

**The Effect of Intensive and Moderate Exercise on Nonalcoholic Fatty
Liver Disease
(A randomized, Open-label, Controlled Trial)**

STUDY PROTOCOL

The First Affiliated Hospital of Xiamen University

Xiamen Diabetes Institute

Shanghai Institute of Endocrinology and Metabolism

ABBREVIATIONS

ABI: Ankle brachial index

CRF: Case report form

cIMT: Carotid artery intima-media thickness

CT: Computer tomography

CVD: Cardiovascular disease

DEXA: Dual energy X-ray assessment

DSMB: Data and Safety Monitoring Board

ECG: Electrocardiogram

GFR: Glomerular filtration rate

HDL-C: High-density lipoprotein cholesterol

HOMA- β : Homeostasis model assessment- β cell function

HOMA-IR: Homeostasis model assessment-insulin resistance

IHTG: Intrahepatic triglyceride content

LDL-C: Low-density lipoprotein cholesterol

METs: Metabolic equivalents

MRS: Magnetic resonance spectrometry

NAFLD: Nonalcoholic fatty liver disease

PWV: Pulse wave velocity

TC: Total cholesterol

TG: Triglyceride

1. Background and Significance

Non-alcoholic fatty liver disease (NAFLD) has become one of the most common public health problems. The prevalence of NAFLD is high and increasing. It affects 10-45% of the general population and 60-70% of obese adults in Western countries.^{1,2} NAFLD represents excessive accumulation of triglycerides (TG) in the liver tissues. Due to synthesis of triglycerides in hepatocytes, excessive triglycerides are stored in the liver and lead to hepatic steatosis. It can progress to steatohepatitis, cirrhosis, or even liver cancer.³⁻⁵ Furthermore, NAFLD is considered a risk factor for type 2 diabetes and cardiovascular disease.⁶

Diet, sedentary lifestyle, and genetic predisposition have been associated with increased risk of NAFLD.⁶ With the progressive global epidemic of obesity, it is expected that the prevalence of NAFLD will rapidly increase. Previous studies indicated that obesity was a major risk factor for NAFLD, and the prevalence of NAFLD among subjects with BMI>30 kg/m² was four times higher than that among normal-weight subjects⁷. Obesity is closely related to insulin resistance. Furthermore, it is associated with hyperinsulinemia and high inflammatory cytokine levels, which can promote the development of hepatic steatosis⁸. On the other hand, NAFLD is considered to be a hepatic manifestation of metabolic syndrome, and is an independent risk factor for diabetes and cardiovascular disease.⁹⁻¹² Patients with NAFLD have an adverse proatherogenic lipid profile with elevated small dense LDL levels and reduced HDL levels, increased levels of inflammatory cytokines, and altered cardiac metabolism.¹³ Therefore, seeking an optimal treatment for NAFLD and related metabolic disorders has become a hot topic for researchers.

Currently, evidence-based treatment options for NAFLD are lacking. A number of studies suggest that insulin-sensitizing agents, antioxidants, lipid-lowering drugs, anti-obesity medication, and anti-hypertensive drugs might improve NAFLD and related metabolic disorders.¹⁴⁻¹⁹ For example, recent data suggested that the thiazolidinedione class of insulin sensitizers might improve NAFLD. However, due to small sample sizes in the previous studies and a lack of high-quality randomized controlled trials (RCTs), there is limited evidence to support that those agents are safe and effective for patients with NAFLD.^{20,21} In addition, side effects and safety concerns prevent these agents from being extensively utilized in clinical practice. On the other hand, there is an increasing interest in lifestyle interventions as an effective and cost-effective treatment for NAFLD.¹⁴

Lifestyle interventions play a role in improving NAFLD by reducing weight and insulin resistance. Some studies have suggested that lifestyle interventions could reduce hepatic fat accumulation and serum liver enzyme levels when achieving a 6.5 -10% weight loss.^{22,23} Other studies have indicated that diet interventions have a significant effect on NAFLD only when more than 7% weight loss was achieved.^{14,24} A large amount of evidence supports that weight loss, achieved through diet and exercise, plays a key role in improving NAFLD. Furthermore, physical exercise, a crucial component of behavioral interventions, has been shown to be effective in reducing serum liver enzyme levels and hepatic lipid content, independent of weight loss^{25,26}. A recent study showed that four weeks of aerobic exercise at 50-70% maximum oxygen consumption reduced hepatic triglyceride concentration by 21% in obese individuals, independent of weight loss²⁷. However, the effect and optimal dose of exercise for improving fatty liver disease have not yet been determined.

