

# ENDOCRINE RESPONSES TO PHYSICAL TRAINING AND TRIBULUS TERRESTRIS SUPPLEMENTATION IN MIDDLE-AGE MEN

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**Abstract.** The aim of this study was to evaluate the effects of steroidal saponin supplementation on blood concentration of T, GH and IGF-1. The research involved 14 men between the age of 45 and 60 years. The duration of the experiment was 12 weeks. There were two series of laboratory tests. Independent tests were conducted at the beginning and after 12 weeks of the intervention.

A two-way repeated measures ANOVA revealed a statistically significant effect of the intervention on the following variables: T-Ch ( $\eta^2 = 0.542$ ), HDL-Ch ( $\eta^2 = 0.522$ ), LDL-Ch ( $\eta^2 = 0.587$ ), T ( $\eta^2 = 0.603$ ), IGF-1 ( $\eta^2 = 0.512$ ) and GH ( $\eta^2 = 0.621$ ). Thus, FFM significantly increased while TBF and BM decreased in comparison to pre-intervention levels. The analyzed results indicate that treatment or supplementation of individual hormone deficiencies can be a successful form of counteracting the aging process. Nevertheless, the effects of TT supplementation on the concentration of T as well as GH and IGF-1, requires further studies, especially in middle-aged and older subjects, along with different exercise programs. The analyzed results indicate that treatment or supplementation of individual hormone deficiencies can be a major form of counteracting the aging process.

**Key words:** steroidal saponins, testosterone, supplementation, growth hormone, insulin-like growth factor

## Introduction

The neuroendocrine system plays a prominent role in controlling the rate of muscle tissue degradation and the efficiency of metabolic processes. Among the main morphological symptoms of aging the most obvious include: a decrease in lean body mass (LBM), an increase in body fat content (FM), disorders in lipid and lipoprotein profile, and a reduction in bone mineral density. The endocrine system, particularly anabolic hormones, such as growth hormone (GH) and testosterone (T), as well as the insulin-like growth factor (IGF-1) determine the anabolic processes occurring in the human body to a large extent. Hypogonadism and hiposomatropism are factors which

directly contribute to a decrease in muscle mass. There are many conflicting results regarding the effects of supplementation on the concentration of particular anabolic and catabolic hormones. The use of supplements before, during and after exercise has a significant impact on post-exercise blood hormone concentration (Bird, Tarpenning, Marino, 2006). The effect of changes in the concentration of various hormones, as a result of supplementation, is distinctly observed among older individuals, diagnosed with hormonal deficiencies.

Tribulus Terrestris (TT) is a supplement derived from the fenugreek plant. Specific properties of saponins, such as interaction with cell membranes, combining with sterols, as well as cholesterol, leads to partial damage and greater permeability. Steroidal saponins have a high capacity for erythrocyte hemolysis. By affecting the metabolism of male androgens saponins influence the increase of T concentration (Huang, Tan Jiang, Zhu, 2003). The exact mechanism of TT is not recognized, however, steroidal saponins inhibit the activity of key enzymes responsible for the unwanted conversion of testosterone to estrogen and dihydrotestosterone (DHT). Reduced production of unfavorable metabolites stimulates the secretion of testosterone by the body, and enhances its activity within the muscle tissue. Other researchers suggest that the ergogenic effect of saponins is primarily associated with the increase in the concentration of luteinizing hormone (LH), which stimulates the secretion of testosterone (De Combarieu, Fizzati, Lovati, Mercalli, 2003). Gauthaman, Mohamed Saleem, Ravi, Sita, Niranjali (2008) showed that a TT dose of 900 to 2500 mg per day stimulates the synthesis of T in rats. Despite theoretical basis and research on animals, which confirm that TT increases the concentration of LH and T, the results of human studies are inconclusive. Studies in a group of healthy subjects with normal levels of GH and T do not confirm the impact of TT on anabolic functions (Rogerson et al., 2007).

Nonetheless, several studies have shown that TT, as well as vitamin and mineral supplementation does not increase muscle strength or muscle hypertrophy as a result of resistance training (Rogerson et al., 2010; Antonio, Uelmen, Rodriguez, Earnest, 2010). There were no significant changes in body composition and body mass; among men who were engaged in regular resistance exercise and TT supplementation (Antonio et al., 2010). A different situation occurs in case of TT supplementation among subjects with known hypogonadism and hisposomatropism. Adimoelja (2000) showed on a group of middle-aged subjects that TT has a significant impact on the concentration of LH and T.

