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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 (1250 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 (1591.50 ± 909.55 *vs.* 2719.86 ± 832.47 U/L) and IGFBP-3 (3075.53 ± 1072.45 *vs.* 3950.83 ± 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 (2946.38 ± 974.07 *vs.* 3632.67 ± 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 1250 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 equal or 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 (1250 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 4000 V for positive mode and 3000 V for negative mode.

Table 1
The baseline parameters of the participants (mean ± SD).

Parameter	E (n = 7)	E + TT (n = 6)
Age (year)	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 (1RM 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; TT = *Tribulus terrestris*; RM = repetition maximum.

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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Table 2
Training protocol of the boxers with high intensity and high volume training.

	High intensity training	High volume training	Note
Monday	1. Barbell bench press, horizontal press, and power clean (40%–50% of 1RM, 6–8 sets × 12–15 repetition); 2. Boxing (HR: about 190 b/min, 2 min × 8 times); 3. Punching sandbag (HR: about 190 b/min, 2.5 min × 4 times); 4. 3000 m race (HR: about 170 b/min); 5. Simulated actual combat (2.5 min × 10 times).	1. 10,000 m race (less than 42 min); 2. Barbell bench press, horizontal press and power clean (40%–50% of 1RM, 6–8 sets × 12–15 repetition); 3. Boxing (HR: about 190 b/min, 2 min × 8 times); 4. Punching sandbag (HR: about 190 b/min, 2.5 min × 4 times).	For training used barbell: 1st week: 40% of 1RM, 6 sets × 12; 2nd week: 40% of 1RM, 8 sets × 15; 3rd week: 50% of 1RM, 8 sets × 15.
Tuesday	1. Barbell squat and bench press (85% of 1RM, 6–8 sets × 2–3 repetition); 2. Boxing (HR: about 190 b/min, 2 min × 8 times); 3. Punching sandbag (HR: about 190 b/min, 3 min × 5 times); 4. Rope skipping (HR: about 190 b/min, 2 min × 4 times); 5. 600 m race (HR: about 190 b/min, <2 min × 4 times).	1. 10,000 m race (less than 42 min); 2. Rope skipping (HR: about 150 b/min, 30 min); 3. Boxing (HR: about 190 b/min, 2 min × 8 times); 4. Punching sandbag (HR: about 190 b/min, 3 min × 5 times); 5. Simulated actual combat (2.5 min × 10 times).	For training used barbell: 1st week: 6 sets × 2; 2nd week: 8 sets × 2; 3rd week: 8 sets × 3.
Wednesday	1. Rope skipping (HR: about 190 b/min, 2 min × 4 times); 2. 10,000 m race (less than 42 min); 3. Simulated actual combat (2.5 min × 10 times).	1. 10,000 m race (less than 42 min); 2. Simulated actual combat (2.5 min × 10 times).	
Thursday	1. Barbell bench press, horizontal press and power clean (50%–75% of 1RM, 6–8 sets × 8–10 repetition); 2. Boxing (HR: about 190 b/min, 2 min × 4 times); 3. Punching sandbag (HR: about 190 b/min, 3 min × 5 times); 4. Football (60 min).	1. 10,000 m race (less than 42 min); 2. Barbell bench press, horizontal press and power clean (50%–75% of 1RM, 6–8 sets × 8–10 repetition); 3. Football (60 min).	For training used barbell: 1st week: 50% of 1RM, 6 sets × 8; 2nd week: 60% of 1RM, 8 sets × 10; 3rd week: 75% of 1RM, 6 sets × 8.
Friday	1. Barbell squat and bench press (85% of 1RM, 6–8 sets × 2–3 repetition); 2. Boxing (HR: about 190 b/min, 2 min × 8 times); 3. Punching sandbag (HR: about 190 b/min, 3 min × 5 times); 4. Simulated actual combat (2.5 min × 10 times).	1. 10,000 m race (less than 42 min); 2. Rope skipping (HR: about 150 b/min, 30 min); 3. Boxing (HR: about 190 b/min, 2 min × 8 times); 4. Punching sandbag (HR: about 190 b/min, 3 min × 5 times).	For training used barbell: 1st week: 6 sets × 2; 2nd week: 8 sets × 2; 3rd week: 8 sets × 3.
Saturday	Same as Wednesday	Same as Wednesday	

Abbreviations: HR = heart rate; RM = repetition maximum.

