Clinical Trial Protocol
Iranian Registry of Clinical Trials

22 Nov 2026

The effect of 5-methyltetrahydrofolate supplementation on serum folate and homocysteine level, metabolic, nutritional status, liver function, and PPARα and TNFα gene expression in patients with metabolic dysfunction–associated steatotic liver disease: a double-blind, parallel randomized controlled trial study

Protocol summary

Study aim

To determine the effect of MTHF supplementation on serum folate and homocysteine level, metabolic, nutritional status, liver function, and PPARα and TNFα gene expression in patients with MASLD

Design

A parallel randomized, double-blinded, controlled trial on 44 MASLD patients (22 in each group). Stratified block-randomization based on sex and body mass index will be used.

Settings and conduct

Study will be conducted on 44 MASLD patients. Patients will be randomly divided into 2 equal groups and will receive MTHF/ placebo for 90 days. To blind all researchers and participants, supplement and placebo are similar in appearance and color and a third person outside the study knows their content.

Participants/Inclusion and exclusion criteria

Inclusion criteria: Adults in the age range of 18-55 years, have a BMI index of 25-35 kg/m2, with diagnosis of MASLD based on liver ultrasound by a gastroenterologist, and willing to participate in the study. Exclusion criteria: History of alcohol and tobacco use, pregnancy, breastfeeding, diagnosed pathological conditions affecting the liver, regular use of nonsteroidal anti-inflammatory drugs, metformin, using folate supplements and multi-vitamin supplements in the past 3 months, having undergone weight loss surgery in the past year.

Intervention groups

Two groups: intervention group (1 tablet containing 800 mcg 5-methyltetrahydrofolate per day) and placebo group (1 tablet per day of corn starch/cellulose).

Main outcome variables

Primary outcome: serum level of folate and homocysteine and gene expression of PPARα and TNFα. Secondary outcome: liver fibrosis, ALT, AST, GGT, FBG and insulin, HOMA-IR, QUICKI, lipid profile, nutritional status, quality of life and adverse events.

General information

Reason for update

Acronym

IRCT registration information

IRCT registration number: **IRCT20100123003140N26**
Registration date: **2026-06-01, 1405/03/11**
Registration timing: **prospective**

Last update: **2026-06-01, 1405/03/11**
Update count: **0**

Registration date

2026-06-01, 1405/03/11

Registrant information

Name

Bahram Pourghassem Gargari

Name of organization / entity

Health and Nutrition Faculty, Tabriz University of medical sciences

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Recruitment status

recruiting

Funding source

Expected recruitment start date
2026-07-23, 1405/05/01
Expected recruitment end date
2027-03-20, 1405/12/29
Actual recruitment start date
empty
Actual recruitment end date
empty
Trial completion date
empty

Scientific title
The effect of 5-methyltetrahydrofolate supplementation on serum folate and homocysteine level, metabolic, nutritional status, liver function, and PPARα and TNFα gene expression in patients with metabolic dysfunction–associated steatotic liver disease: a double-blind, parallel randomized controlled trial study

Public title
The effect of 5-methyltetrahydrofolate supplementation on the control and treatment of metabolic dysfunction–associated steatotic liver disease
Purpose
Treatment
Inclusion/Exclusion criteria
Inclusion criteria:
Adults (male and female) in the age range of 18-55 years Having a body mass index of 25-35 kg/m2 With diagnosis of metabolic dysfunction–associated steatotic liver disease (MASLD) based on liver ultrasound by a gastroenterologist Willing to participate in the study
Exclusion criteria:
Regular use of nonsteroidal anti-inflammatory drugs, antibiotics, steroids (excluding topical and inhaled drugs), metformin, colchicine, hormonal drugs such as high doses of estrogen within the past 3 months, use of chemical or herbal weight loss drugs and use of hepatotoxic drugs such as phenytoin, amiodarone, levothyroxine, lithium, statins, blood pressure drugs, lipid-lowering drugs, use of multivitamin-mineral supplements, folate, antioxidants such as vitamin E and omega-3 supplements Diagnosed pathological conditions affecting the liver such as viral hepatitis, acute or chronic liver failure, cholestasis, liver transplantation Having hemochromatosis, Wilson disease, Alpha-1 antitrypsin, diabetes, heart failure, renal, intestinal, thyroid dysfunction, biliary disease, PCOS, autoimmune diseases and malabsorption diseases such as Crohn's, the presence of any diagnosed neoplasia or malignancy, having symptoms of infectious or inflammatory disease Acute systemic disease, cystic fibrosis, history of gastrointestinal surgery within the past year, irritable bowel syndrome, celiac disease Pregnancy, breastfeeding, menopause History of alcohol and tobacco use
Age
From **18 years** old to **55 years** old
Gender
Both

