J Arch Mil Med. 2017 September; 5(3):e14902. doi: 10.5812/jamm.14902.

Published online 2017 September 27. **Research Article**

Lack of Effect of Choline Supplement on Inflammation, Muscle Endurance and Injury Indices, and Shooting Accuracy Following Simulated Army Ranger Operation

Ahmad Azad,[1,*] Rasoul Parsa,[2] and Aghaali Ghasemnian[2]

[1]Department of Physical Education Bandar Anzali Branch, Islamic Azad University, Bandar Anzali, IR Iran

[2]Department of Sport Sciences, Faculty of Humanities, University of Zanjan, Zanjan, IR Iran

[*]*Corresponding author*: Ahmad Azad, Department of Physical Education Bandar Anzali Branch, Islamic Azad University, Bandar Anzali, IR Iran. Tel: +98-9121413799, Fax: +98-1344400486, E-mail: azad@znu.ac.ir

Received 2017 June 15; **Revised** 2017 August 23; **Accepted** 2017 August 27.

Abstract

Background: Sleep deprivation, weighted road march combined with short term high intensity physical activities are important aspects of army ranger operations, which can lead to inflammation, muscular injury, and physical and shooting performance loss. On the other hand, animal and human studies documented that choline supplement relieves inflammation and improves physical and mental performances. The aim of this study was to examine the effect of choline supplementation on serum creatine kinase and IgA, macular endurance, and shooting accuracy of army rangers following one-day simulated operation.

Methods: Twenty army rangers voluntarily participated in this double-blind, placebo controlled quasi-experimental study. The subjects were divided to 2 equal groups and randomly assigned as choline (n = 10, age = 21.2 $\pm$ 3.2 years, Vo2max = 37.7 $\pm$ 4.9 mL/kg/minute) and placebo (n = 10, age = 21.2 $\pm$ 2.2 years, Vo2max = 38.7 $\pm$ 6.6 mL/kg/minute) groups. Choline supplement (2 gr choline bitartrate/day) or placebo (a tea spoon of acid citric/day) was administrated one week before the day of simulated operation, which included partial sleep deprivation (22 p.m.-1a.m.), 19.3 km weighted (12 kg) road march, and 4.8 km competitive run test without extra weight. Serum CK and IgA, muscle endurance (upper body, lower body, and abdomen), and shooting accuracy were assessed before and on the day following the simulated operation. The data were analyzed using independent samples T test (subtracting pretest from posttest) and paired samples t test.

Results: There were no significant changes in serum CK and IgA, abdominal muscles endurance and shooting performance in choline and placebo treatments from pre to the post test, while the posttest upper and lower body endurances were significantly ($P < 0.05$) lower than the pretest in both study groups. Following the operation, no significant differences were found between serum CK and IgA, muscle endurance (upper body, lower body, and abdomen), and shooting performance changes.

Conclusions: The results of this study showed that choline supplement does not induce any positive effects on post operation outcomes of inflammation, muscle injury, muscle endurance, and shooting accuracy.

Keywords: Choline, Inflammation, Sleep Deprivation

1. Background

Army ranger operation is a very demanding activity, which requires considerable physical and mental capabilities. Energy sources depletion due to huge energy expenditure, sleep deprivation, and dangerous operational environment could negatively affect hormonal and immune systems, and destroy muscle mass, physical and mental performance (1). Army rangers conduct prolonged load bearing marches with an approximate load of 30 kg followed by ambush fast attack on the target during a typical night operation (2). The lack of sufficient time to replenish energy sources due the dangerous environmental conditions could induce about 40% energy intake reduction in army rangers (3). Researches show that energy deficit combined with prolonged physical activity result in physical and mental performance decline, cortisol elevation, immune system inhibition, and immunoglobulin's reduction (4). Immunoglobulin A (IgA) is the most important anti-body in human saliva and plays a protective role against toxic agents and bacteria in the oral duct and upper respiratory tract (5). Research findings indicate that the army rangers' upper respiratory tract infection in bottle field is related to IgA supersession (6).

