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Investigation of the Acute Effect of Beetroot Supplement on Certain Physiological Indices in National Mountaineering Athletes

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Abstract:

Research has shown that beetroot supplementation improves performance in endurance sports. However, the effect of beetroot juice supplementation on the performance of mountaineers is still unclear. This study aimed to investigate a single acute dose of 70 milliliters of beetroot supplement rich in 3NO (containing 400 milligrams of 3NO) on a Bruce treadmill test compared to a placebo. Twelve trained male mountaineers aged 25 ± 6 years were divided into two groups (supplement and placebo) using a crossover, double-blind design. One hundred and fifty minutes after consuming the beetroot supplement and placebo, the number of Bruce test stages was recorded along with NIRS equipment. Independent t-tests were used for data analysis. Statistical analysis indicated that lactate dehydrogenase levels were significant at less than 0.05, while ANCOVA results showed that a single session of beetroot consumption had a significant impact on the athletes' VO2 max (P=0.09). Additionally, total hemoglobin (THb) levels in the hamstring and quadriceps muscles were significant (P=0.001), as well as oxygen levels in these two muscles (P=0.003 and P=0.001, respectively). Overall, it appears that the consumption of beetroot juice can improve the functional performance of hamstring and quadriceps muscles in mountaineers. However, further research is needed for definitive conclusions.

Objectives: This research aims to investigate the acute effect of beetroot supplementation on certain physiological indices in Iranian national mountaineering athletes.

Findings: The results indicated no significant differences in age, height, and body mass index between the two groups (P>0.05), suggesting that the groups were homogeneous. Lactate dehydrogenase data also showed significance at less than 0.05, while ANCOVA results indicated that a single session of beetroot consumption significantly affected the athletes' VO2 max (P=0.09). Furthermore, total hemoglobin (THb) levels in the hamstring and quadriceps muscles were significant (P=0.001), as were oxygen levels in these muscles (P=0.003 and P=0.001).

Conclusion: The findings of this study demonstrate the positive effect of beetroot supplementation on the physiological performance of national mountaineering athletes, suggesting that this supplement may help improve the performance of mountaineers.

Keywords: Beetroot supplement, SMO2, lactate dehydrogenase, total hemoglobin, muscle oxygen

Introduction:

High-altitude mountaineering and hypoxic conditions impose unique stress and pressure on the human body, making this distinctive environment an excellent research setting for examining the physiological limitations of humans. High-altitude climbing generally requires a very high level of respiratory fitness;

professional climbers often pause for breath recovery after every two steps during ascent. The primary cause of this significant decrease in exercise capacity is hypoxemia due to the reduced partial pressure of oxygen in inhaled air at high altitudes. This consequently decreases the oxygen delivered to active muscles during activity, leading to increased pulmonary ventilation even at rest. This increased ventilation functions similarly to hyperventilation at sea level, resulting in a reduced carbon dioxide level. Carbon dioxide follows a pressure gradient; thus, more carbon dioxide is expelled from the blood into the lungs and exhaled. Such a decrease in carbon dioxide causes an increase in blood pH, a condition known as respiratory alkalosis (1). Although the proportion of oxygen in the atmosphere remains approximately 21% of total barometric pressure with increasing altitude, the partial pressure of oxygen significantly drops. This lower overall pressure means fewer oxygen molecules are present in each liter of inhaled air (2). Respiratory alkalosis increases the affinity of hemoglobin for oxygen, thus enhancing oxygen loading in pulmonary capillaries under limited diffusion conditions. An alkaline pH shifts the hemoglobin dissociation curve to the left, facilitating oxygen loading in the lungs but reducing oxygen release at the tissue level (3). The result is hypoxia, which can lead to symptoms such as headaches, nausea, lethargy, insomnia, mood changes, altered breathing patterns, seizures, and even coma indicating acute mountain sickness. In addition, subtle neurophysiological disturbances such as impaired comprehension, judgment, and vision, decreased learning ability, increased fatigue, weakness, or drowsiness may begin to occur at altitudes between 1500 and 2500 meters (2).

