International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:6, No:6, 2012

Association between Serum Concentrations of Anabolic Hormones and their Binding Proteins in Response to Graded Exercise in Male Athletes

A. Żebrowska, A. Kochańska-Dziurowicz, A. Stanjek-Cichoracka

Abstract**—**We investigated the response of testosterone (T), growth hormone (GH), cortisol (C), steroid hormone binding globulin (SHBG), insulin-like growth factor (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), and some anabolic-catabolic indexes, i.e.: T/C, T/SHBG, and IGF-1/IGFBP-3 to maximal exercise in endurance-trained athletes (TREN) and untrained subjects (CG). The baseline concentration of IGF-1 was higher in athletes (TREN) when compared to the CG ($p<0.05$). The GH concentration and GH/IGF-1 ratio increased after exercise in all subjects compared to respective values at rest. The resting IGF-1/IGFBP-3 ratio was significantly higher in athletes. The maximal exercise test induced an increase in post-exercise T/SHGB ratio in athletes compared to CG ($p<0.05$). These results indicate that elevation of baseline serum IGF-1/IGFBP-3 and T/SHGB ratio after exercise might suggest that free fractions of these hormones may act as a potent stimulant of muscle hypertrophy in trained endurance athletes.

Keywords**—**anabolic hormones, endurance training, exercise, growth factors

I. Introduction

THE skeletal muscle undergoes morphological and functional changes in response to physical training. As previously described, the muscle is able to adapt by increasing the size and amount of contractile proteins, leading to an increase in the size of muscle fibers and resultant force production [1]-[3]. Muscle hypertrophy in athletes depends on several determinants including type of exercise, intensity and duration of training. Cross sectional studies confirmed the hypothesis that divergent skeletal muscle adaptations do occur in athletes performing purely dynamic or static sports, and combined dynamic and static sports (cycling and rowing). In fact, training regimens of athletes may result in different muscle adaptation in response to both mechanical load and endocrine system reaction [1], [4]. Circulating levels of both anabolic (T, GH), and catabolic hormones increase with exercise intensity [5]-[7]. Testosterone is known to promote hypertrophy while increased corticosteroids accelerate protein degradation. As a result, insulin and growth factors levels become increased to regulate muscle protein synthesis [8], [9].

A. Żebrowska is with Department of Physiology and Medicine of Sport, Academy of Physical Education, Katowice, Poland (phone: 48 32 2075145; e-mail: olazebrowska@yahoo.com)

A. Kochańska-Dziurowicz is with Department of Radioisotope Diagnosis and Radiopharmaceuticals, Medical University of Silesia, Poland (phone: 48 32 364 1593; email izotop@sum.edu.pl)

A. Stanjek-Cichoracka is with Department of Radioisotope Diagnosis and Radiopharmaceuticals, Medical University of Silesia, Poland (phone: 48 32 364 1593; email izotop@sum.edu.pl)

Intensive endurance training can induce beneficial effects on aerobic performance and prevent muscle loss. It has been found that an acute increase in serum concentration of cortisol (C) accelerates the mobilization of free fatty acids for energy during exercise. This mechanism is believed to be responsible for controlling the balance between carbohydrate and fat metabolism during and after exercise [10], [11].

Recent investigations suggest that the equilibrium between anabolic and catabolic process depends not only on greater stimulation of endocrine factors, but also on paracrine mechanisms. Insulin-like growth factor-1 (IGF-1) has been shown to be the most important growth factor involved in muscle hypertrophy [12]-[14]. Moreover, important factors that regulate IGF-1 and testosterone (T) affinity for target cells are high-affinity binding proteins [15]-[17]. Insulin like binding protein-3 (IGFBP-3) and steroid hormone binding globulin (SHBG) are considered important factors modulating the interaction of IGF-1 and T with theirs receptors [18]-[19]. It was hypothesized that exercise-induced binding proteins proteolysis might contribute to anabolic effects of exercise by increasing the concentrations of free-fraction hormones [20].