TT supplementation in subjects with known hypogonadism is also diabetogenic, prevents cancer, reduces total cholesterol (Ch-T) and low-density lipoprotein (LDL) (De Combarieu et al., 2003). Steroidal saponin supplementation in men with physiologically reduced concentrations of T and GH may be a reasonable alternative for hormone therapy, and as research indicates an increase in the concentration of T in patients with GHD or impaired secretion of testosterone significantly affects the changes of body composition and improves the physical abilities of the organism (Zajac et al., 2010).

The aim of this study was to evaluate the effect of steroidal saponins supplementation on blood concentration of T, GH and IGF-1 and changes in body mass and body composition, as well as in the lipid and lipoprotein profile in middle-aged and slightly overweight men.

## Methods

### Subjects

The research involved 14 men between the age of 45–60 years, body mass index of 25–33, and body fat content between 23–30%. The subjects were randomly divided into two groups – an experimental (exp) and

a control group (contr). The exp. group received steroidal saponins; for the first six weeks three capsules (900 mg) per day in split doses. Two capsules were ingested in the morning on an empty stomach (600 mg) and one at bedtime (300 mg). From weeks 6 to 12, 6 capsules (1,800 mg) were ingested per day in split doses. Four capsules in the morning on an empty stomach (1,200 mg) and two at bedtime (600 mg). The contr. group received a placebo in the form of gelatin capsules. During the 12 weeks of the experiment all subjects participated in a physical activity program. Prior to testing, as well as in the course of the experiment, participants followed an isocaloric mixed diet (55% carbohydrate, 20% protein, 25% fat).

## Research methods

### *Test procedure*

The duration of the experiment was 12 weeks. There were two series of laboratory tests. Independent tests were conducted at the beginning and after 12 weeks of the intervention. The study evaluated such morphological, physiological and biochemical variables as body mass and body composition (BM, FFM, FM, TBW, BMI), the concentration of chosen hormones and growth factors (T, GH, IGF-1), as well as the lipid and lipoprotein profile including: triglycerides (TAG), total cholesterol (CH-T), high density cholesterol (HDL-Ch), and low-density cholesterol (LD-Ch).

Determination of body mass and body composition was performed by electrical impedance using the 220 InBody apparatus.

Venous blood samples were taken from all participants (10 ml) to be assayed for the concentrations of T, GH, and IGF-1. IGF-1 concentration in the serum was determined by immunoradiometric (IRMA) methods using a diagnostic kit DSL-2800 Active IGF-1 (Diagnostic System Laboratories, Webster, Texas, USA). Determination of the concentration of T was performed using the radioimmunoassay (RIA) analysis of blood serum using DSL-2100 assay. Biochemical analysis of the lipid and lipoprotein profile TAG, T-Ch, LDL-Ch, HDL-Ch was performed with an enzymatic method by a fully automated analyzer (Siemens Dimension). All biochemical assays were performed in duplicate with the ICC between 0.92 and 0.97.

### *Physical activity program*

The physical exercise program applied to all the participants included 4 training sessions per week, with 2 sessions directed at the improvement of anaerobic power (resistance exercise), while 2 consisted of aerobic endurance exercise.

Aerobic training was performed on a stationary cycle ergometer, starting with 30 minutes of continuous exercise at an intensity of 70–75% of maximum heart rate (HR max). Every two weeks, the work volume was increased by 5 minutes in order to reach 60 minutes in the last two weeks of the experiment. Strength training had a holistic approach, involving all major muscle groups (the back, chest, abdomen, arms and lower limbs). For the first four weeks, exercises were performed in 3 sets of 8–12 reps with the resistance equal to 60–70% of 1RM and 2 min rest periods between sets. During the experiment, the number of sets of each exercise increased from 3 to 4 sets in weeks 5–8, and respectively to 5 sets in weeks 9–12 for each exercise.

The research project was approved by the Ethics Committee for Scientific Research at the Academy of Physical Education in Katowice, Poland.

## Statistical analysis

The data were analyzed using the Statistica 9.1 software. The descriptive analyses consisted of the mean and standard deviation. For all measured variables, the estimated sphericity was verified according to the Mauchly's W test, and the Greenhouse–Geisser correction was used when necessary. Before using parametric tests, the assumption of normality was verified using the Kolmogorov-Smirnov test (Maszczyk et al., 2012). The comparison of analyzed values before and after the introduction of the experimental factor, was carried out with a two-way repeated measures ANOVA. When significant differences were found, Tukey HSD post-hoc tests were used. The effect size (eta-squared;  $\eta^2$ ) of each test was calculated for all analyses. Effect size was classified according to Hopkins (Hopkins, 2010). Statistical significance was set at  $p < 0.05$ .

