Research Journal of Biological Sciences 6 (11): 590-596, 2011
ISSN: 1815-8846

The Effect of Different Intensities of Sub Maximal Aerobic Exercise on Proteinuria in Young Football Players

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Abstract: The purpose of this study was to determine the effects of different intensities of submaximal aerobic exercise on proteinuria in young football players. Ten volunteers of young football players (Age: 18±0.5 years, weight: 64.42±3.64 kg, height: 174±4 cm) were selected and participated in three sessions of aerobic activities including 30 min of running at three different intensities, 55, 70 and 85% of maximal heart rate. Before and 20 min after all three sessions, the amounts of urinary albumin, total protein, creatinine, β_2-microglobuline and protein to creatinine ratio were measured and investigated using analysis of variance with repeated measurements and paired-samples student t-test. With increasing exercise intensity, the protein content of urine increased. β_2-microglobuline significantly increased only for 85% of maximal heart rate. It seems that tubular proteinuria does not occur at lower intensities of activity. At lower intensities albuminuria and glomerular proteinuria take place and no proteinuria was observed for <70% of maximal heart rate intensities. Urine protein to creatinine ratio in before and after each exercise was <0.1, respectively. Thus, proteinuria after aerobic sub maximal activity with three different intensities has the physiological range.

Key words: Proteinuria, aerobic exercise, albumin, β_2-microglobuline, protein to creatinine ratio, Iran

INTRODUCTION

Large proteins, such as globulin and albumin cannot pass through glomerular membrane and therefore are observed in very small amounts in urine but in case of glomerular damages their amounts in urine increase, a condition called glomerular proteinuria (Poortmans and Ouchinsky, 2006). Proteins with lower molecular weights such as β_2-microglobulin and lysosome easily pass through glomerule but due to sufficient tubular reabsorption, these proteins are also found in very small amounts in urine (Poortmans and Ouchinsky, 2006; Poortmans *et al*., 1996). In medical conditions accompanied by tubular disorders the presence of these proteins increases, a condition called tubular proteinuria. It is observed that the proteinuria is increased following heavy physical activities (Kocer *et al*., 2008).

Renal disorders caused by sports activities first were reported in 1878 after observing the incidence of proteinuria in soldiers who had hard physical activities. Post-exercise proteinuria is a well-recognized phenomenon among human athletes, the severity of which has depended on the intensity of the exercise (Poortmans *et al*., 1996; Poortmans, 1984). It has been suggested that the presence of excess protein excretion in urine may be due to a disturbance in the selective permeability in the glomerulus associated or not with a saturation process in the reabsorption of the filtered protein load (Poortmans *et al*., 1996; Hardwicke *et al*., 1970). These assumptions have been based on renal clearance of high and low molecular mass plasma proteins (Poortmans *et al*., 1996). In renal disease in which glomerular permeability is increased, a larger excretion of proteins with high molecular mass has been observed (Poortmans *et al*., 1996). It has been reported that when proteinuria is a consequence of tubular dysfunction, the amount of protein filtered by the glomeruli remains stable and the proteins with low molecular mass appear in larger

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quantity due to their incomplete tubular reabsorption (Poortmans *et al*., 1996; Peterson *et al*., 1969). During exercise the plasma flow rate in kidneys decrease and this decrease is directly proportional to exercise intensity. It seems that sympatic nerves and also endocrine systems play a role in this process. As a result of decreasing renal blood flow rate, the glomerular filtration rate decreases too (Poortmans, 1984). There exists a close relation between plasma decline and the presence of albumin in urine, i.e., albuminuria (Estivi *et al*., 1992). Alyea and Parish Jr. (1958) reported that most sportsmen attending either contact or non-contact sports activities are observed to have a variety of proteins in their urine (Alyea and Parish Jr., 1958). Glomerular type and both glomerular and tubular types have been observed after moderate and heavy exercises, respectively (Boileau *et al*., 1980; Poortmans, 1985). Poortmans and Vancalck (1978) reported excretion of proteins after short strenuous exercise. They reported increase in total protein and albumin content as well as renal clearance increase after exercise (Poortmans and Vancalck, 1978). Carroll and Temte (2000) reported that proteinuria is a common problem among adults attending sports activities. It was also reported that fever, high intensity physical or sports activities, body water loss, mental stress and serious diseases are benign and indangerous factors that may cause proteinuria. According to him proteinuria may be categorized as glomerular, tubular and overflow of which glomerular type is most pronounced (Carroll and Temte, 2000). Post-exercise proteinuria is a transient process, lasting approximately 1 h and the maximum proteinuria occurs in 20-30 min after exercise (Poortmans *et al*., 2006).

