Effect of Intermittent Calorie Restriction on Metabolic Dysfunction-Associated Steatotic Liver Disease Patients with Abnormal Glucose Metabolism

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Protocol

1. Background

Metabolic dysfunction-associated steatotic liver disease (MASLD) often coexists with type 2 diabetes (T2DM) because of their common risk factors[1]. With the global epidemic trend of obesity and T2DM, MASLD has become an important cause of chronic liver disease in developed countries and rich areas in China. At present, the prevalence of MASLD in Chinese adults is 6-27%[2-4]. Compared with non-MASLD patients, the relative risk of type 2 diabetes in patients with MASLD is 1.86[5]. For patients with elevated blood glucose, the strategy of reducing liver fat content could also alleviate insulin resistance, contribute to better glucose control, and delay the occurrence of diabetes complications[6]. Due to the unclear pathogenesis, there is a lack of effective medication for MASLD. Lifestyle intervention is still the main treatment. Weight loss of 7-10% can effectively reduce liver steatosis and inflammation.

Calorie restriction (CR) is an effective method to reduce weight and severity of obesity related diseases[7,8], but traditionally it requires constant effort to cut down calorie intake every day (continuous calorie restriction, CCR) and may lead to potential adverse effects on the body. Intermittent calorie restriction (ICR) refers to maintaining no or very-low calorie intake periodically, which means alternating normal diet and fasting for a certain period to prevent and treat various diseases. The most common ICR schemes include time restrict fasting (TRF) or time restrict eating (TRE), alternate day fasting (ADF) and fasting for two days per week (5:2 diet). ICR can effectively reduce weight, lower blood pressure, improve lipid profile and reduce oxidative stress[9-11]. It also facilitates glucose control by increasing insulin sensitivity and improving β Cell function[12,13]. Recent studies have proved the effectiveness of TRE and ADF on reducing weight and improving cardiometabolic risk factors[9,14-18]. However, effect of ICR, especially 5:2 diet, on MASLD patients has been rarely studied. A few studies have found ICR could reduce liver enzymes in MASLD patients[14,19,20], but they were less accurate because fat content of the whole liver, as well as of different liver segments were not quantitatively evaluated before and after treatment. More importantly, no study directly compared the therapeutic effect of ICR and CCR on MASLD currently.

2. Aim of the study

In the present study, we carried out a randomized, controlled, open-label clinical trial to investigate the efficacy and safety of ICR in MASLD patients with abnormal glucose metabolism. For ICR group, we adopt the 5:2 scheme which was related to stronger patient compliance[12]. The control group received traditional CCR. Two groups were instructed to keep overall calorie restriction to similar degree during 12 weeks of treatment. The changes of LFC of the whole liver, as well as of different liver segments measured by MRI-Proton Density Fat Fraction (MRI-PDFF) were compared between two groups. We hypothesize that ICR will be more effective than CCR in reducing LFC and other metabolic risk factors.

3. Research design

3.1 Overall design

This 12-week, open-label, randomized, controlled, parallel-group trial was conducted at Zhongshan Hospital Fudan University between July 2020 and July 2021 (ClinicalTrials.gov, NCT04283942). The trial was initiated by the researcher, performed in accordance with the ethical principles of the Declaration of Helsinki and approved by the Ethics Committee of Zhongshan Hospital Fudan University (approval number: B2019-256). A total of 60 patients were enrolled in the trial, and written informed consent was obtained from all patients.

3.2 Randomization and masking

The simple randomization method was used to ensure similar baseline characteristics between two groups. The randomization scheme will be provided by the Statistics Department of School of Public Health of Fudan University (generated by SAS software), and randomization will be completed by staff unrelated to this trial. According to the randomization scheme, a random envelope marked with the treatment number will be made and assigned for each subject according to the order of enrollment. The treatment will be carried out according to the arrangement of corresponding scheme.

4. Study participants

4.1 Inclusion Criteria

- Age 18-70
- Diagnosed as fatty liver by ultrasound or magnetic resonance imaging
- BMI ≥ 24 kg/m^2
- Abnormal glucose metabolism: (meeting at least one):
    1) Impaired glucose regulation: fasting blood glucose ≥ 100 mg/dL, postprandial blood glucose ≥ 140 mg/dL or HbA1c ≥ 5.7%
    2) Diabetes mellitus: diabetic symptoms + plasma glucose concentration ≥ 200 mg/dL at any time or fasting plasma glucose concentration ≥ 100 mg/dL or OGTT 2 h plasma glucose concentration ≥ 200 mg/dL