Eccentric muscular contractions due to load bearing activities combined with energy deficit create a catabolic condition and cause the breakdown of structural proteins in army rangers (7). Skeletal muscle proteins catabolism and eccentric contractions along with high oxidative stress could lead to muscular injury in army rangers (8). Plasma Creatine Kinase (CK) is usually considered a muscle injury index and documented results are an indication of CK level

elevation following vigorous muscle contraction or muscle injury (9). Therefore, plasma CK could be considered to monitor muscular injury in army rangers.

On the other hand, successful army ranger operations depend on cognitive performance, alertness, and correct decision-making. Studies show that sleep deprivation could adversely affect immune function, hormone secretion, and physical and mental performance (10). A wide range of tasks, such as cognitive ability, dealing with rapidly changing conditions, reaction time, care and attention, and shooting performance are also negatively associated with sleep deprivation (11).

Respect to the multi-stressor nature of army rangers' operation, the committee on nutritional needs of army forces declares that nutritional interventions play a key role in army ranger operations (12). Knapik et al. in one study reported top 10 supplements (sports drink, multivitamin, protein powder, creatine, sports bar, vitamin, meal replacement drinks, vitamin E, antioxidants, androstenedione) among elite US army populations (13). Unfortunately, despite worldwide research efforts, no study was found in the literature related to the physiological and psychological effects of supplement use during Iranian army ranger operations.

Choline was discovered in 1988 (14). It is essential for neurotransmitter synthesis (acetylcholine), lipid transport, and cell membrane signaling (14). Choline could be absorbed from choline containing foods, yet evidences suggest that body choline content could be decreased during prolonged physical activity (14). A previous study by the authors showed that choline supplementation could improve endurance performance and metabolic fatigue in elite cyclists during 2 hours of laboratory cycling tests (15). Choline could also induce anti-inflammatory effects, since inflammatory indices (C-reactive protein, homocysteine, interleukin-6, and tumor necrosis factor) were found to be lower in subjects with choline rich diets (16). Animal studies suggest that sleep deprivation decreases choline-containing compounds in the brain and this may explain the cognitive and mental concentration impairments in sleep deprived subjects (17).

Despite extensive researches in this area, to date, our knowledge of the physiological and functional aspects of Iranian army ranger operations is limited. Therefore, this study aimed at investigating the effects of choline supplementation on some functional and physiological indices, following a day of simulated army ranger operation.

2. Methods

Twenty volunteer army rangers from a ranger unit participated in this double-blind, placebo controlled randomized study. The subjects singed informed consents and the study was approved by the ethics committee of the University of Zanjan. All procedures performed in this study were in accordance with the ethical standards of the 1964 Helsinki declaration. Inclusion criteria included no history of musculoskeletal injury and inflammatory diseases. The subjects were divided to 2 equal groups, based on age, VO2max, weight, muscle endurance, and marksmanship accuracy, and randomly assigned as placebo and choline supplement groups (Table 1).

The study protocol was carried out during 11 days in the following order:

2.1. Day 1

Fasting (10 to 12 hours) blood samples were collected from antecubital vein after 24 hours of active recovery (8 am). Weight and height were measured using a digital scale and stadiometer, respectively. Upper and lower body endurance was assessed by push-ups, squats and sit-up tests (4 pm).

2.2. Day 2

Cooper 12-minute run test and related formula was used to calculate VO2max (4 pm).

2.3. Day 3

Shooting performance was assessed at a prone position. The targets were placed 200 m far from shooters (4 pm). Each subject fired 10 shots. Scores ranged from zero for hitting the outside zone, to 10 for hitting in the zone.

2.4. Day 4 - 10

The choline supplement group ingested 2 gr/day choline bitartrate with 200 mL of fruit juice. The placebo group received a tee spoon of citric acid/day with 200 mL of juice. The subjects conducted army ranger workouts, except on the last day, which was considered as the active recovery day. In order to control the choline content of the diet, all the subjects were required to use military ration.

2.5. Day 11

The simulated one day operation protocol was conducted as follow:

1- Standard breakfast combined with either placebo or choline was served at 8 a.m. after an overnight partial sleep deprivation (3 hours).

