Jawatankuasa Etika Penyelidikan Manusia USM (JEPeM)
Human Research Ethics Committee USM (HREC)

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10th August 2015

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JEPeM Code : USM/JEPeM/15040117
Protocol Title : The Effect of Modified Alternate Day Calorie Restriction on Non-Alcoholic Fatty Liver Disease (NAFLD).

Dear Dr.,

We wish to inform you that your study protocol has been reviewed and is hereby granted approval for implementation by the Jawatankuasa Etika Penyelidikan Manusia Universiti Sains Malaysia (JEPeM-USM). Your study has been assigned study protocol code **USM/JEPeM/15040117**, which should be used for all communication to the JEPeM-USM related to this study. This ethical clearance is valid from **August 2015** until **July 2016.**

The following documents have been approved for use in the study.

1. Research Proposal

In addition to the abovementioned documents, the following technical document was included in the review on which this approval was based:

1. Patient Information Sheet and Consent Form (English version)
2. Patient Information Sheet and Consent Form (Malay version)
3. Data Collection Sheet

Attached document is the list of members of JEPeM-USM present during the full board meeting reviewing your protocol.

While the study is in progress, we request you to submit to us the following documents:

1. Application for renewal of ethical approval 60 days before the expiration date of this approval through submission of **JEPeM-USM FORM 3(B) 2014: Continuing Review Application Form.** Subsequently this need to be done yearly as long as the research goes on.
2. Any changes in the protocol, especially those that may adversely affect the safety of the participants during the conduct of the trial including changes in personnel, must be submitted or reported using **JEPeM-USM FORM 3(A) 2014: Study Protocol Amendment Submission Form.**
3. Revisions in the informed consent form using the **JEPeM-USM FORM 3(A) 2014: Study Protocol Amendment Submission Form.**
4. Reports of adverse events including from other study sites (national, international) using the **JEPeM-USM FORM 3(G) 2014: Adverse Events Report.**
5. Notice of early termination of the study and reasons for such using **JEPeM-USM FORM 3(E) 2014.**
6. Any event which may have ethical significance.
7. Any information which is needed by the JEPeM-USM to do ongoing review.
8. Notice of time of completion of the study using **JEPeM-USM FORM 3(C) 2014: Final Report Form.**

Research Protocol

Protocol title

A Randomized Controlled Trial on the Effectiveness and Adherence of Modified Alternate-day Calorie Restriction (MACR) in Improving Activity of Non-Alcoholic Fatty Liver Disease

Sponsor

Universiti Sains Malaysia, Health Campus, 16150, Kubang Kerian, Kota Bharu, Kelantan, Malaysia

- USM short term grant (reference no: 304/PPSP/61313173)
- Research University Individual (RUI) grant (reference no: 1001/PPSP/812151)

Investigators

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[A]Study Design
[B]Data Collection
[C]Statistical Analysis
[D]Data Interpretation
[E]Manuscript Preparation
[F]Literature Search

PROJECT SUMMARY

Rationale: There is no effective therapy for non-alcoholic fatty liver disease (NAFLD), although intensive calorie restriction is typically recommended but dietary adherence is an issue. The current study aimed to determine the effectiveness and adherence of eight weeks of modified intermittent fasting in the control of NAFLD activity.

Objective: Primary objective are to determine the effectiveness of 8 weeks of MACR in changes of BMI, biochemical and ultrasound parameters in NAFLD. The secondary objective is to evaluate the adherence rate of MACR in NAFLD patients.

Method: This is a randomized, single-blind controlled trial with 8-weeks modified alternate-day calorie restriction (MACR) as the active intervention and normal habitual diet as control in Hospital Universiti Sains Malaysia with study duration for 24 months. The outcome measures are included changes in BMI, blood lipids, fasting blood sugar, liver enzymes and ultrasonographic measurements of liver steatosis and shear wave elastography (SWE). Per-protocol (PP) and intention-to-treat (ITT) analysis are performed within and between-groups with $P < 0.05$ as significant.

