Effect of Submaximal Aerobic Exercise in Hypoxic Conditions on Proteinuria and Hematuria in Physically Trained Young Men

Mohammad-Ali Kohanpour,[1] Suzan Sanavi,[2] Maghsoud Peeri,[1] Amir-Hamzeh Zare,[3] Mona Mirsepasi[1]

[1]Department of Exercise Physiology, Islamic Azad University, Tehran, Iran
[2]Clinical Department, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
[3]Department of Exercise Physiology, Shiraz University of Medical Sciences, Shiraz, Iran

Keywords. physical exercises, hypoxia, hematuria, proteinuria

Original Paper

Introduction. Exercise-induced proteinuria is predominantly caused by alterations in renal hemodynamics. The present study was conducted to determine the effect of aerobic exercise in hypoxia on proteinuria and hematuria.

Materials and Methods. The study population consisted of 17physically trained healthy young men. They were asked to attend in 4 sets of 30-minute running sessions, separated by 72-hour resting intervals, to attain 70% of maximal heart rate in normoxia (fraction of inspired oxygen of 0.21) and 3 different levels of hypoxia (lower fraction of inspired oxygen equivalent to the heights of 2750 m, 3250 m, and 3750 m above the sea level). Urine samples were collected before exercise and immediately and 1 hour after each session to measure total protein, albumin, β2-microglobulin, and erythrocyte count.

Results. Postexercise urinary total protein, albumin, and β2-microglobulin showed significant increases compared to basline values, while no significant difference was found in urinary total protein between hypoxia and normoxia conditions. However, there was a significant positive correlation between the amount of albuminuria and the height (P = .01), and a significant difference in β2-microglobulinuria between normoxia and the simulated 2750-m altitude (P = .007), which disappeared at higher elevations. None of the participants developed hematuria.

Conclusions. Aerobic exercise with moderate intensity in trained men might induce mixed proteinuria with glomerular predominance correlated with height, while tubular component loses this relation at altitudes above 2750 m. Further research on the influence of exercise on urinary abnormalities, particularly in different environmental conditions, is recommended.

IJKD 2012;6:192-7
www.ijkd.org

INTRODUCTION

Most people develop physiological and biochemical changes at the altitude of 3000 m above the sea level. However, high altitude sickness may supervene at the height as low as 2000 m. Although, high altitude has not been precisely defined, it is arbitrarily predicated to the height above 2500 m.[1] The most common renal complications of the high altitude are related to hypoxia, but additional deterioration may also result from dehydration.

Acute hypoxia decreases renal blood flow (RBF) and glomerular filtration rate (GFR) which potentiates exercise-induced renal ischemia.[2-4] There is a close relation between RBF decline and occurrence of albuminuria.[5] Alyea and Parish reported that most athletes attending either contact or noncontact sports had some degree of proteinuria.[6] In addition, proteinuria has been observed after rapid ascent to the heights above 3000 m.[7-11]

The responsible mechanisms could be attributed to increasing filtered proteins due to the size or charge selectivity changes of the glomerular barrier, hemodynamic alterations, and reduced tubular reabsorptive capacity of proteins.[7,12] Acute hypoxia results in renal arterial blood pressure elevation with resultant increased intraglomerular hydrostatic pressure, which eventually leads to rising of the filtration fraction.[7,13] Moreover, Pines found hematuria in some of his entourage that was further suggestive of direct kidney damage at altitudes.[8] However, it seems that the high-altitude–induced proteinuria continues to be a subject of controversy; some researchers have suggested significant increase in proteinuria at high altitudes, while others have not.[10,14] Furthermore, the minimal height requiring for detection of proteinuria has not been precisely defined. This study was conducted to determine the effects of exercise in a hypoxic condition at different altitudes on urinary protein excretions following submaximal aerobic exercise in trained young men.

