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Comparison of the Effects of Dietary Intake of Carbohydrate and Protein Carbohydrate on Serum Testosterone and Cortisol Following Two Competitions in Karate Men

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Abstract: *The purpose of this study was to investigate the effect of dietary intake of carbohydrate and protein carbohydrate on testosterone and serum cortisol following two karate competitions in karateurs. For this purpose, 10 premiers Shabestar Karate shots were randomly selected based on physical characteristics (weight, fat percentage, height) as the subjects in two groups of five. The first group received a carbohydrate drink and the second group received a protein carbohydrate protein after a crossover blind crossover activity they made. sports carbohydrate drink 10% solution (6 ml /kg). The test was performed in two separate stages, one-week interval. On the test day, the subjects held two karate competitions with 10 minutes of rest between the competitions. To compare the test pressure, the Borg pressure was used. Blood samples were taken in two stages in the first and second weeks and each stage once a day before the match and 30 minutes, and the third time 3 hours after the competition and total six times. The serum testosterone and cortisol levels were measured and the normalization of the data was measured by the Shapiro-Wilk test and the analysis Data analysis was performed using repeated ANOVA method. The results of this study showed that the response of testosterone, cortisol, and testosterone to cortisol ratio was not similar in karate men for 30 minutes and 3 hours after two matches, and the type of supplementation used on the pattern of response of these variables was effective. Moreover, the levels of each of these indices in two the group did not make any difference before the competitive activity. Also, the results showed significant differences in the levels of testosterone and cortisol at different stages. That is, the implementation of two karate competitions led to a change in the amount of testosterone and cortisol. There was a significant difference in the ratio of testosterone to cortisol in different stages. That is, exercise has led to a change in the ratio of testosterone to cortisol. Regarding the results of this study, we concluded that there is a significant difference between the effects of carbohydrate supplementation with carbohydrate supplementation on testosterone and cortisol, and protein supplementary carbohydrate supplementation is more suitable for recovery.*

Keywords: *Cortisol, Carbohydrate Drink, Protein Carbohydrate, Testosterone, Karat*

INTRODUCTION

Karate is a martial art that stimulates and strengthens most of the body's muscles (Karate Federation, 2011, p. 16). It is an interdisciplinary field, putting a lot of pressure on aerobic and anaerobic energy systems. And it is considered as the short-term field in terms of the competition period. The energy contribution of the

anaerobic system and the aerobic system is 90% and 10%, respectively. The duration of the karate match is three minutes according to the rules of the Karate Federation, which, if the winner is not defined, will continue for 1 and 2 minutes. (Karate Federation, 2011). From the above information, we conclude that today athletes in various sports fields, compared to their past counterparts have become faster, more powerful and more endurance. A part of these achievements have been achieved in the light of dietary manipulation, the use of various nutritional supplements and energetic agents. The use of supplements and sport drinks has been widespread among athletes. Athletes believe that the use of sport drinks due to the use of raw materials can be useful in restoring their energy resources (Hemmatfar, limoie, 2011; Araazi, Demirchi and Asadi, 2014). When exercising, the body must respond quickly to the increasing demands imposed on it, in other words, the muscular activity requires the regulation and coordination of many physiological and bio-chemical systems. Such integration is created by the complex interaction between the autonomic nervous system and specialized tissues of the body, glands (which secrete hormones). Organizing known hormonal responses to exercise activities is important in biological functions for the ability to withstand activity pressure. (Shahbazi and Maleknia, 2000). In fact, these hormonal fluctuations can be considered as a reaction of the body to exercise stresses in order to establish a homeostasis condition (Aslan, 1998). One of the steroid hormones is testosterone. This important steroid hormone, in the body of mammals including humans (both sexes) produce androgenic (gender) and anabolic (constructive, growth) effects that is produced and secreted in men primarily in testicular leydig cells. (Jack Wilmore, Gaieni translation, 2007). Cortisol or hydrocortisone is also the most famous glucocorticoid in the body that is secreted from the adrenal gland and is also affected by physical and emotional shocks, such as pain, fear, anxiety, worry, etc. Each with the effect on messages, by inactivating the negative regulation system, and also by disrupting the all the time program of secretion, increase the rate of rapid cortisol secretion and provide immediate body care. (Ghasemian, Gaieni, Choobineh, Ghorbaniyan, 2012, p. 299)

