



The effect of arginine supplementation following sleep deprivation on carbohydrate and fat metabolism, balance and fatigue index in female athlete students

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Article Info	Abstract
<p>Original Article</p> <p>Article history:</p> <p>Received: 19 August 2020</p> <p>Revised: 25 August 2020</p> <p>Accepted: 4 October 2020</p> <p>Published online: 1 January 2021</p> <p>Keywords: arginine, exercise, fatigue, lactate, sleep.</p>	<p>Introduction: Sleep deprivation is one of common phenomena in athletes. The aim of the present study was to investigate the influence of arginine supplementation following sleep deprivation on carbohydrate and fat metabolism, balance and fatigue index in female athletic students.</p> <p>Methods: The study population included all undergraduate female students of Sport Sciences at Imam Khomeini International University (age: 22.2 ± 2.16 years, BMI: 230.15 ± 2.59 kg/m²) of whom 8 volunteered took part in the research. All experiments were performed in laboratory of university in 2020. Subjects were classified into four groups: Control, Sleep deprivation, Arginine and Arginine-sleep deprivation. Lactometer was used to measure blood lactate. Best Balance device was used to measure balance and GAS Analyzer was used to measure metabolism. Data was analyzed by analysis of covariance (ANCOVA).</p> <p>Results: Sleep deprivation significantly decreased static balance ($P=0.01$) and dynamic balance ($P= 0.004$) while arginine consumption resulted in improved static balance in sleep deprivation arginine group ($P=0.03$). It was also shown that lactic acid levels were lower in the two groups that took arginine than in the groups that did not take arginine ($P \leq 0.05$).</p> <p>Conclusion: Arginine consumption can improve static and dynamic balance as well as mitigate side effects of sleep deprivation on static balance.</p>

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1. Introduction

Several factors like physical fitness [1, 2], nutrition [3, 4] and sleep [5, 6] have shown the significant effect on high levels of performance in athletes. Sleep plays an important role as an essential component of recovery and optimal performance [6, 7, 8]. In recent years, many researches have suggested that quality and quantity of sleep are so important in athletes and their medical teams and aid to improve function and psychosocial characteristics in competition events [9, 10, 11]. There are several studies suggesting that 19, 24, and 48 hours of sleep deprivation is capable of fluctuation of primary motor control and postural stability.

The overall conclusion of such studies is that postural control is negatively affected by sleep deprivation and appropriate interventions are required to improve balance control [12, 13]. In molecular studies, it has been shown that sleep regulates key molecular mechanisms (i.e. regulation of amino acids of proteins) and plays key role in metabolic homeostasis [14]. Several cognitive function impairments have been reported in athletes with sleep disorders such as sleepiness, fatigue, decreased vigor, low alertness and slower reaction time [8, 15, 16]. Research evidences have suggested that enough sleep would increase muscle glycogen store in athletes and improve their performance [17].

Based on research evidences, there are some nutrients which affect quality of sleep (e.g., antioxidants, tryptophan, carbohydrate, melatonin, micronutrients and fruit) [6, 18, 19, 20]. Quality of sleep can be optimized by some strategies such as nutritional interventions [21]. Arginine (Arg) is an amino acid that directly produces the signaling molecule nitric

oxide (NO). NO is a molecule that causes vasodilation, increasing blood flow, potentially delivering more nutrients and metabolic substrates while removing more waste products. ARG is taken up by endothelial cells through a family of cationic amino acid transporter proteins where NO synthase enzymes then use the newly introduced ARG as its substrate [22]. NO is then released into vascular smooth muscle cells, causing relaxation and consequently vasodilation [22]. Therefore, increasing blood flow to the myocardium and skeletal muscle possible is of advantageous of Arg supplementation.

In this regard, Arg plays key role in the removal of excess ammonia from the body and the synthesis of muscle protein, other amino acids, and creatine [23, 24]. Arg can decrease exercise-induced increases in plasma lactate and ammonia in healthy individuals [25, 26]. On the other hand, other researches have shown that oral supplementation with Arg had no influence on time to exhaustion and treadmill walking [23]. Furthermore, it has been suggested that the chronic oral ingestion of Arg had no significant effect on plasma lactate and ammonia responses during fatiguing workouts on a cycle ergometer. There are contradictions in results of previous studies about the effect of Arg on fatigue, physical performance and metabolic process in healthy adults.

