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Original Article

Tribulus terrestris extracts alleviate muscle damage and promote anaerobic performance of trained male boxers and its mechanisms: Roles of androgen, IGF-1 and IGF binding protein-3

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Abstract

Purpose: To investigate the effects of *Tribulus terrestris* (TT) extracts on muscle mass, muscle damage, and anaerobic performances of trained male boxers and its mechanisms: roles of plasma androgen, IGF-1, and IGF-1 binding protein-3 (IGFBP-3).

Methods: Fifteen male boxers were divided into exercise group (E, $n = 7$) and exercise plus TT group (E + TT, $n = 8$). The two groups both undertook 3-week high intensity and 3-week high volume trainings separated by a 4-week rest. TT extracts (1,250 mg/day) were orally administered by boxers in E + TT group. TT extract compositions were detected by UHPLC–Q-TOF/MS. Before and at the end of the two trainings, muscle mass, anaerobic performance, and blood indicators were explored.

Results: Compared with E group, decreases of plasma CK (1,591.50 ± 909.55 *vs.* 2,719.86 ± 832.47 U/L) and IGFBP-3 (3,075.53 ± 1,072.45 *vs.* 3,950.83 ± 479.25 ng/mL) as well as increases of mean power (MP, 459.42 ± 122.25 *vs.* 434.60 ± 69.47 W) and MP/body weight (MP/BW, 7.54 ± 0.85 *vs.* 7.07 ± 1.09 W/kg) were detected in E + TT group after a high intensity training. For high volume training, reduction of IGFBP-3 (2,946.38 ± 974.07 *vs.* 3,632.67 ± 470.06 ng/mL) and increases of MP (508.71 ± 103.21 *vs.* 477.81 ± 49.90 W) and MP/BW (8.24 ± 0.29 *vs.* 7.52 ± 0.92 W/kg) were detected in E + TT group. Muscle mass, blood levels of testosterone, dihydrotestosterone (DHT), and IGF-1 were unchanged between the two groups.

Conclusion: Taking 1,250 mg capsules containing TT extracts did not change muscle mass and plasma levels of testosterone, DHT, and IGF-1 but significantly alleviated muscle damage and promoted anaerobic performance of trained male boxers, which may be related to the decrease of plasma IGFBP-3 rather than androgen in plasma.

Keywords: IGF binding protein-3; Muscle damage; Performance; Testosterone; *Tribulus terrestris*

1. Introduction

Tribulus terrestris (TT) is a famous traditional Chinese medicine that has been widely used in many countries for thousands of years.[1] TT revealed many compounds including steroidal saponins, flavonoids, alkaloids, and amino acids. TT saponins are considered the most important active components that possess a broad range of biological effects such as relieving sexual dysfunction and improving erectile function in rabbits and males,[2] protecting myocardium against ischemia/reperfusion injury and treating hypertension and coronary heart disease.[3]

TT, claimed to be a testosterone booster, is a popular nutritional supplement in athletes and physically active men for enhancing gain in muscle mass, strength, and performance. Supplement of TT extracts increased serum testosterone levels on male rats,[4–7] primates, rabbits and castrated rats.[8] Our previous studies demonstrated that TT extracts improved exercise performance of rats with high intensity endurance training[9] and overload training[10] by increasing plasma level of testosterone. However, different views still exist. TT has no significant influence on serum testosterone concentrations, strength, lean body mass, and exercise performance in elite rugby league players,[11] resistance-trained males,[12] and normal females[13,14] as well as intact and castrated rats.[13,14] Although there is little strong evidence to prove that TT truly has the effects of testosterone booster and muscles anabolism promoter,[3] TT extracts are still used constantly by many athletes. More clinical trials should be

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carried out to get a clear conclusion about the effectiveness of TT extracts.

Skeletal muscle is a highly dynamic tissue that responds to endogenous and external growth factor stimuli, among which insulin growth factor (IGF-1) is one of the primary regulators affecting muscle growth, damage, repair, and regeneration. IGF-1 reduced aged-related wasting of skeletal muscle,[15] and resistance training which is the most useful treatment for the loss of muscle mass and strength in elderly people upregulated the expression of IGF-1.[16] IGF-1 injected soleus muscles of C57BL6 mice resulted on average 19% larger than the contralateral muscles and produced 16% more force.[17] Local upregulation of IGF-1 has been also observed during muscle repair and regeneration in a variety of animal models of muscle damage.[18] The action of IGF-1 is modulated by high-affinity binding proteins known as IGF binding proteins (IGFBPs), and until now seven of IGFBPs (IGFBP1–7) have been found, among which IGFBP-3 is the most abundant in blood and tissue fluid combined with more than 80% IGF-1 normally.[19] As the most important inhibitor of IGF-1 activity, the changes of IGFBP-3 level may have an indirect effect on muscle mass, muscle damage, and performance.

