

EFFECT OF SENSORY STIMULATION ON SALIVARY IgA SECRETION RATE IN KARATE PLAYERS

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AUTHORS: Baralic I.¹, Miletic I.¹, Djordjevic B.¹, Terzic T.², Radojevic-Skodric S.², Nikolic G.³

¹ Institute of Bromatology, Faculty of Pharmacy, University of Belgrade, Serbia

² Institute of Pathology, School of Medicine, University of Belgrade, Serbia

³ Blood Transfusion Institute, Belgrade, Serbia

Reprint request to:

Ivana Baralic

Vatroslava Lisinskog 19/17

11000 Belgrade, Serbia

E-mail: i.baralic@beotel.rs

Tel: +381 11 2764224

Fax: +381 11 3090740

ABSTRACT: Objectives: Secretory IgA is predominant immunoglobulin in secretions of the mucosal immune system. Low concentration of salivary IgA is associated with a higher risk of respiratory tract infections episodes in athletes during the training season. The purpose of this study was to determine whether orange-flavored whey based drink has beneficial effect on level of IgA in saliva after the training. Methods: Twenty healthy, young karate players participated in this study. They were divided into two groups. One group rinsed a mouth with 20 ml of liquid whey, two times in 30-min period: 15 and 30 min after training. Second group rinsed a mouth with 20 ml of whey based orange flavored soft drink, at the same time. Saliva was collected before the training, just after the training and after application of fluids. Results: We observed decrease in salivary flow after physical activity. The salivary flow was higher after the application of flavored drink compared to salivary flow after the application of whey. The absolute concentration of sIgA and sIgA secretion rate decreased just after exercise compared to pre exercise values. Application of whey elevated sIgA levels on day 1, while application of flavored drink caused increasing in sIgA levels on day 3. In all other cases sIgA level was decreasing even after applied stimuli. Conclusions: The exercise induces decreasing in salivary flow, sIgA absolute concentration and sIgA secretion rate. Application of fluid whey and flavored whey-based drink elevated salivary flow, but had little effect on absolute concentration of sIgA and sIgA secretion rate in young karate players.

KEY WORDS: salivary IgA, physical training, sensory stimulation

INTRODUCTION

Secretory IgA is predominant immunoglobulin in secretions of the mucosal immune system. It is found in saliva, intestinal secretions, bronchoalveolar lavage fluid, urine, tears, and other mucosal fluids. It inhibits attachment and replication of pathogenic microorganisms, preventing colonization by these pathogens. It is also capable of neutralizing toxins and viruses. Since the mucosal surfaces are the areas involved in the most frequent contact with foreign antigens, secretory IgA is the probably the most important antibody in first line of defense [33]. It has been suggested that low concentration of salivary IgA is associated with a higher risk of upper respiratory tract infections (URTI) episodes [11]. However, this direct link between sIgA and incidence of URTI remains to be confirmed.

Mucosal immunity has come under more consideration with respect to the impact of exercise on salivary IgA concentration and secretion rate [26]. Salivary IgA concentrations have been shown to be reduced after intense prolonged and chronic exercise and after interval of intense exercise in elite athletes in a variety of endurance sports [8, 14].

In recent years, researchers have attempted to identify nutritional countermeasures to exercise-induced changes in IgA concentration in saliva. Effect of different kind of supplementation both on exercise induced decreases in salivary IgA and incidence of URTI, was investigated, such as glutamine [13,14], carbohydrate [9], protein [14], nucleotide [21], vitamin C [25], and bovine colostrum supplementation [5,22].

The present study was designed to examine effect of training on salivary flow, absolute concentration of IgA and salivary IgA secretion rate in young karate players. In addition, we investigated the effect of orange-flavored whey based drink had on level of IgA in saliva after the training. We wanted to show whether orange aroma had any influence on secretion of IgA, so whey base was used as standard.

MATERIALS AND METHODS

Subjects. Twenty karate players (8 men and 12 women) participated in this study. That was the group of recreative karate players that shared the same training habits. They were between 20-25 years of age. The participants were in good health and regularly took part in

TABLE 1. SUBJECTS CHARACTERISTICS.