By respect to the aforementioned reductions of sleep deprivation on physical and physiological performance of athletes, the aim of this study is to determine the effect of Arg supplementation following sleep deprivation on carbohydrate and fat metabolism, balance and fatigue index in female athlete students.

2. Materials and Methods

2.1. Participants

Eight trained female sport sciences undergraduate students of Imam Khomeini International University (age: 22 ± 2.16 years; BMI: 230.15 ± 2.59 kg/m²; mean \pm SD) were volunteer to participate in the study. The sample size was estimated based on G*Power software (G*Power for Windows, Version 3.1, Dusseldorf, Heinrich-Heine University) with a statistical power of 95%, effect size of 0.5, and alpha of 0.05 [4]. All participants had background of championship in sports in their city and province (martial arts). The inclusive criteria included:

- a) BMI scores between 18-25;
- b) Not smoking and alcohol use;
- c) The lack of significant difference in body composition parameters (body fat% < 28);
- d) Lack of medical treatment for two months prior to the study;
- e) The Pittsburgh Sleep Quality Index (PSQI) >5;
- f) The lack use of special food regime;
- g) Living in dormitory;
- h) Being a member of sport teams.

Volunteers were given food programs one week prior to the study. All participants were explained about the study procedure and signed an informed consent form prior to the study. PSQI was given to fill up by the subjects before and after intervention. In scoring the PSQI, seven component scores are derived, each scored 0 (no difficulty) to 3 (severe difficulty). The component scores are summed to produce a global score (0 to 21). Higher scores indicate worse sleep quality. This index is a valid and reliable tool in Persian version [3, 14]. The research has been confirmed by ethical committee of Qazvin Medical University (IR.QUMS.REC.1398.

029). The study was conducted in accordance to follow the guidelines set forth in the declaration of Helsinki.

2.2. Study design

The present study applied a cross over design with pre/post-test design. Subjects were randomly studied in four conditions of Experiment 1 (24 h of Total Sleep Deprivation-TSD); Experiment 2 (Arg); Experiment 3 (taking Arg following 24 hours of sleep deprivation- Arg+TDS); and Control group. Each experiment was conducted by one week apart. One week was considered for sleep control as 8 hours from 23.30 pm to 7.30 am; then sleep deprivation for 24 hours done after one week sleep control. Their activities within the experiment included studying, watching TV, playing tennis table, reading newspaper during sleep deprivation [6].

Taking caffeine drugs were prohibited in the experiment. The subjects in experiment condition were administered 1.4 mg/day⁻¹ of Arg orally in capsules before posttest 1 and 2. The last night before pretest, participants had 8 hours sleep duration. As seen in Figure 1, blood lactate and balance control were measured after 1 hour of Bruce test and Gas analysis. Then, post-test 1 and 2 were evaluated. TDS group did all instructions like other groups with 24 hours sleep deprivation before pre-test. In Arg+TDS group, all subjects participated in pretest then used 1.4 mg/day⁻¹ of Arg [21] and followed mentioned instructions. (Figure 1).

2.3. Measures

Fat metabolism was evaluated by a graded treadmill exercise test. The protocol included a warm-up exercise of treadmill (3 min of walking at the speed of 2.5 km/h with an incline of 1%, followed by 3 min of stretching exercises and a 2-minute rest).