Some clinical studies support that vigorous exercise intensity could reduce hepatic fat content.^{28,29} However, because of their small sample size and short-term duration, the evidence from clinical studies is inconclusive. There is no evidence from clinical trials to determine the long-term effects of exercise on NAFLD. Intensive physical activity is usually adopted, but it is difficult for obese individuals to adhere to vigorous intensity exercise. In fact, obese individuals are more prone to adopt moderate intensity exercise, but the effect of moderate intensity exercise on NAFLD has not been established. In addition, the efficacy of current physical activity guidelines (150 minutes of moderate intensity activity per week) recommended by the Department of Health and Human Services, the American College of Sports Medicine, and the American Heart Association in reducing liver enzyme levels and hepatic fat content is uncertain. Therefore, the optimal dose, intensity, and mode of exercise for improving NAFLD need to be tested in future clinical studies.

2. Specific Aims

In the proposed study, we aim to identify an optimal level of exercise intensity, which is practical and effective in improving fatty liver disease and relevant metabolic disorders. We propose to conduct a 12-month randomized controlled trial to determine the effect of intensive exercise and moderate exercise on NAFLD and metabolic risk factors in obese subjects. We hypothesize that both intensive and moderate exercise would improve NAFLD as compared with no-exercise control, and that intensive exercise would be more effective than moderate exercise in improving NAFLD and metabolic risk factors.

The primary specific aims are:

- 2.1. To test the effect of moderate exercise (brisk walking 150 minutes/week at 45-55%

maximum heart rate) over 6 months and 12 months on changes in intrahepatic triglyceride content compared to no-exercise control;

- 2.2. To test the effect of intensive exercise (jogging 150 minutes/week at 65-80% maximum heart rate) over 6 months on changes in intrahepatic triglyceride content compared to no-exercise control and moderate exercise;
- 2.3. To test the effect of transitioning from intensive exercise to moderate exercise over 6 months on changes in intrahepatic triglyceride content.

The secondary specific aim is:

- 2.4. To compare the effect of intensive exercise, moderate exercise, and no-exercise control on body weight, waist circumference, fat composition, and metabolic risk factors.

3. Research Design and Methods

3.1. Overall designs

This study is a randomized, open-label, controlled clinical trial. After screening, all eligible subjects will be randomly assigned to the intensive/moderate exercise group, moderate exercise group, and control group (Figure 1). A total of 216 participants will be enrolled. The intensive/moderate exercise group will receive the intensive exercise intervention for six months, followed by moderate exercise for six months. The moderate exercise group will receive the moderate exercise intervention for 12 months. In addition, all participants in intensive exercise, moderate exercise, and no-exercise control will receive identical health education for one year (Figure 2). The study will be conducted in a community-based health center in Lianqian District, Xiamen City, China.