2- The subjects started weighted road march (19.3 km) at a rate of 6 km/hr, while carrying a load of approximately 12 kg (water bottle, backpack, and weapon). They were allowed to drink water every 30 minutes.

Table 1. Characteristics (Mean ± SD) of the Study Groups

Group (N = 10)	Age, y	Height, cm	Weight, kg	VO2max, mL/kg/min
Placebo	21.2 ± 2.2	175.4 ± 4.4	71.3 ± 9.3	38.7 ± 6.6
Choline	21.2 ± 3.2	179 ± 3.8	72.6 ± 12.2	37.7 ± 4.9

3- After completion of a road march, each subject rested for 30 minutes and they were allowed to drink 500 mL of water.

4- Two study groups preformed a 4.8-km competitive run test (without weight).

5- Muscle endurance tests (squat, push-up and sit-up) were conducted after a 30-minute rest interval (500 mL of water was allowed).

6- Prone position shooting test was preformed immediately after muscle endurance tests (ad libitum drink of water was allowed).

7- Blood samples were taken immediately after the shooting test.

Serum IgA was measured by nephelometry and Bioassay technology kit (China). The Enzyme Linked Immunosorbent Assay (ELISA) method and Bioassay technology kit (China) were used to assess serum CK.

2.6. Statistical Analysis

Shapiro-Wilk test verified the normal distribution of the data for all independent variables. Within group changes were assessed by paired samples t test. By subtracting the pre-operation measure from post-operation, the Δ of each variable was calculated. Then, between group differences were evaluated using independent samples t test. Statistical analyses were performed using the SPSS version 22 statistical software, and $P \leq 0.05$ was considered significant.

3. Results

No significant changes were found in CPK, IgA, shooting performance, and sit-up scores from pre- to post-test in choline and placebo conditions (Table 2). After the simulated operation, both groups showed a significant ($P < 0.05$) decrease in squat and push up test scores compared with the pretest (Table 2). There were no significant differences between groups on post-operation changes of CPK, IgA, shooting, sit-up, push-up, and squat tests (Table 3).

4. Discussion

In the present study there was no significant change in serum IgA from pre to post operation under either choline or placebo conditions (Table 2) and no significant difference was found for serum IgA changes between the 2 conditions, following simulated ranger operation (Table 3).

The IgA response to acute exercise appears equivocal. In contrast to the current findings, Babaei et al. reported serum IgA reduction after an acute high intensity aerobic test (4). They suggest that serum IgA reduction might be related to immune system suppression due to the cortisol and epinephrine increased secretion (4). However, it has been shown that increased serum IgA level in elite male athletes following an exhaustive test is related to the plasma volume reduction (18). In line with the current findings, no significant alterations in blood immunoglobulin concentrations were reported in male distance runners after a 12.5-km run (18). Evidences suggest that regular exercise training down regulates biological processes of inflammation in athletes (19). It seems that lack of IgA levels change in army rangers after simulated operation stems from inflammatory processes down regulation due to regular army ranger training program.

Contrary to the current findings, Hui et al. in their study of the immunoglobulin response to sleep and sleep deprivation, found that serum IgA level increased following 24 hours of sleep deprivation in human subjects (20). They related this elevation to production and release of cytokines during sleep deprivation (20). These study subjects were exposed to 3 hours of sleep deprivation, which seems was not long enough to evoke IgA response in the placebo and choline conditions.

Studies show that choline rich diets are related to lower inflammatory indices in human subjects (16). For this reason, the current researchers expected lower serum IgA level in the choline supplement group after simulated operation. Regarding the magnitude of IgA elevation (Δ IgA) after simulated operation (Table 3), it is clear that the magnitude is none significantly higher in the choline intervention than in the placebo (27 ± 4.3 versus 14 ± 12.4). The researchers did not find any conclusive evidence for anti-inflammatory effect of choline supplements.