2.5. Detection of muscle mass and fat mass

Muscle mass and fat mass of male boxers in the two groups were determined by dual-energy X-ray absorptiometry (DEXA) (Lunar Prodigy; GE, Madison, WI, USA). The test included a complete body scan of the boxers, in supine position, with the apparatus always regulated and operated by a technically trained professional. Lean soft tissue mass including total, appendicular, and trunk as well as fat mass and body fat percentage were obtained. The mass of total skeletal muscles was calculated by the formula: 1.115 × mass of appendicular lean soft tissues (kg) −1.135.[20]

2.6. Determination of anaerobic performance

No. 894E Wingate anaerobic power bicycle (Monark Company, Vansbro, Sweden) was used to test anaerobic performance before and after the two trainings. After the rest period followed by a 5-min warm-up, the subject began to pedal maximally at the signal "go". When the subject's cadence reached 100 rpm at no resistance, the electromagnet released the weight pan and the 30 s test began. Computer software (Monark Anaerobic Test Software version 3.0.1) was used to calculate PP, PP/BW, MP, MP/BW and fatigue index $((PP - P_{min})/PP \times 100\%)$ throughout the 30 s test.

2.7. Statistical analysis

All values were expressed as mean ± SD and statistical significance was set as $p < 0.05$. Data were analyzed using SPSS 20.0 for windows (IBM Corporation, Armonk, NY, USA) and entered into a two-way repeated measure ANOVA model with group (E *vs.* E + TT) as the between-subject effect and experimental condition (high intensity and high volume trainings) as the within-subject effect. The correlations of the change of IGFBP-3 with the changes of CK, MP, and MP/BW in E + TT boxers were evaluated by the correlation of Pearson's chi-squared test.

3. Results

3.1. TT extract components by UHPLC–Q-TOF/MS

Alcohol-soluble components of TT extracts were separated by UPLC, and their accurate molecular weights and molecular formula were identified through the information of positive ion and negative ion determined by Q-TOF/MS (Fig. 1). Then, names of the components of TT extracts were obtained by referring to literatures about TT extracts. As shown in Table 3, 22 constituents were identified from the TT extracts and the most abundant constituents were six kinds of TT saponins (25(R)-Spirostan-3,6,12-trione/25(R)-Spirostan-4-ene-3,12-dione; TT

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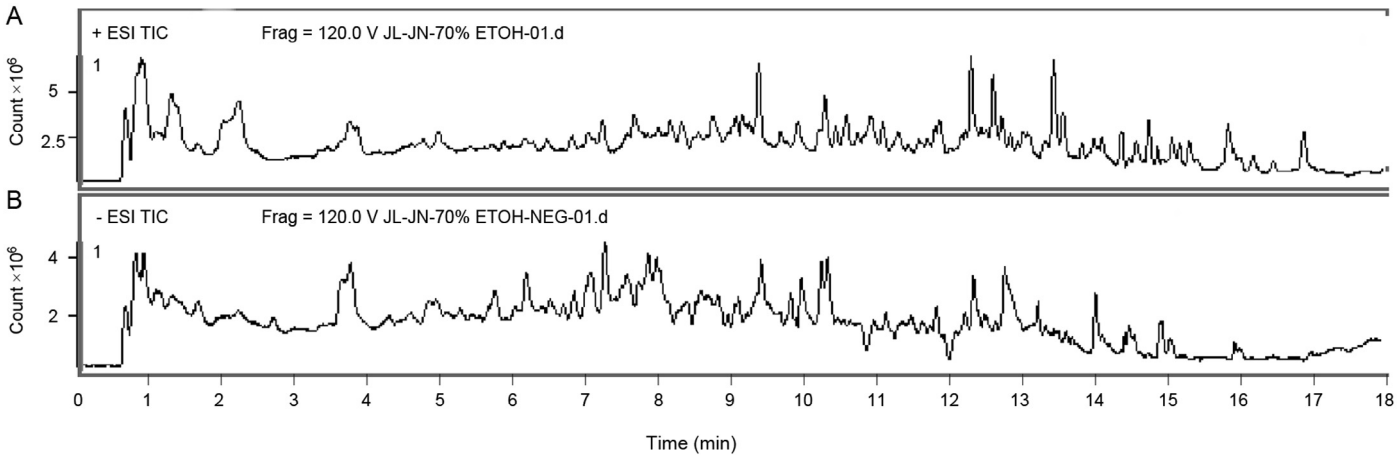

