



# Nonalcoholic steatohepatitis Fitness Intervention in Thrombosis (NASHFit): Study protocol for a randomized controlled trial of a supervised aerobic exercise program to reduce elevated clotting risk in patients with NASH

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## ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease worldwide affecting upwards of one third the global population. For reasons not fully understood, individuals with NAFLD and its more severe variant, nonalcoholic steatohepatitis (NASH), are at increased risk for venous thromboembolism which significantly increases morbidity and mortality. Lifestyle changes centering around exercise training are the mainstay of treatment for NAFLD/NASH. While exercise training can lessen venous thromboembolic risk in healthy persons and those with cardiovascular disease, whether or not this benefit is seen in patients with NAFLD/NASH remains unknown. In order to better understand how exercise training impacts thrombosis risk in NAFLD, we present the design of a thirty-two week randomized controlled clinical trial of 42 sedentary subjects age 18–69 with biopsy proven NASH. The main aim is to determine the impact of an aerobic exercise training program on the abnormal hemostatic system unique to NAFLD/NASH. The main outcome is change in plasminogen activator inhibitor one level, an established marker for venous thromboembolism. Secondary outcomes include body composition, cardiorespiratory fitness, control of comorbid metabolic conditions (e.g., obesity, hypertension, hyperlipidemia, diabetes), dietary composition, health related quality of life, liver enzymes and histology, NAFLD/NASH disease activity (e.g., biomarkers, clinical decision aids), microbiome, other markers of hemostasis, and PNPLA3 gene expression. The study represents the first clinical trial of an exercise training program to reduce elevated clotting risk in subjects with NAFLD/NASH.

## 1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in the United States, affecting 100 million adults and costing the healthcare system \$32 billion annually [1–4]. Patients

with NAFLD have inferior survival, independent of metabolic comorbidities, although this effect appears to be driven largely by the presence of fibrosis [5,6]. Nonalcoholic steatohepatitis (NASH), the more severe variant of NAFLD, is characterized by inflammation and/or fibrosis [7–9]. The development of NAFLD and its progression to NASH is a multifactorial process that is in part due to physical inactivity [10,11].

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Currently, there are no approved pharmacologic treatments that are universally effective in reversing fibrosis in NASH. Consequently, dis-

NASH; 30–45 min of exercise, five days a week is recommended for all patients with NAFLD/NASH [26–33]. Exercise has a favorable effect on

### Abbreviations:

ABW	adjusted body weight	IPAQ	International Physical Activity Questionnaire
ACSM	American College of Sports Medicine	IRB	institutional review board
AE	adverse event	ITT	intention to treat
AF	activity factor	LDL	low density lipoprotein
ALT	alanine aminotransferase	MRI	magnetic resonance imaging
AST	aspartate aminotransferase	NAFLD	nonalcoholic fatty liver disease
BMI	body mass index	NAS	NASH Activity Score
CAT	computerized adaptive testing	NASH	nonalcoholic steatohepatitis
CK	cytokeratin	NFS	NAFLD Fibrosis Score
DXA	dual-energy x-ray absorptiometry	NIH	National Institutes of Health
ECG	electrocardiogram	PAI	plasminogen activator inhibitor
ELISA	enzyme-linked immunosorbent assay	PDFF	proton density fat fraction
EP	exercise physiologist	PI	principal investigator
FIB-4	Fibrosis-4 Index	PNPLA3	patatin like phospholipase-3
GAQ	Get Active Questionnaire	PROMIS	Patient-Reported Outcomes Measurement Information System
GRE	gradient echo sequence	RD	registered dietitian
HDL	high density lipoprotein	REE	resting energy expenditure
HOMA-IR	homeostatic model assessment of insulin resistance	RPE	rating of perceived exertion
HR	heart rate	TEE	total energy expenditure
HRQOL	health-related quality of life	TEG	thromboelastography
INR	international normalized ratio	VO <sub>2</sub> max	maximal oxygen consumption
IBW	ideal body weight	VTE	venous thromboembolism

ease progression to cirrhosis and end-stage liver disease occurs not infrequently [10]. Five million persons have NASH cirrhosis [7–9]. NASH cirrhosis is expected to become the number one reason for liver transplantation in the imminent future [12]; NASH is already the leading indication in women [13].

The effect of NASH is not limited to the liver. Patients with NASH have multiple extrahepatic manifestations including abnormalities in the hemostatic system [14,15]. Hemostasis can be broken down into primary (clot formation), secondary (clot stabilization) and tertiary (clot breakdown) stages, all of which are impaired in patients with NASH. Increased platelet aggregation and reactivity (primary hemostasis) is observed in NASH. This is mediated through elevated levels of leptin and von Willebrand factor and low levels of adiponectin [15–17]. NASH also leads to ubiquitous endothelial dysfunction which promotes additional abnormality in primary hemostasis [15–17]. NASH is a hypercoagulable state (secondary hemostasis) due to increased Tissue Factor, Factor VII and VII. These factors initiate the coagulation cascade and stabilize clot formation [14,15]. Plasminogen activator inhibitor (PAI)-1, is the best described prohemostatic factor in NASH. Elevated PAI-1 in parallel with decreased tissue-activating factor antigen and tissue plasminogen activator leads to a chronic state of impaired fibrinolysis (tertiary hemostasis) [15]. Elevated PAI-1 independently promotes thrombotic risk and may accelerate liver disease progression due to intrahepatic thrombi [18]. The presence of at least one thrombotic risk factor is associated with a nearly two-fold fibrosis stage increase in NASH [19]. From a clinical standpoint, patients with NASH are independently predisposed to venous thromboembolism (VTE) through adiposity-dependent impaired fibrinolysis [20]. Independent of metabolic comorbidities, liver transplant recipients with NASH have over a two-fold increased risk of portal vein thrombosis [21,22]. Hospitalized patients with NASH cirrhosis have a two and a half greater risk of deep vein thrombosis and/or pulmonary embolism [23]. Portal vein thrombosis, deep vein thrombosis and pulmonary embolism all significantly increase morbidity and mortality in patients with cirrhosis [24,25].

Increased physical activity is the first-line treatment for NAFLD/

the hemostatic system in healthy persons [34–36]. Chronic exercise training improves primary hemostasis in patients with vascular disease by improving endothelial dysfunction and lessening platelet activation and aggregation [37]. Exercise training also activates fibrinolysis [34, 35,38–41]. Reductions in PAI-1 following aerobic exercise programs 12 weeks or longer range from 23 to 37% [38,40–43]. Whether or not aerobic exercise training lowers thrombotic risk by favorably modulating the hemostatic system in patients with NASH remains unknown. We aimed to determine if supervised, structured aerobic exercise training reduces PAI-1 level in patients with biopsy-proven NASH when compared to standard clinical care.