In the study groups, no significant changes were found in serum CK measures from pre to post operation (Table 2). It could be said that combination of prolonged physical exercise and partial sleep deprivation did not produce any significant raise in serum CK. It has been shown that 72

Table 2. Blood Variables, Performance and Shooting Scores (Mean ± SD) Changes From Pre to Post Test

Group	Choline (N = 10)				Placebo (N = 10)			
Variable	Pretest	Post test	T	P Value	Pretest	Post test	T	P Value
CPK, U/L	223.6 ± 104	206.6 ± 54.1	0.61	0.55	187.4 ± 69.9	253.2 ± 87.1	-2.13	0.06
IgA, mg/dL	156.7 ± 82.2	184.7 ± 92.4	-1.90	0.08	189.9 ± 29.9	204.9 ± 58.8	-1.19	0.26
Pushup (repetitions)	33.7 ± 13.5	24 ± 10	3.84	0.004[a]	40 ± 18	31.4 ± 13.2	3.20	0.04[a]
Sit-up (repetitions)	41.9 ± 14.8	33.9 ± 7.3	1.60	0.14	38.8 ± 11.5	37.1 ± 9.3	0.50	0.62
Squat (repetitions)	28.3 ± 12	15.7 ± 6.4	4.42	0.01[a]	29.3 ± 15.1	10.8 ± 6.8	3.93	0.03[a]
Shooting (score)	44 ± 21.18	48 ± 18.4	-1.39	0.22	36.5 ± 14.5	34 ± 14.2	0.62	0.55

[a] Significant differences among pre and posttest values ($P \leq 0.05$).

Table 3. Comparison of Blood Variables, Muscle Endurance and Shooting Scores Changes (Subtracting Pre From Posttest) Between Choline and Placebo Groups Following the Simulated Operation[a]

Variables	Group		
	Choline (n = 10)	Placebo (n = 10)	P Value
ΔCPK, U/L	17 ± 27.5	-65 ± 30	0.06
ΔIgA, mg/dL	-27 ± 4.3	-14 ± 12.4	0.5
ΔPush-up (repetitions)	9.7 ± 3	8.6 ± 3.9	0.81
ΔSit-up (repetitions)	8 ± 4.9	10.7 ± 3.3	0.30
ΔSquat (repetitions)	12 ± 4.1	18.5 ± 4.7	0.35
ΔShooting (score)	-4 ± 3	2.5 ± 4.3	0.21

[a] Δ = pre operation- post operation.

hours of sleep deprivation could induce a rise in serum CK due to mild to moderate multi-organ damage in mice (21). However, Gopalakrishnan et al. found that short-term (8 hours) and long-term (3 to 14 days) sleep deprivation could not induce oxidative damage for peripheral tissues, such as skeletal muscle in rats (22). The researchers did not find evidence form the literature for relations of partial sleep deprivation and peripheral tissue damage in human subjects, yet the current results indicate that oxidative stress due to partial short-term sleep deprivation does not seem to be the case in the present study.

Some studies have indicated the vigorous exercise (especially after resistive physical activity) could induce blood CK elevation (9). It has been well-established that heavy exercise leads to sarcolemma disruption and Z discs injury with consequent CK leakage from muscle cells into circulation (9). Serum CK increase has also been shown after prolonged aerobic exercise, which results from free radical reaction and loss of cellular membrane integrity (23). Warhol et al. reported peak serum CK in marathon runners 6 hour following a marathon race (24). On the other hand, Warburton et al. showed that a high level of physical fitness could suppress muscle catabolic possesses in endurance runners, which results in slower rate of CK leakage in physically fit athletes (25). I could be suggested that high levels of physical fitness in the current subjects and lack of oxidative stress from partial sleep deprivation were the main factors, which attenuated serum CK increase during the simulated operation.

Serum CK changes (ΔCK) were not significantly different between choline and placebo conditions, following the simulated operation. Based on evidences (15), the researchers hypothesized that choline supplement could attenuate oxidative stress through reducing fatigue during the simulated operation. Muscular endurance measures (ΔPush-up, ΔSit-up, and ΔSquat) were similar between placebo and choline trials after the operation (Table 3). Therefore, the of this study hypothesis was not true in this case. Warber et al. reported that weighted road march followed by exhaustive run test did not cause plasma choline reduction and choline supplement did not induce any fatigue attenuating effect when there was no plasma choline reduction (2). With respect to the operation protocol similarity between this study and Warbler's word, it could probably be said that similar muscular fatigue and serum CK levels in the study groups rooted from the fact that there was no plasma choline depletion in the current study subjects.Following one day of simulated operation, lower body and upper body endurance were significantly lower than the pre-operation measures in both study groups (Table 2). Dziados et al. studied the effect of muscular endurance on 16 km weighted road march ability in soldiers and found a close relationship between road march ability and hamstring muscles group endurance (26). Findings from other studies show that upper and lower body strength and endurance and also abdominal muscles endurance play a central role in weighted road march and

