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EVALUATION OF GAMMA-GLUTAMYL TRANSFERASE CHANGING IN URINE RELATED TO THE TRAINING LOAD IN THE RHYTHMIC GYMNASTS COMPETITORS AGED 7-10

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Abstract. Post– exercise proteinuria and increased urinary gamma-glutamly transferase (GGT) levels can be predictive of exercise induced renal damage. In the literature, there exists numerous studies on exercise induced proteinuria, but studies investigating the effects of exercise on urinary GGT levels are quite few. The purpose of this study was to determined that the changes in urinary GGT activity, urinary creatinine and protein levels, in order to asses any potential exercise induced tubular damage of rhythmic gymnasts competitors aged 7-10. The study was performed on 11 rhythmic gymnasts who were in elementary category as competitors of the same sport club in Istanbul - Turkey. The training load was 3 hours 30 min for the same loading and same duration for all gymnasts. Urine samples which were collected before, after and 24 hours after the training were analyzed for urinary GGT, creatinine and protein levels. Significant difference was observed between before and after training urinary GGT levels (U/L and U/g creatinine) of rhythmic gymnasts ($p<0.01$, $p<0.05$). No statistically significant difference was observed between before, after and 24 hours after training in urinary GGT level (U/L and U/g creatinine), urinary creatinine and protein levels ($p>0.05$). As a result, the level of GGT urine increased depending on the exercises and decrease during resting but the training programme did not give any damage in kidneys.

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Key words: Gamma-glutamyl transferase - Urine - Training load - Rhythmic gymnast

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Introduction

Rhythmic gymnastic is a sport which demands both the coordination of handling various apparatus (rope, hoop, ball, clubs, ribbon) and motoric features. The evaluation of routine is based on grace, precision, originality, coordination to music and must contain some technical difficulties. That's why conditioning and coordinative elements, physical features, training principles and mastery of apparatus technique which affect the compositions performance are very important [2,3,11,18,23,24,33,34]. To attain perfection and reproducibility of their routines, the gymnasts must practice and repeat the basic elements of their routines several times. Therefore preparing and executing the proper intensity of training program is compulsory and that kind of program should be executed in order to keep the level of the composition performance for each age group.

Although several studies have been published describing the physiological characteristics of elite rhythmic gymnasts [2,3,11,18,24,33] few studies have been carried out on the contribution of the energy systems in rhythmic gymnasts performance [1,6,19]. But it is also very important what the effect of the loading to the organism. There have not been published any studies about the effects of training load to the kidneys damage in rhythmic gymnastics.

Exercise induces profound changes in the renal haemodynamics and in electrolyte and protein excretion. Effective renal plasma flow is reduced during exercise. Strenuous work leads to a more over excretion of erythrocytes and leucocytes in urine. Cylindirurie has been regularly found in post exercise urine in different sports. Post exercise proteinuria urine is a common phenomenon in humans. It seems to be directly to the intensity of exercise, rather than to its duration. The increased clearance of plasma proteins suggests in an increased glomerular permeability and a partial inhibition of tubular reabsorption of macromolecules [29].

Exercise intensity and duration can also affect the type of proteinuria. High-intensity short-duration exercise has been shown to induce both glomerular and tubular originated proteinuria, while moderate-intensity long-duration exercise causes proteinuria [12,29,35,40] partially due to reduced tubular protein reabsorption [32].

Gamma-glutamyl transferase (GGT) is a membrane bound enzyme. GGT is a key enzyme of the gamma-glutamyl cycle involved in glutathione on degradation and synthesis [10]. The highest activity of this enzyme is found in the kidney where it is primarily located on the luminal surface of membranes of proximal tubule epithelial cells [37,38].

Increase in the urinary excretion of renal enzymes is a manifestation of kidney damage. Urinary enzymes have the potential to determine the primary site of renal damage because different sections of the nephron have a characteristic complement of enzymes. These enzymes are N-acetyl beta-D-glucosaminidase (NAG), leucine amino-peptidase, alkaline phosphatase and gamma-glutamyl transferase [5,39].

The purpose of this study was to determine the changes in urinary GGT activity, urinary creatinine and protein levels in order to assess any potential exercise-induced tubular damage of rhythmic gymnasts.

