

Official Title:

**The Efficacy of Aerobic Exercise Training on ANS and Endothelial Function
in Compensated Cirrhosis**

NCT Number: NCT06288828

Document Date: January 01, 2026

Study Protocol and Statistical Analysis Plan

This study was conducted at King Chulalongkorn Memorial Hospital, Bangkok, Thailand). The study was approved by the Institutional Review Board (IRB) of Faculty of Medicine, Chulalongkorn University (IRB No. 569/66). The study protocol was registered under the Clinical Trial Registry (<https://clinicaltrials.gov/>) (NCT06288828).

1. Study design

Prospective, Randomized controlled study

2. Study participants

1) Target population: Compensated cirrhosis

2) Sample population: Patients who visited Liver unit of Division of Gastroenterology, Department of Medicine at King Chulalongkorn Memorial Hospital

3) Inclusion criteria:

- (1) Diagnosis of cirrhosis confirmed by either liver biopsy or compatible features on imaging (e.g., ultrasonography or MRI).
- (2) Age range between 45 and 75 years
- (3) Child Pugh score class A cirrhosis
- (4) Reporting a sedentary lifestyle, defined as engaging in physical activity for less than 150 minutes of moderate-intensity physical activity per week, or less than 75 minutes of vigorous-intensity physical activity per week, or an equivalent combination of both
- (5) Abstinence from alcohol consumption for at least six months.

4) Exclusion criteria:

- (1) Active hepatocellular carcinoma (HCC) or remission of HCC within the past 3 months prior to the enrollment date
- (2) Untreated large esophageal varices (grade \geq F2), severe grade portal hypertensive gastropathy, and a history of upper gastrointestinal bleeding from portal hypertension (PHT).
- (3) Active or history of hepatic encephalopathy

- (4) Recent hospitalization in the past 3 months
- (5) Portal vein thrombosis occurring in any segment or branch of the portal vein
- (6) Trans-jugular intrahepatic portosystemic shunt
- (7) Presence of exercise contraindications and relative contraindications (Appendix A.)
- (1)
- (8) Presence of severe orthopedic limitations
- (9) Have body mass index (BMI) exceeding 35 kg/m²
- (10) Use of alpha or beta-blocker
- (11) Inability to communicate with the research team via telemedicine system

3. Sample size calculation

The sample size was calculated to compare the mean difference of SDNN change between two groups, using a two-sided significance level of 0.05 and a power of 0.80. From prior studies in patients with chronic kidney disease (CKD), which share hyperammonemia-related mechanisms with cirrhosis, an expected mean difference of 10.9 units with a standard deviation of 11.89 was applied (2). Using the t-distribution method with non-centrality parameter, the required number of participants was calculated as follows:

$$n = \frac{2 \times (Z_{1-\alpha/2} + Z_{1-\beta})^2 \times S^2}{E^2}$$

where $Z_{1-\alpha/2} = 1.96$, $Z_{1-\beta} = 0.84$, $S = 11.89$, and $E = 10.9$.

$$n = \frac{2 \times (1.96 + 0.84)^2 \times 11.89^2}{10.9^2}$$

$$\approx 19.8 \Rightarrow 20 \text{ participants per group.}$$

Thus, the total required sample size was 40 participants (20 per group). To account for an anticipated dropout rate of 20%, the adjusted sample size was calculated as $40 \div (1 - 0.20) = 50$. Therefore, the final target enrollment was set at 25 participants per group, for a total of 50 participants.

4. Patient enrollment

Cirrhosis patients attending the Liver Unit of the Gastroenterology Clinic, Department of Medicine, King Chulalongkorn Memorial Hospital, Bangkok, Thailand, during routine appointments and meeting the eligibility criteria were invited to participate in the study. In addition, the study was publicized through posters displayed in the clinic. These posters included the researcher's contact information, enabling interested patients to directly communicate with the research team for further details and potential enrollment.