The physiology of physical activity is associated with the body's physiological adaptations to short-term and long-term pressures from exercise and physical activity. Research has reported significant effects of transient bodily responses to exercise and

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physiological adaptations resulting from sustained aerobic training (4). Acute physical activity increases oxidative stress and muscle damage, particularly when exercise intensity is high. Oxidative stress in muscle and adipose tissue may lead to oxidative damage, resulting in reduced immune system function, increased fatigue, and decreased athletic performance (6). The determinants of human tolerance to exercise pressure remain unclear. However, depending on the intensity, duration, and extent of training, it may relate to central and peripheral factors and the balance between oxidative and non-oxidative energy contributions (7). Prolonged endurance activities with moderate intensity can increase the production of reactive species and free radicals, leading to oxidative stress. Oxidative stress reflects an imbalance between reactive species and the body's antioxidant system, favoring reactive species and free radicals. Increasing the intensity and duration of physical training, particularly endurance exercises, raises oxygen consumption, oxidative stress, and inadequacy of the antioxidant defense system. This results in the production of free radicals in skeletal muscles and body tissues. For this reason, many elite athletes and even ordinary individuals turn to antioxidant supplements to prevent the negative effects of physical activity, oxidative stress, and muscle damage (8). Beetroot juice is rich in nitrates and antioxidant compounds, which can enhance skeletal muscle function by regulating blood flow, contractility, glucose and calcium homeostasis, respiration, and mitochondrial biogenesis. The nitrate in beetroot is converted into nitric oxide (NO) in the body. Nitric oxide dilates blood vessels, increasing blood and oxygen delivery to active muscles, thereby reducing oxygen cost and fatigue and improving endurance performance. In cases of prolonged and intense exercise, when inflammatory and oxidative factors increase, antioxidant factors such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and malondialdehyde (MDA) are activated. GPX protects the organism from oxidative damage by converting hydrogen peroxide to water. The enzyme CAT, as a hydrogen donor, catalyzes the reaction of converting hydrogen peroxide into water and oxygen. SOD enzymes catalytically convert superoxide radicals into oxygen and hydrogen peroxide. MDA, formed through the breakdown of primary and secondary lipid peroxidation products, is a reliable marker for determining oxidative stress clinical conditions due to its high toxicity and reactivity (9). Nitric oxide (NO) has garnered attention from sports nutrition experts due to its ability to stimulate vascular dilation. Previously, scientists believed NO was only generated through the oxidation of L-arginine, catalyzed by a family of enzymes known as NO synthases (NOS), referred to as the L-arginine-NOS-NO pathway. This NO production pathway is oxygen-dependent. However, in recent decades, a nitrate-nitrite-NO pathway has been discovered, which functions under hypoxic conditions as a complementary pathway to NOS (10). Furthermore, based on the findings of more recent research, the question arises: Does the improvement in athletic performance stem from the dosage of beetroot juice supplementation or the waiting