## 2. Methods

### 2.1. Overview

NASH Fitness Intervention in Thrombosis (NASHFit) is a single center, randomized controlled clinical trial (NCT03518294). NASHFit seeks to randomize 42 subjects between age 18–69 years who have a liver biopsy diagnosing NASH within the preceding six months and a lack of secondary causes of steatosis including significant alcohol intake to 16 weeks of either: 1) Exercise training including supervised aerobic exercise sessions with an American College of Sports Medicine (ACSM) certified Exercise Physiologist (EP) and dietary counseling from a Registered Dietitian (RD) or 2) Standard of care where subjects maintain their current activity level verified by weekly phone calls and remote monitoring with fitness activity trackers with heart rate monitor (Fitbit ChargeHR2, Fitbit Inc, San Francisco, CA, USA). The primary aim of this study is to compare changes in serum PAI-1 level between conditions. We hypothesize that exercise subjects will have a 23% reduction in PAI-1 while control subjects will have no change after 16 weeks [38,40–42]. The exercise intervention did not include strength training sessions as the impact of strength training on PAI-1 is unknown in either healthy persons or those with chronic vascular disease. Secondary outcomes include other markers of hemostasis, control of metabolic comorbidities,

hepatic fat, liver fibrosis, NASH Activity Score (NAS), fitness level as measured by maximal oxygen consumption (VO<sub>2</sub> max) testing, body composition, weight and body mass index (BMI), microbiome, metabolomics, Patatin like phospholipase-3 (PNPLA3) rs738409 polymorphism genotype and gene activity, macronutrients and health-related quality of life (HRQOL). All study procedures are approved by the Institutional Review Board (IRB) at Pennsylvania State University College of Medicine.

## 2.2. Eligibility criteria

Table 1 describes the inclusion and exclusion criteria for NASHFit. This trial was designed to select participants with NASH who would derive the greatest benefit of intervention without an exercise training related adverse event (AE) including musculoskeletal injury or a cardiac event. NASH is a well-established clinical and histologic diagnosis [44, 45]. The six-month window wherein a liver biopsy was obtained prior to randomization was chosen to ensure the most accurate histologic assessment of NASH; this time period is standard of care across multiple landmark NASH clinical trials [46,47].

Similar to previous landmark exercise training trials in subjects with NASH [48,49], we exclude potential participants who over the previous three months reported >90 min/week of at least moderate intensity exercise in order to select sedentary individuals. To assure that informed consent was obtained, we exclude people who cannot complete the consent in English. To ensure safety of VO<sub>2</sub>max testing, we exclude subjects with BMI >45 kg/m<sup>2</sup> [50] or decompensated cirrhosis. If there is a positive response to any question asked by the Get Active Questionnaire (GAQ) including active cardiac symptoms, the subject is referred back to their primary healthcare provider to determine whether or not exercise is safe [51]. We also exclude potential participants who report being unable to walk ¼ mile or >2 blocks independently, active substance abuse or smoking, due to concerns this would limit their ability to meaningfully participate in the intervention. We exclude potential subjects participating in a weight-loss program or using a weight-loss supplement to avoid bias from additional weight loss beyond what would be expected from study intervention. For

**Table 1**  
Inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
Adults age ≥18 or <70 years	>90 min/week of at least moderate intensity exercise over the previous three months
Liver biopsy ≤6 months prior to enrollment	Pregnancy
Biopsy proven NASH [45]	BMI <18 or >45 kg/m <sup>2</sup> [50]
Lack of secondary causes of hepatic fat accumulation:	Uncontrolled diabetes [31] <sup>c</sup>
• Significant alcohol consumption <sup>a</sup>	Active cardiac symptoms
• Chronic hepatitis C	Severe medical comorbidities/psychiatric illness
• Wilson disease	Decompensated cirrhosis <sup>d</sup>
• Lipodystrophy	Cancer with life expectancy <6 months
• Parenteral nutrition	MRI contraindications
• Long-term use of steatogenic medications <sup>b</sup>	Other liver disease
• Monogenic hereditary disorders	Active weight-loss program participation or weight-loss supplement use
	Active substance abuse/smoking
	Inability to provide informed consent
	Institutionalized/prisoner
	Inability to walk >2 blocks or 1/4 mile
	Non-English-speaking patients <sup>e</sup>
	Anticoagulants excluding 81 mg of aspirin daily

BMI = body mass index; MRI = magnetic resonance imaging; NASH = non-alcoholic steatohepatitis.

<sup>a</sup> <21drinks/week for men and <14 drinks/week for women.

<sup>b</sup> Mipomersen, lomitapide, amiodarone, methotrexate, tamoxifen, corticosteroids.

<sup>c</sup> Changes in medication dosing over the previous three months or hemoglobin A1c >9%.

<sup>d</sup> Bleeding gastroesophageal varices, ascites or hepatic encephalopathy.

<sup>e</sup> Due to unavailability of translators for all visits/sessions.

individuals whose baseline visit reveals pregnancy, uncontrolled diabetes [31] or an abnormal electrocardiogram (ECG) after review by a board-certified cardiologist, study enrollment is placed on hold and the patient is referred back to their primary medical provider for further clinical management. We also exclude subjects on anticoagulants or antiplatelet agents except for aspirin at a dosage of 81 mg once daily.

## 2.3. Recruitment and screening procedures

The NASHFit Trial is in the process of enrolling 42 subjects at one tertiary care academic medical center in the United States. There are three main recruitment methods. First, recruitment letters are mailed to gastroenterology and hepatology providers in our clinical referral basis. Second, we use a range of mass communication methods (e.g., posters, working with our public relations department to develop stories for local television stations, newsletters, clinical trial brochures, hospital on-hold message). Social media is also used as a standard recruitment element. StudyKIK (<https://studykik.com>), an online social media recruitment platform, allows patients to find and sign up for clinical trials with ease through Facebook, Twitter, Instagram, Pinterest, Google, Bing, and Yahoo. Daily recruitment messages and advertisements are placed on these platforms. Patient traffic is filtered using targeting software within a 25-mile radius of Penn State Milton S. Hershey Medical Center matching the inclusion and exclusion criteria of the study. For other trials utilizing StudyKIK at Penn State, on a monthly basis, there are over 1000 site views, 150 subject contacts, 50 subject phone interviews, 2–5 screening visits and 2–5 conversions with study enrollment. Third, we deliver multiple in-person presentations at local medical practices, hospital seminars and local medical conferences. All interested participants are provided with a study description and screened for eligibility in-person or by phone. Eligible participants are scheduled for a baseline data collection and randomization visit.

