ACUTE PHASE PROTEIN INCREASE IN HIGH ALTITUDE MOUNTAINEERS

AUMENTO DA PROTEÍNA DE FASE AGUDA EM MONTANHISTAS DE GRANDES ALTITUDES

AUMENTO DE LA PROTEÍNA DE FASE AGUDA EN MONTAÑEROS DE GRAN ALTITUD

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Tolga Saka[1]
(Sports Medicine Physician)
Mehmet Karakus[2]
(Sports Medicine Physician)

1. Bezmialem Vakif University Medical Faculty Department of Sports Medicine, İstanbul, Turkey.
2. Erciyes University Medical Faculty Department of Sports Medicine, Kayseri, Turkey.

Correspondence:

Department of Sports Medicine, Bezmialem Vakif University Medical Faculty Hospital, Adnan Menderes Bulvarı, Vatan Caddesi, 34093 Fatih, Istanbul, Turkey.
tolgasakamd@gmail.com

ABSTRACT

Introduction: Many middle-aged Turks go hiking in mountains to breathe some fresh air or to maintain fitness. Objective: This study investigated the effects of regular high altitude mountain climbing on the metabolic and hematological responses of mountaineers. Methods: Hematological and biochemical parameters were studied, as well as some hormonal values of 21 mountaineers and 16 healthy age-matched sedentary volunteers. Results: The neutrophil to lymphocyte ratio (NLR) was significantly lower ($p<0.04$) in mountaineers compared with the sedentary group. Total protein ($p<0.001$) and albumin ($p<0.001$) were lower, while the levels of ferritin ($p<0.04$), creatine ($p<0.03$) and creatine phosphokinase ($p<0.01$) were higher in mountaineers. Other hematological and biochemical parameters, i.e., erythrocytes, leukocytes, hemoglobin and hematocrit, did not change significantly. Conclusion: Our results show that regular exposure to high altitude increased the serum levels of some acute phase proteins with anti-inflammatory properties.

Keywords: mountaineering; sedentary lifestyle; altitude; altitude sickness; neutrophils; lymphocytes.

RESUMO

Introdução: Muitos turcos de meia-idade fazem caminhadas em montanhas para respirar ar puro ou manter a boa forma física. Objetivo: Este estudo pesquisou os efeitos da escalada regular em grandes altitudes sobre as respostas metabólicas e hematológicas dos montanhistas. Métodos: Foram estudados parâmetros hematológicos e bioquímicos, assim como alguns valores hormonais de 21 montanhistas e 16 voluntários sedentários de mesma idade. Resultados: A razão entre neutrófilos e linfócitos (RNL) foi significantemente menor ($p<0,04$) nos montanhistas, em comparação com o grupo sedentário. A proteína total ($p<0,001$) e a albumina ($p<0,001$) foram mais baixas, enquanto os níveis de ferritina ($p<0,04$), creatina ($p<0,03$) e creatina fosfoquinase ($p<0,01$) foram mais altos nos montanhistas. Outros parâmetros hematológicos e bioquímicos, isto é, eritrócitos, leucócitos, hemoglobina e hematócrito não tiveram mudanças significantes. Conclusão: Nossos resultados mostram que a exposição regular a grandes altitudes aumentou os níveis séricos de algumas proteínas de fase aguda com propriedades anti-inflamatórias.