MATERIALS AND METHODS

Participants

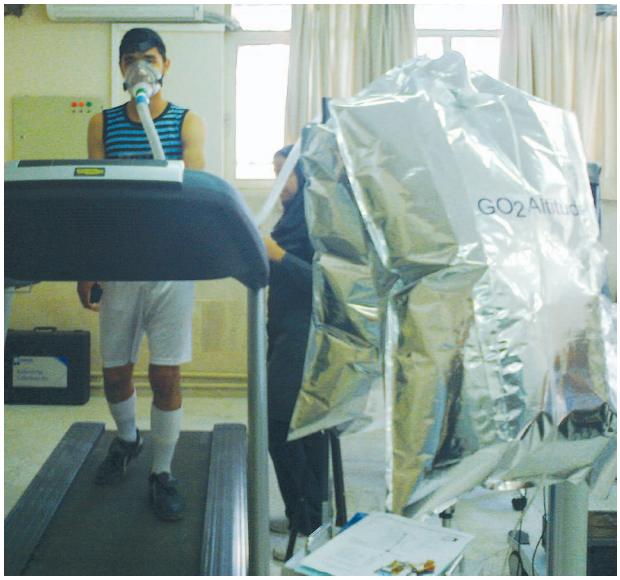
Following announcing call at Universities of Tehran and clearly defining the study purposes, 17 eligible volunteers were recruited from among 23 healthy men. Volunteers with no medical history, no abnormal finding on physical examination and laboratory records, and no history of smoking or regular alcohol consumption were enrolled in the study. They were physically trained young males (age range, 20 to 24 years) who had regular physical training of 6.0 ± 0.55 h/w during the past 2 years.

Exercise Protocol

First, aerobic power of the participants was measured, using the Bruce test on treadmill,[15] and 5 days later, they participated in the first exercise session. The exercise protocol consisted of 4 sessions of 30-minute running on treadmill at the intensity of 70% of age-predicted maximal heart rate in normoxia (fraction of inspired oxygen [FiO2] of 0.21) and 3 different levels of simulated normobaric hypoxic conditions (FiO2 of 0.13, 0.14, and 0.15, equivalent to the hypoxic conditions at 3750-m, 3250-m, and 2750-m altitudes), provided by a high-altitude simulator device (Go2Altitude Hypoxicator, Biomedtech, Victoria, Australia; Figure). The maximal heart rate (MHR) was calculated using the following equation[16]:

MHR = 208 - (0.7 × age)

The sessions were interfered with 72-hour resting periods, and to avoid misleading results, the order of the sessions was chosen on a random basis. The participants were asked to avoid caffeine intake at nights before the exercise days.



Simulation of high-altitude hypoxia.

Urine Analyses

Urine samples were taken before and immediately and 1 hour after each exercise session. The samples were measured for proteinuria indexes including total protein, albumin (glomerular proteinuria), β2-microglobulin (tubular proteinuria), and creatinine. Urinary total protein, albumin, and β2-microglobulin were assayed using the Bradford protein assay (enzyme-linked immunosorbent assay, Coomassie-blue), immunoturbidometric assay, and chemiluminescense methods (with a sensitivity of 1 µg/dL, 3 mg/L, and 0.12 mg/L), respectively. In addition, urinary sediment of all participants was

prepared to evaluate postexercise hematuria, using erythrocyte counting in light microscopy (normal range, 1 to 3 erythrocytes per high-power field).

Statistical Analyses

Normality of the distributions of the variables was determined by the Kolmogorov-Smirnov test. Continuous variables were presented as mean ± standard deviation. The 1-way analysis of variance with repeated measurements for evaluation of variability among the four exercise sessions was used. If a significant difference was observed, the paired *t* test was used with Bonferroni correction. The paired *t* test was applied to check the variability within each of the exercise sessions. Data analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Ill, USA), and significance was accepted at the level of a *P* value less than .05.

RESULTS

Characteristics of the participants are shown in Table 1. The mean values of pre-exercise and postexercise urinary total protein, albumin, β2-microglobulin, erythrocyte count, and protein-creatinine ratio are summarized in Tables 2 to 4.