Materials and Methods

Subjects: Eleven female gymnasts aged 7-10 participated in this study as volunteers after informed consent forms were signed by their parents. The study was limited with the subjects involving in elementary category as a competitor in rhythmic gymnastics for two years Besiktas Rhythmic Gymnastics Sport Club in Istanbul, Turkey. The gymnasts who participated in this study were same height, weight, age and training age. In table 1 the means (±SD) of age, height, weight and training age of subjects are shown.

Table 1
Means and ± SD for age, height, weight of gymnasts

Variables	Subject group (n=11)		
	Mean±SD	min	max
Age (years)	8.7273±0.91	7.00	10.00
Height (cm)	131.27±5.48	120.00	141.00
Body weight (kg)	24.82±3.06	20.00	30.00
Training year (month)	26.09±18.37	6.00	48.00

Body weight measurement: Weight was measured to the nearest 0.1 kg. using an electronic scale. The measurement was made with the naked feet and with leotard [25].

Height measurement: Height was measured to the nearest 0.1 mm. with an anthropometric plane and all the subjects were without shoes an in maximal inspiration [25].

Urinary samples: Urine samples were collected before, after and 24 hours after the exercises were analyzed for urinary GGT, creatinine and protein levels. The samples were stored in deep-freezer (-20°C) until the day of assay.

Assays:

Urinary GGT: Urine samples were centrifuged (Fete, clinical centrifuge) at 2500 g for 10 min. Supernatants were diluted with distilled water (1:10). GGT activity was determined by the Szasz method [36] using gamma-glutamyl p-nitroanilide as the substrate and glycylglycine as acceptor. The nitroaniline released was measured at 405 nm using spectrophotometer (Boehringer Manheim, photometer 4010). One unit (U) represents the amount of enzyme catalyzing the release of 1 μmol of nitroaniline/min. at 37^{o}C.

Urinary creatinine: Urinary creatinine levels were measured using the Jaffé reaction (8). In order to minimize errors arising from the effects of changes in urine flow (14) GGT levels are given as ratios against urinary creatinine levels.

Urinary protein: Protein levels were determined according to the Bradford method using bovine serum albumin as the standard [9].

Training programme: Subject group consisted of 11 active gymnasts were trained for 3 hours 30 min for the same loading and same duration in all of the training periods. During the starting period of training, general warm-up (10 min) and flexibility exercises (30 min) were performed. In the main part, American lesson (20 min) including straight and flexibility elements in the floor exercises was studied and then basic body movements (jumps/ leaps, pivots, balances, flexibilities/ 25 min), acrobatic elements (10 min), a composition with ball and without apparatus with the music (105 min) were performed and relaxation exercises (10 min) were executed.

To determine the points, elements and intensity of the compositions: The technical value (TV) and artistic value (AV) points were taken into consideration for each routine. The total point of each composition was determined by adding technical and artistic value points. Special artistic characteristics points were taken into consideration to determine artistic value points. In order to determine the composition elements, technical and artistic value forms were used for each gymnast. All the body and apparatus elements in the ball and without apparatus compositions were designed for each gymnast.

The intensity of the competitive composition was determined according to the number of difficulty risk elements related to the duration of the composition. The following formula was used to calculate the parameters of the loading and the relative intensity of the competitive composition [22].

B=A/C B= number of difficulty elements per min (intensity of composition).
A= number of the elements.
C= length of the loading in min.

Number of the technical value elements was determined counting each body movement difficulties written in the TV forms according to the Code of Points of rhythmic gymnastics [21]. Number of the AV of elements was designated from special artistic characteristics (SAC). SAC and also the other body movement elements which were not included in the technical value forms were determined counting each body movement difficulties from the AV forms and each element was counted as only one movement.

Statistical analysis: Group means and standard deviations, minimum and maximum values were calculated for each variable. Urine samples were collected three times from each gymnast at the beginning of the training, at the end of the training and 24 hours after the end of the training. The differences of pre test and post-test measurements were determined collecting three different urine samples taken from each gymnast before training and after training, also before training and 24 hours after training, also after training and 24 hours after training.

We used Anova tests for each variable to estimate differences between the samples. And also Tukey test were performed to determine the differences between the two paired samples. Statistical significance was accepted for p<0.05 and was presented as the means (±SD). SPSS 12.0 for Windows was used in all statistical analyses.