	Soft drink	Liquid whey
Age (years)	22.9±0.9	22.2±0.7
Weight (kg)	73.6±3.8	74.8±2.6
Height (m)	1.80±0.1	1.83±0.05
$\dot{V}O_2$ max (ml/kg/min)	56±4.1	58.6±8.2
HR rest (beat/min)	63±5	61±4

exercise-3 days per week for 60min per session. They were non-smokers, reported no significant oral, dental or other symptoms of infection and were not taking any medication or dietary supplement in the month prior to the experiment. Training habits did not change during the study period. Subjects are also instructed not to disrupt sleeping patterns. The subject's general characteristics are summarized in Table 1. The protocol was fully explained to each subject before he or she signed a consent form indicating his or her willingness to take part in an experiment on saliva. The experimental procedures were in accordance with the principles set forth in the Helsinki Declaration.

Experimental design. Prior to the experimental period subjects were assessed for aerobic power using an incremental test to determine maximal oxygen consumption ($\dot{V}O_2$ max). During the test, heart rate was monitored.

The experiment was performed on three days during one week, when subjects trained (D1, D2, D3). Exercise trials were scheduled for the same time of day to negate the effects of circadian variation. Participants were not allowed to drink water during the training. Each training was preceded by general warm-up consisting of general stretching and approximately 10 min of running, followed by the standard karate training. Each training lasted for 1 hour.

Heart rate was monitored four times during exercise. Ratings of perceived exertion (RPE) were obtained at 15 min intervals using Borg scale.

The subjects were divided into two groups. Group 1 rinsed a mouth with 20 ml of liquid whey: 15 and 30 min after training. Group 2 rinsed a mouth with 20 ml of whey based orange flavored soft drink, at the same time. They held fluid in mouth for 20 s for sensory stimulation and then swallowed. Subjects were required to avoid food or fluid intake for 1 h prior to experiment.

Whey based soft drink consisted of 87% liquid whey and 12 % of fruit preparation. Also citric acid and stabilizer were added.

Saliva sampling. Saliva was collected before the training, just after the training and after application of fluids.

Collections after applying the stimuli were made within 1 minute after swallowing the fluids. The subjects were seated in a comfortable position, with slightly lowered head, allowing spontaneous saliva flow in the mouth. Subjects expectorated three times during 2 minutes, at 0, 60 s and 120 s into sterile plastic tubes containing an enzyme inhibitor cocktail (30 mM Epsilon-aminocaproic acid, 10 mM ethylene diamine tetraacetic acid and 6 mM benzamidine-HCl;

Sigma Chemical, St. Louis, MO). Subjects were asked not to make any special efforts to gather saliva in their mouths, but rather to allow the saliva to flow as it naturally would.

Immediately after collecting saliva, the tubes were agitated so the enzyme inhibitor cocktail could mix with the saliva, and then centrifuged to remove cellular debris. The supernatant fluid was frozen at -80°C for later analysis of IgA.

Before and after saliva collection, plastic tubes with an enzyme inhibitor cocktail were weighted on an analytical balance. The amount of saliva in grams was converted to milliliters assuming that the specific gravity of saliva is 1, and divided by 2 to express salivary flow in ml/min.

ELISA for salivary IgA determination. Saliva samples were analyzed for IgA concentrations by using an enzyme-linked immunosorbent test. Goat anti – human IgA antibodies (α -chain specific, Sigma Chemical, St. Louis, MO) was used as capture antibodies and absorbed onto Immulon plates (NUNC, Denmark) in 0.1 M bicarbonate buffer, pH9.5, overnight at 4°C. After washing with TTBS buffer (Tween 20 Tris Buffered saline, pH7.5), 3% gelatin in TTBS was added to the wells as blocking agent. The plate was incubated for 2 h at room temperature. After the washing, human IgA standards (secretory IgA, isolated from human colostrum, Sigma Chemical, St. Louis, MO) and saliva samples were added to the wells. The plate was incubated for 1h at room temperature. Serial samples from the same person were always analyzed on the same plate to avoid influence of interassay variability. Then, wells were washed with TTBS buffer and filled with anti-IgA HRP labeled antibody (Sigma Chemical, St. Louis, MO). After an additional 1h, incubation wells were washed and filled with σ -phenyldiamine in 0.05 M phosphate-citrate buffer and 0.03% H_2O_2 and incubated in the dark for 0.5 h. The reaction was stopped with 2.5M HCl. Developed color was measured at 490nm (Victor² Multilabel Counter, Wallac).

All samples were assayed in triplicate and the average of absorbance values was used as representative value. Regression analysis using the relation of standard sIgA concentrations and amount of absorbance (nm) was used to interpolate the concentration of sIgA in the samples. Salivary IgA secretion rate (mg/min) was determined by multiplying the absolute sIgA concentration (mg/ml) with saliva flow rate (ml/min).

