Alternate day fasting combined with exercise for the treatment of non-alcoholic fatty liver disease (NAFLD)

Principal investigator:
Krista A. Varady, Ph.D.
Associate Professor
Department of Kinesiology and Nutrition
University of Illinois, Chicago
1919 West Taylor Street, Room 532
Chicago, Illinois, 60612
Tel: 312-996-7897
Email: varady@uic.edu

Study location:
Human Nutrition Research Unit
1919 West Taylor Street, Room 121C
Chicago, Illinois, 60612

Sponsor:
NIH (NIDDK) R01 DK119783

Version 13

June 15, 2023

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ADF	Alternate day fasting
BIA	Bioelectrical Impedance analysis
CRP	C-Reactive Protein
DXA	Dual-energy X-ray Absorptiometry
FGF-21	Fibroblast growth factor-21
IL-6	Interleukin-6
MRS	Magnetic resonance spectroscopy
NAFLD	Non-alcoholic fatty liver disease
OGGT	Oral glucose tolerance test
PI	Principal investigator
RBP4	Retinol binding protein 4
SHBG	Sex hormone binding globulin
TNF-a	Tumor necrosis factor-alpha

1.0 Project Summary/Abstract

Background: Approximately 65% of obese individuals have non-alcoholic fatty liver disease (NAFLD), and this condition is strongly related to the development of insulin resistance and diabetes. Innovative lifestyle strategies to treat NAFLD are critically needed. Alternate day fasting (ADF) has been shown in animals to reduce hepatic steatosis and improve hepatic insulin sensitivity, but these findings have yet to be confirmed in human subjects. ADF consists of a "feast day" where individuals are permitted to consume food *ad libitum*, alternated with a "fast day" where individuals consume 25% of their usual intake (~500 kcal). We performed a pilot study to evaluate the effects of ADF combined with exercise, versus ADF or exercise alone, on hepatic parameters in obese patients. Our results show that the combination of ADF plus exercise produced greater reductions in alanine aminotransferase (ALT; an indirect marker of hepatic steatosis), compared to ADF alone, or exercise alone, after 12 weeks. Greater decreases in insulin resistance, HbA1c, LDL cholesterol, and more pronounced increases in HDL cholesterol, were observed in the combination group versus individual interventions. Data from our pilot trial also suggest that these decreases in insulin resistance may be mediated in part by changes in hepatocyte-derived hormones (hepatokines) that occur with liver fat reduction. Although these pilot findings are very promising, these data still require confirmation by a well powered longer-term (24 week) clinical trial. **Hypotheses:** The present proposal will test the following hypotheses: **(1)** The combination group (ADF plus exercise) will experience greater reductions in hepatic steatosis (measured by magnetic resonance spectroscopy; MRS) when compared to ADF or exercise alone; **(2)** The combination group will experience greater improvements in hepatokine profile (fetuin-A, fetuin B, FGF-21, RBP4, selenoprotein P, SHBG, adropin) when compared to ADF or exercise alone; **(3)** The combination group will experience greater improvements in hepatic insulin sensitivity, insulin resistance and HbA1c and other metabolic disease risk variables (fasting glucose, fasting insulin, triglycerides, LDL cholesterol, blood pressure, inflammatory parameters) when compared to ADF or exercise alone. **Methods:** To test these objectives, a 24-week randomized, controlled, parallel-arm feeding trial will be implemented. Obese individuals with NAFLD (n = 360) will be randomized to 1 of 4 groups: (1) ADF (fast day: 25% energy intake, feed day: ad libitum fed, no exercise), (2) exercise (ad libitum fed, training 5 days/week), (3) combination (ADF plus exercise), and (4) control (ad libitum fed, no exercise). **Significance:** If the aims of this application are achieved, this study will be the first to show that the combination of alternate day fasting plus aerobic exercise is an effective non-pharmacological therapy to reduce hepatic steatosis, improve hepatic insulin sensitivity and prevent the progression to type 2 diabetes in NAFLD patients.

2.0 Background/ Scientific rationale

Nonalcoholic fatty liver disease (NAFLD) is characterized by an accumulation of fat in the liver (not resulting from excessive alcohol consumption). Approximately 65% of obese individuals have NAFLD, and this condition is strongly related to the development of insulin resistance and type 2 diabetes.[1-4] While certain pharmacological agents have been shown to reduce liver fat (i.e. thiazolidinediones), there is mounting concern regarding the safety and weight-gaining effects of these compounds.[5,6] In light of this, recent research has focused on non-pharmacological lifestyle therapies to reduce hepatic steatosis, such as daily calorie restriction combined with aerobic exercise. Evidence from clinical trials suggest that this combination is an effective lifestyle therapy improve liver fat content and hepatic insulin sensitivity.[7,8]

More recently, it's been shown that **intermittent fasting** may produce *even greater* improvements in hepatic steatosis and hepatic insulin sensitivity, when compared to conventional calorie restriction. For instance, intrahepatic lipid accumulation was lower and insulin sensitivity was higher in mice fasted every other day, when compared to mice who were energy restricted every day.[9] Moreover, data from human trials show that obese adults experience greater decreases in insulin and insulin resistance with intermittent fasting versus daily restriction.[10,11] These findings suggest that intermittent fasting may be a more effective diet therapy to reduce hepatic steatosis and improve insulin sensitivity, when compared to daily calorie restriction.