To test this hypothesis we have investigated serum concentrations of growth hormone, insulin growth factor-1, insulin growth factor binding protein-3, testosterone, steroid hormone binding globulin, and cortisol in response to graded maximal exercise in chronically endurance trained athletes and untrained male subjects.

II. Material And Methods

Forty-nine professional endurance trained athletes (TREN) volunteered to participate in the experiment. Eleven healthy but untrained men, students of the Physical Education Faculty age-matched to the study subjects, served as the control group (CG). All athletes had been training for about 8.2 ± 2.4 years and underwent medical evaluations at the same point of the pre-season training process. The experiment was approved by the Ethics Committee of the Academy of Physical Education in Katowice and conforms to the standards set by the Declaration of Helsinki.

All subjects performed incremental maximal intensity treadmill exercise (over the anaerobic threshold AT), which had been recognized to stimulate a higher increase in blood concentrations of anabolic hormones than did low intensity exercise (below AT) [25]. Pulmonary ventilation (VE), oxygen uptake (VO_2), and carbon dioxide output (CO_2) were measured continuously from minute 6 prior to exercise test and throughout each stage of the exercise load using the Oxycon Apparatus (Jaeger, Germany).

TABLE I
ANTHROPOMETRIC AND PHYSIOLOGIC CHARACTERISTICS OF THE CONTROL GROUP (NT) AND ATHLETES (TREN) (MEAN ± SD)

Variable	CG n=11	TREN n=49
Age (years)	20.4 ± 1.0	21.7 ± 0.5
Body height (cm)	180 ±1.8	181 ± 0.8
Body mass (kg)	73.7 ± 2.3	71.9 ± 1.1
BMI (kg/m$^{2)}$	22.7 ± 0.5	21.8 ± 0.2
FAT (%)	13.3 ± 0.8	10.4 ± 0.4**
FM (kg)	9.8 ± 0.6	7.4 ± 0.3***
LBM (kg)	64.4 ± 2.1	64.4 ± 1.0
TBW (kg)	46.8 ± 1.6	47.2 ± 0.7
Training status (years)	0	8.2 ± 2.4
VO_2max (ml/kg/min)	51.4 ± 1.9	61.5 ± 0,9***
Power output max (Wat)	263 ± 14.8	389 ± 10.3***
LA (mmoL/L)	9.3± 1.3	10.6±1.8*

BMI- body mass index; FAT- percent of body fat; FM-fat mass; LBM-lean body mass; TBW-total body water: VO_2max-maximal oxygen uptake; LA-lactate concentration Statistical significance: * *p<0.05* ** *p<0.01*, *** *Mp<0.001 TREN vs. CG*

After 3 days of standardized normocaloric (37kcal/kg/day) diet with 50-60% carbohydrate, 15-20% protein, and 20-30% fat, bioelectric impedance analysis under resting conditions was used to measure percent body fat and total body water. The values (mean ± SD) of age, height, and body mass and body mass index of the participants are presented in Table 1. Venous blood samples were collected at rest (pre-exercise) for the assessment of baseline serum insulin-like growth factor (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), growth hormone (GH), testosterone (T), cortisol (C), and steroid hormone binding globulin (SHGB) concentrations. The sampling was repeated immediately after cessation of exercise and after 15 minutes of recovery. Additionally, 0.1 mL of blood was taken from the finger tip at the end of each work load for the assessment of hematocrit and blood lactate concentration. Serum somatomedin IGF-1, GH and IGFBP-3 were determined in duplicate using an immunoradiometric assay (IRMA) kit (RIACT, Cisbio). Serum testosterone (T) and cortisol (C) were also determined in duplicate with an immunoradiometric assay kit (TESTO-CT-2, Cisbio), while SHBG was measured by immunoradiometric assay kit (SPECTRIA SHGB IRMA, ORIONdiagnostica).

Blood lactate concentration (LA) was measured enzymatically using commercial kits (Boehringer, Manheim, Germany).