4.2 Exclusion Criteria

- Type 1 diabetes, gestational diabetes and other special types of diabetes
- Poor blood glucose control, HbA1c > 8.5% within 3 months
- Taking antidiabetic drugs in the past month
- Serum ALT > 6 times of normal upper limit
- Excessive alcohol consumption (alcohol intake: men > 140 g, women > 70 g in the past 6 months)
- Other liver diseases: such as acute and chronic viral hepatitis, drug-induced hepatitis, immune hepatitis, liver cirrhosis, liver cancer, etc
- Taking drugs that may affect MASLD in the past three months, such as vitamin E
- Biliary obstructive diseases
- Other diseases affecting glucose and lipid metabolism: hyperthyroidism, hypothyroidism, Cushing's syndrome, etc
- Chronic kidney disease (serum creatinine ≥ 2.0 mg/dL)
- Life expectancy of no more than 5 years
- Already pregnant or plan to be pregnant in the near future
- Mental illness
- Other conditions affecting follow-up

- Have participated in other clinical trials in the past 4 weeks
- Absent of informed consent

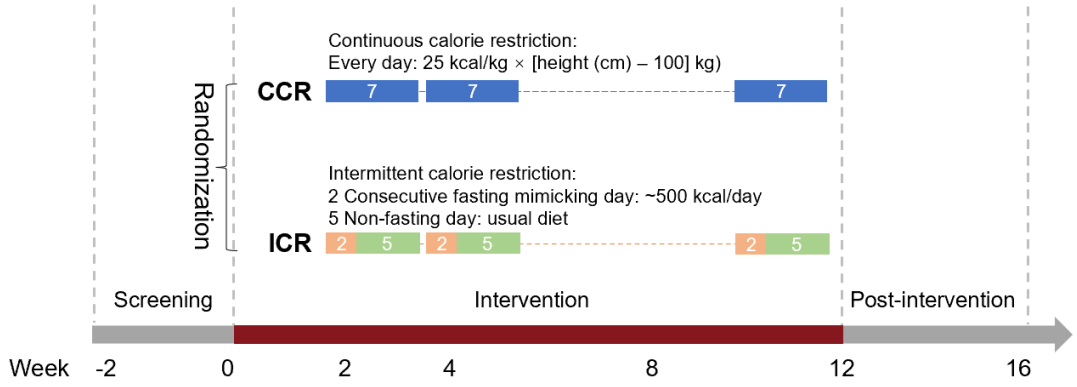
4.3 Termination criteria

- Request to withdraw informed consents
- Pregnancy
- Systolic blood pressure ≥ 180 mmHg and diastolic blood pressure ≥ 110 mmHg
- Liver enzymes (ALT or AST) increase to ≥ 6 times of the upper normal limit and last for 4 weeks
- Fasting blood glucose increases to > 200 mg/dL and lasts for 4 weeks
- Serum creatinine increases to ≥ 1.5 mg/dL
- During the treatment, drugs which may affect liver fat content are added or adjusted for dosage: such as hypoglycemic drugs, lipid-lowering drugs and liver protecting drugs

5. Intervention

After a 2-week screening phase, a total of 60 eligible patients will be randomized 1:1 into ICR and CCR group.

Figure 1. Study design



5.1 Dietetic intervention

- ICR group: Participants in ICR group were instructed to ensure 2 successive days of fasting-mimicking and 5 days of recovery per week. On fasting-mimicking days, the participants were instructed to consume approximately 500 kcal/day and they were provided with plant-based meal replacement (ZhenBaiNian nutrition bar, Beijing Winlife Research Institute of Nutrition, Health, Food Science, and Technology, China) to improve adherence and ensured adequate intake of micronutrients. In the rest 5 days of recovery per week, participants were allowed to consume their usual diet. Main ingredients of this meal replacement are as follows:

  - Main ingredients: isomaltooligosaccharide solution, high-protein and high-fiber crispy grain (soy protein isolate, rice, polydextrose, calcium carbonate), Chia seed, resistant dextrin, coconut potato, salty vegetable grain, etc.

Table 1. Information of nutrition bar used in this trial

	Per 100g	%NRV

energy	355.4 kcal	18%
protein	15.4 g	26%
Fat	12.4 g	21%
Trans fats (acids)	0 g	
Polyunsaturated fatty acids	3.6 g	
carbohydrate	34.0 g	11%
dietary fiber	23.5 g	94%
sodium	261 mg	13%
vitamin A	565 μg	71%
vitamin D	2.5 μg	50%
vitamin E	8.05 mg	58%
Vitamin B1	1.30 mg	93%
Vitamin B2	1.30 mg	93%
Vitamin B6	1.30 mg	93%
Vitamin B12	0.80 μg	33%
vitamin C	67.7 mg	68%
nicotinic acid	13.50 mg	96%
folic acid	328 μg	82%
Pantothenic acid	3.17 mg	63%
Biotin	3.8 μg	13%
phosphorus	257 mg	37%
potassium	318 mg	16%
magnesium	72 mg	24%
calcium	433 mg	54%
iron	6.7 mg	45%
zinc	5.25 mg	35%
selenium	10.0 μg	20%
copper	0.44 mg	29%
fluorine	0.06 mg	6%
manganese	1.00 mg	33%
ω~ 6 Linoleic acid	1.6 g	
ω~ three α- linolenic acid	2.0 g	