A trial of a wrestling match effects on testosterone and cortisol levels, Dailamy (2001), reported in a research. These two hormones increase linearly in response to a sport that suddenly reaches a particularly high threshold to reach a concentration plate (Bionja and LG, 1970, p. 293). Exercise with 60% or more of the maximum oxygen consumption is one of the Physiological stresses, it can increase cortisol secretion. (Jorma and Normkou 2004). Cortisol release, by trying to maintain blood glucose levels during physical activity, affects metabolism, and the amount of its secretion in exercise greatly depends on the duration of exercise. (Jorma and Norm Koi 2004).

In Araazi research and his colleagues, one week after determining a repeat of the maximum chest pressure, a moderate acute resistance training, including 4 repetitions of 12 repetitions with an intensity of approximately 70%, with 1 repetition maximum and 2 minutes' rest interval between the turns was performed. Blood samples were taken before, immediately and 30 minutes after exercise, and it was stated that there is a negative relationship between cortisol and testosterone hormones in certain conditions (Araazi et al., 2014). In researches about the results of the testosterone and cortisol tests have shown that testosterone and cortisol is also tangible in these fields (Shahbazi and Maleknia, 2000). However, these studies have so far not been able to explicitly examine this view (Bionja and LG, 1970, p. 294), and also in the review of a fatigue training session on testosterone and serum cortisol and the study of the ratio of free testosterone to cortisol in the basketball elite have been shown to have no significant effect. (Agha Alinezhad, 2013). Significant changes in serum testosterone and cortisol are reported when exercises are intense and painful. The study of these hormones reveals contradictory results. (Diakin, 2004). Researchers evaluated the effect of hormonal responses on moderate to high intensity exercises that two testosterone and cortisol hormones produced a significant increase after exercise with high intensity versus medium intensity exercise. In a study that investigated the effect of 11-week strength training on cortisol and testosterone hormones in young men of soccer players, it was found that serum cortisol and testosterone did not differ significantly in resting state after 11 weeks of strength training. (Grosstiga and et al., 2005, p. 507) found that there was no

significant change in cortisol level in a study that was conducted during a vigorous exercise session. (Nimen and et al. 2004). Reducing the testosterone ratio to cortisol also indicates that the anabolic and catabolic balance is suppressed following a disabling activity and thus can keep the body in a catabolic state. In addition, increasing cortisol level and catabolic conditions can lead to weakening of the immune system. (Azarbaijani and Nickbakht, 2002 p. 101).

Similar results were reported in James (1999) and Azarbaijani research (1999). In these studies, after wrestling, cortisol level increased and testosterone level decreased even up to 90 minutes after recovery (Azarbaijani and Nikbakht, 2002, p. 101). In a research found that the response of serum testosterone and cortisol to the effect of 8 weeks of severe resistance training in women, there was no change in the total concentration of testosterone, but the cortisol concentration decreased after 8 weeks of training, thus the testosterone / cortisol ratio (T / C) increased, which was 20% resting time. (Ghasemian et al., 2012, p. 299). To avoid catabolic conditions and reduce testosterone-to-cortisol ratio, researchers have used a variety of strategies, including the use of sports drinks such as carbohydrate and protein carbohydrate (Roland Translated by Farajzadeh, 2001). In a study by researchers on the effect of diet with exercise on testosterone and cortisol concentration, have found interesting results that concentration of these hormones is moderated depending on the athlete's diet, and after the resistance training, the concentration of testosterone is further increased. Therefore, the combination of carbohydrates and protein leads to increased testosterone. (Nimen et al., 2004)