## 2.4. Randomization

We are using blocked randomization. Once a subject has completed their screening and baseline data collection, they are randomized 2:1 into either the exercise and control group respectively using a computer-generated randomized scheme in blocks of six. Randomization is automatically performed by the study database (REDCap, Vanderbilt University), a secure, web-based application that supports data capture for research studies and serves as a data repository [52].

## 2.5. Measures

Table 2 lists the study measures and their timing. All self-reported measures are completed by participants via iPad, web browser or smartphone based FitBit application. Stool collection kits are distributed and the subject obtains and submits the sample from home. Subjects are called at week 32 to assess if they are continuing to exercise independently after intervention conclusion.

## 2.6. Primary outcome: plasminogen activator inhibitor (PAI)-1 level

NASHFit is designed and statistically powered to detect a difference in serum PAI-1 level. 0.5 mL of fresh or frozen (−80°C) serum are utilized to perform enzyme-linked immunosorbent assay (ELISA) before and after the intervention. PAI-1 present in the sample or standard binds to the PAI-1 antihuman monoclonal antibody coated onto microwells. A biotin-conjugated anti-human PAI-1 polyclonal antibody is next added to the wells and it binds to the PAI-1 captured by the first antibody. The plate is then incubated for 2 h at room temperature (18–25 °C) on a microplate shaker (400 rpm). Following incubation, the plate is washed three times with wash buffer to remove the unbound biotin-conjugated antihuman PAI-1 antibody. Streptavidin-HRP anti-human PAI-1 antibody is added to the dry wells and the plate is incubated for 1 h at room

**Table 2**  
Schedule of study measures.

Assessment	Baseline In-person	Every week In-person or phone	Every month In-person	End-of-program In-person	12-week follow-up Phone
<b>Primary outcome</b>					
PAI-1	X			X	
<b>Secondary outcomes</b>					
Blood pressure and heart rate	X		X	X	
BMI and weight	X	X	X	X	
Body composition	X		X	X	
Cardiopulmonary fitness	X			X	
Cholesterol	X			X	
Dietary composition	X	X	X	X	
Glycemic control <sup>a</sup>	X			X	
HRQOL	X		X	X	
Inflammation <sup>b</sup>	X			X	
Liver enzymes	X			X	
Liver histology <sup>c</sup>	X			X	
				(optional)	
Markers of hemostasis <sup>d</sup>	X			X	
MRI-PDFF/liver volume	X			X	
Microbiome	X			X	
NASH biomarkers <sup>e</sup>	X			X	
NASH Clinical Decision Aids <sup>f</sup>	X			X	
PNPLA3 genotype	X				
PNPLA3 expression	X			X	
<b>Covariates</b>					
Activity level	X	X	X	X	X
Medical/surgical/social history	X		X	X	
NASH and other medications	X		X	X	
Sociodemographics	X				
Sleep quality	X	X	X	X	

BMI = body mass index; HRQOL = health-related quality of life; MRI-PDFF = magnetic resonance imaging proton density fat fractionation; PNPLA3 = Patatin like phospholipase-3.

<sup>a</sup> Hemoglobin A1c, Insulin level, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

<sup>b</sup> Ferritin and White blood cell count.

<sup>c</sup> NASH Activity Score and Fibrosis assessment.

<sup>d</sup> Antithrombin, Factor VII, ADAMTS-13, D-dimer, Factor VIII, Fibrinogen, International Normalized Ratio (INR), P-selection, Platelet count, Protein S, Protein C, Thromboelastography (TEG), von Willebrand Factor.

<sup>e</sup> Adiponectin, Cytokeratin (CK)-18.

<sup>f</sup> NAFLD Fibrosis Score, Fibrosis-4 Index (FIB-4).

temperature at 400 revolutions per minute. After the incubation, the wells are washed three times again to remove the unbound Streptavidin-HRP. Immediately after the wash step a TMB (tetramethyl-benzidine) Substrate Solution is added to all the wells which is reactive to HRP. The plate is once again incubated at room temperature for 10 min and a blue color product is formed in proportion to the amount of human PAI-1 present in the sample of standard. The reaction is terminated by adding Stop Solution (1 M Phosphoric Acid) to all the wells which turns the mixture yellow. The absorbance of each well is then read in a BIO-TEK EL311 plate reader set at 450 nm. Assay data is quantified using the Gen5 All-In-One Microplate Reader Software and the concentrations of PAI-1 in the samples are determined from a standard curve generated from seven human PAI-1 standards.

## 2.7. Secondary study outcomes

### 2.7.1. Blood pressure and heart rate

Blood pressure (mmHg) and heart rate (bpm) are measured at the in-person baseline and monthly visits after the subject sits at rest for at least 5 min. We use an automated, portable blood pressure and heart rate monitor (Omron HEM 907XL) [53] or a manual sphygmomanometer (Welch Allyn) and fitness activity tracker with heart rate monitor (FitBit Charge HR2).

### 2.7.2. Body mass index (BMI) and weight

At baseline, weekly during the intervention, and at week 20, weight (kg) is measured with either a Scale-Tronix oversized wheelchair scale or Detecto scale. Height (cm) is measured with a portable stadiometer. BMI is calculated as weight in kilograms divided by the square of height in meters.

### 2.7.3. Body composition

Body composition is assessed by both dual-energy X-ray absorptiometry (DXA) and skin-fold measurements. DXA is completed at baseline and end of intervention. Skin-folds are measured monthly. Using the enCore-based X-ray Bone Densitometer system, the General Electric Healthcare Lunar iDXA® DXA scanner allows for accurate assessment of bone density and advanced body composition testing including data visualization, trending and reporting tools. CoreScan allows for visceral fat quantification. Each subject lays flat on the machine surface and remains completely still while breathing normally. The scan lasts for 7–13 min depending on body thickness, which is automatically calculated by the DXA scanner based on input height and weight. Skin folds will be completed with a Harpenden Skinfold Caliper (Baty International, England) which is calibrated using metrics traceable to National Standards (e.g., measuring range 0–80 mm, accuracy 99%). Skinfolds are obtained at seven sites: chest (diagonal fold taken one half of the distance between the nipple and the anterior axilla), abdominal (vertical skinfold measurement taken 2.5 cm to the right of the umbilicus), thigh (vertical skinfold measurements taken half the distance between the patella and the inguinal crease with the leg straight and relaxed), triceps (vertical fold parallel to the long axis of the arm midway between the acromion process and the olecranon process), subscapular (diagonal fold taken on the upper back, just below the inferior angle of scapula at a 45-degree angle approximately parallel to the inferior angle of the scapula), suprailiac (diagonal fold following the natural line of the iliac crest), and midaxillary (vertical fold taken on the midaxillary line at the level of the nipple). Measurements are completed by a trained ACSM-EP and then assessed using the Harpenden Skinfold Caliper Body Assessment Software (Baty International, England).

