

The Effect of Glutamine Supplementation on Exercise-Induced Oxidative Stress

B. Nakhostin-Roohi

Department of Exercise Physiology, Ardabil Branch, Islamic Azad University, Ardabil, Iran

Email: b.nakhostinroohi@iauardabil.ac.ir

R. Javanamani

Science and Research Branch, Islamic Azad University, Guilan, Iran

Email: r.jamani@yahoo.com

Abstract—The shift in balance between oxidant/antioxidant in favor of oxidants is termed oxidative stress. Physical exercise may increase accumulation of free radicals and induce oxidative stress. The aim of this study was to evaluate effect of 7 day glutamine supplementation on exercise-induced oxidative stress. Nineteen healthy, nonsmoking, young men were recruited to participate in this study. Participants were randomized in a double-blind placebo-controlled fashion into 2 groups: Glutamine (G group) (n = 9) and placebo (P group) (n = 10). Subjects consumed daily either placebo (1.5 g/kg glutamine + 250ml water + 15g sweetener) or glutamine (250ml water + 15g sweetener) for 7 days. Then, Participants ran 14 km. Blood samples were taken before supplementation, before exercise, immediately, and 1h after exercise. TAC significantly increased immediately after exercise compared with pre-exercise just in G group ($P<0.05$). There was significant GHS increase in G group after supplementation, immediately, and 1h after exercise, but just 1h after exercise in P group compared with baseline ($P<0.05$). MDA-TBARS significantly increased 1h after exercise compared with pre-exercise just in P group ($P<0.05$). It seems 7day glutamine supplementation has been able to affect oxidative stress markers via possibly effect on antioxidant agents.

Index Terms—glutamine, supplementation, oxidative stress, reduced glutathione.

I. INTRODUCTION

Regular physical activity and exercise are recommended for the maintenance of an optimal health status and the prevention or management of chronic diseases. However, physical exercise may increase accumulation of free radicals and induce oxidative stress as a response to the increased oxygen consumption. Under normal physiological conditions, the cellular antioxidant system removes reactive oxygen species (ROS) and other inflammatory molecules [1]. However, oxidative stress occurs when there is an imbalance between the production of free radicals and antioxidant defense [2]. The cells in our body continuously produce free radicals and reactive oxygen species (ROS) as part of metabolic processes [3]. Oxidative stress can occur as a

consequence of a general increase in ROS generation, a depression of the antioxidant defense system (enzymatic and non-enzymatic), or both [4]. Oxidative stress causes damage to biologic macromolecules such as nucleic acids, membrane lipids, and proteins, and hence disrupts normal physiological function [5]. Evidence for increased ROS production during and following exercise is provided by numerous investigations that have noted an increase in various oxidative stress biomarkers following both acute aerobic and anaerobic exercise [6], [7]. During strenuous exercises, an insufficiency of endogenous antioxidants may cause antioxidant defense systems to be temporarily overwhelmed. Supplementation of these systems with antioxidants may therefore reduce oxidative stress [8]. Measurement of various antioxidant or oxidant parameters can be used to determine the risk of oxidative stress or the effectiveness of antioxidant supplementation [9]-[12]. Glutamine is an important constituent of proteins and is a precursor for the synthesis of amino acids, nucleotides, nucleic acids, amino sugars, and several other biologically important molecules [13], [14]. It is the most abundant amino acid in plasma as well as skeletal muscle and accounts for more than 60% of the total intramuscular free amino acid pool [15], [16]. Glutamine is largely synthesized in skeletal muscles and precursors to gluconeogenesis in the liver. Physical exercise is known to affect glutamine synthesis and to modulate glutamine uptake [17]. Glutamine, a nonessential amino acid, has received increasing attention because it becomes essential during stress and catabolic conditions [18]. Moreover, According to some researches, glutamine has antioxidant capacity. Its administration can result in an enhanced antioxidant capacity in various situations, such as critical illness or sepsis [19]. It seems glutamine exerts its antioxidant property through promotion of reduced glutathione synthesis [19]. Strenuous physical exercise as well as prolonged endurance-like programs leads to glutamine depletion due to lowered synthesis and enhanced uptake by liver and immune cells [20]. Nevertheless, based on a literature survey, the alleviating or augmenting action of glutamine on oxidative stress following acute strenuous exercise in humans has not been reported.

Manuscript received August 29, 2014; revised December 23, 2014.