Fig. 1. Total ion chromatogram from UHPLC–Q-TOF/MS of *Tribulus terrestris* extracts in positive (A) and negative (B) modes.

saponin A; gitogenin; TT saponin; tigogein; and diosgenin) which covered about 58.86% of the total peak area. Amino acids (valine and phenylalanine) and flavonoids (rutin; kaempferol-3-glucoside; quercetin; tricin; and kaempferol) comprised about 20.67% and 10.24% of the total peak area, respectively.

3.2. Effect of TT extracts on muscle mass and muscle damage

No significant post-training change was found in total muscle mass, total fat mass, and the percentages of total muscle and fat between the main effects for groups, experimental condition, or their interaction ($p > 0.05$) (Table 4).

For the change in CK after high intensity training, the ANOVA showed significant main factors of group ($F(1, 11) = 5.53$, $p = 0.038$), and experimental condition ($F(1, 11) = 53.33$, $p < 0.001$). In addition, the interaction between the above two main effects indicated that the CK in the E + TT group following high intensity training was significantly lower than that in the E group ($F(1, 11) = 5.00$, $p = 0.047$) while there was no significant difference between groups after high volume training. Finally there was no significant post-training change in BUN between the main effects for groups, experimental condition, or their interaction ($p > 0.05$) (Table 5).

3.3. Effect of TT extracts on anaerobic performance

There was no significant post-training change in PP, PP/BW, and fatigue index between the main effects for groups,

Table 3
Q-TOF/MS analysis results for components of *Tribulus terrestris* (TT) extracts.

