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Evaluation of high-intensity interval training on MMP-9 and Galectin-3 indices in male judokas of Shushtar City

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Abstract---Interval exercises are used as an effective approach to improving fitness in a short course of time. The effect of such exercises on functional and physiological adaptations as well as inflammatory responses in athletes is still unclear. The effect of such exercises on functional and physiological adaptations as well as inflammatory responses in athletes is still unclear. Therefore, the present study aimed to investigate high-intensity interval training on MMP-9 and Galectin-3 indices in male judokas in Shushtar city. Materials and methods: For this purpose, among male judokas in Shushtar, 12 people were randomly selected (with an age range of 18-25 years, mean weight of 64.5 ± 6.52 , and body mass index of 22.03 ± 2.15) to conduct the research. Subjects underwent a training session that included a combination of judo and bodybuilding exercises under the supervision of an instructor and by controlling the intensity of the exercises by measuring the heart rate during the training. Blood samples were taken from the subjects in 3 shifts (before the camp, after one training course, and after seven training sessions) to measure the levels of metalloproteinase matrix-9 and galectin-3. Descriptive statistical methods were used to describe the collected information, to evaluate the significant difference between the effect of one course and seven courses of selected exercises on dependent variables, repeated measures analysis of variance was used at a significant level ($p \leq 0.05$), if significant, Bonferroni post hoc test was used. Using Shapiro-Wilk test, the normality of data distribution for each variable was investigated. Conclusion: Findings from the study showed that; A course of selected exercise has no significant effect on the levels of matrix metalloproteinase 9 as well as galectin 3 ($p \leq 0.05$). Also, according to the research findings, the levels of matrix

metalloproteinase 9 and also galectin 3 have decreased after seven selected training sessions.

Keywords---Exercises, Interval, High Intensity, MMP-9 and Galectin-3 indices, Judokas, Male, Shushtar city.

Introduction

The effectiveness of sports training depends on the intensity, volume, time, and frequency of training and the athlete's ability. Therefore, many efforts have been made objectively to reduce the balance between training load and athlete endurance. Instructors try to adjust these essential factors to maximize optimal adaptations (Mackel et al., 2009). Athletes, on the other hand, often need a training program to achieve maximum fitness in a short course of time, especially after a short course of training (Rodas et al., 2000). In such cases, intense HIT intermittent exercise is considered, although there is no comprehensive definition of HIT, HIT is mostly performed in repetitive sessions with relatively short intermittent activities with full intensity or near intensity equal to ($VO_{2max} > 90\%$). Depending on the intensity of the workout, performing a HIT may take from a few seconds to several minutes, with different areas being separated by a few minutes of rest or low-intensity activity (Gibala -Gee, 2008). The ability of HIT programs to rapidly improve exercise capacity and skeletal muscle energy metabolism has been investigated by various researchers (Gibala et al., 2006; Laursen et al., 2005; Burgomaster et al., 2005; MacDougall et al., 1998).

Judo is known as a high-intensity physical activity that requires a high level of aerobic and anaerobic fitness. In a study, McInnes et al. (1995) reported that in a real game, players make the maximum effort repeatedly over a 30-minute time frame. Sprinting activities occur on average every 21 seconds, lasting 2-3 seconds per activity (McInns et al., 1995).

Regular physical activity combined with proper nutrition is a simple way to prevent disease, maintain good health and improve quality of life. In this regard, the results of studies indicate that; High-intensity aerobic exercise can be beneficial for non-athletes and even the sick. However, some researchers believe that performing certain sports activities by applying mechanical metabolic pressures can cause cell damage, inflammatory processes, and thrombotic processes (Mackey et al., 2006).

Intense exercise is associated with increased indicators of damage to cells and muscle tissue. It is possible that the nature of the impact and the frequent contractions during exercise increases the activity of the matrix metalloproteinase (MMPs) and causes injury and inflammatory conditions. In a study by Mackey et al. (2006), MMP9 activity and CK index and analysis by type IV collagen synthesis (as MMP9 substrate) were examined after two types of contact activity (10 km road running) and non-contact activity (one session of running in water) in 8 adult males. Despite the increase in CK after traumatic activity, the level of MMP9 did not change (McKay et al., 2004).