Phase
2-3
Groups that have been masked

- Participant
- Care provider
- Investigator
- Outcome assessor

Sample size
Target sample size: **44**
Randomization (investigator's opinion)
Randomized
Randomization description
At the beginning of the study and before the intervention, random assignment of individuals to intervention groups will be performed using the Stratified Block Randomization method. Initially, individuals will be placed in four strata (overweight men, obese men, overweight women, obese women) based on sex (male/female) and body mass index (overweight/obese). In each stratum, individuals will be randomly assigned to the intervention or placebo group in a 1:1 ratio using blocks of 2. Randomization will be done using the random allocation software. Given the total sample size (44 individuals), each study group (intervention or placebo) will be approximately 22 individuals. Blocking and stratification ensure that balance is maintained in each stratum, even if the number of individuals in the strata is not exactly equal.
Blinding (investigator's opinion)
Double blinded
Blinding description
The present study will be conducted in a double-blind manner, meaning that the participants, the principal investigator, and the evaluators will not be aware of the content (supplement/ placebo) they receive. The appearance, color, taste, and odor of the MTHF supplement and the placebo will be similar. As a result, the study participants will not know which supplement/placebo they were taking. Also, the supplement/placebo will be coded, and a person outside the study will know the codes and the type of supplement/placebo. The evaluators will deliver the supplement/placebo to the participants based on the code labeled on them and will not know the content of each supplement.
Placebo
Used
Assignment
Parallel
Other design features

Secondary Ids

empty

Ethics committees

1

Ethics committee
Name of ethics committee
Ethics committee of Tabriz University of Medical

Sciences

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No 2 Central Building, Tabriz University of Medical Sciences, Golgasht Street, Tabriz

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East Azarbaijan

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5166614711

Approval date
2026-04-27, 1405/02/07

Ethics committee reference number
IR.TBZMED.REC.1405.059

Health conditions studied

1

Description of health condition studied
Metabolic dysfunction–associated steatotic liver disease

ICD-10 code
K76.0

ICD-10 code description
Fatty (change of) liver, not elsewhere classified

Primary outcomes

1

Description
Serum level of folate

Timepoint
Before and after the intervention

Method of measurement
ELISA assay

2

Description
Serum level of homocysteine

Timepoint
Before and after the intervention

Method of measurement
Enzymatic method

3

Description
PPARα gene expression

Timepoint
Before and after the intervention

Method of measurement
Real-time PCR assay

4

Description
TNFα gene expression

Timepoint
Before and after the intervention

Method of measurement
Real-time PCR assay

Secondary outcomes

1

Description
Liver fibrosis (FIB-4 Score)

Timepoint
Before and after the intervention

Method of measurement
Formula: FIB-4 Score = (Age × AST) / (Platelet count × √ALT) (age in years, ALT and AST in U/L, and platelet count in 109/L)

2

Description
Alanine transaminase

Timepoint
Before and after the intervention

Method of measurement
IFCC (International Federation for Clinical Chemistry) method

