Clinical Trial Protocol
Iranian Registry of Clinical Trials

23 Sep 2026

The combination effects of Beetroots Juice and Caffeine with High-Intensity Interval Training on cardiovascular risk factors in Middle-Aged Obese Men

Protocol summary

Study aim

This study aims to investigate the metabolic profile of middle-aged obese individuals through the incorporation of beetroot juice (BRJ), caffeine, and high-intensity interval training (HIIT).

Design

The clinical trial will have a control group, with parallel groups, double-blind, and a sample size of 80 with a random sequence using the randomizeR package in R software and will be divided into four groups.

Settings and conduct

All groups perform the HIIT program, which is conducted at the university gym of Halabja, Iraq, under the guidance of an exercise physiologist, utilizing a motorized treadmill. Participants are unaware of which group they are assigned to and whether they are receiving active supplements, placebos, or a combination.

Participants/Inclusion and exclusion criteria

The inclusion criteria comprise men with obesity (BMI ≥30 kg/m²), stable weight, a sedentary lifestyle, no history of cardiovascular or metabolic disorders, and no consumption of nutritional supplements.The exclusion criteria include chronic illnesses, tobacco use, recent nitrate supplementation, or regular caffeine consumption.

Intervention groups

Participants are categorized into four groups: (1) placebo, (2) beetroot juice, (3) caffeine, and (4) beetroot juice combined with caffeine.All groups engage in identical high-intensity interval training programs.

Main outcome variables

Anthropometric (body composition, VO_2 peak), blood analysis (IL-6, TNF-α, CRP, IL-10, NOx, NO, and eNOS, OxLDL, TOS, OSI, and TBARS, troponin I and T, CK-MB and CK-T, lipid profile, glucose, and insulin).

General information

Reason for update

Acronym

IRCT registration information

IRCT registration number: **IRCT20250531066002N1**
Registration date: **2025-09-28, 1404/07/06**
Registration timing: **prospective**

Last update: **2025-09-28, 1404/07/06**
Update count: **0**

Registration date

2025-09-28, 1404/07/06

Registrant information

Name

Dara Latif Saifalddin

Name of organization / entity

University of Halabja

Country

Iran (Islamic Republic of)

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Recruitment status

Recruitment complete

Funding source

Expected recruitment start date

2025-10-23, 1404/08/01

Expected recruitment end date

2025-10-23, 1404/08/01

Actual recruitment start date

empty

Actual recruitment end date

empty

Trial completion date

empty

Scientific title
The combination effects of Beetroots Juice and Caffeine with High-Intensity Interval Training on cardiovascular risk factors in Middle-Aged Obese Men

Public title
Effects of exercise training and dietary supplements (beetroot juice and caffeine) on cardiovascular risk factors in obese men

Purpose
Basic scienece

Inclusion/Exclusion criteria

Inclusion criteria:
Obese men (BMI ≥30 kg/m²) Stable weight (±2 kg) for three months A sedentary lifestyle (<60 minutes/week of structured exercise in the past 6 months) No history of cardiovascular or metabolic illnesses, No use of nutritional supplements

Exclusion criteria:
Chronic illnesses Tobacco use Recent nitrate supplementation Habitual caffeine intake above 200 mg per day

Age
From **30 years** old to **50 years** old

Gender
Male

Phase
0

Groups that have been masked

- Participant
- Care provider
- Outcome assessor
- Data analyser

Sample size
Target sample size: **72**

Randomization (investigator's opinion)
Randomized

Randomization description
A stratified randomized block design was implemented, considering peak oxygen consumption and body mass index, with two degrees of fitness and body fat %. Randomly interspersed permuted blocks of sizes 4 and 8 were utilized to reduce predictability. Randomization was conducted at the individual level, with each participant independently assigned to one of four groups. Randomization sequences were produced with the randomizeR program within the R software environment. Within each stratum, groups of 4 or 8 allocations were established. Strict confidentiality was kept through the utilization of sealed, opaque, serially numbered envelopes (SNOSE), which were arranged in a sequence tailored to each stratum. Every envelope included a folded allocation card. Following baseline assessments, recruitment personnel accessed the subsequent envelope in the strata relevant to the participant. The envelopes were stored in a secured cabinet accessible just to the trial coordinator.