No.	Name	RT (min)	Formula	M ± X	Expected m/z	Experimental m/z	Error (ppm)	Area (%)
1	Rutin	7.727	$C_{27}H_{30}O_{16}$	M + H	611.1624	611.1607	−2.87	3.659
2	Kaempferol-3-glucoside	7.931	$C_{21}H_{20}O_{11}$	M + H	449.1092	449.1078	−3.04	1.542
3	Quercetin	7.010	$C_{15}H_{10}O_7$	M + H	303.0503	303.0499	−1.24	1.674
4	Valine	0.790	$C_5H_9NO_2$	M + H	116.0713	116.0706	−5.73	18.438
5	Valine acid	7.206	$C_8H_8O_4$	M + H	169.0481	169.0495	8.62	1.805
6	TT amide	9.064	$C_{18}H_{17}NO_5$	M + H	328.121	328.1179	−9.13	4.977
7	Tricin	10.010	$C_{17}H_{14}O_7$	M + H	331.0821	331.0912	−2.77	1.957
8	25(R)-Spirostan-3,6,12-trione/ 25(R)-Spirostan-4-ene-3,12-dione	13.458	$C_{27}H_{38}O_4$	M + H	427.2881	427.2843	−8.69	36.807
9	Diosgenin	11.836	$C_{27}H_{42}O_3$	M + H	415.3215	415.3207	−3.15	0.260
10	Tigogein	12.553	$C_{27}H_{44}O_3$	M + H	417.3369	417.3363	−1.34	0.431
11	TT saponin A	13.630	$C_{27}H_{42}O_4$	M + H	431.3196	431.3156	−9.10	18.584
12	Gitogenin	13.950	$C_{27}H_{44}O_4$	M + H	433.3325	433.3312	−3.00	1.946
13	Palmitic acid monoglyceride	14.477	$C_{19}H_{38}O_4$	M + H/M + Na	331.2847/353.2666	331.2843/353.2662	−1.47/−1.17	0.070
14	Phenylalanine	5.731	$C_9H_{11}NO_2$	M + COOH	210.0767	210.0772	2.67	2.228
15	N-trans caffeoyltyramine	8.511	$C_{17}H_{17}NO_4$	M − H	298.1076	298.1085	3.11	1.325
16	TT amide	9.098	$C_{18}H_{17}NO_5$	M − H	326.1026	326.1179	2.50	1.613
17	TT saponin	9.253	$C_{30}H_{26}O_{13}$	M − H	593.1295	593.1301	0.88	0.831
18	Kaempferol	9.375	$C_{15}H_{10}O_6$	M − H	285.0405	285.0405	−0.15	0.398
19	Physcione	10.044	$C_{16}H_{12}O_5$	M + COOH	329.0667	329.0612	−0.21	0.374
20	7-Hydroxy-4′-methoxyisoflavone	10.582	$C_{16}H_{12}O_4$	M − H	267.0655	267.0663	2.85	0.201
21	Emodin	12.000	$C_{15}H_{10}O_5$	M − H	269.0454	269.0455	0.40	0.071
22	Kaempferol	14.006	$C_{20}H_{34}O_3$	M + COOH	367.2488	367.2490	0.60	0.809

Abbreviations: RT = retention time; m/z = mass-to-charge ratio; M = molecule; X = uncertain material.

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Table 4
Effect of *Tribulus terrestris* (TT) extracts on muscle mass and fat mass of male boxers trained with high intensity or high volume (mean ± SD).

	Baseline		High intensity training		High volume training	
	E	E + TT	E	E + TT	E	E + TT
Total skeletal muscles (kg)	28.5 ± 2.9	27.6 ± 4.8	29.0 ± 2.9	27.9 ± 4.7	29.3 ± 3.4	28.6 ± 5.0
Skeletal muscles/body weight (%)	44.4 ± 1.9	44.5 ± 2.4	45.3 ± 1.5	45.3 ± 2.2	44.9 ± 1.3	45.5 ± 1.8
Total body fat (kg)	6.2 ± 2.5	6.9 ± 1.7	5.7 ± 2.2	6.1 ± 1.8	6.1 ± 2.3	6.4 ± 1.8
Body fat percentage (%)	9.6 ± 3.2	9.8 ± 2.4	8.8 ± 2.6	9.0 ± 2.3	9.2 ± 2.5	9.2 ± 2.3

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

Table 5
Effect of *Tribulus terrestris* (TT) extracts on plasma levels of CK and BUN of male boxers trained with high intensity or high volume (mean ± SD).

	Baseline		High intensity training		High volume training	
	E	E + TT	E	E + TT	E	E + TT
CK (U/L)	313.2 ± 170.6	357.8 ± 151.3	2719.9 ± 832.5*	1591.5 ± 909.6*#	297.3 ± 100.3	345.0 ± 191.6
BUN (mmol/L)	4.5 ± 0.9	4.8 ± 0.8	5.2 ± 1.5	4.8 ± 0.7	5.2 ± 0.7	5.3 ± 0.3

* $p < 0.01$, compared with the baseline values in the same group; # $p < 0.05$, compared with E group after the same training.
Abbreviations: E = exercise; E + TT = exercise plus TT; CK = creatine kinase; BUN = blood urine nitrogen.

