EFFECT OF ACUTE EXERCISE ON SERUM GROWTH HORMONE AND FATTY ACID LEVELS IN ELITE MALE WATER POLO PLAYERS

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Abstract - The aim of study was to estimate the effect of acute exercise on serum growth hormone (GH) and fatty acid (FFA) levels in elite water polo players. Twelve male water polo players (20.50 ± 2.02 years) and eleven non-athletic male subjects (20.55 ± 1.04 years) participated in this study. In order to determine GH and FFA responses to acute exercise, a treadmill-running test was performed following an incremental protocol. Pre-exercise blood samples for both athletes and non-athletes were taken at 9 AM. Post-exercise samples were taken immediately after and 30 min after the treadmill running test. Water polo players had significantly lower baseline values of serum GH concentration compared to controls, whereas serum FFA concentration was significantly higher in water polo players compared to controls ($p<0.01$; $p<0.05$, respectively). In both groups, concentration of GH was significantly higher immediately after and after the 30-min of recovery compared to baseline levels ($p<0.05$). In water polo players, the concentration of FFA was significantly decreased immediately after and after the 30-min of recovery compared to baseline levels ($p<0.05$). No significant response to maximal exercise test was observed in the control group for serum FFA concentration. Our research indicates that acute exercise resulted in a significant increase in serum GH and reduction in fatty acid levels in elite water polo players.

Key words: athletes, acute exercise, growth hormone, free fatty acids

INTRODUCTION

Regular physical activity confers considerable benefit and affects many of the homeostatic mechanisms with which the endocrine system is almost immediately involved (Jenkins, 1999; Petrović-Oggiano et al., 2009). With regard to the abuse of growth hormone (GH) by competitive athletes as a performance-enhancing agent, there has been considerable interest into the response of the GH/IGF-I axis to exercise (Gibney et al., 2007). Because of this, the link between the physiological role of GH and exercise could be examined in elite athletes who are exposed to prolonged, regular physical activity. Moreover, Deuster et al. (1989) showed that the adaptation to physical training results in a more efficient hormonal mechanism, which in turn makes it possible to increase the workload. However, contradictory results have been reported in elite athletes' GH adaptation (Gibney et al., 2007). Studies have shown that resting GH levels might be elevated in trained individuals (Vasankari et al., 1993; Manetta et al., 2002). After a typical aerobic swimming set during a training season, Bonifazi et al. (1998) found that GH response was enhanced. In contrast, another study found a lower GH response to exercise in training subjects than in controls (Howlett, 1987).

Growth hormone is known to increase significantly in the blood in response to acute exercise stimuli, given its potential physiological roles in metabolism (Gibney et al., 2007; Widdowson et al., 2009). GH levels start to increase 10-15 min after the onset of exercise, peak either at the end or shortly after exercise, and remain elevated for up to 2 h following exercise (Widdowson et al., 2009). The need for energy supply (glucose and FFA) is increased during exercise and is associated with adaptive hormonal changes in elite athletes, possible with GH. In fact, numerous studies in the area of pituitary-derived GH observed an increase in lipolysis, sparing glucose and enhancing amino acid uptake in skeletal muscle (Milosevic et al., 2012). With such diverse roles of GH in the metabolism, it is interesting to study GH and FFA response to acute endurance exercise sessions in athlete populations with high body fat percentage, as seen in water polo players.

The aim of this study was to investigate the effect of acute exercise on serum growth hormone and fatty acid levels in elite male water polo players, and to compare this response with sedentary controls.

MATERIALS AND METHODS

Participants

Twelve elite male water polo players and eleven non-athletic male subjects participated in this study. The non-athletes were recruited from the student population of the University of Belgrade. They exercised less than 3 h/week (Table1). The subjects were healthy, nonsmokers, normotensive, did not take any drugs or medication and had no history of any endocrine disorders before or during this study. None of them had any family history of diabetes or obesity. All participants in this study were fully familiarized with the procedures before providing written informed consent to participate in the experiment as approved by the Ethics Committee of the School of Medicine, University of Belgrade.

Experimental design

All participants in this study completed a questionnaire about their training history and a body composition test before exercise testing. The pre-exercise blood samples (Pre-Ex) for both athletes and non-athletes were taken at 9 AM. All participants had eaten a high-carbohydrate meal about two hours before the exercise testing. Exercise tests were performed on a treadmill (Treadmill T200, Cosmed, Italy) following an incremental protocol targeting duration between 8 and 12 min. The treadmill protocol used for the VO_2 max test began at a speed of 4 km/h at 0.0% incline. The speed of the treadmill was increased by 1 km/h each minute. A maximal level of effort was considered to have been attained if there was a plateau in VO_2 despite increased exercise intensity and a RER value > 1.10. VO_2 was monitored continuously, and the average of the three highest 10-s consecutive values was defined as the VO_2max. Post-exercise blood samples were taken immediately after (Post-Ex) and 30 min (30 min rec) during recovery after the treadmill running test.

