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Effect of Glutamine Supplementation and Leech Therapy on Blood Lactate Level and Pain Index in a Single Bout Exhaustive Exercise in Young Athletes

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Abstract

Background: Glutamine supplementation for recovery is common in sports. Leech therapy has also been used as complementary medicine in traditional Iranian medicine.
Objectives: The purpose of this study was to investigate the effect of glutamine intake and leech therapy on blood lactate level and pain index in a single bout exhaustive exercise in athletes.
Methods: Thirty-two male athletes (22.2 $\pm$ 2.06 years) were selected and randomly divided into four groups (n = 8) of control, glutamine (0.6 g/kg body weight), leech therapy (2 leeches), and combined leech therapy + glutamine. The subjects participated in an exhaustive test. The levels of lactate and muscle pain perception were measured before, immediately after, and a half and an hour after the test. The significance level was considered P $\leq$ 0.05.
Results: In all groups, lactate increased immediately after the activity, the highest increase was observed in the control, leech therapy, glutamine, and glutamine + leech therapy groups, respectively. Also, the most reduction in lactate level was observed in 1 hour and a half hour after the activity, which was related to the glutamine + leech therapy group. The highest reduction in the pain was observed at 1 hour and a half hour after the activity, which was related to the glutamine + leech group.
Conclusions: The results showed that glutamine supplementation prior to a single bout exhaustive exercise followed by leech therapy led to a decrease in the lactate level and pain index during the recovery period compared to the other three groups, suggesting that this method may be effective. However, more research is needed to prove the benefits of this method to reduce the lactate and pain subsequent exhaustive exercise.

Keywords: Physical Activity, Blood Lactate, Pain Index, Glutamine Supplement, Leech Therapy

1. Background

One of the supplements used by athletes is glutamine. Glutamine is a precursor of glutathione, increase plasma antioxidant capacity (1). On the other hand, laboratory sampling after intensive exercise shows that muscles have bleeding and disconnected filaments, causing muscle pain due to erosion along with the muscle contraction (2). Also, the use of leeches (Hirudo medicinalis) is still popular in modern medicine. Leeches can suck around 5 - 15 mL of blood per latching. Usually, 1 to 2 leeches are used for the treatment (3). Leeches usually unlatch spontaneously after sucking blood, and this time is variable (4).

Hirudin causes dilution of the blood, the opening of the arteries and, consequently, increased blood circulation and localized oxygenation (5). It seems that at least one hundred types of special compounds with different therapeutic effects are secreted from leeches. The Hirudin in saliva and some of the enzymes secreted by the leech also enter the host body (3, 5). Leech prefers carbon monoxide-enriched blood to oxygenated arterial blood and selects vessels that have thicker blood. As the leech sucks the blood, it locally injects its saliva containing Hirudin and possibly more than 100 other types of substances (e.g., prostacyclin) that perform blood purification and dilution simultaneously, and as a result, healthy blood will lead to increased tissue oxygenation (6).

Also, high-intensity exercise training causes damage to the muscle tissue that causes myalgia and discomfort. These effects can negatively impact on the exercise performance (7). Lactate acid decomposition to lactate causes the accumulation of hydrogen ions in muscle cells, which results in metabolic acidosis (8). Physiologists have investigated the amount of blood lactate in athletes at different times. The most important stage is the recovery. Sometimes the interval between the two rounds of a competition or training is not long enough for recovery (9). Failure to complete the recovery will slowly lead to a reduction in the ability to perform further physical functions (10).

There is a limited number of studies on the impact of glutamine prior to exercise and its effects on recovery. According to studies, the leech has great therapeutic effects, and its anti-inflammatory (6), anesthetic, vasodilatory (11), and analgesic (12) effects have been documented.

2. Objectives

Considering the undesirable effects of lactate on the muscle cells and the development of muscle pain, especially during intensive physical activities, and since there has been no research on leech therapy in sports, the aim of this study was to evaluate the effect of glutamine supplementation and leech therapy on the blood lactate level and pain index in a single bout exhaustive exercise.

3. Methods

3.1. Participants

The participants were chosen using the convenience sampling method. After an announcement, 32 athletes (18 to 24 years) from university sports teams with at least three years of regular physical exercise training were recruited. The subjects randomly divided into four groups of eight participants: (1) control, (2) leech therapy, (3) glutamine, and (4) glutamine + leech therapy (Table 1). Prior to performing the research, all the research conditions were precisely explained in a briefing session and a consent form, as well as a personal characteristic form, were completed. The subjects were asked not to use any energy boosters or supplements or other drugs and not to do any exercise activity 48 hours before the test. One week before the study all the subjects assigned to the leech groups were referred to an evaluation of relative prothrombin time (PT), partial thromboplastin time (PTT), and international normalized ratio (INT). All the measurements were performed at the Sports Physiology Laboratory of IKIU University.