Total proteins. Total proteins were determined by BCA test (Sigma) using bovine serum as a standard and manufacturer instruction.

Statistical analysis. All data were expressed as the mean \pm SD. The data were examined using two-way analysis of variance (ANOVA) and T test. For analysis, a P value of 0.05 or less was accepted as statistically significant.

RESULTS

Heart rate and rating of perceived exertion during exercise. The mean heart rate for the exercise trials were 143±11/min, 139±11/min and 144±11/min, respectively. The mean heart rate for the D1, D2 and D3 trials did not differ. Ratings of perceived

TABLE 2. CHEMICAL COMPOSITION, pH VALUE AND ENERGY VALUE OF SOFT DRINK AND LIQUID WHEY

Component (g/100g)	Soft drink	Liquid whey
Total solids	14.48	12.25
Sugar	13.27	6.85
Proteins	0.64	0.71
Fat	0.30	0.35
Ash	0.27	0.21
Ph	4.0	4.3
Energy value (kcal/100 g)	60.01	54.20

TABLE 2. THE MINERAL COMPOSITION OF WHEY-BASED ORANGE-FLAVORED SOFT DRINK AND LIQUID WHEY

Minerals	Soft drink	Liquid whey
Ca (g/kg)	0.36	0.29
Na (g/kg)	0.35	0.70
K (g/kg)	1.00	1.16
Mg (g/kg)	0.10	0.05
Zn (mg/kg)	1.27	0.1
Fe (mg/kg)	0.51	0.29
Cu (mg/kg)	0.24	0.05

exertion (RPE) that were recorded at 15 min interval averaged 12.6 ± 1.2 , 11.8 ± 1.0 and 12.8 ± 1.5 for the D1, D2 and D3 sessions, respectively.

Chemical analysis of whey and soft drink. Chemical composition, pH value, energy value and mineral composition of liquid whey and whey base soft drink were determined and presented in Tables 2 and 3.

Salivary flow rate: Salivary flow rate appears to be modified during and after physical training. Karate training significantly reduced the quantity of saliva produced in every three sessions in both groups. Application of whey and flavoured drink increased salivary flow above preexercise values. Elevation of salivary flow after the application of flavoured drink was statistically significant after the training on the first and second day.

The salivary flow was higher after the application of flavoured drink compared to salivary flow measured after the application of whey, but this difference was not statistically significant.

Figures. 1 and 2 show average salivary flows in karate players before the training, after the training and after the sensory stimulation. *IgA concentration:* There was no statistical difference between preexercise absolute concentration of sIgA on three sessions. The absolute concentration of sIgA decreased just after exercise compared to preexercise values. Decline in sIgA reached statistical significance on D1 and D3. The greatest decreasing in sIgA concentration was observed on the D1. In the group 2 after 30 min sIgA concentration dropped to 24% of pre exercise value, while in the group 1 sIgA concentration raised after second application of whey. On the day 2 we observed constant, but not significant decrease in sIgA concentration in both groups.

On the D3, in group 2 we observed mild, but not significant increase in sIgA concentration after application of flavoured drink, while in the group 1 we observed constant decrease in salivary IgA, after the training and application of liquid whey.

Figures. 3 and 4 show average absolute concentrations of sIgA in karate players before the training, after the training and after the sensory stimulation.

Salivary IgA secretion rate: sIgA secretion rate changed in response to exercise. We observed decrease in sIgA secretion rate just after the exercise, every day. These changes reached statistical significance on the first and third day in both groups. Application of whey nor drink did not elevated sIgA secretion rate to preexercise level.

The most pronounced decrease of sIgA was observed on the D1, in group 2. After two application of flavoured drink sIgA output was lower compared to preexercise values. These changes showed statistical significance. Salivary IgA secretion rate raised after 30 min and two application of whey in group 1, but still did not reach preexercise values.

