Clinical Trial Protocol
Iranian Registry of Clinical Trials

11 Sep 2026

Effects of arginine supplementation along with spirulina and HIIT training on athletic performance, inflammatory indices, and oxidative stress in female water polo players.

Protocol summary

Study aim

Determining the effect of L-arginine supplementation with spirulina and HIIT training on athletic performance, inflammatory markers, and oxidative stress in female water polo players

Design

A randomized parallel study with a simple randomization method on 27 people.

Settings and conduct

In this semi-experimental study, 27 subjects were divided into three groups. Before starting the training protocols and consuming the sports drink, a 5 cc blood sample was taken from each player, and the same conditions were repeated for the next 8 weeks.

Participants/Inclusion and exclusion criteria

Participants must be selected from female athletes in the youth age group (16-18 years). At least 2 years of professional and competitive water polo experience and membership in teams in the first division of the country. Participants must be members of the national champion team in the under-20 age group in the year of the study. No musculoskeletal injuries (such as muscle tears or shoulder or knee injuries) that would prevent them from performing heavy training or physical tests. No use of any anti-inflammatory drugs (steroidal or non-steroidal), antibiotics, orthogenic supplements (such as creatine or beta-alanine), or synthetic antioxidants during the past 3 months until the end of the study period. Commitment to attend training sessions according to the team program. Completion of the informed consent form by the participant (or legal guardian if under 18 years of age) after receiving a full explanation of the objectives and procedure of the study.

Intervention groups

Participants were divided into three groups: L-arginine + spirulina supplementation, HIIT training, and L-arginine + spirulina supplementation + HIIT training.

Main outcome variables

Plasma creatine kinase concentration, plasma lactate dehydrogenase concentration, IL1b, Nfkb gene expression levels

General information

Reason for update

Acronym

IRCT registration information

IRCT registration number: **IRCT20250517065774N3**
Registration date: **2026-08-13, 1405/05/22**
Registration timing: **prospective**

Last update: **2026-08-13, 1405/05/22**
Update count: **0**

Registration date

2026-08-13, 1405/05/22

Registrant information

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Navid Abedpoor

Name of organization / entity

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Iran (Islamic Republic of)

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Recruitment status

recruiting

Funding source

Expected recruitment start date

2026-08-22, 1405/05/31

Expected recruitment end date

2026-09-22, 1405/06/31

Actual recruitment start date

empty

Actual recruitment end date

empty

Trial completion date

empty

Scientific title

Effects of arginine supplementation along with spirulina and HIIT training on athletic performance, inflammatory indices, and oxidative stress in female water polo players.

Public title

Arginine supplementation along with spirulina and HIIT training affects athletic performance, inflammatory indices, and oxidative stress in female water polo players.

Purpose

Basic scienece

Inclusion/Exclusion criteria

Inclusion criteria:

Participants must be selected from among female athletes in the youth age group (16 to 18 years old). Having at least 2 years of professional and competitive experience in water polo and membership in teams in the country's first division league. Participants must be members of the national championship team in the under-20 age group in the year of the study. Not having any musculoskeletal injuries (such as muscle tears or shoulder or knee injuries) that would prevent you from performing strenuous exercise or physical tests. Absence of chronic metabolic (such as diabetes), cardiovascular, respiratory, or inflammatory diseases that would affect physiological responses to training and supplementation. No use of specific medications and supplements: No use of any anti-inflammatory drugs (steroidal or non-steroidal), antibiotics, orthogenic supplements (such as creatine or beta-alanine), or synthetic antioxidants during the past 3 months until the end of the study period. Commitment to attend training sessions according to the team schedule, such that the percentage of training attendance during the study period is at least 85% (based on the average attendance of 87 ± 11% mentioned in the main text). Completion of an informed consent form by the participant (or legal guardian if under 18 years of age) after receiving full explanations about the research objectives and process.

