Clinical Trial Protocol
Iranian Registry of Clinical Trials

07 Nov 2026

Effect of Synbiotic Supplementation on Liver Fibrosis Indices, Inflammatory and Oxidative Stress Biomarkers, Appetite, Metabolic Parameters, and Expression of PPAR-α, UCP1, and UCP2 Genes in Overweight or Obese Adolescents with Metabolic Dysfunction–Associated Steatotic Liver Disease (MASLD): A Randomized Double-Blind Placebo-Controlled Trial

Protocol summary

Study aim
To evaluate the effects of a 16-week synbiotic supplementation on liver, metabolic, inflammatory, oxidative, and gene expression outcomes in adolescents with Metabolic Associated Steatotic Liver Disease.

Design
Randomized, double-blind, placebo-controlled, parallel-group trial (n=126). Randomization (1:1) uses blocks of four.

Settings and conduct
Multi-center trial conducted at Imam Khomeini and Shahid Motahari hospitals. Participants, parents, investigators, and data analysts are blinded.

Participants/Inclusion and exclusion criteria
Overweight or obese adolescents (13–18 years) with Metabolic Associated Steatotic Liver Disease (CAP ≥ 248 dB/m). Exclusions include chronic heart, kidney, or liver diseases, endocrine disorders, and use of antibiotics, probiotics, or prebiotics within 3 months.

Intervention groups
Intervention group receives one daily synbiotic capsule (Familact 2Plus; eight probiotic strains and 38.5 mg FOS). Control group receives identical placebo capsules.

Main outcome variables
Primary: Change in Controlled Attenuation Parameter. Secondary: Liver stiffness; metabolic, inflammatory, and oxidative indices; appetite; and expression of PPAR-α, UCP1, and UCP2 genes.

General information

Reason for update

Acronym

IRCT registration information
IRCT registration number: **IRCT20260722070298N1**
Registration date: **2026-10-15, 1405/07/23**
Registration timing: **prospective**

Last update: **2026-10-15, 1405/07/23**
Update count: **0**

Registration date
2026-10-15, 1405/07/23

Registrant information
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Recruitment status
recruiting

Funding source

Expected recruitment start date
2026-10-22, 1405/07/30

Expected recruitment end date
2027-09-16, 1406/06/25

Actual recruitment start date
empty

Actual recruitment end date
empty

Trial completion date
empty

Scientific title

Effect of Synbiotic Supplementation on Liver Fibrosis Indices, Inflammatory and Oxidative Stress Biomarkers, Appetite, Metabolic Parameters, and Expression of PPAR-α, UCP1, and UCP2 Genes in Overweight or Obese Adolescents with Metabolic Dysfunction–Associated Steatotic Liver Disease (MASLD): A Randomized Double-Blind Placebo-Controlled Trial

Public title

Effect of Synbiotic in MASLD

Purpose

Treatment

Inclusion/Exclusion criteria

Inclusion criteria:

Age 13 to 18 years. Overweight or obesity, defined as BMI-for-age ≥85th percentile according to WHO growth reference. Diagnosis of MASLD confirmed by FibroScan with CAP ≥248 dB/m. Stable dietary and physical activity patterns during the 3 months prior to enrollment. Written informed consent from the parent or legal guardian and assent from the participant.

Exclusion criteria:

History of cardiovascular, renal, or autoimmune diseases. Use of hepatotoxic medications within 3 months before the study. Participation in another clinical trial within the previous 3 months. History of probiotic, prebiotic, or antibiotic supplement use within the past 3 months. Use of medications affecting metabolism, such as statins, glucocorticoids, metformin, or other insulin sensitizers. Presence of chronic liver diseases, including viral hepatitis, Wilson’s disease, autoimmune hepatitis, or genetic and metabolic liver disorders. Diagnosis of renal disease, thyroid disorders, or other endocrine abnormalities. Pregnancy at baseline in post-menarcheal female participants.