Matrix metalloproteinases are a group of endopeptidases (proteinases) that contain zinc ions (Zn) and calcium (Ca). These enzymes can break down various components of the extracellular matrix.

The family of MMPs in humans has at least 18 members, which are divided into 5 subgroups of collagenases, gelatinases, stromilizens, membrane metalloproteinases, and other types of proteinases based on structural similarity and specificity of substrates. (Murphy, Carbbe, 1995; Woessner, Nagase, 2000; Okada, 2001). Among these 5 subgroups, the highest release and activity are related to gelatinase A or MMP-2 as well as gelatinase B or MMP-9 (Amirsasan et al., 2011; Borden and Heller, 1997).

Donley et al. (2014) reported a decrease in MMPs following 8 weeks of exercise (Danley et al., 2014). Cook et al. (2013) also observed a decrease in MMP-9 levels after 6 weeks of resistance training in men aged 18 to 35 years (Cook et al., 2013). Dastani et al. (2014) showed that eight weeks of aerobic exercise, three sessions per week, and 50 minutes per week reduced MMP-9 and increased TIMP-1 in diabetic women. (Dastani et al., 2014)

Galectin-3 (Gal-3) is a biomarker secreted by cardiac macrophages and plays an important role in altering the pathophysiological status of the left ventricle in animal models (Felker et al., 2012; Sharma et al., 2004). Studies have shown that; Gal-3 expression was significantly increased in myocardial macrophages and mice with cardiac hypertrophy, even before the first symptoms of heart failure. (Sharma et al., 2004 Shah et al., 2012). The mechanisms responsible for the most common heart injuries include cardiac necrosis and cardiac fibrosis, which are now reflected by various measurements, including Galectin-3.

In a study by Hatch et al. (2014) on the relationship between endurance exercise and galectin-3 plasma levels in twenty-one healthy amateur marathon runners, plasma glactin-3 levels rose significantly after the exercise. (Hattasch et al., 2014) Summarizing research findings from various studies and considering that so far the effect of these exercises on functional adaptation and inflammatory responses of judokas is unclear, therefore, in the present study, the effect of intense intermittent exercise on Galectin-3 and MMP-0 levels was investigated to answer the following question, Do high-intensity interval training effect MMP-9 and Galectin-3 indices in male judokas in Shushtar city?

Methodology

The research is applied in terms of type and purpose and because it has been implemented on human samples and lacks control of all influencing factors, it is of semi-experimental type. The research design is pre-test-post-test with an experimental group without a control group. Since the researcher in the following research manipulates the independent variable and examines its results independent variables, the research is prospective. The statistical population of male judokas in Shushtar city is in the age range of 18-25 years. Among the judokas of Shushtar city, 12 people interested in participating in this research are randomly selected as a statistical sample by available sampling.

Measurement tools include Health Questionnaire 1, Syringe, test tubes, and blood storage chamber, SECA217 mobile gauge made in Germany, STABILA-BM40 tape meter made in Germany, BEURER cup scale with an error rate of 100, grams made in Germany, Special laboratory kits: ZellBio GmbH kit for measuring the matrix factor, metalloproteinase 9 made in Germany, ZellBio GmbH kit for measuring the factor galactin-3 made in Germany.

First, 12 players from the club are randomly selected to conduct the research. Subjects then completed a health questionnaire to assess general health status and satisfaction with the research process. Blood samples were taken from the subjects 3 times (before the start of the camp, after one training course, and after seven training sessions). Each time, 10 ml is taken from their vein while sitting, and in a special storage container, the samples are transferred to the laboratory. The first time is on the first day of the judokas entering the club before the start of training at 8 am and while all the subjects are fasting. The second round is taken from the subjects one week after the training at the Ross Club at 8 in the morning and fasting and the third time blood sampling is taken from the subjects seven weeks after performing the exercises at 8 a.m. and fasting. Anthropometric measurements are also taken in 3 steps:

1-Weight measurement, 2- body circumference measurement, 3-height measurement.