Descritores: *montanhismo; estilo de vida sedentário; altitude; doença da altitude; neutrófilos; linfócitos.*

RESUMEN

Introducción: Muchos turcos de mediana edad hacen caminatas en montaña para respirar aire puro o mantener la buena forma física. Objetivo: Este estudio ha investigado los efectos de la escalada regular en gran altitud sobre las respuestas metabólicas y hematológicas de los montañeros. Métodos: Se estudiaron parámetros hematológicos y bioquímicos, así como algunos valores hormonales de 21 montañistas y 16 voluntarios sedentarios de la misma edad. Resultados: El índice neutrófilo/linfocito (INL) fue significativamente menor ($p<0,04$) en los montañeros, en comparación con el grupo sedentario. La proteína total ($p<0,001$) y la albúmina ($p<0,001$) fueron más bajas, mientras que los niveles de ferritina ($p<0,04$), creatina ($p<0,03$) y creatina fosfoquinasa ($p<0,01$) fueron más altos en los montañistas. Otros parámetros hematológicos y bioquímicos, es decir, eritrocitos, leucocitos, hemoglobina y hematocrito no cambiaron significativamente. Conclusión: Nuestros resultados muestran que la exposición regular a grandes altitudes aumentó los niveles séricos de algunas proteínas de fase aguda con propiedades anti-inflamatorias.

Descriptores: *montañismo; estilo de vida sedentario; altitud; mal de altura; neutrófilos; linfocitos.*

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INTRODUCTION

'Erciyes Tigers' are a group of mountaineers who do this sport regularly. They have been climbing Mount Erciyes (3500 m, in Kayseri, Turkey) once a week at least for ten years. When they climb Erciyes in winter, they take a snow bath. Walking in mountains covered with snow at a high altitude in windy and freezing temperatures is stressful. Mountaineers are obliged to carry dry clothes about 15 kg, and their climbing boots have to weigh at least five kg. Since boots are worn and not carried on the back, their burden equipment is almost five times their mass. Exposure to cold causes a reduction in the sense of thirst thus a reduced voluntary water intake. This decreased water intake is usually accompanied by reduced food intake and a negative nitrogen balance[1]. This study

investigated the effects of regular mountaineering on the metabolic and hematological responses of ***mountaineers*** (Erciyes Tigers) and sedentary (age 40-60 yr) men at resting condition in winter.

METHODS

Twenty one athletes that climbed regularly and 16 healthy age-matched sedentary volunteers participated in the study at resting condition.

Approval from the Ethical Committee of Erciyes University and informed consents were obtained before the study (2010/69). All the subjects participated in the study voluntarily and provided written informed consent. The mountaineers were allowed free intake of their usual diets during the study period. A medical and training history was obtained before the study.

The sedentary group comprised participants whose physical activity had not exceeded two hour per week in the previous two years. None of the participants had a history of metabolic bone disease and everyone denied taking corticosteroids, which are known to affect bone and calcium metabolism. Furthermore, none had a history of arthritis, fatigue fractures or foot pathology in the previous two years. Other exclusion criteria included smoking, excessive alcohol intake, a body mass index 43 kg/ m^2, eating disorders, diabetes mellitus, liver disease and renal disorders. Height (m) and weight (kg) were measured in exercise clothing with shoes removed and body mass index (BMI) was calculated.

Blood analysis

Routine hematological and biochemical tests were performed (Fasting blood glucose, renal and hepatic function tests, full blood count and urinary analysis). A venous blood sample was taken from each individual between 07:00 and 08:00 am after fasting for at least 10 hours overnight to determine serum cholesterol and triglycerides concentrations instantly. Each sample was obtained with the subject in seated position in the laboratory. Blood analysis was processed using Coulter Counter ZF6 to determine hemoglobin (Hb), hematocrit (Hct) and red cell indices as mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) and red cell distribution width (RDW). Plasma glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatine levels were analyzed using spectrophotometric method with the auto analyzer (Architect 1600, Abbott). Serum iron concentration was measured by ascorbic acid reduction on a Boehringer Mannheim- Hitachi 917 analyzer. Total iron binding capacity was calculated from serum transferrin. Ferritin concentration was analyzed by immunoassay measurement.

Statistical analyses

All statistical analyses were performed using SPSS for Windows Version 10.0 (SPSS Inc., Chicago, IL). Data were presented as means ± SE. Unpaired Student's ***t***-test was used to compare the two groups. A ***P*** value less than 0.05 was considered statistically significant.