Age

From **13 years** old to **18 years** old

Gender

Both

Phase

N/A

Groups that have been masked

- Participant
- Care provider
- Investigator
- Outcome assessor
- Data analyser

Sample size

Target sample size: **126**

Randomization (investigator's opinion)

Randomized

Randomization description

This is a single-center, randomized, double-blind, placebo-controlled trial conducted at a clinical center affiliated with Urmia University of Medical Sciences. Eligible participants will be randomly allocated in a 1:1 ratio to either the synbiotic supplementation or placebo group. To ensure a balanced distribution of baseline confounding factors across both arms, stratified block randomization will be performed based on four key variables: sex (female vs. male), age (13–15 vs. 16–18 years), body mass index (<95th percentile vs. ≥95th percentile), and baseline Controlled Attenuation Parameter (CAP score: <260 dB/m vs. ≥260 dB/m), yielding a total of 16 distinct strata. For each stratum, an independent randomization sequence will be generated using STATA software (version 17) with the blockrand package and a fixed block size of 4, applying a predefined random seed to ensure reproducibility. Allocation concealment will be strictly maintained using sequentially numbered, opaque, sealed envelopes (SNOSE) prepared separately for each stratum. The envelopes and uniquely coded, identical-looking supplement/placebo containers will be prepared and managed by an independent researcher/pharmacist who has no involvement in participant recruitment, clinical care, or outcome assessment. Envelopes will only be opened sequentially after formal participant enrollment.

Blinding (investigator's opinion)

Double blinded

Blinding description

The trial is double-blind. Participants, parents, investigators, clinicians/care providers, study personnel involved in outcome assessment, and data analysts will remain unaware of group assignments. Synbiotic and placebo capsules will be identical in appearance, color, smell, and taste. Capsule containers will be coded according to the allocation list by a pharmacist who will not be involved in data collection or analysis. The randomization code will be broken only after data collection or when potentially required for a serious adverse event. In the event of a serious adverse event, the need for unblinding will be jointly reviewed by the Principal Investigator and the clinical hepatologist.

Placebo

Used

Assignment

Parallel

Other design features

Standardized Lifestyle and Dietary Counseling:To isolate the specific effect of the synbiotic supplementation and control for potential confounding factors, both the intervention and placebo groups will receive identical, standardized dietary and lifestyle counseling throughout the 16-week trial. This counseling will be provided by a trained clinical nutritionist to both the adolescent and their parent (preferably the mother) at baseline and reinforced during follow-up visits.The dietary plan is designed based on established guidelines for the staged treatment of pediatric obesity, with the goal of achieving safe, gradual weight loss and improving metabolic parameters. The daily energy deficit is calculated individually based on the severity of obesity: For overweight adolescents (BMI-for-age between 85th and 95th percentile): A diet with a daily energy deficit of 300 kcal relative to their estimated energy requirement (EER). For obese adolescents (BMI-for-age ≥ 95th percentile): A diet with a daily energy deficit of 500 kcal relative to their EER.This approach aims for a gradual, safe, and sustainable weight loss of 0.5 to 1 kg per week. The dietary composition will follow standard recommendations for pediatric MASLD: Carbohydrates:

50-55% of total daily energy (with emphasis on complex, high-fiber sources like whole grains, vegetables, and legumes). Protein: 15-20% of total daily energy (from lean sources such as skinless poultry, fish, legumes, and low-fat dairy). Fat: 25-30% of total daily energy (with emphasis on unsaturated fats like olive oil, nuts, and seeds, while limiting saturated and trans fats).Behavioral and Dietary Recommendations: Complete elimination of sugar-sweetened beverages. Increased consumption of fresh fruits and vegetables to at least 5 servings per day. Controlled portion sizes and avoidance of fast food. Encouragement of at least 5 family meals per week. Self-monitoring of daily food intake using a provided diary.Monitoring Dietary Intake:To ensure that any observed changes in metabolic and inflammatory markers are primarily due to the synbiotic intervention and not unintended dietary changes, dietary intake will be rigorously monitored at three time points (baseline, week 8, and week 16) using three 24-hour dietary recalls at each time point. This data will be analyzed using Nutritionist IV software and will be included as a covariate in the final statistical analysis. Physical activity will also be assessed at baseline and week 16 using the validated Persian version of the Physical Activity Questionnaire for Adolescents (PAQ-A) and adjusted for in the analysis. This comprehensive approach ensures that the true effect of the synbiotic can be isolated from lifestyle modifications.