Exclusion criteria:

Absence of more than 15% of training sessions or failure to complete the 8-week training intervention period. Failure to comply with the supplementation protocol: Failure to regularly take L-arginine and spirulina supplements according to the study instructions (such as forgetting more than 20% of the prescribed doses). Any acute injury during the study that prevents the participant from continuing with training or testing, or acute illness (such as severe flu or viral infections) that requires rest or medication. Dietary or medication changes: Sudden and significant changes in diet, initiation of specific diets (such as ketogenic or vegan diets), or initiation of new medications that may interfere with study variables (such as inflammatory or hormonal markers). Unwillingness or inability to provide blood or saliva samples at designated times (pre-test, mid-test, or post-test). The willingness of the participant to leave the study at any point in time without having to provide a reason. Performing strenuous exercise outside of the team's training program or participating in informal competitions that affect training load and oxidative stress.

Age

From **16 years** old to **18 years** old

Gender

Female

Phase

1

Groups that have been masked

- Participant
- Care provider
- Investigator
- Data analyser

Sample size

Target sample size: **27**

Randomization (investigator's opinion)

Randomized

Randomization description

In order to use the random number table, the researcher will first determine the reading direction of the table numbers. Then the numbers will be considered for different groups. For example: numbers 14-00 for group A, numbers 15-29 for intervention B, numbers 30-44 for group C. Then we will place our hand on one of the numbers and move in one of the predetermined directions. The numbers will be recorded and assigned to three groups: L-arginine + spirulina supplement consumption, HIIT training, and L-arginine + spirulina supplement consumption + HIIT training. In order to conceal the random assignment, the coded box method with a random sequence will be used. In this method, a number of boxes that are numbered based on a random sequence are used. It will be ensured that the boxes are adequately sealed and waxed, that they are similar in appearance, and that they are of the same weight.

Blinding (investigator's opinion)

Double blinded

Blinding description

In this study, the analyst, statistician, assistant professor, trainer, and exercise physiologist were blinded to the entire study process. The blinding process included concealment of allocation through sealed envelopes and supplement intake, standardized exercise protocols, and outcome assessment by an independent, blinded team with automated digital devices, along with a controlled disclosure mechanism designed for emergencies. To blind the intake of supplements, all participants were given dark bottles during the interventions so that the color of the drink was not visible.

Placebo

Used

Assignment

Parallel

Other design features

Secondary Ids

empty

Ethics committees

1

Ethics committee

Name of ethics committee
Ethics Committee on Research Islamic Azad University - Isfahan Branch (Khorasgan)

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اصفهان، خیابان جی شرقی، ارغوانیه، بلوار دانشگاه ، اصفهان

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Approval date
2026-07-28, 1405/05/06

Ethics committee reference number
IR.IAU.KHUISF.REC.1405.223

Health conditions studied

1

Description of health condition studied
Sports performance, inflammatory markers, and oxidative stress in female water polo players.

ICD-10 code

ICD-10 code description

Primary outcomes

1

Description
Repeated sprint ability test

Timepoint
At the beginning and end of 8 weeks of study

Method of measurement
To assess specialized fitness, the protocol was performed according to Tan et al. (2010). This protocol consisted of 4 sets of 6 10-meter swim sprints with 17 seconds of rest between sprints and 4 minutes of rest between sets. The timing of the sprints was recorded and analyzed with high precision using a digital camera and Kinovea software to calculate the best time, average time, and absolute and percentage reduction.

2

Description
Expression levels of inflammatory genes and oxidative stress

Timepoint
At the beginning and end of 8 weeks of study

Method of measurement
Real-time qPCR

3

Description
Functional and physiological assessments

Timepoint
At the beginning and end of 8 weeks of study

Method of measurement
Performance tests are conducted in a 50-meter indoor pool with controlled temperature (29.5 ± 1.3°C). Before the test, participants perform a standard warm-up (10 minutes of low-to-moderate intensity swimming and short high-intensity stimulations) under the supervision of technical staff.

4

Description
Plasma glutathione, plasma superoxide dismutase, and plasma catalase concentrations.

Timepoint
At the beginning and end of 8 weeks of study

Method of measurement
Elisa Kit

Secondary outcomes

1

Description
Heart Rate Variability - HRV

Timepoint
At the beginning and end of 8 weeks of study

Method of measurement
In order to monitor the state of the autonomic nervous system and assess the level of recovery and physiological fitness of the athletes, the heart rate variability (HRV) index was measured during the study period. According to the research protocol, HRV assessments were performed three times a week and before any physical activity during the assessment weeks (the time interval between measurements was set between 24 and 48 hours).