3.2. Research Design

This was a semi-experimental study performed using an experimental and control group.

3.2.1. Glutamine Supplementation Group

The subjects in this group received 0.6 g/kg glutamine plus 5 cc/kg of body weight with 10% dextrose one hour before the Bruce protocol. Thirty minutes after the supplementation, the Bruce protocol was executed.

3.2.2. Leech Therapy Group

In leech therapy groups, immediately after the completion of the test, two medicinal leeches were used. The site of placement of medicinal leeches was selected along the cephalic vein. The leeches were separated after 30 minutes.

3.2.3. Glutamine + Leech Therapy Group

This group was a combination of the first two groups. Before the exercise, they received glutamine, and immediately after finishing the Bruce test, leech therapy was performed.

3.2.4. Control Group

This group did not receive any interventions and only performed the test.

3.3. Bruce Protocol

After the assessment of the pre-test, the subjects in the four groups (after applying the intervention according to the group) performed the Bruce test on a laboratory treadmill to the level of exhaustion (13).

3.4. Lactate Measurement

A blood sample was taken from the index finger of the non-dominant hand by the lactometer (Sense Lab, Lactate Scout) to evaluate the pre-test data on the lactate level. This measurement was performed immediately, 30 minutes and 1 hour after the end of the test for the assessment of the post-test data.

3.5. Pain Index Measurement

Numeric rating scale (NRS) was used to assess the pain index. On this scale, numbers less than 1 are reported as "lack of pain", numbers between 1 - 4 are equivalent to "mild", between 4 - 7 denote to "moderate", and numbers greater than 7 are equivalent to "severe pain" (14). The pain level of the subjects was first measured by the NRS questionnaire. Then immediately, 30 minutes and 1 hour after the end of the Bruce test, the pain was assessed in each group.

3.6. Statistical Analysis

After assessing the distribution of data by Kolmogorov-Smirnov test, repeated measures ANOVA and Bonferroni's post hoc test were run. $P \leq 0.05$ was considered significant.

Table 1. The Participants' Characteristics[a]

	Glutamine	Leech Therapy + Glutamine	Leech Therapy	Control
Age, y	23 ± 3.01	21.50 ± 1.68	21.57 ± 1.48	22 ± 2.07
Weight, kg	75.49 ± 5.84	77.53 ± 4.50	73.93 ± 5.50	71.37 ± 6.54
Height, cm	182.57 ± 2.24	185.25 ± 3.10	175.75 ± 2.91	181.87 ± 2.64

[a]Values are expressed as mean ± SD.

3.7. Ethical Considerations

The present study was approved by the Ethics Committee of Imam Khomeini International University with the Code of Ethics (code: 17682).

4. Results

In all groups, lactate increased immediately after the activity, the highest increase was observed in the control, leech therapy, glutamine, and glutamine + leech therapy groups, respectively. Also, the most reduction in the lactate level was observed in 1 hour and a half hour after activity, which was related to glutamine + leech therapy group. The highest reduction in the pain was observed at 1 hour and a half hour after activity, which was related to the glutamine + leech group (Table 2).

Lactate level and pain were significantly different between the groups (Table 3).

The values obtained from the mean changes in the lactate levels in the four groups at different times indicated that the highest reduction was observed in the glutamine + leech therapy group (Figure 1). Also, the values obtained from the mean changes in pain index in the four groups at different times showed that the greatest reduction was observed in the glutamine + leech therapy group (Figure 2).

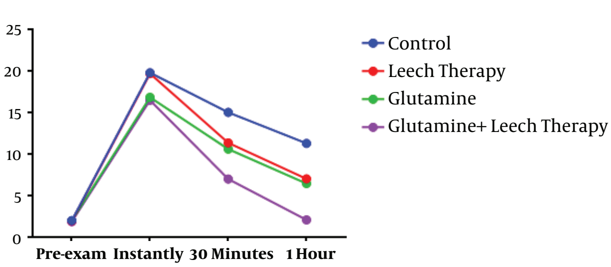

Figure 1. Changes in the blood lactate at different levels are shown.

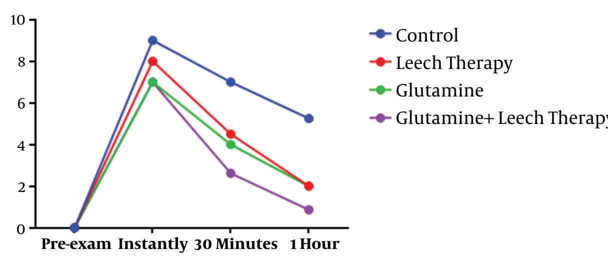

Figure 2. Changes in the pain index at different levels are shown.