We recently performed a pilot study to evaluate the effects of alternate day fasting (ADF) and exercise, alone or combined, on various hepatic parameters in obese adults. ADF consists of a "fast day", in which 25% of energy needs (~500 kcal) are consumed, alternated with a "feed day", in which food is consumed ad libitum. Results from our pilot study show that the combination of ADF plus exercise produced greater reductions (-21 $\pm$ 9%) in alanine aminotransferase (ALT; an indirect marker of hepatic steatosis), compared to ADF alone (-13 $\pm$ 4%), or exercise alone (-16 $\pm$ 7%) after 12 weeks. Greater decreases in insulin resistance, HbA1c, LDL cholesterol, and more pronounced increases in HDL cholesterol, were observed in the combination group versus individual interventions. Data from our pilot trial also suggest that these decreases in insulin resistance by ADF plus exercise may be mediated in part by changes in hepatocyte-derived hormones (fetuin-A and FGF-21) that occur with liver fat reduction.[12] Although these pilot findings are very promising, these data still require confirmation by a larger-scale (n = 360), longer-term (24 week) clinical trial. Accordingly, the specific aims of this study are:

3.0 Objective/Aims

Specific aim 1: To compare the effects of a combination intervention (ADF and aerobic exercise) versus ADF or exercise alone on hepatic steatosis over 24-weeks in ~~prediabetic~~ obese NAFLD subjects.

Hypothesis 1: The combination group will experience greater reductions in hepatic steatosis (measured by MRS) when compared to the ADF group and the aerobic exercise group.

Specific aim 2: To compare the effects of a combination intervention (ADF and aerobic exercise) versus ADF alone or aerobic exercise alone on hepatokine profile over 24-weeks in ~~prediabetic~~ obese NAFLD subjects.

Hypothesis 2: The combination group will experience greater decreases in hepatokines with negative metabolic actions (fetuin-A, fetuin B, FGF-21, RBP4, selenoprotein P) and greater increases in hepatokines with positive metabolic actions (SHBG, adropin) when compared to the ADF group and the exercise group.

Specific aim 3: To compare the effects of a combination intervention (ADF and aerobic exercise) versus ADF alone or aerobic exercise alone on hepatic insulin sensitivity, insulin resistance, HbA1c, and other metabolic disease risk variables over 24-weeks in ~~prediabetic~~ obese NAFLD subjects.

Hypothesis 3: The combination group will experience greater improvements in hepatic insulin sensitivity, insulin resistance and HbA1c and other metabolic disease variables (fasting glucose, insulin, plasma lipids, blood pressure, TNF-α, IL-6) when compared to the ADF group and the exercise group.

4.0 Eligibility

Subject eligibility will be assessed and determined by Kelsey Gabel M.S., R.D., or key research personnel identified in Appendix P. Screening will be conducted over Zoom and at the Human Nutrition Research Unit (HNRU) located in the Applied Health Sciences Building (1919 West Taylor St, Room 121C).

4.1 Inclusion criteria:

- Age between 18 to 65 years old
- BMI between 30.0 and 59.9 kg/m^2
- NAFLD (hepatic steatosis ≥ 5% previously diagnosed) and ALT > 15 U/L
- Prediabetic (fasting glucose: 100-125 mg/dl, HbA1c: 5.7-6.4%) or normoglycemic (fasting glucose: <100 mg/dl, HbA1c: <5.7%) [13]
- Sedentary (<20 min, 2x/week of light activity at 3-4 metabolic equivalents (METs) for 3 mo prior to study) [14]

4.2 Exclusion criteria:

- Have chronic liver disease other than NAFLD (hepatitis B or C, primary biliary cirrhosis, sclerosing cholangitis, autoimmune hepatitis, hemochromatosis, Wilson's disease, α1-antitrypsin deficiency)
- Consume excessive amounts of alcohol (Michigan Alcohol Screening Test score > 4) [15]
- Have a history of known cardiovascular, pulmonary or renal disease
- Diagnosed T1DM or T2DM
- Are not weight stable for 3 months prior to the beginning of study (weight gain or loss > 4 kg)
- Are claustrophobic or have implanted metallic/electrical devices (e.g. cardiac pacemaker, neuro-stimulator)
- Are not able to keep a food diary or activity log for 7 consecutive days
- Are taking drugs that induce steatosis (e.g. corticosteroids, estrogens, methotrexate, Ca channel blockers)
- Are taking drugs that benefit NAFLD (e.g. betaine, pioglitazone, rosiglitazone, metformin, or gemifibrozil)
- Are taking drugs that influence study outcomes (weight loss, lipid-lowering, glucose-lowering medications)
- Are perimenopausal or have an irregular menstrual cycle (menses that does not appear every 27-32 days)
- Are pregnant, or trying to become pregnant
- Are smokers

5.0 Subject enrollment

Independently living subjects from the Chicago area will be recruited by flyers posted around the University of Illinois at Chicago (UIC) campus. ResearchMatch.org will also be used to recruit participants. Subjects will also be recruited from the Hepatology Clinic at the University of Illinois Medical Center by Dr. Sean Koppe MD. During clinical visits with these NAFLD patients, Dr. Koppe will describe the study to each of these individuals and distribute a flyer and contact information to those who are interested. Study procedures will be conducted at the subject's home.