## Results

Table 1 presents pre- and post-intervention values of morphological variables under analysis. A two-way repeated measures ANOVA revealed a statistically significant effect of the intervention program on the following variables: T-Ch ( $\eta^2 = 0.542$ ), HDL-Ch ( $\eta^2 = 0.522$ ), LDL-Ch ( $\eta^2 = 0.587$ ), T ( $\eta^2 = 0.603$ ), IGF-1 ( $\eta^2 = 0.512$ ), GH ( $\eta^2 = 0.621$ ) in the exp. group. Tukey's HSD post-hoc test revealed a statistically significant decrease of T-Ch ( $p = 0.002$ ) and LDL-Ch ( $p = 0.001$ ), while other post-hoc tests revealed statistically significant increases of HDL-Ch ( $p = 0.002$ ), T ( $p = 0.001$ ), IGF-1 ( $p = 0.003$ ) and GH ( $p = 0.001$ ) in comparison to pre-intervention values.

The increase trend of T ( $\eta^2 = 0.071$ ) and HDL ( $\eta^2 = 0.057$ ) was observed only in the contr. group. However, the effect of the intervention program was not statistically significant ( $p = 0.052$ ,  $p = 0.064$  respectively).

**Table 1.** The lipoprotein profile and hormone concentrations before and after supplementation with steroidal saponins with results of a two-way repeated measures ANOVA

Variables	Group	Before X ± SD	After X ± SD	F	P
T-Ch (mg/dl)	I exp	189.64 ±28.98	178.53 ±25.05	15.417	0.003
	II cont	196.42 ±26.85	192.36 ±24.89		
HDL-Ch (mg/dl)	I exp	65.44 ±16.80	70.01 ±14.50	14.261	0.003
	II cont	68.90 ±4.33	65.57 ±4.30		
LDL-Ch (mg/dl)	I exp	103.01 ±21.20	98.64 ±11.31	17.231	0.002
	II cont	127.63 ±11.35	128.78 ±8.75		
GH (ng/ml)	I exp	0.47 ±0.15	0.52 ±0.16	23.261	0.001
	II cont	0.58 ±0.14	0.57 ±0.12		
IGF-1 (ng/ml)	I exp	150.01 ±30.17	180.17 ±27.38	13.250	0.003
	II cont	191.98 ±25.87	191.57 ±23.85		
T (nmol/l)	I exp	13.19 ±5.34	14.74 ±4.06	18.510	0.001
	II cont	17.88 ±5.14	17.64 ±4.01		

Table 2 presents the pre- and post-intervention values of body composition and body mass variables. A two-way repeated measures ANOVA revealed a statistically significant effect of the intervention on BM ( $\eta^2 = 0.644$ ), FFM ( $\eta^2 = 0.417$ ), and TBF ( $\eta^2 = 0.817$ ) in the exp. group. Not significant post-intervention differences were observed in

TBW ( $\eta^2 = 0.094$ ) value. Thus, FFM significantly increased while TBF and BM decreased in comparison to the pre-intervention levels ( $p = 0.001$ ,  $p = 0.001$  and  $p = 0.003$ , respectively).

Similarly, not significant post-intervention differences were observed in the contr. group. The increase of FFM ( $\eta^2 = 0.058$ ) and FAT ( $\eta^2 = 0.044$ ) values, and decrease of BM ( $\eta^2 = 0.084$ ) value ( $p = 0.061$ ,  $p = 0.071$ ,  $p = 0.581$  respectively), were observed only.

**Table 2.** Pre- to post-intervention changes in body composition and body mass, before and after the saponins treatment with results of a two-way repeated measures ANOVA

Variables	Group	Before X $\pm$ SD	After X $\pm$ SD	F	P
BM (kg)	I exp	89.17 $\pm$ 8.62	87.02 $\pm$ 8.58	19.551	0.011
	II cont	99.54 $\pm$ 7.24	99.38 $\pm$ 7.57		
TBF (kg)	I exp	20.22 $\pm$ 7.57	18.87 $\pm$ 7.44	69.921	0.011
	II cont	20.82 $\pm$ 5.86	20.22 $\pm$ 5.93		
FFM (kg)	I exp	70.42 $\pm$ 6.18	70.88 $\pm$ 5.55	9.591	0.026
	II cont	85.84 $\pm$ 8.78	84.77 $\pm$ 8.26		
TBW (kg)	I exp	51.75 $\pm$ 4.49	53.37 $\pm$ 5.04	1.243	0.772
	II cont	62.74 $\pm$ 6.16	61.71 $\pm$ 14.39		