5. Informed consent

To invite patients into the study, the study protocol, including potential risks and benefits, was explained in detail to ensure that each patient had sufficient information to make an informed decision. Patients were encouraged to ask questions and were given unlimited time to consider participation. They were also provided with the researcher's contact information to request additional details before making a decision. To minimize any possibility of coercion, patients were reassured that their standard medical care would not be affected by their decision to participate or decline. Patients who expressed interest in participating provided written informed consent at the Sports and Exercise Medicine Research Laboratory, Physiology Laboratory, Department of Physiology, King Chulalongkorn Memorial Hospital, prior to the start of the study. All participants continued to receive standard treatment according to routine clinical practice. Data collected during the study were kept strictly confidential and were analyzed and reported anonymously.

6. Study flow and allocation

6.1 Randomization procedure

This study was designed as a randomized controlled trial. For group allocation, randomization was performed by a research assistant who was not involved in participant recruitment or assessment. Block randomization with a block size of four was applied using the online tool <https://randomizer.org>. The randomization results were placed into sealed, opaque envelopes to maintain allocation concealment until the point of assignment.

6.2 Methods

The study was initiated following approval from the Institutional Review Board (IRB). Data collection and study procedures were conducted according to the approved protocol:

1. Baseline characteristics were obtained from electronic medical records and supplemented with patient interviews using a structured paper-based record form. These included height, underlying comorbidities, current medications, and smoking status. In addition, nutritional status was assessed using a 3-day food record.
2. Blood samples were collected for complete blood count (CBC), liver function tests (LFTs), coagulation profile, blood urea nitrogen (BUN), creatinine (Cr), serum ammonia, fasting blood sugar (FBS), hemoglobin A1c (HbA1c), and lipid profile.
3. Participants underwent body composition assessment using a dual-energy X-ray absorptiometry (DXA) scanner (Horizon W, Hologic™) and a bioelectrical impedance analysis (BIA) device (InBody 720™). These assessments provided measurements of body weight, body mass index (BMI), fat mass, and muscle mass. In addition, physical performance was evaluated using the 6-minute walk test (6MWT), and muscle strength was assessed with a handgrip dynamometer.
4. Participants underwent baseline assessments of the primary outcomes of interest, including autonomic nervous system function measured by the Ewing autonomic battery test and heart rate variability (HRV), as well as endothelial function assessed by flow-mediated dilation (FMD), prior to entering the study protocol. Detailed protocols for each group are provided in Section 3.6.2.1. After these assessments, participants were randomized into either the intervention or control group. Randomization was performed by a research assistant, who opened sealed envelopes containing the group assignments after the baseline tests. Detailed protocols for each group are provided in Section 3.6.2.2.
5. Participants followed the assigned protocol for 16 weeks. To ensure adherence and safety, the researcher contacted them once per week via telephone or a messaging application to monitor compliance and record any adverse events.
6. At the end of the 16-week intervention, participants underwent repeat assessments corresponding to baseline measures, including including blood test, body composition (DXA and BIA), muscle strength (handgrip strength) and physical performance (6MWT), autonomic function (Ewing autonomic battery test), and endothelial function (FMD).

7. Data were then analyzed according to the pre-specified statistical plan.

6.2.1 Control group protocol

Table 1 Timetable of the control group protocol	
Weeks	Details
0	<ul style="list-style-type: none"> - Baseline & outcome measurements (autonomic tests, HRV, FMD, body composition, muscle strength, 6MWT) - Nutritional assessment and dietitian consultation
1–16	<ul style="list-style-type: none"> - Weekly follow-up (phone/app) to monitor physical activity and adverse events - Nutritional assessment and dietitian consultation at week 8
Study completion (16-18)	<ul style="list-style-type: none"> - Repeat outcome measurements - Final nutritional assessment and dietitian consultation

Participants in the control group were advised to maintain their usual physical activity levels throughout the study. To monitor adherence, their activity was evaluated through weekly contact via telephone or messaging application. Exercise recommendations were offered after study completion.

Nutritional status was assessed by a dietitian using a 3-day food diary at baseline, week 8, at study completion. Participants received individualized dietary counseling aimed at meeting daily energy and protein targets of 35 kcal/kg/day and 1.2 g/kg/day of body weight, respectively. If participants were unable to meet these targets, the nutritionist provided tailored advice to help them achieve adequate nutrition (Figure 1 and Table 1).