3

Description
Aspartate aminotransferase

Timepoint
Before and after the intervention

Method of measurement
IFCC (International Federation for Clinical Chemistry) method

4

Description
Gamma-glutamyl transferase

Timepoint
Before and after the intervention

Method of measurement
Enzymatic method

5

Description
Fasting blood glucose

Timepoint
Before and after the intervention

Method of measurement
Enzymatic method

6

Description
Fasting serum insulin

Timepoint
Before and after the intervention

Method of measurement
ELISA assay

7

Description
Insulin resistance (HOMA-IR)
Timepoint
Before and after the intervention
Method of measurement
Formula: HOMA-IR = (fasting insulin (mU/L) × fasting glucose (mg/dL))/405

8

Description
Insulin sensitivity (QUICKI)
Timepoint
Before and after the intervention
Method of measurement
Formula: QUICKI= 1/[log fasting insulin (µU/mL)+log fasting glucose (mg/dL)]

9

Description
Serum lipid profiles (HDL-C، LDL-C، TC و TG)
Timepoint
Before and after the intervention
Method of measurement
Enzymatic methods for TC,TG and HDL-C and for LDL-C : Freidwald's formula: LDL-C = TC- HDL-C - (TG/5)

10

Description
Nutritional status (calorie and nutrients intake)
Timepoint
Before and after the intervention
Method of measurement
Food record questionnaire

11

Description
Anthropometric index (weight, height, body mass index, waist circumference and waist to hip ratio)
Timepoint
Before and after intervention
Method of measurement
Digital scale for weight, measurement tape for height, waist and hip circumference, body mass index with formula (weight(kg)/square height (m))

12

Description
Body composition
Timepoint
Before and after intervention
Method of measurement
Bioelectrical impedance analysis

13

Description
Quality of life
Timepoint
Before and after intervention
Method of measurement
Questionnaire

14

Description
Adverse events
Timepoint
Before and after intervention
Method of measurement
Questionnaire

Intervention groups

1

Description
Intervention group: Patients in this group will receive 5-methyltetrahydrofolate tablets (800 mcg) once a day for 90 days. Tablets are manufactured by Ashbal Chemi pharmaceutical company.
Category
Treatment - Other

2

Description
Control group: Patients in this group will receive placebo for 90 days. The placebo is corn starch/ cellulose and will be consumed once a day.
Category
Placebo

Recruitment centers

1

Recruitment center
Name of recruitment center
Tabriz University of Medical Sciences
Full name of responsible person
Dr. Manouchehr Khoshbaten
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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
Tabriz University of Medical Sciences

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
Tabriz University of Medical Sciences

Full name of responsible person
Dr. Bahram Pourghassem Gargari

Position
Professor

Latest degree
Ph.D.

Other areas of specialty/work
Nutrition

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Sharing plan

Deidentified Individual Participant Data Set (IPD)

Yes - There is a plan to make this available

Study Protocol

Yes - There is a plan to make this available

Statistical Analysis Plan

No - There is not a plan to make this available

Informed Consent Form

No - There is not a plan to make this available

Clinical Study Report

Yes - There is a plan to make this available

Analytic Code

No - There is not a plan to make this available

Data Dictionary

No - There is not a plan to make this available

Title and more details about the data/document

The study protocol will be written and published in the form of an article. The clinical report of the study will be published in the form of an article.

When the data will become available and for how long

8 months after the end of the study

To whom data/document is available

The data will only be available for people working in academic institutions.

Under which criteria data/document could be used

To use the findings in the clinic or to write other articles, including review articles. In the case of original articles, researchers will be allowed to do so.

From where data/document is obtainable

Data and documents related to the present study will be available via email from the study researcher, Dr. Bahram Pourghassem Gargari (pourghassemb@tbzmed.ac.ir/bahrampg@yahoo.com).

What processes are involved for a request to access data/document

After receiving the request from the person in charge of updating, the study will be provided to the researcher in consultation with the scientific officer.

Comments