5. Discussion

5.1. Lactate Changes in the Glutamine Group

The results showed that blood lactate decreased in the glutamine group compared to the control group. Also, immediately after exercise activity, lactate in the supplement group showed a lower increase compared to the control group. Half an hour and 1 hour after the exercise, the level of lactate in the supplement group reduced more compared to the control group. At 1 hour after the activity. The results of this study were consistent with Cruzat and Tirapegui's study (15). However, they are not congruent with the findings of Falk et al. (16). The discrepancy between our results and those of Falk et al. could be attributed to the dosage of glutamine or the presence of glutamine with other supplements, such as creatine and ribosomes, the effects of which cannot be differentiated from each other (17). Acceptable mechanisms in relation to these results suggest that because of an increased hepatic glutamine absorption for the enhancement of renal ammoniagenesis and gluconeogenesis, it creates an adaptive response that results in metabolic acidosis. Therefore, glutamine plays an important role in regulating the acid-base balance in the body. This process helps to produce bicarbonate ions to neutralize lactic acid (18).

As amino acids migrate into the muscle, they absorb water to help maintain muscles hydrated. This hydrated condition will prevent muscles from moving to the catabolic condition and increase anabolic growth. As long as glutamine reserves are not depleted, the recovery time will be shorter (19). Other factors are likely to affect the amount of lactate reduction, including the amount of lactate release from muscle cells, the intake of lactate by tissues such as the liver and the heart, and the rate of its distribution in the body fluids. Also, because lactate can easily permeate across the membrane, it can enter organs whose

Table 2. Condition × Groups Timing Interaction[a]

Time	Glutamine	Leech Therapy	Leech Therapy + Glutamine	Control	P Value
Lactate (mmol/L)					0.00
Pre-exam	1.95 ± 0.2	1.90 ± 0.2	1.84 ± 0.2	1.97 ± 0.2	
Instantly	16.80 ± 1.4	19.66 ± 1.2	16.45 ± 1.06	19.77 ± 1.2	
30 minutes	10.58 ± 0.7	11.33 ± 0.4	6.96 ± 0.8	14.98 ± 0.7	
1 hour	6.43 ± 0.61	6.98 ± 0.7	2.06 ± 0.70	11.26 ± 0.5	
Pain					0.00
Pre-exam	0 ± 0	0 ± 0	0 ± 0	0 ± 0	
Instantly	7 ± 0.9	8 ± 1.1	7 ± 1.3	9 ± 0.7	
30 minutes	4 ± 0.7	4.5 ± 1.1	2.62 ± 0.9	7 ± 0.7	
1 hour	2 ± 0.5	2 ± 1.0	0.87 ± 0.8	5.25 ± 1.03	

[a]Values are expressed as mean ± SD.

Table 3. Tests of Between-Subjects Effects

Source, Measure	df	Mean Square	F	P Value
Intercept				
Pain	1	1785.031	2009.281	0.000
Lactate	1	11398.801	14956.960	0.000
Group				
Pain	3	42.198	47.499	0.000
Lactate	3	148.162	194.411	0.000

density are less than the density of the blood (10). Owing to the buffer role of glutamine and its effect on lactic acid, the fatigue caused by the inhibition of the product can be prevented and the activity time can be increased. This could be probably due to an increased hepatic glutamine uptake for gluconeogenesis and staged protein synthesis or renal elevation of buffering acidosis. Moreover, after exhaustive exercises, oral or intravenous glutamine increases glycogen sources of skeletal muscles (20).

5.2. Changes in Pain Index in the Glutamine Group

The results of this study indicate that the use of glutamine at all times reduced pain more than the control group, which had the greatest effect on reducing the amount of the pain half an hour after the exercise. Many researchers have suggested that the beginning of muscle damage and the subsequent pain and stiffness following unconventional exercises may be due to the effects of free radicals. In fact, extrovert contractions are an unconventional type of muscle training that cause muscle damage (21). One of the results of extrovert training is an increase in the number of neutrophils. The number of neutrophils in the bloodstream is said to be several times greater after soreness and muscle damage (21).

Glutamine plays an important role in increasing host defense. In other words, it reduces the period of inflammation and fiber necrosis (22). Cruzat et al. in a study on mice showed that glutamine supplementation reduces inflammatory responses from long-term exercise (19). This result is consistent with our findings. However, considering that the protocol used was different and the prostaglandins were not evaluated in this study, the measurement of the pain and muscle soreness in this study was estimated only by NRS. The probability of involvement of different mechanisms in subjects' responses to the pain is very high. Since there is no similar study on this protocol, it is not possible to compare the consistency or inconsistency of the results of this study with other findings, and this is the first study on glutamine supplementation in this field.