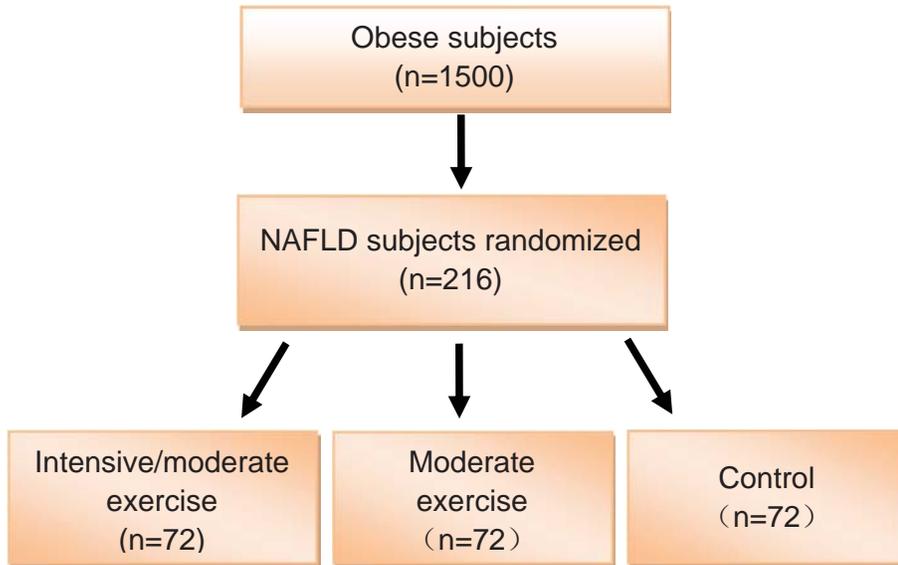


Figure 1. The schedule for screening

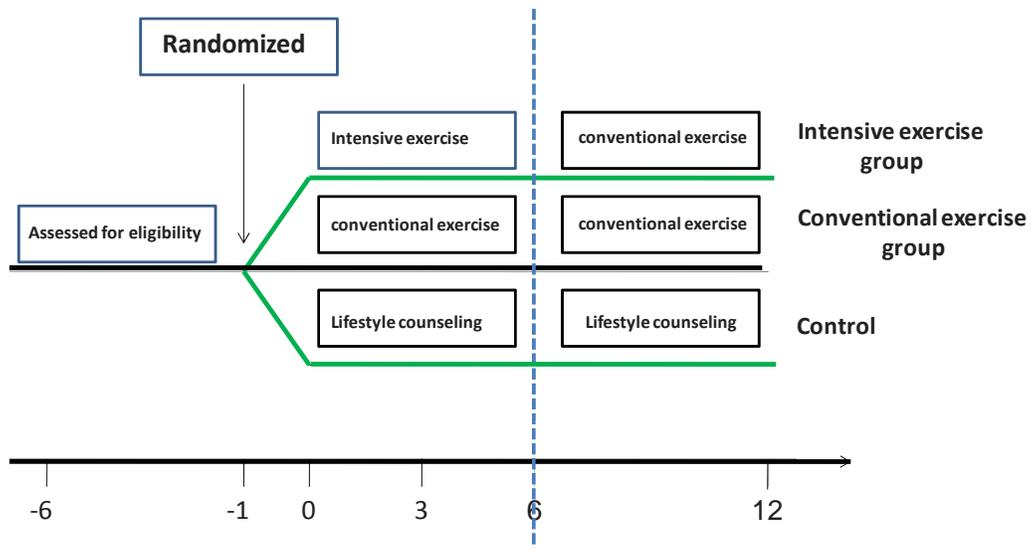


Figure 2. The procedure for the exercise intervention

3.2 Statistical power and sample size

The study will enroll 216 participants, who will be randomly allocated to three groups with an equal number of individuals per group.

- No-exercise control
- Moderate exercise

- Intensive/moderate exercise (vigorous-intensity exercise followed by moderate-intensity exercise)

The primary outcome (intrahepatic triglyceride content) will be compared among three groups at 6 months and 12 months. The sample size calculation was based on the following assumptions:

- Statistical power 90%
- Significance level of 0.0083 (0.05/6 for the Bonferroni correction of multiple comparisons) using a 2-tailed test
- Detectable effect size of intrahepatic triglyceride content = 1.76%^{27,29}
- Standard deviation of intrahepatic triglyceride content = 2.35%^{27,29}

Thus, 57 subjects in each group are required. Assuming a dropout rate of 20%, we will recruit 216 participants and randomly allocate 72 subjects to each group in the proposed clinical trial.

4. Inclusion and Exclusion Criteria

4.1. Inclusion criteria

- Subjects with NAFLD determined by ¹H MRS (intrahepatic triglyceride content $\geq 5\%$);
- 40-65 years old;
- Waist circumference >90cm for men and >85cm for women.³⁰

4.2. Exclusion criteria

- Consumed more than an average of 140 grams of ethanol (10 alcoholic drinks) per week in men and 70 grams of ethanol (five drinks) in women during the past six

months;

- A history of acute or chronic viral hepatitis, drug-induced liver diseases, and autoimmune hepatitis;
- Myocardial infarction in the past six months;
- Biliary obstructive diseases;
- Uncontrolled hypertension (i.e. systolic BP>180 mmHg, and/or diastolic DBP>100 mmHg);
- Chronic kidney disease (serum creatinine ≥ 1.5 mg/dL in men and ≥ 1.3 mg/dL in women);
- Heart failure (New York Heart Association III or IV);
- Currently participating in weight loss programs;
- Currently pregnant or planning to be pregnant;
- Having any medical condition that would affect metabolism (i.e. diabetes, known hyperthyroidism or hypothyroidism);
- Having a medical condition that would limit exercise participation and taking medication that would affect metabolism or weight loss (i.e. thyroid medication and glucocorticoids) or would alter the heart rate response during exercise (i.e. β -blockers);
- Unable to participate in the follow-up examination.

5. Recruitment

Participants will be recruited from the Lianqian District, Xiamen City, China, from December, 2011 to December, 2012. A total of 1500 adults aged 40-65 year old with central

obesity (waist circumference greater than 90 cm for men and 85 cm for women) from previous community screening programs will be invited to attend a screening abdominal ultrasound examination at the study clinic. Those who have ultrasound-diagnosed nonalcoholic fatty liver disease will be invited to confirm their diagnosis by proton magnetic resonance spectroscopy (intrahepatic triglyceride content $\geq 5\%$) at the First Affiliated Hospital of Xiamen University. Once a patient is confirmed to be eligible, the physician will review the study procedures and requirements with the patient and discuss his or her commitment to the study and acceptance of randomization. Written informed consent will be obtained from each participant, and a baseline evaluation will be performed at the next visit, which is immediately scheduled by the physician assistant.

6. Randomization and masking

6.1. Randomization

Randomization will be conducted by the Epidemiology Research Unit at the First Affiliated Hospital of Xiamen University, China. Patients will be randomly assigned to moderate exercise, intensive/moderate exercise, or no-exercise control using a block design. The randomization scheme is generated and concealed until an eligible participant is ready to be randomized. Prior to randomization, the study coordinator should confirm that all screening procedures have been completed, the participant meets all eligibility criteria, and all required baseline data have been collected. Any individual lacking required documents and data will not be randomized.