Weight measurement: Weight was measured with a beurer cup scale made in Germany with an accuracy of 0.1 kg with the least amount of clothing, without shoes, standing in the middle of the scale, and without assistance.

Measurement of body environments: Waist circumference was measured with a tape measure made in Japan in the narrowest area while the person was at the end of his normal exhalation. In this study, subjects were asked not to wear clothes that lead to body deformation.

Pelvic circumference was measured using a tape measure in the most prominent area without imposing any pressure on the body with an error of 0.1 cm. 1WHR was then obtained through the waist-to-pelvic ratio. A ratio greater than or equal to 0.8 was considered the limit for defining abdominal obesity in women.

Height measurement: The heights of the subjects were measured with a Seca gauge with an accuracy of 0.1 cm with barefoot conditions, placing the hands next to the body, eyes and face forward the contact of the spine, and the back of the heels with the gauge surface.

The subjects in this research are judokas of Shushtar city. Their exercises are performed 2 times a day, in the morning and the afternoon. Judokas have benefited from the same conditions during the week, including bedtime, nutrition, and so on. Exercises are performed in the morning from 9.30 to 11.30 and the afternoon from 17.30 to 19.30. The type of training is such that bodybuilding exercises, both tactical techniques are performed. Bodybuilding includes cardiovascular endurance, aerobic power, anaerobic power, agility, reaction speed, and speed are performed in a planned manner in training sessions or with a warm-up. The intensity of training in different steps of training is measured by counting the heart rate.

Table 1. Examples of subjects' exercises

Intensity of training	Description	Days	
%75 VO2max	Exercises include 15 minutes of warm-up and technical and tactical exercises aimed at physical fitness and improving technique and 1 hour of intensive play	Morning	Saturday
	Exercises include warm-ups and general exercises and physical fitness tests such as lying down and sitting, sergeant jumping, 30-meter sprints, specialized judoka test	afternoon	
%75 VO2max	Warm-up and bodybuilding: Cardiovascular fitness training by running and bodybuilding with the device	Morning	Sunday
	20 minutes of general warm-up and in-game technical exercises	afternoon	
%75 VO2max	20 minutes of warm-up and technical training, tactical training on attack and defense points, a combination of agility training and reaction speed and accuracy	Morning	Monday
	20 minutes of warm-up and bodybuilding exercises	afternoon	
%75 VO2max	Warm-up and bodybuilding: Cardiovascular fitness training by running and bodybuilding with the device	Morning	Tuesday
	20 minutes of warm-up tactical attack and defense exercises	afternoon	
	Exercises include warm-ups and specialized and general exercises	Morning	Wednesday
%75 VO2max	20 minutes of warm-up and aerobic exercise with running and exercise	afternoon	
%75 VO2max	Start training with specialized warm-ups and agility and speed exercises	Morning	Thursday
	Bodybuilding exercises with the device	afternoon	

ZellBio GmbH kit was used to measure metalloproteinase matrix factor 9 made in Germany (Appendix 3) and also ZellBio GmbH kit was used to measure galactin-3 factor made in Germany (Appendix 4). Raw data obtained from the measurement process were analyzed in two parts: descriptive (mean and standard deviation) and inferential (repeated measures analysis of variance, Bonferroni post hoc test, and Shapiro-Wilk test). In the descriptive part, the individual characteristics of the subjects and the descriptive information of the research variables, and in the inferential part, the information related to the dependent variables in the pre-and post-test is compared. The significance level in the test of relevant hypotheses is considered 0.05. SPSS software version 16 was used to analyze the data and Excel computer software was used to draw the graphs.