experimental condition, or their interaction ($p > 0.05$). For the change in MP after high intensity training, the ANOVA yielded significant main effect of MP ($F(1, 11) = 31.81$, $p < 0.001$), but not group ($F(1, 11) = 0.07$, $p = 0.799$). However, a significant group × MP interaction ($F(1, 11) = 7.79$, $p = 0.018$) was found, and *post hoc* test revealed a significant decrease in MP for E group ($p < 0.001$) with no difference for E + TT group ($p = 0.078$). After high volume training, there was also an interaction of group × MP ($F(1, 11) = 4.81$, $p = 0.050$) but not group ($F(1, 11) = 0.06$, $p = 0.819$). *Post hoc* test showed a decrease in MP for E group ($p = 0.017$) with no difference for E + TT group ($p = 0.702$) (Table 6). These results indicated that the decrease of MP after the two trainings was modulated by TT supplement ($p < 0.05$).

Similar results were found in MP/BW between groups. After high intensity training, the ANOVA showed significant main effect of MP/BW ($F(1, 11) = 30.78$, $p < 0.001$) and interaction of group × MP/BW ($F(1, 11) = 6.64$, $p = 0.026$). Although there was no group discrepancy of MP/BW ($F(1, 11) = 0.17$, $p = 0.685$), *Post hoc* test revealed a significant decrease in MP/BW for E group ($p < 0.001$) with no difference for E + TT group ($p = 0.068$). After high volume training, significant main effects of MP/BW ($F(1, 11) = 6.97$, $p = 0.023$) and group × MP/BW interaction ($F(1, 11) = 7.82$, $p = 0.017$) were found. Although there is no group difference in MP/BW ($F(1, 11) = 0.03$, $p = 0.867$), *post hoc* test revealed a significant decrease in MP/BW for E group ($p = 0.002$) with no difference for E + TT group ($p = 0.917$) (Table 6). These results indicated that the decrease of MP/BW after the two trainings was modulated by TT supplement ($p < 0.05$).

3.4. Effect of TT extracts on plasma levels of testosterone, DHT, IGF-1, and IGFBP-3

No significant post-training change was found in testosterone (T), DHT, and IGF-1 between the main effects for groups, experimental condition or their interaction ($p > 0.05$). For the change in plasma IGFBP-3 after high intensity training, the ANOVA showed significant group × IGFBP-3 interaction ($F(1, 11) = 14.73$, $p = 0.003$), but not group ($F(1, 11) = 0.57$, $p = 0.467$). *Post hoc* test revealed a decrease in IGFBP-3 for E + TT group ($p = 0.005$) with no difference in E group ($p = 0.091$). For high volume training, significant main effects of IGFBP-3 ($F(1, 11) = 5.07$, $p = 0.046$) and group × IGFBP-3 interaction ($F(1, 11) = 5.78$, $p = 0.035$) but not group ($F(1, 11) = 0.35$, $p = 0.568$) were found. *Post hoc* test revealed a significant decrease in IGFBP-3 for E + TT group ($p = 0.009$) with no difference for E group ($p = 0.912$), indicating that the significant main factor of IGFBP-3 was modulated by TT supplement ($p < 0.05$) (Table 7).

Table 6
Effect of *Tribulus terrestris* (TT) extracts on anaerobic performances of male boxers trained with high intensity or high volume (mean ± SD).