6.2. Masking

Although this is an open-label trial, the research assistants who collect study outcome

data will be masked to participants' intervention assignment. In addition, the adjudicators for end-points will not be aware of study-group assignments.

7. Intervention

This trial aims to test the effect of different exercise intensities on the improvement of NAFLD and metabolic risk factors. The duration of the intervention is one year.

7.1. Intervention programs

A. Intensive exercise

The participants in the intensive/moderate exercise group will engage in vigorous-intensity exercise for 6 months, and subsequently moderate physical activity for another 6 months.

A1. Mode of exercise: Running on treadmill (-1 to 6 months)

A2. Exercise intensity: The participants in the intensive exercise group will engage in vigorous-intensity exercise training at 65-80% of their maximum predicted heart rate (8.0-10.0 METs) 30 minutes a day and 5 days a week. The maximum predicted heart rate was calculated as 220 (210 for women) minus the subject's age. Intensive exercise will take place in a gym of a local health center and will be supervised by study staff. Exercise intensity and duration will be recorded. The heart rate will be monitored throughout the exercise sessions by nurses and the target heart rate will be adjusted based on exercise capacity.³¹ The intensive exercise program starts with 2-4 week training sessions (-1 to 0 month) to prepare participants. Initially, the participants will run on a treadmill for 15-30 minutes at a heart rate equivalent to 45-50% of their maximum predicted heart rate. The duration and intensity will be progressively increased at the end of the first 2-4 weeks to 30 minutes of vigorous intensity

exercise at 65-80% of their maximum predicted heart rate per day and 5 days a week.

Compliance with exercise intensity will be assessed by recording the participant's heart rate throughout the exercise sessions.

A3. Exercise place: The Gym of Lianqian Community Health Center, Xiamen, China.

B. Moderate exercise

B1. Mode of exercise: Brisk walking (7-12 months in intensive/moderate exercise group and -1 to 12 months in moderate exercise group)

B2. Exercise intensity: The intensity of moderate exercise will be set at approximately 45-55% of their maximum predicted heart rate. Participants will take brisk walks (120 steps a minute) for 30 minutes per day and five days a week. A target of at least 10,000 steps each day will be set for participants engaging in moderate exercise. Daily physical activity will be recorded using a pedometer and evaluated by nurses every week.

B3. Exercise place: Parks or sidewalks in or near the participants' communities.

C. Health education

The control group will not change their usual physical activity habits. They will receive the health education that the other two exercise intervention groups will also receive throughout the intervention session. Health education includes a meeting for health behavior education every two weeks in the first 6 months and every month during months 7-12. In addition, participants will receive a phone call every two weeks from the study team throughout the duration of the study. Food consumption will be recorded for three continuous days, including total energy intake and macronutrient composition for all listed foods³².

Compliance with the study protocol will be reviewed every two weeks.

7.2. Intervention monitoring and quality control

In the intensive exercise group, all exercise sessions will be supervised by research staff. The exercise intensity, duration, heart rate and blood pressure before and after exercise, and adverse events during exercise will be recorded by study staff. In the moderate exercise group, participants will be instructed to record their exercise every day in a log and return it to the study team every week. In order to increase compliance, pedometers will be provided to the participants receiving the moderate exercise intervention by the study team. Study staff will call participants twice weekly to assess their compliance and provide advice on adherence to the exercise program. Participants will be required to have an extra week of exercise if they are unable to complete 4 sessions of exercise in any week.

8. Study Outcomes

8.1 Primary outcome:

Changes in intrahepatic triglyceride content from baseline at six months and 12 months after intervention.

8.2 Secondary outcomes:

- 1). Changes in body weight, waist circumference, and body fat composition
- 2). Changes in metabolic risk factors
- 3). Change in blood pressure, lipids, and glucose
- 4). Changes in insulin resistance and beta cell function
- 5). Changes in urinary albumin and kidney function
- 6). Change in carotid intima-media thickness and pulse wave velocity

9. Participant Termination and Retention

9.1 Termination criteria

- 1). Adverse events or other unexpected reasons (e.g., fracture, myocardial ischemia, etc.)
- 2). Inability to complete required exercise program (i.e., exercise intensity or duration <80%)
- 3). Unwillingness to be followed-up

9.2 Study Participants Retention

The study participant retention is critically important and every effort will be made to increase participants' adherence to their intervention and follow-up visit schedule. At weekly staff meetings, recruitment and retention will be discussed. Visits will be scheduled at the convenience of the patients. Personalized birthday, holiday, and anniversary cards will be sent to the participants. Small gifts will be used to improve participants' connection to the study and research staff.