RESULTS

Averages of age and body mass index of the groups involved in the study are shown in Table1. There were no statistically significant differences between the groups in terms of both age and BMI levels.

When the study results were evaluated for both groups, significant differences were found between sedentary subjects and mountaineers in the NLR ($p<0.04$) (Table 2). There were no significant differences in other full blood count parameters (RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, and RDW). There were also no significant differences between the groups in the white blood cells, platelet, neutrophile, eosinophile, lymphocyte, basophile, monocyte, and sedimentation values (Table 2).

Table 1. Descriptive statistical values of groups (mean± S.E.).

	Sedentary	Mountaineer	p	
Age (year)	48.83 ± 2.0	50.40 ± 1.69	0.35	NS
BMI	27.41 ± 1.37	29.26 ± 0.93	0.26	NS

Table 2. Comparison of hematologic values of sedentary and mountaineer groups (mean± S.E.).

	Sedentary (n=16)	Mountaineer (n=21)	t	p
RBC (10^6µl)	5.24 ± 0.13	5.14 ± 0.12	0.51	0.6
Hb (g/dl)	15.3 ± 0.39	14.9 ± 1.64	0.93	0.3
Hematocrit, (%)	45.82 ± 1.21	44.89 ±0.74	0.68	0.4
MCV (fl)	87.48 ± 1.51	87.90 ± 0.84	0.36	0.7
MCH (pg)	29.41 ± 0.44	29.20 ± 0.40	0.32	0.7
MCHC (g/dl)	33.62 ± 0.21	33.60 ± 0.36	0.02	0.9
RDW (%)	13.13 ± 0.19	13.17 ± 0.14	-0.15	0.8
WBC count, x 10^9/L	7.25 ± 0.88	6.65 ± 0.30	0.79	0.4
Platelet count, x 10^9/L	281.1 ± 12.6	251.8± 11.7	0.12	0.1
White cell distrubition, %				
Neutrophile	60.01 ± 2.85	56.5 ± 1.33	1.2	0.2
Eosinophile	1.86 ± 0.26	2.14 ± 0.22	-0.7	0.6
Lymphocyte	30.3 ± 2.58	32.97±1.19	-1.0	0.6
Basophile	0.53 ± 0.09	0.75 ± 0.10	-1.3	0.3
Monocyte	5.51 ± 0.27	5.60 ± 0.25	-0.21	0.5
Sed (mm/hr)	9.46 ± 1.58	9.86 ± 1.64	-0.1	0.8
NLR	2.26 ± 0.30	1.80 ± 0.10*	1.75	<0.04

RBC: Red blood cell, Hb:hemoglobin, MCV: mean cell volume, MCH: mean cell hemoglobin, MCHC:mean cell hemoglobin concentration, RDW: red cell distribution width, WBC:White blood cells; Sed: Sedimentation; *p<0.05 compared with controls.

Creatine, creatine phosphokinase and ferritin levels were significantly higher in the mountaineer group when compared with the sedentary group (respectively, $p<0.03$, $p<0.01$, $p<0.04$). There were no significant differences in the glucose triglyceride, cholesterol, HDL, BUN, LDL, uric acid, ammonia, iron, iron-binding, Vitamin B12 levels between the groups (Table 3).

Table 4 shows the biomedical parameters of the groups. Total protein and albumin were lower in the mountaineer group when compared with the sedentary group (respectively $p<0.000$, $p<0.001$). There were no significant differences in other parameters (Table 4).

Only the differences in the magnesium levels of the electrolyte tests were significantly different between the groups. Magnesium levels were higher in the sedentary group ($p<0.03$). There were no statistically significant differences between some hormone levels (Table 5).

Table 3. Comparison of biochemical parameters of sedentary and mountaineer groups (mean± S.E.).