COVID screening precautions: The study coordinator will call the subject prior to each on-campus appointment and screen the subject using the UI Health COVID screening form. If the subject passes this screening, they will be invited for an in-person visit. When they arrive on campus, the subject will be once again screened using the UI Health COVID screening form. Their temperature will also be checked using a no-touch digital thermometer. All research personnel and participants will be required to wear masks during each appointment. Only one research staff will be present to limit exposure. This screening procedure will be implemented every time the subject comes to campus for a study visit.

Each subject will attend an online screening appointment and an in-person appointment. **During the online appointment:** Subjects will review the consent form with the Study Manager, Kelsey Gabel, MS, RD, over Zoom. The subject will then sign the consent form using Adobe Sign. After signing the consent form, the subject will be screened via the approved questionnaire, which will assess eligibility based on the requirements listed above. If

the subject is eligible for the study based on the questionnaire, they will be invited for an in-person appointment. **During the in-person appointment:** The subject will visit the HNRU to have these measurements taken: body weight and height (for BMI), a blood draw to assess HbA1c, ALT and fasting glucose, a pregnancy test, and the Michigan Alcohol Screening Test (for alcohol consumption). The Study Manager will also email a 7-day food record to the subject, and provide detailed instructions on how to complete the record. The subject will be asked to return the food record within 10 days.

Subjects who meet all the inclusion/exclusion criteria will be invited to participate in the study. Eligible subjects will be randomized by way of a stratified random sample. The sample frame will be divided into strata based on BMI, sex, and age. Subjects from each stratum will then be randomized to 1 of 4 groups: 1) ADF, 2) aerobic exercise, 3) combination (ADF plus aerobic exercise), or 4) control. **A total of n = 360 subjects (n = 90 per intervention group) will be recruited.** All the subjects will have the right to withdraw from the study at any time.

6.0 Study design and procedures

A 24-week randomized, controlled, parallel-arm feeding trial will be implemented to test the effects of ADF and exercise, alone and in combination, on hepatic steatosis/fibrosis, hepatokines, hepatic insulin sensitivity, and other metabolic disease variables. Obese individuals with NAFLD (n = 360) will be randomized to 1 of 4 groups: (1) ADF (fast day: 25% energy intake, feed day: ad libitum fed, no exercise), (2) exercise (ad libitum fed, training 5 days/week), (3) combination (ADF plus exercise), and (4) control (ad libitum fed, no exercise). Changes in hepatic steatosis will be measured by MRS, and hepatic insulin sensitivity will be assessed by OGTT testing and the Matsuda Index. All study activities will be carried out at the Human Nutrition Research Unit (HNRU) located in the Applied Health Sciences Building (1919 West Taylor St, Room 121C).

Diet and exercise interventions

Group	Diet protocol	Exercise protocol
Combination (n = 45)	Fast day: 25% energy intake Feed day: Ad libitum fed	5 days/week, supervised 60 minutes, Intensity: 85% HRmax
ADF (n = 45)	Fast day: 25% energy intake Feed day: Ad libitum fed	No exercise
Exercise (n = 45)	Every day: Ad libitum fed	5 days/week, supervised 60 minutes, Intensity: 85% HRmax
Control (n = 45)	Every day: Ad libitum fed	No exercise

Diet protocol

ADF group and combination groups: These participants will consume 25% of their baseline energy needs on the "fast day" and eat ad libitum at home on alternating "feed days".

Fast day (week 1-4): All fast day meals will be provided to subjects during week 0-4. Meals will be prepared in the metabolic kitchen of the HNRU. The diets will be provided as a 3-d rotating menu comprised of typical American foods. Meals will be formulated on the basis of the American Heart Association (AHA) guidelines (30% kcal from fat, 15% kcal from protein, and 55% kcal from carbs). All meals will be consumed outside of the research center. **Fast day (week 5-24):** At week 5, fast day meals will no longer be provided to subjects. Instead, subjects will meet one-on-one with a dietician for 60 min to learn how to self-select healthy foods

to stay within the calorie limits on the fast day. **Feed day:** On the feed day, subjects will eat ad libitum at home.

Exercise group and control groups: These participants will be asked to maintain their regular eating habits during the trial. They will eat ad libitum at home every day, and will not receive any packaged meals or dietary counseling.