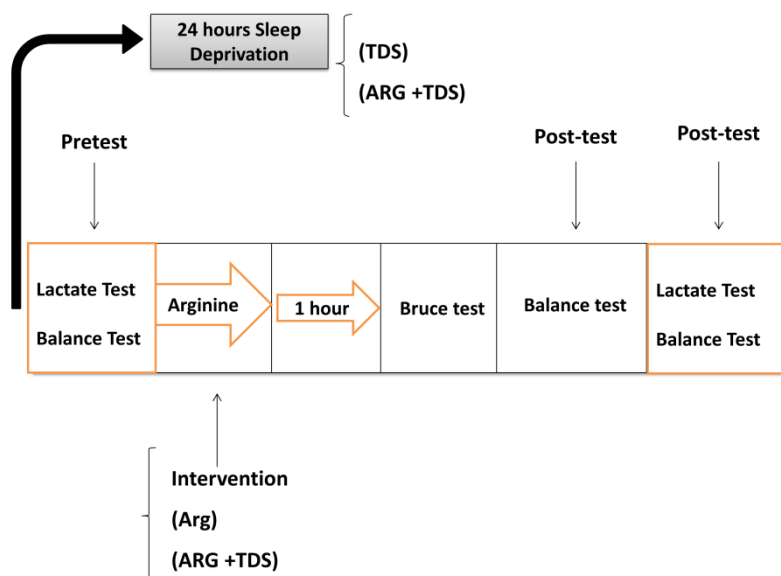


Figure 1. Scheme of experimental design

The exercise was primarily set at a speed of 4.0 km/h with an incline of 1% for 3 min. Then it was increased to 4.5 km/h for 3 min, 5.0 km/h for 3 min and finally 5.5 km/h for 3 min, until the respiratory exchange ratio (RER) reached 1.0. Oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were measured using an open-circuit indirect gas analyzer (Cortex Metalized 3B Gas Analyzer, CORTEX Biophysics GmbH, Germany) [27, 28]. For blood lactate, a lactometer (Lactate Scout Model) device related to the finger tactical test was used. Good Balance device was used to measure static and dynamic balance. All the tests were repeated in posttest [4].

2.4. Statistical analysis

At the level of descriptive statistics, indicators such as mean and standard deviation were used, and at the level of inferential statistics, tests such as the Shapiro–Wilk test, Levin test, ANCOVA test (covariance) and repeated analysis of variance (Repeated Measure ANOVA) were used. These tests were also performed using SPSS 21 software at a significance

level of $P < 0.05$.

Those students who acquire inclusion criteria were examined in pre-test and post-test. The general characteristics of the subjects at baseline included (age: 22 ± 2.16 years, height: 178.3 ± 4.14 cm, weight: 73.74 ± 9.56 kg, BMI: 23.15 ± 2.59 kg/m²). The distribution of data was normal based on the Kolmogorov–Smirnov test. Heart rate, VO_2 and Bruce treadmill test time after exhaustive exercise in four groups are shown in Table 1.

The results of ANCOVA for main effects is shown in Figure 2. Post-static balance scores in TSD group showed more significant reduction than Arg ($P = 0.001$) and control ($P = 0.001$) groups (Figure 2A). The results of dynamic balance identify improvement in traveled speed scores of Arg and Arg + TSD groups. Dynamic balance score of Arg group had significant increase compared with TSD ($P = 0.004$) and control ($P = 0.02$) groups (Figure 2B).

The results of ANOVA analysis showed that there were no differences in VO_2 and RER scores between groups (Figure 3A, B). But there were significant

differences between post- Lactate scores of Arg and other groups ($P \leq 0.01$) (Figure 3C). It was suggested that Arg consumption would significantly decrease lactate levels ($P = 0.001$).

3. Discussion

As mentioned in the results section, sleep deprivation significantly decreased static and dynamic balance while Arg consumption resulted in improved static balance in sleep deprivation + Arg group. It was also suggested that lactic acid levels were lower in the two groups who took Arg than in the groups that did not take Arg. Arg group and sleep deprivation group had lower level of lactic acid compared to sleep deprivation group additionally. No significant differences were found in V_{O_2} and RER of groups.

This result contradicted some of Furtado research, which stated that postural control in healthy individuals was not affected by insomnia. In their study, people with poor sleep quality performed the open-eye postural control test like those with better sleep quality, while those with the eyes closed and on an unstable surface had lower balance scores than those with poor sleep quality.

Perhaps, one possible reason for the differentiation of present study and Furtado's study was due to the tests used, in which the balance was performed in two states of the eye. On the other hand, both genders participated in Furtado's study, and although the age range was similar to the present study [29]. Our participants were all students and lived in a dormitory.