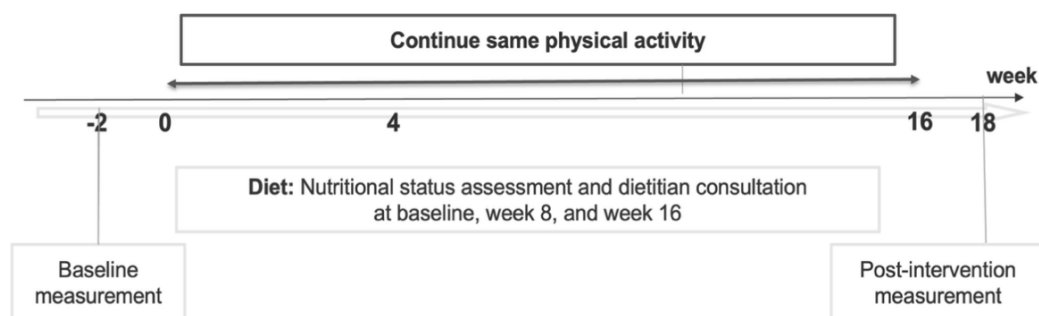


Figure 1 Timeline of the control group protocol: Participants were advised to maintain their usual physical activity with weekly follow-up (1 time/week). Nutritional status was assessed, and dietitian consultations were conducted at baseline, week 8, and week 16.

6.2.2 Intervention group

As in the control group, participants in the intervention group also received nutritional assessments and consultations with a dietitian at baseline, week 8, and at study completion. They were guided to achieve dietary targets of 35 kcal/kg/day for energy and 1.2 g/kg/day for protein intake. If participants were unable to meet these targets, individualized dietary recommendations were provided to help ensure adequate nutrition.

In part of physical activity, participants in the intervention group followed a 16-week aerobic exercise program that divided into two phases (Table 2 and Figure 2).

Table 2 Timetable of the intervention group protocol	
Weeks	Details
0	<ul style="list-style-type: none"> - Baseline & outcome measurements (autonomic tests, HRV, FMD, body composition, muscle strength, 6MWT) - Nutritional assessment and dietitian consultation
1–4	<ul style="list-style-type: none"> - Hospital-based exercise (1 session/week) + home-based exercise following protocol
5–16	<ul style="list-style-type: none"> - Home-based exercise following protocol - Weekly follow-up (phone/app) to monitor compliance and any adverse events - Nutritional assessment and dietitian consultation at week 8
Study completion (16-18)	<ul style="list-style-type: none"> - Repeat outcome measurements - Final nutritional assessment and dietitian consultation

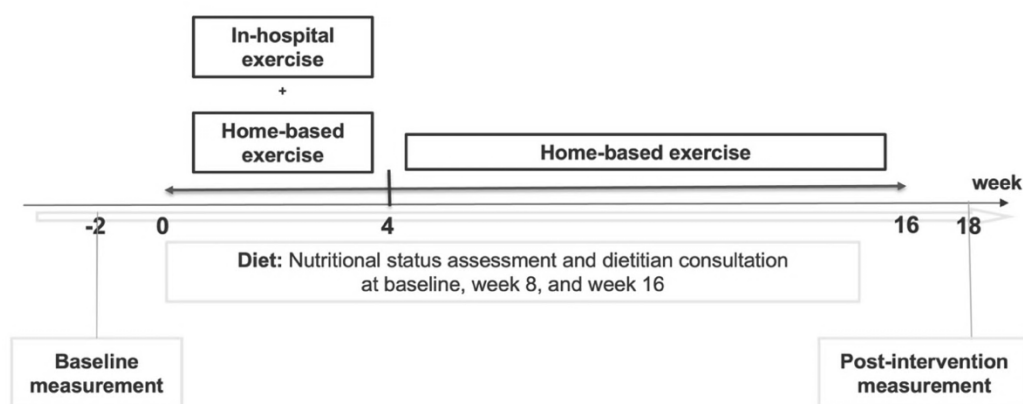


Figure 2 Timeline of the intervention group protocol: Participants completed supervised hospital-based exercise plus home exercise during weeks 1–4, followed by home-based exercise with weekly follow-up (1 time/week) during weeks 5–16. Nutritional status was assessed, and dietitian consultations were conducted at baseline, week 8, and week 16.