Results

Some individual and physiological characteristics of the subjects are divided into groups in Table 2.

Table 2. Personal and physiological characteristics of participants

Judokas men (12 people) 10 people	Variables
18.2±1.03	Age (years)
64.5±6.52	Weight (kg)
171.25±4.7	Height (cm)
22.03±2.15	Body mass index (kg / m ²)
24	Exercise rate (hours per week)

Test research hypotheses

Research Hypothesis 1: A high-intensity interval training course has a significant effect on Galectin-3 levels in male judokas in Shushtar. Research Hypothesis 2: Seven high-intensity interval training sessions have a significant effect on serum GALECTIN-3 levels in male judokas in Shushtar. Research Hypothesis 3: Between one and seven-course of high-intensity interval training has a significant effect on serum levels of GALECTIN-3 in male judokas in Shushtar.

Table 3. Comparison of galactin levels in different steps, in general

Variable statistics	sum of squares	Degrees of freedom	average of squares	F ratio	Significance level
GALECTIN-3	183046/222	2	92023.111	30.18	0.001*

* It is a sign of significance ($P < 0.05$).

As can be seen in Table 3, the significance level is 0.001. Therefore, because its value is less than 0.05%, in general, there is a statistically significant difference between the mean of GALECTIN-3 in different steps of research. Accordingly, the null hypothesis, which emphasizes the absence of a statistically significant difference, is rejected. Table 4 shows the exact location of the differences between the different steps. Significant differences are indicated by *.

Table 4. Comparison of GALECTIN-3 serum in different steps of research, in pairs

	Statistics Steps		Mean difference	standard error	Significance level
GALECTIN-3	Before the course	After a course After seven-course	152.667	23.421 28.147	0.001 0.002*
	After a course	After seven-course	150.667	13.56	0.001*

The results of Bonferroni test indicate a significant difference between the pre-training phase the post-training phase and also the post-training phase with the post-training phase ($P < 0.05$). According to these results, Hypothesis 2 and Hypothesis 3 of the research were accepted and Hypothesis 1 was rejected.

Figure 1 also clearly shows the general trend of galactin changes in male judokas during different steps of testing. According to the figure, the difference between the pre-workout step and the post-seven-step step and also the post-one-step step with the post-seven-step training course ($p \geq 0.05$) is significant while between the pre-training step and after a training course, this difference is not significant ($P \leq 0.05$).

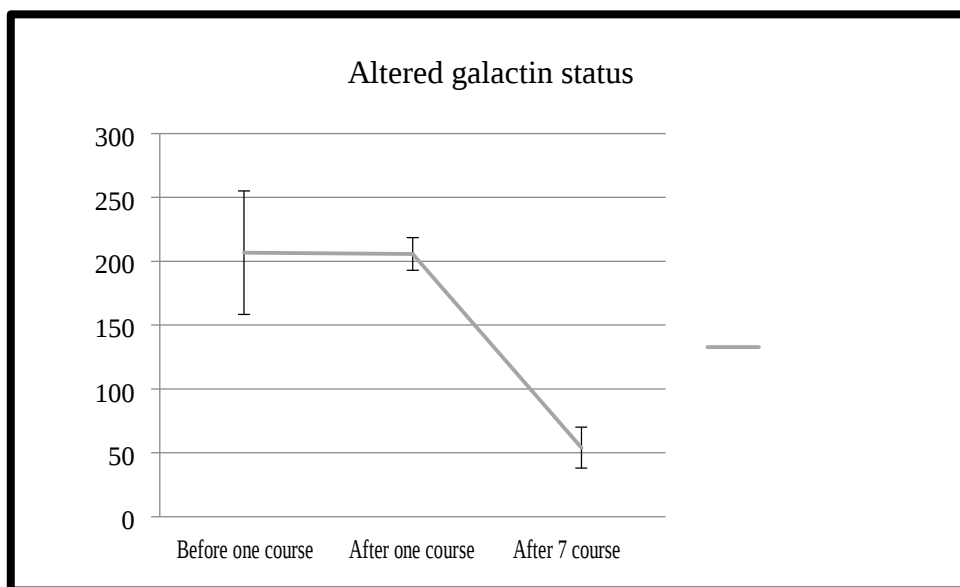


Figure 1. A general trend of galactin changes during 3 steps of testing in male judokas

Hypothesis 4,5,6:

Research hypothesis 1: A high-intensity interval training course has a significant effect on 9-MMP in male judokas in Shushtar.