Exercise protocol

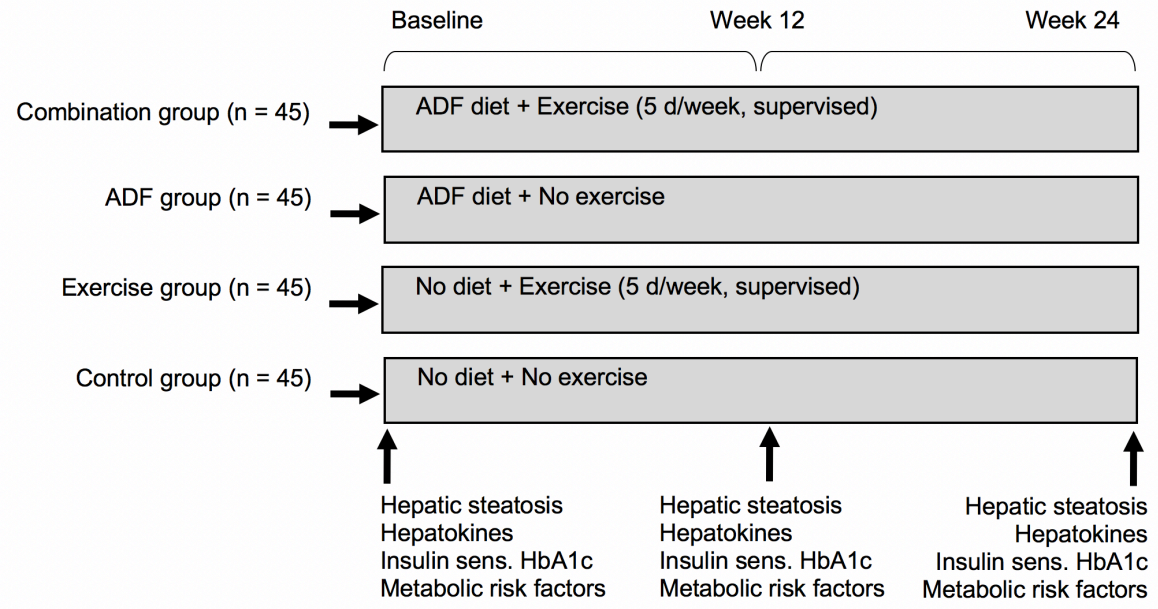
Exercise group and combination groups: Only combination and exercise groups will partake in the exercise training. All training sessions will be conducted at the subject's home. Subjects will participate in a supervised aerobic exercise program 5 times per week, 40-60 min/d, for 24 weeks.[16] Exercise will be performed by watching YouTube aerobic exercise (Zumba) videos at home. The video links will be provided by the study coordinator prior the exercise session. The coordinator conducting the study session on Zoom will have immediate knowledge of the subject's location so that they can relay this information to emergency personnel if needed. During the week 1 sessions, subjects will exercise for 40 minutes at a moderate intensity (65% heart rate max (HRmax)). During the week 2 sessions, subjects will train for 50 minutes at a higher intensity (75% HRmax). For weeks 3-24, subjects will train for 60 minutes at an even higher intensity (85% HRmax). The participants will wear heart rate monitors (Fitbit Alta HR, Boston, MA) during each training session to provide visual feedback of their individualized target heart rate. Fitbit monitors will be distributed to subjects so that they can monitor their heart rate at home. **Compliance with the training:** A research assistant will be present to supervise all the training sessions over Zoom. All sessions will be pre-scheduled with the research assistant so the participant knows when to enter the Zoom meeting to perform the activity. Exercise will be carefully documented through regular online attendance at the training sessions. Subjects will be permitted to choose the day and time slot that works best for them. If a subject misses their session, they will be required to make up for it that same week. Subjects will be permitted to miss 5 sessions total during the 24-week trial.

ADF group and control groups: ADF and control subjects will be asked to maintain their regular daily activity habits during the study. They will not participate in the exercise protocol.

Control group protocol: Controls will be instructed to maintain their weight throughout the trial, and not to change eating or physical activity habits. Controls will not receive packaged meals or dietary counseling. Controls will visit the research center at the same frequency as the treatment groups (for outcome measurements) to control for investigator-interaction bias between groups. Controls will be offered free weight loss diet counseling (4 sessions) at the conclusion of the study by Kelsey Gabel, MS, RD.

Subject compensation. Each subject will receive $500 upon completion of the trial. Compensation will be prorated (please see Initial Review application for details).

Summary of intervention groups and key outcome measures



Study activities

Study activities	Study visits (week of study)																							
	Screen	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Informed consent	●																								
Screening questionnaire	●																								
Michigan Alcohol Screening test	●																								
Pregnancy test	●	●												●											●
Body weight (at home)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Blood draw	●	●												●											●
Blood pressure/ heart rate		●												●											●
Body composition (BIA)		●												●											●
Waist circumference		●												●											●
Body composition (DXA)		●												●											●
Activity monitor		●												●											●
Hepatic insulin sensitivity (OGTT)		●												●											●
Hepatic steatosis (MRS)		●												●											●
Food record	●	●												●											●
Sleep questionnaires		●												●											●
Time per visit:	**1**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**

Body weight, blood pressure, heart rate: Body weight measurements will be taken weekly with subjects wearing light clothing and without shoes using a balance beam scale at home. Digital scales will be provided to each subject. Height will be assessed using a wall-mounted stadiometer at week 1. BMI will be assessed as kg/m^2. Blood pressure and heart rate will be assessed at week 1, 13, 24.