Results

In Table 2, without apparatus and ball compositions technical value points, artistic value points and total points of routines are shown.

Table 2
Technical value, artistic value and total points of the gymnasts

Variables	Subject group (n=11)		
	Mean ±SD	min	max
WATVP (point)	3.48±0.74	3	4.70
WAAVP (point)	0.57±0.09	0.30	0.60
WATP (point)	4.05±0.76	3.60	5.30
BTVP (point)	3.43±0.66	3.00	4.70
BAVP (point)	2.70±0.89	2.20	4.60
BTP (point)	6.13±1.49	5.20	8.60

WATVP: without apparatus technical value point; WAAVP: without apparatus artistic value point; WATP: without apparatus total point; BTVP: ball technical value point; BAVP: ball artistic value point; BTP: ball total point

Number of the elements in technical value, artistic value and number of the total elements and the loading intensity of compositions in without apparatus and ball routines are shown in Table 3.

Table 3
Number of the elements in technical value, artistic value and total elements and loading intensity of compositions for each routine

Variables	Subject group (n=11)		
	Mean ±SD	min	max
WATVNE (number)	13.27±0.79	12	15
WAAVNE (number)	6±0.00	6	6
WANTE (number)	19.27±0.79	18	21
WAIC (%)	14.93±0.75	13.85	16.41

BATVNE (number)	12.82±1.40	12	15
BAVNE (number)	7.82±4.85	5	16
BNTE (number)	20.64±6.25	17	31
BIC (%)	15.94±4.92	13.08	24.22

WATVNE: without apparatus technical point elements; WAAVNE: without apparatus artistic point elements; WATNE: without apparatus number of total elements; WACI: without apparatus intensity of composition. BTVNE: ball technical point elements; BAVNE: without apparatus artistic point elements, BTTE: ball number of total elements, BIC: ball intensity of composition.

Urine variables collected before training are shown in Table 4.

Table 4
The urine variables of the gymnasts before training

Before training (n=11)			
Variables	Mean±SD	min	max
BTGGT (U/L)	2.31±0.743	1.20	3.90
BTGGT (U/g creatinine)	3.27±1.841	1.10	7.58
BTCRT (mg/dl)	91.46±44.18	23.00	148.40
BTPR (µg/ml)	68.00±44.20	10.00	140.00
BTPR/CR (µg protein/mg creatinine)	76.15±34.86	16.40	118.80

BTGGT: before training gamma-glutamyl transferase; BTCRT: before training creatinine; BTPR: before training protein; BTPR/CR: before training protein/creatinine

Urine variables collected after training are shown in Table 5.

Table 5
The urine variables of the gymnasts after training

After training (n=11)			
Variables	Mean±SD	min	max
ATGGT (U/L)	5.36±3.13	1.20	11.10
ATGGT (U/g creatinine)	12.72±14.46	1.73	46.25
ATCRT (mg/dl)	90.53±46.20	24.00	148.00
ATPR (µg/ml)	81.18±51.21	20.00	150.00
ATPR/CR (µg protein/mg creatinine)	83.58±33.05	15.74	137.50

AT: after training

Urine variables collected after training are shown in Table 6.

Table 6
The urine variables of the gymnasts 24 hours after training

24 hours after training			
Variables	Mean±SD	min	MAX.
24 AT GGT (U/L)	4.01±2.53	1.20	11.10
24 AT GGT (U/g creatinine)	5.26±5.53	1.30	19.50
24 AT CR (mg/dl)	102.63±51.49	41.00	178.00
24 AT PR (µg/ml)	98.54±56.78	20.00	158.00
24 AT PR/CR (µg protein/mg creatinine)	106.66±65.58	26.70	254.00

24 AT: Twenty four hours after training

Arithmetic means, standard deviation and significant levels of the differences of urine samples taken from each gymnasts before training and after training, and also before training and after 24 hours after training and also at the end of the training and 24 hours after training were shown in Table 7.