### 2.7.4. Cardiopulmonary fitness

All participants are asked to complete cardiopulmonary fitness testing at baseline and again at end of intervention. Subjects complete a VO2max test on the Trackmaster Treadmill using the ParvoMedics TrueOne 2400 metabolic measuring system and the QuintonQ-Stress ECG monitor [54]. The Quinton Q-Stress ECG machine monitors heart rhythms. The Parvo Medics TrueOne 2400 measures indirect calorimetry and VO2max and includes a paramagnetic oxygen analyzer (range 0–100%, accuracy 0.1%, response 200 ms), infrared carbon dioxide analyzer (range: 0–15%, accuracy 0.1%, response:100 ms), and Rudolph heated pneumotach flow/volume measurement (range 0–800 L/min, accuracy ± 2% with Precision “Yeh” Algorithm).

Following a 1 min calibration period, each subject then begins the VO2max test and follows the Bruce treadmill ramp protocol. The Bruce treadmill ramp protocol has previously been demonstrated to be safe and feasible in subjects with NASH [55,56]. The Bruce treadmill ramp protocol includes: Stage 1 = 1.7 mph at 10% Grade, Stage 2 = 2.5 mph at 12% Grade, Stage 3 = 3.4 mph at 14% Grade, Stage 4 = 4.2 mph at 16% Grade, Stage 5 = 5.0 mph at 18% Grade, Stage 6 = 5.5 mph at 20%

Grade, Stage 7 = 6.0 mph at 22% Grade, Stage 8 = 6.5 mph at 24% Grade and Stage 9 = 7.0 mph at 26% Grade. Blood pressure is taken with a manual blood pressure cuff at the end of each stage until the subject starts to run. The subject is instructed on how to use the Borg Rated of Perceived Exertion (RPE) scale (range 6–20) which will be measured at the end of each stage [57]. Based on our previous experience [58], during the incremental protocols VO<sub>2</sub>max was assessed as the highest oxygen uptake with maximal HR within 10 beats per minute of age-predicted HR max and/or a respiratory exchange ratio (RER) value > 1.05, and or an RPE score of >18. Age-predicted VO<sub>2</sub>max levels were calculated using standard formulas for untrained normal weight individuals: VO<sub>2</sub>max (men) = 57.8 - (0.445 × age in years); VO<sub>2</sub>max (women) = 42.3 - (0.356 × age in years) [59]. VO<sub>2</sub>max values are provided in standard units of mL O<sub>2</sub>/kg/min.

After completion of the VO<sub>2</sub>max test, a 2–5 min cool down period ensues with continuous heart rhythm monitoring and occasional blood pressure measures. Once the subject has recovered to a heart rate near baseline, the treadmill is stopped. For all maximal tests, discontinuation is symptom limited. A physician and an ACSM-EP are present for all VO<sub>2</sub>max tests.

#### 2.7.5. Cholesterol, glycemic control, inflammation, liver associated enzymes, NASH biomarkers and clinical decision aids

Fasting total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides are measured from serum at week 0 and after intervention. For glycemic control, hemoglobin A1c, fasting glucose and fasting insulin level are measured at baseline and at week 20. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) [60] is calculated to evaluate insulin resistance as (Fasting insulin)\* (Fasting glucose)/405. To evaluate for inflammation, baseline and end of intervention assessment is completed by measuring serum levels of white blood cells (10<sup>9</sup> cells per liter) and ferritin (ng/mL). Liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are measured in IU/L at baseline and week 20 as is total bilirubin (g/dL). NASH biomarkers adiponectin and cytokeratin (CK)-18 as well as NASH clinical decision aids NAFLD Fibrosis Score (NFS) and Fibrosis-4 index (FIB-4) will be assessed at similar time points [7,61]. Paired Enhanced Liver Fibrosis (ELF) tests will also be completed pre- and post-intervention in order to non-invasively assess the impact of intervention on fibrosis stage [62].

#### 2.7.6. Dietary composition

Dietary composition is measured at baseline for all subjects, weekly during the intervention period starting at week 5 and then again at week 20. Subjects are instructed to self-report their daily dietary intake via the secure FitBit application. In the event a subject does not report with this technology, a paper 24-h dietary recall is completed at the direction of the study RD. Total energy, macronutrient (fat, protein and carbohydrate), fiber and sodium intake are automatically calculated by the Fitbit application. The study RD reviews dietary intake weekly utilizing Fitabase, a secure data management platform utilized by > 400 clinical trials, and provides individualized education and counseling on following a general, healthy diet including low saturated fat, high-fiber and low-sodium recommendations. This is completed in-person, by phone or by secure video conference on a weekly basis.

#### 2.7.7. Health-related quality of life (HRQOL)

As NAFLD/NASH patients have inferior HRQOL across multiple domains of health when compared to other etiologies of chronic liver disease [63], subjects will complete the Patient-Reported Outcomes Measurement Information System (PROMIS) Computerized Adaptive Testing (CAT) instrument via tablet computer (iPad). PROMIS-CAT is accessed through a pre-loaded website from the National Institutes of Health (NIH) through a web browser via a secure wireless internet connection. Subjects are asked to report their experience over the preceding week for each of nine health domains including anxiety/fear,

cognitive function, depression/sadness, fatigue, instrumental support, pain interference, physical function, sleep disturbance and social roles. A standardized protocol is used to describe the assessment process to the subjects as well as to provide direct assistance with any technical difficulties with the tablet computer. We have previously shown both the validity and security of this methodology using tablet computers in medical patients with chronic disease, many of whom have impairments comparable to patients with NASH [64,65]. Real-time scores for each health domain are automatically calculated and scaled to the underlying population distribution obtained from responses through the 2000 U.S. Census [66] using the T-score algorithms provided by the PROMIS Assessment Center software. The median T-score is 50, with one standard deviation equivalent to ±10 units. Scores are automatically stored on the secure PROMIS server from NIH.