Although, quantitative assessment of proteinuria from 24 h urine collection still remains a gold standard for urinary protein estimation, besides protein to creatinine ratio provided a very useful, simple and convenient method for quantitative assessment of protein and can replace 24 h urine collection method which used in present study (Garg *et al*., 2004). As a result of the fact that proteinuria increases following intense physical activities and that excessive excretion of proteins in urine may cause renal damage. One of the main issues that many researchers are concerned with around the world is the impact of physical activities on renal performance in particular the proteinuria. The purpose of the present study was to investigate, the response of different intensities of aerobic exercise on proteinuria in young football players.

MATERIALS AND METHODS

Subjects: The subjects of this study consisted of 10 young male soccer players competing that their characteristics are shown in Table 1. These football players in the past 3 years had playing experience in the premier league the Fars province of Iran. Announcing call for youth football teams in the Premier League of province and elaborating the purposes of the present study, 10 soccer players eligible to participate in the study after receiving informed consent and medical examinations and tests were selected. The subjects were non-smokers with no background of renal, heart and liver, etc., disorders and had not a surgery or medical treatment in 6 months before conducting the experiments of this study. Indeed, they were healthy and athletic people who had regular practice at least 3 days week^{-1}. All of them had no experiment of playing in a better or worse league than the best league of Fars province. Then use the personal information questionnaire, information such as age, sex, sports background obtained and healthy of kidney and urinary system was confirmed by physician.

Table 1: Average and standard deviation of subjects characteristics

BMI (kg m^{-2})	VO_2max (mL/kg/min)	Height (cm)	Weight (kg)	Age (years)	N
21.27±2.14	47.6±4.83	174±4	64.42±3.64	18±0.5	10

Exercise program: The 5 days before the research protocol, aerobic power of the subjects was measured using Bruce test on treadmill (Maud and Foster, 1995). Then, they met in the first exercise session. Three sessions of aerobic exercise concluded 30 min running on treadmill with three different intensity. These three exercise sessions were interfered with 48 h rest periods. In order to avoid misleading results, caused by the effects of exercise sessions on each other, the order of exercise sessions was chosen on a random basis. Each participant attended 30 min of running on treadmill at 55, 70 and 85% of maximal heart rate. Maximum heart rate using the Eq. 208 (0.7 age) were obtained (Tanaka *et al*., 2001). During the study, the subjects were kept in a house prepared for them and they were asked to rest and avoid any physical activity. They were also asked to avoid take meals full of fat, proteins and caffeine at nights before sampling days. In the morning of sampling days, each subjects should empty his bladder and then rest sitting without any physical activity. They should be present at exercise location 2 h before each test. They drink enough water before and after each exercise session in order to produce urine for sampling. All protocols were approved by the Graduate Council of Faculty of Physical Education and Sports Science, Islamic Azad University, Central Tehran.

Taking urine samples and analysis: Urine samples were taken before and 20 min after each exercise session. The samples were stored in special containers at 4°C and all samples study 30 main proteinuria indices were measured and analyzed. Albumin, total protein and creatinine as glomerular damage indices and β_2-microglobuline as tubular damage index. The concentration of urinary albumin was measured by Immunoturbidomentric Method using American Diasorin kit with a sensitivity of 3 mg L^{-1}, urinary total protein concentration by Elisa Comasi-blue (Brad Ford) Method using American Diasorin kit with a sensitivity of 1 mg dL^{-1}, urinary creatinine concentration by Spectophotometry Method (based on the Jaffe Model) using American Diasorin kit with a sensitivity of 31 mg dL^{-1} and urinary β_2-microglobuline concentration by Chemiluminescense Method using American Diasorin kit with a sensitivity of 0.12 mg L^{-1}. Urinary protein to creatinine ratio also was calculated by dividing the total protein and creatinine in each sample by units of mg dL^{-1}.

Statistical methods: In the present study in order to make sure that the data distribution is normal and in order to determine the parametric or non-parametric statistical test, the resulted data was first investigated using Kolmogorov-Smirnov test and it was determined that the data posses normal distribution. So for variability between three exercise sessions with three different intensity. Analysis of Variance (ANOVA) with repeated measurements was used. In case of observing significant difference, in order to determine the difference location and reduce the error paired-samples Student t-test with Bonferroni corrections was also utilized. Also, check the variability within each of the three exercise sessions paired-samples Student t-test was used.