Blinding (investigator's opinion)
Double blinded

Blinding description
Participant blinding: Subjects remain uninformed about their group assignment and whether they are getting active supplements, a placebo, or a combination of both. They are aware that the supplements are probably caffeine, beetroot juice, a combination of both, or a placebo. They are also unable to discern their supplement kind from flavor, look, or packaging. Moreover, practitioners engaged in direct engagement with participants (e.g., conducting training sessions, administering supplements, collecting performance data, evaluating results, and analyzing data) remain uninformed about the group type, therefore identifying groups solely by code (Group A, B, C, or D). To preserve blindness, beetroot juice placebos typically consist of nitrate-free beetroot juice or an inert beverage that resembles the color and flavor of beetroot juice. The caffeine and placebo are indistinguishable tablets, frequently opaque. All supplements were made by individuals not participating in the intervention. All participants, irrespective of group affiliation, got two items (a beverage and a pill) every day or prior to exercise.

Placebo
Used

Assignment
Parallel

Other design features

Secondary Ids

empty

Ethics committees

1

Ethics committee

Name of ethics committee
The ethical committee Ethical Committee for Human Studies at the University of Halabja

Street address
Research Center University of Halabja, Zanko Street

City
Halabja

Postal code
46018

Approval date
2026-09-24, 1405/07/02

Ethics committee reference number
09 2025/01

Health conditions studied

1

Description of health condition studied
Obesity

ICD-10 code
E66

ICD-10 code description
Overweight and obesity

Primary outcomes

1

Description
Interleukine-6
Timepoint
Before starting intervention, and 48 hours after the last training session.
Method of measurement
Elisa

2

Description
Interleukine-10
Timepoint
Before starting intervention, and 48 hours after the last training session.
Method of measurement
Elisa

3

Description
TNF-α
Timepoint
Before starting intervention, and 48 hours after the last training session.
Method of measurement
Elisa

4

Description
C-reactive protein
Timepoint
Before starting intervention, and 48 hours after the last training session.
Method of measurement
Elisa

5

Description
Leptin
Timepoint
Before starting intervention, and 48 hours after the last training session.
Method of measurement
Elisa

6

Description
Adiponectin
Timepoint
Before starting intervention, and 48 hours after the last training session.
Method of measurement
Elisa

7

Description
Nitric oxide
Timepoint
Before starting intervention, and 48 hours after the last training session.
Method of measurement
Elisa

8

Description
Endothelial nitric oxide synthase
Timepoint
Elisa
Method of measurement
Before starting intervention, and 48 hours after the last training session

9

Description
Oxidized low-density lipoprotein
Timepoint
Before starting intervention, and 48 hours after the last training session
Method of measurement
Elisa

10

Description
Plasma nitrate + nitrite (NOx) concentrations
Timepoint
Before starting intervention, and 48 hours after the last training session.
Method of measurement
Griess assay and immunoassay techniques

11

Description
Insulin
Timepoint
Before starting intervention, and 48 hours after the last training session.
Method of measurement
Elisa

12

Description
Total antioxidant status
Timepoint
Before starting intervention, and 48 hours after the last training session
Method of measurement
Using a microplate reader on serum samples with a commercial kit

13

Description

Total oxidant status (TOS)

Timepoint

Before starting intervention, and 48 hours after the last training session

Method of measurement

Using a microplate reader on serum samples with a commercial kit

14

Description

Plasma thiobarbituric acid-reactive substances

Timepoint

Before starting intervention, and 48 hours after the last training session

Method of measurement

Fluorescent thiobarbituric acid (TBA) assay.

15

Description

Total cholesterol

Timepoint

Before starting intervention, and 48 hours after the last training session

Method of measurement

Utilizing an auto-analyzer and suitable kits

16

Description

Triglycerides

Timepoint

Before starting intervention, and 48 hours after the last training session.

Method of measurement

Utilizing an auto-analyzer and suitable kits

17

Description

Low-density lipoprotein cholesterol

Timepoint

Before starting intervention, and 48 hours after the last training session.

Method of measurement

Utilizing an auto-analyzer and suitable kits

18

Description

High-density lipoprotein cholesterol

Timepoint

Before starting intervention, and 48 hours after the last training session.

Method of measurement

Utilizing an auto-analyzer and suitable kits

19

Description

Troponin I

Timepoint

Before starting intervention, and 48 hours after the last training session.

Method of measurement

Utilizing an auto-analyzer and suitable kits

20

Description

Troponin T

Timepoint

Before starting intervention, and 48 hours after the last training session.