#### 2.7.8. Liver histology

A liver biopsy within six months prior to enrollment is required to confirm the diagnosis of NASH and to stage fibrosis. We have an independent blinded liver pathologist calculate the NASH Activity Score (NAS) [45] and fibrosis stage. This will be compared to the clinical biopsy report. Interobserver agreement will be determined with a formal Kappa calculation (>0.7 will be considered agreement). Ten subjects from the exercise group will be sub-randomized to undergo an optional end of program biopsy after exercise training intervention as part of a pilot feasibility study. Where available, NAS and fibrosis stage will again be determined utilizing similar methods.

#### 2.7.9. Markers of hemostasis

Primary, secondary and tertiary hemostasis will be assessed at baseline and again at week 20 as will a dynamic assessment of the entire coagulation cascade. Antithrombin, Factor VII, ADAMTS-13, D-dimer, Factor VIII, Fibrinogen, International Normalized Ratio (INR), P-selection, Platelet count, Protein S, Protein C, Thromboelastography (TEG) and von Willebrand Factor will be measured. TEG will be performed within 2 h of a fresh blood draw to ensure reliable results. We anticipate the novel TEG results may be used to power future large-scale study.

#### 2.7.10. Magnetic resonance imaging proton density fat fractionation (MRI-PDFF) and liver volumetric assessment

Subjects undergo a non-contrasted MRI of the abdomen at baseline and after intervention to assess changes in hepatic fat content and liver size. Data will be acquired on a Siemens 3T PrismaFit system (Siemens Healthineers, Erlangen, Germany). Signal reception will utilize an 18-channel body flex array and the system 32-channel spine coil. A 2D breath-held multi-echo gradient echo sequence (GRE) will be acquired for calculation of Proton Density Fat Fraction (PDFF) maps. The sequence parameters are TR = 175 ms, TE = 2.30–10.35 ms, echo spacing = 1.15 ms, resolution = 2.08 × 2.08 \* 8 mm, and slices = 14. A separate 3D breath-held GRE Dixon-based sequence is to be acquired for calculation of total anatomical liver volume. Sequence parameters are TR = 3.97 ms, TE = 1.29/2.52 ms, resolution = 1.1875 × 1.1875 \* 2.5 mm, and slices = 82 to 96. Fat, water, in-phase, and opposing phase images will be reconstructed automatically at the system console. PDFF maps will be reconstructed in Matlab (Mathworks, Inc, Natick, MA) with in-house software to determine amount of hepatic steatosis [67]. The liver is then manually segmented from surrounding tissue with the ITK-SNAP package [68]. Liver segmentation is performed separately on both the PDFF maps and the 3D anatomical images. Care will be taken to exclude major vessels and visceral fat at the boundary of the liver. PDFF will be quantified as the mean PDFF value over the entire segmented liver. The volume of the segmented liver from the 3D anatomical images will be taken as the total liver volume.

#### 2.7.11. Microbiome

Home stool collection kits are submitted at baseline and after intervention. We will determine taxonomic composition of gut

microbiota using Illumina MiSeq 16S ribosomal RNA gene sequencing [69]. Bioinformatics analysis will include amplicon sequence variant determination, alpha diversity analysis, beta diversity analysis, relative abundance summaries and taxa enrichment analysis. Microbiome network analysis will also be completed.

2.7.12. *PNPLA3 genotype and gene expression*

PNPLA3 genotyping will be performed by purifying genomic DNA from whole blood by QIAasympphony DNA Midi Ki (Qiagen., Germantown, MD) following normal isolation protocol and concentrations obtained via NanoDrop ND-1000 (ThermoFisher Scientific, Wilmington, DE USA). Samples are normalized to 5 ng/uL. Using predesigned and validated TaqMan® SNP Genotyping Assay (AppliedBiosystems, Foster City, CA), DNA will be amplified in 384-well plate reactions with total volume of 5 µL using an ABI QuantStudio 12KFlex and Genotyping setup, with the following conditions: 50 °C for 2 min; 95 °C for 10 min; and 40 cycles of 95 °C for 15 s and 60 °C for 1 min followed by post-read stage. Reagents include Taqman Genotyping Master Mix (1 × final concentration), 900 nM for each primer, 200 nM for each probe, and 10 ng of DNA. Negative controls (no DNA template) are run on every plate. Genotypes are assigned by the automatic calling feature of the allelic discrimination option in QuantStudio 12 K Flex Software v1.2.2 (Applied Biosystems, Foster City, CA).

For PNPLA3 gene expression, total RNA will be isolated from whole blood by QIAasympphony PAXgene Blood RNA Kit (Qiagen., Germantown, MD). RNA quality and concentrations will be determined using the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). First strand cDNA will be produced from 1000 µg of total RNA using the standard High Capacity cDNA Reverse Transcription kit with RNase Inhibitors (ThermoFisher, Waltham, MA) protocol. cDNA concentrations will be quantified by absorbance using a NanoDrop-100 (ThermoFisher, Waltham, MA). Quantitative RT-PCR will be performed for seven targets of interest using an ABI QuantStudio 12KFlex Sequence Detection System (ThermoFisher, Waltham, MA). Assays will be prepared using 50 ng cDNA reaction product, 2X TaqMan Gene Expression Master Mix Master Mix and Assay-on-Demand primers and probes (900 nM unlabeled PCR primers; 250 nM FAM dye-labeled TaqMan MGB probe) (ThermoFisher, Waltham, MA) in a final reaction volume of 10 µl. Quantitative RT-PCR conditions are 2 min at 50 °C, 10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C. ABI SDS 2.2.2 software and the 2-ΔΔCt analysis method be used to quantitate relative amounts of product using 18S as endogenous control [70].

2.8. *Covariates*

2.8.1. *Activity level*

Baseline activity level is assessed by the validated International Physical Activity Questionnaire (IPAQ) [71]. We use the long version of the IPAQ which asks about five activity domains including job-related activity, transportation physical activity, home (e.g., housework, house maintenance and caring for family), sport (e.g., recreation, sport and leisure-time), and sitting. The IPAQ is administered monthly during the intervention period. Weekly review of additional activity as recorded by the fitness activity tracker is completed by study personnel. The fitness activity tracker records total steps, flights of stairs climbed, distance walked, total duration of activity based on HR zones established from VO2 max testing, calories expended from activity and resting HR in a 24-h period. A week 32 assessment (12 weeks after the end of the intervention) will be completed by phone to determine if subjects continue to exercise outside of the structured research environment to verify sustainability of the intervention as this is a well-established time frame to assess adherence following the end of structured exercise training intervention [72].