Hashemi et al. (2011) stated that the use of protein-carbohydrate-based drinks would improve recovery and better preservation of energy sources. Evans et al. (2001) stated that the addition of carbohydrates (6 percent glucose, sucrose) to provide energy and to maintain blood glucose at normal levels, and the use of sodium and potassium to maintain water and better absorption of fluids, without preservatives and carbonates is a suitable drink and without gastric problems. Using carbohydrates immediately before the exercise creates an anabolic condition in the body and reduces the catabolism in the body. Bionja and Diss (1970 p. 293); Hickson et al. (1994, p. 663) stated that the consumption of protein immediately before and after exercise increased the anabolic environment of the body and reduced the amount of catabolism (Nimen et al., 2004). In research of Hashemi, Amanie, khazeni, Talebi & Faramarzi, 2012, p. 88, 24 elite male soccer players were selected selectively and divided equally into three groups of carbohydrate drink (carbohydrate CHO) - Protein Carbohydrate Drinks (CHO-PRO) and Protein Drinks (PRO). The results showed that carbohydrate combination with protein, before, during and 1 hour after activity did not have a significant effect on HSP72 insulin and glucose in blood relative to carbohydrate intake alone.

Many studies have been conducted in which there is a high degree of variation in the response of cortisol and testosterone to exercise. Investigating the research about hormone shows a contradictory response due to differences in severity, volume, duration, rest time, age, and physical fitness of subjects. Regarding the lack of resources related to the effect of different types of drinks on different hormones and contradictory results, the present study aims to compare the use of sport protein carbohydrates and carbohydrates drinks on testosterone and serum cortisol following two competitions of male karate to eliminate existing contradictions and ambiguities.

Research Method:

Present research is one of the practical researches and in terms of data collection method is based on semi experimental. The participants were selected from Shabestar male karate. At first, the subjects completed the form of the health questionnaire, as well as the written consent form. The initial measurements included height, weight, and fat percentage of the subjects, as outlined in table one.

Table 1. Mean standard deviation in descriptive indices of karate men (n = 10)

Variable	Maximum value	Minimum value	Standard deviation	Average value
Age(years)	31/2	20/8	2/79	23/72
Height (cm)	179	168	3/76	174
Weight (kg)	78	59/5	6/17	68/55
Body fat	22/31	10/9	3/83	14/35

The height and weight were measured using a height gauge and digital scales. The percentage of fat resulted from the three points equation of Pollack Jackson and body mass index (BMI) were used for sampling of the research groups. Due to the limitations in this cross-over study, 10 karate men from 13 volunteers were selected randomly. In a cross-over research project for two consecutive weeks after two karate competitions, a solution of 10% carbohydrate drink (6 ml / kg) and 10% soluble protein carbohydrate drink (8% carbohydrate and 2% protein, 6 ml / kg) were consumed. In the first week of the competition, 5 karate men consumed the carbohydrate and 5 karatemens consumed another carbohydrate + protein drink, and in the second week by changing the drink type, the test steps were repeated. 8 Blood sampling according to the stages for these two weeks before, 30 minutes and three hours later were collected from the left hand antecubital venous vein (1.8 cc for CBC and 3cc for clotting and serum isolation in two separate tube in less than one minute after Tourniquet closure was taken and collected in the test tubes. After the completion of the work, samples were centrifuged for 15 minutes in vitro. The serum was separated from the clot and placed in a separate numbered test tubes. Then, it was kept in a special freezer at -80 ° C until the final measurement. The radioimmunoassay and Gama canther device were used to evaluate the level of testosterone and cortisol hormones in the laboratory.

All statistical calculations were performed using Excel and SPSS soft wares version 20 and ANOVA statistical method. The significance level of the hypotheses was also considered as 0.05.