Secondary Ids

empty

Ethics committees

1

Ethics committee

Name of ethics committee
Ethics Committee of National Institute for Medical Research Development (NIMAD)

Street address
No. 21, Besat Ave., West Fatemi Ave., Tehran, Iran

City
Tehran

Province
Tehran

Postal code
1419693111

Approval date
2026-06-16, 1405/03/26

Ethics committee reference number
IR.NIMAD.REC.1405.066

Health conditions studied

1

Description of health condition studied
Metabolic dysfunction-Associated Steatotic Liver Disease (MASLD) / Non-Alcoholic Fatty Liver Disease (NAFLD) in adolescents

ICD-10 code
K76.0

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

Primary outcomes

1

Description
Change in controlled attenuation parameter measured by FibroScan from baseline to week 16 (end of the intervention).

Timepoint
At baseline and at week 16 (end of the intervention).

Method of measurement
Controlled attenuation parameter will be measured using FibroScan transient elastography.

2

Description
Expression of Peroxisome Proliferator-Activated Receptor Alpha in peripheral blood mononuclear cells.

Timepoint
At baseline and at week 16 after the intervention.

Method of measurement
Quantitative real-time polymerase chain reaction using Beta-actin as the reference gene and the 2 to the power of negative delta delta Ct method.

Secondary outcomes

1

Description
Expression of Uncoupling Protein 1 in peripheral blood mononuclear cells.

Timepoint
At baseline and at week 16 after the intervention.

Method of measurement
Quantitative real-time polymerase chain reaction using Beta-actin as the reference gene and the 2 to the power of negative delta delta Ct method.

2

Description
Expression of Uncoupling Protein 2 in peripheral blood mononuclear cells.

Timepoint
At baseline and at week 16 after the intervention.

Method of measurement
Quantitative real-time polymerase chain reaction using Beta-actin as the reference gene and the 2 to the power of negative delta delta Ct method.

3

Description
Alanine aminotransferase.

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Enzymatic colorimetric method using a commercial kit and calibrated autoanalyzer.

4

Description

Aspartate aminotransferase

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Enzymatic colorimetric method using a commercial kit and calibrated autoanalyzer.

5

Description

Gamma glutamyl transferase

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Enzymatic colorimetric method using a commercial kit and calibrated autoanalyzer.

6

Description

Fasting blood glucose

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Enzymatic colorimetric method using a commercial kit and calibrated autoanalyzer after 12 hours of fasting.

7

Description

Fasting insulin

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Enzyme linked immunosorbent assay after 12 hours of fasting.

8

Description

Homeostatic Model Assessment of Insulin Resistance

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Calculated from fasting blood glucose and fasting insulin.

9

Description

Triglyceride

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Enzymatic colorimetric method using a commercial kit and calibrated autoanalyzer.

10

Description

Low density lipoprotein cholesterol

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Enzymatic colorimetric method using a commercial kit and calibrated autoanalyzer.

11

Description

High density lipoprotein cholesterol

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Enzymatic colorimetric method using a commercial kit and calibrated autoanalyzer.

12

Description

Total cholesterol

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Enzymatic colorimetric method using a commercial kit and calibrated autoanalyzer.

13

Description

Neutrophil to lymphocyte ratio

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Calculated from complete blood count.

14

Description

Platelet to lymphocyte ratio

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Calculated from complete blood count.

15

Description

C reactive protein

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Enzyme linked immunosorbent assay.

16

Description

Tumor necrosis factor alpha

Timepoint

At baseline and at week 16 after the intervention.