Phase 1 (Weeks 1–4, Hospital- and home-based exercise):

This orientation phase combined hospital-based and home-based exercise. Participants attended one supervised session per week at the hospital, guided by a physical therapist, to ensure effective exercise and safety. They were then required to continue the exercises at home according to the protocol guidelines. This phase also allowed gradual titration of exercise intensity to ensure tolerance and to achieve the individualized target prescription.

Phase 2 (Weeks 5–16, Home-Based):

The subsequent 12 weeks consisted of a fully home-based aerobic training program, with a total exercise dose equivalent to 150 minutes per week.

The detailed exercise protocol is outlined in Table 3, while the specific aerobic exercise prescription is provided in Table 4.

Table 3 Hospital-based and Home-based Exercise protocol

Home based exercise protocol:

- **Weekly Hospital Visits**

Participants attended the hospital once per week.

- **Exercise Instruction**

At each visit, physical therapists instructed participants on how to perform the prescribed exercises correctly and safely at home. The first four weeks served as a titration phase, allowing exercise intensity to be gradually adjusted to reach the target prescription.

- **Safety supervision**

Physical therapists closely observed participants during supervised sessions for complications such as severe dyspnea, chest pain, dizziness, or falls. If any severe complication occurred, exercise was discontinued immediately. Participants also received counseling on how to manage potential adverse events.

- **Vital signs monitoring**

Vital signs, including blood pressure (BP), heart rate (HR), respiratory rate (RR), and

arterial oxygen saturation (SpO₂), were measured before, during, and after each exercise session. In addition, participants were instructed to measure their heart rate manually by finger palpation immediately after home exercise sessions to monitor exercise intensity.

- **Exercise Intensity Assessment**

Participants reported their Rating of Perceived Exertion (RPE) before and after each session to assess dyspnea and ensure exercise intensity remained moderate intensity.

Hospital based exercise protocol:

- **Exercise guidance**

Participants received an instructional brochure outlining the prescribed exercises on how to properly and safely perform the home-based exercises according to the protocol and clear indications for when to stop exercising (e.g., chest pain, dizziness, severe dyspnea).

- **Logbook (Diary)**

Each participant was required to record the duration of every exercise session, manually measured heart rate (HR) counts, and their Rating of Perceived Exertion (RPE).

- **Weekly follow-up**

The researcher contacted participants once per week (via telephone or messaging application) to monitor compliance, address possible difficulties or doubts or document any adverse effects related to exercise

Table 4 Aerobic Exercise Prescription

- **Form (Type):** Brisk walking
- **Intensity:** Moderate intensity, determined by
 - **Rating of Perceived Exertion (RPE; Borg 6–20 scale):**
Target 12–15 (“moderate” to “somewhat hard”)
- **Duration and frequency**
 - **Week 1:** Exercise for 15 minutes per day, 5 days per week
 - **Week 2:** Exercise for 20 minutes per day, 5 days per week
 - **Week 3 onward:** Exercise for 30 minutes per day, 5 days per week
- **Warm-up and cool-down:** All participants performed warm-up and cool-down sessions for 10 minutes before and after each exercise session.

Compliance Criteria:

- Those who complete at least 80% ($\geq 1,820$ minutes) were classified as having **good compliance**.
- Participants in the intervention group who complete less than 80% of the total prescribed exercise duration (calculated as exercise duration per session \times number of sessions = 2,275 minutes in total; 80% = 1,820 minutes) were classified as having **poor compliance**.
- Participants in the intervention group who complete less than 50% of the total prescribed exercise duration (calculated as exercise duration per session \times number of sessions = 2,275 minutes in total; 50% = 1,138 minutes) were classified as having **very poor compliance**.