## Discussion and conclusions

Ergogenic aids, such as dietary supplements, are essential elements of competitive sports, yet they can also improve metabolic efficiency in recreationally active subjects of all ages. Milasius, dadeliene, Skernevicius (2009), showed a significant positive effect of supplements containing TT on acid-base equilibrium after short-term, high intensity anaerobic exercise in competitive athletes. This effect can also be observed among middle aged and elderly subjects. In our research project, steroidal saponin supplementation in a group of middle-aged men caused a statistically significant increase in resting T, GH and IGF-1. The results of the study partially confirmed Brown's et al., reports (2001), which showed an significant effect of saponin supplementation on serum testosterone concentration in men aged 30–59 years. However, in Brown's et al., study (2001) the ergogenic effect was not caused only by TT, as the reserchers used a complex supplement called DION. Milasius et al. (2009) also observed an increase in blood T concentration, but only during the first 10 days of the experiment with TT supplementation. The test results obtained in the experiment conducted are contrary to Neychev, Mitev's research (2005), which showed no change in concentration of testosterone, androstenadiol and luteinizing hormone (LH) after supplementation with TT. The differences between the results of our experiment and those obtained by Neychev, Mitev (2005), may be the result of different dosages used, different duration of the experiment, and most importantly, different criteria for selecting subjects for the research. In most studies where no effect of saponins on T concentration was observed, young, physically active men with a physiologically high resting concentration of T were involved (Neychev, Mitev, 2005; Poprzecki, Zebrowska, Cholewa, Zając, Waskiewicz, 2005). In our study, the research group included middle-aged men with physiologically low levels of resting T, which seems to be a key factor in the effectiveness of such supplementation. An important finding of the study includes the fact that the TT supplementation also caused an increase in resting concentrations of GH and IGF-1 in the experimental group compared to the placebo group.

Studies indicate that treatment with recombinant GH in elderly subjects significantly affects body composition (Poprzecki et al., 2005; Zajac, Wilk, Socha, Maszczyk, Chycki, 2014). This effect is particularly important in case of increased concentrations of both GH and T (Blackman et al., 2002), which took place in the current study. Apart from the significant increase of GH concentration in this study, an increase in resting IGF-1 was also observed. Our research indicates significant increase in FFM and a decrease of BF with a concomitant increase in TBW. Similar results were obtained in a project by Poole et al. (Poole et al., 2010). Although statistically significant changes in the concentration of T were not shown in the Poole et al., study (2010), there was a trend for increased FFM ( $p < 0.001$ ) and a decrease in BF ( $p < 0.001$ ). Adverse changes in the lipid profile which increase the risk of coronary heart disease are typical for middle-aged men. Our study, in which men aged 45–60 were supplemented with steroidal saponins, revealed significant changes in the lipid and lipoprotein profile after 12 weeks of the experiment. A significant decrease of total cholesterol (T-Ch), an increase in high density cholesterol (HDL-Ch), a decrease in low density lipoprotein (LDL-Ch) and a reduction in plasma triglycerides (TAG) were observed. It may be assumed that TT supplementation is not necessarily directly responsible for changes in the lipid and lipoprotein profile. We hypothesize that it is the result of higher resting concentrations of T and GH, which was also observed in studies by Zajac et al. (2014). It seems that both GH as well as T have a profound effect on the lipid and lipoprotein profile. Testosterone metabolism inhibits the uptake of triglycerides and lipoprotein lipase activity (Amore, 2005). Studies indicate that testosterone treatment reduces the levels of LDL-Ch (Hare et al., 2014), and exerts additional effects on lipids, depending on the dose and form of treatment. Munzer, Harman, Sorkin, Blackman (2009), also describe the impact of recombinant GH treatment on decreases in LDL-Ch, but not T-Ch. Combined treatment with rGH and T affects the decrease in T-Ch and LDL-Ch as well. Research conducted by Zajac et al. (2014) on a similar group of middle-aged men indicate that injections of T and GH resulted in a decrease of T-Ch, an increase in HDL-Ch, a marked reduction in LDL-Ch. This justifies the assumption that it is the increase in the concentration of T and GH which affects the changes in lipid profile and not the direct effect of TT supplementation. The increase in the concentration of resting T and GH, stimulates changes in the lipid and lipoprotein profile in overweight subjects, which was also observed in the current study. The ergogenic effects of TT supplementation are not limited to increased concentrations of T and GH, what affects the lipid and lipoprotein profile, but also related to increased physical activity. In this research, contrary to most studies in this area, the subjects involved in the study were physically active prior to the experiment, so regular exercise was a continuation of the current lifestyle and the changes in the lipid profile were mainly caused by the TT supplementation. The analyzed results indicate that treatment or supplementation of individual hormone deficiencies can be a major form of counteracting the aging process.

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