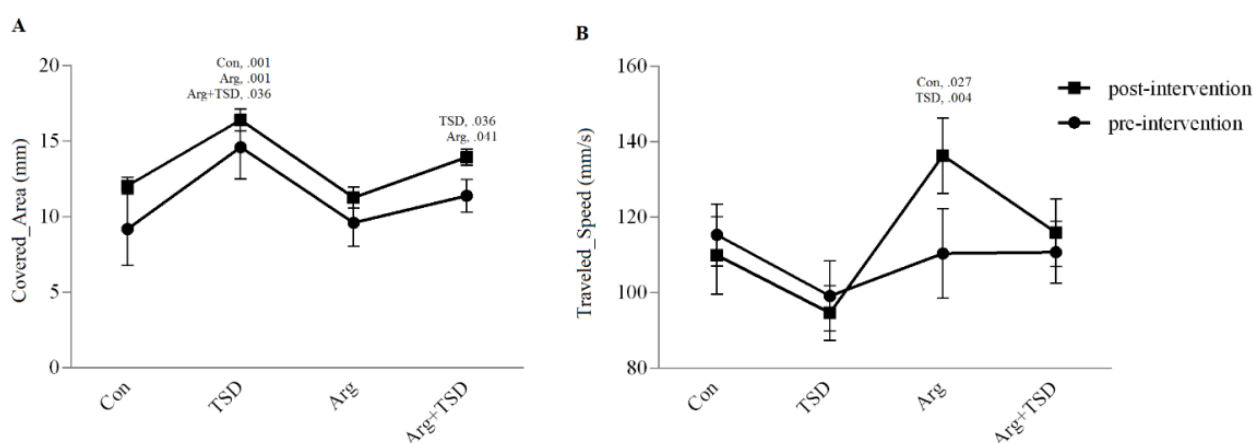
Table 1. Heart rate, V_{O_2} and Bruce treadmill test time after exhaustive exercise in four groups

	Con	TSD	Arg	Arg+TSD	P-value
Heart rate (bpm)	186.2±8.2	186.8±3.71	188±8.78	191.8±2.29	0.296
V_{O_2} (L/min)	76.82±9.56	81.04±4.41	70.51±8.44	84.79±18.89	0.054
Bruce Time (min)	13.88±2.17	12.17±0.89	16.10±2.22 ^{ab}	14.22±1.18	< 0.001

Con: Control group, TSD: 24 h of Total Sleep Deprivation, Arg: Arginine.

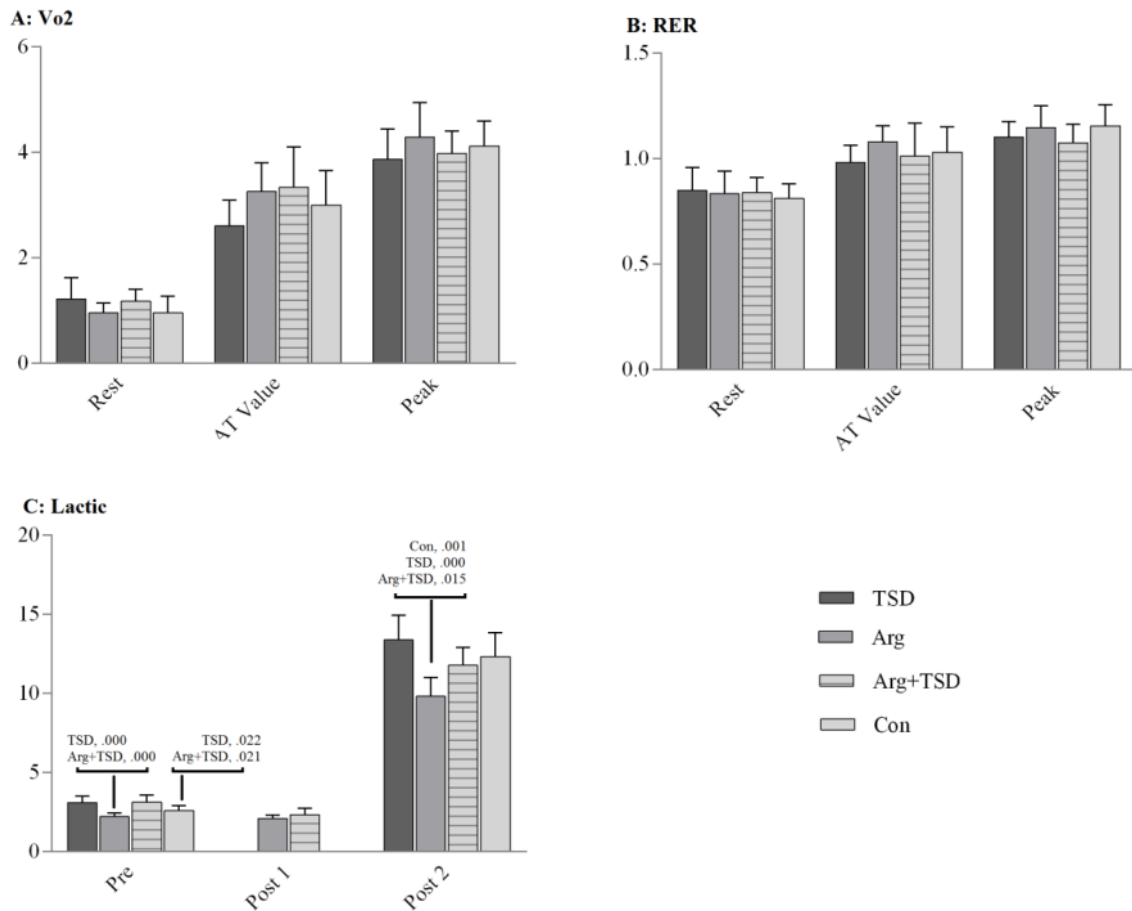
^a $P \leq 0.05$ significant differences with Con