Assessment of body composition

The height (Seca 214 Portable Stadiometer, Cardinal Health, Dublin, Ohio, USA) and body mass (SECA709, Hamburg, Germany) of the participants were measured to the nearest 0.1 cm and 0.05 kg, respectively. Body mass index (BMI) was calculated for all the participants as the ratio of body mass (kilograms) divided by body height (meters) squared. The participants percentage of body fat (BF%) was measured using the bioimpedance segmental body composition analyzer (BC-418 Segmental Body Composition Analyzer, Tanita, Illinois, USA).

Blood analysis

A 10 ml blood sample was obtained from a superficial forearm vein using standard venipuncture techniques with the participant in an upright position. Separated sera were separated into aliquots and immediately frozen at -20°C until use. Serum GH concentration was determined by an enzyme linked immunosorbent assay (ELISA) kit (Diagnostic System Laboratories, Texas, USA). The assay intra- and inter-assay coefficients of variation were less than 10% and the minimum detectable concentration was estimated to be 0.01 pg/ml. The plasma concentrations of FFAs were determined using a Randox NEFA test kit (Randox Laboratories, Crumlin, UK) scaled for use in a colorimetric method.

Statistical analysis

Statistical analysis was carried out using the statistic software package (SPSS software, version 10.0; SPSS, Chicago, IL, USA). All values are presented as mean ± SD or median. All numeric variables were tested for normality of distribution using the Kolmogorov-Smirnov test and, if necessary, were subjected to logarithmic transformation before applying parametric tests. Comparisons between the groups were done with Student's t-test (anthropometric parameters) and Mann-Whitney U test (GH, FFA). Correlations were tested with Spearman's correlation test. Results with p values below 0.05 were regarded as significant.

RESULTS

Descriptive body composition characteristics and the training history of the participants are summarized in Table 1. There were no significant differences in the ages of the water polo players and controls. The water polo players had a significantly higher body mass ($p<0.05$) and body height ($p<0.01$) compared to the untrained controls, but there was no statistical difference in body mass index and body fat percentage between the groups. Furthermore, the whole lean body mass was significantly higher in the water polo players compared to the untrained controls ($p<0.05$).

The GH and FFA responses to the maximum exercise test are shown in Figs. 1 and 2. All pre- and post-exercise GH and FFA concentrations were within the population reference range. Water polo players had significantly lower baseline values of serum GH concentration compared to the controls, whereas the serum FFA concentration was significantly higher in the water polo players compared to the controls ($p<0.01$; $p<0.05$, respectively). No differences were found between water polo players and controls for measured serum GH and FFA concentrations immediately after and after 30 min of recovery (Figs. 1 and 2).

In water polo players, the concentration of GH was significantly higher immediately after and after the 30-min of recovery compared to baseline levels (+84.2 %; +107.0 %, respectively; $p<0.05$; Fig. 1). In the same group, the concentration of FFA was significantly lower immediately after and after the 30 min of recovery compared to baseline levels (-50.0%; -25.0%, respectively; $p<0.05$; Fig. 2). In the controls, the concentration of GH was significantly increased (+22.9%; $p<0.05$; Fig. 1) immediately after the exercise and remained high 30 min after the exercise; this was also significantly different from the corresponding GH values obtained in rest (+62.5%; $p<0.05$; Fig. 1). No significant response to maximal exercise test was observed in the control group for serum FFA concentration (Fig. 2).

In water polo players, GH concentration at baseline were negatively correlated to BF% ($\rho=-0.608$; $p<0.05$), while in the control group the GH concentrations at baseline were positively correlated to the FFA baseline level ($\rho=0.882$; $p<0.01$).

DISCUSSION

Because of the known anabolic and lipolytic effects of GH and the observed exercise-associated increase in GH, it was hypothesized that GH might play an important metabolic role during exercise. The major finding of this study was that for both groups the concentration of GH was significantly higher immediately after and after the 30 min of recovery com-

Table 1. Descriptive characteristics of water polo players and controls

Variable	Water polo Players	Controls	p value
Age (years)	20.50 ± 2.02	20.55 ± 1.04	ns
Training (h/week)	24.75 ± 8.40	2.50 ± 0.60	0.000
Years of practice	11.08 ± 3.45	1.20 ± 0.80	0.000
Body mass (kg)	100.10 ± 12.54	88.95 ± 8.42	0.025
Body height (cm)	194.17 ± 4.53	183.82 ± 6.08	0.000
Body mass index (kg/m^2)	26.54 ± 2.78	26.28 ± 1.37	ns
Total body fat percentage (%)	17.28 ± 2.72	16.93 ± 1.75	ns
Whole lean body mass (kg)	82.63 ± 8.97	73.83 ± 6.43	0.020

All values are means ± SD. ns, not significant

pared to baseline levels, while the concentration of FFA was significantly lower immediately after and after the 30 min of recovery compared to baseline levels only in the elite water polo players.