RESULTS

The amounts of urinary albumin, total protein, Creatinine, β_2-microglobuline and protein to creatinine ratio before and after all three exercise sessions are shown in Table 2. Table 3 showed the results of Analysis of Variance (ANOVA) with repeated measurements between the three exercise sessions also Table 4 showed paired-samples Student t-test for each exercise session.

Based on current research findings with increased intensity of exercise, urinary albumin excretion increased significantly ($p = 0.038$ and $p = 0.036$ for 20 min after and the mean difference before and after exercise, respectively). The maximum differences was observed in range of the 55-70% of maximum heart rate ($p = 0.012$ and $p = 0.014$ for 20 min after and the mean difference before and after exercise, respectively) and also between 55-85% of maximum heart rate ($p = 0.027$ and $p = 0.026$ for 20 min after and the mean difference before and after exercise, respectively). As well as, aerobic activity with the intensity of the 55% of maximum heart rate yields to non-significant increase of urinary albumin ($p = 0.248$) and activities with the intensity up to the 70 and 85% of maximum heart rate yields to significant increase of urinary albumin ($p = 0.018$ and $p = 0.046$, respectively).

Table 2: Average and standard deviation of proteinuria in before and after exercise sessions

	Exercise with 55% MHR		Exercise with 70% MHR		Exercise with 85% MHR	
Variables (mg dL^{-1})	Before	After	Before	After	Before	After
Albumin	0.810±0.630	1.150±1.090	0.830±0.550	3.960±3.450	0.780±0.640	5.420±5.93
Total protein	2.750±2.390	2.550±1.200	2.720±2.320	4.580±2.870	2.710±2.410	10.050±7.86
Creatinine	95.000±56.07	116.000±30.07	95.500±55.65	188.500±51.31	95.000±56.07	219.500±70.25
β2 microglobuline	0.054±0.030	0.057±0.030	0.057±0.040	0.059±0.030	0.056±0.030	0.108±0.05
Protein to creatinine ratio	0.032±0.028	0.023±0.013	0.031±0.026	0.024±0.018	0.031±0.028	0.040±0.021

Table 3:Results of Analysis of Variance (ANOVA) with repeated measurements between the sixth exercise sessions

Variables	Pre/Post	Sum of squares	df	Mean squares	F-test	p-values
Albumin	Pre	0.009	1.224	0.007	0.2450	0.678
	Post	94.202	2.000	47.101	3.9570	0.038*
	D	95.181	2.000	47.59	4.0300	0.036*
Total protein	Pre	0.009	1.224	0.007	0.2450	0.678
	Post	300.973	1.089	276.303	8.4420	0.015*
	D	303.752	1.086	729.714	8.4180	0.015*
Creatinine	Pre	1.667	1.000	1.667	1.0000	0.343
	Post	56431.667	2.000	28215.833	9.2790	0.002*
	D	56295	2.000	28147.5	9.1850	0.002*
β_2-microglobuline	Pre	0.00005807	1.280	0.00004535	1.0810	0.339
	Post	0.016	1.017	0.016	5.5330	0.042*
	D	0.084	1.001	0.084	3.1280	0.111
Protein to creatinine ratio	Pre	0.00002885	2.548	0.00001132	1.0880	0.367
	Post	0.004	1.655	0.002	4.6470	0.032*
	D	0.003	1.398	0.0002	3.5010	0.078

*The mean difference is significant at the 0.05 level; D = Means difference before and after activity (Delta)

Table 4: Results of paired-samples Student t-test for each exercise session

Variables	Exercise sessions	t-test	df	p-values
Albumin	55% MHR	1.237	9	0.248
	70% MHR	2.888	9	0.018*
	85% MHR	2.311	9	0.046*
Total protein	55% MHR	0.245	9	0.812
	70% MHR	1.440	9	0.184
	85% MHR	2.742	9	0.023*
Creatinine	55% MHR	1.036	9	0.327
	70% MHR	3.284	9	0.009*
	85% MHR	4.212	9	0.002*
β_2-microglobuline	55% MHR	2.055	9	0.070
	70% MHR	1.869	9	0.094
	85% MHR	2.492	9	0.034*
Protein to creatinine ratio	55% MHR	1.156	9	0.277
	70% MHR	0.650	9	0.532
	85% MHR	1.179	9	0.269

*The mean difference is significant at the 0.05 level

Based on current research findings with increased intensity of activity, urinary excretion of total protein significantly increased (p = 0.015 and p = 0.015) for 20 min after and the mean difference before and after exercise, respectively. The maximum differences was observed in range of the 55-70% of maximum heart rate (p = 0.013 and p = 0.012) for 20 min after and the mean difference before and after exercise, respectively and also between 55-85% of maximum heart rate (p = 0.013 and p = 0.013 for 20 min after and the mean difference before and after exercise, respectively). As well as, aerobic activity with the intensity of the 55 and 70% of maximum heart rate yields to non-significant increase of total protein (p = 0.812 and p = 0.184, respectively) and activities with the intensity of 85% of maximum heart rate yields to significant increase of total protein (p = 0.023).

