseline), ii) after supplementation and before the RE protocol (pre-test), and iii) after RE session (post-test), to determine the effect of regular *TT* intake on testosterone, cortisol, and thyroid hormones at rest and in response to RE protocol. To date, although a number of animal and human studies evaluating anabolic and catabolic effects of *TT* have been carried out, no data exists about the impact of this herbal plant on the hormonal responses after exercise and the available literature describe these changes at rest. Therefore, any comparison between the present results and those of the other studies should be interpreted cautiously. In accordance with the present findings, Neychev and Mitev reported that *TT* extract had no effect on resting serum testosterone in healthy young men during a 4-week supplementation [20].

Van Eenoo et al. [32] concluded that *TT* is not effective in normal healthy males, because they did also observed no significant changes in endogenous steroid concentration (e.g. testosterone and luteinising hormone) in volunteers who ingested 500 mg *TT* three times a day for four and half days [32]. Similar results were also reported by other researchers who investigated the impact of *TT* supplement in trained athletes [16, 24, 27]. Likewise, short-term administration of *TT* to intact male rats did not

change testosterone concentrations during a 4-week period [9, 17].

In contrast, the concentration of blood testosterone increased statistically reliable during the first 10 days of the experiment in which the influence of *TT* extract (1875 mg per day) on the parameters of the functional preparedness and athletes' organism homeostase was investigated [19]. A more recently research showed that, compared with *TT* extract or RE alone, combination of regular RE and *TT* extract with a dosage of 1000 mg per day for 8 weeks might to induce more increases in resting serum testosterone concentrations in obese men with type 2 diabetes [10]. These findings were also supported by two animal trials, which have been reported that *TT* extract (120 mg/kg) increased serum levels of testosterone of rats undergoing high-intensity exercise training [35, 36]. Additionally, another study conducted by Wang and colleagues revealed that a 4-week treatment with *TT* saponins could significantly decrease serum cortisol levels in male rats [34]. The observed contradictions in the results may be partially attributed to the study subjects (i.e. healthy non-athlete men vs. obese diabetic men, and Sprague-Dawley rats), different exercise protocols (i.e. high-intensity RE vs. low-intensity RE, and high-intensity aerobic training), training-induced adaptations in the mentioned studies [10, 35, 36], and shorter duration of supplementation and lower dosage of *TT* supplement in the present research. It should be taken into consideration that although *TT* seems not to increase androgen levels in healthy subjects, it could be effective in those with androgen deficiency [31].

This study is the first to investigate the effect of *TT* on the responses of thyroid hormones after exercise. Thyroid hormones play a crucial role in the homeostasis of muscle function, such as myogenesis and energy metabolism [6]. The obtained findings revealed that although the average level of thyroid hormones after *TT* supplementation was lower (T3, 173.9 ± 38.8 vs. 184.4 ± 25.8 ng/dL) and higher (T4, 10.4 ± 1.5 vs. 9.4 ± 1.7 µg/dL) in response to the high-intensity RE protocol, there were no significant differences either in different time points or the groups (Figure 2). The role of these hormones during RE sessions is not yet fully understood but may have some interactions with other hormones. T3 has been proposed to up-regulate androgen receptors mRNA, and higher androgen levels could potentiate this effect [5]. Moreover, administration of testosterone enanthate together with regular RE for 8 weeks significantly decreased the serum concentration of T4 in male adult Wistar rats [37]. Our results were in line with those reported previously [1, 3]. However, significant reductions in T3 and T4 have been reported as a consequence of one intensive week of RE in elite weightlifters [21]. The observed

inconsistency between the studies seems to be due in part to remarkable high RE volume (two workouts per day for 1 week vs. a single session). In support of this claim, it is well established that the endocrine release of body hormones is sensitive to the volume of total RE program [7]. Taken together, our findings suggest that a single session of high-intensity RE has no effect on thyroid hormones and short-term *TT* supplementation does not affect these responses. These observations are in line with the conclusion of a literature review [15] that elevations in thyroid hormones during RE protocol are not expected, because of the tight homeostatic control.

Strengths and limitations

The novelty of this study is assessing the effect of a popular herbal supplement among strength/power athletes on the less studied hormonal responses (i.e. T3 and T4) following a session of high-intensity RE. Moreover, a double-blind placebo-controlled randomized design, inclusion of non-athlete subjects to overcome the possible effects of previous exercise experience and control of dietary intake during the days before the exercise are the most important strengths of the present study.

However, this research has some limitations which need to be taken into account when interpreting the results. The generalizability of current findings may affect by the small sample size, as the main limitation. Hence, more research with larger sample size is warranted. Despite some instructions to maintain the total level of normal daily physical activity, the exact amount of this factor was not controlled separately throughout the study. It is relevant because the popularity of dietary supplements with *TT* among athletes is very high. However, in the light of the most scientific data, the risk of *TT* being contaminated or adulterated with doping substances appears to outweigh the benefits of potentially improved sports performance induced by natural *TT* ingredients [23]. Due a risk this plant could lead to a positive doping control test there are recommendations which clearly advise athletes against using dietary supplements based on *TT* [28].

Conclusions

The hypothesis that supplementation with *TT* would improve anabolic state in non-athlete young men after exercise was not confirmed. The findings presented in this experiment revealed that the daily use of two 250-mg capsules of Chinese *TT* plant for 2 weeks does not seem to affect the responses of testosterone and cortisol in non-athlete young men following an intensive session of circuit RE. Moreover, despite a tendency toward higher levels of thyroid hormones, this effect elicited by *TT* supplement did not reach a statistically significant level.

Conflict of interest: Authors state no conflict of interest.

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Received 18.10.2021

Accepted 06.01.2022

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Acknowledgments

The authors would like to thank the participants in this study.