Preparatory training attenuates drastic response of the insulin-like growth factor binding protein 1 at the point of maximal oxygen consumption in handball players

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Received 30 March 2015; revised 3 July 2015; accepted 10 October 2015

Available online

Abstract

Background: Intensive exercise changes physiological need for glucose and several biochemical pathways responsible for its metabolism response. Among them are those which involve insulin, insulin-like growth factor (IGF)-1, and IGF-binding proteins (IGFBPs). Different types and degrees of exercise, as well as an athlete’s fitness, may induce a range of responses regarding concentrations and time needed for the alteration. The idea of the work was to find out whether and how insulin/IGF axis responds to additional physical activity in the already trained subjects and if so, is the adaptation potentially beneficial from the aspect of metabolic control.

Methods: The effect of 4-week intensive training on campus (preparatory training) on the levels of insulin, IGF-1, and IGFBPs during maximal progressive exercise test (MPET) on a treadmill was compared to the results obtained during MPET conducted after a regular training season of a female elite handball team (n = 17, age 17 ± 1 years, height 171 ± 8 cm, weight 65 ± 8 kg, and BMI 22 ± 1 kg/m² at the beginning of the study; there were no significant changes at the end). Serum samples were obtained from players immediately before the test (basal), at the end of the test after reaching the point of maximal oxygen consumption (VO2max), and after recovery.

Results: The concentration of insulin decreased at VO2max, but remained higher in players after preparatory training (12.2 ± 2.5 vs 8.9 ± 4.4 mU/L, p = 0.049). The level of IGFBP-1 decreased in players at VO2max in either case of training, but it remained much higher in tests performed after the preparatory regime than before (p = 0.029). Concentrations of IGF-1, IGFBP-2, -3, and -4 did not change significantly.

Conclusion: The inverse relation between insulin and IGFBP-1 was lost during MPET, as these two molecules changed in the same direction. The results obtained suggest less severe stress-induced depression of insulin and IGFBP-1 after preparatory training. But another metabolic mechanism cannot be excluded, and that is potentially impaired insulin sensitivity resulting in higher level of IGFBP-1.

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Keywords: Female; Insulin; IGF-binding proteins (IGFBPs); Insulin-like growth factor I (IGF-1); Progressive exercise test; VO2max

1. Introduction

Active training induces alterations of hormones and cytokines involved in the regulation of glucose concentration. In general, long-term training leads to reduced insulin concentration1 and increased secretion of growth hormone (GH).2 Results on the concentration of insulin-like growth factor I (IGF-1), a mediator of GH activity,2 and IGF-binding proteins (IGFBPs) are, however, contradictory. These molecules are involved in the regulation of glucose concentration, its utilization, and muscle growth and tissue repair.1

The close relationship between insulin and GH has been reviewed previously.4 The physiological role of insulin is seen in lowering the concentration of glucose in blood, which is performed by two principal mechanisms—inhibition of glucose

http://dx.doi.org/10.1016/j.jshs.2016.01.014
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production in the liver and stimulation of intracellular transport of glucose via specific membrane transporters. Glucose transport is mainly driven by the concentration gradient, but insulin is responsible for controlling translocation of transporters on the cell surface. Intracellular glucose is metabolized and the metabolites further enter the Krebs citric acid cycle, enabling energy release. During exercise, this mechanism, together with the one stimulating glycogen breakdown to insure additional glucose, is activated. After exercise, with reduced need for energy release, insulin stimulates processes favoring conversion of glucose into “energy storage molecules” such as glycogen and fat. Insulin is responsible for the intracellular transport of amino acids as well, essential for protein synthesis and muscle recovery. Contrary to insulin, glucagon, epinephrine, cortisol, and GH are known as glucose-raising hormones. IGF molecules can be seen as specifically positioned in this network, as they are mediators of the anabolic function of GH and yet, they exert insulin-like activity.