Data are presented as means and standard deviation (± SD). The data from two groups of subjects were analyzed by two-way ANOVA test with the use of Statistica 9.0 (StatSoft) software. When significant differences in F ratio were found, post-hoc Tukey's test was used. Statistical significance of intra-group differences (TREN and CG) was analyzed using Bonferroni test for independent variables. Between groups, correlation coefficients were determined with Pearson's rank order test. Statistical significance was set at $p<0.05$

III. RESULTS

Table I shows the difference in the body composition variables and the results of the graded exercise test. There were significant differences in fat mass ($p<0.001$) and maximal oxygen uptake (VO_{2max}) ($p<0.001$) of the TREN group compared to CG. The mean values of the maximum power output and lactate concentrations (LA) determined in TREN and CG are presented in Table 1. Resting IGF-1 concentration was higher in TREN athletes compared to CG ($p<0.05$).

Graded exercise tests had a significant effect on serum IGF-1 concentration at maximal intensity of exercise ($p<0.05$). Furthermore, at maximal exercise, higher values of serum IGF-1, IGFBP-3, and IGF-1/IGFBP-3 ratio were found in athletes compared to CG (Table II).

TABLE II
TESTOSTERONE (T), CORTISOL (C), TESTOSTERONE/CORTISOL RATIO (T/C), STEROID HORMONE BINDING GLOBULIN (SHGB), GROWTH HORMONE (GH), INSULIN-LIKE GROWTH FACTOR-1 (IGF-1), INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 (IGFBP-3), GH/IGF-1, AND IGF-1/IGFBP-3 IN CONTROL SUBJECTS (CG) AND ATHLETES (TREN), (MEAN ± SD) (RESTING VALUES, VALUES REACHED AT MAXIMAL EXERCISE INTENSITY - EX, AND 15 MINUTES AFTER EXERCISE - POST-EX)

Variable	CG n=11			TREN n=49		
	rest	Ex.	post -Ex.	rest	Ex	post-Ex
T (nmoL/L)	20.4	23.6	22	19	23.3**	22.1*
C (nmoL/L)	506	574	704**	561	686**	778**
T/C	0.04	0.04	0.03	0.04	0.04	0.03*
SHBG (nmoL/L)	38.4	49.4	63	43	42	39.9#
T/SHGB	61.6	59	55	51.3#	73.2*#	66
GH (μIU/mL)	0.8	51.5*	49.7**	3.7	61.1**	55.4**
IGF-1(ng/mL)	234	271	275	338#	362#	351
GH/IGF-1	0.02	0.2*	0.2**	0.02	0.2**	0.2**
IGFBP-3 (ng/mL)	2484	2447	2649	2515	3229**#	3129**
IGF-1/IGFBP-3	0.1	0.1	0.1	0.2#	0.2#	0.1

Statistical significance: * p<0.05, ** p<0.01 Ex and post-Ex vs. rest.
# p<0.05 TREN vs. CG

These results indicate that incremental physical exercise caused a significant increase in circulating GH and cortisol concentrations in all study participants. Endurance exercise had little effect on testosterone, SHBG, and T/C ratio, except for a significant increase in anabolic steroids after exercise compared to resting values, and higher T/SHBG in male athletes compared to untrained subjects (Table II).

Analysis of variance revealed a significant effect of endurance training and exercise on serum SHGB level $p<0.05$ ($F=3.6$) (Fig. 1), but no significant difference was seen in T/C ratio ($p>0.05$) (Fig. 2). ANOVA also revealed a significant effect of training on IGF-1/IGFBP-3 ratio $p<0.05$ ($F=2.9$) (Fig.3).

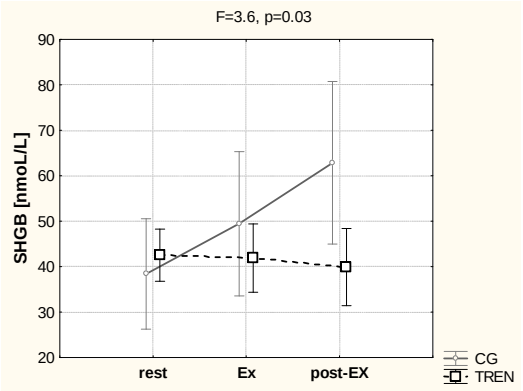

Fig. 1 Effect of training and exercise on serum SHBG concentration in all subjects (mean ± SD)