Based on current research findings with increased intensity of exercise, urinary creatinine excretion increased significantly (p = 0.002 and p = 0.002) for 20 min after and the mean difference before and after exercise, respectively. The maximum differences was observed in range of the 55-70% of maximum heart rate (p = 0.002 and p = 0.003) for 20 min after and the mean difference before and after exercise, respectively and also between 55-85% of maximum heart rate (p = 0.003 and p = 0.003) for 20 min after and the mean difference before and after exercise, respectively. As well as, aerobic activity with the intensity of the 55% of maximum heart rate yields to non-significant increase of urinary albumin (p = 0.327) and activities with the intensity up to the 70 and 85% of maximum heart rate yields to significant increase of urinary albumin (p = 0.009 and p = 0.002, respectively). Based on current research findings with increased intensity of activity, β_2-microglobolinuria significantly increased for 20 min after exercise (p = 0.042) but there was observed no significant difference between mean value of β_2-microglobolin in after and before of exercise (p = 0.111). Most of the difference was observed between 55 and 85% of maximum heart rate about 20 min after activity (p = 0.035). As well as, aerobic activity with the intensity of the 55 and 70% of maximum heart rate yields to non-significant increase of urinary β_2-microglobolin (p = 0.070 and p = 0.094, respectively) and activities with the intensity of 85% of maximum heart rate yields to significant increase of urinary β_2-microglobolin (p = 0.034).

DISCUSSION

In the present study albuminuria, total proteinuria, creatininuria and β_2-microglobulinuria were observed to increase significantly with increasing exercise intensity. Poortmans and Labilloy (1988) reported that the post-exercise proteinuria is more related to activity intensity. Poortmans and Vancalck (1978) showed that intense activity result in urinal excretion of albumin and total protein.

Kramer *et al.* (1988) reported that albuminuria increases following heavy sports activities. This indicates glomerular origin of proteinuria (Poortmans and Vancalck, 1978). Delforge also showed that the more the exercise intensity the more serious is proteinuria. This conclusion was then confirmed by Poortmans and Labilloy (1988). In the present study increasing exercise intensity resulted in increasing β_2-microglobulinuria. This is in agreement with previous studies which indicated that tubular proteinuria occurs with increasing physical activity intensity (Poortmans and Vancalck, 1978). Poortmans and Vancalck (1978) and Montelpare *et al.* (2002) showed that periodic physical load increases β_2-microglobulinuria. After exercise high renal clearance of β_2-microglobulinuria has been observed. This indicates that post-exercise proteinuria has also a tubular origin (Poortmans and Vancalck, 1978).

Poortmans *et al.* (1988) showed β_2-microglobuline along with increasing blood lactate (Portmans *et al.*, 1988). Suzuki and Ikawa (1991) stated that decreasing blood pH as a result of organic acids may change glomerular permeability and prevent tubular absorption (Suzuki and Ikawa, 1991). This may be one of the reasons of increasing proteinuria as a result of heavy physical activities because we know that heavy physical activities make body environment more acidic. These findings have been confirmed with Turgut *et al.* (2003). However, the exercise protocol was not related to increasing body acidity. β_2-microglobulinuria significantly increased at an activity intensity of 85%. Perhaps several amino acids contribute to tubular reabsorption disorder (Naderi and Reilly, 2008). Under rest conditions, >95% of filtered proteins are reabsorbed by proximal tubular cell and converted to amino acids (Naderi and Reilly, 2008). All amino acids are present in overflow proteinuria and tubular reabsorption is prevented as a result of absorption capacity completion (Naderi and Reilly, 2008).

In addition, the result of the present study suggest that aerobic activity at 55% of maximal heart rate increases albuminuria but not significantly. Some researcher have shown that following light physical activities proteinuria is observed to increase only in some unhealthy for example diabetic, sedentary people and this is not the case for healthy ones (Poortmans and Labilloy, 1988).