	Sedentary (n=16)	Mountaineer (n=21)	t	p
Glucose (mg/dL)	99.38 ± 4.84	99.21 ± 4.47	0.13	0.9
Triglyceride (mg/dL)	152.42 ± 24.33	161.08 ± 16.39	0.12	0.7
Cholesterol (mg/dL)	211.07 ± 11.29	201.60 ± 6.32	0.76	0.4
HDL (mg/dL)	50.64 ± 2.95	48.50 ± 3.61	0.32	0.6
LDL (mg/dL)	145.11 ± 17.90	121.51 ± 6.12	0.70	0.5
BUN (mg/dL)	14.00 ± 7.32	15.08 ± 3.61	-1.15	0.3
Creatine (mg/dL)	0.88 ± 0.03	0.98 ± 0.04*	2.27	<0.03
Uric acid(mg/dL)	6.20 ± 0.31	5.84 ± 0.22	0.84	0.3
Ammonia (mcg/dL)	30.67 ± 3.01	28.92 ± 2.96	0.51	0.5
Iron (µg/dL)	108.42 ± 12.60	97.92 ±16.18	0.59	0.5
IBC (µg/dL)	361.5 ± 25.19	360.69 ± 15.85	-0.10	0.9
Ferritin (ng/mL)	83.00 ± 12.16	110.90 ±15.21*	1.82	<0.04
CK (U/L)	113.38 ± 9.45	164.93 ± 15.79	-2.64	<0.01
Fibrinogen(mg/dL)	293.66 ± 10.66	299.8 ± 10.62	-3.03	0.70

HDL: High density lipoproteine, LDL: Low density lipoproteine, BUN: Blood Urea Nitrogen
IBC: Iron binding capacity, CK: Creatine kinase *p<0.05 compared with controls.

Table 4. Comparison of biochemical parameters of sedentary and mountaineer groups (mean± S.E.)

	Sedentary (n=16)	Mountaineer (n=21)	t	p
Total bilirubin (mg/dL)	0.78 ± 0.20	0.80 ± 0.27	-0.17	0.8
D. bilirubin (mg/dL)	0.20 ± 0.03	0.20 ± 0.08	-0.06	0.9
Amylase (U/L)	69.00 ± 8.29	82.95 ± 5.10	-1.6	0.1
Lipase (U/L)	28.84 ±1.93	34.60 ± 2.19	-2.15	<0.08
AST (U/L)	26.76 ± 6.66	29.17 ± 2.41	-0.48	0.4
ALT (U/L)	32.46 ± 2.81	37.78 ± 4.64	-0.53	0.4
ALP (U/L)	83.76 ± 6.41	76.69 ± 3.85	1.05	0.2
Total protein (g/dL)	7.69 ± 0.40	7.19 ± 0.31**	4.09	<0.001
Albumin (g/dL)	4.70 ± 0.21	4.39 ± 0.05**	3.43	<0.001
UIBC (µg/dL)	263.61 ± 25.26	275.95 ± 11.85	-0.74	0.5

D: Direct, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: Alkaline phosphatase, UIBC: Unsaturated iron binding capacity p<0.001 compared with controls.

Table 5. Comparison of Electrolyte tests and Hormone Levels.

Electrolytes	Sedentary (n=16)	Mountaineer (n=21)	t	p
Calcium (mg/dL)	9.60 ± 0.27	9.57 ± 0.10	0.24	0.8
Phosphorus (mg/dL)	3.12 ± 0.47	3.35 ± 0.09	1.29	0.1
Magnesium (mmol/L)	0.83 ± 0.17	0.78 ± 0.02	1.65	**<0.03**
Sodium (mmol/L)	143.15±10.18	142.43 ±0.43	1.15	0.3
Potassium (mmol/L)	4.46 ± 0.85	4.42 ± 0.08	0.42	0.7
Chlorine (mmol/L)	109.07 ± 7.66	102.65 ± 4.18	1.15	0.2
fT3 (ng/dL)	3.22 ± 0.7	3.21 ± 0.06	0.05	0.9
fT4 (pg/mL)	1.19 ± 0.04	1.22 ± 0.06	-0.67	0.5
TSH (mIU/L)	1.44 ± 0.16	1.42 ± 0.13	0.08	0.9

fT3: Free Triiodothyronine fT4: Free Thyroxine TSH: Thyroid-stimulating Hormone
*p<0.05 compared with controls.