To the best of our knowledge, there is a paucity of investigations about the prophylactic effects of glutamine supplementation on exercise-induced oxidative stress and muscle damage. Therefore, the aim of this study was to evaluate influence of one week glutamine supplementation on selected markers of oxidative stress after 14 km running.

II. MATERIAL AND METHODS

A. Subjects

TABLE I. PHYSICAL CHARACTERISTICS OF SUBJECTS IN PLACEBO (P) AND GLUTAMINE (G) GROUPS. VALUES FOR EACH GROUP REPRESENT MEANS \pm SEM

Groups	Age (year)	Stature (cm)	Body mass (kg)	VO_{2max} ($ml.kg^{-1}.min^{-1}$)	Running records (min)
P (n=10)	22.40 \pm 0.97	177.8 \pm 2.21	74.60 \pm 3.32	44.71 \pm 1.83	83.40 \pm 5.49
G (n=9)	24.55 \pm 0.80	182.3 \pm 3.22	82.56 \pm 2.63	44.07 \pm 1.75	86.67 \pm 4.90

B. Experimental Design

All procedures were completed at laboratory of Islamic Azad University, Ardabil branch. Two weeks prior to main test, participants underwent Cooper test for determining their VO_{2max} [21]. Participants warmed up for 10-min and then ran around the track for 12-min, and the distance covered was recorded. The participants must have been encouraged to push themselves as hard as they could. The formula used to calculate VO_{2max} is:

$$VO_{2max}: \text{Speed (meter/min)} * 0.2 + 3.5$$

Then, participants were randomized in a double-blind, placebo-controlled fashion into two groups: Glutamine (G group) (n = 9), and placebo (P group) (n = 10). They arrived at laboratory after an overnight fasting. A baseline blood draw was taken. Then, subjects consumed daily either placebo (250ml water + 15g sweetener) or the glutamine supplement (1.5 g/kg glutamine + 250ml water + 15g sweetener) for 7days. Afterwards, on the day of the test, subjects attended at athletic arena after an overnight fasting. After second blood taking, participants were allowed to eat a standard breakfast consisting of bread and jam. Two and half hours later, participants warmed up for 15 minutes consisting of running at 50% VO_{2max} (10min) and stretching (5min). Then, Participants ran 14 km. Participants were allowed to consume water ad libitum throughout the exercise. Blood samples were taken immediately and 1hour after exercise.

C. Blood Sampling and Analysis

Approximately 10 ml of blood was withdrawn at each time point. Three milliliters of blood was placed in heparinized tubes and centrifuged at 4000 rpm for 10 min. Plasma was transferred to micro-tubes and stored at -80 °C for subsequent analysis. The rest of the blood was allowed to clot and centrifuged at 4000 rpm for 20 min. Serum was removed and aliquoted in 0.2 ml volumes and stored at -80 °C until analysis. Total antioxidant capacity (TAC) was analyzed by Varga et al. method [22], reduced glutathione (GHS) was measured by Ellman

This was a double-blind, placebo-controlled study. Nineteen healthy, nonsmoking, young men were recruited to participate in this study. The protocol of the study was approved by the university ethics committee in accordance with the Helsinki Declaration. All participants were informed verbally and in writing about the nature and demands of study, and subsequently completed a health history questionnaire and gave their written informed consent. Subjects were free of vitamin/mineral and other types of antioxidants supplementation for 3 weeks prior to the study. Physical characteristics were similar in the both groups (Table I).

method [23], and Malondialdehyde (MDA-TBARS) was analyzed using a spectrophotometric method [24].

D. Statistical Analysis

Results are expressed as mean \pm standard error of mean (SEM). Data were analyzed for time and group inter-variability using two way repeated measures analysis of variance (Two-way ANOVA). When appropriate, significant differences among means were tested using Bonferroni post hoc test. Between groups comparison for subject characteristics was done using unpaired t-test. Differences between groups were considered to be significant when $P < 0.05$.

III. RESULTS

There were no significant differences between physical characteristics of both groups ($P > 0.05$) (Table I).