Increased concentration of IGF-1 was measured by several researchers after resistance training, alternating resistance exercise and running, long-term training in competitive swimmers, endurance exercise and in women involved in army physical training. Decreased concentration of IGF-1 was reported in handball players and in healthy women after strength training. Finally, Eliakim and colleagues found no effect of treadmill running on IGF-1 in handball players and Nindl and his group denied an effect of exercise on total, free, and bioactive IGF-1 in women.

Similar inconsistency may be seen in the published results on IGFBPs. Intensive physical activity was found to increase, decrease, or not to affect IGFBP-3 concentration. The concentration of IGFBP-1 was detected to increase, decrease, or remain unchanged. Less data are available on other IGFBPs, but they are opposing as well. IGFBP-2 was measured to increase, decrease, or stay unaltered due to exercise, while IGFBP-4 and IGFBP-6 were reported to increase in army women. Such enormous variation in the results on the IGF/IGFBP axis in athletes indicates exercise-specific adaptation. Therefore, results obtained for one type of athlete cannot be extrapolated to others.

Handball is a globally popular team sport played by more than 30 million athletes all over the world. It is a strenuous, intermittent physical activity that requires both aerobic and anaerobic power and endurance. In spite of these facts, hormonal changes in handball players have been poorly studied, especially in female players. Thus, the aim of this study was to investigate changes in the insulin/IGF/IGFBP axis during maximal progressive exercise test (MPET) on treadmill performed by female elite handball team players after intensive training on campus (preparatory training) and to compare them with the response recorded after regular training. In accordance, it seemed relevant to find out whether and how insulin/IGF axis responds to additional physical activity in the already trained subjects and, if so, is the adaptation potentially beneficial from the aspect of metabolic control. The first test was carried out immediately after the competition season, at the beginning of preparatory training and the second one after preparatory training, which lasted 4 weeks. We determined additional influence of preparatory training on the insulin/IGF/IGFBP axis, especially at the point of maximal oxygen consumption (VO2max), which is assumed to be the best single measure of aerobic fitness.

2. Materials and methods

2.1. Participants

The study included 17 young female handball players, aged 16–18. The players were members of the Serbian national team, playing at national and international competitions. All of them were healthy, non-smokers and reported regular menstrual cycles (26–32 days). None of them received any medications or supplements for at least 1 month before the study. All participants (or their parents if they were under 18) were fully informed about the protocol before the start of the study and gave a written informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Ethical Committee of the Faculty of Medical Sciences, University of Kragujevac.

2.2. Protocol

The study started immediately after regular competition season with the first MPET and lasted 4 weeks terminating with the second MPET. During this period, players were subjected to preparatory training in a closed campus in Serbia and they all had the same diet. The regular regime before the study included training once a day for 1.5 h, as a combination of aerobic, conditioning and strength exercise. The campus regime included two training sessions per day, lasting a total of 3 h, with the same combination of exercises, but of higher intensity.

The research protocol started in the morning, between 8:00 and 9:00 a.m., after overnight rest and fast. A blood sample was taken from each subject’s antecubital vein and a small catheter was inserted for further blood sampling. A routine medical examination was performed to confirm that all participants were healthy and without any acute or chronic diseases. A study protocol was carefully explained to each of them. Handball players were subjected to the same dietary protocol 3 days before the study. During those 3 days, they were asked to keep a diary of daily food intake. According to their data and the food composition database of the Italian National Institute of Nutrition, the average dietary intake was calculated. Athletes were instructed to avoid any heavy physical activity 24 h before the research and the consumption of alcohol and caffeine 48 h before the test, as well as not to have breakfast before the examination.

The exercise was performed in continuation on a treadmill (HP Cosmos, Nussdorf-Traunstein, Germany). The starting velocity for the maximal test was the one at which subjects reached the heart rate of 150 beats/min during the 15-min warm-up period. The workload was increased by 2 km/h every 3 min with constant elevation of 3%. This type of protocol was chosen to reach maximal oxygen consumption according to the
Serum samples were prepared from JSHS258_proof and no 19.0 Appropriate HRP-conjugated horse anti-goat checked by the Shapiro–Wilk test, differences between groups mean IGFBP obtained by densitometry were also expressed as

(Version 16.0; IBM, Armonk, NY, USA). Relative amounts of exercise, the VO2 values were recorded as average measures for brated with high precision. During sub-maximal and maximal each test and the gas analyzer and the flow meter were cali-

Rome, Italy). The ambient conditions were recorded before using an automated metabolic cart (Quark b2; CosmedSrl, expired minute ventilation (VE) were measured continuously until analysis.