Research Hypothesis 2: Seven courses of high-intensity interval training have a significant effect on serum levels of 9-MMP in male judokas in Shushtar.

Research Hypothesis 3: Between one and seven-course of high-intensity interval training has a significant effect on serum 9-MMP levels in male judokas in Shushtar.

Table 5. Comparison of 9-MMP levels in different steps, in general

Variable statistics	sum of squares	Degrees of freedom	Mean squares	F ratio	Significance level
-9MMP	0.746	2	0.373	8.77	0.002 *

* Is a significant sign ($P < 0.05$).

As can be seen in Table 5, the significance level is 0.002. Therefore, because its value is less than 0.05%, in general, there is a statistically significant difference between the mean of 9-MMP in different steps of the research. Accordingly, the null hypothesis, which emphasizes the absence of a statistically significant

difference, is rejected. Table 6 shows the exact location of the differences between the different steps. Significant differences are indicated by *.

Table 6. Comparison of serum 9-MMP of different research steps, in pairs

	Steps statistics		Geometric mean ratio	Significance level
9-MMP	Before the course	After a course	1.142	0.447
		After seven-course	2.139	0.019*
	After a course	After seven-course	1.873	0.062 *

The results of Bonferroni test indicate a significant difference between the pre-training phase and the post-training phase ($P < 0.05$). According to these results, Hypothesis 2 was accepted and Hypotheses 1 and 3 were rejected.

Figure 4-15 also clearly shows the general trend of 9-MMP changes of judokas during different steps of testing. According to the figure, the difference between the pre-training phase and the post-seven phase ($P \geq 0.05$) is significant. While between the pre-training phase and the post-training phase and the post-training phase with the post-seven phase is not significant ($p \leq 0.05$).

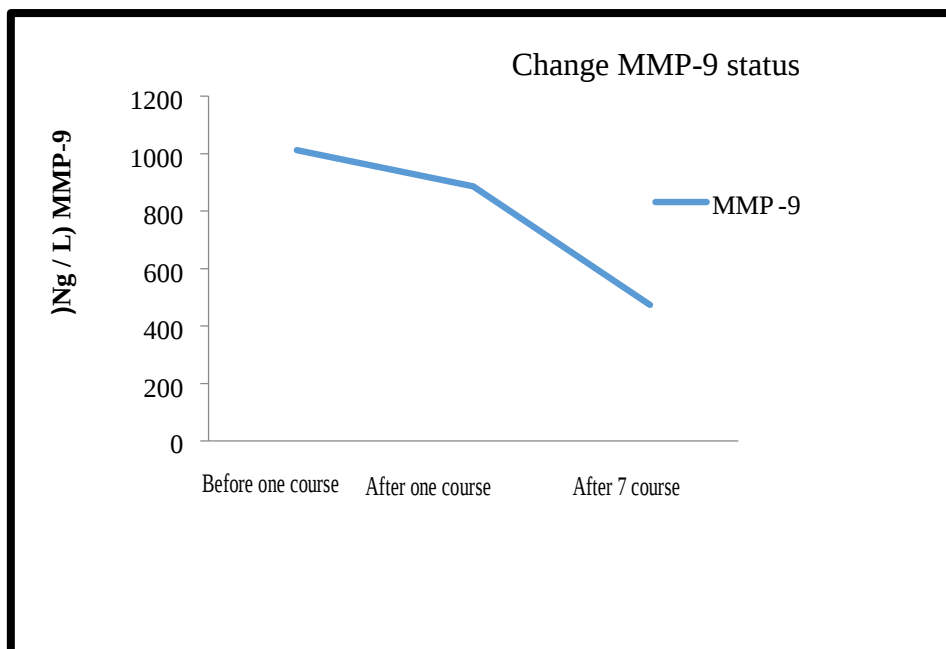


Figure 2. A general trend of 9-MMP changes during the 3 steps of testing in male judokas