Body composition: Dual energy X-ray absorptiometry (DXA) will be performed on all subjects at week 1, 13, and 24 (iDXA, GE Inc) to assess fat mass, fat free mass, and visceral fat mass. Percentage body fat will be assessed in triplicate using a tetra-polar bioelectrical impedance analyzer each week (Omron HBF-500; Omron Health Care, Bannockburn, IL). Waist circumference will be measured weekly.

Hepatic steatosis by magnetic resonance spectroscopy (MRS): MR scans will be performed at the Center for Magnetic Resonance Research (CMRR). MR images and spectrum will be acquired using a 3.0 T MR scanner. MR examinations will be performed by experienced MR technologists. Subjects will arrive at the CMRR in the morning following an overnight (8 h) fast. Total hepatic triglyceride content will be assessed at week 1, 13 and 24 by ^{1}H MRS. A body array MRI coil will be affixed to each subject's back and aligned with the subject's spine and shoulders for accurate repositioning of repeat scans. Each subject will be positioned face down and head first in the scanner on a memory foam mattress to minimize respiratory motion. An 8 cm^3 voxel is positioned within the right posterior lobe of the subject's liver with guidance from the high-resolution localization images. Manual shimming will be performed to a line width of ~40Hz to ensure high quality spectra. MR spectra with and without water suppression will be acquired with a single-voxel PRESS acquisition with a long repetition time (TR=5000ms), and a short echo time (TE=30ms) to limit the effects of magnetic relaxation. [17,18] The acquisition will be obtained with 32 averages to obtain sufficient signal, and the data will be Fourier-Transformed, filtered, baseline corrected, and phased. Areas of resonances from protons of water and methylene groups in fatty acid chains of the hepatic triglyceride will be evaluated with a line-fit procedure and commercial software (NUTS-ACORNNMR, CA).

Blood sampling: Twelve-hour fasting blood samples will be collected at week 1, 13, and 24 consecutive feed and fast days (20 ml at each time point). All blood draws will be performed at the HNRU by Kelsey Gabel. Blood will be centrifuged for 15 min at 520 x g and 4°C to separate plasma from red cells, and will be stored at -80°C at the HNRU. Specimens will be labeled with the subject ID and study week, and will be stored until the study is completed. After the study is over, all specimens will be destroyed.

Hepatic insulin sensitivity by OGTT and Matsuda Index: Hepatic insulin sensitivity will be assessed at week 1, 13 and 24 by the oral glucose tolerance test (OGTT) and Matsuda Composite Index. [19] For this procedure, subjects will arrive at the research center in the morning after a 12-h fast. They will have their blood drawn (20 ml), and then be given a 75 g dose of glucose (Thermo Scientific Glucose Tolerance Test Beverage, Waltham, MA). Four blood samples (20 ml each) will be taken 30, 60, 90, and 120 min after the glucose dose for the measurement of glucose and insulin. Hepatic insulin sensitivity will be calculated as Matsuda's Insulin sensitivity index (ISI) = [10000/(fasting glucose x fasting insulin x mean glucose during OGTT x mean insulin during OGTT)$^{1/2}$].

Activity monitor: All subjects will be instructed to maintain their regular daily activity habits (i.e not join a gym or participate in other exercise classes). Alterations in physical activity habits will be quantified by an activity monitor (Fitbit Alta HR, Boston, MA)) that will be worn on the wrist for 7 days at week 1,13, and 24.

Food record: Dietary intake will be assessed in all groups by a 7-day food record at week 1, 13 and 24. Subjects will be given detailed guidelines on how to complete the records by the Dietician at baseline. Dietary intake data will be assessed by Nutritionist Pro software (Axxya, TX).

Michigan Alcohol Screening test: Alcohol consumption will be assessed by the Michigan Alcohol Screening Test at the screening appointment. [15]

Metabolic disease risk factors: Plasma metabolic disease risk factors will be assessed at baseline week 1,13, and 24. Plasma total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations will be measured in duplicate using enzymatic kits, standardized reagents, and standards (Biovision Inc, Mountainview, CA) and analyzed using a microplate reader (iMark Microplate Reader; Bio-Rad Laboratories Inc, Richmond, CA). HbA1c will be assessed by ELISA (Mybiosource, San Diego, CA). Fasting plasma glucose concentrations will be measured with a hexokinase reagent kit in duplicate (A-gent glucose test, Abbott, South Pasadena, CA). Fasting insulin will be measured as total immunoreactive insulin (Coat-A-Count Insulin, Los Angeles, CA). Insulin resistance (IR) will be calculated by the HOMA (Homeostasis Model Assessment) method: [HOMA-IR = Fasting insulin (μIU/ml) × Fasting glucose (mg/dL) / 405].

Proteomics and metabolomics: Plasma-derived proteomic and metabolomic outcomes will be measured using immunoassay and mass spectrometry, as described previously.[20, 21]

Hepatokine plasma levels: Plasma hepatokines will be measured at week 1, 13, and 24. Commercially available ELISA kits will be used to measure concentrations of Fetuin-A, fetuin-B, FGF-21, SHBG, and RBP4, (R&D Systems, Minneapolis), and adropin and selenoprotein P (Mybiosource, San Diego, CA). All samples will be assessed in duplicate.