Intervention groups

1

Description
Intervention group: Supplement (L-arginine + Spirulina): L-arginine, as a precursor of nitric oxide (NO) synthesis, plays a key role in vasodilation (widening of blood vessels) and oxygen transport. Based on standard protocols of similar studies on athletes and to achieve optimal physiological effects, the daily dose of L-arginine is considered to be 6 grams per day. This amount is usually divided into two doses of 3 grams to avoid possible digestive problems. Participants should dissolve L-arginine powder or capsules with 300 ml of water and consume. The first dose (3 grams) is consumed 60 minutes before the training session (to increase blood flow and nutrient transport during training) and the second dose (3 grams) is consumed at breakfast or

before bedtime (to improve tissue repair and recovery). On non-training days, both doses are consumed with the morning and evening meals. Spirulina, a red algae rich in protein, phycocyanin, and potent antioxidants, is prescribed to modulate oxidative stress and inflammatory responses induced by high-intensity exercise. The daily dose of spirulina is considered to be 3 grams per day. This dose has been shown to be effective in several studies for its protective effects against muscle damage. Spirulina powder or standardized tablets should be taken with a full glass of water. Due to its absorption nature and antioxidant effects, it is recommended to take spirulina 30 minutes before breakfast. This timing allows for better absorption of phytonutrients and provides systemic antioxidants throughout the training day. To reduce bias, this study will be double-blinded, meaning that neither the participants nor the researchers responsible for conducting the tests will be aware of the contents of the capsules or powders (the main supplement or the placebo, which is usually maltodextrin). To ensure adherence to the protocol, participants receive a logbook to record when supplements are taken and any potential gastrointestinal side effects. Regular (weekly) meetings are held to retrieve used supplement packages and count the number of capsules remaining to assess athlete compliance with the treatment protocol.

Category

Other

2

Description

Intervention group: HIIT training: Participants will perform HIIT training in water three times a week for 8 weeks. The intensity of the training is adjusted based on maximum heart rate (HRmax) and monitored with a monitoring device. The training program is designed based on the physiological principles of water polo. The intervention training groups will perform HIIT training for 8 weeks, 6 days a week. Each training session will consist of two parts: land-based training and water-based training. The first part will consist of strength training (about 4 times a week) and the second part, which will be performed about 30 minutes later, will consist of swimming training (about 5 times a week) and specialized water polo training. The intensity of swimming training will be determined based on the Maglischo classification into four zones: below the anaerobic threshold (End-1), above the anaerobic threshold (End-2), above the anaerobic threshold (End-3), and maximum speed effort (Sprint). Internal training load is monitored in all sessions using the rated perceived exertion (sRPE) method.

Category

Other

3

Description

Intervention group: Combination group (L-arginine supplementation + spirulina + HIIT training)

Category

Other

Recruitment centers

1

Recruitment center

Name of recruitment center

استخر ساحل جی اصفهان

Full name of responsible person

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Sponsors / Funding sources

1

Sponsor

Name of organization / entity

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Full name of responsible person

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Grant name

Grant code / Reference number

Is the source of funding the same sponsor organization/entity?

Yes

Title of funding source

Islamic Azad University

Proportion provided by this source

100

Public or private sector

Public

Domestic or foreign origin

Domestic

Category of foreign source of funding

empty

Country of origin

Type of organization providing the funding
Academic

Person responsible for general inquiries

Contact
Name of organization / entity
Islamic Azad University
Full name of responsible person
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Position
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Sharing plan

Deidentified Individual Participant Data Set (IPD)
Undecided - It is not yet known if there will be a plan to make this available
Study Protocol
Undecided - It is not yet known if there will be a plan to make this available
Statistical Analysis Plan
Undecided - It is not yet known if there will be a plan to make this available
Informed Consent Form
Undecided - It is not yet known if there will be a plan to make this available
Clinical Study Report
Undecided - It is not yet known if there will be a plan to make this available
Analytic Code
Undecided - It is not yet known if there will be a plan to make this available
Data Dictionary
Undecided - It is not yet known if there will be a plan to make this available