PennState

HRP-591 - Protocol for Human Subject Research

Protocol Title:

NASH Fitness Intervention in Thrombosis (NASHFit) Trial

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Abbreviations:

AASLD=American Association for the Study of Liver Diseases
ACSM=American College of Sports Medicine
AE=adverse event
BMI=body mass index
CAT=computerized adaptive testing
DVT=deep vein thrombosis
DXA= dual-energy x-ray absorptiometry
FIB-4=Fibrosis-4 index
HCAR= Hershey Center for Applied Research
HR=heart rate
HRQOL=health related quality of life
IHTG=intrahepatic triglyceride
IPM=Institute for Personalized Medicine
IRB=institutional review board
MRI=magnetic resonance imaging
NAFLD=nonalcoholic fatty liver disease
NASH=nonalcoholic steatohepatitis
NASHFit=NASH Fitness Intervention in Thrombosis
PAI=plasminogen activator inhibitor
PAR-Q= Physical Activity Readiness Questionnaire
PDFF= proton density fat fractionation
PE=pulmonary embolus
PI=principal investigator
PMR=Physical Medicine and Rehabilitation
PNPLA3=patatin like phospholipase-3
PROMIS=patient reported outcomes measurement information system

PVT=portal vein thrombosis
RCT=randomized controlled trial
SNP= single nucleotide polymorphism
TAF= tissue activating factor
tPA= tissue plasminogen activator
US=United States
VTE=venous thromboembolism
VO2 max=maximal oxygen consumption
vWF=vonWillebrand Factor

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1.0 Objectives

1.1 Study Objectives

Often comorbid with obesity, nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in the United States affecting 75-100 million adults, of which 15-20 million have the more severe variant nonalcoholic steatohepatitis (NASH).(1-3) Conservative estimates project a doubling in NASH by 2025.(4-6) The most advanced forms of NAFLD are associated with increased liver-related mortality and lower overall survival. The most effective treatment for NAFLD remains adopting healthy dietary and exercise patterns,(7-14) however NAFLD patients are among the least physically active individuals.(15) Predicting exercise behavior on an individual level is highly complex due to differing motivation, physiologic response to and subjective experience of exercise as well as emerging genetic evidence.(16, 17) The human genome and regulation of gene expression is influenced by physical activity. Patatin like phospholipase-3 (PNPLA3) rs738409 polymorphism (GG, GC and CC genotypes) plays a crucial role in the development of NAFLD.(18-24) The GG genotype is both associated with advanced NAFLD,(18, 25-27) and predicts response to physical activity.(10, 28) Patients with NASH have extensive extrahepatic disease(29) and are hypercoagulable.(30-42) NASH is a prothrombotic state with fibrinolytic dysfunction through elevated plasminogen activator inhibitor (PAI-1), an independent risk factor for venous thromboembolism (VTE). Consequently, patients with NASH are predisposed to VTE;(30-32) the risk of portal vein thrombosis (PVT) in NASH is 210% greater than in other liver disease.(30, 31) NASH patients are also at increased risk for pulmonary embolism (PE) and deep vein thrombosis (DVT). The most advanced forms of NASH have the greatest thrombotic risk. While studies observe that change in diet, weight and physical activity patterns improve NASH, it is not clear whether these lifestyle changes also reduce the elevated clot risk, however, moderate-intensity exercise leads to improved fibrinolysis in healthy persons.

Developing an effective treatment modality to lessen thrombotic risk is imperative for this highly prevalent condition. In this proposal, we seek to begin a line of work to answer the question "Can lifestyle changes effectively mitigate the increased risk of VTE in patients with NASH?" focusing initially on the at-risk population genetically susceptible to advanced disease. We hypothesize that fibrinolysis as measured by PAI-1 will be improved with exercise in patients with NASH. We expect this effect to be determined by changes in body composition as PAI-1 levels are dependent on adiposity.

1.2 Primary Study Endpoints

The primary outcome of interest is change in fibrinolysis as measured by PAI-1 level immediately following completion of the exercise and nutritional counseling program.

1.3 Secondary Study Endpoints

Secondary Outcomes are as follows: (1) Change in PAI-1 after 16-week exercise regimen; (2) Change in other markers of hemostasis [von Willebrand Factor (vWF), P-selection, Protein S, Protein C, Factor VII, Factor VIII, Fibrinogen, Antithrombin,] after 16-week exercise regimen; (3) Change in PAI-1 stratified by PNPLA3 genotype after 16-week exercise regimen; (4) Correlation between loss of hepatic fat as measured by magnetic resonance imaging proton density fat fractionation (MRI-PDFF) and changes in hemostatic markers after 16-week exercise regimen; (5) Correlation between maximal oxygen consumption (VO2 max) and hemostatic markers after 16-weeks of exercise; (6) health-related quality of life (HRQOL) change following exercise intervention

2.0 Background

2.1 Scientific Background and Gaps

Significance: NAFLD is the leading cause of chronic liver disease in the United States (US). The epidemic of metabolic disease has reached substantial portions with widespread public health impact. Nearly 70% of US adults are overweight or obese. NAFLD is a spectrum of chronic liver disease that is often comorbid with obesity. NASH is the more severe variant of NAFLD, characterized by inflammation and/or fibrosis. NAFLD is the leading cause of chronic liver disease in the US affecting 75-100 million adults *(~40% of the adult population*); 15-20 million adults have NASH.(1-3) NAFLD rates are projected to double by 2025; conservative estimates suggest 30 million individuals will have been diagnosed with or progressed to NASH.(4-6)

The most advanced forms of NASH are associated with increased liver-related mortality and lower overall survival. NASH is staged based on the degree of fibrosis and ranges from stage 0 (no fibrosis) to stage 4 (cirrhosis). Patients with simple steatosis (no fibrosis) have a favorable prognosis with a 1-2% risk of developing cirrhosis over the subsequent 15-20 years.(43, 44) The risk of progression with NASH (presence of fibrosis) is much greater with cirrhosis rates of 12% over the eight years following diagnosis.(43, 44) NASH with significant fibrosis (stage ≥2) is associated with increased liver-related mortality and lower overall survival rates due to cardiovascular events and malignancy.(4-6)