Inflammation: Inflammatory markers will be measured at week 1, 13, and 24. C-reactive protein (CRP) will be measured in duplicate using Immulite 1000 High Sensitivity CRP kits (Diagnostic Products Corpor- ation, Los Angeles, CA). Adiponectin, leptin, TNF-a, and IL-6 will be measured using ELISA (R&D Systems, Minneapolis, MN).

Sleep questionnaires: Sleep quality and duration will be measured by validated questionnaires at week 1, 13, and 24. The severity of insomnia in the past week will be measured by the Insomnia severity index (ISI), which is a 7-item questionnaire. Each item is rated by a 5-point Likert scale (where 0 indicates no problem, and 4 indicates a very severe problem) yielding a total score of 0-28. The total score for the ISI is interpreted as follows: no clinically significant insomnia (0-7), sub-threshold insomnia (8-14), moderate severity insomnia (15-21), and severe insomnia (22-28). Sleep quality, timing and duration will be measured by the Pittsburgh Sleep Quality Index (PSQI). This 19-item self-report questionnaire measures total sleep quality in the past month, yielding a total score of 0-21. A PSQI total score >5 indicates poor sleep quality. The questionnaire also asks for usual bedtime, usual wake time, and hours of actual obtained sleep. Risk of obstructive sleep apnea will be estimated using the Berlin Questionnaire at baseline.

7.0 Expected risk and benefits

Risks: Blood draws: Blood drawing may cause temporary discomfort from the needle stick, bruising, and infection. A total of 18 blood samples (20 ml each) will be collected over 24 weeks = 360 ml total. **Alternate day fasting:** Alternate day fasting is generally well tolerated and has no harmful effects. Subjects may feel hungry, however, which is unpleasant. **DXA scans:** DXA scans will be performed to assess body composition (3 scans total). Subjects who participate in this study will receive a small amount of radiation from the DXA scanning. The amount is similar to that received in many standard x-ray procedures, but is considerably more than subjects would receive from natural daily exposure or in the normal course of treatment, and it carries at least a theoretical risk. **Magnetic resonance spectroscopy (MRS):** There are no known hazards to MRS examination other than the discomfort associated with confinement and remaining stationary for the duration of the study. Testing of all subjects will be terminated immediately should they become distressed. We will carefully screen out individuals who have an implanted electrical device (such as a cardiac pacemaker or a neurostimulator), or a certain type of metallic clip (i.e., an aneurysm clip in the brain). These devices can malfunction and cause harm to the patient in the magnet environment. We will also carefully screen individuals who are claustrophobic to reduce any adverse events. **Confidentiality:** To protect subject identity, only code numbers (i.e. randomly generated numbers) will identify all laboratory specimens, evaluation forms, reports, and other records. **Exercise program:** Risks of participating in the

exercise program include: muscle/joint strains, sprains, or injury. If a subject is injured during the exercise session, they will be asked to stop exercising immediately and rest. The muscle/joint will be iced and elevated, and the subject will be sent home with a small ice pack and asked to ice/elevate the area for 20 minutes every hour to alleviate pain and inflammation. The subject will refrain from participating in the exercise session until the pain has significantly improved. If the pain does not improve in 48 hours, it will be recommended that the subject follow-up with their doctor. A cardiac event may also occur as a result of participating in the exercise program. If this occurs, 911 will be immediately called by the research assistant who is supervising the exercise session and the subject will be treated by a medical professional.
Risk of coercion: Some subjects will be recruited from the Hepatology Clinic at the University of Illinois Medical Center by Dr. Sean Koppe MD (Co-investigator). Thus, there is a risk that subjects may feel coerced by Dr. Koppe to participate in the research. To mitigate this, each subject will be clearly informed that they are not obligated to participate in this research study offered by Dr. Koppe. Subjects will also be informed that participating in the study is completely voluntary and they are free to not participate. They will also be told that their decision to not participate will not affect your clinical care now or in the future.

Benefits: Subjects may lose weight as a result of treatment. Since weight loss has been shown to improve some metabolic disease risk factors, subjects partaking in the treatment may experience these benefits to their overall health. No benefits can be guaranteed, however.

8.0 Data collection and management procedures

Sources of data include body weight and body composition measurements, hepatic steatosis measurement by MRS, hepatic insulin sensitivity by OGTT, blood pressure measurements, biochemical analyses of blood draws, activity monitor measurements, and questionnaire responses. Questionnaires will be stored in locked filing cabinets at the HNRU. To protect subject identity, code numbers only will identify all laboratory specimens, evaluation forms, reports, and other records. Subject identities will not be used in any reports or publications resulting from this study. All patient records will be kept in locked files; code sheets linking the patient's name to a patient identification number will be stored separately in a locked file cabinet. Only study personnel will have access to the files. Blood specimens will be stored at the HNRU in a locked -80C freezer. Specimens will not be labeled with any personal identifiers. Only the subject ID and study week will be on the label of the blood specimens. Code sheets linking the patient's name to a patient identification number will be stored separately in a locked file cabinet.