Findings

The results indicate that the implementation of two karate competitions led to a change in the amount of testosterone and cortisol. There was a significant difference in the ratio of testosterone to cortisol at different stages. That is, exercise has led to a change in the ratio of testosterone to cortisol. Table 2 shows the results of in-person analysis of variance with repeated measurements 2 * 3 on serum cortisol response to two types of drinks

Table 2: Results of in-person analysis of variance with repeated measurements 2 * 3 on serum cortisol response to two types of drinks

effect	Level	f	df	Edf	significant Level
First series	0/048	179.478	1.000	9.000	.000
Second series	.010	409.331	2.000	8.000	.000
Third series	.023	166.264	2.000	8.000	.000

The first series is before the competition, the second and third series are 30 minutes and 180 minutes after competition. The results of the analysis of variance in the subjects with repeated measurements of 2 * 3 showed that the main effect of the type of drink was significant: $F = 48/179$ $p = (001)$.

Also, the main effect of repetition of the test is also significant $F = 409/33$, $P = (001)$. However, the interaction between the type of drinks and the repetition of the test is also significant: $F = 166/24$, $P = (001)$.

Table 3: Results of in-person analysis of variance with repeated measurements of 2 * 3 on serum testosterone response to two types of drinks

effect	Level	f	df	Edf	significant Level
First series	.015	610.239	1.000	9.000	.000

Second series	.090	40.439	2.000	8.000	.000
Third series	.012	338.859	2.000	8.000	.000

The results of the above table indicate that there is a significant difference between the three stages of serum testosterone test in terms of carbohydrate sport drink $f=238/85$ $p = (001)$

Table 4: Results of the analysis of variance of the subjects in a repeated measurements (2 types of drinks * 3 repetitions of test) on the testosterone ratio to cortisol in different time series

effect	value	f	df	Edf	sig
First series	0/048	179.478	1.000	9.000	.000
Second series	.010	409.331	2.000	8.000	.000
Third series	.023	166.264	2.000	8.000	.000

The results of the above table indicate that there is a significant difference between three series of repeated test on testosterone to cortisol in terms of two types of drinks ($F = 96.22$, $p = (001)$)

Discussion

The results of statistical analysis showed a significant difference in testosterone and cortisol values at different stages ($p = 001$). Namely, the implementation of two karate competitions has led to a change in the amount of testosterone and cortisol. There was a significant difference between the two conditions of carbohydrate supplementation and carbohydrate and protein supplementation ($p = 001$). Also, the results of statistical analysis showed that there is a significant difference between the effect of measurement steps and type of supplementation ($P = 0.001$). In other words, there is a significant difference between the patterns of changes in testosterone levels in the two complementary groups at different stages of the measurement. In addition, in examining the testosterone levels with repeated ANOVA, the results showed that there was a difference in the testosterone levels in the pre-test, after 30 minutes and after 180 minutes of two karate competitions. Therefore, the effect of two complementary carbohydrates and carbohydrates-proteins is not the same. In the study of cortisol values with repeated ANOVA, the results showed that there is a difference in the levels of cortisol in the pre-test, after 30 minutes and after 180 minutes of two karate competitions. Therefore, the effect of two complementary ones was different. Conjugated with the current cross-sectional study by Speering (2009) and colleagues, the effect of two types of low strength and high intensity exercise training on serum testosterone responses was measured. High intensity resistance training had increased 16% serum testosterone levels in comparison with the pre-test, while there was no significant increase in the low intensity exercise group. In line with the present study, Hickson et al. (1994) examined the response of cortisol and testosterone to men and women for 8 weeks paid the heavy resistance training. In this study, there was a significant increase in serum cortisol levels after exercise only in men, while in women testosterone levels increased 4 to 18% immediately after the last session. Compared to the first day, there was a similar change in men (Hickson et al., 1994, p. 670). According to Salvador (1998), a wrestling match has effects on testosterone and cortisol. These two hormones in response to exercise that suddenly reach a particularly high threshold, increases linearly, usually at the end of the activity (Azerbaijani and Nick Bakht, 2002, p. 101). Arazi, Demirchi and Asadi (2014) in a study conducted on 26 elite runners of 19-years -old, cortisol levels increased in endurance runners. Mirzaei (1997) measured the amount of cortisol hormone changes in athletes in a single training session. Exercise maximizes the level of cortisol in the subjects in the morning and evening. However, this difference was not statistically significant. The cause of inconsistent results with this study can be related to the type of practice, age, and gender of the subjects (Aslan, 1998, p. 414). The decrease in testosterone-to-cortisol ratio also indicates that there is an overlap in the anabolic and catabolic equilibrium is followed by an exhaustive activity and thus can hold the body to a catabolic state. (Resaei et al., 1996)