army rangers operation (27). The current results were also an indication of the meaningful involvement of the upper and lower body muscle groups in army rangers operation. No study found no effects for partial sleep deprivation and army ranger operation on muscular endurance measures. Mejiri et al. observed that partial sleep deprivation exerted no effect on intermittent aerobic performance in taekwondo athletes (28). Based on the above-mentioned findings, it is probable that muscle endurance loss in the current study subjects resulted from simulated operation rather than sleep deprivation.

In the present study, Posttest muscle endurance changes (ΔPush-up, ΔSit-up, and ΔSquat) were not significantly different between the two study groups. Therefore, it could be said that muscle endurance reductions were similar in the study groups and choline supplementation did not positively affect muscular endurance in army rangers. A previous work conducted on cyclists showed that choline supplementation reduced metabolic and mayo electric fatigue indices following a 2-hour laboratory cycle test (29). Rezagholizadeh et al. reported increased lipid oxidation and endurance performance improvement in triathlons after choline supplementation (30).

Consistent with the current results, in a study of the effect of choline supplementation on physical performance, Warber et al. administrated choline supplements to male soldiers before a laboratory protocol, which was Similar to the current field setting protocol (2). They found that choline supplementation had no positive effect on fatigue indices following laboratory prolonged weighted road march (2). They did not find any plasma choline depletion under placebo conditions. Plasma choline has been observed to decrease during prolonged physical activities, such as marathon and ultra-marathon, which could negatively affect acetylcholine synthesis at neuromuscular junction and in turn muscular performance (2). The rational for choline supplementation is that the supplement improves endurance performance by compensating plasma choline depletion (2). In agreement with Warder's results, it could be suggested that the lack of choline ergogenic effect in the current study was due to the fact that plasma choline does not appear to be affected by a one-day simulated operation.

In this study, the study groups showed no significant change in shooting scores from pre to post test (Table 2) and shooting performances (Δshooting = pretest-posttest) were not significantly different between the two groups after the simulated operation (Table 3). These results indicated that fatigue due to the operation and partial sleep deprivation did not negatively effect shooting performance in the placebo group and choline supplementation had no positive effect on post operational shooting scores in sleep deprived rangers. Results of a previous study showed that shooting performance were negatively affected by fatigue. It has been reported that, after weighted road march, marksmanship scores in standing position decreased due to upper body muscles fatigue (31). Evidences suggest that sleep deprivation could also negatively impact shooting performance. Haslam et al. showed that 90 hours of sleep deprivation significantly reduced marksmanship in soldiers (31).

In line with the current findings, knapik et al. showed that fatigue did not induce any significant effect on shooting performance in prone position, because the rifle is supported by the ground rather than muscles of the arms, shoulders, and back (32). It has been also reported that 90 hours of sleep deprivation does not induce any reduction in shooting accuracy when soldiers fire at stationary target without a time constraint (33). It could be suggested that the lack of negative effects of simulated operation and partial sleep deprivation on shooting scores in placebo and choline conditions was due to the fact that 1) the subjects were army rangers, who were adapted to partial sleep deprivation due to frequent sleep deprivation exposition during their ranger training course and 2) the subjects fired at stationary targets from prone position with no time limitation.

In the present study, compared with the placebo, choline supplementation appeared to be ineffective on shooting performance after simulated operation and sleep deprivation. To the best of our knowledge the interactive effects of simulated operation, partial sleep deprivation, and choline supplementation on shooting has not yet been studied in army rangers. However, in agreement with our findings, Warber et al. did not find a significant effect regarding choline supplements on shooting scores after simulated laboratory operation in non-sleep deprived army rangers (2). They stated that the effects of simulated operation were not high enough to decrease plasma choline and consequently acetylcholine synthesis to the level, which could disrupt shooting performance (2). Although plasma choline levels were not assessed in this study, yet based on a pervious study, which documented no plasma choline depletion after simulated operation (2), it could probably be said that the lack of choline supplements positively effected shooting performance results in the current subjects.