The body composition of water polo players in this study is in good agreement with earlier reports on athletes' specific body composition (Mazic et al., 2009; Ferragut et al., 2011). According to Mazic et al. (1996), relative body fat content is significantly higher in water-sport competitors than in land competitors, and this higher percentage of fat results in lower body density, which also has a beneficiary effect in water sports (Ferragut et al., 2011). Since the water polo players had the same body fat percentage as the sedentary controls, and as the athletes were not habituated to laboratory testing on a treadmill, they were an excellent group to study the acute responses of GH and FFA to exercise.

Reports on the influence of prolonged regular physical activity on GH and FFA levels are inconsistent (Zaccaria et al., 1999; Frisch, 1999; Wideman et al., 2002). Some studies have demonstrated an increased GH response to exercise after 3 and 6 weeks of training (Wideman et al., 2002; Frisch, 1999). On the other hand, Zaccaria et al. (1999) did not find GH response after 4 months of intensive endurance training in middle-aged men and young competition cyclists. In our study, water polo players had significantly lower baseline values of serum GH concentration compared to the controls. These differences might be related to differences in training intensity and to the nature of the stimulus for GH release, as well as to the training season. Mejri et al. (2005) confirmed this statement, showing that GH levels were highest at the beginning of the season and then reduced. There are several explanations for this decrease in GH response. First, an adaptive response to continuous physical activity is an increased sensitivity of target tissues to GH, which despite lower levels of GH increases the release of energy substrates in the liver and adipose tissue (Godfrey et al., 2003). Second, chronic prolonged physical activity increases muscle glycogen reserves, which reduces the need for the intervention of metabolic hormones in order to mobilize substrate from other organs (Mejri et al., 2005). This hypothesis is supported by the presented data showing a higher baseline FFA level in water polo players compared to controls.

Our results are in good agreement with many previous studies that have found that an acute exercise bout increases serum GH concentrations in young elite athletes (Bunt et al., 1986; Manetta et al., 2002; Ehrnborg et al., 2003). A peak of GH concentration is detected at the end of exercise, with a minor gender difference in the timing of the peak GH response which occurs earlier in female athletes (Bunt et al., 1986). Ehrngborg et al. (2003) have specifically investigated the influence of gender on exercise-induced GH release and showed that women exhibit an anticipatory response to exercise and a more rapid attainment of peak GH levels with exercise. Never-

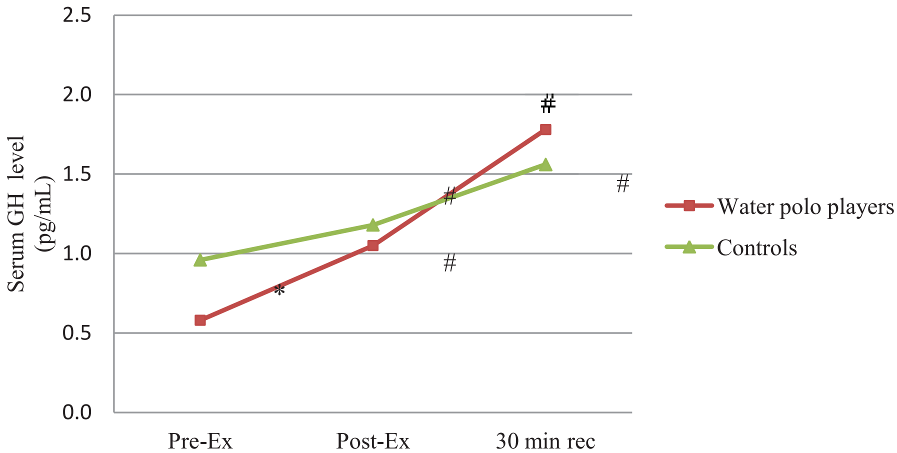

Fig. 1. Serum growth hormone (GH) concentrations before (Pre-Ex), immediately after (Post-Ex), and after 30 min of recovery (30 min rec) in water polo players and controls. * Significantly different from Controls; # Significantly different from Pre-Ex.