On the D2, we observed mild, but not significant decrease in sIgA secretion rate just after the training, and after 15 and 30 min. There was no difference between two groups. On the day 3, sIgA secretion rate increased after the application of flavoured drink, while sIgA

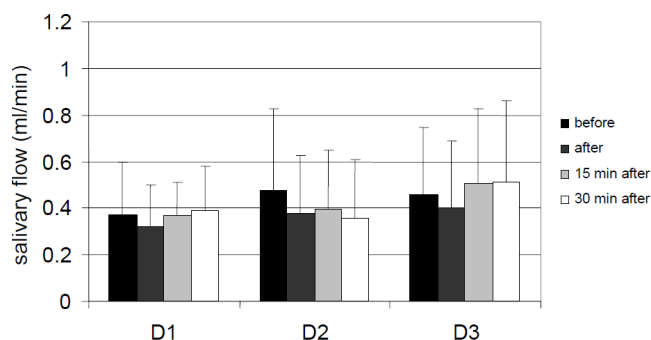


FIG. 1. MEAN SALIVARY FLOW (ml/min) FOR KARATE PLAYERS BEFORE THE TRAINING, AFTER THE TRAINING AND AFTER APPLICATION OF LIQUID WHEY AT 15 MINUTES AND AT 30 MINUTES AFTER THE TRAINING, DURING THREE DAYS.

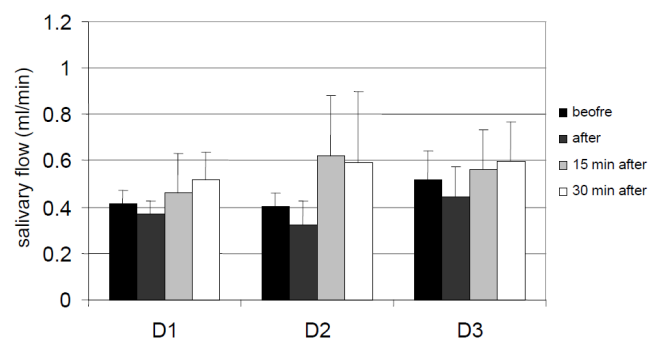


FIG. 2. MEAN SALIVARY FLOW (ml/min) FOR KARATE PLAYERS BEFORE THE TRAINING, AFTER THE TRAINING AND AFTER APPLICATION OF WHEY BASED ORANGE FLAVORED SOFT DRINK AT 15 MINUTES AND AT 30 MINUTES AFTER THE TRAINING, DURING THREE DAYS.

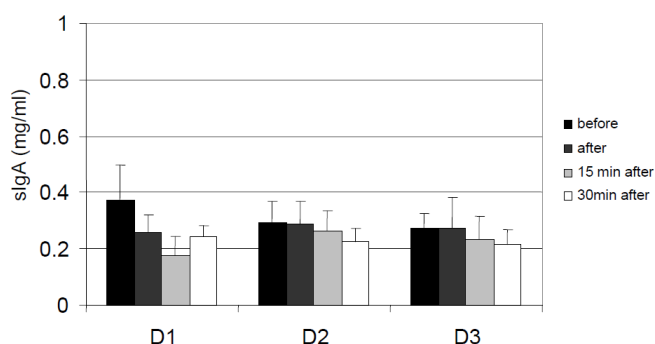


FIG. 3. MEAN ABSOLUTE CONCENTRATION OF sIgA (mg/ml) FOR KARATE PLAYERS BEFORE THE TRAINING, AFTER THE TRAINING AND AFTER APPLICATION OF LIQUID WHEY AT 15 MINUTES AND AT 30 MINUTES AFTER THE TRAINING, DURING THREE DAYS.

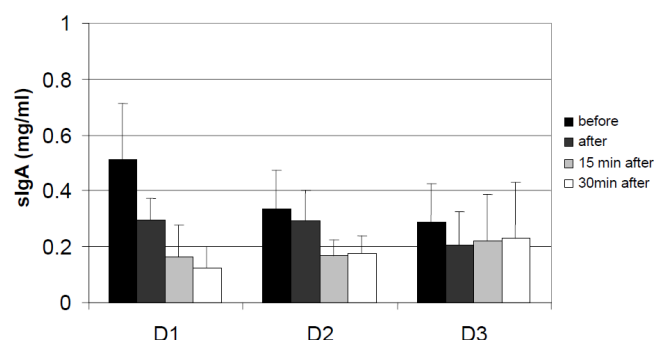


FIG. 4. MEAN ABSOLUTE CONCENTRATION OF sIgA (mg/ml) FOR KARATE PLAYERS BEFORE THE TRAINING, AFTER THE TRAINING AND AFTER APPLICATION OF WHEY BASED ORANGE FLAVORED SOFT DRINK AT 15 MINUTES AND AT 30 MINUTES AFTER THE TRAINING, DURING THREE DAYS.