4.1. Conclusion

Choline supplementation before a simulated army ranger operation did not significantly affect measures of inflammation, muscle injury endurance, and shooting at

the end of the operation. These findings need to be verified in a crossover study monitoring plasma choline levels.

References

1. O'Hara R, Henry A, Serres J, Russell D, Locke R. Operational stressors on physical performance in special operators and countermeasures to improve performance: a review of the literature. *J Spec Oper Med.* 2014;**14**(1):67–78. [PubMed: 24604441].
2. Warber JP, Patton JF, Tharion WJ, Zeisel SH, Mello RP, Kemnitz CP, et al. The effects of choline supplementation on physical performance. *Int J Sport Nutr Exerc Metab.* 2000;**10**(2):170–81. [PubMed: 10861337].
3. Montain S. Nutrient Composition of Rations for Short-Term, High-Intensity Combat Operations. ; 2006. pp. 169–79.Physiological demands of combat operations.
4. Babaei P, Damirchi A, Assarzadeh M. The Effect of a single maximal aerobic training on serum IgG and IgA [In Persian]. *J Guilan Univ Med Sci.* 2003;**12**(46):1–6.
5. Mehdivand A, Sari Sarraf V, Barzegari A, Asgari B. Changesof mucosal immune responses in soccer players in different positionsin a single bout of soccer [In Persian]. *J Mazandaran Univ Med Sci.* 2010;**20**(75):46–53.
6. Pacqué P, Booth C, Dwyer D. Salivary immunoglobulin A (sIgA) as a biomarker of immune suppression following the combat fitness assessment. DTIC Document; 2002.
7. Henning PC. Leucine metabolite, beta-hydroxy beta-methylbutyrate (HMB) as an intervention to maintain lean body mass and performance during sustained military operations. The Florida State University; 2010.
8. Appell HJ, Soares JM, Duarte JA. Exercise, muscle damage and fatigue. *Sports Med.* 1992;**13**(2):108–15. doi: 10.2165/00007256-199213020-00006. [PubMed: 1561506].
9. Kuipers H. Exercise-induced muscle damage. *Int J Sports Med.* 1994;**15**(3):132–5. doi: 10.1055/s-2007-1021034. [PubMed: 8005725].
10. Wolkow A, Ferguson S, Aisbett B, Main L. Effects of work-related sleep restriction on acute physiological and psychological stress responses and their interactions: A review among emergency service personnel. *Int J Occup Med Environ Health.* 2015;**28**(2):183–208. doi: 10.13075/ijomeh.1896.00227. [PubMed: 26182918].
11. Jovanović M. , Sporiš G. , Šopar J. , Harasin D. , Matika D. . The effects of basic military training on shooting tasks in conditions of sleep deprivation. *Kinesiology.* 2012;**44**(1):169–77.
12. Skiller B, Booth C, Coad R, Forbes-Ewan C. Assessment of nutritional status and fatigue among army recruits during the army common recruit training course: part A: catering services and diet. 2005.
13. Knapik JJ, Steelman RA, Hoedebecke SS, Farina EK, Austin KG, Lieberman HR. A systematic review and meta-analysis on the prevalence of dietary supplement use by military personnel. *BMC Complement Altern Med.* 2014;**14**:143. doi: 10.1186/1472-6882-14-143. [PubMed: 24884570].
14. Zeisel SH, da Costa KA. Choline: an essential nutrient for public health. *Nutr Rev.* 2009;**67**(11):615–23. doi: 10.1111/j.1753-4887.2009.00246.x. [PubMed: 19906248].
15. Azad A, Gharakhanlou R, Gaeini A, Ghojaie M. The effect of choline supplement and carbohydrate solution on endurance performance, glucose and blood lipids of trained cyclists [In Persian]. *Olympic.* 2004;**12**(2):53–72.