time before exercise? Previous studies used doses of 70, 140, 250, 300, 340, 420, 490, and 500 milliliters, with some research indicating improved performance and others not (11). In any tissue undergoing aerobic metabolism, the adequate delivery of oxygen ($\dot{Q}o2$) to meet metabolic demands ($\dot{V}o2$) is vital for the tissue's long-term survival (12). To determine tissue oxygenation, invasive or expensive techniques are usually required (such as MRI, MRS, PET, or arterial-venous catheterization) (13). However, these methods are often problematic and inaccessible, depending on the tissue and organ under study. Therefore, a non-invasive method to determine relative or absolute tissue oxygenation would be highly valuable and practical, particularly in protocols requiring repeated measurements or for patients with limited testing opportunities. Unlike visible light (~400-650 nm), where shorter wavelengths cannot penetrate tissues, light in the near-infrared region (~700-900 nm) can potentially penetrate several millimeters or more into body tissues. In this region, the primary absorptive chromophores in skeletal muscles include hemoglobin (Hb), myoglobin (Mb), and cytochrome oxidase (CYTOX). At longer wavelengths in this range, water and lipids within tissues also exhibit significant absorption, allowing light to pass through. Hemoglobin and myoglobin contain iron cores within their heme groups, whose absorption characteristics depend on whether oxygen is bound (12). Near-infrared spectroscopy (NIRS) devices monitor tissue oxygenation (O2) and muscle oxygen saturation (SmO2) and describe changes in tissue blood volume at the site of oxygen exchange through total hemoglobin and myoglobin mass measurements. Since the recovery of the phosphocreatine (PCR) pathway in muscle depends on ATP production via the aerobic (mitochondrial) system, a relationship between PCR phosphorylation and desaturation has been demonstrated in resting muscles (14). The toxic effects of free radicals (molecules with one or more unpaired electrons in their outer orbit) result in oxidative damage to cells. When free radical production, reactive oxygen species (ROS) generation, or the body's antioxidant capacity decreases, the likelihood of tissue damage increases, referred to as oxidative damage (15). The primary reason for this occurrence is increased oxygen consumption. While oxygen is essential for life, it can also pose risks to living organisms because physical activity can increase oxygen consumption in muscles by up to 100 times. Thus, free radical production rises post-exercise (16). This suggests a relationship between oxygen consumption during physical activity and oxidative damage (17). Beetroot juice is also considered a promising therapeutic agent for a range of clinical conditions associated with oxidative stress and inflammation. Its components, particularly betalain pigments, exhibit strong antioxidant and anti-inflammatory properties (18). Overall, evidence suggests the potential beneficial effects of nitrates on inflammatory markers, especially in individuals at risk with elevated markers such as CRP (19). One of the objectives of this study is to examine the effect of a single session of beetroot supplementation on total hemoglobin levels, muscle oxygenation in the hamstring and quadriceps, lactate dehydrogenase levels, and VO2max in national-level mountaineers. Thus, this research seeks to answer the question: Can acute beetroot supplementation significantly impact certain physiological markers in male members of Iran's national mountaineering team?