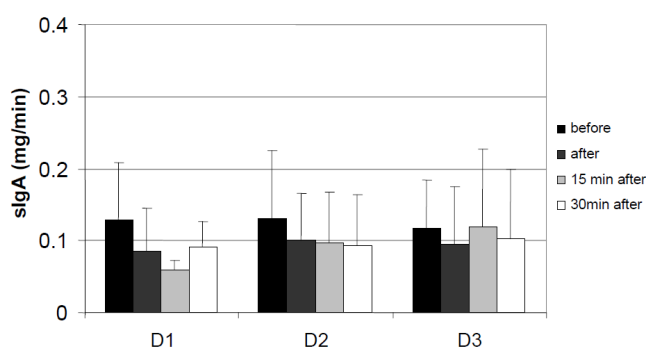


FIG. 5. MEAN SALIVARY IgA SECRETION RATE (mg/min) FOR KARATE PLAYERS BEFORE THE TRAINING, AFTER THE TRAINING AND AFTER APPLICATION OF LIQUID WHEY AT 15 MINUTES AND AT 30 MINUTES AFTER THE TRAINING, DURING THREE DAYS.

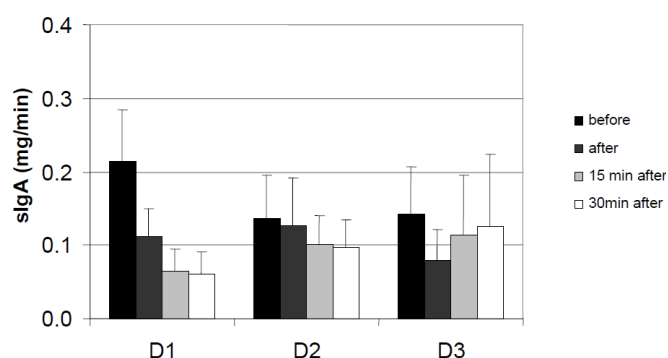


FIG. 6. MEAN SALIVARY IgA SECRETION RATE (mg/min) FOR KARATE PLAYERS BEFORE THE TRAINING, AFTER THE TRAINING AND AFTER APPLICATION OF WHEY BASED ORANGE FLAVORED SOFT DRINK AT 15 MINUTES AND AT 30 MINUTES AFTER THE TRAINING, DURING THREE DAYS.

output decreased after application of whey, but these changes did not show statistical significance.

There was no statistical difference between preexercise sIgA secretion rate on three sessions.

Figures 5 and 6 show average sIgA secretion rate in karate players before the training, after the training and after the sensory stimulation. *Total protein.* There was a significant interaction between exercise and saliva protein concentration. Saliva protein concentration measured on three sessions varied from 0.73 ± 0.25 g/l to 1.54 ± 0.44 g/l on D1, 0.74 ± 0.4 g/l to 1.45 ± 0.50 g/l on D2, and 0.75 ± 0.2 g/l to 1.60 ± 0.4 g/l on D3.

DISCUSSION

The secretory immune system of the upper respiratory tract is the first barrier to colonization by pathogens, with IgA the major effector of host defense [2]. The regulation of secretion and synthesis of sIgA is not only dependent on prior antigenic stimulation but also under strong neuroendocrine control. Thus, alterations in neuroendocrine functioning (such as induced by stress, exercise, pregnancy, menstrual cycle and pharmacological interventions) may affect secretory IgA levels [30].

Results of the present study showed a significant decrease in salivary flow immediately after 1 hour duration of training. The decline of salivary flow showed statistical significance on every session. That is in agreement with other studies [6,15,25]. In one study, the largest decline in flow rate occurred after the most intense session [17]. An increase in sympathetic nervous system (SNS) activity may explain the decrease in saliva flow rate during prolonged exercise by causing vasoconstriction of blood vessels of the salivary glands which in turn may limit water availability for saliva production [4]. Laing et al. [15] have shown that prolonged exercise with sufficient fluids to offset fluid losses prevents the decrease in salivary flow. Taking into account that participants did not take water during and 30 min after the training, it is likely that fluid loss may explain the decrease in unstimulated salivary flow after exercise in this study.

Our results indicate that one hour of karate training decreased absolute sIgA concentration as well as sIgA output in young karate players. The decrease in absolute concentration of IgA and secretion rate in response to exercise is in keeping with some previous findings [9,14,15], but is not confirmed with others [6,15,31]. Secretion rate of sIgA and sIgA concentration decreased during and following

the ultramarathon [25]. Krzywkowski et al. observed that absolute concentration of IgA and IgA output declined after 2 h of exercise [14]. Novas et al. showed that sIgA secretion rate dropped significantly after 1 hour of tennis play [24]. The decrease in the saliva values observed after exercise is dependent on both the duration and intensity of the exercise [25]. In addition, preexercise sIgA concentration and secretion rate are directly associated with the amount of training undertaken during the previous day and week [24]. That is because the duration of the exercise-induced lowering in sIgA concentration has been found to last for some time after intense prolonged exercise [16]. However, collection of saliva samples differs between studies (resting saliva, stimulated saliva, whole saliva, and stimulated parotid saliva) which make the comparison of studies difficult.