Postexercise urinary total protein, 1 hour after the exercise, significantly increased compared to the baseline values for the sessions with normixia and hypoxia at the levels of 2750 m, 3250 m, and 3750 m ($P = .005$, $P < .001$, $P = .002$, and $P = .004$,

Table 1. Characteristics of Participants

Characteristic	Value
Age, y	23.33 ± 1.56
Weight, kg	67.16 ± 3.14
Height, cm	176.00 ± 1.76
Body mass index, kg/m^2	21.60 ± 0.91
VO2max, mL/kg/min	48.60 ± 3.96
Resting heart rate, /min	68.55 ± 3.74

Table 2. Pre-exercise and Postexercise Urinary Proteins Following Aerobic Exercise in Normoxia Condition*

Variable	Pre-exercise	Immediate Postexercise	1-hour Postexercise
Total protein, mg/dL	1.587 ± 0.454	1.650 ± 0.200	2.687 ± 0.953
Albumin, mg/24 h	91.375 ± 10.225	84.75 ± 11.937	112.880 ± 26.319
B2-microglobulin, mg/dL	0.987 ± 0.364	1.037 ± 0.150	1.362 ± 0.362
Creatinine, mg/24 h	606.500 ± 156.459	952.250 ± 151.577	930.250 ± 153.677
Erythrocyte, /HPF	1.0 ± 0.7	1.1 ± 0.6	3.0 ± 1.7

*HPF indicates high-power field.

Table 3. Pre-exercise and Postexercise Urinary Proteins Following Aerobic Exercise at Three Levels of Hypoxia*

Variable	Pre-exercise	Immediate Postexercise	1-hour Postexercise
Total protein, mg/dL			
2750 m	1.587 ± 0.451	1.687 ± 0.429	2.100 ± 0.498
3250 m	1.575 ± 0.416	1.625 ± 0.319	2.050 ± 0.453
3750 m	1.550 ± 0.453	1.762 ± 0.570	2.10 ± 0.738
Albumin, mg/24 h			
2750 m	91.250 ± 9.453	89.000 ± 16.656	116.500 ± 23.543
3250 m	91.250 ± 10.067	86.250 ± 14.675	130.120 ± 26.427
3750 m	91.125 ± 9.657	87.875 ± 13.271	182.500 ± 57.547
B2-microglobulin, mg/dL			
2750 m	0.987 ± 0.304	1.050 ± 0.534	1.437 ± 0.641
3250 m	0.987 ± 0.364	0.900 ± 0.325	1.375 ± 0.373
3750 m	0.987 ± 0.365	0.787 ± 0.383	1.175 ± 0.319
Creatinine, mg/24 h			
2750 m	606.500 ± 156.226	1036.400 ± 289.912	1204.100 ± 377.427
3250 m	606.500 ± 156.537	882.880 ± 67.718	1047.600 ± 181.162
3750 m	606.500 ± 156.196	1114.900 ± 265.572	1126.100 ± 270.182
Erythrocyte, /HPF			
2750 m	0.7	1.0 ± 0.7	2.1 ± 1.3
3250 m	1.0 ± 0.5	1.2 ± 0.7	2.0 ± 1.2
3750 m	1.0 ± 0.9	0.8 ± 0.6	2.5 ± 1.5

*HPF indicates high-power field.

Table 4. Pre-exercise and Postexercise Urinary Protein-Creatinine Ratio Following Aerobic Exercise in Different Conditions.