Studies have shown that testosterone levels have increased in speed runners. (Diakin, 2004). In Moradi's research (2011) on nine male non-athletes including twelve weeks training with a weight of 3 sessions per week, ten stations, 3 sets, 12 -8 repetitions in these station, with an intensity of 60-80% of a maximum repetition, Rest time between sessions for one minute and between stations was two minutes, duration of training was 60 minutes. Cortisol and testosterone levels did not change significantly (Fattah Moradi, 2012, p. 135). The reason for this conclusion is that the lack of the same diet for two endurance and speed groups has expressed. Hosseini et al stated that eight weeks of strength training in immovable young girls did not affect serum cortisol concentrations (Jormay & Normakway, 2004). No changes in serum cortisol levels following a strength training period can be similarly studied with studies have investigated the acute and chronic effects of endurance training on the hypothalamus- hypophysis -super-kidney axis. There was also a difference in the amount of these two hormones in the present study. Faraji et al. (2016) stated that the use of carbohydrates and proteins in cortisol and testosterone levels is not meaningful. (Faraji et al., 2016). The reason for this conclusion is the difference in the field of sports.

There is a significant difference in the ratio of testosterone to cortisol at different stages ($p = 0.000$). That is, exercise has led to a change in the ratio of testosterone to cortisol. There is a significant difference between the two conditions of carbohydrate supplementation and carbohydrate and protein supplementation. In addition, the results of testosterone-cortisol test with repeated ANOVA test showed that there was a significant difference between testosterone and cortisol levels after 30 minutes and after 180 minutes of two karate competitions. Therefore, the effect of two complementary carbohydrates and carbohydrates-proteins is not the same.

Together with this research, Troules Rosted et al. (2000), by studying reactions to moderate and high intensity exercises, concluded that two testosterone and cortisol hormones showed a significant increase after training with high intensity in comparison with the moderate intensity training. In the studies on the effect of testosterone and cortisol on martial arts, the results show that changes in testosterone and cortisol levels are also evident in these disciplines (Azarbaijani and Nikbakht, 2002, p. 101). The ratio of these two hormones in this study was altered and, with regard to carbohydrate supplementation and protein, these changes were significant. In Tesung research (2011), which was performed on nine wrestlers of 19 years old, each wrestler participated in three races and consumed of two types of carbohydrate, carbohydrate and branched-chain amino acids and arginine, as long as enough carbohydrates were available for recovery, the consumption of amino acids does not have an effect on energy recovery. The reason for this contradiction in the conclusion can be expressed as the intensity and duration of the race. In line with this research, in the study of Aghaalienejad (2013), 36 women aged 20 years who experienced moderate or continuous exercise in 8 weeks of exercise, increased cortisol and dehydroepiandrosterone, and stated that young women can have different types of exercise to improve their physical function (Aghaalienejad, 2013, p. 16). In the Bustani research (2013), on 17 karate athletes 20 aged over one hour of karate training at 85% of maximum heart rate, testosterone and serum cortisol, and the ratio of testosterone to cortisol ,the reduction of both testosterone and cortisol significantly decreased immediately after exercise and expressed, Serum testosterone concentrations decreased after exercise with 85% of maximum heart rate, but this decrease was not statistically significant (Boustani and Fatholahzadeh, 2013, p. 10). The reason for this conclusion and the contradiction with the result of this study can be expressed by different Exercise pressure. Along with this research, in Asgarpour research (2015), 34 non-athlete men were 33 years old divided into three groups: Experimental A (four training sessions), Experimental B (three sessions per week) and control. Resistance training (12 21 sessions) based on the Cramer training protocol, which included 3 sets of 8 to 10 repetitions with 60 to 70 percent of 1 maximum repeat in the large muscles of the body. It Showed that resistance training increased muscle strength, lean body mass, testosterone, and decreased cortisol and myostatin levels in both groups. This increase and decrease in experimental group A was more than that of experimental group B, as well as an increase in serum levels of myostatin and cortisol levels in the experimental group B after idle period