Table 7
The differences and significant levels between the means related to paired samples

VARIABELS	Deferences Mean +SD	Q	p
BTGGT1 – ATGGT1 (U/L)	-3.04±2.85	4.871	p<0.01
BTGGT1-24ATGGT1 (U/L)	-1.70±2.62	2.727	p>0.05
AFGGT1-24ATGGT1 (U/L)	1.34±3.28	2.144	p>0.05
BTGGT2-ATGGT2 (U/g creatinine)	-9.45±14.10	3.659	p<0.05
BTGGT2-24ATGGT2 (U/g creatinine)	-1.98±4.32	0.735	p>0.05
ATGGT2-24ATGGT2 (U/g creatinine)	7.46±14.74	2.924	p>0.05
BTCR – ATCR (mg/dl)	0.92±53.64	0.069	p>0.05
BTCR-24ATCR (mg/dl)	-11.17±57.84	0.841	p>0.05
ATCR –24ATCR (mg/dl)	-12.10±73.67	0.911	p>0.05
BTPR - ATPR (µg/ml)	-12.27±38.15	1.550	p>0.05
BTPR – 24ATPR (µg/ml)	-29.64±33.13	3.743	p<0.05
ATPR – 24ATPR (µg/ml)	-17.36±39.78	2.193	p>0.05
BTPR/CR - ATPR/CR (µg protein/mg creatinine)	-7.43±52.64	0.562	p>0.05
BTPR/CR – 24ATPR/CR (µg protein/mg creatinine)	-30.51±64.80	2.307	p>0.05
ATPR/CR - 24ATPR/CR (µg protein/mg creatinine)	-23.07±67.61	1.745	p>0.05

BTGGT1: before training gamma-glutamyl transferase (U/L); ATGGT1: after training gamma-glutamyl transferase (U/L); 24ATGGT1: 24 hours after training gamma-glutamyl transferase (U/L); BTGGT2: before training gamma-glutamyl transferase (U/g creatinine); ATGGT2: after training gamma-glutamyl transferase (U/g creatinine); 24ATGGT2: 24 hours after training gamma-glutamyl transferase (U/g creatinine); BTCR: before training creatinine (mg/dl); ATCR: after training creatinine (mg/dl); 24ATCR 24 hours after training creatinine (mg/dl); BTPR: before training protein (μg/ml); ATPR: after training protein (μg/ml); 24ATPR: 24 hours after training (μg/ml); BTPR/CR: before training protein/creatinine (μg protein/mg creatinine); ATPR/CR: after training protein/creatinine (μg protein/mg creatinine); 24ATPR/CR: 24 hours after training protein/creatinine (μg protein/mg creatinine).

GGT (U/L) levels were increased after training and after 24 hours of training than before training and after training GGT (U/L) level was increased then before training GGT (U/L) level and only this increase was significant statistically ($p<0.01$). GGT (U/g creatinine) values were increased after training and after 24 hours of training than before training and only increase of GGT (U/g creatinine) was found significantly only between before training and after training ($p<0.05$). Creatinine levels (mg/dl) were increased after training and after 24 hours training than before training but those increases were not found significantly ($p>0.05$). A proteinuria level were higher after training and after 24 hours of training then before training but increase of proteinuria level was found significantly only between after 24 hours of training and before training. It has been determined that proteinuria / creatinine levels (μg protein/mg creatinine) were increased after training and after 24 hours of training than before training but those increases were not found significantly ($p>0.05$).

It has been found that there was a significant increase statistically in urine GGT excretion between the beginning of the training and after the training. Also it was seen that there was a significant decrease in GGT level after 24 hours. But it has been determined that GGT excretion of the sample collect after the exercises decreased significantly 24 hours after training.

Although the training time was longer then the suggested time for that group, the level of GGT urine increased depending on the exercises decrease during resting and the training programme did not gave any damage in kidneys.

Discussion

Several authors have reported the importance of the condition of post-exercise proteinuria. In 1956, Gardner introduced the term"athletic pseudo-nephritis" to indicate that the protein pattern of post-exercise urine was initially believed to be different from that of the nephrotic syndrome because of the preponderance of the albumin peak. However, the changes were benign, transient and reversible. In

1958, Alyea and Parish observed that 70% to 80% of athletes had proteinuria after severe exercise not only contact sports (football, boxing) but also in sports that are nontraumatic and involve no body contact (track, rowing, swimming) [30].

Poortsmans *et al*. [28] showed that the type of activity influences the post-exercise proteinuria in humans. Subjects engaging in maximal short-term running excrete more proteins than in bicycling, swimming or rowing.