Exercise Sessions	Protein-Creatinine Ratio, mg/mg		
	Pre-exercise	Immediate Postexercise	1-hour Postexercise
Normoxia	0.828 ± 0.334	0.533 ± 0.154	0.910 ± 0.439
Hypoxia (2750 m)	0.833 ± 0.351	0.511 ± 0.194	0.560 ± 0.230
Hypoxia (3250 m)	0.832 ± 0.346	0.553 ± 0.139	0.597 ± 0.147
Hypoxia (3750 m)	0.816 ± 0.326	0.498 ± 0.207	0.577 ± 0.226

respectively) with no significant difference between hypoxic and normoxic conditions ($P = .17$). In addition, postexercise urinary albumin (after 1 hour) showed a significant increase ($P = .012$, $P = .007$, $P < .001$, and $P = .002$, respectively), while there was a significant positive correlation between the amount of albuminuria and height ($P = .014$). Postexercise urinary β2-microglobulin had also a significant increase at 1 hour ($P = .04$, $P = .007$, $P = .005$, and $P = .007$, respectively), and a significant difference was only seen between the values for normoxia and 2750 m altitude ($P = .007$), which was disappeared at higher altitudes (Tables 2 and 3). None of participants showed significant hematuria in any of the conditions.

DISCUSSION

Proteinuria, predominantly of glomerular origin, following rapid ascent to elevations has been hitherto reported by many researchers.[9,17] A time lag of 1 to 3 days has been suggested before the appearance of significant changes in protein excretion; however, even on the next day of acute ascent, this changes may be apparent.[1] Rennie and colleagues found that residents of high altitudes excrete more urinary protein, usually in physiological ranges, than subjects living at the sea level.[11,18] However, this proteinuria may reach to levels as high as 1000 mg/d or more.[1] In a study of Khan and colleagues, the proteinuria had a decreasing fluctuation along with staying at high altitudes and reached baseline after return.[1] Similarly, it was demonstrated by Pines that repetitive ascent to the same altitude induced less degree of proteinuria.[8] Hypoxia-induced kidney injury has been thought to be the possible etiological factor.[1] Patients with cyanotic heart disease who are chronically hypoxic from birth also have increased proteinuria, which is directly related to the degree of polycythemia and hence hypoxia.[19]

The suggested mechanisms for high-altitude proteinuria may be increased glomerular permeability to protein, reduced tubular reabsorption of protein, or both in response to hypoxic injury.[1] Several studies have reported that both acute and chronic altitude hypoxia induce significant proteinuria.[8-10,20] Moreover, an association between the amount of proteinuria and the severity of acute mountain sickness has been demonstrated in some,[8] but not all studies.[21] A confounding parameter in the majority of acute altitude experiments is the concomitance of exercise while climbing to high altitudes, which per se results in proteinuria.[8-10,21,22]

The narrowing of renal arteries due to sympathetic excitement and catecholamine release during exercise may be one of the reasons of postexercise proteinuria. When renal blood flow decreases during exercise, glomerular filtration rate concomitantly reduces (which is smaller than renal blood flow reduction), and the filtration fraction increases, and as a result, passing through glomerular membrane becomes easier for high-molecular weight proteins.[4,23,24] The mediation of kallikrein, an enzyme of the kinin system which is closely related to the rennin-angiotensin system, may increase the permeability of glomerular membrane. Furthermore, the loss of capillary wall negative charge may be an effective factor.[23,24] Zambraski and coworkers studied variations of renal sialic acids in response to exercise and indicated that exercise decreases glomerular electrostatic resistance and may justify part of the increase in passage of macromolecules.[25] On the other hand, Hansen and colleagues and Winterborn and colleagues suggested that the elevated urinary albumin, strongly dependent on the degree of hypoxia at high altitude, is most likely related to a generalized increase in the capillary permeability and is not caused by a hypoxia-induced elevation in blood pressure, an increase in renal filtration fraction, or changes in renal tubular function.[7,10]

Normally, the considerable quantity of low-molecular weight proteins filtered through Bowman space is almost entirely reabsorbed in the proximal

tubule.[12] Although no precise measures of changes in tubular protein reabsorption exists, urinary β2-microglobulin can be used as a marker of proximal tubular dysfunction, as it is filtered freely across the glomerular membrane.[26,27] Hansen and colleagues reported that urinary excretion of β2-microglobulin remained unchanged at high altitude, and neither normoxia nor hypoxia induced further changes.[7] In addition, Winterborn and colleagues could not demonstrate any tubular malfunction due to hypoxia.[10] On account of these unchanged excretion rates of β2-microglobulin, tubular dysfunction seems not to be a proper explanation of hypoxia-induced proteinuria.[7]