DISCUSSION

The benefits of regular physical activity in adults are extensive. As noted in the adult recommendation, regular physical activity reduces the risk of cardiovascular disease, thromboembolic stroke, hypertension, type 2 diabetes mellitus, osteoporosis, obesity, colon cancer, breast cancer, anxiety, and depression[2].

In this study, we investigated the effects of regular mountaineering on the metabolic and hematological responses of mountaineer (Erciyes Tigers) and sedentary men. We found that NLR, total protein and albumin were lower, while ferritin, creatine and creatine phosphokinase levels were higher in mountaineers.

NLR reflects the balance between the neutrophil and lymphocyte levels in the body and is an indicator of systemic inflammation[3]. It has been recently reported that NLR is an indicator of overall systemic inflammatory status in the Asian population[4]. The NLR was evaluated in numerous studies on cardiovascular or other diseases. It was also associated with an increased risk of long-term mortality[5,6]. It has been also suggested that the NLR can provide a good measure of exercise stress and subsequent recovery since in the hours following recovery from exercise the blood neutrophil count continues to increase and the blood lymphocyte count decreases[7]. NLR has been reported to increase significantly following exercise stress test with subjects who had coronary artery disease, whereas in subjects with a positive exercise test outcome this increase has been even more significant[8].

The NLR usually returns to normal within six to nine hours after exercise, but where the exercise has been particularly prolonged and stressful, the NLR may still be elevated at 24 hours post-exercise[9].

In the present study, we observed that the NLR was lower in mountaineers. NLR measurements were realized under resting conditions. So, this measurement showed us that prolonged moderate exercise might decrease the risk of long-term mortality. Makras et al.[10] found that military physical training was followed by a significant decrease in the NLR, which was estimated approximately at 30% of pre-training values. The noradrenaline/adrenaline ratio appeared first in studies

concerning race horses[11], while the neutrophil/lymphocyte ratio was suggested by Nieman et al.[12] as a useful indication of exercise stress in runners because of its three hour recovery correlation with recovery cortisol concentrations. They hypothesized that changes in the secretion of catecholamines in favor of noradrenaline might affect the mobilization of neutrophils and consequently their depletion in such areas as the lungs, spleen or other areas of tissue damage. In the present study, we were unable to confirm the aforementioned cortisol-related leukocyte alterations. But we think that acute exercise may affect cortisol-related leukocyte alterations. Our mountaineers have been doing this sport for at least 10 years.

Acute phase proteins

The acute phase response (APR) is a prominent systemic reaction of the organism to local or systemic disturbances in its homeostasis caused by infection, tissue injury, trauma or surgery, neoplastic growth or immunological disorders[13].

The decrease of serum zinc, iron and albumin, a decrease of transferrin, cortisol-binding globulin, transthyretin (TTR) and retinol-binding protein (retinol=vitamin A) have been described as negative acute phase proteins[14].

Positive acute phase proteins (APPs) can generally be listed in three major groups: (1) with an increase of about 50%: ceruloplasmin and complement factor-3 (C3), (2) with an increase of two-three fold: haptoglobin, fibrinogen, ferritin, α-globulins with antiprotease-activity and lipopolysaccharide binding protein, and (3) with a rapid increase of up to five-fold to 1000-fold: creatine phosphokinase (CK) and serum amyloid A[15].

In our study, we didn't examine the acute effects of high altitude and physical exercise. But, our mountaineers have been doing this sport once every week and at least for 10 years in a regular way. So, total protein and albumin levels were lower as a result of negative acute phase reaction. Fibrinogen level was not different between the groups, while ferritin, creatine and creatine phosphokinase levels were higher in the mountaineers.

