Metabolic Response to a High-Fat Challenge in Children and Adolescents.

NCT05230433

VERSION 8.28.2023

PROTOCOL TITLE: Metabolic Response to a High-Fat Challenge in Children and Adolescents.

D-HH IRB OVERSIGHT:

☒ The Principal Investigator is employed by D-H
☐ The study will utilize any D-H data or specimens
☒ The study will enroll D-H patients or recruit from D-H sites
☒ The study will utilize any D-H resources, e.g. study procedures will occur at D-H locations and/or use of D-H equipment or shared resources

PROTOCOL TITLE:

Metabolic Response to a High-Fat Challenge in Children and Adolescents.

PRINCIPAL INVESTIGATOR:

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VERSION NUMBER/DATE:

Version 2.0 / January 3, 2022

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
	8.28.2023	Revision to consent & assent language throughout for consistency	No

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1.0 Study Summary

Study Title	Metabolic Response to a High-Fat Challenge in Children and Adolescents.
Study Design	Clinical trial
Primary Objective	The objective of this study is to classify the metabolome and gene expression response to a high-fat (HF) challenge in children, identifying predictors of the homeostatic response, such as insulin resistance (IR) and cardiorespiratory fitness (CRF).
Secondary Objective(s)	To phenotype body composition, daily physical activity, and habitual dietary intake to quantify modifiable behaviors associated with CRF, with the long-term goal of developing clinical interventions aimed at improving CRF in adolescents to preserve metabolic health.
Research Intervention(s)/ Investigational Agent(s)	High-fat Challenge (Boost Glucose Control with palm oil)
IND/IDE #	N/A
Study Population	DHMC Pediatrics
Sample Size	50
Study Duration for individual participants	Two visits separated by 1-2 weeks
Study Specific Abbreviations/ Definitions	AC – acylcarnitine ADA - American Diabetes Association AED - automated external defibrillator AHA - American Heart Association ASA-24 – Automated Self-Administered 24-h dietary recall BIA - bioelectrical-impedance analyses BMI – body mass index CHaD - Children's Hospital at Dartmouth-Hitchcock cMetS - continuous metabolic syndrome score CRC – clinical research coordinator CRF – cardiorespiratory fitness CVD – cardiovascular disease DG – diacylglycerols DHMC – Dartmouth-Hitchcock Medical Center FA- fatty acid FA-COOH – dicarboxylic fatty acid GINA - The Genetic Information Non-Discrimination Act HbA1c - hemoglobin A1c HDL – high-density lipoproteins

	HEI - Healthy Eating Index HF – high-fat HOMA-IR - homeostatic model assessment for insulin resistance IGF-1 – insulin growth factor 1 IR – insulin resistance LDL- low-density lipoproteins MAP - mean arterial blood pressure OGTT - oral glucose tolerance test OLTT – oral lipid tolerance test PA – physical activity PBMC - peripheral blood mononuclear cells PC – phosphocholine PHI – protected health information SM – sphingomyelin T2D – type 2 diabetes TG – triglycerides VO_2max – maximal oxygen consumption WC – waist circumference WWC – Weight & Wellness Center 6MWT - 6-minute walk test