The present study showed that moderate exercise (70% of MHR) accompanied by different levels of simulated hypoxia can induce glomerulotubular (mixed) proteinuria with albuminuria predominance. The magnitude of albuminuria was closely related to hypoxia levels so that higher degrees of hypoxia induced more albuminuria. This finding is in accordance with other studies which performed at altitudes of 2500 m above the sea level and higher and may be attributed to renal inability in acute restoring of the causative factors such as the loss of negative charge of capillary wall.[10] In our previous research, like other studies, we noted that moderate exercise could induce albuminuria, which was significant with the intensity of 70% of MHR and greater and at the intensity of 85% MHR (heavy exercise) tubular proteinuria (β2-microglobulin) as a mixed pattern supervened that represented tubular dysfunction.[28] Whereas, these studies had not been performed in an appropriate hypoxic conditions (altitudes > 2500 m), we decided to conduct the present study to evaluate the virtual effects of higher altitudes on renal physiology. Surprisingly, despite above studies, we found increasing levels of urinary β2-microglobulin following exercise which intensified only at 2750 m (FiO2, 0.15) and resolved at higher elevations. Indeed, this height resulted in tubular proteinuria at a lesser exercise intensity, which may be related to additive influence of comparatively severe hypoxia on tubular transport. We concluded that resolving of this effect, at higher altitudes, may be due to renal adaptation (autoregulation) against more vigorous hypoxia. Why this adaptable mechanism is not considered for continuing albuminuria at higher altitudes may be related to more easiness of ameliorating hypoxia-induced changes in intraglomerular hemodynamics, while charge selectivity disorders needs to return to sea level and hypoxia-induced changes in intraglomerular hemodynamics are not responsible for the albuminuria.[7]

CONCLUSIONS

It seems that alterations of kidney function play a part in acclimatization in the high-altitude particularly in certain conditions (eg, altitudes above 2750 m) and for specific substances such as β2-microglobulin. An explanation for discrepancy between the findings of the current study and our previous research may be related to different studied populations. The present study included healthy young men under simple training and not professional athletes who have more prolonged heavy training programs with expected influences on renal physiology. We recommend further investigations surrounding this issue.

CONFLCT OF INTEREST

None declared.

REFERENCES

1. Khan I, Javed Sial SH, Khan JH, Waris S, Iqbal Z, Khan FA. Renal excretory response at high altitude. J Postgrad Med Inst. 1998;12:64-71.
2. Raff H, Shinsako J, Keil LC, Dallman MF. Vasopressin, ACTH, and corticosteroids during hypercapnia and graded hypoxia in dogs. Am J Physiol. 1983;244:E453-8.
3. Zillig B, Schuler G, Truniger B. Renal function and intrarenal hemodynamics in acutely hypoxic and hypercapnic rats. Kidney Int. 1978;14:58-67.
4. Poortmans JR. Exercise and renal function. Sports Med. 1984;1:125-53.
5. Estivi P, Urbino R, Tetta C, Pagano G, Cavallo-Perin P. Urinary protein excretion induced by exercise: effect of a mountain agonistic footrace in healthy subjects. Renal function and mountain footrace. J Sports Med Phys Fitness. 1992;32:196-200.
6. Alyea EP, Parish HH, Jr. Renal response to exercise: urinary findings. J Am Med Assoc. 1958;167:807-13.
7. Hansen JM, Olsen NV, Feldt-Rasmussen B, et al. Albuminuria and overall capillary permeability of albumin in acute altitude hypoxia. J Appl Physiol. 1994;76:1922-7.
8. Pines A. High-altitude acclimatization and proteinuria in East Africa. Br J Dis Chest. 1978;72:196-8.
9. Rennie ID, Joseph BJ. Urinary protein excretion in climbers at high altitudes. Lancet. 1970;1:1247-51.
10. Winterborn MH, Bradwell AR, Chesner IM, Jones GT. The origin of proteinuria at high altitude. Postgrad Med J. 1987;63:179-81.