We found decreased total protein and albumin levels in the mountaineers. This may indicate that rather than the role of protein absorption in the digestive tract, muscle protein functions as a major storage for the amino acids required for APP synthesis. Since the amino acid composition of the APPs differs from that of muscle protein, the demands for phenylalanine, tryptophan and tyrosine together necessitate the mobilization of an amount of muscle protein that is considerably exceeding (thrice) the quantity of the APP synthesized[16].

There have been few previous studies of albumin and fibrinogen turnover at high altitude. Imoberdorf et al.[17] examined the acute effects of active and passive ascent to high altitude on plasma volume (PV) and rates of synthesis of albumin and fibrinogen. They found that increases in plasma protein synthesis at high altitude result mainly from the physical exercise associated with climbing[17]. Surks[18] studied albumin turnover in five men at the summit of Pikes Peak, Colorado (4.300 m), by means of 131I-labeled albumin and reported an increased degradation rate.

Serum ferritin concentration increased compared with the healthy subjects. This is similar to the study of Seiler et al.[19], who found an increase during the first three days, followed by a slight decrease without reaching initial values during a 1000 km run.

Fallon et al. studied the serum of participants before, after four and 11 days in a 1600 kilometers ultra-marathon running. Ferritin increment was found only on Day four. Serum ferritin concentration is often used as a single variable for the diagnosis of deficient iron stores in athletes[20]. An increment in ferritin is consistent with an acute phase response. The acute phase response of this protein should be recalled in this situation, and therefore determination of serum ferritin should be performed at a time remote from strenuous exercise of long duration. In a recent study, mitochondrial ferritinin found abundantly in the heart cells has been reported to have a cardioprotective effect against oxidative stress and to prevent muscle cell apoptosis[21]. The same study emphasized that mitochondrial ferritin expression markedly increased with exhaustive exercise.

There were significant differences between sedentary controls and mountaineers in terms of the creatine values in our study. We found that the level of serum creatine was higher in mountaineers.

Indeed, similar results have been obtained in many studies of different exercise duration and intensity; significant increases in the levels of creatine after exercise have been reported in these studies. According to Celenk[22], there were significant differences between sedentary controls and branches; the creatine levels of sedentary individuals are low, but there are no differences among branches.

Although there are findings on the rapid improvement of creatine excretion with exercise, some studies[23,24] also indicate that improvement has not been full even after 48 h of exercise.

There are no comprehensive studies investigating the clinical factors associated with differences in CK[25].

A practical index of muscle damage in athletes performing heavy training is elevation of muscle proteins (e.g. myoglobin, creatine kinase or lactate dehydrogenase) in the blood plasma. However, well-trained athletes who perform eccentric muscle actions do not usually show large increases in plasma creatine kinase activity, though they still experience soreness, perhaps as a result of damage and inflammation of connective tissue structures in muscle[26].

Assessment of plasma creatine kinase activity is therefore potentially useful, not as a marker of impending overtraining, but as a means of identifying a state of recent muscle damage or temporary overreaching[9]. We hypothesize that creatine kinase activity in mountaineers increases as a result of positive acute phase reaction**.**

High altitude is a multi-stress environment constituting, apart from hypoxia, several other stressors, such as extreme cold, ionizing radiations, physical exertion, and mental stress[27].