This is in agreement with the fact that all participants of the study were young, healthy sportsmen. However, aerobic activities at 70 and 85% of maximal heart rate caused significant increase in albuminuria in the present study and this increase was maximum for exercise with 85% maximal heart rate. This is in agreement with findings of Clerico *et al.* (1990), Feldt-Rasmussen *et al.* (1985), Poortmans and Vancalck (1978) and Kramer *et al.* (1988). In the present study, aerobic activities at 55 and 70% of maximal heart rate were not strong enough to increase total proteinuria significantly but the increase was significant at 85% of maximal heart rate.

Poortmans and Vancalck (1978), De Palo *et al.* (2002) and Turgut *et al.* (2003) showed that urinary excretion of proteins increases following physical activities and this may be related to renal clearance increase. According to the results of the present study, creatinuria increased as a result of exercise at 70 and 85% of maximal heart rate. This is in agreement with Turgut *et al.* (2003) who reported significant post-exercise proteinuria in both young men and women.

The narrowing of renal arteries due to epinephrine and norepinephrin increase duing exercise may be one of the reasons of post-exercise proteinuria increase (Poortmans, 1984).

As a result of renal blood flow decrease during exercise glomerular filtration rate also decreases and regarding that this decrease is smaller that renal blood flow decrease, the filtration fraction increases and as a result passing through glomerular membrane becomes easier for high molecular weight proteins (Poortmans, 1984). Increase in plasma rennin activity that is observed during hard physical activities and is a result of glomerular sympathetic excitement may affect post-exercise proteinuria (Creeth *et al.*, 1963; Poortmans, 1985). The mediation of kallikrein, an enzyme of Kinin System which is closely related to Renin-Angiotensin System may increase the permeability of glomerular membrane. The loss of capillary wall negative charge may also be effective (Creeth *et al.*, 1963; Poortmans, 1985). Zambraski *et al.* (1981) studied variations of renal ciallic acids in response to exercise and stated that exercise decreases glomerular electrostatic resistance and may justify part of increase in passage of macromolecules. The role of factors like prostaglandins is also of importance and if people take medicines that block prostaglandins production during exercise, proteinuria decreases significantly provided that there is no renal hemodynamic change (Feldt-Rasmussen *et al.*, 1985). Gunduz *et al.* (2003), Senturk *et al.* (2007) and Kocer *et al.* (2008) observed increase in post-exercise proteinuria. The findings of Poortmans and Vancalck (1978) and Clerico *et al.* (1990) suggest that post-exercise proteinuria is very transient. Although, the main factor affecting post-exercise proteinuria is activity intensity (Kramer *et al.*, 1988; Poortmans *et al.*, 1978), the activity duration is also effective (Boileau *et al.*, 1980; Clerico *et al.*, 1990).

Based on the findings of this study, although the amounts of urinary protein to creatinine ratio were observed after three meeting of 20 min of activity with three different intensity. But researchers observed no significant difference when reseirachers compared the mean value of these three meetings in before and after exercise. This finding is also approved in previous studies (Kristal *et al.*, 1998). Kristal *et al.* (1998) found significant linear relationship between proteins to creatinine ratio and proteinuria of 24 h. Protein to creatinine ratio of <0.1 and 0.1-1 and >1 may be used in order to identify a range of physiologic, pathologic and nephrotic proteinuria (Kristal *et al.*, 1998). It has been concluded that the urine protein to creatinine is more reliable to estimate the quantitative proteinuria (Kristal *et al.*, 1998). It is also know that ratio <0.2 shows proteinuria in limited range and proteinuria >3.5 is the scope of nephrotic proteinuria (Garg *et al.*, 2004). Thus, the protein to creatinine ratio is used to detect significancy of proteinuria (Saikul *et al.*, 2006). Thus, the results show that proteins to creatinine ratio had physiologic domain and has significant difference with the pathologic and nephritic values thus it is not malicious (Kristal *et al.*, 1998).

CONCLUSION

In the present study increasing exercise intensity was significantly effective on proteinuria. β_2-microglobulinuria was only significant in exercise at 85% of maximal heart rate and this suggests that tubular proteinuria does not take place at lower intensities. At lower intensities albuminuria and glomerular proteinuria occur and at intensities $<70\%$ of maximal heart rate no proteinuria takes place. As well as was found that none of the training sessions has any significant change in the ratio of urinary protein to creatinine and values obtained from all samples indicating a <0.1, respectively. Accordingly, the maximum aerobic activity in the present study yields to physiological proteinuria in young soccer players that is not malicious. Post-exercise proteinuria could not be a limiting factor for physical activities. Furthermore, in order to come up with more thorough conclusions it seems that more researches are required.

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