11. Rennie D, Lozano R, Monge C, Sime F, Whittembury J. Renal oxygenation in male Peruvian natives living permanently at high altitude. J Appl Physiol. 1971;30: 450-6.
12. Rosenberg ME, Hostetter TH. Proteinuria. In: Seldin DW, Giebisch G, editors. The kidney: physiology and pathophysiology. New York: Raven; 1992. p. 3039-61.
13. Peterson PA, Evrin PE, Berggard I. Differentiation of glomerular, tubular, and normal proteinuria: determinations of urinary excretion of beta-2-macroglobulin, albumin, and total protein. J Clin Invest. 1969;48:1189-98.
14. Soylu A, Kavukcu S, Yilmaz O, et al. Renal failure in high altitude: renal functions, renal pathology and bone mineralization in rats with ablation nephropathy at 1200 m altitude. Pathol Res Pract. 2007;203:795-800.
15. Maud PJ, Foster C. Physiological assessment of human fitness. 2nd ed. Champaign, IL: Human Kinetics Publishers; 2006. p. 19-39.
16. Tanaka H, Monahan KD, Seals DR. Age-predicted maximal heart rate revisited. J Am Coll Cardiol. 2001;37:153-6.
17. Bradwell AR, Delamere JP. The effect of acetazolamide on the proteinuria of altitude. Aviat Space Environ Med. 1982;53:40-3.
18. Rennie D, Frayster R, Gray GW, Houston CS. Urine and plasma proteins in men at 5400 m. J Appl Physiol. 1972;32:369-73.
19. Dittrich S, Haas NA, Buhrer C, Muller C, Dahnert I, Lange PE. Renal impairment in patients with long-standing cyanotic congenital heart disease. Acta Paediatr. 1998;87:949-54.
20. Rennie D, Marticorena E, Monge C, Sirotzky L. Urinary protein excretion in high-altitude residents. J Appl Physiol. 1971;31:257-9.
21. Bradwell AR. Birmingham Medical Research Expeditionary Society 1977 Expedition: serum and urine proteins during a high altitude trek. Postgrad Med J. 1979;55:478-82.
22. Poortmans JR, Brauman H, Staroukine M, Verniory A, Decaestecker C, Leclercq R. Indirect evidence of glomerular/tubular mixed-type postexercise proteinuria in healthy humans. Am J Physiol. 1988;254:F277-83.
23. Creeth JM, Kekwick RA, Flynn FV, Harris H, Robson EB. An ultracentrifuge study of urine proteins with particular reference to the proteinuria of renal tubular disorders. Clin Chim Acta. 1963;8:406-14.
24. Poortmans JR. Postexercise proteinuria in humans. Facts and mechanisms. JAMA. 1985;253:236-40.
25. Zambraski EJ, Bober MC, Goldstein JE, Lakas CS, Shepard MD. Changes in renal cortical sialic acids and colloidal iron staining associated with exercise. Med Sci Sports Exerc. 1981;13:229-32.
26. Piscator M. Early detection of tubular dysfunction. Kidney Int Suppl. 1991;34:S15-7.
27. Rashad FA, Vacca CV, Speroff T, Hall PW, 3rd. Altered urinary beta 2-microglobulin excretion as an index of nephrotoxicity. Kidney Int Suppl. 1991;34:S18-20.
28. Peeri M, Kohanpour MA, Sanavi S, et al. Effects of different intensities of aerobic exercise on proteinuria in hypoxia and normoxia conditions in young football players. Dial Traspl. 2012. In press.

Correspondence to:
Suzan Sanavi, MD
University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
E-mail: s2sanavi@yahoo.com

Received November 2011
Revised February 2012
Accepted February 2012