Expected outcome: Specifically, we expected here that MACR regimen resulted in a mean weight loss between 3-5% from baseline after 8 week of intervention thus it is also expected to decreases in several key biomarkers for CAD risk, such as total cholesterol, LDL cholesterol, triacylglycerols and blood pressure. Liver parameter which included liver enzymes, steatosis and fibrosis would also expect to improve after 8 weeks MACR. Additionally, a similar adherence rate is expected to achieve during the MACR regime when compared with other alternate day calorie restriction regime.

Funding: USM short term grant (reference no: 304/PPSP/61313173) and Research University Individual (RUI) grant (reference no: 1001/PPSP/812151).

Keywords

Non-alcoholic fatty liver disease; fatty liver; calorie restriction; liver steatosis; liver fibrosis

Rationale & background information

Disease activity and progression of non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH) and cirrhosis can be highly variable (1), where 2-3% will eventually progress to end-stage liver diseases. With the rising prevalence of metabolic syndrome and obesity, NAFLD has become the most frequent form of chronic liver disease in the West but also in Asia (2, 3).

There are good evidence that weight loss is effective in improving liver histology in NAFLD, for example, Pomrat et al randomised 31 obese patients with NASH into intensive lifestyle changes over 48 weeks versus structured basic education only, and the intensive lifestyle group showed significant improvements in steatosis, necrosis, and inflammation (4). Intense calorie restriction is the recommended form of dietary strategy for management of NAFLD. Even though such intense dietary strategy has proven to be effective (4, 5), some patients find it difficult to adhere and maintain.

On the other hand, intermittent fasting achieves more consistent weight loss by improving adherence (6, 7), as intermittent fasting only requires calorie restriction every other day compared to conventional form of daily calorie restriction (8). Alternate day calorie restriction can be divided into two components, a 'feed day' and a 'fast day' where food is consumed ad libitum for 24 hours period alternating with either complete or partial (modified) calorie restriction for the next 24 hours. Modified alternate-day calorie restriction (MACR), the dietary strategy employed in our study, restricts 70% of an individual's daily requirement of calorie per day (8). There are other forms of intermittent fasting, for example, 2-4 days of ad libitum feeding alternating with 2-4 days of calorie restriction (8-11).

Currently, there are no approved pharmacological therapies for NAFLD, and many guidelines advocate recommendation with a focus on controlling risk factors and lifestyle interventions that include dietary and physical activities. No specific NAFLD trials have evaluated the effectiveness of modified form of intermittent fasting in the control of NAFLD activity. Therefore, our study aimed to determine the effectiveness of 8 weeks of MACR in changes of BMI, biochemical and ultrasound parameters in NAFLD and to evaluate the adherence rate of such dietary strategy.

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Study goals and objectives

Research question

1. Does of MACR associated with improvement of mean liver steatosis and elastrography using shear wave elastography in NAFLD patients?
2. Does MACR associated with improvement of biochemical parameter (lipid profile, liver chemistries) in NAFLD patients?
3. Does MACR have a good adherence rate for dietary strategy

Study goals
To determine the effectiveness of 8 weeks of MACR in changes of BMI, biochemical and ultrasound parameters in NAFLD and to evaluate the adherence rate of such dietary strategy.

Primary objective
To determine the effectiveness of 8 weeks of MACR in changes of BMI, biochemical and ultrasound parameters in NAFLD

Secondary objective
To evaluate the adherence rate of MACR in NAFLD patients

<u>Study Design</u>

Type of study
Randomized control trial

Study Duration and location
Study Location : Gastroenterology Clinic and Radiology Department Hospital USM.
Study Duration : 24 months

Sampling Population and sample
Reference Population:
- Non-Alcoholic Fatty Liver Disease (NAFLD)

Source Population
Gastroenterology Clinic in Hospital Universiti Sains Malaysia (HUSM), Kota Bharu, Kelantan NAFLD patient

Sampling Frame
Inclusion criteria
1. Age ranges 18 to 70 years old
2. Baselines Ultrasound show evidence of fatty liver
3. Elevated alanine aminotransferase (ALT) or aspartate aminotransferase (AST) values (ALT>41 or AST>34U/L)

Exclusion criteria
1. Medically or surgically ill patients who cannot consent
2. Complicated chronic liver disease with portal hypertension
3. Significant alcohol consumption (1 standard drink per day)
4. Contraindications to calorie restriction
5. Pregnancy
6. Engagement in an active weight loss program
7. Taking weight-loss medication, substance abuse
8. Significant psychiatric problems

Withdrawal criteria
1. Subject who unable to tolerate alternate daily fasting intervention during the trial
2. Patient own choice

Methodology

Participants

NAFLD patients who attending the Gastroenterology Clinic at Hospital Universiti Sains Malaysia, a tertiary referral centre for the north-eastern Peninsular Malaysia, will be screen from August 2015 till July 2016.