	Baseline		High intensity training		High volume training	
	E	E + TT	E	E + TT	E	E + TT
PP (w)	667.1 ± 112.6	616.1 ± 135.7	572.9 ± 84.8	551.2 ± 136.5	603.1 ± 54.3	632.9 ± 147.5
PP/BW (w/kg)	10.8 ± 1.2	10.2 ± 1.4	9.3 ± 1.1	9.1 ± 1.1	9.5 ± 0.9	10.2 ± 0.9
MP (w)	550.6 ± 75.5	498.7 ± 112.8	434.6 ± 69.5*	459.4 ± 122.3*#	477.8 ± 49.9*	508.7 ± 103.2#
MP/BW (w/kg)	8.9 ± 0.7	8.2 ± 0.9	7.1 ± 1.1*	7.5 ± 0.9*#	7.5 ± 0.9*	8.2 ± 0.3#
Fatigue index (%)	32.9 ± 7.5	35.1 ± 5.5	45.5 ± 12.9	36.9 ± 8.7	42.4 ± 12.5	35.7 ± 13.8

* $p < 0.05$, compared with the baseline values in the same group; # $p < 0.05$, compared with E group after the same training.
Abbreviations: E = exercise; E + TT = exercise plus TT; PP = peak power; BW = body weight; MP = mean power.

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Table 7
Effect of *Tribulus terrestris* (TT) extracts on plasma levels of T, DHT, IGF-1, and IGFBP-3 of male boxers trained with high intensity or high volume (mean ± SD).

	Baseline		High intensity training		High volume training	
	E	E + TT	E	E + TT	E	E + TT
T (nmol/L)	15.8 ± 3.0	15.4 ± 6.8	18.1 ± 4.5	16.0 ± 4.0	18.3 ± 4.6	16.9 ± 7.1
DHT (ng/mL)	817.0 ± 298.3	880.3 ± 348.9	776.2 ± 157.5	719.2 ± 231.3	788.8 ± 180.5	774.1 ± 272.0
IGF-1 (ng/mL)	224.4 ± 29.1	243.0 ± 46.9	179.1 ± 29.9	202.0 ± 52.0	208.5 ± 39.5	215.5 ± 53.8
IGFBP-3 (ng/mL)	3605.0 ± 807.2	3784.0 ± 1,049.6	3950.8 ± 479.3	3075.5 ± 1,072.5*#	3632.7 ± 470.1	2946.4 ± 974.1*#

* $p < 0.05$, compared with the baseline values in the same group; # $p < 0.05$, compared with E group after the same training.
Abbreviations: E = exercise; E + TT = exercise plus TT; T = testosterone; DHT = dihydrotestosterone; IGF-1 = insulin growth factor-1; IGFBP-3 = insulin growth factor binding protein-3.

3.5. No correlation between the change of IGFBP-3 with the changes of CK, MP, and MP/BW

The correlation coefficients of the change of IGFBP-3 with the changes of CK, MP, and MP/BW in E + TT boxers were not statistically significant after the two trainings (data not shown).

3.6. No role of TT extracts on RBC and Hb

There was no statistical difference in RBC and Hb between E and E + TT groups after high intensity or high volume training (data not shown), which was similar to our previous results in rats[9] and report of Milasius et al.[21]

4. Discussion

4.1. Bioactive components of the TT extracts

Q-TOF mass spectrometry can provide high resolution and accurate mass measurement of both the precursor and product ions, thus it has become more and more popular for identification of drug components and metabolites.[22] Twenty-two constituents were identified from the TT extracts, among which the most abundant constituents were TT saponins (25(R)-Spirostan-3,6,12-trione/25(R)-Spirostan-4-ene-3,12-dione and TT saponin A). The compositions and quantitative contents of TT extracts are not stable, depending on geographical region, climate[23] and part of herb, which may partly explain the divergent results of TT extracts from different studies.