9.0 Data analysis

Data will be analyzed by Sally Freels PhD (Biostatistician) and Krista Varady by SPSS software (SPSS v24).

10.0 Quality control and quality assurance

The PI will be responsible for the evaluation of data quality, and this will be done on a weekly basis by evaluating biochemical analyses output and reviewing questionnaire responses.

11.0 Data and safety monitoring

Assessment of Risk: The risk of significant adverse events to participants in this study is low. During the 24-week study, participants will undergo 18 blood draws, 3 DXA scans, 3 MRS scans, alternate day fasting and exercise.
Anticipated Adverse Events: Blood drawing may cause temporary discomfort, bleeding or bruising at the needle site and in rare cases fainting and infection. As for the DXA scan, the radiation dose associated with DXA measurements is very low or even insignificant in comparison with background radiation levels. Subjects may feel hungry as a result of the ADF diet, which may be unpleasant. Other forms of psychological stress may result from the dietary restriction or the demands of participating in and complying with a study. The subjects will be clearly informed of the expectations of the study at their

screening appointment (e.g. dietary protocol, exercise intervention, time commitment, etc.), and will be free to withdraw from the study at any point. There are no known hazards to MRS examination other than the discomfort associated with confinement and remaining stationary for the duration of the study. Testing of all subjects will be terminated immediately should they become distressed. We will carefully screen out individuals who have an implanted electrical device (such as a cardiac pacemaker or a neurostimulator), or a certain type of metallic clip (i.e., an aneurysm clip in the brain). These devices can malfunction and cause harm to the patient in the magnet environment. We will also carefully screen individuals who are claustrophobic to reduce any adverse events. Loss of confidentiality may also be a risk. To protect subject identity, only code numbers (i.e. randomly generated numbers) will identify all laboratory specimens, evaluation forms, reports, and other records. Risk of participating in the exercise program includes: muscle/joint strains, sprains, or injury. If a subject is injured during the exercise session, they will be asked to stop exercising immediately and rest. The muscle/joint will be iced and elevated, and the subject will be sent home with a small ice pack and asked to ice/elevate the area for 20 minutes every hour to alleviate pain and inflammation. A cardiac event may also occur as a result of participating in the exercise program. If this occurs, 911 will be immediately called by the research assistant who is supervising the exercise session and the subject will be treated by a medical professional.

Adverse Event Grading Scale: Because only minor adverse events related to blood drawing, DXA scanning, MRS scanning, alternate day fasting and exercise may occur, no grading scale is necessary.

Reporting of Adverse Events: Reporting of adverse events will follow requirements mandated by the University of Illinois Office for Protection of Research Subjects (OPRS) and the NIH. The OPRS will receive a verbal or e-mail report of any serious adverse event (SAE) that occurs during the conduct of the study within 48 hours. This will be followed by a detailed written report within 10 working days. In addition, any incidents or problems involving the conduct of the study or patient participation, including problems with the recruitment and/or consent processes will be reported within 10 working days. The NIH Program Official will be provided with a detailed written report of fatal, life threatening SAEs and unanticipated problems (UP) within 7 calendar days of the event. The NIH Program Official will be notified of any UPs that pose greater risk of harm to study participants than was previously known or recognized within 30 calendar days of the event.

Safety Monitoring Plan: Sterile procedures will be employed for all blood drawing procedures. Subjects will be provided with a 24-hour contact phone number to report any persistent problems. The OPRS will be notified of any adverse events as described above.

Frequency of Safety Reviews: Safety reviews will be conducted annually in conjunction with the protocol renewal to OPRS.

Persons to Perform Safety Reviews: The PI will be responsible for reporting any adverse events and conducting the annual safety review of study data.

12.0 Statistical considerations

All continuous variables will be examined for distributions and the presence of outliers. Variables that are not normally distributed will be transformed and if normality cannot be achieved, will be analyzed using non-parametric tests. Standardized descriptive statistics including measures of means, median, standard deviations, ranges, and standard errors for continuous variables within each group will be calculated to describe the groups at fixed time points. Differences between groups at baseline will be assessed by a one-way ANOVA. A repeated measures ANOVA will be used to evaluate the effects of diet, exercise, and their interaction (diet x exercise) on dependent variables; a significant interaction will be interpreted with a Bonferroni correction for multiple comparisons. A multivariate analysis that adjusts for the effects of confounding variables (age, sex, race, and baseline hepatic steatosis, hepatic insulin sensitivity, hepatokine profile, body weight, and body composition) will also be performed. Relations between continuous variables will be assessed by Pearson's or Spearman's correlation coefficients as appropriate. Data will be analyzed using SPSS software (SPSS, Chicago, IL).

13.0 Regulatory requirements

13.1 Informed consent: Consent will be obtained prior to the administration of the screening questionnaire by a member of the study team (Kelsey Gabel or key research personnel identified in Appendix P). The informed consent document will be stored in a locked filing cabinet at the Human Nutrition Research Center (1919 W Taylor St, Room 121C). All study team members have completed the CITI and HIPAA training courses.