2.8.2. *Sociodemographics and medical history*

At baseline, participants directly report or verify self-reported

information in the electronic medical record documenting their demographics (e.g., age, gender, race, ethnicity, educational attainment). All participants also have their past medical, surgical and social history reviewed, including smoking status and alcohol consumption. Of particular interest are comorbid diabetes, hypertension, hyperlipidemia, hypothyroidism, polycystic ovarian syndrome, and psoriasis.

2.8.3. *NASH and other medications*

At baseline and monthly during the intervention, subjects are asked to report which prescription drugs, over the counter medications and herbal-dietary supplements they use. Of particular interest are medications used to treat hypertension, hyperlipidemia, diabetes and anticoagulants. Medications prescribed for these indications are grouped into these classes. In addition, subjects are specifically asked about medications that may be used to treat NASH including vitamin E, metformin, obeticholic acid, pentoxifylline and pioglitazone.

2.8.4. *Sleep quality*

Sleep quality is recorded by Fitbit Charge HR2 with established validated cutoffs [73]. This will be reviewed with each weekly download of information from Fitabase. Sleep quality is well known to impact exercise capacity and VO2max testing [74], and may affect thrombosis risk [75].

3. *Study interventions*

NASHFit is a randomized, controlled interventional trial designed to test the hypothesis that structured aerobic exercise training will improve fibrinolysis as measured by PAI-1 and lessen the increased risk of clotting events in subjects with biopsy-proven NASH when compared to standard medical care. Table 3 provides details of the study conditions by group.

3.1. *Standard of care*

Subjects in the standard of care group will be instructed to continue their medical care at the discretion of their treating medical professional. They will be informed to maintain their current physical activity level. They will be given information from the American Liver Foundation to provide a basic understanding of NASH and to reinforce the counseling from their treating medical professional. We expect this information may result in a subject choosing to exercise, however, similar information-only interventions have led to only small effects, which are accounted for in the sample size calculation [76]. Weekly phone calls will be performed by study personnel to ensure adherence to the protocol (e.g., no changes in activity). Subjects will report for anthropometric assessment on a monthly basis to confirm their self-reports. Study investigators will perform an interim history and physical examination

**Table 3**  
Details of conditions.

	Standard of care	Supervised exercise training
Safety of exercise assessment	Baseline	Baseline
NASH education	Baseline	Baseline
Nutritional assessment	Baseline	Baseline
Nutritional counseling	No	Weeks 5–20; weekly
Instructional exercise lead-in period	No	Weeks 1–4; three to five days a week
Aerobic exercise sessions	No	Weeks 5–20; five days a week
Review of FitBit and IPAQ for activity outside of supervised exercise session	Weeks 1–16; weekly	Weeks 5–20; weekly
Phone calls to assess for activity outside of supervised exercise session	Weeks 1–16; weekly	No
Phone call after exercise intervention to assess for exercise sustainability	No	Week 32

at that time. Subjects will also be given a Fitbit Charge HR2 and downloaded data review will be performed monthly at the in-person site visits. These methods of compliance have previously been validated [48]. Smoking status will be reviewed with the subject as this can influence VO<sub>2</sub>max testing. This study will be a delayed treatment trial. Subjects randomized to the control condition will be given five months of fitness center membership and access to an ACSM certified fitness professional for training sessions following completion of the study protocol.

### 3.2. Supervised exercise training

The aerobic exercise protocol is adapted from the landmark NASH-exercise trials by Sullivan et al. [48] and Bacchi et al. [49] that demonstrated the efficacy of exercise in improving intrahepatic fat and body composition but did not evaluate the effect on hemostasis and thrombosis risk. Subjects in the aerobic exercise group will be supervised and exercise 30 min, five times per week at a moderate intensity (heart rate target corresponding to 45–55% of their VO<sub>2</sub>max) as this is the current recommended activity for patients with NASH according to the AASLD Practice Guidance on The Diagnosis and Management of Nonalcoholic Fatty Liver Disease [77]. Formal exercise instruction and supervision will be provided by an ACSM certified fitness professional at the Penn State University Fitness Center.

Subjects will initiate their exercise program with a four-week lead-in period to ensure acclimation to exercise and injury prevention as subjects are sedentary at baseline. They will begin by walking on a treadmill for 15 min at their target HR goal monitored by Fitbit Charge HR2 and progressively increase the duration until the goal of 30 min of moderate intensity exercise five times a week is reached. The following schedule will be used: week one (15 min per session), week two (20 min per session), week three (25 min per session) and week four (30 min per session). Aerobic exercise can be completed on either the treadmill, exercise bike, rowing machine or the elliptical machine.

Subjects will then exercise five times a week under direct supervision for 16-weeks. Aerobic exercise can be completed on either the treadmill, exercise bike, rowing machine or the elliptical machine. Additional home exercise beyond the in-person sessions will be assessed by downloading data from the Fitbit Charge HR2. Review of downloaded information will take place once a week following an in-person exercise session. Subjects will progress in intensity at the discretion of the supervising ACSM-EP as their conditioning improves. Any subject who performs <3 sessions in any given week during the exercise training period will be required to extend their training by two additional weeks to offset loss of physical conditioning [78]. They can be extended no longer than four weeks. If a subject misses more than two weeks of exercise sessions, they will be discontinued from the study.

Study investigators will perform an interim history and physical examination on a monthly basis during the exercise protocol. If a subject is required to extend the protocol, interim history and physical examinations will continue to be performed on a monthly basis. Smoking status will be reviewed with the subject as this can influence VO<sub>2</sub>max testing. At the conclusion of the exercise protocol, subjects will be provided assistance in developing a plan to continue to exercise after the trial ends.

### 3.3. Warm-up and cool-down

The ACSM Guidelines for Exercise Testing and Prescription for Obese Persons with Metabolic Disease will serve as the framework for the ACSM-EP to teach proper warm up, use of equipment, exercise form, mode of activity, intensity of exercise, flexibility exercises, and cool down. Each exercise session will be preceded by a 10 min warm-up with 5 min of walking on the treadmill at 30–40% of target HR followed by five dynamic exercises including knee to chest, 10-yard lateral shuffle, bent over twist, calf sweeps and leg swings. Each exercise session will

end with a 5-min cool down on the treadmill at 30–40% of target HR.