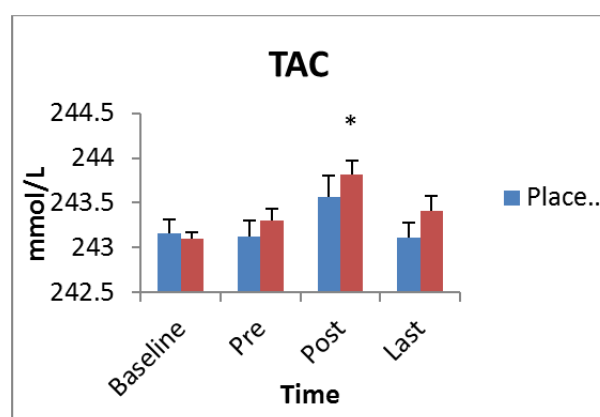


Figure 1. TAC before and after exercise. * Values represent within group significant increase compared with pre-exercise in G groups ($P < 0.05$). Values represent means \pm SEM.

A. Antioxidant MARKER

TAC increased immediately after exercise compared with pre-exercise just in G group ($P < 0.05$). There were no significant differences between groups before and after exercise (Fig. 1).

B. Oxidative Stress Markers

GHS increased immediately after supplementation, immediately, and 1h after exercise compared with baseline in G group ($P < 0.05$). There was significant increase in P group just 1h after exercise compared with baseline ($P < 0.05$) (Fig. 2). MDA-TBARS significantly increased 1h after exercise compared with pre-exercise just in P group ($P < 0.05$) (Fig. 3).

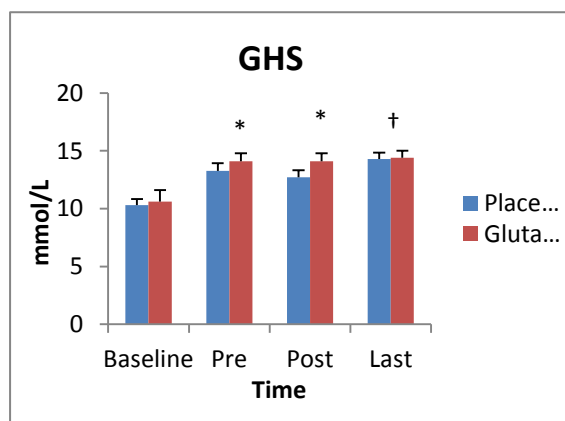


Figure 2. GHS before and after exercise. * Values represent significant increase in G group compared with Baseline ($P < 0.05$). † Values represent significant increase compared with baseline in both groups ($P < 0.05$). Values represent means \pm SEM.

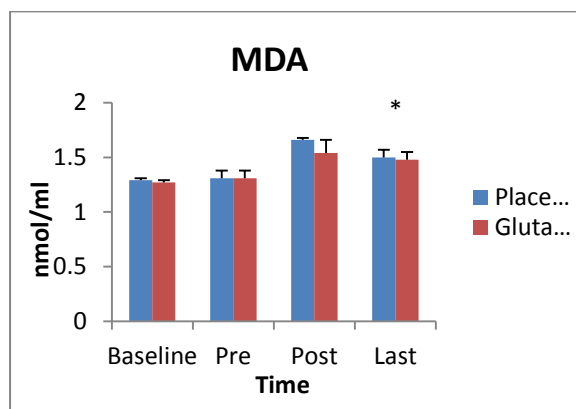


Figure 3. MDA-TBARS before and after exercise. * Values represent significant increase just in P group compared with pre-exercise ($P < 0.05$). Values represent means \pm SEM.

IV. DISCUSSION

The purpose of this study was to evaluate effect of 7day glutamine supplementation on exercise-induced oxidative stress markers.

Exercise is known to have many benefits, including preventive and therapeutic effects on a variety of chronic disorders such as diabetes mellitus, dislipidemia, hypertension, obesity, cardiovascular and pulmonary diseases, muscle, bone and joint diseases, cancer, and depression [25]. While regular exercise training is associated with numerous health benefits, it can be viewed as an intense physical stressor leading to increased oxidative cellular damage, likely due to enhanced production of ROS [26].

The majority of research in the area of oxidative stress and acute exercise in humans has utilized aerobic

exercise protocols. Acute exercise-induced oxidative stress has been well documented over the last decade. A single bout of physical exercise has been shown to induce formation of ROS and nitrogen species and the related oxidative damage. Previous studies have identified elevations in blood oxidative stress markers after acute exercise, indicating that oxidative stress is not limited to the cellular compartment. Furthermore, very high intensity exercise appears to exaggerate the blood oxidative stress response [27]. A number of potential pathways exist for exercise-related oxidant production [28]:

1) Oxygen consumption increases several-fold with exercise. Electron leak from the mitochondrial electron transfer chain results the production of superoxide anions. Free radical production measured by electron spin resonance spectroscopy correlates strongly with maximal oxygen consumption.