Research Methodology

This research is an applied and semi-experimental study that includes two experimental groups and two phases of pre-test and post-test. The statistical population of this study consists of men aged 18 to 31 who are members of the Iranian national mountaineering team with at least 5 years of continuous mountaineering experience in the years 2023-2024. Among the 15 individuals who expressed their readiness through a call for participation, 12 were selected based on entry criteria and randomly divided into two groups: one receiving supplements and the other a placebo (6 individuals in each group).

Research Protocol

After completing the consent form and questionnaire, participants were invited to the laboratory for an introductory session and two main sessions. They were provided with the same breakfast two hours before the test. In the first session, individual characteristics and body composition were measured using the IN BODY270 device, and oxygen consumption was assessed using a gas analyzer. In the second and third sessions, participants underwent the Bruce test, and data including VO2MAX, SMO2, THB, and serum levels were collected using LDH laboratory kits.

After completing the Bruce test, 10 cc of venous blood was drawn from participants, and samples were stored at -20 degrees Celsius. Data were analyzed using descriptive and inferential statistics. The Shapiro-Wilk test was used to assess the normality of data distribution, while paired t-tests and one-way ANOVA were employed to examine within-group and between-group variations. A significance level of $P \leq 0.05$ was considered.

Statistical Methodology: The statistical investigation involved 15 men from the national mountaineering team, out of which 12 were selected as participants following team training camps and a call for participation. After conducting an orientation session and filling out health forms and consent documents, participants were randomly assigned to either the supplement or placebo group. Following anthropometric measurements, participants consumed a standardized breakfast and then had 5 minutes for warm-up. Subsequently, NIRS sensors were placed on the muscles under study, and the Bruce test protocol was executed until exhaustion. At the end of the protocol, blood samples were taken to assess serum LDH levels. These steps were repeated on the second day, during which participants consumed either the supplement or placebo. Data analysis was performed using descriptive and inferential statistical methods. The Shapiro-Wilk test was used to check for normal distribution of data, and paired t-tests along with one-way ANOVA were utilized to examine intergroup variations. The data from this study were analyzed using SPSS version 16 with a significance level of (P>0.05).

Findings

Table 1. Demographic Characteristics of Research Participants (Mean ± Standard Deviation)

P	Placebo	Supplement	Group
0.68	27.33 ± 1.75	26.66 ± 3.55	**Age (years)**
0.28	180.50 ± 7.68	176.66 ± 3.14	**Height (m)**
0.74	77.35 ± 13.44	75.36 ± 4.86	**Weight (kg)**
0.71	23.63 ± 3.03	24.13 ± 0.96	**BMI (kg/m²)**
0.23	15.31 ± 2.97	13.40 ± 2.20	**Body Fat %**

Table 2. Descriptive Statistics of Test Variables in Two Groups

Placebo		**Supplement**		**Group / Variable**
Post-Test	Pre-Test	Post-Test	Pre-Test	
64.95 ± 13.01	62.96±12.44	76.68±8.57	64.90±4.09	**VO2max (ml/kg/min)**
483.00 ±31.50	433.83±9.76	482.50±13.99	471.16±0.53	**Lactate Dehydrogenase (LDH)**
12.30±0.22	12.40±0.26	13.42±0.65	12.47±0.53	**Total Hemoglobin in Quadriceps Muscle (g/dL)**
12.23±0.50	12.20±0.33	13.05±0.46	12.32±0.52	**Total Hemoglobin in Hamstring Muscle (g/dL)**
54.11±5.91	54.16±5.93	57.05±10.23	51.76±11.71	**Oxygen Consumption in Quadriceps Muscle**
49.68±14.79	44.33±11.09	55.05±16.20	51.15±17.16	**Oxygen Consumption in Hamstring Muscle**

Table 3. Results of the Kolmogorov-Smirnov Test for Assessing the Normal Distribution of Data Related to Research Variables

P (Post-Test)	P (Pre-Test)	Variable

0.20	0.20	**VO2max (mL/kg/min)**
0.20	*0.001	**Lactate Dehydrogenase**
0.20	0.20	**Hemoglobin Concentration (Quadriceps)**
0.20	0.20	**Hemoglobin Concentration (Hamstrings)**
0.20	0.20	**Oxygen Saturation (Quadriceps)**
0.20	0.17	**Oxygen Saturation (Hamstrings)**

Table 4. Results of ANOVA for Comparison of Post-Test Performance Between the Two Groups (n=12)

Effect Size (η^2)[1]	P	df	F	Mean ± SD (Post-Test)	Mean ± SD (Pre-Test)	Group	Variable
0.28	0.09	1	3.58	76.68 ± 8.57	64.90 ± 4.09	Suppleme nt	**VO2max**
				64.95 ± 13.01	62.96 ± 12.44	Placebo	

1. Effect size interpretation: (0 to 0.19) trivial, (0.20 to 0.59) small, (0.60 to 1.10) moderate, (1.11 to 1.19) large, (1.20 to 3.90) very large, and (greater than 3.90) extremely large.

Chart 1 compares VO2max between the supplement and placebo groups in national mountaineering athletes.