Reductions in renal blood flow and glomerular filtration rate have been documented to occur during exercise; the extent of this reduction depends on the exercise intensity. In addition to this, during exercise glomerular capillary wall permeability increases leading to “glomerular proteinuria”, while protein removal or reabsorption rate from the renal tubule decreases leading to “tubular proteinuria” [13].

In renal damage, the urinary excretion of some renal enzymes such as NAG, leucine aminopeptidase, alkaline phosphatase increases. GGT is also considered the indicator of proximal tubule damage [39].

Several studies showed that exercise causes an increase in urinary albumin and protein excretion in healthy subjects. However, studies failed to demonstrate a great increase in urinary albumin excretion after submaximal exercise in healthy subjects. Many of the studies were performed on healthy subjects who participated in short-duration high-intensity exercises.

In short-duration exercises, the degree of urinary protein excretion is related with the intensity of exercise. Albumin secretion was noted to increase 20-25 times in short-duration exercises, while it increased only 2-6 times during long-duration exercises. This can suggest the transient and thus benign nature of the exercise proteinuria. While all kinds of exercise can cause an increase in protein secretion, it can be concluded that the significance of increase depends on the duration and intensity of the exercise [32].

Protein secretion may increase due to the increased glomerular permeability or diminished tubular reabsorption or a combination of both. Exercise intensity and duration may be indicative of the type of proteinuria. High-intensity short-duration (supramaximal) exercise has been shown to induce both glomerular (albuminuria) and tubular (beta-2 microglobulinuria) proteinuria [30,31]. Moderate-intensity exercise (<75% VO_{2max}) has been shown to induce a glomerular proteinuria [26,30]. Huttunen [20] and Campenecci [10] failed to demonstrate any post-exercise increase in the beta2-microglobulin secretion.

In a study performed on 122 long-distance runners, post-exercise proteinuria and microscopic hematuria were observed in 95% of the runners. Urine samples were collected just before and after the exercise and at post-exercise 6 th, 12 th, 24 th, 36 th and 48 th hours. A significant increase was observed in the urinary GGT levels. The urinary abnormalities were reported to resolve in 24-36 hours after the exercise. It was concluded that post-exercise abnormal urinary finding is a frequently encountered situation [17].

Ayça *et al.* [4] failed to demonstrate a significant difference between urinary GGT levels measured before and after taekwondo competitions. This finding was suggested to be due to the short-duration of the competitions. The potential trauma-associated renal damage could have been prevented by the use of body protectors.

There have been a few studies about urine GGT level changing related training load. In our study, the distribution of the training period was formed as follows 46.12 % body movements (4.85 % general warm-up, flexibility exercises 14.56 %, American lesson and acrobatic elements 14.56%, jumps/leaps, balances, pivots and flexibility elements which are basic body movement elements 12.13 %) performing ball and without apparatus composition 50.97 % and conditioning programme 2.91 %. All the gymnasts executed total 684 elements during the training period and the loading duration in daily training program was 210 min.

According to the features of the training load of Ukrainian rhythmic gymnasts the daily repetition numbers of elements are 100-500 and the duration of training period is 90-180 min [7]. But in most of the countries the duration of training load is suggested as 90-120 min for this age in rhythmic gymnastics [16]. We used longer time than suggested time for the training load.

According to the competition evaluation for this category the maximum TV point should be 5.20 for without apparatus and 4.80 for the ball routine. The maximum AV point should be 0.60 point for special artistic characteristics for without apparatus and 6.00 for ball. It was determined that TV mean of without apparatus of our gymnasts was 3.48±0.74 and the AV of mean was 0.57±0.09. The TV mean of ball routine was 3.43±0.66 and AV was 2.70±0.89. In this case it was determined that the point of TV was 2.00 point lower then the maximum points in without apparatus and 1.50 points lower in ball exercises.

It was determined that the special artistic characteristic point was the maximum in without apparatus but this value was much lower in ball routine. The total number of elements in TV was 19.27±0.79 for without apparatus and the composition intensity was 14.93±0.75 (%), TV was 20.64±6.25 (point) and 15.94±4.92 (%) ball exercises.

Although the duration of training period was longer then the suggested time for that group and much more elements were used during the training period, it was found that the loading was not higher because the repetition number of composition were low and the intensity of composition wasn't high.

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