2. Materials and methods

2.1. Subjects

Fifteen male boxers (national second-level athletes, 2–3 years of training) were recruited from boxer team of Shanghai University of Sport Affiliated School of Sports in China. The boxers were randomly divided into exercise (E) group and exercise plus TT (E + TT) group, and two subjects in the E + TT group quit the experiment for leaving the school after 6 weeks. The baseline parameters of the participants are shown in Table 1. The experiment was approved and supervised by the Ethics Committee of Shanghai University of Sport (No. 2014002). An informed consent form was signed by the boxer who was older than 18 or by the guardian of the boxer who was younger than 18.

2.2. Administration and composition determination of TT extracts

The capsules of TT extracts (TT saponin > 40%) were purchased from Pronova Biocare company of Sweden. Two TT capsules a day (1,250 mg, recommended dosage) were orally administered by male boxers of E + TT group every morning during 3-week high intensity training and 3-week high volume training, while placebo capsules of starch were taken by E group boxers. The capsules were administered in a double-blind fashion.

The compositions of TT extracts were detected by ultra-high performance liquid chromatography–quadrupole-time of flight mass spectrometry (UHPLC–Q-TOF/MS; Agilent Technologies, Santa Clara, CA, USA). Briefly, the powder of TT extracts from a capsule was dissolved in 70% alcohol and the supernatant was obtained to identify the compositions of TT extracts after ultrasonic extraction and centrifugation. An Agilent 1290 infinity UHPLC (Agilent Technologies) with binary pump, auto-sampler, thermostatted column compartment coupled with 6538 Q-TOF/MS system was used for the study on MS characterization of TT extracts. The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) with the following gradient: 0–1 min, 5% B; 1–6 min linearly increased B from 5% to 20%; 6–9 min, linearly increased B from 20% to 50%; 9–13 min, linearly increased B from 50% to 95%; 13–18 min, 95%. Dual Agilent jet stream electrospray ion source was used and ran at both positive and negative modes. The temperature of gas was set as 350°C and the flow rate was 11 L/min. The nebulizer was 45 psi and the capillary voltage was set at 4,000 V for positive mode and 3,000 V for negative mode.

Table 1
The baseline parameters of the participants.

Parameter	E (n = 7)	E + TT (n = 6)
Age (y)	16.6 ± 1.9	16.1 ± 1.8
Height (cm)	172.7 ± 4.0	174.0 ± 8.1
Weight (kg)	64.1 ± 6.6	62.8 ± 15.2
Body fat percentage (%)	9.6 ± 3.2	9.8 ± 2.4
Maximum strength (1 RM of barbell bench press, kg)	72.0 ± 2.0	71.0 ± 2.5
Aerobic endurance (10,000 m race, min)	41.8 ± 2.5	42.2 ± 2.5
Anaerobic endurance (peak power/body weight, W/kg)	8.6 ± 1.3	8.3 ± 1.1
Anaerobic endurance (mean power/body weight, W/kg)	7.2 ± 0.8	6.7 ± 0.7

Abbreviations: E, exercise; E + TT, exercise plus TT.

2.3. Exercise protocol

All athletes received similar 3-week high intensity training and 3-week high volume training separated by a 4-week rest. Besides special technical training, the main part of the high intensity training was strength training including maximum strength training (twice a week, on Tuesday and Friday) and speed strength training (twice a week, on Monday and Thursday). For high volume training, the boxers undertook endurance training (10,000 m race every day and low to moderate intensity rope skipping twice a week, on Tuesday and Friday), and special technical training and speed strength training similar to high intensity training. Table 2 showed the training protocol of the boxer with high intensity training and high volume training.

2.4. Blood index assays

Fasting blood samples were collected before and at 40 h after the last training session to avoid the potential acute influence of the training on the levels of humoral factors. Blood levels of CK, BUN, and Hb were detected by colorimetry (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Plasma testosterone was determined using chemiluminescence immunoassay, while plasma DHT (ALPCO, New Hampshire, NH, USA), IGF-1 (total IGF-1 rather than free IGF-1) and IGFBP-3 were examined in duplicate by ELISA (R&D, Minneapolis, MN, USA) according to the manufacturers' instruction. The intra- and inter-assay coefficients of variation (CV) were less than 7% and 12% for DHT, and less than 5% and 8% for IGF-1 and IGFBP-3.

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