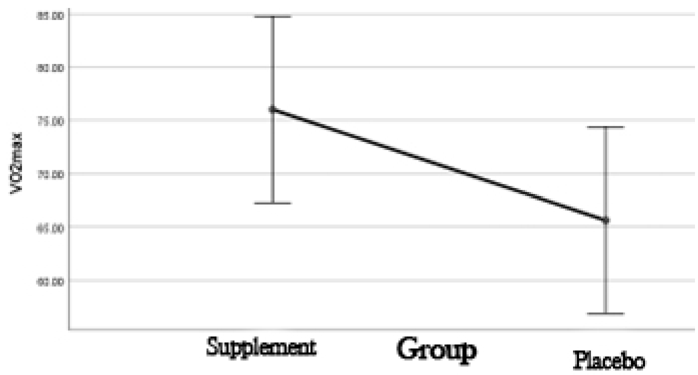


Chart 1. VO2max of the supplement and placebo groups in national mountaineering athletes.

Table 5. Results of the Kruskal-Wallis test for examining intergroup differences in the post-test for lactate dehydrogenase level (n=12).

P value	Z Statistic	Mann-Whitney U Statistic	Variable
0.37	-0.88	12.50	Lactate Dehydrogenase

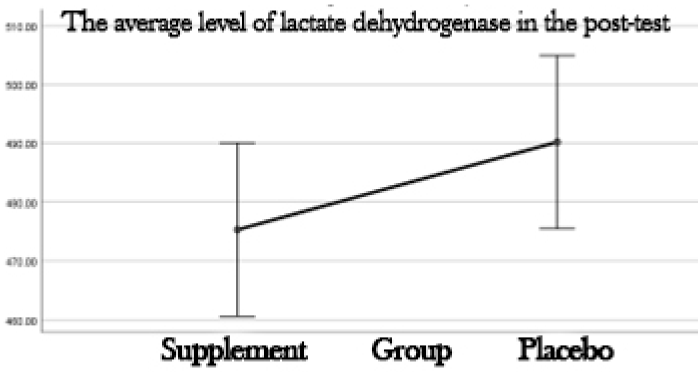
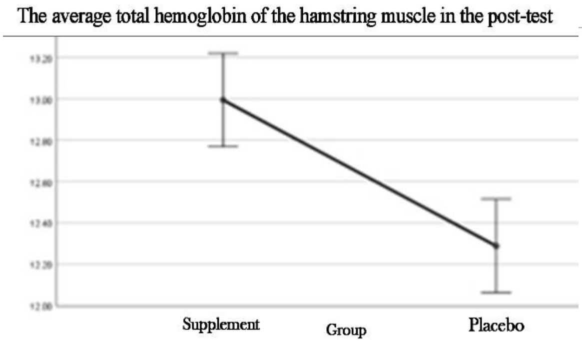


Chart 2. Lactate dehydrogenase levels in the supplement and placebo groups in national mountaineering athletes.

Effect Size	P	df	F	Mean (Post-test)	Mean (Pre-test)	Group	Variable
0.75[2]	*0.001	1	27.08	13.24 ± 0.65	12.47 ± 0.53	Supplement	**Total Hemoglobin Level of Quadriceps**
				12.30 ± 0.22	12.40 ± 0.26	Placebo	
0.73	*0.001	1	24.75	13.05 ± 0.47	12.32 ± 0.52	Supplement	**Total Hemoglobin Level of Hamstring**
				12.23 ± 0.50	12.20 ± 0.33	Placebo	

Table 6. ANCOVA test for comparing quadriceps and hamstring muscle oxygen levels between the two groups in the post-test (n=12).

2. Effect size interpretation: (0 to 0.19) trivial, (0.20 to 0.59) small, (0.60 to 1.10) moderate, (1.11 to 1.19) large, (1.20 to 3.90) very large, and (greater than 3.90) extremely large.