These stress factors may have an effect on APPs. Yildirim et al.[28] showed that high-altitude exposure was altering serum levels of some acute phase proteins in mountaineers. Their study was realized at the end of day seven at high altitude. They found higher a1-antitrypsine and ceruloplasmin and lower lipoprotein and prealbumin levels in serum samples on day seven when compared to those of day one. C-reactive protein levels were higher in the serum on day seven than onset values, but this increase was not significant. Transferrin levels have not shown a statistically significant difference between the groups. Mountaineers have a more active lifestyle on a day-to-day basis than sedentary subjects which could have accounted for significantly higher mean CK in our study. Although CK values were found higher than in sedentary subjects they were within the mean baseline range for Caucasians[25,29]. AST and ALT are also expected to increase when CK is higher than normal[30]. In our study AST and ALT of mountaineers and controls were normal which confirms that CK increase was within the normal range. On the other hand, CK levels are impacted by many clinical factors. Decreases in muscle mass with age may explain lower CK in elderly patients[25].

Our study had a few limitations. Firstly, we were unable to confirm the aforementioned cortisol-related leukocyte alterations. Secondly,

urinary tests were not performed to determine whether or not albuminuria causes the serum albumin reduction.

Future studies are needed to elucidate the correlation and reliability of non-invasive serum NLR and acute phase protein levels and invasive MDCT or angiography used to assess cardiovascular stroke risk. Serum NLR and acute phase protein levels could be personalized and used for non-invasive cardiovascular stroke risk and general health assessment.

CONCLUSION

Our results show that long term high-altitude exposure is altering serum levels of some acute phase proteins in mountaineers as an adaptive mechanism. Compared to a sedentary lifestyle an active physical lifestyle has an anti-inflammatory effect on the body.