### 3.4. Dietary assessment and counseling

All subjects, regardless of treatment group assignment, receive dietary assessment and counseling at their baseline visit. Nutrition counseling will be provided by a study RD to promote a hypocaloric, low-saturated fat dietary intake pattern. In the event RDs are unavailable in person, counseling will be completed by phone or video conference. Food Frequency Questionnaire will be used to assess baseline dietary practices. Individual caloric needs will then be calculated using the Mifflin St. Jeor equation, providing resting energy expenditure (REE), multiplied by the appropriate activity factor (AF) to yield total energy expenditure (TEE). The Mifflin St. Jeor Equation is calculated as follows: for males  $10(\text{wt. (kg)}) + 6.25(\text{ht. (cm)}) - 5(\text{age (yr)}) + 5 = \text{REE} \times \text{AF} = \text{TEE}$  and for females  $10(\text{wt. (kg)}) + 6.25(\text{ht. (cm)}) - 5(\text{age (yr)}) - 161 = \text{REE} \times \text{AF} = \text{TEE}$ . AF are as follows: seated work with no option of moving around and little or no strenuous leisure activity (1.4–1.5), seated work with discretion and requirement to move around but little or no strenuous leisure activity (1.6–1.7), standing work such as housework (1.8–1.9), strenuous work or highly active leisure (2.0–2.4). Ideal body weight (IBW) is calculated as follows: for males  $106 + 6 \times (\text{Ht. (in)} - 60)$  and for Females  $= 100 + 5 \times (\text{Ht. (in)} - 60)$ . Adjusted body weight (ABW) will be used when the participant's BMI is equal to or greater than 30 kg/m<sup>2</sup>. ABW is calculated as follows: for males  $\text{IBW} + 0.38 \times (\text{BW} - \text{IBW})$  and for females  $\text{IBW} + 0.32 \times (\text{BW} - \text{IBW})$ .

Macronutrient distribution will be recommended at 50–60% carbohydrate, 15–20% protein, and 20–30% fat with <10% total calories attributable to saturated fat. A sodium intake of <2000 mg daily will be encouraged. For the exercise group, weekly review (weeks 5–20) of dietary logs obtained via self-report through the FitBit application and secure web based platform with directed feedback by study RD will also be provided either in-person, telephone interview or video conference. Additionally, participants will be given dietary education handouts. In the event that the subject does not self-report their dietary intake through the FitBit platform, a 24-h dietary recall data collection form will be utilized.

### 3.5. Adherence monitoring and measurement

For participation in the exercise training group, adherence is closely monitored in several ways. One, each exercise session is directly supervised by an ACSM-EP and HR is measured real-time via FitBit ChargeHR2 to ensure appropriate intensity. Following each exercise session, study staff complete a tracking sheet. The tracking sheet is reviewed by study personnel and entered into the database (REDCap). The goal of the monitoring is to achieve five exercise sessions each week lasting 30 min in duration at the HR corresponding to 45–55% of VO<sub>2</sub>max. Additional home exercise beyond the recommended in-person sessions is not allowed by the study protocol and is assessed weekly via data download acquired from remote activity tracking with the fitness tracker device. If a subject is suspected to be completing additional exercise based on this data review, they are interviewed by study personnel to confirm and re-education is provided if necessary to ensure adherence to the protocol.

For participants in the standard of care group, weekly phone calls are completed by study personnel to ensure there has been no significant increase in their activity level. A tracking sheet is completed during each phone call and reviewed by study personnel and entered into the study database. If the subject self-reports increased activity, re-education about the study protocol is provided by study personnel. Subjects also report on a monthly basis for an in-person visit with study investigators at which time downloaded data acquired from remote tracking with fitness activity devices is reviewed. Smoking status will be reviewed with the subject as this can influence VO<sub>2</sub>max testing. The IPAQ and PROMIS are administered as well. These methods of adherence have

previously been validated [48].

### 3.6. Effect size

The study is powered to detect a 23% difference (9 ng/mL assuming baseline level between 35 and 45 ng/mL) in PAI-1 as exercise training regimens lasting at least three months in healthy persons and those with vascular disease reduce PAI-1 between 23 and 37% [38,40–42]. This is clinically significant because for each 1 ng/mL decrease in PAI-1, the risk of VTE decreases by 18–60% [79,80]. This is similar to the reduction of VTE risk by 55% with chemical prophylaxis (e.g., low molecular weight heparin) based on a recent Cochrane Review of eight studies and 3680 outpatients with limb immobilization at high-risk for VTE [81]. We expect no change in PAI-1 level in the control arm as activity should remain the same.

### 3.7. Sample size calculations

In order to have 80% statistical power to detect a difference of 9 ng/mL in PAI-1 following 16-weeks of exercise training intervention, we will enroll 42 subjects at baseline (28 exercise training and 14 standard of care given the 2:1 enrollment ratio). We assume a Type I error rate ( $\alpha$ ) of 5% (0.05), statistical power of 80.74%, and a two-sided, two-sample unequal variance *t*-test, which allows us to detect differences in either direction. We expect a 15–20% attrition rate [82].

### 3.8. Adverse events (AEs) and data and safety monitoring plan

All AEs are captured on a rolling basis by study staff and reviewed by the Principal Investigator (PI) to determine their possible relationship to the intervention. AEs are screened for after each exercise session by the ACSM-EP by answering if an AE occurred or if there are any barriers to the subject continuing in the exercise protocol and again at each monthly check-in with a study investigator. This process is overseen by an independent medical monitor who is a senior clinical researcher with over 30 years of experience. The medical monitor is charged with monitoring the safety of the study protocol. The PI and medical monitor meet bi-annually. Prior to each meeting, the medical monitor is provided an AE log. Within one week of the meeting, the medical monitor presents the meeting conclusions to the investigators. If serious AEs occur at a threshold that is more than expected as determined by the independent medical monitor, the PI will place on hold further enrollment in the study until review by an independent local regulatory board composed of a Hepatologist, a Gastroenterologist, and another uninvolved physician vote unanimously to continue the study with appropriate binding modifications made and approved by the local IRB.

A trained study team member is responsible for data quality checks in REDCap. These are performed bi-weekly and reviewed quarterly by an independent data quality monitor in the Department of Public Health Sciences. The data quality study team member and the independent data quality monitor are responsible for advising the research team on issues of participant accrual, retention, and completeness of the data.