10. Data collection

10.1 Measurements

- 1). Medical history: personal information (age, gender, marital status, occupation, education, household income, etc.), medical history including medications, and lifestyle risk factors (cigarette smoking, alcohol drinking, physical activity, and dietary habits).
- 2). Anthropometric measurements: height, weight, waist circumference, hip circumference, and blood pressure.
- 3). Biochemical measurements: fasting plasma glucose, 2-hour post-load plasma glucose, serum triglycerides, total cholesterol, LDL- and HDL-cholesterol, liver enzymes, serum creatinine, and urine albumin.
- 4). Insulin sensitivity: HOMA-insulin resistance, HOMA- β , insulin secretion index ($\Delta 30\text{Ins} / \Delta 30\text{Glu}$).

5). Body fat composition: body fat composition determined by dual energy X-ray assessment (DEXA) and visceral fat by CT scanning.

(6) Other measurements: pulse wave velocity (PWV) by automatic arteriosclerosis analyzer, carotid intima-media thickness by ultrasound.

10.2. Methods

A. Questionnaire

- **Demographic information:** A self-report demographic questionnaire will be used to collect participant characteristics at baseline, including age, race/ethnicity, socioeconomic status (employment, education, and income), internet usage, and health status.
- **Medical history:** Medical history including medication use will be self-reported by participants at the screening visit and subsequent follow-up visits. The medical recordings will be reviewed at times to ensure all the data are complete.
- **Diet:** Three 24-hour dietary recalls will be conducted at baseline and during follow-up visits. Nutrient intake will be calculated using the China Food Composition table.
- **Physical activity:** Physical activity will be recorded at each visit using the International Physical Activity Questionnaire, which allows for calculations of self-reported leisure time and work-related physical activity, and kilocalorie expenditure per week³³

B. Clinical examination

B1. Weight, waist circumference, and blood pressure

Height will be measured to the nearest 0.5 cm at baseline and follow-up visits using a wall-mounted stadiometer. Body weight will be measured to the nearest 0.1 kg in duplicate at each visit using a digital scale with participants dressed in light indoor clothes and without shoes. A gulick tape measure will be used to obtain duplicate measurements of waist and hip girths to the nearest 0.1 cm. Blood pressure will be measured in triplicate at each visit using

the Omron HEM-907-XL automated sphygmomanometer. Participants will avoid exercise, smoking, coffee or tea for 30 minutes. Participants will be seated quietly for 5 minutes before measurement.

B2. Oral glucose tolerance test (OGTT)

Participants will fast for at least 10 hours before the test. After a fasting blood sample is drawn, participants will drink 75g glucose in 250-300 ml water within 5 minutes. Blood samples will be drawn at 30' and 120' after glucose loading to measure glucose and insulin.

B3. Body fat composition

The bioelectrical impedance method will be used to measure body fat content using the Human Body Composition Analyzer BC-420 (Tanita, Japan).

B4. Visceral and subcutaneous fat area

Visceral and subcutaneous fat will be measured using an abdominal CT scan (Siemens Medical Systems, Germany). Participants will lie in supine position and be scanned by single transected CT at the level of the navel in breath-holding status.³⁴

B5. Hepatic fat content

3.0-T Siemens magnetic resonance (Siemens Verio, Germany) will be used to measure intrahepatic triglyceride content. The spectrum line will be analyzed using special spectrum processing software (Syngo spectroscopy VB15, Siemens AG). Intrahepatic triglyceride content = 100% × area under curve of lipid peak / area under curve of the sum of water and lipid peak.

B6. Brachial ankle pulse wave velocity (PWV)

We will use an automatic arteriosclerosis analyzer (Omron Corporation, Japan) to measure pulse wave velocity.

C. Biochemical tests

Overnight blood samples and spot urine will be collected to measure metabolic risk

factors (plasma glucose, insulin, triglycerides, total cholesterol, LDL- and HDL-cholesterol, liver enzymes, serum creatinine, and urine albumin) using standard methods at the clinical laboratory of the First Affiliated Hospital of Xiamen University.

11. Baseline and Follow-up Visits

11.1. Screening visit (V0)

A. Questionnaire

- Demographic information: age, gender, ethnicity, education, occupation, and income.
- Medical history: personal history of biliary obstructive diseases, acute or chronic viral hepatitis, drug-induced liver diseases, autoimmune hepatitis, Wilson's disease, cancer, myocardial infarction, hyperthyroidism, hypothyroidism, and diabetes, or having a medical condition or taking medication that would limit exercise participation.
- Medications: lipid-lowering, anti-hypertension, and liver-protection medication (specific drugs dosage and termination time).
- Lifestyle risk factors: alcohol drinking and cigarette smoking.