Discussion

Physical activity is associated with a reduction in cardiovascular disease. Studies also show that regular physical activity can improve the pathological changes caused by metabolic disorders. In a type of pathological processes, such as exposure to physical and mechanical stressors, the inflammatory response begins with proinflammatory cytokines and MMPs. In recent years, much attention has been paid to extracellular degradation enzymes (MMPs) or matrix metalloproteinases. There has also been much research on the importance of the 3-Galectin biomarker in patients with heart disorders. In the present study, seven HIT training sessions significantly reduced the levels of MMP9 and -Galectin3 factors. Also, in different studies, the levels of these factors have decreased.

In this regard, Danley et al. (2014) reported a decrease in MMPs after 8 weeks of exercise, which is consistent with the present study. Cook et al. (2013) also observed a decrease in 9-MMP levels after 6 weeks of resistance training in men aged 18 to 35 years. Dastani et al. (2014) showed those eight weeks of aerobic exercise, three sessions per week and 50 minutes per week reduced 9-MMP and increased 1-TIMP in diabetic women, which is consistent with the present study. Also, in a study of mouse models of renal fibrosis, it was shown that reducing 3-Gal expression leads to protection against fibrosis so that depletion of macrophages from 3-Gal severely reduces fibroblast activity and ultimately reduces Collagen expression. The consistency of the results may be due to the timing of sample collection, the same measurement method, as well as the similarity of training protocols in terms of physiological changes.

In a study by Ramin Amirsasan et al. (2007) The effect of debilitating aerobic activity on matrix metalloproteinases in athletes and non-athletes on 11 top endurance runners who had 5 years of experience in regular club training and national team camps and 10 men who did not train. The results showed a significant increase in 9-MMP and 2-MMP levels of athletes following a session of debilitating aerobic activity ($P \leq 0.001$). Showed that, of course, was still significantly higher than the baseline value (30 minutes before the test) ($P \leq 0.001$), which is not consistent with the present study.

(3-Galectin-3) Gal is a biomarker secreted by cardiac macrophages and plays an important role in changing the pathophysiological status of the left ventricle in animal models (41, 42). Studies have shown that 3-Gal expression is significantly increased in myocardial macrophages and mice with cardiac hypertrophy, even before the first symptoms of heart failure. In a study by Hatsch et al. (2014) on the relationship between endurance exercise and plasma levels of 3-galectin in 21 healthy male marathon runners, plasma levels of glactin 3 increased significantly after exercise which is inconsistent with the present study.

In a study by McKay et al. (2006), MMP9 activity and CK index, and type IV collagen degradation (synthesis as MMP9 substrate) were examined following two types of activity (10 km road running) and (one water running session) in 8 adult males. Despite the increase in CK after activity two, the level of MMP9 did not change. The inconsistency of the present results with the results of these studies

can be due to the nature of the training course, duration of physical activity, age, sex, level of physical fitness of the subjects, and methods of measurement.

Conclusion

There are conflicting results from various studies on changes in the levels of 9-MMP and 3-GALECTIN due to physical activity. Various studies have reported an increase, decrease or no change in 9-MMP and 3-GALECTIN levels after exercise. In the present study, seven courses of exercise significantly reduced 9-MMP and 3-GALECTIN levels. On the other hand, there was no significant change in 9-MMP and 3-GALECTIN levels after a course of exercise. Based on the findings of various studies, the response of 9-MMP, as well as 3-GALECTIN, is dependent on exercise intensity.

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