The current standard of care for NAFLD is lifestyle changes through diet and exercise. The American College of Sports Medicine (ACSM) recommends all persons participate in ≥30 minutes of moderate-intensity physical activity 3-5 times/week and to perform strength training two times/week.(45) The mainstay of NASH treatment is lifestyle change through adopting healthy diet and exercise routines,(7-14) an effect that may be independent of weight loss.(46, 47) A recent meta-analysis by Katsagoni *et al.*(48) comprised of twenty high-quality randomized controlled trials (RCTs) (n=1,043) found a favorable pooled effect of exercise (improved liver associated enzymes and intrahepatic fat), independent of dietary counseling, however, it is unknown if exercise improves fibrosis. The authors found no discernable difference between aerobic exercise and strength training, however, intensity of training was associated with different results and moderate-to-high volume intensity resulted in better response.(48) Sustainable exercise remains challenging in clinical practice owing to factors that are often outside the control of the treating physician. NASH patients are among the least physically active individuals.(15, 49) Only 20% of patients with NASH achieve the recommended daily physical activity amount and intensity. NASH patients have greater ratings of perceived exertion at similar exercise thresholds,(50) lower physical function and lower performance status.(49, 51) A sedentary lifestyle promotes NASH progression; lower levels of activity are associated with greater intrahepatic fat and inflammation.(52, 53)

The human genome and regulation of gene expression is influenced by physical activity. Over time, humans have become increasingly sedentary and as a consequence, our current genome has become maladapted resulting in abnormal gene expression and manifestation of chronic disease, including NASH.(54) Genes that were once favorable during times of starvation have now become overexpressed and pathologic resulting in metabolic dysfunction and sarcopenia.(54) Restoration of this perturbed metabolic homeostasis can be accomplished through habitual exercise in our sedentary culture.(54) To date, the expression of thirty plus genes important to chronic inflammation have been found to be regulated by exercise.(55) The PNPLA3 rs738409 polymorphism (a missense variant resulting in cytosine to guanosine substitution which encodes for an isoleucine to methionine substitution at amino acid position 148) leads to abnormal protein production and subsequent accumulation of triglycerides in the liver, independent of obesity.(18-21) PNPLA3 plays a crucial role in NASH development and progression to cirrhosis via interaction with behavioral risk factors including physical inactivity.(22-24, 56) And while PNPLA3 activity is modulated by hyperinsulinemia and carbohydrate deficiency, the effect of exercise on PNPLA3 gene expression is unknown.

PNPLA3 genotype is associated with advanced NASH and predicts response to physical activity. The PNPLA3 GG genotype is associated with the most advanced forms of NASH.(18, 25-27) PNPLA3 predicts

response to lifestyle intervention(10, 28) with the greatest benefit being derived by GG homozygotes.*(28)* In their RCT of 154 subjects, Shen *et al.* (10) found GG homozygotes had the greatest decrease in body weight, body mass index (BMI) and intrahepatic triglyceride (IHTG) even when adjusting for higher baseline IHTG percentages. Additionally, compound GC heterozygotes were more likely than CC homozygotes to experience weight loss, decrease in BMI and reduction in IHTG. In fact, for each G allele, a nearly 3% more absolute reduction in IHTG was observed with exercise intervention. G-allele substitution occurs 40-45% in NAFLD.(19)

NASH is a prothrombotic state with fibrinolytic dysfunction through elevated PAI-1.
Chronic inflammation from hepatic steatosis(34-37) culminates in activation of the coagulation system and abnormalities in all three phases of hemostasis. (38, 39) Hemostasis can be broken down into primary, secondary and tertiary components.(57) Increased platelet activation (primary hemostasis) has been observed in NASH as have elevated levels of vWF.(39, 40, 58) Hypercoagulability (secondary hemostasis) in NASH manifests through increased levels of Factor VIII and fibrinogen.(38, 39) Levels of anticoagulants antithrombin and protein C are decreased in NASH, tipping the hemostatic balance towards clotting. The most severe prothrombotic imbalance is seen in parallel with the most advanced disease (including NASH cirrhosis).(38, 39)

PAI-1 is elevated in NASH while tissue activating factor antigen and tPA are decreased. This leads to a chronic state of hypofibrinolysis (tertiary hemostasis).(39) Similar to the Factor VIII-to-Protein C ratio, PAI-1 correlates histologically with increasing severity of steatohepatitis and fibrosis.(41) Additionally, patients with NASH have longer clot lysis times and greater clot density.(58) Elevated PAI-1 promotes thrombotic risk and may accelerate liver disease progression due to local tissue ischemia stemming from intrahepatic thrombi, known as *parenchymal extinction*.(59) Clinically, the presence of at least one thrombotic risk factor is associated with a nearly two-fold fibrosis stage increase in NASH, further supporting the notion of thrombosis and disease progression.(42, 60)

Patients with NASH are predisposed to VTE. The hemostatic environment in patients with chronic liver disease is a precarious balance between pro- and anti-thrombotic factors. Patients with NASH cirrhosis are at increased risk for thrombosis. Independent of metabolic comorbidities, liver transplant recipients with NASH have a 55% greater risk of PVT. The odds of PVT increase exponentially for high-risk NASH (age >60 years with diabetes, hypertension and obesity) [OR 2.1 (95% CI 1.6-2.8)].(30-32, 61) Our preliminary data [accepted, 2017 American Association for the Study of Liver Diseases (AASLD) Liver Meeting] documents further coagulation derangement in NASH. We found the thrombotic state extends from the portal venous to the systemic circulation as the risk of VTE (PE/DVT) was two and a half-fold greater in hospitalized patients with NASH cirrhosis, supporting work by Di Mino *et al.*(33)

PA1-1 modulates fibrinolysis and is an independent risk factor for VTE. Decreased fibrinolytic activity is a risk factor for VTE.(62, 63) PAI-1, which is produced by adipose tissue and consequently elevated in obesity and NASH and reduced by weight loss,(64) inhibits breakdown of fibrin based clots through physiologic inhibition of plasminogen activators (e.g. urokinase). Elevated PAI-1 levels lead to longer clot lysis times.(65) Multiple studies have documented PAI-1 to be an independent predictor of PE, DVT and PVT when adjusting extensively for prothrombotic confounders.(65-67) PAI-1 is one of the stronger independent risk factors for PVT [OR 6.4 (95% CI 2.5-16.1).(66) The 4G/5G PAI-1 polymorphism confers additional risk for VTE.