13.2 Subject confidentiality: To protect subject identity, code numbers only will identify all laboratory specimens, evaluation forms, reports, and other records. Subject identities will not be used in any reports or publications resulting from this study. All patient records will be kept in locked files; code sheets linking the patient's name to a patient identification number will be stored separately in a locked file cabinet. Only study personnel will have access to the files.

13.3 Unanticipated problems: Heart disease, diabetes and fatty liver disease risk parameters will be measured during the study. If the lab tests indicate that disease risk has increased as a result of participating in the study by week 12 or 24, the subject will be immediately informed. A printout of the lab values will be given to the subject and they will be asked to follow up with their doctor. Reporting of adverse events will follow requirements mandated by the University of Illinois Office for Protection of Research Subjects (OPRS) and the NIH. The OPRS will receive a verbal or e-mail report of any serious adverse event (SAE) that occurs during the conduct of the study within 48 hours. This will be followed by a detailed written report within 10 working days. In addition, any incidents or problems involving the conduct of the study or patient participation, including problems with the recruitment and/or consent processes will be reported within 10 working days. The NIH Program Official will be provided with a detailed written report of fatal, life threatening SAEs and unanticipated problems (UP) within 7 calendar days of the event. The NIH Program Official will be notified of any UPs that pose greater risk of harm to study participants than was previously known or recognized within 30 calendar days of the event.

14.0 References

1. Lazo M, Hernaez R, Eberhardt MS, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. Am J Epidemiol 2013;178:38-45.
2. Le MH, Devaki P, Ha NB, et al. Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States. PLoS One 2017;12:e0173499.
3. Bugianesi E, Moscatiello S, Ciaravella MF, Marchesini G. Insulin resistance in nonalcoholic fatty liver disease. Curr Pharm Des 2010;16:1941-51.
4. Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Nutrients 2013;5:1544-60.
5. Bell LN, Wang J, Muralidharan S, et al. Relationship between adipose tissue insulin resistance and liver histology in nonalcoholic steatohepatitis: a pioglitazone versus vitamin E versus placebo for the treatment of nondiabetic patients with nonalcoholic steatohepatitis trial follow-up study. Hepatology 2012;56:1311-8.
6. Musso G, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. Hepatology 2010;52:79-104.
7. Goodpaster BH, Delany JP, Otto AD, et al. Effects of diet and physical activity interventions on weight loss and cardiometabolic risk factors in severely obese adults: a randomized trial. JAMA 2010;304:1795-802.
8. Shah K, Stufflebam A, Hilton TN, Sinacore DR, Klein S, Villareal DT. Diet and exercise interventions reduce intrahepatic fat content and improve insulin sensitivity in obese older adults. Obesity (Silver Spring) 2009;17:2162-8.

9. Baumeier C, Kaiser D, Heeren J, et al. Caloric restriction and intermittent fasting alter hepatic lipid droplet proteome and diacylglycerol species and prevent diabetes in NZO mice. Biochim Biophys Acta 2015;1851:566-76.
10. Harvie M, Wright C, Pegington M, et al. The effect of intermittent energy and carbohydrate restriction v. daily energy restriction on weight loss and metabolic disease risk markers in overweight women. Br J Nutr 2013;110:1534-47.
11. Harvie MN, Pegington M, Mattson MP, et al. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women. Int J Obes (Lond) 2011;35:714-27.
12. Meex RCR, Watt MJ. Hepatokines: linking nonalcoholic fatty liver disease and insulin resistance. Nat Rev Endocrinol 2017;13:509-20.
13. Diabetes Management Guidelines. American Diabetes Association. 2017. Available from: http://www.ndei.org/ADA-diabetes-management-guidelines-diagnosis-A1C-testing.aspx.html.
14. Pate RR, O'Neill JR, Lobelo F. The evolving definition of "sedentary". Exerc Sport Sci Rev 2008;36:173-8.
15. Connor JP, Grier M, Feeney GF, Young RM. The validity of the Brief Michigan Alcohol Screening Test (bMAST) as a problem drinking severity measure. J Stud Alcohol Drugs 2007;68:771-79.
16. Edition. ACoSMt. ACSM's Guidelines for Exercise Testing and Prescription. : Lippincott Williams and Wilkins.; 2017.
17. Fealy CE, Haus JM, Solomon TP, et al. Short-term exercise reduces markers of hepatocyte apoptosis in nonalcoholic fatty liver disease. J Appl Physiol (1985) 2012;113:1-6.
18. Haus JM, Solomon TP, Kelly KR, et al. Improved hepatic lipid composition following short-term exercise in nonalcoholic fatty liver disease. J Clin Endocrinol Metab 2013;98:E1181-8.
19. Selimoglu H, Duran C, Kiyici S, et al. Comparison of composite whole body insulin sensitivity index derived from mixed meal test and oral glucose tolerance test in insulin resistant obese subjects. Endocrine 2009;36:299-304.
20. Perry A, Tanriverdi K, Risitano A, et al. The inflammatory proteome, obesity, and medical weight loss and regain in humans. Obesity (Silver Spring). 2023;31:150–158.
21. Murthy V, Reis J, Pico AR, et al. Comprehensive metabolic phenotyping refines cardiovascular risk in young adults. Circulation. 202;142:210.