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REFERENCES

1. Dann EJ, Gillis S, Burstein R. Effect of fluid intake on renal function during exercise in the cold. Eur J Appl Physiol Occup Physiol. 1990;61(1-2):133-7.
2. Nelson ME, Rejeski WJ, Blair SN, Duncan PW, Judge JO, King AC, et al. Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc. 2007;39(8):1435-45.
3. Kalay N, Dogdu O, Koc F, Yarlioglues M, Ardic I, Akpek M, et al. Hematologic parameters and angiographic progression of coronary atherosclerosis. Angiology. 2012;63(3):213-7.
4. Imtiaz F, Shafique K, Mirza SS, Ayoob Z, Vart P, Rao S. Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. Int Arch Med. 2012;5(1):2.
5. Porrata LF, Ristow K, Habermann T, Inwards DJ, Micallef IN, Markovic SN. Predicting survival for diffuse large B-cell lymphoma patients using baseline neutrophil/lymphocyte ratio. Am J Hematol. 2010;85(11):896-9.
6. Shen XH, Chen Q, Shi Y, Li HW. Association of neutrophil/lymphocyte ratio with long-term mortality after ST elevation myocardial infarction treated with primary percutaneous coronary intervention. Chin Med J (Engl). 2010;123(23):3438-43.
7. Nieman DC. Influence of carbohydrate on the immune response to intensive, prolonged exercise. Exerc Immunol Rev. 1998;4:64-76.
8. Korkmaz A, Yıldız A, Türker Duyuler P, Duyuler S, Yılmaz S, Basyigit F, et al. Combination of change in hematological parameters with exercise stress test to predict coronary artery disease. J Clin Lab Anal. 2017 Mar 20. doi: 10.1002/jcla.22205. [Epub ahead of print]
9. Gleeson M. Biochemical and immunological markers of over-training. J Sports Sci Med. 2002;1(2):31-41.
10. Makras P, Koukoulis GN, Bourikas G, Papatheodorou G, Bedevis K, Menounos P, et al. Effect of 4 weeks of basic military training on peripheral blood leucocytes and urinary excretion of catecholamines and cortisol. J Sports Sci. 2005;23(8):825-34.
11. Wong CW, Smith SE, Thong YH, Opdebeeck JP, Thornton JR. Effects of exercise stress on various immune functions in horses. Am J Vet Res. 1992;53(8):1414-7.
12. Nieman DC, Simandle S, Henson DA, Warren BJ, Suttles J, Davis JM, et al. Lymphocyte proliferative response to 2.5 hours of running. Int J Sports Med. 1995;16(6):404-9.
13. Gruys E, Toussaint MJ, Landman WJM, Tivapasi M, Chamanza R, van-Veen L. Infection, Inflammation and Stress Inhibit Growth. Mechanisms and Non-specific Assessment of the Processes by Acute Phase Proteins. In: Wensing T, editor. Production Diseases in Farm Animals. 10th International Conference, 1998. Wageningen: Wageningen Press; 1999. p. 72–87.
14. Ingenbleek Y, Young V. Transthyretin (prealbumin) in health and disease: nutritional implications. Annu Rev Nutr. 1994;14:495-533.
15. Gruys E, Toussaint MJ, Niewold TA, Koopmans SJ. Acute phase reaction and acute phase proteins. J Zhejiang Univ Sci B. 2005;6(11):1045-56.
16. Reeds PJ, Fjeld CR, Jahoor F. Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? J Nutr. 1994;124(6):906-10.
17. Imoberdorf R, Garlick PJ, McNurlan MA, Casella GA, Peheim E, Turgay M, et al. Enhanced synthesis of albumin and fibrinogen at high altitude. J Appl Physiol (1985). 2001;90(2):528-37.
18. Surks MI. Metabolism of human serum albumin in man during acute exposure to high altitude (14,100 feet). J Clin Invest. 1966;45(9):1442-51.
19. Seiler D, Nagel D, Franz H, Hellstern P, Leitzmann C, Jung K. Effects of long-distance running on iron metabolism and hematological parameters. Int J Sports Med. 1989;10(5):357-62.
20. Fallon KE, Sivyer G, Sivyer K, Dare A. Changes in haematological parameters and iron metabolism associated with a 1600 kilometre ultramarathon. Br J Sports Med. 1999;33(1):27-31.
21. Wu W, Chang S, Wu Q, Xu Z, Wang P, Li Y, et al. Mitochondrial ferritin protects the murine myocardium from acute exhaustive exercise injury. Cell Death Dis. 2016;7(11):e2475.
22. Çelenk Ç. Investigation of biochemical and hematologic parameters of elite female athletes in different branches with sedanters. Afr J Pharm Pharmacol. 2012;6(19):1405-9.
23. Genc O, Turgut G, Ozguner MF, Kaptanoglu B, Vural G. Effects of exercise on urinary creatinine excretion in adults and children. SDÜ Tip Fakültesi Dergisi. 1999;6:15-8. Disponível em: http://edergi.sdu.edu.tr/index.php/sdutfd/article/view/658/765
24. Huang LL, Huang CT, Chen ML, Mao IF. Effects of profuse sweating induced by exercise on urinary uric acid excretion in a hot environment. Chin J Physiol. 2010;53(4):254-61.
25. George MD, McGill NK, Baker JF. Creatine kinase in the U.S. population: Impact of demographics, comorbidities, and body composition on the normal range. Medicine (Baltimore). 2016;95(33):e4344.
26. O'Reilly KP, Warhol MJ, Fielding RA, Frontera WR, Meredith CN, Evans WJ. Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. J Appl Physiol (1985). 1987;63(1):252-6.
27. Vij AG, Dutta R, Satija NK. Acclimatization to oxidative stress at high altitude. High Alt Med Biol. 2005;6(4):301-10.
28. Yildirim A, Şahin YN, Turhan H, Şen H, Kaplan İ. The effect of high-altitude exposure on serum levels of some acute phase proteins in mountaineers. Fırat Tıp Dergisi. 2008;13(4): 239-42.
29. Santos VC, Sierra AP, Oliveira R, Caçula KG, Momesso CM, Sato FT, et al. Marathon race affects neutrophil surface molecules: role of inflammatory mediators. PLoS One. 2016;11(12):e0166687.
30. Laufs U, Scharnagl H, Halle M, Windler E, Endres M, März W. Treatment options for statin-associated muscle symptoms. Dtsch Arztebl Int. 2015;112(44):748-55.