Moderate-intensity exercise leads to improved fibrinolysis in healthy persons. Exercise has a favorable effect on the coagulation system across all three phases of hemostasis.(68-70) Habitual exercise improves primary hemostasis via endothelial-dependent vasodilation and nitric oxide production leading to less platelet activation and aggregation.(71) Moderate intensity exercise improves hemostasis efficiency by activating fibrinolysis in concert with improving coagulation.(68, 69) Specifically, chronic aerobic based training leads to improved fibrinolytic activity in both healthy subjects and those with

cardiovascular disease. This occurs during exercise and persists at rest.(68, 69, 72-75) Reductions in PAI-1 following aerobic exercise programs lasting 3-8 months range from 23-37%.(72, 74-76) When comparing subjects who are aerobically trained to those who are not, further benefit has been observed in fibrinolytic activity via skeletal muscle tPA efficiency.(73) Patients with higher baseline PAI-1 experience the greatest benefit from exercise through both weight and fat loss.(72, 75, 77)

As the obesity epidemic continues to worsen, prevalence rates of NASH are increasing and threaten to overwhelm the healthcare system. The most effective treatment for NASH remains exercise however, predicting which patients will achieve a response to exercise remains more of an art than a science. Genetics may play a role with this response and can be used to identify the patients most likely to derive benefit. NASH patients have significant extrahepatic disease including thrombotic events. Despite the longstanding knowledge that inflammation leads to thrombosis, exercise improves NASH *(a thrombotic state)*, and chronic aerobic exercise improves fibrinolysis through PAI-1 (*independent risk factor for VTE)*, the effect of exercise on thrombosis in this at-risk population remains unknown. We hypothesize that increased physical activity will decrease hepatic fat and adipose tissue leading to less PAI-1 associated thrombotic risk.

2.2 Previous Data

(1) Definition of clinically important thrombotic events in patients with NASH. Dr. Stine published two large cross-sectional studies documenting the increased risk of PVT in NASH.(30, 31) Of the 33,368 subjects who underwent liver transplantation between 2003-2012, 6.3% (n=2,096) had PVT. 12% of recipients with NASH had PVT compared to 7% in all other etiologies of liver disease ($p<0.001$), a finding that remained statistically significant when adjusting for metabolic confounders. Additionally, liver transplant recipients with high-risk NASH have even greater risk for PVT at the time of liver transplantation with prevalence rates of 14%.

(2) Feasibility of administration of Patient Reported Outcomes Measurement Information System Computerized Adaptive Testing (PROMIS-CAT) to patients with chronic liver disease. Dr. Stine completed a pilot study of 109 adult liver transplant candidates enrolled over three months. Through iPad platform, subjects completed the PROMIS-CAT. All subjects completed the PROMIS-CAT (100% conversion) and average completion times were ~15 minutes. The results of this pilot are helpful in that this novel, technologically advanced model is readily completed by subjects with advanced medical illness.

[illegible] NASH cirrhosis, whereas

(3) Development of an exercise training model. Dr. Stine developed and led a 16-week aerobic exercise program training both sedentary and moderately active adults to increase their cardiopulmonary fitness culminating in competition in an amateur sprint triathlon. Dr. Stine was previously a certified indoor cycling instructor, team captain and varsity collegiate lightweight rower at Penn State and an elite amateur triathlete. He has a Bachelor's of Science Degree from Penn State in Nutritional Sciences, awarded with honors in 2005.

(4) Previous principal investigator of a small clinical trial. Under the mentorship of Dr. Patrick Northup (collaborator), Dr. Stine studied twelve patients in a RCT enrolling hospitalized subjects with decompensated cirrhosis and hepatorenal syndrome. Dr. Stine served as principal investigator (PI) and administrative contact for the study for two years. The manuscript was recently accepted and published in *Annals of Hepatology.* During this experience, Dr. Stine obtained the skillset necessary to be an effective PI for clinical trials.

2.3 Study Rationale

Despite the longstanding knowledge that inflammation leads to thrombosis, exercise improves NASH *(a thrombotic state)*, and chronic aerobic exercise improves fibrinolysis through PAI-1 (*independent risk*

factor for VTE), the effect of exercise on thrombosis in this at-risk population remains unknown. Similarly, while exercise is known to influence pro-inflammatory gene expression, modulation of PNPLA3 activity through exercise is unknown.

3.0 Inclusion and Exclusion Criteria

3.1 Inclusion Criteria

- Adults age >=18 or <70 years
- Liver biopsy <= 6months prior to enrollment
- Biopsy proven NASH(78)
- Lack of secondary causes of hepatic fat accumulation:
    - Significant alcohol consumption (<21 drinks/week for men and <14 drinks/week for women)
    - Chronic hepatitis C
    - Wilson disease
    - Lipodystrophy
    - Parenteral nutrition
    - Long-term use of steatogenic medications (mipomersen, lomitapide, amiodarone, methotrexate, tamoxifen, corticosteroids)
    - Monogenic hereditary disorders

3.2 Exclusion Criteria

- >90 minutes/week of at least moderate intensity exercise over the previous three months as assessed by the Get Active Questionnaire (GAQ).
- Pregnancy
- BMI <18 or >45 kg/m^2(16)
- Uncontrolled diabetes (changes in medication dosing over the previous three months or hemoglobin A1c >9%)(12)
- Active cardiac symptoms
- Severe medical comorbidities/psychiatric illness
- Decompensated cirrhosis (history of esophageal varices, ascites or hepatic encephalopathy)
- Cancer with life expectancy <6 months
- MRI contraindications (severe claustrophobia, implanted ferrous metal)
- Other liver disease (positive hepatitis B surface antigen)
- 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 ¼ mile
- Concurrent use of antiplatelet medications excluding aspirin 81 mg once daily as aspirin
- Non-English-speaking patients due to unavailability of translators for all visits/sessions

3.3 Early Withdrawal of Subjects

3.3.1 Criteria for removal from study

- Development of severe adverse event
- Subject consents to withdrawal
- Development of decompensated cirrhosis
- Failure to complete two successive weeks of exercise protocol.
    - Any subject who performs <3 sessions in any given week during the 16-week study period will be required to extend their training by two additional weeks to

offset loss of physical conditioning. The power calculation accounts for expected study dropout (15-20% in exercise protocols).

3.3.2 Follow-up for withdrawn subjects

This will be an intention to treat analysis and subjects who are withdrawn will be followed at the same time points as who are enrolled still. E.g., they will receive a phone call at the completion point of the 16-week exercise program.

4.0 Recruitment Methods

4.1 Identification of subjects

Potential subjects will be recruited from the surrounding area by a referral basis through both print marketing ("Dear Doctor" outreach letter) as well as in-person presentations by Dr. Stine. StudyKIK (https://studykik.com), an online recruitment service, will be utilized as well on a consultative basis in order to increase recruitment using a social media platform. The social media ad is submitted as an attachment to this protocol. Also, we will be using the Penn State Marketing Department to send targeted emails through the patient portal to patients that meet the basic study criteria. The email script is submitted as an attachment to this protocol.

Once potential subjects are referred to Penn State, they will be pre-screened by phone and/or in-person after their visit in hepatology clinic. Activity level will be assessed by the GAQ (attached). StudyFinder will be utilized.

The phone screen procedure is described in the supplemental attachment.

4.2 Recruitment process

StudyFinder, Penn State email blast through the patient portal and print materials will be utilized. StudyKIK will be used as a consultative service.