- Manufacturer: Beijing Wanlaikang Nutrition and Health Food Science and Technology Research Institute Co., Ltd
- Product standard No.: Q/CPKWC 0085
- Food production license No.: SC10611141312769

- CCR group: Participants in CCR group were instructed to consume the prescribed calories (25 kcal / kg × [height (cm) – 100] kg[21]) every day by eating conventional food without time restriction.

Both groups received dietary counseling from experienced nutritionists during the trial. Kitchen scales were distributed to participants, and they were encouraged to weigh food to ensure accurate reporting of their caloric intake. During the trial, all the participants were required to write daily dietary log. Using each log, nutritionists assessed their calorie intake each day based on the nutrient content shown on the Chinese Food Composition

Table[22].Participants received face-to-face instruction or app messages every week to assess their adherence to the prescribed diet.

5.2 Physical activity

During the trial, all participants were required to maintain their exercise routines and record their daily steps using a unified pedometer (Redmi Smart Band, Xiaomi Corporation, China). The use of drugs affecting blood glucose and fatty liver were avoided.

6. Study outcomes

6.1 Primary outcome

- Change in liver fat content from baseline to 12 months

6.2 Secondary outcomes

- Change of weight
- Change of blood glucose: fasting blood glucose,2h postload blood glucose, HbA1c
- Change of liver enzymes: alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase
- Change of lipid profile: total cholesterol, triglyceride, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, free fatty acid

6.3 Other pre-specified outcomes

- Change of fat mass and lean mass measured by a bioimpedance analyzer
- Change of abdominal adiposity measured by MRI: visceral adipose tissue and subcutaneous adipose tissue
- Change of liver stiffness measured by liver transient elastography
- Change of insulin sensitivity: fasting insulin and HOMA-IR (fasting glucose in mmol/L x fasting insulin in μU/mL)/22.5)

7. Follow-up visits

(1) Screening visit (V0): The purpose of this visit is to confirm subjects' eligibility and interest in the proposed study, and obtain informed consent, relevant medical history (including diabetes, fatty liver, alcohol drinking history, hepatitis history, drug use, etc.), physical measurements (weight, height, waist circumference and blood pressure), and laboratory measurements (OGTT, HbA1c, liver and renal functions, electrolytes, hepatitis markers, etc). ^{1}H-Proton Magnetic Resonance Spectroscopy (^{1}H-MRS), MRI-Proton Density Fat Fraction (MRI-PDFF) and liver transient elastography were applied to evaluate the fatty liver. Body composition analysis and other examinations were performed. Relevant laboratory and imaging examination results within 2 weeks before screening can be approved.

(2) Baseline visit (V1): The baseline follow-up was scheduled within 2 weeks after the screening period. Physical examination was conducted. Subjects will be required to record their diet and steps for 3 days before day 0 and the average of calorie consumption and steps will be used as baseline reference. Randomization will be conducted.

(3) Follow up and termination visits (V2 - V9): At each visit, diet and step records will be collected. At week 2, 4, 8 12, physical measurements, fasting glucose and adverse events will be recorded. Nutritionists will provide dietary advice and help subjects to keep calorie

restriction and a balance of nutrients. On this basis, liver and renal functions, electrolytes, fasting blood glucose and lipids profiles will be measured at week 4. At week 12 (termination visit), OGTT, ^{1}H-MRS, MRI-PDFF, liver transient elastography and body composition analysis will be added. At week 1, 3, 6, 10, only telephone follow-up will be conducted.

(4) Post-intervention visit (V10): At 4 weeks after the intervention (week 16), body weight will be investigated by telephone.