2) Xanthine dehydrogenase oxidizes hypoxanthine to xanthine and xanthine uric acid using NAD^+ as the electron acceptor forming NADH. During ischemia, active muscles xanthine is formed via anaerobic metabolism of ATP and xanthine dehydrogenase is converted to xanthine oxidase. During reperfusion, with the resulting increase in oxygen load, xanthine oxidase still converts hypoxanthine to uric acid, but utilizes oxygen as the electron acceptor forming superoxide.

3) Tissue damage resulting from exercise may induce the activation of inflammatory cells such as neutrophils, with the subsequent production of free radicals by NADPH oxidase.

4) Catecholamine concentrations are increased during exercise, and ROS can result from their auto-oxidation.

5) Muscle mitochondria undergo increased uncoupling and superoxide generation with increasing temperatures. Therefore, exercise-induced hyperthermia may cause oxidative stress.

6) Auto-oxidation of oxyhemoglobin to methemoglobin results in the production of superoxide and the rate of formation of methemoglobin can increase with exercise.

In present study same as our previous studies; we used 14km continuous aerobic running protocol to induce oxidative stress [29], [30]. According to our previous studies, 14km continuous exercise could increase production of free radicals and enhance oxidative stress markers in health, active, but non-athlete subjects.

Measurement of the body's antioxidant capacity is utilized as a marker of oxidative stress. In response to conditions of strenuous physical work the body's antioxidant capacity may be temporarily decreased as its components are used to quench the harmful radicals produced. On the other hand, Increase in TAC following exercise was reported [8], [29]. Our study demonstrates an increase in plasma TAC level immediately after exercise just in G group showing possible effect of glutamine supplementation as an antioxidant. The reason of enhancement of TAC in G group may be due to increase of GHS contents of cells after supplementation and exercise (Fig. 2).

The measurement of redox changes in glutathione (the major non-enzymatic endogenous antioxidant) has also been routinely performed as a representation of exercise induced oxidative stress. It plays an important role in the elimination of organic peroxide and hydroxide peroxide. Decrease in GSH level after exercise as a marker of oxidative stress has been reported [31]. Our study showed significant increase in plasma GSH after supplementation, immediately, and 1h after exercise in G group but just 1h after exercise in P group, demonstrating the GSH synthesis promotion possibly from glutamine sources [19].

Lipid peroxidation has been the subject of extensive studies for several decades, and its mechanisms, dynamics, and products are now fairly well established. The most common method utilized to indicate exercise induced oxidative damage in regards to non-eccentric aerobic exercise has been the assessment of lipid peroxidation, with MDA and thiobarbituric acid reactive substances (TBARS) representing the most commonly used assays. MDA is a three carbon chain aldehyde produced during decomposition of a lipid hydroperoxide. Current study also presents the MDA-TBARS level of plasma as a marker of lipid peroxidation. The increase of lipid peroxidation following 14km running was confirmed in our previous works [7], [25], [32]. Current study shows significant increase just in P group 1h after exercise compared with pre-exercise, demonstrating the possible effect of glutamine supplementation as an antioxidant. The possible explanation for no within-group enhancement of MDA-TBARS in G group compared with P group is possibly promotion of GSH contents and antioxidant capacity due to glutamine supplementation.

V. CONCLUSION

The present study suggests acute strenuous bout of exercise could lead oxidative stress on healthy young men. One week supplementation of glutamine has some alleviating effects on lipid peroxidation and may augment intracellular antioxidant system. Nevertheless, the exact mechanism of glutamine on attenuating the markers of oxidative stress is not well established and further exploration is needed.

ACKNOWLEDGMENTS

We would like to thank the subjects that participated in this study as well as exercise physiology laboratory assistant (Miss Razieh Ramazan-zadeh) of Ardabil branch, Islamic Azad University who assisted with data collection and analysis.