(Aghaalinezhad, 2013, p. 16). Limoei et al. (2011) in their research on twelve volleyball players of 24 years old who performed Bruce's Treadmill test in the morning and afternoon showed that there was no significant difference between testosterone level and testosterone to cortisol at these two times, but one session of an exhausting exercise in the morning and afternoon on cortisol levels showed a significant decrease, and it can be argued that a session of exhausting exercise cannot make an effect on the level of testosterone hormone and its ratio to serum cortisol, while it may have a significant effect on the reduction of cortisol alone, and may reduce the amount of cortisol (Rasayee et al., 1994). In Ghasemian et al. (2012) research on 10 male athletes, 23 years old, subjects were given a dose of 6 ml / kg of body weight, 40 minutes after consuming an exercise drink (experimental group) or placebo (control group) 81% of the maximum heart rate (70% of maximal oxygen consumption) ran in the experimental environment on treadmill until they reached fatigue, while running in every 15 minutes, at a dose of 2 ml per kilogram of body weight, consumed a sport drink or a placebo. Blood samples were taken to measure glucose before and immediately after exercise. It was stated that in the experimental group, there was no significant difference in endurance function, activity heart rate and BG overview in the experimental group, but the level of blood glucose from the pre-test to post-test in the experimental group was significantly increased compared to the control group. Found and stated that ergogenic compounds in sports drinks do not have an effect on improving exercise performance (Ghasemian et al., 2012, p. 299). But in the present study, there was a difference between two types of carbohydrate and carbohydrate exercise drinks. The reason for this contradiction in the results of this research was different training exercises. Along with this research in Arazi and Partners survey (2013), subjects were 10 young men 21 years old and 8 middle-aged men 49 years old. One week after determining a maximum repetition of chest pressure, moderate acute resistance training performed four sets, 12 repetition with an intensity of approximately 70% of a maximum repetition with a 2 minute rest interval between the sets. Both groups experienced significant increases in testosterone, hormone Growth, cortisol and blood lactate immediately and 30 minutes after resistance training (Moradi, 2012, p. 125). The Gaviglyu study (2014) was conducted on 22 male 28-year-old rugby players who ran 3 days a week for 6 consecutive weeks. The ratio of testosterone to cortisol was higher in the matches that were held and won in the middle of the week, and this ratio was lower in lost games due to physical fatigue and lack of time for proper physical and mental recovery. Gaviglyu, 2014, p3447). In line with this research, protein synthesis during carbohydrate-protein and carbohydrate consumption was 6 and 4 times higher than water in 60 minutes of high-intensity cycling, consistent with protein synthesis with insulin response. Paradoxes in existing reports can be used to differentiate between the studied groups of measurement and evaluation methods, intensity and duration, and the type of exercise program for sampling and relaxation between two practices.

Conclusion

Regarding the results of this study, we concluded that there is a significant difference between the effects of carbohydrate supplementation with carbohydrate supplementation on testosterone and cortisol, and protein carbohydrate supplementation is more suitable for recovery.

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