Table 2. Study visits and data collection

	Screening	Baseline								Termination	Post-intervention
Visit	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
Week	<-2	0	1	2	3	4	6	8	10	12	16
Day		0	7±3d	14±7d	21±3d	28±7d	42±3d	56±7d	70±3d	84±7d	112±3d
Inform consent	X										
Randomization		X									
Demographic information	X										
History about fatty liver and hyperglycemia	X										
Comorbidity and concomitant drug use	X										
blood pressure	X	X		X		X		X		X	
Height	X									X	
Weight	X	X		X		X		X		X	X
Waist circumference	X	X		X		X		X		X	
Diet and steps records for 3 days		X									
OGTT	X									X	
HbA1c	X									X	
liver and renal functions	X					X				X	
Lipid profile	X					X				X	
Fasting plasma glucose	X			X		X		X		X	
Electrolytes	X					X				X	
Routine blood test	X					X				X	
Hepatitis markers and autoantibody	X										
Urinalysis	X									X	
Liver transient elastography	X									X	
Liver ^{1}H-MRS	X									X	
Liver MRI-PDFF	X									X	
Brain functional MRI	X									X	
Body composition	X									X	
Activity (steps)		X	X	X	X	X	X	X	X	X	
Diet guidance		X	X	X	X	X	X	X	X	X	
Blood sample		X		X		X		X		X	
Urine and feces sample		X								X	

Adverse events		X	X	X	X	X	X	X	X	X	
Distribute/Collect diet diary	X	X		X		X		X		X	
Distribute/Collect research food (only for ICR group)		X		X		X		X		X	

8. Measurements and investigations

Questionnaire: The research staff will apply a questionnaire obtaining information on history about fatty liver and hyperglycemia, comorbidity and concomitant drug use (such as fatty liver, diabetes, hypertension, dyslipidemia and cardiovascular disease), lifestyle risk factors (cigarette smoking, alcohol drinking and physical activity).

Dietary record: The research staff will provide an electronic kitchen scale to subjects enrolled. Subjects are required to record the name and weight of food they eat in a whole day on a distributed form. After the diet record is finished, they should take a photo of form and send to dietitian in research group. The form is as followed. They are required to record food intake for 3 days before day 0 and the average of calorie consumption will be used as baseline reference. During intervention, subjects in both groups will get distributed blank forms in each visit and are required to record their diet every day.

Table 3. Dietary form

		Date							
		Breakfast		Lunch		Dinner		Extra meals	
Item		Food	Weight (g)	Food	Weight (g)	Food	Weight (g)	Food	Weight (g)
Staple food	Rice								
	Noodles								
	Coarse grain								
	Tubers								
Meat	Pork								
	Beef and mutton								
	Chicken and duck								
	Fish and shrimp								
	Others								
Eggs									
Dairy food	Milk								
	Yogurt								
	Others								
Bean products	Bean curd								
	Soybean milk								
Vegetables	1								

	2								
	3								
	4								
Fruits	1								
	2								
	3								
Nuts									
Alcoholic drinks	Beer								
	Grape wine								
	Millet wine								
	Baijiu								
Non-alcoholic drinks									
Oils									

Activity (steps): During the intervention, subjects need to wear a smart electronic wearable device (Redmi Smart Band, Xiaomi Corporation, China) to record the daily steps automatically.

Physical measurements: At the clinic visit, trained staff will take measurements of body weight, height, waist circumference, blood pressure according to standard procedures. Subjects should avoid wearing shoes and heavy clothes when measuring body weight. Mid-way between the lower border of rib margin and the upper border of iliac crest will be measured as waist circumference. The blood pressure will be measured after resting for 5 minutes in a sitting position and alcohol, cigarettes, coffee/tea and physical exercise will be avoided within 30 minutes before the measurement. Automatic sphygmomanometer will be applied for standardized detection.

Laboratory measurements: Blood samples will be drawn 10 hours after fasting and be used to measure blood glucose, HbA1c, liver enzymes, serum creatinine, urine acid and lipids.. High performance liquid chromatography (HPLC) method will be used for measuring HbA1c. Total cholesterol, high-density lipoprotein cholesterol, triglycerides, free fatty acids, electrolytes, liver enzymes, serum creatinine and urine acid will be measured by a model 7600 automated bioanalyzer (Hitachi, Tokyo, Japan). Low-density lipoprotein cholesterol will be calculated by the Friedewald equation. The FBG and 2-hour postprandial blood glucose in oral glucose tolerance test will be tested following a standard 75g oral glucose tolerance test and measured using the glucose oxidase method. An electrochemiluminescence immunoassay will be used to measure the fasting and 2-hour postprandial insulin. All parameters are tested in the clinical biochemical laboratory of Zhongshan Hospital affiliated to Fudan University. The clinical testing laboratory will adopt the standardized process and obtain the corresponding certification qualification.

^{1}H-MRS: Whole liver fat content by ^{1}H-MRS was performed with a 1.5T MRI Scanner (Siemens Healthineers, Germany). Firstly, T1-weighted sagittal, coronal, and axial images were acquired for the localization of the spectroscopy acquisition voxel. A single 2×2×2 cm voxel was placed within the right lobe avoiding major vascular structures and subcutaneous fat tissue. The proton spectrum was acquired after shimming over the volume of interest by means of a point-resolved spectroscopy sequence with the following parameters: repetition time = 1500 ms and echo time = 135 ms. Signal intensities of the water peak at 4.8 ppm (Sw) and the fat

peak at 1.4 ppm (Sf) were measured, and the hepatic fat percentage was calculated as a ratio of the signal from the methylene group to the total signal of methylene and water[23].