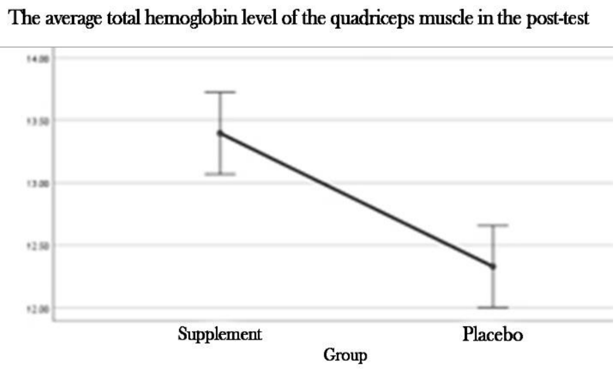
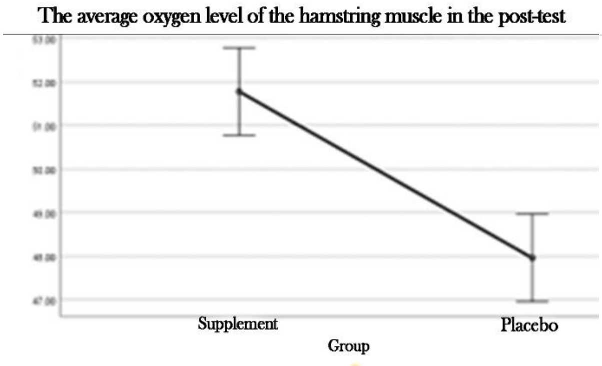


Chart 3. Total hemoglobin levels in the quadriceps muscle (right) and Chart 4. Total hemoglobin levels in the hamstring muscle of the supplement and placebo groups in national mountaineering athletes.

Effect Size	P	df	F	Mean (Post-test)	Mean (Pre-test)	Group	Variable
0.65	*0.003	1	17.00	57.05 ± 10.23	51.76 ± 11.71	Supplement	**Oxygen Level of Quadriceps Muscle**
				54.11 ± 5.91	54.16 ± 5.93	Placebo	
0.79	*0.001	1	35.75	55.05 ± 16.20	51.15 ± 17.16	Supplement	**Oxygen Level of Hamstring Muscle**
				44.68 ± 11.18	44.33 ± 11.09	Placebo	



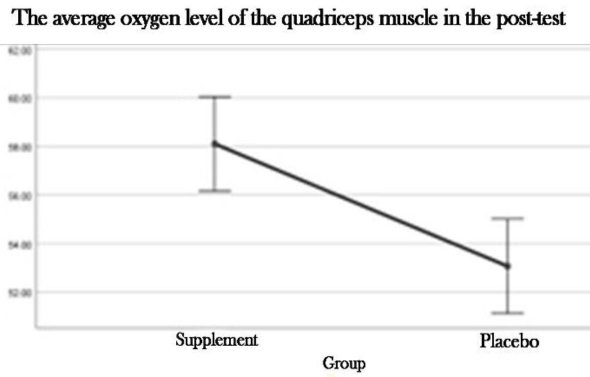


Chart 5. Oxygen levels in the quadriceps muscle (right) and Chart 6. Oxygen levels in the hamstring muscle of the supplement and placebo groups in national mountaineering athletes (left).

Discussion and Conclusion

In this topic, the results of previous consistent and inconsistent studies can be summarized as follows:

1. Rad et al. (2021): This study examined the short-term effects of caffeine and beetroot juice supplementation on antioxidant and functional factors in ten male runners. The results showed that beetroot juice and caffeine consumption significantly affected serum levels of antioxidant enzymes (GPX, CAT, SOD), while no significant difference was observed in MDA levels. (24)

2. Hassanpour et al. (2022): This research investigated the acute effect of beetroot juice consumption on anaerobic performance in trained individuals. The results indicated that beetroot juice had no significant impact on athletes' performance in anaerobic tests. (25)

3. Hashemi et al. (2022): This study examined the acute effects of red beetroot juice on aerobic and anaerobic power in non-professional female karate athletes. The results demonstrated a significant reduction in peak power and fatigue index, but no significant difference was found in the KAST aerobic test. (26)