B. Physical examination

Height, weight, waist circumference, hip circumference, thigh circumference, and blood pressure;

C. Laboratory examination

- Regular blood and urine tests
- Liver enzymes
- Serum triglycerides, total, LDL- and HDL-cholesterol, and plasma glucose
- Electrocardiograph (ECG)

D. Other examinations

- DEXA for body fat content and bone density
- Doppler ultrasonography for carotid artery intima-media thickness

11.2. Randomization visit (V1)

A. Questionnaires

- Assessment of three 24-hour dietary recalls
- Assessment of baseline physical activity (for 5 continuous days recorded by pedometer)

B. Measurements of the primary and secondary outcomes

- Vascular function and anthropometric examination: PWV, ABI, CIMT, and ambulatory blood pressure
- Evaluation of myocardial ischemia by treadmill exercise test
- Measurement of body fat distribution by DEXA
- Measurement of hepatic fat content using MRS
- Measurement of visceral and subcutaneous fat area by abdominal CT

C. OGTT (0, 30, 120min) for plasma glucose and insulin levels.

D. Collection of blood, urine, and stool specimens;

E. Randomization: Participants will be allocated into the intensive exercise group, moderate exercise group, or control group using a computer program.

Intensive exercise group: The participants in the intensive exercise group will start vigorous-intensity exercise training after randomization. The participants will initiate the exercise program by running on a treadmill. Adjustment to exercise intensity will be performed in the first month per protocol. The participants will enter the intervention period

once the set exercise intensity is achieved. Exercise information will be recorded, including date, starting and ending time of each exercise session, heart rate in the first five minutes after exercise, heart rate in the middle of exercise, heart rate after exercise, intensity of exercise, and speed of treadmill.

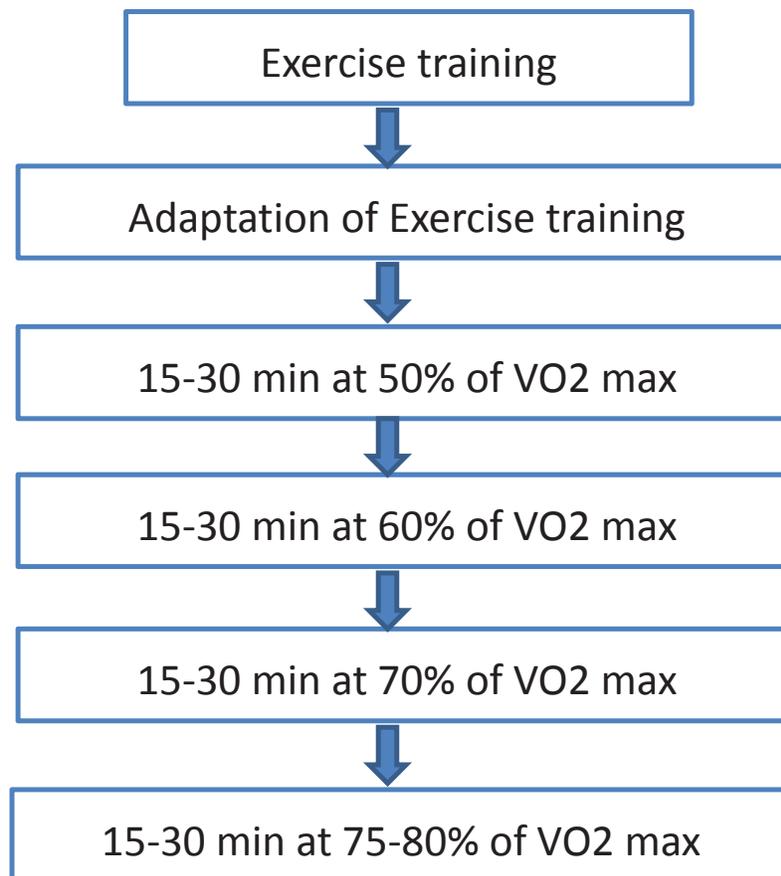


Fig 3. Protocol of exercise intensity and duration titration

Conventional exercise group: Participants will be instructed to take brisk walks for moderate intensity exercise during the adjustment period in the training center. After they complete the training, participants will continue to exercise at the same moderate intensity on sidewalks or in parks in or near their communities. A pedometer will be used to record daily physical activity, which will be reported to the study team every week. Exercise information

will be recorded regarding the date, start time and ending time, exercise duration, and daily step count.

Control group: Group and in-person meetings for health education will be provided to all participants in control, moderate exercise, and intensive exercise groups. Participants in the control group will be instructed to not change their physical activity.

11.3. Month 0 visit (V2)

A. Exercise records will be reviewed.

B. Exercise intensity evaluation and adjustment: The exercise intensity will be calculated as $100\% \times \text{heart rate during exercise} / \text{maximum predicted heart rate}$.

Compliance will be calculated as $100\% \times \text{actual exercise times} / \text{required exercise times}$.