MRI-PDFF: MRI examinations were performed on a 3.0T MRI scanner (Ingenia CX 3.0 T, Philips Healthcare, USA)[24-26]. The quantitative calculation of fat fraction requires the use of MRI mDIXON-Quant_BH or FF sequences, because the dicom header information of these sequences already contains the value of fat fraction. However, the profile of the liver is not clear in these sequences, so we first use the deep learning neural network to predict the outline of the liver and the eight segments of the liver on the in-phase of dixon as it has a clear view of the liver. Then resample these two labels to the mDIXON-Quant_BH or FF sequence, and correspond to the location of each slice of these two sequences. In this way, the liver profile label and liver Couinaud segment label on the fat sequence are obtained. Afterwards, the corresponding matrix is obtained by multiplying the mDIXON-Quant_BH or FF sequence with the liver profile and liver Couinaud segment label matrix. Finally, the corresponding fat fraction mean and upper-lower percentiles of every liver segment can be calculated according to different needs. It should also be noted that the fat fraction values in different machines or sequences are in different units and need to be divided again by the corresponding ratios to obtain the final results. The neural network used for predicting liver profile and liver Couinaud segment is a 2.5D UNet. The input of this network is a multi-layer 2D matrix, and residual modules and channel attention mechanism modules are added on the basis of UNet. The training dataset Including more than 200 cases with manually corrected liver profile and liver Couinaud labels and from Quanjing Imaging Center and Zhongshan Hospital Affiliated to Fudan University. The ratio of training, verification and test datasets is 4:1:1, and the inference dice of the model test set is respectively 0.981 for liver profile and 0.804 for liver Couinaud segment.

Transient elastography: The controlled attenuation parameter (CAP) in dB/m and Liver stiffness measurement (LSM) in kPa will be measured using FibroScan (FibroScan 502 Touch, Echosens, France)[27]. All subjects will be detected when holding breath in supine position by using the M probe. Each subject collected at least 10 valid measurements. Only when the proportion of effective measurement times to the total measurement times is ≥ 60%, the interquartile range (IQR) of repeatedly collected CAP value is ≤ 40 dB / m, and the IQR of repeatedly collected LSM value is ≤ 30%, can the result be considered reliable. The median value will be kept as a representative result.

Body composition: Lean mass and fat mass were measured by a bioimpedance analyzer Inbody520 (Biospace, Seoul, Korea). Specifically, for the area of abdominal adipose tissue, MRI (1.5 T, Siemens Avanto, Erlangen, Germany) were applied to conduct abdominal positioning scanning first, and then ^{1}H-MRS detection. Sections will be acquired through the abdomen at the level of umbilicus to L4. Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) will be measured with NIH image software (ImageJ 1.41o; National Institutes of Health, Bethesda, MD, USA)[28].

Blood samples in weeks 0 and week 12 were collected for metabolomics analysis. Feces samples in weeks 0 and week 12 were collected for detection of gut microbiome and gut microbiota-derived metabolites. Resting state cerebral functional magnetic resonance imaging was conducted before and after intervention to explore the effects of intervention on brain structure, network connectivity, and other parameters of brain functions.

9. Adverse events (AE)

9.1 Definition

Non-Serious Adverse Event (NSAE) refers to an unforeseeable medical condition that occurs during or after the treatment, or an event that makes the original condition worse. The occurrence of NSAE does not necessarily have a causal relationship with the intervention. Adverse medical conditions include symptoms (such as nausea and chest pain), physical signs (such as tachycardia and hepatomegaly) or abnormal examination results.

Serious adverse event (SAE) refers to the adverse event that meets one or more of the following criteria caused by intervention in screening method, treatment period, cleaning period or follow-up period:

1) Causing death;
2) Life-threatening injury;
3) Leading to longer hospitalization;
4) Causing continuous or obvious disability;
5) Congenital malformation/defect;
6) Major medical events that may cause injury to the subject or require medical intervention to prevent its occurrence.

Severity grading of non-serious adverse events:

1=mild (with symptoms or signs, but tolerable)
2=moderate (feeling unwell and affecting normal activities)
3=severe (inability to perform normal activities)

The unresolved NSAE at the end of the trial need to be followed up. The follow-up interval and duration depend on the medical needs. For the unsolved adverse events at the end of the trial or when the subjects withdraw from the study, the follow-up shall be conducted until the NSAE returns to normal, or until the investigator believes that further follow-up is not necessary.