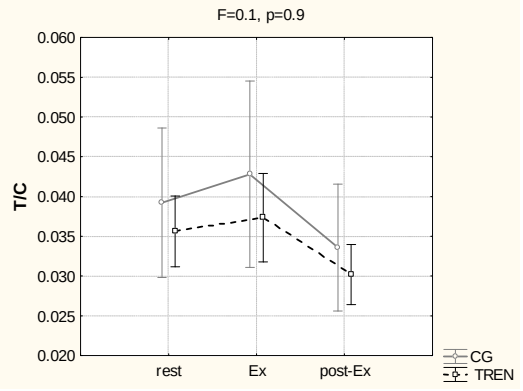

Fig. 2 Effect of training and exercise on T/C ratio in all subjects (mean ± SD)

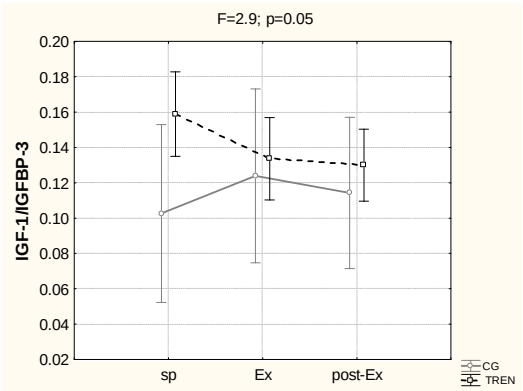

Fig. 3 Effect of training and exercise on IGF-1/IGFBP-3 ratio in all subjects (mean ± SD)

IV. Discussion

Data concerning the effects of endurance training on anabolic-catabolic imbalance are still sparse [3], [21]-[24]. Most studies report beneficial effects of this type of exercise on physical performance and metabolism in endurance athletes compared with control subjects [8], [25], [26]. However, no change in muscle mass and/or muscle degradation was found in master athletes [21], [27], [28]. Despite well-matched age groups, endocrine responses following endurance training were different in male athletes and untrained subjects, which may have both metabolic and hypertrophic implications. Our subjects also demonstrated differences in their body composition parameters, clearly indicating the significant effect of endurance training on their metabolism.

We found that athletes had significantly higher resting concentrations of circulating IGF-1 than age-matched controls. Moreover, IGF-1 was significantly elevated after incremental exercise in athletes compared to the control. The significance of our study lies in the fact that the ratio between IGF-1/IGFBP-3 was higher in trained compared to untrained subjects. Since no difference in growth hormone and testosterone level has been detected between athletes and control subjects, all aforementioned results imply that serum IGF-1 elevation during endurance training may induce anabolic effects in the muscle [29]-[31]. According to previous data, we speculated that during acute and repeated exercise, the proteolysis of IGFBP-3 might increase the bioavailability of free IGF-1 [8], [32], [33]. It should be pointed out that the elevation of resting and post-exercise concentrations of IGF-1 observed in our study was associated with a markedly increased IGF-1 to IGFBP-3 ratio in athletes.

The equilibrium between anabolic and catabolic processes depends on testosterone and cortisol blood concentrations [10], [18], [34]. Therefore as the so called anabolic-catabolic index, ie., the T/C ratio, has been analyzed [35]. Several studies have demonstrated that free testosterone, unbound to the albumin and SHBG, has greater biological activity [17], [22]. An increase in free anabolic-androgenic steroid concentrations in the blood may stimulate muscle hypertrophy, both in training-related and pathological conditions [17], [18], [22, [36]. Our study revealed lower resting serum T/SHGB ratio in athletes compared to untrained subjects. However, post-exercise T/SHGB levels were higher in TREN compared to CG group, suggesting that testosterone was involved in anabolic processes in our experienced athletes.

V. Conclusion

Our results indicate that resting and post-exercise serum IGF-1 concentrations were higher in trained compared to untrained subjects. We conclude that the greater IGF-1/IGFBP-3 and T/SHGB elevation after exercise might suggest that free fractions of these hormones may act as potent stimulants of muscle hypertrophy in trained endurance athletes.

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