Method of measurement

Utilizing an auto-analyzer and suitable kits

21

Description

Creatine kinase-MB

Timepoint

Before starting intervention, and 48 hours after the last training session.

Method of measurement

Utilizing an auto-analyzer and suitable kits

22

Description

Creatine kinase-T

Timepoint

Before starting intervention, and 48 hours after the last training session.

Method of measurement

Utilizing an auto-analyzer and suitable kits

23

Description

Glucose

Timepoint

Before starting intervention, and 48 hours after the last training session.

Method of measurement

Utilizing an auto-analyzer and suitable kits

Secondary outcomes

1

Description

Body composition

Timepoint

Before starting intervention, and 48 hours after the last training session.

Method of measurement

Body composition analyzer

2

Description

VO_2 peak

Timepoint

Before starting intervention, and 48 hours after the last

training session.

Method of measurement

A graded exercise test on a treadmill

Intervention groups

1

Description

Intervention group 1: beetroot juice: a daily dosage of 70 mL of concentrated beetroot juice (Beet-It Sport Shot 400; James White Ltd, Ashbocking, UK), which contains 400 mg (6.4 mmol) of NO_3^- and capsules containing maltodextrin comparable to caffeine capsules. In addition, they perform high-intensity interval training (on a motorized treadmill for 4 × 4-minute intervals at 70-90% of maximum heart rate (HRmax), interspersed with 3 minutes of active recovery) 3 times a week.

Category

Other

2

Description

Intervention group 2: Caffeine, at a dosage of 6 mg/kg of body weight daily, using pharmaceutical-grade capsules, and a beverage consisting of 1 g of powdered beetroot juice mixed with 1 L of mineral water and lemon juice, akin to the beetroot juice bottle. In addition, they perform high-intensity interval training (on a motorized treadmill for 4 × 4-minute intervals at 70-90% of maximum heart rate (HRmax), interspersed with 3 minutes of active recovery) 3 times a week.

Category

Other

3

Description

Intervention group 3: caffeine plus beetroot juice; caffeine, a dosage of 6 mg/kg of body weight daily, using pharmaceutical-grade capsules. Moreover, a daily dosage of 70 mL of concentrated beetroot juice (Beet-It Sport Shot 400; James White Ltd, Ashbocking, UK), which contains 400 mg (6.4 mmol) of NO_3^-, and a placebo capsule similar to a caffeine-containing capsule. In addition, they perform high-intensity interval training (on a motorized treadmill for 4 × 4-minute intervals at 70-90% of maximum heart rate (HRmax), interspersed with 3 minutes of active recovery) 3 times a week.

Category

Other

4

Description

Intervention group 4: (control-placebo); the placebo group will administer capsules containing maltodextrin comparable to caffeine capsules, along with a beverage consisting of 1 g of powdered beetroot juice mixed with 1 L of mineral water and lemon juice, akin to the beetroot juice bottle, at a specific time.

Category

Placebo

Recruitment centers

1

Recruitment center

Name of recruitment center

University of Halabja

Full name of responsible person

Dara Latif Saifalddin

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Sponsors / Funding sources

1

Sponsor

Name of organization / entity

The university of Halabja

Full name of responsible person

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+964 770 282 7151

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hassan.abdulla@uoh.edu.iq

Grant name

Grant code / Reference number

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

Yes

Title of funding source

The university of Halabja

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
The University of Halabja

Full name of responsible person
Dara Latif Saifalddin

Position
Assistant professor

Latest degree
Ph.D.

Other areas of specialty/work
Physiology

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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
Yes - There is a plan to make this available

Statistical Analysis Plan
Yes - There is 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
Yes - There is a plan to make this available

Analytic Code
Not applicable

Data Dictionary
Not applicable

Title and more details about the data/document
There is a plan to share the results of this study in a manuscript which may publish finally.

When the data will become available and for how long
After finishing the intervention and analyzing data, and writing the manuscript the data will be published as the journal allows.

To whom data/document is available
To the reviewers of journals which publishes our manuscript.

Under which criteria data/document could be used
There is no restricted condition except the private information regarding the subjects.

From where data/document is obtainable
The person responsible to this document is Dara Latif Saifalddin which can be available via email address: dara.saifaddin@uoh.edu.iq

What processes are involved for a request to access data/document
Academic person can request the document after publishing the data, and the will get answer as soon as possible.

Comments