4.3 Recruitment materials

StudyFinder and print materials will be utilized. The print materials are recruitment letters to outside physician practices and also within Penn State as well as the email blast template letter. StudyKIK will develop IRB approved recruitment material in concert with Penn State and all IRB requirements.

4.4 Eligibility/screening of subjects N/A

5.0 Consent Process and Documentation

5.1 Consent Process

5.1.1 Obtaining Informed Consent

5.1.1.1 Timing and Location of Consent

Subjects will be invited for initial consultation performed by the study PI. Informed consent will take place at this consultation. Investigators must ensure that patients are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which they volunteer to participate. Freely given written informed consent must be obtained from every subject prior to clinical study participation, including informed consent for any screening procedures conducted to establish subject eligibility for the study. The rights, safety, and well-being of the study patients are the most important

considerations and should prevail over interests of science and society. The consent process will occur in a private room in the Penn State Hershey Medical Center General Clinical Research Center (GCRC). The PI will carefully discuss the risks and benefits of study participation and review study procedures. The PI will make it clear that the subject's standard clinical care will not be affected in any way through participation or non-participation in the study. All questions will be answered prior to signing the informed consent document and the subject will verbalize understanding at the time of consent. The consent process will take approximately 15 minutes.

5.1.1.2 Coercion or Undue Influence during Consent

Investigators will explain the consent and allow for ample time to read and ask questions. Study procedures will be fully explained, voluntariness will be emphasized as well as the fact that no care will be denied regardless of the subject's decision.

5.1.2 Waiver or alteration of the informed consent requirement

Due to the extensive inclusion/exclusion criteria, patients will be asked to provide verbal consent to allow the study team to review their PHI to determine their eligibility for the study.

5.2 Consent Documentation

5.2.1 Written Documentation of Consent

Informed consent will be provided by a standardized process and informed consent form.

5.2.2 Waiver of Documentation of Consent (Implied consent, Verbal consent, etc.)

A waiver of consent documentation is requested for the phone screen. This is needed to allow for collection of PHI while screening potential subjects. Full and written consent will be recorded before any interventions begin.

5.3 Consent – Other Considerations

5.3.1 Non-English Speaking Subjects

Not planned at the time.

5.3.2 Cognitively Impaired Adults

5.3.2.1 Capability of Providing Consent

N/A

5.3.2.2 Adults Unable To Consent

N/A

5.3.2.3 Assent of Adults Unable to Consent

N/A

5.3.3 Subjects who are not yet adults (infants, children, teenagers)

5.3.3.1 Parental Permission

N/A

5.3.3.2 Assent of subjects who are not yet adults

N/A

6.0 HIPAA Research Authorization and/or Waiver or Alteration of Authorization

6.1 Authorization and/or Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

Check all that apply:

☐ **Not applicable, no identifiable protected health information (PHI) is accessed, used or disclosed in this study.** *[Mark all parts of sections 6.2 and 6.3 as not applicable]*

☒ **Authorization will be obtained and documented as part of the consent process.** *[If this is the only box checked, mark sections 6.2 and 6.3 as not applicable]*

☒ **Partial waiver is requested for recruitment purposes only (Check this box if patients' medical records will be accessed to determine eligibility before consent/authorization has been obtained).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Full waiver is requested for entire research study (e.g., medical record review studies).** *[Complete all parts of sections 6.2 and 6.3]*

☒ **Alteration is requested to waive requirement for written documentation of authorization (verbal authorization will be obtained).** *[Complete all parts of sections 6.2 and 6.3]*

6.2 Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

6.2.1 Access, use or disclosure of PHI representing no more than a minimal risk to the privacy of the individual

6.2.1.1 Plan to protect PHI from improper use or disclosure

Information is included in the "Confidentiality, Privacy and Data Management" section of this protocol.

6.2.1.2 Plan to destroy identifiers or a justification for retaining identifiers

Identifiers will be destroyed per standard Penn State operating protocol after the required time frame has been met.

6.2.2 Explanation for why the research could not practicably be conducted without access to and use of PHI

This is a randomized, controlled trial.

6.2.3 Explanation for why the research could not practicably be conducted without the waiver or alteration of authorization

This is a randomized, controlled trial.

6.3 Waiver or alteration of authorization statements of agreement

Protected health information obtained as part of this research will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other permitted uses and disclosures according to federal regulations.

The research team will collect only information essential to the study and in accord with the 'Minimum Necessary' standard (information reasonably necessary to accomplish the objectives of the research) per federal regulations.

Access to the information will be limited, to the greatest extent possible, within the research team. All disclosures or releases of identifiable information granted under this waiver will be accounted for and documented.

7.0 Study Design and Procedures

7.1 Study Design

The aims of this research proposal are to better understand the potential for lifestyle interventions to reduce the elevated clot risk of patients with NASH without needing to take an anticoagulant (e.g. warfarin, low-molecular weight heparin). This proposal includes a RCT to determine the efficacy of supervised exercise on future clot risk. A RCT (n=42) of subjects with NASH will be conducted with a supervised 16-week moderate intensity aerobic exercise program as the intervention and the effect on fibrinolysis will be measured by change in PAI-1**.**

7.2 Study Procedures

Subjects will be invited for initial consultation performed by the study PI. This will be completed at the GCRC. If subjects are not currently in the Penn State system, they will be registered prior to this consultation through the Penn State Gastroenterology/Hepatology clinic staff (x28-2664 or x4950). For all individuals who provide signed, informed consent, the following baseline measures will be completed:

- Comprehensive history and physical examination
- Anthropometric assessment [height, weight, BMI calculation]
- Physical activity questionnaire (IPAQ)
- Urine Pregnancy Test
- Nutrition counseling will be provided by a Registered Dietitian (RD) to promote a hypocaloric, low-fat dietary intake pattern. In the case that Dieticians are unavailable in person, counseling will be completed by phone or video chat using the Penn State Health-branded Zoom. Food Frequency Questionnaire (FFQ) will be used to assess their current dietary practices. Individual caloric needs will be calculated at baseline using the Mifflin St. Jeor equation, providing resting energy expenditure (REE), multiplied by the appropriate activity factor (AF) to yield total energy expenditure (TEE). Adjusted body weight (ABW) will be used when the participant's BMI is equal to or greater than 30. Five-hundred to 750 calories will be subtracted to promote an approximate weight loss of 1 pound per week for participants whose BMI classifies as overweight or obese. 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 <2,000 mg daily will be encouraged.