4.2. TT extracts mitigated muscle damage and increased anaerobic performance while unchanged muscle mass

The levels of serum or plasma CK and BUN are commonly used to judge the severity of muscle damage and muscle protein catabolism, respectively.[24,25] In the present study, mitigating muscle damage was found in male boxers who took TT extracts during high intensity training. Decreasing contents of CK induced by TT extracts in rats were also found by another study.[26]

TT extracts have been regarded as natural substances that can be added in diets in order to improve exercise performance and increase lean body mass.[27] Our previous studies demonstrated the increased effect of TT extracts on rat performance.[9,10] In the present study, similar results were found in male boxers, that TT capsules (1250 mg, about 20 mg/kg a day for 3 weeks) significantly increased anaerobic performance (absolute and relative mean power). The promoting effect of TT on anaerobic performance was also demonstrated by Milasius et al.,[21] who reported that anaerobic alactic muscular power and single muscular contraction power were significantly increased among youth men after consuming TT capsules (1875 mg, about 25 mg/kg a day) for 20 days. In contrast to the above data, other studies did not confirm the increased performance effect of TT on athletes, such as on resistance-trained men[12] and rugby players.[11] In addition, no significant discrepancy of muscle mass and fat mass with or without TT extracts was demonstrated during training, similar to the results of our previous work in rats[9] and other researchers,[11,12] suggesting that the increased anaerobic performance by TT extracts was not mediated by increasing muscle mass.

4.3. TT extracts play no role on plasma testosterone and DHT

Testosterone is an important androgenic anabolic hormone for its long-term anabolic actions[28] and a close connection between testosterone and hypertrophy-type training was demonstrated.[29] So far, the results of TT extracts on blood androgens among humans and animals were both contradictory, and limited animal studies displayed a significant increase in blood testosterone levels after TT administration,[4,5] but this effect was not found in humans except that TT was part of a combined supplement administration.[3] The present study also demonstrated that TT extracts did not affect the blood testosterone and DHT of trained male boxers, indicating that plasma androgens were irrelevant to the enhanced effect of TT extracts on anaerobic performance.

Nutritional supplements recommended for competitive athletes to enhance their performance may be contaminated intentionally by androgenic-anabolic steroids,[30] which may lead to inadvertent doping in competitive sports. However, taking TT without any contaminations did not cause positive anti-doping tests.[14] We believe that two things should be done for using TT safety and effectively among athletes and physically active men, one is to purify and separate TT bioactive components and the other is to avoid contamination of TT by androgenic-anabolic steroids.

4.4. Changes of plasma IGF-1 and IGFBP-3: possible mechanisms for TT extracts?

IGF-1 has great effect on muscle hypertrophy, muscle repair, and alleviating muscle damage.[31] Over-expressed IGF-1 exhib-

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ited dramatically enlarged skeletal muscles in a number of animal models;[17] in contrast, decreased IGF-1 in circulation and skeletal muscle was found to be dramatically reduced in muscle mass.[32] Recently, a potential role of IGF-1 in protecting unloaded skeletal muscles from damage and accelerating muscle repair and regeneration was reported.[17] In addition, the change of IGFBP-3 is considered a crucial aspect in modulating IGF-1 bioactivity. Serum concentrations of total IGF-1 decreased and IGFBP-3 increased were found after 16 weeks of resistance training in young women[33] and in endurance-trained elite athletes at the end of exercise.[34] However, increases, decreases, and no changes of circulating total IGF-1 and IGFBP-3 after both acute and chronic exercises have been reported[35,36] because of the heterogeneity in subject characteristics, physical activity (type, intensity, and duration), and training state. In the present study, supplement of TT extracts decreased the plasma level of IGFBP-3 in male boxers after the two trainings. But there was no statistical significant correlation of the change of IGFBP-3 with the changes of CK, MP, and MP/BW in E + TT boxers, which may related to the small sample size ($n = 6$). These results suggested that the effects of TT in trained male boxers may be mediated by decreasing IGFBP-3, ultimately increasing the bioactivity of IGF-1.

5. Conclusion

Taking 1250 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 associated with the decrease of IGFBP-3 rather than androgen in plasma.

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Authors' contributions

MYM carried out ELISA and DEXA studies, participated in statistical analysis and drafted the manuscript; GZC carried out Wingate test and UHPLC-Q-TOF/MS analysis; WXH conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

None of the authors declare competing financial interests.

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