5.3. Changes in Lactate in the Leech Therapy Group

Because this is the first research in the field of leech therapy in sports, it is not possible to compare the results, but it can be explained that when performing exercise activities, glucose is used for the metabolism of muscle cells and turns into pyruvate. When exercise is more intense, enough oxygen is not available to convert all pyruvate, and as a result, a part of pyruvate is converted to acid lactic. Excessive production of this compound in the body causes fatigue and muscle pain (23). Possible mechanisms for leech therapy associated with lowering the level of blood lactate is that the leech drains deoxygenated blood, and on the other hand, it injects its saliva, which contains an anticoagulant (Hirudin) and more than 100 other substances. Hirudin dilutes the blood, opens clogged vessels, and increases blood circulation and oxygenation. Owing to the fact that leech performs blood purification and dilution simultaneously, it can increase tissue oxygenation (6). One of the salivary enzymes is Eglin, which has anti-oxidant and anti-inflammatory properties (12). Since antioxidants reduce fatigue (24), it may be possible to associate with a decreased level of blood lactate to the function of Eglin enzyme.

5.4. Changes in the Pain Index in the Leech Therapy Group

The results of this study showed that the effect of leech therapy reduced the amount of pain compared to the control group. Many researchers have suggested that initiating muscle damage and the pain and stiffness following unconventional exercises may result in increased free radicals (25). Furthermore, it has been proposed that the number of neutrophils in the bloodstream is several times greater after the onset of muscular soreness and destruction. This action increases the oxidation of cell membrane fat and ultimately leads to the breakdown of muscle proteins (25). In empirical studies, a number of chemical compounds in the leech saliva have shown analgesic (12) and anti-inflammatory properties (6). Recent studies on Hirudin and thrombin inhibitors, in addition to their known anticoagulant effects, have highlighted the direct anti-inflammatory effects of these agents. An Empirical study at the University of Lausanne has attracted considerable attention (26). In this study, researchers first developed an antigen-induced joint inflammation in laboratory animals and then treated animals with subcutaneous doses of recombinant PEG-Hirudin for 13 days. Within 7 days, an obvious decrease in inflammation and a histologic reduction in the thickness of the synovial tissue was detected by the scintigraphic method. With these findings, it comes to the mind that the inhibitory effect of Hirudin not only affects the thrombin system but also it affects inflammation at the cellular level. In another study, they showed that Hirudin inhibited some proinflammatory cytokines in the synovial fluid (27).

Also, Eglin enzymes, which are secreted from leech saliva, inhibit alpha-chymotrypsin, subtilisin, neutrophil protease elastase, and capsaicin G. Similarly, Eglin inhibits neutrophil activity and inhibits inflammation (12). The free radical increment is one of the causative agents of exercise-related pain. Today, the effects of leech therapy in the treatment of the pain and inflammation of painful syndromes due to the spinal cord, Tennis elbow syndrome (28), rheumatoid arthritis (4), regional effects, and sensitivity reduction in painful stimulants have been proven (29). However, still no area for a leech bite to create such mechanisms has been identified and it is difficult to be determined in empirical models (12). Considering the fact that the protocol was different and the prostaglandins were not evaluated in this study, the measurement of the pain and muscle soreness in this study was estimated only by NRS. The probability of the involvement of different mechanisms in the subjects' response to pain is very high. Since no studies have been conducted on exercise and leech therapy, it is not possible to compare our results, and this is the first research in the field of leech therapy in sports.

5.5. Conclusion

In total, glutamine supplementation has been shown to reduce pain and lactate levels in subjects during recovery. Leech therapy also reduced the levels of lactate and pain in the subjects during recovery. Regarding the physiological effects of glutamine reported in previous studies as well as the results of this study, probably, it can be argued that since leeches drain deoxygenated blood and increase oxygenation, the combination of leech therapy and glutamine supplementation is effective. However, further controlled trials are warranted to prove the effects of leech therapy and its combination with glutamine in sports.

Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

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Footnotes

Authors' Contribution: Study design, full article review, supervision over all matters, and data analysis: Abbas Sadeghi; preliminary studies, execution of exercise protocol, data collection, and manuscript preparation: Hamed Ghiyami.

Conflict of Interests: The authors declare that they have no competing interests.

Ethical Approval: The present study was approved by the Ethics Committee of Imam Khomeini International University (code: 17682).

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Patient Consent: The study was performed under the supervision of professors in the Imam Khomeini International University's sports physiology laboratory and with complete consent of the subjects. The objectives of the study and the procedures were explained to all of the participants and then written informed consents were obtained. The subjects were aware of all aspects of the research and could withdraw the study at any time.

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