9.2 Recording and assessment of adverse events

Attention should be paid to the collection of AE during the whole research process. The following are the variables that should be recorded for each AE: onset and stop time, severity, measurements, causal relationship with intervention, outcomes and whether it is a serious adverse event.

The severity of adverse events is assessed as follows: Mild: usually temporary, and not affecting daily activities; Moderate: causing discomfort and affecting normal activities, thought tolerable and not requiring participants to take any medication. Severe: disrupting daily activities and intolerable, requiring participants to take medication immediately. 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. The severity assessment of adverse events is as follows:

1) Mild: usually temporary and does not affect daily activities.
2) Moderate: discomfort, affect normal activity and does not require medication to alleviate symptoms.
3) Serious: not endurable, affect daily activities and require medication immediately.

9.3 Treatment of AE

The patients with mild AE can continue the intervention and visits. Those with SAE should stop intervention and take corresponding treatment in time.

Once NSAE happens, the severity, duration, treatment and outcome of adverse reactions should be explained. If other drugs are used at the same time during the trial, it shall be recorded in a timely manner on case report forms.

All SAEs, whether related to the intervention or not, should be reported and recorded in the adverse event report form of the case report form, recording the occurrence time, severity, duration, measures taken, etc. Researchers have the responsibility to notify the Ethics Committee within 24 hours.

10. Data management

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. All data, including questionnaires, physical examinations, laboratory and other examinations, will be filled in the subject's chart. Original documents, participants' charts, and CRF forms will be stored in the study office. All data will be double-entered by researcher staff.

11. Statistics

11.1 Statistical power and sample size

A 12-week ICR has been reported to result in approximately 7% weight loss in overweight and obese participants, while the CCR group caused 30% less weight loss than the ICR group[29]. According to previous studies[28,30], a 7% weight loss was approximately accompanied with a 19.3% reduction in LFC. Thus, a priori power calculation assumed 19.3% and 13.5% reduction in LFC in the ICR and CCR groups, respectively. Assuming an anticipated dropout rate of 15%, we estimated that an enrollment target of 60 participants (30 per group) would provide the trial with 80% statistical power to detect a significant difference of 5.8±8% in absolute LFC change between ICR and CCR group at a significance level of 0.05 using a two-tailed test.

11.2 Data analysis

Data were analyzed according to the intention-to-treat principle using R version 4.0.3. Results are presented as means ±SD unless otherwise noted. Paired t tests or Wilcoxon matched-pairs signed-ranks tests were used for comparison of continuous variables before and after 12-week intervention. While the chi-square or Fisher's exact tests were used for comparison of the categorical variables. Generalized linear models were used to compare the changes of the continuous variables after a 12-week intervention between the two groups, with adjustment for age, sex and baseline measures. All statistical analyses were two-sided, and $P<0.05$ was considered statistically significant.

12. Quality control

The trial will comply with China's Standard for Quality Management of Clinical Trials (GCP) and other laws and regulations related to clinical trials. The personal information of participants will be confidential and shall not be disclosed without permission. We will develop a standardized research procedure manual, including recruitment of subjects, form filling, intervention methods, parameter measurement methods, sample collection and management. All researchers will get relevant training before the start of the trial.

13. Ethics approval

This trial will comply with the Helsinki Declaration (1996 version) and Chinese clinical trial regulations. All amendments to the research protocol and informed consent will be approved by the Ethics Committee of Zhongshan Hospital affiliated to Fudan University. Adverse events must be reported to the Ethics Committee as required. The study will be registered on the NIH Clinical Trial website before enrollment.