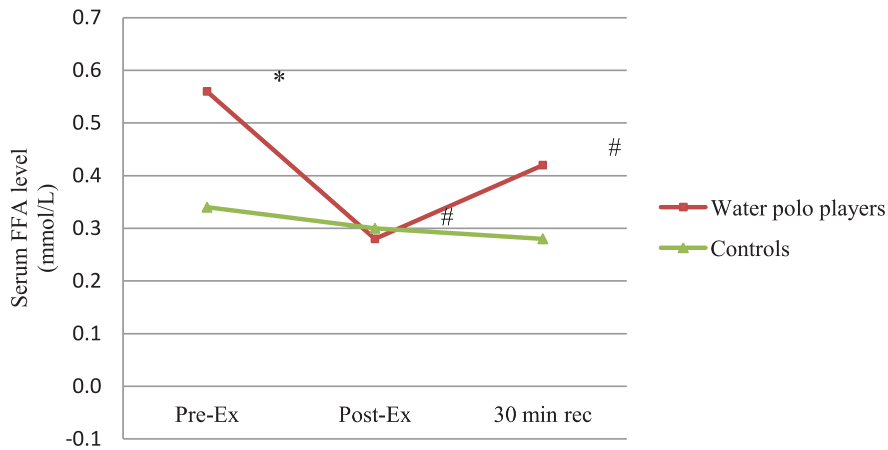

Fig. 2. Serum free fatty acid (FFA) concentrations before (Pre-Ex), immediately after (Post-Ex), and after 30 min of recovery (30 min rec) in water polo players and controls. * Significantly different from Controls; # Significantly different from Pre-Ex.

theless, the response to exercise is likely independent of circadian rhythm, as the time of day does not influence the GH response to exercise in young men (Galassetti et al., 2001).

Our results show that exercise induced a better response of GH in water polo players than in the controls (+84.2 %; +22.9%, respectively) where the increase did not persist after the 30 min recovery period. In a previous report, Manetta et al. (2002) found a three-fold greater GH response in trained subjects and proposed this to be an adaptive mechanism to prolonged physical activity. This result is contradictory to the study of Bunt et al. (1986), who observed a more rapid GH response at the beginning of exercise in subjects with lower levels of physical activity. The authors explained this as due to higher sympathetic sensitivity. Hackney (2006) confirmed the finding that sympathetic activation is an important mediator of the GH response to acute exercise. We also found that untrained individuals show almost the same increases in GH concentration during the 30 min recovery period. This agrees with evidence that physical exercise represents a neuroendocrine-mediated stimulus of somatotropic secretion. McArdle et al. (2001) also observed the maintenance of high serum GH concentrations in untrained subjects for even more hours after cessation of the acute exercise.

The neuroendocrine mechanisms of exercise-induced GH release are still not fully understood. One possible explanation is that during and immediately after physical activity, numerous substances that stimulate GH secretion, such as lactate, catecholamines, hydrogen ions (Chwalbinska-Moneta et al., 1996) are present in the blood. In particular, the scientific community has debated the impact of lactate levels in GH release after physical activity. Numerous studies show no correlation between lactate levels and GH after exercise (Zoladz et al., 2002; Ehrngborg et al., 2003), while Cuneo and Wallace (1994) even suggested that GH levels increased before the lactate threshold is reached. Central cholinergic pathways are also considered responsible for the GH response to acute exercise (Cappa et al., 1993).

The results of numerous studies showed no significant differences in FFA response to acute exercise with respect to differences in the level and duration of physical activities (Cuneo and Wallace, 1994; Wigerness et al., 2001; Gibney et al., 2007). The results of the present study demonstrate an effect of acute exercise on serum FFA levels in elite male water polo players. Our results demonstrated a successive decrease in serum FFA concentration immediately after exercise and during the 30 min of recovery in the water polo players, but not in the sedentary control. The decrease in FFA concentration in the water polo players suggested an adaptation of the metabolism and its increasing reliance on fats as a fuel until they are consumed. It is well established that during exercise ATP, creatine phosphate, glycogen and myoglobin oxygen are partly depleted during the high-intensity work phases (Wigerness et al., 2001). However, the increase in FFA levels in this study was not pronounced even during recovery, because of the short duration of the exercise and recovery period.

Previous studies have reported contradictory results on the relationship between FFA and GH levels after exercise (Wigerness et al., 2001). One study found that GH was not associated with any change in FFA turnover at rest, but resulted in a marked reduction in FFA release into the blood during and following exhaustive exercise (Widdowson et al., 2009). Another study showed that GH levels increased, regardless of the increase in FFA levels (Gibney et al., 2007). In our study, GH and FFA levels were not correlated after acute exercise and recovery period.

In conclusion, the results present herein demonstrate that acute exercise is a potent stimulus for GH release, particularly in water polo players. In this group, the serum FFA concentration decreased immediately after exercise, but not in the sedentary control. These findings could contribute to a better understanding of human endocrine and metabolic physiology in acute exercise conditions.

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