C. Height, body weight, waist circumference, hip circumference, thigh circumference, blood pressure, and heart rate will be recorded at the end of every month.

D. OGTT (0, 30, 120min) for plasma glucose and insulin levels.

11.4. Month 3 visit (V3)

A. Questionnaires

- Assessment of three 24-hour dietary recalls
- Assessment of baseline physical activity (for 5 continuous days recorded by pedometer)

B. Physical examination: height, weight, waist circumference, hip circumference, thigh circumference, blood pressure, and body fat

C. Laboratory examination:

- Routine tests for blood and urine, serum lipids, liver enzymes, urine microalbumin and creatinine;
- Fasting plasma glucose, serum insulin, glycosylated hemoglobin

D. Evaluation and adjustment of exercise intensity.

11.5 Month 6 visit (V4)

A. Questionnaires

- Assessment of three 24-hour dietary recalls
- Assessment of physical activity (for 5 continuous days recorded by pedometer)

B. Physical examination: height, weight, waist circumference, hip circumference, thigh circumference, blood pressure, and body fat

C. Laboratory examination

- Regular blood and urine tests
- Liver enzymes
- Serum triglycerides, total, LDL- and HDL-cholesterol, and plasma glucose
- Electrocardiograph (ECG)

C. OGTT (0, 30, 120min) for plasma glucose and insulin levels;

D. Measurements of the primary and secondary outcomes

- Vascular function and anthropometric examination: PWV, ABI, CIMT, and ambulatory blood pressure
- Evaluation of myocardial ischemia by treadmill exercise test
- Measurement of body fat distribution by DEXA
- Measurement of hepatic fat content using MRS

- Measurement of visceral and subcutaneous fat area by abdominal CT

11.6. Month 12 visit (V5)

A. Questionnaires

- Assessment of three 24-hour dietary recalls
- Assessment of physical activity (for 5 continuous days recorded by pedometer)

B. Physical examination: height, weight, waist circumference, hip circumference, thigh circumference, blood pressure, and body fat

C. Laboratory examination

- Regular blood and urine tests
- Liver enzymes
- Serum triglycerides, total, LDL- and HDL-cholesterol, and plasma glucose
- Electrocardiograph (ECG)

C. OGTT (0, 30, 120min) for plasma glucose and insulin levels;

D. Measurements of the primary and secondary outcomes

- Vascular function and anthropometric examination: PWV, ABI, CIMT, and ambulatory blood pressure
- Evaluation of myocardial ischemia by treadmill exercise test
- Measurement of body fat distribution by DEXA
- Measurement of hepatic fat content using MRS
- Measurement of visceral and subcutaneous fat area by abdominal CT

11.7. Monitoring and quality control

A. Physicians and nurses will receive training on data collection or intervention procedures.

- B. Intensive exercise will be supervised by study staff.
- C. Compliance to moderate exercise will be recorded by a pedometer and accessed by study staff weekly.
- D. Plasma glucose, lipid, and liver enzymes will be determined at the Clinical Laboratory of the First Affiliated Hospital of Xiamen University with stringent quality control.
- E. All study outcomes will be measured by trained staff using a stringent quality control process. Participants will be informed not to eat, smoke, drink, or do any strenuous exercise at least 10 hours before the examination at each visit.

12. Safety and adverse events

12.1. Observation of vital signs and laboratory examinations

All participants will be evaluated for their pulse rate and blood pressure before exercise and will be evaluated for any physical discomfort, including palpitations, chest pain, or other adverse events during exercise. The occurrence, severity, and duration of physical discomfort will be recorded. All participants will receive a screening for undiagnosed cardiovascular disease by undergoing the treadmill exercise test.

12.2. Adverse events

A. Definition of adverse events

All medical adverse events will be recorded and the causal relationship to the exercise intervention will be further evaluated. The relevant adverse events are defined as heart palpitations, chest pain, falls, and hypoglycemia during exercise.

B. Recording of adverse events

Any medical adverse events, including physical discomfort or abnormal

laboratory examinations, will be recorded. Additionally, the nature, severity, and causal-effect relationship will be evaluated by physicians and recorded in a timely manner on case report forms.

C. Definition of a causal-effect relationship

With regard to the causal-effect relationship, medical adverse events are divided into five categories: definite, probable, possible, unlikely, and unrelated. Only "definite" and "probable" are counted as adverse events.

D. Assessment of severity

The severity of adverse events is categorized at three levels as follows:

- Mild: usually temporary and not affecting daily activities.
- Moderate: causing discomfort and affecting daily activities, though tolerable and not requiring participants to take any medication.
- Severe: disrupting daily activities and intolerable, requiring participants to take medication immediately.

E. Treatment

Physicians will observe and record the progress of adverse events and keep track of those participants who have dropped out until the adverse events have completely subsided. Additionally, physicians will evaluate those adverse events for a causal-effect relationship.