4. Souri et al. (2021): This research evaluated the effectiveness of acute beetroot juice supplementation on the performance of taekwondo athletes in a simulated match. The findings showed that the number of successful strikes was higher in the supplement group, but no significant difference was observed in the Borg scale between the two groups. (27)

5. Saadatlou et al. (2022): This study assessed the short-term effects of two different doses of caffeine-beetroot supplementation on plasma TNF-α, GDF-15, and SELECTIN-E levels in runners. The results indicated that these supplements significantly reduced TNF-α and increased GDF-15. (28)

6. Moghaddasi et al. (2023): This study examined the interactive effects of CrossFit training and beetroot juice supplementation on athletic performance. The results showed that beetroot juice supplementation alongside CrossFit training did not enhance athletic performance. (29)

7. Hemmati-Nejad et al. (2021): This study revealed that a six-day beetroot juice supplementation regimen could improve aerobic and anaerobic performance and reduce fatigue levels. (30)

In International Studies:

1. Kent et al. (2019): This study found that beetroot juice supplementation might not improve repeated sprint performance in hypoxic conditions but could reduce VO2 during submaximal exercise. (31)

2. Reynolds et al. (2020): The findings of this study indicated that acute beetroot juice consumption had no significant effect on short-term repeated running performance. (32)

3. Arnold et al. (2015): This research showed that beetroot juice did not enhance running performance at altitude in professional athletes. (21)

4. Jonvick et al. (2018): This study revealed that beetroot juice supplementation did not improve intermittent performance in elite female water polo players. (33)

5. Garnacho et al. (2022): This study reported that beetroot juice increased the number of repetitions in resistance training and elevated plasma NOx levels. (34)

Given the physiological and anatomical nature of mountaineering, which relies heavily on the aerobic and oxygen-based energy system, professional mountaineers frequently pause every two steps during ascents to recover their breathing. The primary cause of the drastic reduction in exercise and activity capacity is hypoxemia caused by the partial pressure reduction of oxygen in the inhaled air at altitude. This reduces the oxygen delivered to active muscles during activity, leading to increased pulmonary ventilation even at rest. This heightened ventilation closely resembles hyperventilation at sea level, where carbon dioxide levels are significantly reduced. Carbon dioxide follows the pressure gradient; therefore, more carbon dioxide leaves the blood, enters the lungs, and is expelled through exhalation. Such a reduction in carbon dioxide leads to an increase in blood pH, a condition known as respiratory alkalosis (1). The role of oxygen in the cardiovascular and muscular systems of athletes is critical. Thus, supplements that can effectively enhance oxygen delivery to tissues can undoubtedly impact the performance of mountaineers. The contraction process itself temporarily reduces blood flow to the muscles, as the contracting muscle compresses the blood vessels within it. Consequently, strong isotonic contractions can cause rapid muscle fatigue during sustained contractions due to a lack of oxygen and nutrients in the muscle. During activity, blood flow to the muscles significantly increases, a result of the direct effects of increased muscle metabolism. The remainder of this increase is due to several factors, the most significant of which is likely a moderate rise in arterial blood pressure, typically around 30%. This rise in pressure not only propels more blood through the vessels but also stretches the walls of the arterioles, further reducing vascular resistance. As a result, a 30% increase in arterial blood pressure can often more than double blood flow. This effect amplifies the sharp increase in blood flow that had already occurred due to metabolic vasodilation, effectively doubling it (5, 20). Long-duration endurance activities of moderate intensity can increase the production of reactive oxygen species and free radicals, leading to oxidative stress (8). Beetroot juice, rich in nitrates and antioxidants, can alter skeletal muscle performance by regulating blood flow, contractility, glucose and calcium homeostasis, respiration, and mitochondrial biogenesis (9). Previously, scientists believed that nitric oxide (NO) was produced solely through the oxidation of L-arginine, catalyzed by a family of nitric oxide synthase (NOS) enzymes a pathway known as the NO-NOS-arginine-L pathway. This pathway is oxygen-dependent. However, in recent decades, a nitrate-nitrite-NO pathway has been discovered, which complements NOS under low oxygen conditions (10). In previous studies, doses of beetroot juice ranged from 70, 140, 250, 300, 340, 420, 490, to 500 milliliters. In some studies, it improved performance, while in others, it did not (11). A non-invasive method for determining relative or absolute tissue oxygenation is highly valuable and practical. Unlike visible light (~400–650 nm), where shorter wavelengths cannot penetrate tissues, light in the near-infrared region (~700–900 nm) can potentially penetrate several millimeters or more into body tissues. In skeletal muscles, the primary absorbing chromophores are hemoglobin (Hb), myoglobin (Mb), and cytochrome oxidase (CYTOX). In the longer wavelengths of this region, water and lipids beneath the tissues also exhibit significant absorption, but the light still passes through. Both hemoglobin and myoglobin contain an iron core that determines oxygen binding (12). Near-infrared spectroscopy (NIRS) devices measure O_2 and sO_2 and describe changes in tissue blood volume at the site of oxygen exchange through total hemoglobin and myoglobin mass. Since the recovery of the PCR pathway in muscles depends on ATP production through the aerobic (mitochondrial) system, studies have shown a relationship between PCR phosphorylation and oxygen saturation in resting muscles (14).