2.3. Serum samples

Three samples were obtained at the start of the study and the other three after 4 weeks of preparatory training. Blood was drawn: (1) immediately before the test (basal), (2) at the end of the test after reaching the point of VO2max, and (3) after a 10-min recovery (without eating). VO2max was reached at the moment when the increase in workload could not further increase oxygen consumption. Serum samples were prepared from blood by centrifugation at 1500 g for 5 min and stored at −20°C until analysis.

2.4. Determination of insulin and IGF-1 concentrations

Concentrations of insulin and IGF-1 in serum were mea-

asured using the commercial immunodiagnostic assays: INS- RIA and IGF-1-RIA (INEP, Belgrade, Serbia).

2.5. Determination of relative amounts of IGFBP-1, IGFBP-2, IGFBP-3, and IGFBP-4 by immunoblotting

Electrophoresis under non-reducing conditions (SDS-PAGE, 12% gel) and immunoblotting with polyclonal anti-IGFBP-1, -IGFBP-2, -IGFBP-3, or -IGFBP-4 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used for IGFBP detection in serum. Appropriate HRP-conjugated horse anti-goat secondary antibody was applied (Biosource, Camarillo, CA, USA) and the chemiluminescent substrate (Pierce, Minneapolis, MN, USA). Densitometric analysis of protein bands on immunoblots was done using Image Master Total Lab v2.01 software (Amersham BioSciences, Buckinghamshire, UK).

2.6. Statistical data analysis

Anthropometric parameters and concentrations were expressed as mean ± SD and statistically analyzed by SPSS (Version 16.0; IBM, Armonk, NY, USA). Relative amounts of IGFBP obtained by densitometry were also expressed as mean ± SD. Since all variables showed normal distribution, as checked by the Shapiro–Wilk test, differences between groups were assessed by the repeated measures ANOVA followed by Tukey post hoc test (p < 0.05).

3. Results

3.1. Alterations in insulin and IGF-1 levels

Ordinary biochemical and hematological parameters (i.e., concentrations of glucose, urea, creatinine, bilirubin, cholesterol, triglycerides, total proteins, albumin, iron, calcium, hemoglobin, activities of transaminases and alkaline phosphatase, blood cells counts) at the start and at the end of the study for each player were very similar (data not shown). Anthropometric parameters of the players are shown in Table 1 and no significant changes were found between two training regimes.

The results on insulin, IGF-1, and IGFBPs were analyzed by comparing values obtained in MPET on treadmill before and after training on campus (Table 2). During MPET on treadmill, concentration of insulin decreased at VO2max compared to basal

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Before</th>
<th>After</th>
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<tr>
<td>Age (year)</td>
<td>17 ± 1</td>
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<td>Height (cm)</td>
<td>171 ± 8</td>
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<td>Weight (kg)</td>
<td>65 ± 8</td>
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<td>Body mass index (kg/m²)</td>
<td>22 ± 1</td>
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<td>Body fat (%)</td>
<td>23 ± 4</td>
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<td>Body water (kg)</td>
<td>36 ± 5</td>
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<tr>
<td>Lean body mass (kg)</td>
<td>50 ± 7</td>
<td>51 ± 7</td>
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Table 2