12.3. Severe adverse events

A. Definition: According to the definition by the Food and Drug Administration, USA, severe adverse events are defined as any of the following:

- Life-threatening experience

- Death
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect

B. Reporting of severe adverse events

If severe adverse events occur in participants, physicians should immediately provide the appropriate care to ensure their safety. Additionally, physicians will report the adverse events and treatment to the Principal Investigator and the Committee within 24 hours and complete the report form.

Unit	Contacts	Phone number
Xiamen Diabetes Institute, the First Affiliated Hospital of Xiamen University	Zhimin Ma	13395030467
Xiamen Diabetes Institute, the First Affiliated Hospital of Xiamen University	Huijie Zhang	15959238193

13. Drop-outs

13.1. Dropout determination

Participants who withdraw from the trial for any reason will be considered a drop-out.

13.2. Common reasons for dropping out

- Poor compliance with the protocol
- Adverse events
- Lack of efficacy
- Withdraw and quit
- Others

13.3. Dropout management

Physicians should complete the case report form and record the reason for dropping

out. Physicians should also assess the causal-effect relationship of the intervention with adverse events and collect detailed data related to the last exercise session. The data from subjects who have dropped out will be stored and used for intention-to-treat analysis.

14. Ethical requirements

This study has been approved by the Ethics Committee of the First Affiliated Hospital of Xiamen University. The study complies with the declaration of Helsinki and Chinese laws and regulations on clinical trials. The study will respect the rights of participants, and written informed consent will be obtained. The study has been registered in ClinicalTrial.gov (NCT0148027).

15. Data management

15.1. Case report form

All case report forms for each participant should be filled out by study staff in a timely manner. The case report form should be double-checked for potential errors or missing data prior to patients leaving the clinic. All data, including screening assessments, questionnaires, physical examinations, and laboratory examinations, will be filed in the participant's chart. Original documents, participants' charts, and CRF forms will be stored in the study office

15.2. Data entry

All data will be double-entered by researcher staff. Two sets of databases will be generated and tested for consistency using the SAS program. Whenever inconsistencies are found, the data will be corrected by re-examination of the original case report forms or laboratory reports.

15.3 Data reports

Several standardized reports will be generated as follows: 1) participant recruitment and follow-up; 2) demographics; 3) data quality and monitoring; 4) adverse events. These reports

will be used for study management. These reports will be blinded to research personnel who collect study outcomes.

16. Data analysis plan

Data will be analyzed according to participants' randomization assignments, regardless of their subsequent status (intention-to-treat).

16.1. Study outcomes

The primary study outcome is net changes in intrahepatic triglyceride content from baseline at six months and 12 months after intervention. The secondary outcomes include net changes in body weight, waist circumference, and body fat composition; metabolic risk factors; blood pressure, lipids, and glucose; insulin resistance and beta cell function; urinary albumin and kidney function; and carotid intima-media thickness and pulse wave velocity.

16.2 Analysis methods

Baseline data will be compared among three groups. Analysis of variance and covariance will be used to test for continuous variables and χ^2 and logistic regression analysis will be used to test for categorical variables.

A mixed-effects model will be used to assess the effects of exercise programs on the change of intrahepatic triglyceride content and secondary outcomes. An autoregressive correlation matrix will be used to correct within-subject correlation for repeated measurements. PROC MIXED of SAS (SAS Institute Inc, Cary, NC) will be used to obtain point estimates and standard errors of the treatment effects and to test for differences between treatments. There are six comparisons in this trial: three group comparisons in month six and three group comparisons in month 12. Therefore, p-value <0.008 (0.05/6) will be considered as statistically significance.

In a sensitivity analysis, multiple imputation for missing data in the multivariable

analyses will be conducted using the Markov chain Monte Carlo method.

17. Training for study staff

All study staff (physicians and nurses) will be trained and certified before the study is initiated. The training program will include instruction on questionnaire administration and anthropometrics (height, weight, waist circumference, and hip girth) and blood pressure measurement. A standard questionnaire will be used, which includes personal information (name, sex, age, place of birth, marital status, occupation, education, household income, etc.), medical history, medication use, family history, lifestyle factors (smoking and drinking), physical activity, dietary habits, and menstrual and reproductive history. Anthropometric measurements and blood pressure will be obtained using a standard protocol. The data, including that for exercise intensity, duration, heart rate, and anthropometric measurements, will be collected and recorded in CRFs by nurses every week.

Intrahepatic triglyceride content will be measured using proton magnetic resonance spectroscopy. Body fat mass will be quantified using the Hologic whole body DXA system (Hologic Inc., Bedford, MA). Abdominal visceral fat and subcutaneous fat areas will be measured by computed tomography (Siemens Medical Solutions, Forchheim, Germany) at the level of the fourth lumbar vertebra. All technologists are experienced and masked to participant randomization.

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