During the first visit, if participants met the selection criteria, informed consent are then taken. Demographic data of participants are recorded. All participants are required to complete a two-week run-in period consisting of maintaining and self-monitoring their usual dietary and daily activities.

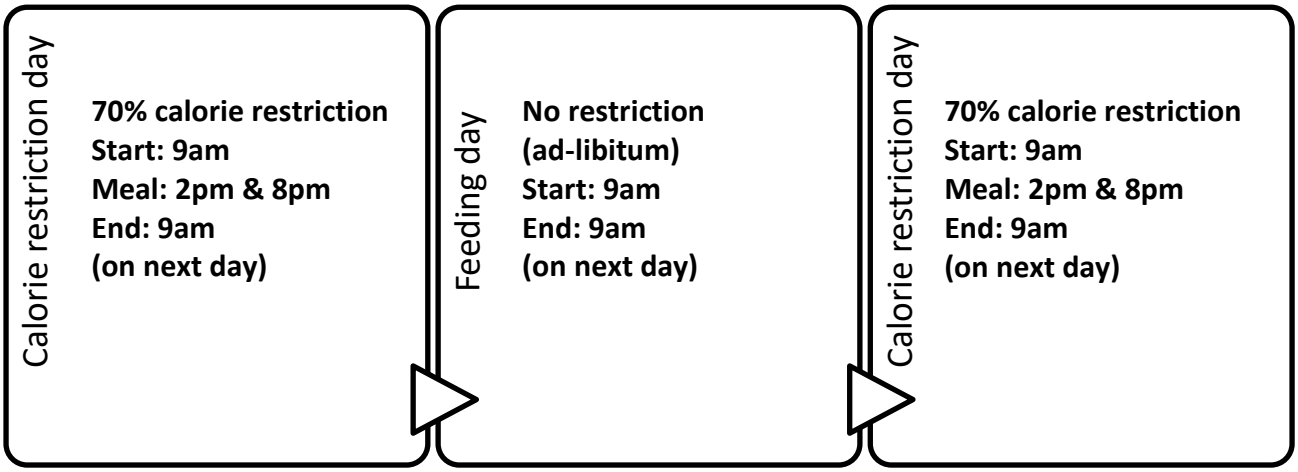
Participants who fulfilled the selection criteria are randomly assign to the MACR group or the control group after a 2 week run-in period. During the 2 weeks, participants are requiring to keep their body weight stable by maintaining their usual eating habits and their daily activities. Randomization is performed using a random number generator with recruitment aimed for three MACR participants for every control participant by principal investigator. Calculated sample size of 46 participants would achieve a significant difference in studied outcome between intervention and control group using a two-sided test with $\alpha = 0.05$ and power = 0.8. Participants are blinded after assignment to interventions or control group.

Interventions

MACR group

For a total of 8 weeks, participants in this group need to restrict 70% of their energy needs over 24 hours on a calorie restriction day alternate with a feeding day for the next 24 hours, where participants allowed eating whatever they wish (ad libitum). The calorie restriction and feeding days begin at 9am each day, and on the calorie restriction day, meals are consumed between 2pm and 8pm to ensure that each participant underwent the same duration of calorie restriction. The MACR protocol is shown in Figure 1. On each calorie restriction day, participants are allowed to take energy-free beverages, tea, coffee, and sugar-free gum and encouraged to drink plenty of water. Diet plans are not provided to participants but are self-selected using detailed individualized food portion lists, meal plans, and recipes. To ensure maximal adherence to dietary plan, participants received phone calls from the investigator (Izzad) and 2-weekly appointments (total four appointments) with a dietician described below. Adverse experiences are assessed every 2 weeks until 1 week after the completion of trial.