  <u>ABW & IBW Calculations</u>:

  - Use ABW to calculate REE when BMI >/=30
    - ABW calculation:
      - Males: IBW + 0.38 x (BW – IBW)
      - Females: IBW + 0.32 x (BW – IBW)
    - IBW calculation:
      - Males = 106 + 6 x (Ht.(in)-60)
      - Females = 100 + 5 x (Ht.(in)-60)

  <u>REE via Mifflin St. Jeor Equation</u>:

  - Male: 10(wt. (kg)) + 6.25(ht. (cm)) - 5(age (yr)) + 5 = REE x AF = TEE
  - Female: 10(wt. (kg)) + 6.25(ht. (cm)) - 5(age (yr)) – 161 = REE x AF = TEE

Activity Factors:	
<u>Lifestyle and Physical Activity Level (PAL)</u>	

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 (e.g. housework, shop assistant)	1.8-1.9
Significant amounts of sport or strenuous leisure activity (30-60 min four to five times per week)	+0.3 (increment)
Strenuous work or highly active leisure	2.0-2.4

Creating a Caloric Deficit for Weight Loss:

- Male and Female:
  - TEE - 500 kcals when BMI 25-29.9
  - TEE – 750 kcals when BMI 30-44.9

- Fasting laboratory testing (including baseline assessment of the hemostatic system). Subjects will be instructed to be fasting for at least nine hours. The following tests will be performed:
  - Complete blood count
  - Hemoglobin A1c
  - Thromboelastography
  - Insulin level
  - Basic metabolic panel
  - Hepatic panel
  - Lipid panel
  - Ferritin
  - ADAMTS13
  - Plasminogen activator inhibitor one
  - D-dimer
  - VonWillebrand Factor
  - Protein C
  - Protein S
  - Factor VII
  - Factor VIII
  - INR
  - Fibrinogen
  - Antithrombin
  - P-selectin
  - Adiponectin
  - Cytokeratin-18
- Calculation of non-invasive markers of fibrosis [NAFLD Fibrosis Score, Fibrosis-4 Index (FIB-4)
- Stool sample collection for 16S ribosomal microbiome assessment. A home stool collection kit and instructions will be provided to the subject. The kit will be labeled with the subject's study code number.
- Additional genetic testing for single nucleotide polymorphisms (SNPs) of interest for exercise response, coagulation or NASH; subjects will not be notified of the results of these tests.
- Assessment of hepatic fat content with MRI-PDFF
- Baseline electrocardiogram (ECG) to be reviewed by study physicians
- HRQOL assessment via the PROMIS-CAT, an individual approach tailored to the health status of each subject offering significant improvements over the existing fixed length questionnaire evaluations of HRQOL (e.g. Short Form-36) that have been used almost exclusively in NASH (http://www.nihpromis.org).(51, 79-90) PROMIS includes gender- and age-matched normative data obtained from extensive testing of the U.S. population. PROMIS will be completed in-person under the direct supervision of trained study personnel.
- The liver biopsy obtained prior to enrollment will be reviewed by the pathology department to confirm the stage of fibrosis and NASH Activity Score.

Whole blood will be drawn in the Penn State Hershey GCRC (hours 730a-4p) by trained GCRC nursing or NASHFit research staff. Whole blood will be obtained and placed into the following tubes in the amounts described below [total nine teaspoons (tsp) of blood]:

- Three 3mL lavender (EDTA) top tubes (1.8 tsp)
- Two 4.5mL green (sodium heparin) top tubes (1.8 tsp)
- Three 6mL red top tube (3.6 tsp)
- Three 2.7mL blue (sodium citrate) top tubes (1.6 tsp)

Samples will then be delivered by NashFit study personnel to both the Clinical Laboratory for processing to serum and platelet poor plasma and also to Susie George in the Institute for Personalized Medicine (IPM) for processing prior to transfer to the Endocrine Core Laboratory. Samples will be frozen and picked up from the Clinical Laboratories by IPM staff and brought to the IPM for storage and also the Genome Sciences and Bioinformatics Core for Taqman assay performance. Coded samples will be shipped through the IPM to Dr. Andrew D. Patterson, Associate Professor in University Park for metabolomics analysis. Results will be sent back to the IPM per standard IPM operating procedure. Stool samples will be obtained from the subject at home with a home collection kit and mailed directly from the subject to Dr. Gina Lamandella (collaborator), Assistant Professor of Medicine, Juniata College. Stool samples will be processed, analyzed with 16S rRNA testing and stored in Dr. Lamandella's laboratory.

Intervention Conditions: The aerobic exercise protocol is adapted from the landmark NASH-exercise trials by Sullivan *et al(91)* and Bacchi *et al(92)* that demonstrated the efficacy of exercise in improving intrahepatic fat and body composition but did not evaluate the effect on hemostasis and thrombosis risk. Once subjects have passed screening, they will be randomized 2:1 into either the exercise or control group respectively (two exercise subjects per one control subject) using a computer-generated randomized scheme in blocks of six by an independent statistician (Dr. Ming Wang- Public Health Sciences).

Following their assessment and randomization, subjects will undergo baseline VO2 max testing at the Penn State Physical Medicine and Rehabilitation (PMR) Research Laboratories under the supervision of ACSM certified trained fitness professionals and study physicians. Anthropometric measurements will be completed at this assessment as well with dual-energy x-ray absorptiometry (DXA) body composition measurement, waist circumference, hip circumference and skin fold measurement. Following this, subjects will be given a Fitbit Charge2 with heart rate (HR) monitor along with an instruction form on how to use dietary log. Formal instruction on how to use the Fitbit Charge2 will be provided by trained study staff. This will include technical support with the Fitbit app that is supported on smartphones, the Fitbit website and Fitabase, a secure comprehensive data management platform for research studies, clinical trials and healthcare innovation projects utilized by >400 clinical trials since its inception. Participants will be instructed to record their food and beverage intake in a daily food diary through the Fitbit app and be provided information on how closely their daily food intake met the goals for macronutrients and energy.

Moderate intensity aerobic exercise: Subjects in the aerobic exercise group will be supervised to exercise 30 minutes, five times per week at a moderate intensity (HR target corresponding to 45-55% of their VO2max). Formal exercise instruction and supervision will be provided by ACSM certified fitness professionals at the Penn State University Fitness Center. Subjects will initiate their exercise program by walking on a treadmill for 15 minutes at their target HR goal and progressively increase the duration and frequency until the goal of 30 minutes of moderate intensity exercise five times a week is reached by the end of the four-week lead-in period. Subjects will then exercise five times a week under direct supervision in one of three locations; the Penn State University Fitness Center, the Cancer Center

Exercise Medicine Unit or the Hershey Center for Applied Research. Aerobic exercise can be completed on either the treadmill, exercise bike, rowing machine or the elliptical machine. Subjects will be given the opportunity to complete these supervised sessions virtually or in-person as described above. If the subject chooses to complete the strength training sessions virtually, the study EP will schedule and complete exercise sessions using Penn State HIPPA complaint Zoom. Additional home exercise beyond the recommended sessions will be assessed by downloading data from the Fitbit Charge2. Review of downloaded information will take place once a week. Any subject who performs *<3 sessions* in any given week during the 16-week study period will be required to extend their training by two additional weeks to offset loss of physical conditioning. They can be extended no longer than 4 weeks. If subject misses more than 2 weeks of exercise sessions, they will be discontinued from the study.