3.6.3 Drop-out criteria

Participants were withdrawn from the study under the following conditions:

- 1) Medication initiation or adjustment

- Initiation or dose adjustment of antihypertensive drugs, including angiotensin-converting enzyme inhibitors (ACEIs), angiotensin-receptor blockers (ARBs), diuretics, or aldosterone antagonists.
 - Initiation of specific treatments for cirrhosis, such as liver transplantation or transjugular intrahepatic portosystemic shunt (TIPS).
- 2) Serious complications
- Development of severe exercise-related complications in the intervention group.
 - Development of severe cirrhosis-related complications in either the intervention or control group.
- 3) Control group protocol violation
- Participants in the control group who increased their physical activity beyond sedentary thresholds, defined as: Performing moderate-intensity activity ≥ 150 minutes/week, or Performing vigorous-intensity activity ≥ 75 minutes/week.
- 4) Intervention group - very poor compliance
- Participants in the intervention group who performed $< 50\%$ of the prescribed total aerobic exercise duration.

7 Data collection

7.1 Baseline Characteristics

1. Demographic and anthropometric data: sex, age, comorbidities, current medications, body weight, height, and body mass index (BMI).
2. Etiology and severity of cirrhosis: determined by the underlying cause of liver cirrhosis and severity assessed using the Model for End-Stage Liver Disease (MELD) score and the Child–Turcotte–Pugh (CTP) score.
3. Laboratory investigations: complete blood count (CBC), liver function tests (LFTs), coagulation profile, fasting blood sugar, albumin, blood urea nitrogen (BUN), serum creatinine (Cr) and serum ammonia.
4. Physical performance: assessed using the 6-minute walk test (6MWT), in which participants are instructed to walk as far as possible in 6 minutes (without running), and the distance covered is recorded.

5. Muscle strength: assessed by handgrip strength using a Takei™ Hand Grip Dynamometer. Participants performed the test twice with their dominant hand, and the average value was recorded.
6. Dietary assessment: A 3-day food record (2 weekdays and 1 weekend day) was collected to evaluate daily caloric intake and protein intake.

7.2 Primary Outcomes assessment

Primary outcomes were assessed by blinded evaluators who were unaware of participants' group allocation. All measurements were conducted in the morning in a temperature-controlled room (25 °C). Participants were instructed to avoid caffeinated or alcoholic beverages, stimulants such as energy drinks, and smoking for at least 12 hours before testing and abstain from food intake for at least 8 hours before testing. They were also asked to stop physical activity or exercise for at least 24 hours prior to assessment.

7.2.1 Autonomic nervous system (ANS) function

7.2.1.1 Ewing autonomic battery test (3)

Before testing, the procedures were explained in detail to each participant. They were allowed to rest for at least 5 minutes prior to the start of testing and again between each test to minimize carryover effects. The complete assessment protocol required approximately 30–45 minutes. Tests included measurement of heart rate (HR) responses using a three-lead electrocardiogram (ECG) and blood pressure (BP) responses using a BP monitoring device (Figure 3)



Figure 3: Comprehensive tests that assess heart rate (HR) and blood pressure (BP) response assessments. The HR tests (upper row) include HR response to the Valsalva maneuver (A), HR response to deep breathing (A), and HR response to standing (30:15 ratio) (B). The BP tests (lower row) include postural systolic blood pressure response and diastolic blood pressure response to sustained handgrip.

1) Parasympathetic Function Tests (HR response):

- **HR response to standing:** After resting supine for 5 minutes, HR was recorded at baseline and again at 6 seconds after standing. HR was then measured at 35 seconds post-standing. The ratio of the R–R interval at the 30th beat to the 15th beat (30:15 ratio) was calculated.
- **HR response to deep breathing:** After resting for 5 minutes in a sitting position, participants performed paced breathing at 6 breaths/min (5 seconds inspiration, 5 seconds expiration). HR was continuously monitored over 30 seconds, with maximum and minimum R–R intervals for each cycle recorded. The average difference between maximum and minimum HR across cycles was calculated.
- **HR response to Valsalva maneuver:** After 5 minutes of seated rest, participants blew into a mouthpiece connected to a pressure gauge, maintaining 40 mmHg pressure

for 15 seconds. HR changes were analyzed, and the Valsalva ratio was calculated as the longest R–R interval divided by the shortest R–R interval.