Figure 1 MACR protocol for the 8 weeks duration



Assessment of dietary plan adherence

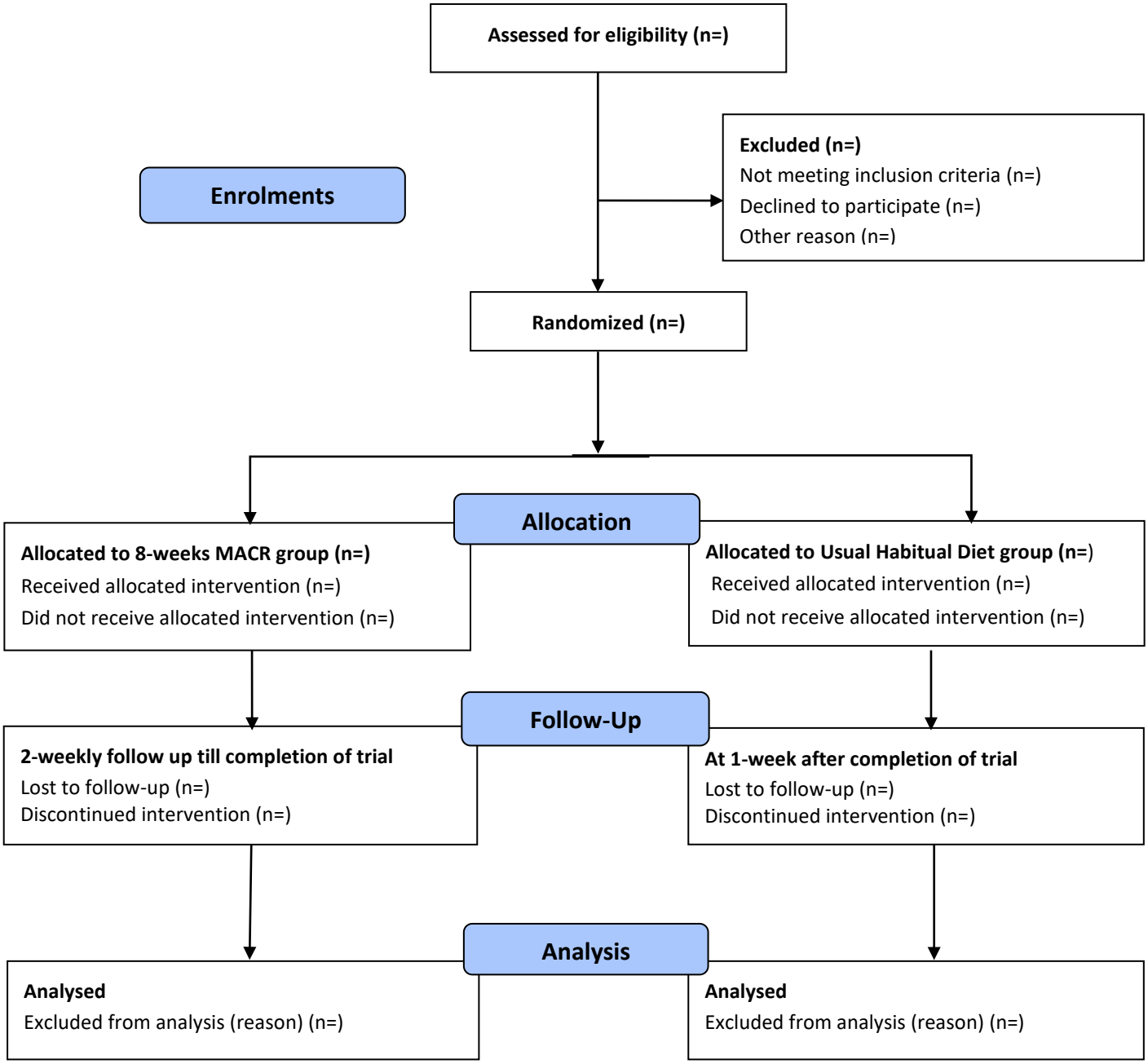
A designated dietician (KhairunNisah) will provide education to participants regarding calorie restriction and help to monitor their dietary compliance. Adherence to intervention is assessed via a diet diary and dietary recall. The diary is collected and reviewed by the dietician every 2 weeks. If the diary indicates that the participants eat an extra calorie on a fasting day, that day is labelled as "not adherent." If the diary revealed that the participant did not eat any extra food item, that day is labelled as "adherent." Adherence data are assessed each week as 1) absolute adherence (number of adherent days with diet) and 2) percentage of adherence calculated by applying the following formula for adherence in percentage. The formula is shown below.