^b $P \leq 0.05$ significant differences with TSD



Con: Control group, TSD: 24 h of Total Sleep Deprivation, Arg: Arginine

Figure 2. Results of Bonferroni post-hoc test for balance. A: The results of static balance test. B: The results of dynamic balance test. Significant differences and significant intergroup values were showed at the top of the group.



Con: Control group, TSD: 24 h of Total Sleep Deprivation, Arg: Arginine.

Figure 3. Results of Bonferroni post-hoc test for Vo_2 , RER and lactic acid levels. Significant differences and significant intergroup values were shown at the top of the group.

Along with the results of our research, Fabbri et al. (2006) stated that insomnia leads to a decline in balance function because changes in sensory integration, especially vision following insomnia, occur in the brain, which inactivates parts of the brain that affect attention and concentration [30]. On the other hand, the results of research on the effect of sleep deprivation on balance contradict the results of Almeida et al. (2017). They reported that insomnia does not upset the balance of individuals [31]. There is evidence that sleep disorders can cause changes in the body's metabolism [32, 33]. Another study reported that acute insomnia altered the metabolite of the DNA genome in adipose tissue, which is rooted in

a link between sleep cycle disorders and daily rhythms, known as body clocks, affecting metabolic function. In this study, it was concluded that sleep deprivation affects amino acid metabolism, which will also affect muscle synthesis [34].

As the results, lactic acid levels in the sleep deprivation and sleep deprivation + Arg groups were higher than the control group, indicating that Arg could not moderate the destructive effect of sleep deprivation and sleep deprivation had a significant negative effect on lactate levels. According to research, blood lactic acid levels rise as a result of strenuous exercise. Lactic acid is known as the end product of glycolysis in the absence of oxygen and is

considered to be the main cause of muscle fatigue because it lowers blood pH and thus increases muscle hydrogen ions, which ultimately leads to a decrease in muscle strength and muscle mass. In addition, increased the levels of lactic acid in the body can lead to decreased blood pH, which leads to the release of calcium and combines with troponin.

Research has shown that Arg would affect aerobic and strength training groups. In the case of aerobic exercise, an increase in L-Arg leads to an increase in endogenous growth hormone, which results in increased fat breakdown and fat oxidation, which improves the performance of endurance athletes [24]. It should be noted that the type of exercise protocol, the duration of supplementation, number of subjects and the supplement dose taken are all items that can justify the difference in results. One of the main limitations of this study was the small number of subjects who participated in this study voluntarily. On the other hand, it might be better to use four independent groups in this study, which is mentioned as a major limitation in this section. The lack of a placebo group due to limited access to subjects was another limitation that is recommended for future research.

4. Conclusion

Finally, it can be concluded that L-Arg in people who have experienced insomnia at night can reduce lactic acid concentration in the blood and thus reduce fatigue. However, the improvement in metabolism was not confirmed in the present study, which was probably due to the acute use of Arg supplementation, while other studies have used Arg in chronic mode. However, the mechanisms by which L-Arg supplementation is performed to increase performance are not fully understood.

Conflict of interest

The authors declared no conflicts of interest.

Authors' contributions

All authors contributed to the original idea, study design.

Ethical considerations

The author has completely considered ethical issues, including informed consent, plagiarism, data fabrication, misconduct, and/or falsification, double publication and/or redundancy, submission, etc. The participants were informed about the purpose of the research and its implementation stages; they were also assured about the confidentiality of their information. Moreover, they were allowed to leave the study whenever they want, and if desired, the results of the research would be available to them. All research processes and methods have been approved by the Ethics Committee in the Research of the Qazvin medical sciences university (Code: IR.QUMS.REC.1397.393).

Data availability

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

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