Weekly review of dietary logs obtained via self-report through the FitBit application and secure web-based platform with directed feedback by trained study personnel (nutritionist) will also be provided either in-person, telephone interview or video chat. Additionally, participants will be given dietary education forms. In the event that the subject does not self-report their dietary intake through the FitBit platform, the 24-hour dietary recall data collection form will be utilized.

Study investigators will perform an interim history and physical examination on a monthly basis during the exercise protocol. If subject is required to extend protocol, interim history and physical examinations will continued to be performed on a monthly basis. The subject may complete these either in person or virtually.

The purpose of the post-exercise program liver biopsy is to observe changes in stage of fibrosis and NASH Activity Score. Liver biopsy is the gold standard to stage chronic liver disease from NASH and the benefit of disease staging and prognostication outweighs the small risk to the subject. Non-invasive methods of diseases staging (serologic scoring systems NFS, FIB-4) and ultrasound with elastography have inferior performance characteristics with much lower sensitivity and specificity and are hindered by significant inaccuracy with misclassification bias observed in upwards of 25%.(1, 93) Sub-

[redacted]

Control condition (standard of care): Subjects in the control condition 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. Weekly phone calls will be performed by study personnel to ensure adherence to the protocol (no changes in activity). Subjects will report to Penn State on a monthly basis for anthropometric assessment in the GCRC to confirm their self-reports and study investigators will perform an interim history and physical examination at that time. Subjects may choose to complete this visit in person or virtually using Zoom. Subjects will also be given a Fitbit Charge2HR and downloaded data review will be performed monthly at study visits visits. These methods of compliance have previously been validated.(91)

This study will be a delayed treatment trial. Subjects randomized to the control condition will be given five months of fitness center membership to UFC and access to an ACSM certified fitness professional for training sessions following completion of the study protocol.

Penn State PM&R Research Laboratories: Housed at Hershey Center for Applied Research (HCAR), the 4,500 ft^2 facility is comprised of Exercise, Body Composition, Locomotor Training and Gait Laboratories

(Director-Dr. Gater, collaborator). While the available exercise and body composition assessment equipment totals well over $1,000,000 and includes multiple gait training devices, this study will utilize the following exercise equipment: Cosmed® K4B2 Portable Metabolic Device and ECG Telemetry system, ParvoMedics TrueMax® 2400 Metabolic Measurement System, and Quinton Q710 Stress Testing/Resting ECG System. Body composition resources include Lunar iDXA® DXA scanner, skinfold and anthropometric calipers.

Penn State University Fitness Center: Housed with the Penn State University Conference Center, the University Fitness Center has a plethora of resources including free weights and cardiovascular equipment necessary for this protocol. In addition, group fitness classes, gymnasium, locker room and shower facilities are available during convenient all-day hours, 7 days a week. In addition to the dedicated NASHFit exercise physiologist, there are an additional seven exercise physiologists on staff. There are private rooms available for study visits allowing for completion of assessment immediately before or after an exercise session by trained study personnel.

Follow-up. Subjects will be seen by a study investigator immediately after the completion of the 16-week exercise intervention. This will be completed at the Penn State Hershey GCRC and the PM&R research laboratory. A focused history and physical exam, anthropometric assessment including DXA scan, IPAQ, dietary log, cardiopulmonary fitness testing, laboratories, stool sample, MRI-PDFF, PROMIS and end of study survey will be completed. A sub-study of ten subjects will be required to complete a liver biopsy following sub-randomization at the completion of the exercise intervention. Samples collected for research purposes will be stored per standard SOP requirements of the Pathology Department. Subjects will be compensated ($25 at baseline, $25 at completion of the exercise regimen, $100 following liver biopsy obtained at the completion of the exercise regimen if selected). They will also be allowed to keep their FitBit Charge2 HR. Finally, participants will receive a list of local fitness facilities available in the area. Twelve weeks after the end of the program, study staff will contact participants in the exercise arm by phone to complete a follow up questionnaire.

Paired liver biopsy samples (enrollment sample and post-exercise sub randomized sample) will be sent to a biotechnology company, Reveal Biosciences, to undergo an automated computerized ImageDx analysis to evaluate the severity of NASH. Samples will be shipped directly from the Pathology (where they are stored) and they will be de-identified.

Schedule of visits and study protocol assessments

Visit	Initial Assessment	Exercise Instruction	Exercise Program or Control	End of exercise program
Day/Week	*Day 0*	*Weeks 0-4*	*Weeks 5-19*	*Week 20*
Initial comprehensive history and physical exam	X			
Focused history and physical exam			X (monthly)	X
Anthropometric assessment	X		X (monthly)	X
Physical activity questionnaire (IPAQ)	X		X (monthly)	X
Dietary Log		X (weekly)	X (weekly)	x
FitBit activity data download		X (weekly)	X (weekly)	X
Cardiopulmonary fitness testing (VO2 max)	X			X
Electrocardiogram (ECG)	X			
Laboratories	X			X
Stool sample	X			X
Urine pregnancy testing (if applicable)	X			
Genetic testing	X			

Liver MRI-PDFF	X			X
PROMIS Quality of Life Assessment via iPad (completed under direct supervision of trained research personnel)	X		X (monthly)	X
DXA body composition testing	X			X
Liver biopsy				X (if sub-randomized)

7.3 Duration of Participation

20 weeks- please see above table.

8.0 Subject Numbers and Statistical Plan

8.1 Number of Subjects

We expect to enroll 42 subjects.

8.2 Sample size determination

Subjects will be randomized into two treatment groups. This is an efficient study design with increased power and is appropriate for the proposed exercise intervention. The primary outcome will be change in PAI-1 following a 16-week exercise course. Secondary outcomes are described above. Baseline levels of PAI-1 are between 35-45 ng/mL.(72, 74-76) Estimates for change in PAI-1 following exercise regimens of between 3-8 months duration range between 23-37% with >30% reduction only reported in regimens >6 months long.(72, 74-76) De Gues *et al.(75)* found an initial 24.5% reduction as measured at four months in PAI-1 levels in thirty healthy adults aerobically exercising 2.5 hours per week. We expect the exercise intervention to result in a 23% reduction (9 ng/dL) in PAI-1. We expect no change in PAI-1 levels in the control arm. We conservatively determined power calculations utilizing the standard deviations in the literature (2.0 for no exercise group and 14.0 for the aerobic exercise group). We expect a 15-20% attrition rate;(94) to account for this we will enroll an additional six subjects.