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<tr>
<th>Parameter</th>
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<th>After</th>
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<tr>
<td>Insulin (mU/L)</td>
<td>11.8 ± 5.4</td>
<td>13.0 ± 5.3</td>
</tr>
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<td>VO₂max</td>
<td>8.9 ± 4.4</td>
<td>12.2 ± 2.5^a</td>
</tr>
<tr>
<td>Recovery</td>
<td>19.0 ± 6.8^b</td>
<td>19.0 ± 7.3^b</td>
</tr>
<tr>
<td>IGFBP-1 (mmol/L)</td>
<td>58 ± 13</td>
<td>57 ± 11</td>
</tr>
<tr>
<td>VO₂max</td>
<td>51 ± 13</td>
<td>54 ± 12</td>
</tr>
<tr>
<td>Recovery</td>
<td>53 ± 9</td>
<td>60 ± 10</td>
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<tr>
<td>IGFBP-3 (DU)</td>
<td>1.4 ± 0.5</td>
<td>1.8 ± 0.6</td>
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<tr>
<td>VO₂max</td>
<td>0.7 ± 0.3^b</td>
<td>1.4 ± 0.5^a</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.8 ± 0.4</td>
<td>1.9 ± 0.6^a</td>
</tr>
<tr>
<td>IGFBP-4 (DU)</td>
<td>2.5 ± 0.7</td>
<td>2.2 ± 0.9</td>
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<tr>
<td>VO₂max</td>
<td>3.2 ± 1.3</td>
<td>2.9 ± 1.0</td>
</tr>
<tr>
<td>Recovery</td>
<td>2.8 ± 1.1</td>
<td>2.6 ± 1.0</td>
</tr>
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</table>

Note: Statistically significant differences between ^two training regimes; *two successive sampling points.

Abbreviations: IGF-1 = insulin-like growth factor 1; IGFBP = IGF-binding protein; DU = densitometric units.
value and recovered afterwards ($p = 0.019$ and $p = 0.021$ in the test performed before and after preparatory training, respectively). Concentrations of insulin before the test and after recovery were similar regardless of the training regime. The concentration of insulin at the moment of VO2max however, was higher in players after more intensive training on campus ($p = 0.049$). Concentrations of IGF-1 were similar before the test and at VO2max in both training regimes. IGF-1 level was slightly lower at VO2max and remained almost unchanged after recovery in regularly exercising players, whereas it raised in players trained on campus (although not statistically significantly).

### 3.2. Alterations in IGFBP levels

The relative amounts of IGFBPs changed as shown in Fig. 1. Representative profiles for six samples obtained from the same person are given (1–3 before and 4–6 after preparatory training). Densitometric evaluation of protein bands was performed and statistically significant changes were seen only for IGFBP-1 when the entire population was analyzed. Results for IGFBP-1, -3, and -4 expressed in densitometric units (DU) are given in Table 2. The level of IGFBP-1 decreased at VO2max in either case of training, but significant only in the test performed before preparatory training ($p = 0.020$). The level of IGFBP-1 remained significantly higher in the test performed after the campus regime, both at the VO2max sampling point ($p = 0.029$) and after the recovery ($p = 0.023$). The level of IGFBP-2 did not seem to be affected by MPET or the exercise mode. The concentration of IGFBP-3 was the highest at VO2max and remained higher at recovery point after the test than before the test. The training regime did not affect the profile of IGFBP-3. Finally, IGFBP-4 exhibited either no change during the test or slight increase at VO2max.

### 4. Discussion

The general feature of MPET on treadmill performed by the female active handball players was a significant reduction in the levels of insulin and IGFBP-1 at VO2max. After preparatory training however, athletes demonstrated a less pronounced decrease in these parameters at VO2max compared to regular training. A reduction in insulin level after endurance exercise was reported earlier, but there are no data on the changes in the IGFBP-1 level at the point of VO2max.

During regular metabolism, concentrations of insulin and IGFBP-1 are inversely proportional, as insulin is one of the regulators (suppressor) of IGFBP-1 synthesis in the liver. Response of the liver to insulin, however, needs time. In oral glucose tolerance test, a decrease in the concentration of IGFBP-1 was found to occur 1 h after the rise in the concentration of insulin. Thus, in the case of MPET, which lasted approximately 15 min, the complete feedback control between insulin and IGFBP-1 could not be established. The more likely explanation for the simultaneous decrease in the concentrations of insulin and IGFBP-1 is the response to stress, which involved activation of pro-inflammatory mechanisms. Since MPET lasted a relatively short period of time, reduced concentrations of the investigated parameters most likely resulted from the increased activity of proteases and other stress-related degradation mechanisms. It was already documented that intensive training up-regulates expression and release of proteolytic enzymes capable of degrading IGFBP-1. IGFBP-1 in the circulation predominantly inhibits the activity of IGF-1 and the concentration of free IGF-1 seems to be primarily determined by the IGFBP-1 level. Reduced IGFBP-1 at VO2max may be seen as a mechanism to redistribute IGF-1 possibly enabling it to perform insulin-like activity and assist in intra-cellular glucose transportation.