### 3.9. Data analysis plan: main outcome measure

We will conduct the primary analyses on the main outcome measure, change in PAI-1 level. Initially, the two randomized groups will be compared qualitatively on important demographic and other baseline variables to ensure successful randomization. For the primary outcome (PAI-1), we will apply intention to treat (ITT) principles with all available data and the covariates of age, gender, race, ethnicity, education, metabolic risk factors, medications, sleep quality, anthropometrics, body composition, cardiopulmonary fitness, laboratories and biomarkers, dietary composition, HRQOL, hemostatic assessment, liver volume and PNPLA3 genotype included in our primary analysis. Chi-squared and Fisher's exact test will be used to analyze our categorical

endpoints. Student's *t*-test and Mann-Whitney Rank Sum test will be used for secondary analysis of differences in numerical data. If we determine that by chance the two groups are significantly different on any of these covariates, we will include those covariates in secondary analyses. To conduct these analyses by ITT, an analysis of covariance (ANCOVA) model with the above covariates included as regressors, will be applied to model change in PAI-1 level. A *p*-value of <0.05 will be significant. Analyses will be conducted using SAS version 9.4 (Cary, NC).

### 3.10. Analysis of secondary outcomes

We will analyze the impact of exercise training intervention on each of the following outcomes: blood pressure and heart rate, BMI and weight, body composition, cardiopulmonary fitness, cholesterol, dietary composition (e.g., macronutrients, fiber and sodium content), glycemic control, HRQOL, inflammation, liver enzymes, NAS and fibrosis stage, markers of hemostasis, hepatic fat % and liver volume, microbiome, NASH biomarkers and clinical decision aids (e.g., NFS, FIB-4) and PNPLA3 gene expression. Paired *t*-tests will be used where appropriate (e.g., comparison of pre- and post-intervention fibrosis parameters such as NFS). ITT will be upheld. A *p* value of <0.05 will be significant. Analyses will be conducted using SAS version 9.4 (Cary, NC).

### 3.11. Planned subgroup analysis

Several subgroup analyses are planned. First, a comparison between diabetics and non-diabetics is planned as diabetes is one of the strongest predictors of more advanced NASH and also treatment response with lifestyle changes [83,84]. Second, a comparison between high-risk NASH subjects (age >60 years with diabetes, hypertension and obesity) and low-risk NASH subjects (all others) is planned because high-risk NASH patients are more likely to have clinically important thrombotic events such as portal vein thrombosis [22]. Third, to examine the influence of potential weight loss which can confound isolating the benefit of exercise training, subgroup analysis will be performed comparing those subjects who lost >7% of their body weight to those who did not, as this is a validated threshold for NASH improvement [85].

## 4. Discussion

### 4.1. Limitations

There are several limitations and potential difficulties with the NASHFit Trial. First, our enrollment criteria are somewhat stringent, however, as they are extrapolated from several previous landmark trials [31,48–50,86] that enrolled larger sample sizes than our projected 42 subjects, we feel these criteria are appropriate.

Second, our definition of protocol adherence, which is a subject attending no fewer than three of the five in-person exercise sessions in each week, may lead to difficulties with subject retention. As Zhang et al. [86] found 68 out of 73 subjects (93%) completed a longer, six-month supervised, in-person exercise protocol five days a week, we feel that subjects will adhere to our shorter protocol. Nonetheless, we have powered our study to expect an additional 15–20% attrition rate [82]. Additionally, stipends will be provided to ensure retention. Based on our previous experience, monetary rewards are effective in increasing retention [87]. In addition to allowing participants to keep their FitBit Charge HR2 devices as well as their involvement in daily exercise sessions, we expect that monthly visits with study investigators will improve retention as our previous experience found greater access to the study team lead to improved retention [87].

Third, our choice of clinical endpoint may be somewhat limiting. We chose PAI-1 as the primary measure of fibrinolysis as it is adiposity-dependent and significantly elevated in NASH, however, we recognize that the fibrinolytic system is complicated and involves other



biomarkers. To offset this, we are measuring global coagulation with TEG, and the individual components of the fibrinolytic system as well as those markers of primary and secondary hemostasis.

Lastly, controlling for the influence of dietary change and weight loss to isolate the effect of exercise may prove challenging. As dietary change and weight loss can impact NASH outcomes, we control for this with the trial design. While recommendations will be individualized, overall dietary information will be standardized by a study RD throughout the protocol. Information on macronutrient intake will be recorded and controlled for with statistical modeling. To examine the influence of potential weight loss, subgroup analysis will be performed as described above. If our study is underpowered to detect a difference in subgroup analysis, we will control for weight loss with additional statistical modeling.

## 5. Conclusion

The NASHFit Trial is being undertaken to determine if exercise training reduces the risk of venous thromboembolism in patients with NASH. Additional evidence for the benefit of exercise training for patients with NAFLD and NASH is of utmost importance given that currently available pharmacologic treatments are largely ineffective and have significant side effects. NASHFit will provide insight about whether an aerobic exercise program that combines widely adoptable simple moderate intensity activities such as walking with fitness tracking device utilization and accountability can reduce clinically significant clotting risk. Exercise training offers superior safety and accessibility when compared to pharmacologic treatments for NASH or prevention of VTE in at risk populations. Additionally, exercise training is known to be equally if not more effective as medications in not only the treatment of NAFLD/NASH, but also the secondary prevention of coronary heart disease, rehabilitation after stroke, treatment of heart failure, and prevention of diabetes [88]. Despite this evidence from a recent meta-analysis of over 300,000 subjects [88], it remains largely ignored by health insurance coverage beyond cardiovascular rehabilitation or programs such as Silver Sneakers, each of which are proven to reduce healthcare utilization and costs [89,90]. More evidence for the exercise benefit in NASH from the NASHFit Trial may offer additional argument for expanding insurance coverage to this highly prevalent disease affecting 100 million American adults.

The timing of the NASHFit study is crucial given the worsening epidemic of metabolic disease. As most NASH patients are asymptomatic and our diagnostic testing is ineffective in discerning which persons will have disease progression, this combination is the perfect milieu for progression to end-stage liver disease which may require lifesaving liver transplantation [7–9]. By 2025, NASH cirrhosis will be the leading indication for liver transplantation [12]; it is already the most common reason for liver transplant in women [13]. Furthermore, end-stage liver disease patients with NASH cirrhosis are at greatest risk for clinically significant clotting events including pulmonary embolism, deep vein thrombosis and portal vein thrombosis [21–23], the latter of which may prevent liver transplantation and significantly shortens post-transplantation survival [91]. Given the continued inequity between supply and demand which leads to a deficit of more than 8000-donor organs per year in the United States, intervening earlier with exercise training is paramount to not only halt progression to end-stage liver disease, but also to simultaneously prevent clinically significant clotting events, offering a widely available, safe and valuable solution to this global public health problem.

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## Declaration of competing interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

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