Based on these assumptions and a 2:1 enrollment ratio with two subjects in the aerobic exercise arm to every one subject in the standard of care arm, a total study enrollment size of 28 subjects in the aerobic exercise arm and 14 in the standard of care arm will achieve 80.74% power to detect a difference in mean PAI-1 at sixteen weeks of at least 9 ng/mL, using a two-sided, two-sample unequal variance t-test with a significance level (alpha) of 0.05. A total study enrollment of **42 subjects** is planned**.**

8.3 Statistical methods

Subjects will be compared across multiple important baseline demographic, laboratory, imaging and histologic data. Results will be expressed as means with standard deviations. 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 lab data. Paired t-tests will be used where appropriate (e.g. comparison of pre- and post-intervention fibrosis parameters such as NFS or FIB-4 Score). A p value of <0.05 will be significant. Intention to treat analysis will be performed. Analyses will be conducted using SAS version 9.4. Multivariable regression models will be constructed.

9.0 Confidentiality, Privacy and Data Management

See the Research Data Plan Review Form

10.0 Data and Safety Monitoring Plan

10.1 Periodic evaluation of data

Dr. Thomas Riley (hepatology) will serve as the independent medical monitor for the proposed research project and will meet with Dr. Stine bi-annually. Under the mentorship of Dr. Sciamanna, Dr. Stine will provide oversight for the conduct of the study and collectively we will follow the protocol proposed and ensure all eligibility criteria and consent requirements are met prior to enrolling a subject. All study

procedures and adverse event (AE) reporting will occur in accordance with the Penn State College of Medicine IRB approved protocol. These monitoring activities will remain in place until all participants have completed the intervention. Dr. Stine will review AEs on a rolling basis and quarterly. He will review study enrollment and any early termination of a subject's participation. Any issues requiring study modification or a change in the risk/benefit to a subject will be reported immediately to the IRB. A summary of AEs, study progress and protocol modifications will be sent to the IRB for review in the annual continuing review report.

10.2 Data that are reviewed

Safety data will be reviewed as will untoward events.

10.3 Method of collection of safety information

Case report forms will be utilized for AEs. Study visits will assess for AEs.

10.4 Frequency of data collection

Dr. Stine will review AEs on a rolling basis and quarterly. He will review study enrollment and any early termination of a subject's participation.

10.5 Individuals reviewing the data

Dr. Stine and Dr. Riley

10.6 Frequency of review of cumulative data

Quarterly

10.7 Statistical tests

Subjects will be compared in their randomized groups according to SAE occurrence. Chi-squared and Fisher's exact test will be used to analyze our categorical endpoints. A p value of <0.05 will be significant. Intention to treat analysis will be performed. Analyses will be conducted using SAS version 9.4.

10.8 Suspension of research

If the SAE's 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.

11.0 Risks

This study is designed to assess the efficacy of exercise in patients with NASH. The main risks of any exercise protocol include physical injury (e.g. musculoskeletal), or an adverse cardiovascular event (myocardial infarction). All exercise sessions will be monitored by ACSM certified fitness professionals and an available physician will be available should any injury or cardiovascular event occur. In addition, there are several other risks related to the measures of secondary outcomes:

<u>MRI scan:</u>

- Discomfort related to claustrophobia from being inside an enclosed space for the MRI scan and loud banging noise during the study for which temporary hearing loss has been reported (wearing earplugs can help prevent this).

<u>DXA scan</u>:

- Radiation exposure from the DXA scan that is being used to determine body composition

Venipuncture (blood draw):

- Discomfort associated with removing blood by venipuncture (by needle from a vein) including mild discomfort and/or a black and blue mark at the site of puncture.
- Less common risks of venipucture include a small blood clot, infection or bleeding at the puncture site, and on rare occasions fainting during the procedure.

Cardiopulmonary Testing:

- Maximal oxygen uptake testing involves exercising to exhaustion. Side effects may include, fainting, chest pains, weakness, vomiting, muscle soreness and headache.
- Risk of falling due to increasing speed and incline of the treadmill during exhaustive exercise.

Liver biopsy:(95)

- 5-20% musculoskeletal pain despite topical and deep anesthetic
- <1% risk of
    - Major bleeding
    - Infection
    - <Inadvertent organ puncture (e.g. kidney, lung, gallbladder, colon)

There is also an inherent risk in randomization as subjects are assigned to a study group by chance. The study program a subject is assigned to may prove to be less effective or have more side effects than the other study groups.

Incidental findings discovered through history & physical exam, pregnancy testing, blood draws, cardiopulmonary testing, imaging (MRI and DXA) and during the exercise protocol will be handled on an individual basis according to standard medical practice at the discretion of the study PI. These results will be communicated to not only the subject but also the subject's primary care physician and/or the appropriate medical specialist best suited for further evaluation.

Beyond this, the risks of this study should be minimal to the subjects. Prior studies in have shown exercise to be safe in this population. The risk benefit ratio is minimized in the study design and safety plan.

12.0 Potential Benefits to Subjects and Others

12.1 Potential Benefits to Subjects

Subjects have the possible benefit to improve their overall and liver-specific health with improved cardiopulmonary fitness.

12.2 Potential Benefits to Others

If the results are as expected, further demonstration of benefit in exercise for NAFLD will allow for generalization in this widely prevalent condition afflicting 40 percent of the adult US population

13.0 Sharing Results with Subjects

Results will be shared with study subjects upon completion of the study protocol and analysis of the data and only upon subject written request. Additionally, subjects in the exercise group will receive a summary letter detailing their results pre and post-exercise intervention.

14.0 Subject Stipend (Compensation) and/or Travel Reimbursements

Subjects will be given a stipend ($25 following completion of the baseline visit, $25 following the week 20 visit, $25 after submission of stool sample kit within a week of completion of the program, $100 following completion of the liver biopsy if selected). They will also be allowed to keep their FitBit Charge2 HR.

15.0 Economic Burden to Subjects

15.1 Costs

Travel

15.2 Compensation for research-related injury

It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Costs for the treatment of research-related injuries will be charged to subjects or their insurance carriers.

16.0 Resources Available

16.1 Facilities and locations

Penn State Physical Medicine & Rehabilitation (PM&R) Research Laboratories: Housed at Hershey Center for Applied Research (HCAR), the 4,500 ft^2 facility is comprised of Exercise, Body Composition, Locomotor Training and Gait Laboratories (Director-Dr. Gater, collaborator). While the available exercise and body composition assessment equipment totals well over $1,000,000 and includes multiple gait training devices, this study will utilize the following exercise equipment: Cosmed® K4B2 Portable Metabolic Device and ECG Telemetry system, ParvoMedics TrueMax® 2400 Metabolic Measurement System, and Quinton Q710 Stress Testing/Resting ECG System. Body composition resources include Lunar iDXA® DXA scanner, skinfold and anthropometric calipers.