2) Sympathetic Function Tests (BP response):

- **Postural systolic BP (SBP) response:** After resting supine for 5 minutes, SBP was measured every minute for 5 minutes; the mean SBP at minutes 4 and 5 was defined as the lying SBP. Participants then stood up quickly (within 3–5 seconds), and SBP was measured immediately and again at 1 minute. The lowest standing SBP was recorded, and the difference from the lying SBP was calculated.
- **Diastolic BP (DBP) response to sustained handgrip:** Participants first performed a maximal voluntary handgrip with their dominant hand. After 5 minutes of seated rest, resting DBP was measured every minute for 5 minutes, and the average was defined as baseline DBP. Participants then sustained handgrip at 30% of maximum strength for 3 minutes, during which BP was recorded each minute. The maximum DBP during this period was compared with the average baseline DBP, and the difference was calculated.

7.2.1.2 Heart rate variability (HRV) test

HRV was measured while participants sat quietly at rest in a relaxed state for approximately 15 minutes. Three chest electrodes were attached to record electrocardiographic (ECG) signals, which were transmitted to the Adinstruments™ system for analysis (4) (Figure 4).

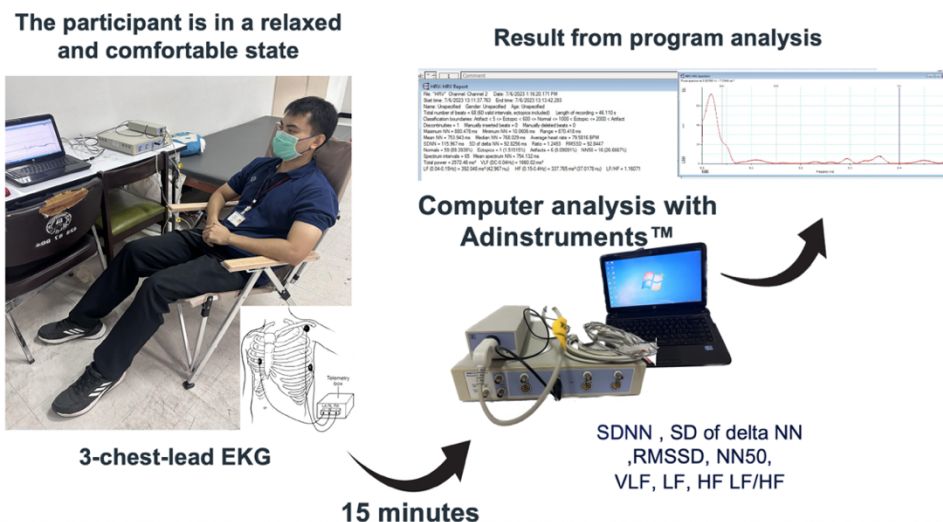


Figure 4 Heart rate variability (HRV) test: Continuous ECG signals were recorded from three chest leads for 15 minutes while the participant remained at rest. The data were subsequently analyzed using ADInstruments™ LabChart software with the HRV Module, which calculates both time-domain and frequency-domain parameter

Instantaneous R–R intervals (NN intervals) were calculated, and both time-domain and frequency-domain parameters were obtained simultaneously. The ECG recording was also reviewed for non-sinus ectopic beats, which were excluded from the HRV analysis.

- **Time-domain parameters:**

- Standard deviation of all normal-to-normal intervals (SDNN)
- Root mean square of successive differences (RMSSD)
- Number of pairs of successive NN intervals differing by more than 50 ms (NN50)

- **Frequency-domain parameters:**

- Very low frequency power (VLF; 0.003–0.04 Hz)
- Low frequency power (LF; 0.04–0.15 Hz)
- High frequency power (HF; 0.15–0.40 Hz)
- LF/HF ratio

7.2.2 Peripheral endothelial function test

7.2.2.1 Flow mediated dilation (FMD) test (5)

On the same day as the ANS tests, participants rested for at least 15 minutes before undergoing FMD measurement. High-resolution ultrasound imaging in B-mode with a linear probe was used to measure the brachial artery of the non-dominant arm, approximately 5–10 cm above the antecubital fossa, with participants in the supine position. A blood pressure cuff was placed just above the olecranon of the measured arm and inflated to approximately 200 mmHg (or at least 50 mmHg above systolic BP) for 5 minutes. Arterial diameter and blood flow velocity were recorded from 1 minute before cuff inflation until 3 minutes after cuff release. The greatest change in vessel diameter and blood flow (peak arterial diameter and flow) after cuff release was used to calculate FMD. Measurements were analyzed using Cardiovascular Suite™ software (6). (Figure 5)