Method of measurement

Enzyme linked immunosorbent assay.

17

Description
Erythrocyte sedimentation rate
Timepoint
At baseline and at week 16 after the intervention.
Method of measurement
Standard clinical laboratory method.

18

Description
Malondialdehyde
Timepoint
At baseline and at week 16 after the intervention.
Method of measurement
Thiobarbituric acid reactive substances assay.

19

Description
Superoxide dismutase activity
Timepoint
At baseline and at week 16 after the intervention.
Method of measurement
Commercial colorimetric assay.

20

Description
Body weight
Timepoint
At baseline and at week 16 after the intervention.
Method of measurement
Calibrated digital scale.

21

Description
Height
Timepoint
At baseline and at week 16 after the intervention.
Method of measurement
Stadiometer.

22

Description
Body mass index
Timepoint
At baseline and at week 16 after the intervention.
Method of measurement
Calculated from body weight and height.

23

Description
Waist circumference
Timepoint
At baseline and at week 16 after the intervention.
Method of measurement
Non stretchable measuring tape.

24

Description
Conicity index
Timepoint
At baseline and at week 16 after the intervention.
Method of measurement
Calculated from waist circumference, height, and body weight.

25

Description
Abdominal volume index
Timepoint
At baseline and at week 16 after the intervention.
Method of measurement
Calculated from anthropometric measurements.

26

Description
Appetite scores
Timepoint
At baseline and at week 16 after the intervention.
Method of measurement
Visual analogue scale.

27

Description
Systolic blood pressure.
Timepoint
At baseline and at week 16 after the intervention.
Method of measurement
Standard calibrated digital sphygmomanometer after 10 minutes of rest in a seated position. The mean of two measurements will be recorded.

28

Description
Diastolic blood pressure
Timepoint
At baseline and at week 16 after the intervention.
Method of measurement
Standard calibrated digital sphygmomanometer after 10 minutes of rest in a seated position. The mean of two measurements will be recorded.

Intervention groups

1

Description
Intervention group: Participants will receive one capsule of Familact 2Plus daily (produced by Zist-Takhmir Co., Tehran, Iran). Each capsule contains 8 probiotic strains (Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus salivarius, Lactobacillus reuteri, Bifidobacterium lactis, Bifidobacterium longum, Bifidobacterium bifidum) with a total of approximately 8×10^9 CFU, and 38.5 mg of

Fructooligosaccharides (FOS) as a prebiotic. The capsules should be taken on an empty stomach, approximately one hour before lunch, with a glass of water, for a duration of 16 weeks. Storage conditions: keep at temperatures below 25°C, in a dry place, away from light and moisture. In addition to the supplement, participants in this group will receive standardized dietary and lifestyle counseling. The dietary plan is designed based on established guidelines for the staged treatment of pediatric obesity, with the goal of achieving safe, gradual weight loss and improving metabolic parameters. The daily energy deficit is calculated individually based on the severity of obesity: For overweight adolescents (BMI-for-age between 85th and 95th percentile): A diet with a daily energy deficit of 300 kcal relative to their estimated energy requirement (EER). For obese adolescents (BMI-for-age ≥ 95th percentile): A diet with a daily energy deficit of 500 kcal relative to their EER.This approach aims for a gradual, safe, and sustainable weight loss of 0.5 to 1 kg per week. The dietary composition will follow standard recommendations for pediatric MASLD (50-55% carbohydrates, 15-20% protein, and 25-30% fat). Behavioral recommendations include elimination of sugar-sweetened beverages, increased consumption of fruits and vegetables (≥5 servings/day), controlled portion sizes, avoidance of fast food, encouragement of family meals, and self-monitoring of food intake using a diary.