REFERENCES

- [1] L. Gravina, F. Ruiz, E. Diaz, J. A. Lekue, *et al.*, "Influence of nutrient intake on antioxidant capacity, muscle damage and white blood cell count in female soccer players," *J. Int Soc Sports Nutr*, vol. 19, pp. 32, Jul. 2012.
- [2] M. Prigol, C. Luchese, and C. W. Nogueira, "Antioxidant effect of diphenyl diselenide on oxidative stress caused by acute physical exercise in skeletal muscle and lungs of mice," *Cell Biochem Funct*, vol. 27, pp. 216-222, Jun. 2009.
- [3] A. Hadžović-Džuvo, A. Valjevac, O. Lepara, S. Pjanić, *et al.*, "Oxidative stress status in elite athletes engaged in different sport disciplines," *Bosn J. Basic Med Sci*, vol. 14, pp. 56-62, May 2014.
- [4] C. K. Roberts and K. K. Sindhu, "Oxidative stress and metabolic syndrome," *Life Sci*, vol. 84, pp. 705-712, May 2009.
- [5] M. Ozata, M. Mergen, C. Oktenli, *et al.*, "Increased oxidative stress and hypozincemia in male obesity," *ClinBiochem*, vol. 35, pp. 627-631, Nov. 2002.
- [6] F. Khoshkharesh, M. Siahkuhain, G. Fisher, and B. Nakhostin-Roohi, "Influence of a low-dose cox-2 inhibitor drug on exercise-induced inflammation, muscle damage and lipid peroxidation," *Biol Sport*, vol. 30, pp. 67-71, Mar. 2012.
- [7] B. Nakhostin-Roohi, S. Barmaki, F. Khoshkharesh, and S. Bohlooli, "Effect of chronic supplementation with methylsulfonylmethane on oxidative stress following acute exercise in untrained healthy men," *J. Pharm Pharmacol*, vol. 63, pp. 1290, Oct. 2011.
- [8] H. K. Vincent, "Antioxidant supplementation lowers exercise-induced oxidative stress in young overweight adults," *Obesity*, vol. 14, pp. 2224-2235, Dec. 2006.
- [9] A. Zembron-Lacny, "Assessment of the antioxidant effectiveness of alpha-lipoic acid in healthy men exposed to muscle-damaging exercise," *J. Physiol Pharmacol*, vol. 60, pp. 139-143, Jun. 2009.
- [10] B. Nakhostin-Roohi, P. Babaei, F. Rahmani-Nia, and S. Bohlooli, "Effect of vitamin C supplementation on lipid peroxidation, muscle damage and inflammation after 30-min exercise at 75% $\dot{V}O_{2\max}$," *J. Sports Med Phys Fitness*, vol. 48, pp. 217-225, Jun. 2008.
- [11] S. Bohlooli, F. Rahmani-Nia, P. Babaei, and B. Nakhostin-Roohi, "Influence of vitamin C moderate dose supplementation on exercise-induced lipid peroxidation, muscle damage and inflammation," *Medicina Dello Sport*, vol. 65, pp. 187-97, June 2012.
- [12] B. Nakhostin-Roohi, Z. Niknam, N. Vaezi, S. Mohammadi, *et al.*, "Effect of single dose administration of methylsulfonylmethane on oxidative stress following acute exhaustive exercise," *Iran J. Pharm Res*, vol. 12, pp. 845-853, Fall, 2013.
- [13] M. Khorshidi-Hosseini and B. Nakhostin-Roohi, "Effect of glutamine and maltodextrin acute supplementation on anaerobic power," *Asian J. Sports Med*, vol. 4, no. 2, pp. 131-136, Jun. 2013.
- [14] Y. O. Fasina, J. B. Bowers, J. B. Hess, and S. R. McKee, "Effect of dietary glutamine supplementation on salmonella colonization in the ceca of young broiler chicks," *Poult Sci*, vol. 89, pp. 1042-1048, May 2010.
- [15] J. Antonio and C. Street, "Glutamine: A potentially useful supplement for athletes," *Can J. Appl Physiol*, vol. 24, pp. 1-14, Feb. 1999.
- [16] R. Kreider, "Dietary supplements and the promotion of muscle growth with resistance exercise," *Sports Med*, vol. 27, pp. 97-110, Feb. 1999.
- [17] F. Agostini and G. Biolo, "Effect of physical activity on glutamine metabolism," *Curr Opin Clin Nutr Metab Care*, vol. 13, pp. 58-64, Jan. 2010.
- [18] T. Etheridge, A. Philp, and P. W. Watt, "A single protein meal increases the recovery of muscle function following an acute eccentric exercise bout," *Appl Physiol Nutr Metab*, vol. 33, pp. 1-6, Jun. 2008.
- [19] Y. P. Fan, "Effects of glutamine supplementation on patients undergoing abdominal surgery," *Chinese Med Sci J.*, vol. 24, no. 1, pp. 55-59, Mar. 2009.
- [20] S. Agostini and G. Biolo, "Effect of physical activity on glutamine metabolism," *Curr. Opin. Clin. Nutr. Metab. Care*, vol. 13, pp. 58-64, Jan. 2010.
- [21] K. H. Cooper, "Aerobics," *Bantam Books*, Jan. 1969.
- [22] S. Z. Varga, B. Matkovic, M. Sasvri, and L. Salgó, "Comparative study of plasma antioxidant status in normal and pathological cases," *Curr. Topics Biophys.*, vol. 22 (suppl), pp. 219-224, Mar. 1998.
- [23] G. L. Ellman, "Tissue sulfhydryl groups," *Arch. Biochem. Biophys.*, vol. 82, pp. 70-77, May 1959.
- [24] N. A. Botsoglou, "Rapid, sensitive, and specific thiobarbituric acid Method for measuring lipid peroxidation in animal tissue, food, and food stuff samples," *J. Agric. Food Chem*, vol. 42, pp. 1931-1937, September 1994.