This decrease in absolute concentration as well as secretion rate reached statistical significance on D1 and D3. On the D2 there was no significant decreasing in absolute concentration of IgA, when we recorded the lowest rate of perceived exertion RPE. So we can conclude that intensity of the training directly affect the IgA secretion.

Some studies showed that exercise of moderate intensity and duration (15-45 min) has no effect on sIgA level [33]. Results of our study showed statistically significant decrease in sIgA secretion rate in karate players after 1h duration of training. Both decreased salivary flow and absolute concentration of sIgA was the reason for decreased availability of sIgA on the mucosal surface after the training. The greatest decline was observed on D1. Decrease in sIgA secretion rate observed after exercise is dependent on both duration and intensity of the exercise. Moderate exercise causes mild, temporary decreasing in sIgA secretion rate. Based on our results we can conclude that this decrease last at least for half an our after the training. Thus, after the exercise athletes may be immunocompromised and more susceptible to infections in the upper respiratory tract.

Salivary concentrations of total protein increased significantly with exercise in each trial. Exercise is known to increase sympathetic activity and the higher protein concentration in saliva following exercise may be due to increased beta –sympathetic activity in the salivary glands [26]. The level of sIgA decreased after 1h duration of training. That indicates specific reduction in synthesis and/or secretion of salivary IgA in response to exercise.

The precise mechanism by which exercise may suppress salivary IgA is currently unknown.

A team of researchers has investigated the role of the α and β -adrenergic pathways of the SNS on mucosal immunity by comparing the effects of different stressors (mental arithmetic test, submaximal exercise) on secretory IgA. These authors demonstrated that sIgA active decrease, but not increase is mediated by α -adrenergic mechanisms [31].

Flavors are the mixtures of odorous molecules that can be extracted directly from natural food or can be synthesized in the laboratory. In addition to the odorous molecules, flavors often contain nonvolatile compound such as amino acids or salts that induce taste or somatosensory stimulation [27]. Schiffman and Warwick found that flavor enhancement of food for elderly retirement home resident resulted in improved immune status as determined by T and B cell levels and these increases appeared to be due to sensory stimulation rather than dietary intake [29]. Miletic et al. reported that taste and odor stimulation could increase the secretion rate of sIgA and hence improve oral immunity. In addition, they found that secretion rate for sIgA for elderly subjects was greater for foods containing MSG than for the same food without monosodium glutamate (MSG) [28]. In our previous study (unpublished data), we found that sIgA secretion rate increased after the application of flavored and non flavored soups and that that flavored soups caused greater elevation in sIgA secretion rate.

Application of fluid whey and flavored drink caused reflex secretion of saliva. After sensory stimulation in both groups, salivary flow was higher comparing to preexercise values. Salivary flow was higher after the application of flavored drink compared to fluid whey but, without significant difference. Reflex secretion of saliva in response to taste and odor stimuli is in part a consequence of the neuroanatomic proximity and interconnections between the salivatory nucleus and the nucleus of the solitary tract in medulla [28].

Application of liquid whey and flavored drink had little effect on absolute concentration of IgA and secretion rate of sIgA. There was no statistically significant difference in absolute concentration of IgA and sIgA secretion rate between two groups. Concentration of IgA and secretion rate of IgA 30 minutes after the training did not reach preexercise values in any of groups.

Exercise induces decreasing in salivary flow, sIgA absolute concentration and sIgA secretion rate and the magnitude of these alterations reflects the intensity, duration and chronicity of the exercise [31]. Application of liquid whey and flavored whey-based drink elevated salivary flow, but had little effect on absolute concentration of sIgA and sIgA secretion rate in young karate players. In addition, application of flavored drink causes significantly higher salivary flow compared to liquid whey, but there was no statistically significant difference in sIgA concentrations or in sIgA secretion rate in these two groups. In response to this apparent decrease in host defense, it would be wise to take precautions immediately following training to minimize athletes contact with cold viruses by isolating them from large groups of people for a brief period after.

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