16. Treede I, Braun A, Sparla R, Kuhnel M, Giese T, Turner JR, et al. Anti-inflammatory effects of phosphatidylcholine. *J Biol Chem.* 2007;**282**(37):27155–64. doi: 10.1074/jbc.M704408200. [PubMed: 17636253].
17. Urrila AS. Sleep deprivation and brain energy metabolism: in vivo studies in rats and humans. 2007.
18. Hoffman J. Physiological aspects of sport training and performance. Human Kinetics; 2014.
19. Liu D, Wang R, Grant AR, Zhang J, Gordon PM, Wei Y, et al. Immune adaptation to chronic intense exercise training: new microarray evidence. *BMC Genomics.* 2017;**18**(1):29. doi: 10.1186/s12864-016-3388-5. [PubMed: 28056786].
20. Hui L, Hua F, Diandong H, Hong Y. Effects of sleep and sleep deprivation on immunoglobulins and complement in humans. *Brain Behav Immun.* 2007;**21**(3):308–10. doi: 10.1016/j.bbi.2006.09.005. [PubMed: 17070668].
21. Periasamy S, Hsu DZ, Fu YH, Liu MY. Sleep deprivation-induced multi-organ injury: role of oxidative stress and inflammation. *EXCLI J.* 2015;**14**:672–83. doi: 10.17179/excli2015-245. [PubMed: 26648820].
22. Gopalakrishnan A, Ji LL, Cirelli C. Sleep deprivation and cellular responses to oxidative stress. *Sleep.* 2004;**27**(1):27–35. doi: 10.1093/sleep/27.1.27. [PubMed: 14998234].
23. Kelle M, Dİken H. , Sermet A. , Atmaca M. , Koçyİğİt Y. . Changes in blood antioxidant status and lipid peroxidation following distance running. *Turkish J Med Sci.* 1998;**28**(6):643–8.
24. Warhol MJ, Siegel AJ, Evans WJ, Silverman LM. Skeletal muscle injury and repair in marathon runners after competition. *Am J Pathol.* 1985;**118**(2):331–9. [PubMed: 3970143].
25. Warburton DE, Welsh RC, Haykowsky MJ, Taylor DA, Humen DP. Biochemical changes as a result of prolonged strenuous exercise. *Br J Sports Med.* 2002;**36**(4):301–3. doi: 10.1136/bjsm.36.4.301. [PubMed: 12145122].
26. Dziados JE, Damokosh AI, Mello RP, Vogel JA, Farmer Jr KL. Physiological determinants of load bearing capacity. DTIC Document; 1987.
27. Kraemer WJ, Vogel JA, Patton JF, Dziados JE, Reynolds KL. The effects of various physical training programs on short duration, high intensity load bearing performance and the Army Physical Fitness Test. DTIC Document; 1987.
28. Mejri MA, Hammouda O, Zouaoui K, Chaouachi A, Chamari K, Rayana MCB, et al. Effect of two types of partial sleep deprivation on Taekwondo players' performance during intermittent exercise. *Biol Rhythm Res.* 2013;**45**(1):17–26. doi: 10.1080/09291016.2013.787686.
29. Azad A, Gharakhanlou R, Gaeini A, Ghojaie M. The effect of choline supplementation and carbohydrate solution on endurance performance and metabolic fatigue of cyclists. *Res Sport Sci.* 2005;**3**(7):1–16.
30. Rezagholizadeh M, EBRAHIM K, Azad A, Karami E. Effect of choline supplement at the level of plasma free fatty acids at the end and recovery period of a long exercise session. *Sport Physiology.* 2013;**5**(17):103–14.
31. Igendia M. Fluctuations in muscle strength, shooting accuracy and serum hormone concentrations in conscripts during a 3-week combat training period. 2015.
32. Knapik J, Staab J, Bahrke M, Reynolds K, Vogel J, O'Connor J. Soldier performance and mood states following a strenuous road march. *Mil Med.* 1991;**156**(4):197–200. [PubMed: 2030843].
33. Haslam DR, Abraham P. Contemporary studies in combat psychiatry. ; 1987. pp. 167–84.Sleep loss and military performance.