- [25] B. K. Pedersen and B. Saltin, "Evidence for prescribing exercise as therapy in chronic disease," *Scand J. Med Sci. Sports*, vol. 1 (Suppl 1), pp. 3-63, Feb. 2006.
- [26] R. J. Bloomer, H. A. Goldfarb, L. Wideman, M. J. McKenzie, *et al.*, "Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress," *J. Strength Cond Res.*, vol. 19, pp. 276-85, May 2005.
- [27] J. C. Quindry, W. L. Stone, J. King, and C. E. Broeder, "The effects of acute exercise on neutrophils and plasma oxidative stress," *Med Sci Sports Exerc*, vol. 35, pp. 1139-45, Jul. 2005.
- [28] C. M. Deaton and D. J. Marlin, "Exercise-associated oxidative stress," *Clin. Tech Equine Prac.*, vol. 2, pp. 278-91, Sep. 2003.
- [29] S. Barmaki, S. Bohlooli, F. Khoshkharesh, and B. Nakhostin-Roohi, "Effect of methylsulfonylmethane supplementation on exercise-induced muscle damage and total antioxidant capacity," *J. Sports Med Phys Fitness*, vol. 52, pp. 170-174, Apr. 2012.
- [30] B. Nakhostin-Roohi, F. Khoshkharesh, K. Parandak, and R. Ramazanzadeh, "Influence of L-carnitine supplementation on exercise-induced muscle damage," *Medicina Dello Sport*, vol. 67, pp. 251-259, June 2014.
- [31] K. Fisher-Wellman and R. J. Bloomer, "Acute exercise and oxidative stress: A 30 year history," *Dyn. Med*, vol. 8, pp. 1. Jan. 2009.
- [32] K. Parandak, H. Arazi, F. Khoshkharesh, and B. Nakhostin-Roohi, "The effect of two-week L-carnitine supplementation on exercise-induced oxidative stress and muscle damage," *Asian J. Sports Med*, vol. 5, pp. 123-128, June 2014.



B. Nakhostin-Roohi was born in Tehran on 24th November 1971. He did B.Sc. in Physical therapy at Tehran University, Tehran, Iran (1991-1995), M.Sc. in Physical Education at Islamic Azad University, Tehran, Iran (1988-2001), and Ph.D in Exercise Physiology at Guilan University, Rasht, Iran (2004-2008). He is assistant professor of Ardabil branch, Islamic Azad University, Ardabil, Iran.

Dr. Babak Nakhostin-Roohi has more than 30 original papers in different areas in scientific journals. His main research interests are:

1. Oxidative stress and antioxidants
2. Obesity and exercise
3. Sport physical therapy
4. Medicine sport
5. Physical fitness



R. Javanamani was born in Ardabil on 15th August 1983. He did B.Sc. in Physical education at Orumieh Branch, Islamic Azad University, Orumieh, Iran (2008-2011). He is doing M.Sc. in Exercise Physiology at Science and Research branch, Islamic Azad University, Guilan, Iran (From 2012). He has been Staff of Ardabil Education Department, Ardabil, Iran for three years.

His main research interests are:

1. Oxidative stress and antioxidants
2. Professional Basketball
3. Physical fitness