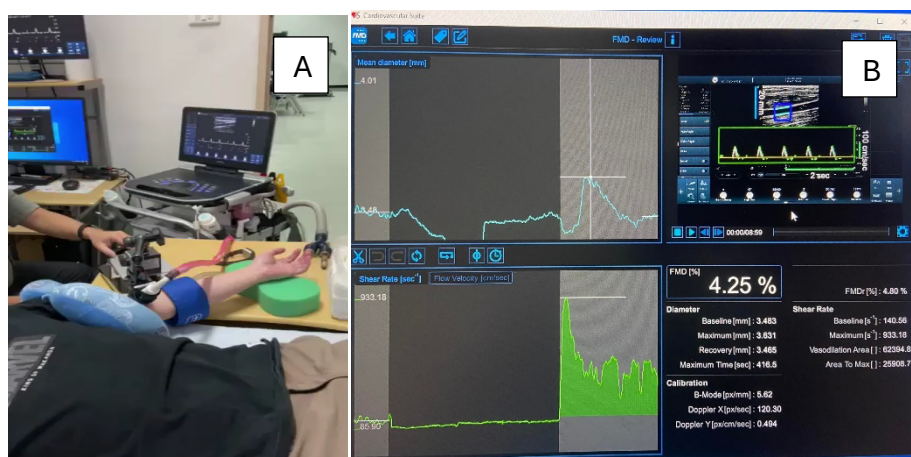


Figure 5 Flow-mediated dilation (FMD) test: (A) Ultrasound measurement of brachial artery diameter is performed at baseline, followed by occlusion with a blood pressure cuff inflated to 200 mmHg (or ≥ 50 mmHg above systolic BP) for 5 minutes. After cuff release, the reactive increase in arterial diameter and blood flow was recorded to calculate the FMD value; (B) Cardiovascular Suite™ software was used to analyze vessel wall changes and calculate the FMD results.

7.3 Secondary outcomes assessment

Secondary outcomes focused on splanchnic hemodynamics, physical performance, muscle strength, and body composition. These assessments were performed at baseline and at the end of the 16-week intervention period.

7.3.1 Splanchnic Hemodynamics (Doppler Ultrasound)

All Doppler ultrasound examinations were performed by a single radiologist. A transcutaneous ultrasound duplex system (Vivid IQ, GE Healthcare) equipped with a curvilinear phased-array transducer was used. The system included a real-time sector scanner operating at 3.5 MHz and a pulsed Doppler flowmeter at 3.0 MHz. The insonation angle between the ultrasound beam and the longitudinal axis of the vessel was maintained at ≤ 60 degrees, guided by B-mode imaging. Participants were positioned in the supine position and instructed to perform brief breath holds during measurements to minimize respiratory motion artifacts (7).

Vessel Identification

1. **Celiac Artery (CA):** The CA was assessed to evaluate blood flow to the upper abdominal organs within the splanchnic circulation. The transducer was placed in the epigastric region using a sagittal view to visualize the origin from the aorta. A transverse view was used to confirm the characteristic “T-shaped” branching pattern of the CA.
2. **Superior Mesenteric Artery (SMA):** The SMA was evaluated to assess mid-abdominal splanchnic blood flow. Measurements were obtained with the transducer placed slightly above the umbilicus in a sagittal plane. The SMA was identified as it emerged anteriorly from the aorta.
3. **Portal Vein (PV):** The PV was examined to assess portal venous inflow to the liver. A right subcostal approach was used to visualize the PV at the confluence of the splenic vein and superior mesenteric vein. Measurements were taken proximal to the bifurcation into the left and right portal branches.