VO_2 max

In the present study, no significant differences were observed in oxygen consumption changes between the supplement and placebo groups, consistent with the findings of study (21).

LDH

In this study, changes in LDH levels between the two groups were not significantly different. However, in contrast to these findings, previous research has reported that beetroot juice can significantly affect serum LDH levels (22, 23).

SMO_2

This study reported significant differences in SMO_2 levels in the hamstring and quadriceps muscles between the supplement and placebo groups.

THb

Significant differences in THb levels were also observed in the hamstring and quadriceps muscles between the supplement and placebo groups in this study.

Today, with the advancement of sports and nutritional sciences, the importance of these subjects is highly regarded by researchers, athletes, and coaches. Based on the results of the present study, beetroot juice supplementation can have a significant effect on SMO_2 and THb, thereby improving athlete performance and delaying fatigue. It can be concluded that beetroot supplementation can serve as an acute supplement before mountaineering programs to enhance performance.

Conclusions and Recommendations

Given the expansion of sports and nutritional sciences and their growing importance among researchers, athletes, and coaches, this topic has gained significant attention. The findings of the present study indicate that beetroot supplementation can significantly impact SMO_2 and THb, thereby enhancing athlete performance and delaying fatigue. Thus, beetroot supplementation can be recommended as an acute supplement before mountaineering programs to improve performance.

Considering the significant effect of beetroot supplementation on muscle oxygen levels and total hemoglobin in national mountaineering athletes, it is recommended that athletes consume beetroot supplements before starting their mountaineering programs. This is likely to play a critical role in delaying athlete fatigue due to the observed changes in muscle oxygen consumption and total hemoglobin levels.

This study aimed to examine the acute effects of 70 milliliters of red beetroot juice (containing 400 mg of NO_3) compared to placebo on mountaineers' performance during the Bruce test. Contrary to our hypotheses, beetroot supplementation did not lead to increased VO_2 max or a reduction in LDH levels in the beetroot group compared to the placebo group. However, it significantly improved SMO_2 levels in the hamstring and quadriceps muscles of mountaineers and enhanced THb levels in these muscles compared to the placebo group.

One limitation of this study was the lack of measurement of blood nitrate and its metabolites following beetroot supplementation. Ultimately, the acute effects of beetroot supplementation on mountaineer performance during a Bruce test were observed. While the 70 milliliters of beetroot supplement did not positively impact LDH levels or VO_2 max, it led to increased THb and SMO_2 in the hamstring and quadriceps muscles during the Bruce test.

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