Penn State University Fitness Center: Housed with the Penn State University Conference Center, the University Fitness Center has a plethora of resources including free weights and cardiovascular equipment necessary for this protocol. In addition, group fitness classes, gymnasium, locker room and shower facilities are available during convenient all-day hours, seven days a week. In addition to the dedicated NASHFit exercise physiologist, there are an additional seven exercise physiologists on staff. There are private rooms available for study visits allowing for completion of assessment immediately before or after an exercise session by trained study personnel.

16.2 Feasibility of recruiting the required number of subjects

Study team has access to >500 potential subjects of which <10% are required for recruitment

16.3 PI Time devoted to conducting the research

Dr. Stine has 60% of his effort protected for clinical research.

16.4 Availability of medical or psychological resources

N/A

16.5 Process for informing Study Team

Weekly study team meetings will take place on Wednesday mornings from 10-11am.

17.0 Other Approvals

17.1 Other Approvals from External Entities

N/A

17.2 Internal PSU Committee Approvals

Check all that apply:

☐ Anatomic Pathology – Hershey only – Research involves the collection of tissues or use of pathologic specimens. Upload a copy of HRP-902 - Human Tissue For Research Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

☐ Animal Care and Use – All campuses – Human research involves animals and humans or the use of human tissues in animals

☒ Biosafety – All campuses – Research involves biohazardous materials (human biological specimens in a PSU research lab, biological toxins, carcinogens, infectious agents, recombinant viruses or DNA or gene therapy).

☒ Clinical Laboratories – Hershey only – Collection, processing and/or storage of extra tubes of body fluid specimens for research purposes by the Clinical Laboratories; and/or use of body fluids that had been collected for clinical purposes, but are no longer needed for clinical use. Upload a copy of HRP-901 - Human Body Fluids for Research Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

☒ Clinical Research Center (CRC) Advisory Committee – All campuses – Research involves the use of CRC services in any way.

☐ Conflict of Interest Review – All campuses – Research has one or more of study team members indicated as having a financial interest.

☒ Radiation Safety – Hershey only – Research involves research-related radiation procedures. All research involving radiation procedures (standard of care and/or research-related) must upload a copy of HRP-903 - Radiation Review Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

☐ IND/IDE Audit – All campuses – Research in which the PSU researcher holds the IND or IDE or intends to hold the IND or IDE.

☒ Scientific Review – Hershey only – All investigator-written research studies requiring review by the convened IRB must provide documentation of scientific review with the IRB submission. The scientific review requirement may be fulfilled by one of the following: (1) external peer-review process; (2) department/institute scientific review committee; or (3) scientific review by the Clinical Research Center Advisory committee. NOTE: Review by the Penn State Hershey Cancer Institute Scientific Review Committee is required if the study involves cancer prevention studies or cancer patients, records and/or tissues. For more information about this requirement see the IRB website at: http://www.pennstatehershey.org/web/irb/home/resources/investigator

18.0 Multi-Site Research

18.1 Communication Plans

N/A

18.2 Data Submission and Security Plan

N/A

18.3 Subject Enrollment

N/A

18.4 Reporting of Adverse Events and New Information

N/A]

18.5 Audit and Monitoring Plans

N/A

19.0 Adverse Event Reporting

19.1 Reporting Adverse Reactions and Unanticipated Problems to the Responsible IRB

In accordance with applicable policies of The Pennsylvania State University Institutional Review Board (IRB), the investigator will report, to the IRB, any observed or reported harm (adverse event) experienced by a subject or other individual, which in the opinion of the investigator is determined to be (1) unexpected; and (2) probably related to the research procedures. Harms (adverse events) will be submitted to the IRB in accordance with the IRB policies and procedures.

20.0 Study Monitoring, Auditing and Inspecting

20.1 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the Penn State quality assurance program office(s), IRB, the sponsor, and government regulatory bodies, of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.).

21.0 Future Undetermined Research: Data and Specimen Banking

21.1 Data and/or specimens being stored

Plasma and whole blood samples will be stored with study identifier. For participants who consent to bio-banking, excess specimens not used in these studies will be placed in a biorepository for future research with related health information. Bio-banking will be completed by the Penn State Institute for Personalized Medicine (IPM) and will be linked to participant medical/demographic data through a unique identification number assigned by the PRIDE IRB protocol. Medical and demographic data collected by the currently proposed study will be stored by the IPM per the PRIDE IRB protocol for future use with bio-banked samples. Specimens within the IPM will be labeled with a second layer Study ID. No PHI will be stored with the samples.

21.2 Location of storage

IPM. Banked specimens will be stored on the PS HMC campus in C2706 per the PRIDE IRB protocol.

21.3 Duration of storage

Per standard IPM operating procedure where specimens and data will be stored indefinitely.

21.4 Access to data and/or specimens

Per standard IPM operating procedure. The honest broker associated with this study will have full access to the master list and database through the PI. These lists will include PHI and information from the MR. Specimen access will be limited to the PI and select members of the IPM staff.

Access to banked specimens will be determined by the IPM staff per the PRIDE IRB protocol.

21.5 Procedures to release data or specimens

Per standard IPM operating procedure. To request a release of banked specimens' investigators will submit, in writing, a copy of the PRIDE Sample Data Request Form. This request will be reviewed by the PRIDE Access Committee per IRB protocol. Penn State investigators meeting PRIDE criteria will be given

access to the specimens, linked by medical record number to clinical data. Medical and demographic data collected by the current study will not be provided.

Following the conclusion of this study, all remaining samples and associated data will become property of the Institute for Personalized Medicine (IPM) and will be governed as described in the PRIDE protocol 40532.

21.6 Process for returning results

Per standard IPM operating procedure. The procedure for returning all results of banked specimens will be carried out in accordance with the IPM's PRIDE IRB outlined in the document, PRIDE Return of Research Results Request. The IPM's PRIDE Program provides a forum for investigators to access and to assume the responsibility of determining what should be done with the research results. This relieves researchers of the individual responsibility for determining what should be done with potentially clinically significant results and ensures that any research results that could impact the clinical care of the participant be appropriately transmitted. The PRIDE Program's Research Results Board will meet following notification by the PRIDE Access Committee that results have been returned and a voting quorum will determine whether results merit a) no further action, b) informing participants with follow-up CLIA genetic testing, c) informing participants with follow-up non-genetic studies, d) inform participants without follow-up testing. If results are returned to participants they will be contacted by a member of the PRIDE Research Results Board.

22.0 References

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