$$\frac{\text{Number of fast day's adherent}}{\text{Number of fast days in week}} \times 100$$

Control group

All participants in the control group will continue their usual habitual diet for 8 weeks. No specific dietary advice or educations are provided throughout the entire trial.

Figure 2 Flow Diagram



Management of diabetes medications during the study period

Previous reports found that Insulin sensitizing agents like thiazolidinediones and metformin might induce biochemical and histological effects similar with NAFLD (9-12). Therefore, participants, who were already taking thiazolidinedione or metformin, must be on a stable regimen for at least 6 months prior to this study enrolment and the regimen must remain stable during the study. All participants are not allowed to start on any of these medications during the entire study period to avoid any potential confounding effects. Participants who continue to have active disease despite on these medications should be allowed to participate in the study trial to maximize generalizability. If medically necessary, participants were allowed new medications for hyperglycaemia and the available options included sulfonylureas, meglitinides and insulin.

Procedures

A standardized calibrated balance (Secca, ZT-120, and Hamburg, Germany) will be used to measure weight of participants in light clothing at each visit. A wall-mounted stadiometer (Secca, ZT-120, Hamburg, Germany) is used to measure height (in m), and together with weight (in Kg), body mass index (BMI) is calculated. Waist circumferences are measured with the participants standing, and with the measuring tape kept horizontal and measured at a level midway between the superior aspect of the iliac crests and the lower lateral margins of the ribs.

Blood samples - 8-10 hours of fasting blood samples are collected at 8-10 am at baseline and within 1 week post intervention for biochemical analysis. High-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), total cholesterol, fasting blood sugar and liver enzymes (alanine aminotransferase, ALT and aspartate aminotransferase, AST) are measured.

Ultra-sonographic measurements including liver steatosis and shear wave elastograhy (SWE) are performed with the SuperSonic Imagine's Aixplorer® Ultrasound machine (Super Sonic Image, Aix-en Provence, France). All measurements are performed by a single sonographer (Khairiah) where the inter-observer agreement level with another experienced sonographer (Juhara) is 85%. Hepatic steatosis is graded as normal (grade 0), mild (grade 1), moderate (grade 2) and severe (grade 3) based on visual analysis of intensity of echogenicity with an appropriate gain setting based on standardised paramete (13-14). Homogenous echo-texture and echogenicity equal or slightly more than the renal cortex and spleen are characteristics of a normal liver. Liver echogenicity would be higher than the renal cortex and spleen if there was hepatic steatosis.

Through the intercostal approach, SWE measurements are performed in the right liver lobe, at the supine position with the right arm in maximal abduction. The sonographer, assist by an ultrasonic time-motion image, located a liver portion of at least 6 cm thick, free of large vascular structures. Once the measurement area had been located, the sonographer presses the probe button to start an acquisition. Patients are asked to hold their breath for about five seconds, while the stiffness of the region of interest was measured and 10 measurements are made for each patient and the median average value of those measurements is recorded in kilopascals (kPa: metric). Based on median liver elastography values, previously published four categories could be determined for different fibrosis stage.

Safety consideration

There may be risks to you if you participate in this study. These tests are fairly safe with litle risk involved. For most people, needle puncture for blood draws will cause discomfort but do not cause any serious problems. If any important new information is found during this study that may affect you wanting to continue to be part of this study, you will be told about it right away.