The inverse correlation between insulin and IGFBP-1 was lost during MPET. Results of this study may be interpreted as less severe stress-induced depression of insulin and IGFBP-1 after preparatory training. But another metabolic mechanism cannot be excluded, and that is potentially impaired insulin sensitivity resulting in higher level of IGFBP-1. Less pronounced changes of insulin and IGFBP-1 at VO2max may be also connected with increased expression of insulin receptors due to training. Studies using biopsies could possibly help, but the approach is not readily applicable on human subjects. Muscle mass and mRNA levels for some growth factors were found reduced in patients on maintenance hemodialysis. When patients were subjected to endurance exercise, the increase in muscle mRNA levels for some IGFBPs was noted, but IGFBP-1 mRNA was undetectable. It may be hypothesized that adjustments in the insulin/IGFBP-1 relationship primarily occur in the liver, yet further studies are needed to resolve differential effects (and possible consequences) of regular and preparatory training.

The limitation of this study was the lack of data on the hormonal status of the participants at the moment of testing and the fact that they were not in the same phase of menstrual cycle. It was shown recently that a decreased progesterone/estradiol

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Please cite this article in press as: Olgica Nedic, et al., Preparatory training at tenuates drastic response of the insulin-like growth factor binding protein 1 at the point of maximal oxygen consumption in handball players, Journal of Sport and Health Science (2016), doi: 10.1016/j.jshs.2016.01.014
ratio may induce a slight increase in insulin levels, thus possibly affecting metabolic pathways dependent on insulin.25

There is little information available on the exercise-induced simultaneous adaptation of the musculoskeletal and endocrine system, especially in adolescents and young athletes, which is surprising considering the importance of that population. The lack of information on biochemical responses to exercise in young individuals can be attributed to ethical concerns which limit invasive research in children and adolescents.26 The data collected in this study and future investigations on the training-induced changes in the insulin/IGF/IGFBP axis could be helpful in preventing overtraining, fatigue, and muscular damage.26 The evaluation of the specific biochemical pathways is necessary to resolve whether the adaptive potential of specific endocrine components is sufficient to protect athletes from the muscular damage and glycogen depletion leading to decreased physical performance.

Taking into account the nature of handball as a game (relatively small playing field, only six players, with very dynamic actions during short periods of time), hormonal changes in MPET on treadmill can be extrapolated to a real situation such as very intensive periods of the game during an ordinary match. Thus, changes that were recorded in this study may be expected to occur regularly in handball professionals. Although preparatory training seems beneficial by enabling smoother insulin/IGFBP-1 alterations upon physical overload, the trend in changes during MPET (and, possibly, during periods of the intensive game) suggests disturbed feedback control. Therefore, one can wonder whether frequent episodes of this type may induce long-lasting metabolic consequences, one of them being reduced insulin sensitivity. It would also be interesting to determine, over the period of a few years, insulin/IGFBP-1 relation during MPET change, as well as the general insulin sensitivity, in the investigated handball players. Finally, it would be interesting to investigate whether similar outcomes can be recorded for other athletes (female/male) involved in similar or completely different type of sports.

Acknowledgment

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (No. 173042 and III41030).

Authors’ contributions

The study was designed by ON and VV; MPET test and sample collection were monitored and supervised by ZV and VJ; laboratory tests were performed by ON, MS, and GM; data were collected and analyzed by ON, VV, and MG; data interpretation and manuscript preparation were undertaken by ON and VV. All authors approved the final version of the paper. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

Authors declare not to have any financial interest or benefit arising from the direct application of the research.

References