Category

Treatment - Other

2

Description

Control group: Participants will receive a placebo capsule, visually identical to the intervention capsule, produced by Zist-Takhmir Co. The placebo capsules do not contain any active bacterial strains or prebiotic content. The dosage and administration schedule will be identical to the intervention group: one capsule daily, on an empty stomach, approximately one hour before lunch, with a glass of water, for a duration of 16 weeks. Storage conditions: keep at temperatures below 25°C, in a dry place, away from light and moisture.Participants in this group will receive standardized nutritional and lifestyle counseling.

Category

Placebo

Recruitment centers

1

Recruitment center

Name of recruitment center

Motahari Hospital, Urmia

Full name of responsible person

Shahsanam Gheibi

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Sponsors / Funding sources

1

Sponsor

Name of organization / entity

Oroumia University of Medical Sciences

Full name of responsible person

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Email

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Grant name

Grant code / Reference number

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

No

Title of funding source

Urmia University of Medical Sciences

Proportion provided by this source

50

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

2

Sponsor

Name of organization / entity

National Institute for Medical Research Development (NIMAD)

Full name of responsible person

NIAMD

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Grant name
Grant code / Reference number
Is the source of funding the same sponsor organization/entity?
No
Title of funding source
National Institute for Medical Research Development (NIMAD)
Proportion provided by this source
50
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
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Full name of responsible person
Rasoul Zarrin
Position
professor
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Other areas of specialty/work
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Other areas of specialty/work
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Person responsible for updating data

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Other areas of specialty/work
Nutrition
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amir.nut89@gmail.com

Sharing plan

Deidentified Individual Participant Data Set (IPD)
No - There is not a plan to make this available
Justification/reason for indecision/not sharing IPD
The participants are adolescents, and the dataset contains sensitive clinical, biochemical, anthropometric, and gene-expression data. The informed consent and the current protocol do not include provisions for sharing individual participant data with external researchers. Therefore, to protect participant confidentiality and minimize the risk of re-identification, deidentified individual participant data will not be shared. Study

findings will be disseminated only in aggregate form through scientific publications and presentations.

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

Yes - There is a plan to make this available

Clinical Study Report

Yes - There is a plan to make this available

Analytic Code

Yes - There is a plan to make this available

Data Dictionary

Yes - There is a plan to make this available

Title and more details about the data/document

Study protocol, statistical analysis plan, informed consent form, final study report, deidentified participant data, STATA analysis code, and data dictionary related to this clinical trial.No directly identifying personal information will be shared.

When the data will become available and for how long

The study documents and deidentified dataset will become available starting 6 months after publication of the main study results or 12 months after completion of the study, whichever occurs first, subject to final approval by the principal investigator and institutional regulations. The materials will remain available for 5 years after the start of data sharing.

To whom data/document is available

The shared materials will be available to qualified researchers, including academic investigators, clinicians, postgraduate students, and other researchers with a methodologically sound research proposal. Requests from non-academic researchers may also be considered, provided that the proposed use is scientifically justified and consistent with ethical and regulatory requirements.

Under which criteria data/document could be used

Access will be granted for scientifically and ethically appropriate purposes, such as secondary analyses, validation studies, systematic reviews with individual-level verification if applicable, and educational or methodological research. Applicants must submit a brief proposal stating the research question, analysis plan, requested materials, and intended outputs. Requests will be reviewed by the principal investigator and the research team with attention to scientific merit, participant confidentiality, and consistency with the original informed consent and institutional ethics requirements. Shared data must remain deidentified, must not be used for participant re-identification, and must not be redistributed to third parties without written permission. A data/material transfer agreement may be required before release.

From where data/document is obtainable

Interested applicants should contact the principal investigator or the designated study contact by email. Requests may also be directed through the affiliated university/research center if applicable.

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

Applicants should submit an email request including their name, affiliation, CV or brief description of expertise, research question, analysis plan, requested materials, and expected timeline. The request will be reviewed by the principal investigator and study team, and if necessary by the relevant institutional authority. Clarifications may be requested from the applicant. If approved, the applicant may be asked to sign a data/material use agreement before receiving the files. The expected review and response time is approximately 2 to 4 weeks, and the approved materials will be shared electronically in a secure manner.

Comments