Data management and statistical analysis

Descriptive statistics are computed for all variables; means with standard deviations (SD) for continuous variables, median with interquartile range (IqR) for ordinal data and frequencies with percentages for categorical variables. Using Kolmogorov-Smirnov analysis, the data were normally distributed if the skewness is within the range of ±2 time standard error (SE)

of skewness. Per-protocol (PP) and intention-to-treat (ITT) analysis are performed where comparison is made within-group (post- vs. pre-intervention for each intervention and control group) and between-group (MACR vs. control). Missing data are imputed using multiple imputation method. Independent *t*-test is used to analyse changes in weight, liver chemistry, lipid profile variables, fasting blood sugar and ultrasonographic changes of liver steatosis and fibrosis between the MACR and control group. SPSS version 22 (SPSS Inc., Chicago, United States) is used for all statistical analysis. All *P* values quoted were two-sided with $P<0.05$ considered as statistically significant. Sample size is 46 participants with 80% power of the study.

Expected outcomes and benefit of the study

This study is the first to determine that modified alternate day calorie restriction (MACR) is an effective dietary intervention compares to control group to help NAFLD individuals to lose weight, lower CAD risk and improving liver status. Specifically, we expected here that MACR regimen, which allowed participants to consume 30% of their energy needs on the fast day, resulted in a mean weight loss between 3-5% from baseline after 8 week of intervention thus it is also expected to decreases in several key biomarkers for CAD risk, such as total cholesterol, LDL cholesterol, triacylglycerols and blood pressure. Liver parameter which included liver enzymes, steatosis and fibrosis would also expect to improve after 8 weeks MACR. Additionally, we expected here that a similar rate of weight loss and adherence rate achieved during the MACR regime when compared with other alternate day calorie restriction regime.

Problems Anticipated

Compliance to dietary modification is one of the problems anticipated while conducting the trial. To overcome this problem, 2 weekly together with designated dietician given to participants in interventional group to monitor the calorie intake as well as education regarding calorie calculations. Besides that, we also will call participants once in a week to ensure the participants comply with dietary modification.

Ethical Issues

This study will be conducted with the highest respect for the subjects according to the study protocol, the ethical principles that have their origin in the Declaration of Helsinki, and the International Conference and Harmonisation (ICH) - Harmonised Tripartite Guideline for Good Clinical Practice (GCP). All researchers of this study will be GCP certified.

The current clinical trial is approved by the Human Research and Ethics Committee of Universiti Sains Malaysia (reference: USM/JEPeM/15040117).

1. Subject vulnerability

1.1 The participants who involved in the study will be provided comprehensive explanation regarding the study procedure, any known adverse reactions, anticipated benefits and potential hazards of the study. If the subject is under my care as a doctor, the patient will be given full freedom to participate or not without affecting her medical condition management and care.

1.2 Participants will not get vulnerable to the study as data obtained from this study does not identify participants individually. Besides that, the data will be independent and will not be used for any achievement assessment and decision related to work

1.3 In addition, participants are given option to terminate their involvement in the study at any time without penalty or loss of benefits if they want to stop. A list of contact number of the investigators will be included in the participants information leaflet and they can directly contact the investigator if they have any queries or opt to cease the study.

2. Declaration of absence of conflict of interest

There is no conflict of interest presence in the study as all the participants are from Gastroentrology Clinic, Hospital USM. The researcher does not hold any supervisory or administrative post over the potential subjects that may influenced the participatory decision making.

3. Privacy and confidentiality

All participants' data including informed consent, medical history, examination will be recorded on the documents prepared for the study. All forms are anonymous and will be entered into SPSS software. Data will be presented as grouped data and will not identify the responders individually.

The medical information will be kept confidential by the study doctor and staff and will not be made publicly available unless disclosure is required by law. Data obtained from this study that does not identify participants individually will be published for knowledge purposes.

4. Community sensitivities and benefits

This study will benefit the participants as well as community. Participants may receive information about their liver status from examination to be done in this study and further treatment will be offer if indicated.

The study allow us to explore the role of MACR in treating NAFLD in addition to exploring feature of NAFLD using new ultrasound techniques. Result and information from the study will provided evidences that modified alternate day calorie restriction (MACR) will be realible alternative ways to improve liver steatosis and fibrosis by weight loss compare to conventional ways.

For community, the study allow us to later educate about NAFLD, including its risk, ultrasound diagnosis and role of modified alternate daily calorie restriction. Furthermore, the findings from study may allow us to apply for larger grants.

5. Honorarium and incentives

Honorarium including transportation cost will be given to the participants during the study.