References

1. Xia, M.F., Bian, H., and Gao, X. (2019). NAFLD and Diabetes: Two Sides of the Same Coin? Rationale for Gene-Based Personalized NAFLD Treatment. Frontiers in pharmacology *10*, 877. 10.3389/fphar.2019.00877.
2. Fan, J.G., Kim, S.U., and Wong, V.W. (2017). New trends on obesity and NAFLD in Asia. Journal of hepatology *67*, 862-873. 10.1016/j.jhep.2017.06.003.
3. Fan, J.G. (2013). Epidemiology of alcoholic and nonalcoholic fatty liver disease in China. Journal of gastroenterology and hepatology *28 Suppl 1*, 11-17. 10.1111/jgh.12036.
4. Wang, F.S., Fan, J.G., Zhang, Z., Gao, B., and Wang, H.Y. (2014). The global burden of liver disease: the major impact of China. Hepatology (Baltimore, Md.) *60*, 2099-2108. 10.1002/hep.27406.
5. Ballestri, S., Zona, S., Targher, G., Romagnoli, D., Baldelli, E., Nascimbeni, F., Roverato, A., Guaraldi, G., and Lonardo, A. (2016). Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. Journal of gastroenterology and hepatology *31*, 936-944. 10.1111/jgh.13264.
6. Bril, F., and Cusi, K. (2016). Nonalcoholic Fatty Liver Disease: The New Complication of Type 2 Diabetes Mellitus. Endocrinology and metabolism clinics of North America *45*, 765-781. 10.1016/j.ecl.2016.06.005.
7. Huang, M.A., Greenson, J.K., Chao, C., Anderson, L., Peterman, D., Jacobson, J., Emick, D., Lok, A.S., and Conjeevaram, H.S. (2005). One-year intense nutritional counseling results in histological improvement in patients with non-alcoholic steatohepatitis: a pilot study. The American journal of gastroenterology *100*, 1072-1081. 10.1111/j.1572-0241.2005.41334.x.
8. European Association for the Study of the, L., European Association for the Study of, D., and European Association for the Study of, O. (2016). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. Journal of hepatology *64*, 1388-1402. 10.1016/j.jhep.2015.11.004.
9. Stekovic, S., Hofer, S.J., Tripolt, N., Aon, M.A., Royer, P., Pein, L., Stadler, J.T., Pendl, T., Prietl, B., Url, J., et al. (2020). Alternate Day Fasting Improves Physiological and Molecular Markers of Aging in Healthy, Non-obese Humans. Cell metabolism *31*, 878-881. 10.1016/j.cmet.2020.02.011.
10. Saad, R. (2020). Effects of Intermittent Fasting on Health, Aging, and Disease. The New England journal of medicine *382*, 1773. 10.1056/NEJMc2001176.
11. Wei, M., Brandhorst, S., Shelehchi, M., Mirzaei, H., Cheng, C.W., Budniak, J., Groshen, S., Mack, W.J., Guen, E., Di Biase, S., et al. (2017). Fasting-mimicking diet and markers/risk

factors for aging, diabetes, cancer, and cardiovascular disease. Science translational medicine *9*. 10.1126/scitranslmed.aai8700.

12. Sundfør, T.M., Svendsen, M., and Tonstad, S. (2018). Intermittent calorie restriction-a more effective approach to weight loss? The American journal of clinical nutrition *108*, 909-910. 10.1093/ajcn/nqy288.
13. Cheng, C.W., Villani, V., Buono, R., Wei, M., Kumar, S., Yilmaz, O.H., Cohen, P., Sneddon, J.B., Perin, L., and Longo, V.D. (2017). Fasting-Mimicking Diet Promotes Ngn3-Driven β-Cell Regeneration to Reverse Diabetes. Cell *168*, 775-788.e712. 10.1016/j.cell.2017.01.040.
14. Johari, M.I., Yusoff, K., Haron, J., Nadarajan, C., Ibrahim, K.N., Wong, M.S., Hafidz, M.I.A., Chua, B.E., Hamid, N., Arifin, W.N., et al. (2019). A Randomised Controlled Trial on the Effectiveness and Adherence of Modified Alternate-day Calorie Restriction in Improving Activity of Non-Alcoholic Fatty Liver Disease. Scientific reports *9*, 11232. 10.1038/s41598-019-47763-8.
15. Liu, D., Huang, Y., Huang, C., Yang, S., Wei, X., Zhang, P., Guo, D., Lin, J., Xu, B., Li, C., et al. (2022). Calorie Restriction with or without Time-Restricted Eating in Weight Loss. The New England journal of medicine *386*, 1495-1504. 10.1056/NEJMoa2114833.
16. Trepanowski, J.F., Kroeger, C.M., Barnosky, A., Klempel, M.C., Bhutani, S., Hoddy, K.K., Gabel, K., Freels, S., Rigdon, J., Rood, J., et al. (2017). Effect of Alternate-Day Fasting on Weight Loss, Weight Maintenance, and Cardioprotection Among Metabolically Healthy Obese Adults: A Randomized Clinical Trial. JAMA internal medicine *177*, 930-938. 10.1001/jamainternmed.2017.0936.
17. Jamshed, H., Steger, F.L., Bryan, D.R., Richman, J.S., Warriner, A.H., Hanick, C.J., Martin, C.K., Salvy, S.J., and Peterson, C.M. (2022). Effectiveness of Early Time-Restricted Eating for Weight Loss, Fat Loss, and Cardiometabolic Health in Adults With Obesity: A Randomized Clinical Trial. JAMA internal medicine *182*, 953-962. 10.1001/jamainternmed.2022.3050.
18. Wilkinson, M.J., Manoogian, E.N.C., Zadourian, A., Lo, H., Fakhouri, S., Shoghi, A., Wang, X., Fleischer, J.G., Navlakha, S., Panda, S., and Taub, P.R. (2020). Ten-Hour Time-Restricted Eating Reduces Weight, Blood Pressure, and Atherogenic Lipids in Patients with Metabolic Syndrome. Cell metabolism *31*, 92-104.e105. 10.1016/j.cmet.2019.11.004.
19. Mari, A., Khoury, T., Baker, M., Said Ahmad, H., Abu Baker, F., and Mahamid, M. (2021). The Impact of Ramadan Fasting on Fatty Liver Disease Severity: A Retrospective Case Control Study from Israel. The Israel Medical Association journal : IMAJ *23*, 94-98.
20. Wilson, R.B., Zhang, R., Chen, Y.J., Peters, K.M., Sawyez, C.G., Sutherland, B.G., Patel, K., Kennelly, J.P., Leonard, K.A., Jacobs, R.L., et al. (2020). Two-Week Isocaloric Time-Restricted Feeding Decreases Liver Inflammation without Significant Weight Loss in Obese Mice with Non-Alcoholic Fatty Liver Disease. International journal of molecular sciences *21*. 10.3390/ijms21239156.
21. Rössner, S. (2007). Paul Pierre Broca (1824-1880). Obesity reviews : an official journal of the International Association for the Study of Obesity *8*, 277. 10.1111/j.1467-789X.2007.00303.x.
22. Y, Y. (2018). China food composition tables
standard edition, 6th ed (Peking University Medical Press).
23. Xia, M.F., Bian, H., Zhu, X.P., Yan, H.M., Chang, X.X., Zhang, L.S., Lin, H.D., Hu, X.Q., and Gao, X. (2018). Serum folic acid levels are associated with the presence and severity of