Doppler Ultrasound Measurements of splanchnic hemodynamics

For CA and SMA Doppler assessments, the following hemodynamic parameters were automatically calculated by the ultrasound system, averaging 2–3 cardiac cycles(8):

1. **Peak Systolic Velocity (PSV)** – maximum velocity during systole (cm/s)
2. **End-Diastolic Velocity (EDV)** – velocity at end-diastole (cm/s)
3. **Time-Averaged Mean Velocity (TAMV)** – mean velocity over the entire cardiac cycle (cm/s)

Vessel diameter (d) was measured using electronic calipers placed perpendicular to the vessel walls.

Calculation of Blood Flow

Blood flow was calculated using the formula:

$$\text{Blood Flow} = \text{TAMV} \times \pi \times d^2 \div 4$$

For the CA and SMA, PSV, EDV, and TAMV were obtained from the spectral Doppler waveform (averaged over X cardiac cycles). The vessel diameter was measured at end-diastole and used to calculate cross-sectional area for blood flow estimation.

For the PV, measurements were obtained during end-expiration (or breath hold) to minimize respiratory variability (9).

7.3.2 Physical performance: Six-Minute Walk Test (6MWT)

Physical performance was evaluated using the Six-Minute Walk Test (6MWT), performed in accordance with American Thoracic Society (ATS) guidelines (10). The test was conducted on a flat, straight course of 15 meters in length. Participants were instructed to walk as far as possible for six minutes at a self-paced speed. The total distance covered was recorded in meters.

7.3.3 Muscle Strength: Handgrip Dynamometer

Upper limb muscle strength was assessed using a Takei™ Hand Grip Dynamometer. Participants were seated comfortably with their elbow flexed at 90 degrees. Measurements were taken from the dominant hand. Participants were instructed to squeeze the dynamometer with maximum isometric effort. The measurement was performed twice, and the average value of the two trials was calculated and recorded in kilograms (kg).

7.3 Body Composition Analysis

(1) Bioelectrical Impedance Analysis (BIA): Participants were assessed using the InBody 720™ body composition analyzer. The assessment required the participant to stand motionless

on the device platform with bare feet, holding the hand electrodes, for approximately 1 to 2 minutes to allow for multi-frequency impedance measurement

(2) Dual-Energy X-ray Absorptiometry (DXA): A whole-body DXA scan was performed using the Hologic™ Horizon W machine. Participants were positioned supine (lying flat) and motionless on the scanning table for approximately 10 minutes while the scan was acquired

8. Data Analysis and Statistics

All analyses were performed using STATA version 15.0, with statistical significance set at $p < 0.05$.

1) Baseline characteristics

- Quantitative data (e.g., age, BMI) was presented as mean \pm standard deviation (SD).
- Qualitative data (e.g., sex, comorbidities) was presented as frequencies and percentages, or described narratively where appropriate.

2) Outcome analysis

- **Qualitative data:** Complications or adverse events occurring during the study were compared between the intervention and control groups to assess differences in incidence and potential effects of the intervention.
- **Quantitative data (continuous variables):**
 - **Correlation analysis:**
 - Pearson's correlation for normally distributed data.
 - Spearman's correlation for non-normally distributed data.
 - **Between-group comparisons (pre- vs post-intervention):**
 - Unpaired t-test for normally distributed data.
 - Mann–Whitney U test for non-normally distributed data.
 - **Within-group comparisons (pre- vs post-intervention):**
 - Paired t-test for normally distributed data.
 - Wilcoxon signed-rank test for non-normally distributed data.
 - **Repeated-measures analysis:**
 - General linear mixed models (LMM) was applied to account for repeated measures over time.

3) Analysis approaches

Three analytical approaches were utilized to assess the effects of the intervention:

(1) Intention-to-treat (ITT): All randomized participants were included in the analysis.

For participants lost to follow-up, missing outcome data were imputed using baseline values to preserve randomization and minimize potential bias.

(2) Modified intention-to-treat (mITT): This approach included all participants who completed the follow-up assessment, regardless of their adherence to the exercise protocol.

(3) Per-protocol (PP): This analysis included only participants who demonstrated good compliance with the exercise regimen and completed all required assessments, allowing evaluation of the intervention effect under optimal adherence conditions.

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