liver steatosis in Chinese adults. Clin Nutr *37*, 1752-1758. 10.1016/j.clnu.2017.06.021.

24. Bonekamp, S., Tang, A., Mashhood, A., Wolfson, T., Changchien, C., Middleton, M.S., Clark, L., Gamst, A., Loomba, R., and Sirlin, C.B. (2014). Spatial distribution of MRI-Determined hepatic proton density fat fraction in adults with nonalcoholic fatty liver disease. Journal of magnetic resonance imaging : JMRI *39*, 1525-1532. 10.1002/jmri.24321.
25. Middleton, M.S., Heba, E.R., Hooker, C.A., Bashir, M.R., Fowler, K.J., Sandrasegaran, K., Brunt, E.M., Kleiner, D.E., Doo, E., Van Natta, M.L., et al. (2017). Agreement Between Magnetic Resonance Imaging Proton Density Fat Fraction Measurements and Pathologist-Assigned Steatosis Grades of Liver Biopsies From Adults With Nonalcoholic Steatohepatitis. Gastroenterology *153*, 753-761. 10.1053/j.gastro.2017.06.005.
26. de Padua, V.A.V., Dillman, J.R., and Trout, A.T. (2022). Retrospective comparison of liver chemical shift-encoded PDFF sampling strategies in children and adolescents. Abdom Radiol (NY) *47*, 3478-3484. 10.1007/s00261-022-03615-0.
27. Yang, X., Chang, X., Wu, S., Sun, X., Zhu, X., Wang, L., Xu, Y., Yao, X., Rao, S., Hu, X., et al. (2021). Performance of liver stiffness measurements obtained with FibroScan is affected by glucose metabolism in patients with nonalcoholic fatty liver disease. Lipids in health and disease *20*, 27. 10.1186/s12944-021-01453-5.
28. Liu, L., Yan, H., Xia, M., Zhao, L., Lv, M., Zhao, N., Rao, S., Yao, X., Wu, W., Pan, B., et al. (2020). Efficacy of exenatide and insulin glargine on nonalcoholic fatty liver disease in patients with type 2 diabetes. Diabetes/metabolism research and reviews *36*, e3292. 10.1002/dmrr.3292.
29. Schübel, R., Nattenmüller, J., Sookthai, D., Nonnenmacher, T., Graf, M.E., Riedl, L., Schlett, C.L., von Stackelberg, O., Johnson, T., Nabers, D., et al. (2018). Effects of intermittent and continuous calorie restriction on body weight and metabolism over 50 wk: a randomized controlled trial. The American journal of clinical nutrition *108*, 933-945. 10.1093/ajcn/nqy196.
30. Yan, H.M., Xia, M.F., Wang, Y., Chang, X.X., Yao, X.Z., Rao, S.X., Zeng, M.S., Tu, Y.F., Feng, R., Jia, W.P., et al. (2015). Efficacy of Berberine in Patients with Non-Alcoholic Fatty Liver Disease. PLoS One *10*, e0134172. 10.1371/journal.pone.0134172.