2.0 Objectives*

Cardiorespiratory fitness (CRF) in youth is an independent risk factor for type 2 diabetes (T2D) (*1*) and cardiovascular disease (CVD) (*2*), and in adulthood, CRF is inversely associated with mortality (*3*). Only 40% of youth in the United States have a healthy CRF (*4*), possibly influenced by increased sedentary time (*5*), decreased moderate-to-vigorous activity (*5*), increased obesity (*6*), and lower dietary quality (*7*). CRF is estimated by measuring maximum oxygen consumption (VO_2max) during intense exercise, challenging the capacity of the respiratory and circulatory systems to supply oxygen to the skeletal muscles for generation of ATP. CRF is directly related to mitochondrial respiratory capacity and is associated with fatty acid (FA) oxidation (*8, 9*). Profiling the response of the FA oxidation intermediates, measured by metabolomics, and FA oxidation gene expression, measured within peripheral blood mononuclear cells (PBMC), to a high-fat (HF) challenge may provide a mechanistic link between CRF and risk for T2D and CVD. The primary objective of this study is to challenge mitochondrial FA oxidation via administering a HF meal, assessing the relationship between FA oxidation and CRF in youth (age 8-17 years). *We hypothesize that low CRF will be associated with inefficient mitochondrial beta-oxidation, profiled by medium-chain acylcarnitines (AC), and increased extra-mitochondrial oxidation, profiled by dicarboxylic FA (FA-COOH).* The secondary objective of this study is to assess the relationship between FA oxidation intermediates and measures of insulin resistance (IR), glucose homeostasis, and cardiometabolic risk. *We hypothesize that incomplete mitochondrial beta-oxidation and increased extra-mitochondrial oxidation will mediate the relationship between CRF with metabolic health.* We will test our hypotheses through these aims:

SPECIFIC AIM 1. Determine if CRF is associated with modifiable behaviors including body composition, daily physical activity (PA), and habitual dietary intake. Children, aged 8-17 years (body mass index (BMI) percentile ≥ 85% for sex/age, matched with leans [5%≤BMI percentile<85%], n=50), will be recruited. Gold-standard stress testing will use a modified Bruce multistage protocol, increasing both the elevation and speed over time, to measure VO_2 max. Body composition will be measured using bioelectrical impedance (Seca, Hamburg, Germany). Over seven days, participants will be asked to wear an ActiGraph accelerometer (ActiGraph, Pensacola, FL) and complete three dietary recalls using Automated Self-Administered 24-h dietary recall (ASA-24). We will assess the interaction between PA and habitual dietary intake, measured by the Healthy Eating Index (HEI) score (*10*), with VO_2 max. We will consider if the interaction between PA and HEI score modifies the linear association between percent fat mass and VO_2 max.

SPECIFIC AIM 2. Following a HF-challenge, assess the relationship between mitochondrial and extra-mitochondrial FA oxidation with CRF. After an overnight fast, participants will consume the HF-challenge, composed of palm oil supplemented Boost® (15% kcal carbohydrates, 15% kcal protein, 70% kcal lipid). Blood will be sampled via intravenous line at baseline (t0) and after consuming the challenge (t30, t60, t120, and t180 minutes) and analyzed for fluctuations in glucose and insulin. Plasma targeted metabolomics will be conducted, profiling AC and FA-COOH metabolites. PBMC will be isolated and mRNA levels of genes related to beta- and omega-oxidation will be determined by qPCR. Fold change calculations and longitudinal trajectories, measured by empirical Bayes time-series analysis (*11*), will quantify the response of metabolites and expression of genes to the challenge. Mixed

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regression models will determine the association between CRF with FA oxidation metabolite and gene (1) absolute levels, (2) longitudinal trajectories, and (3) ratios (*12*), considering the influence of age, sex, pubertal status, and BMI percentile.

SPECIFIC AIM 3. Identify if mitochondrial and extra-mitochondrial oxidation of the HF-challenge mediates the relationship between CRF and metabolic health. In fasting plasma, we will profile the homeostatic model assessment for IR (HOMA-IR), hemoglobin A1c (HbA1c), and a continuous metabolic syndrome score (cMetS), composed of waist circumference (WC), mean arterial blood pressure (MAP), HOMA-IR, HDL-C, and triglycerides (TG) (*13*). Linear regression will assess the relationship between VO_2 max and the metabolic markers. Mediation analyses will be used to identify if FA oxidation metabolite and gene (1) absolute levels, (2) longitudinal trajectories, and (3) ratios (*12*) mediates the relationship between VO_2 max and the metabolic markers, considering the influence of age, sex, pubertal status, and BMI percentile.

The proposed aims will identify modifiable behaviors associated with CRF in youth and will assess if FA metabolism mediates the relationship between CRF and metabolic health, with the long-term goal of developing clinical interventions aimed at improving CRF in youth to preserve metabolic health (**Figure 1**).

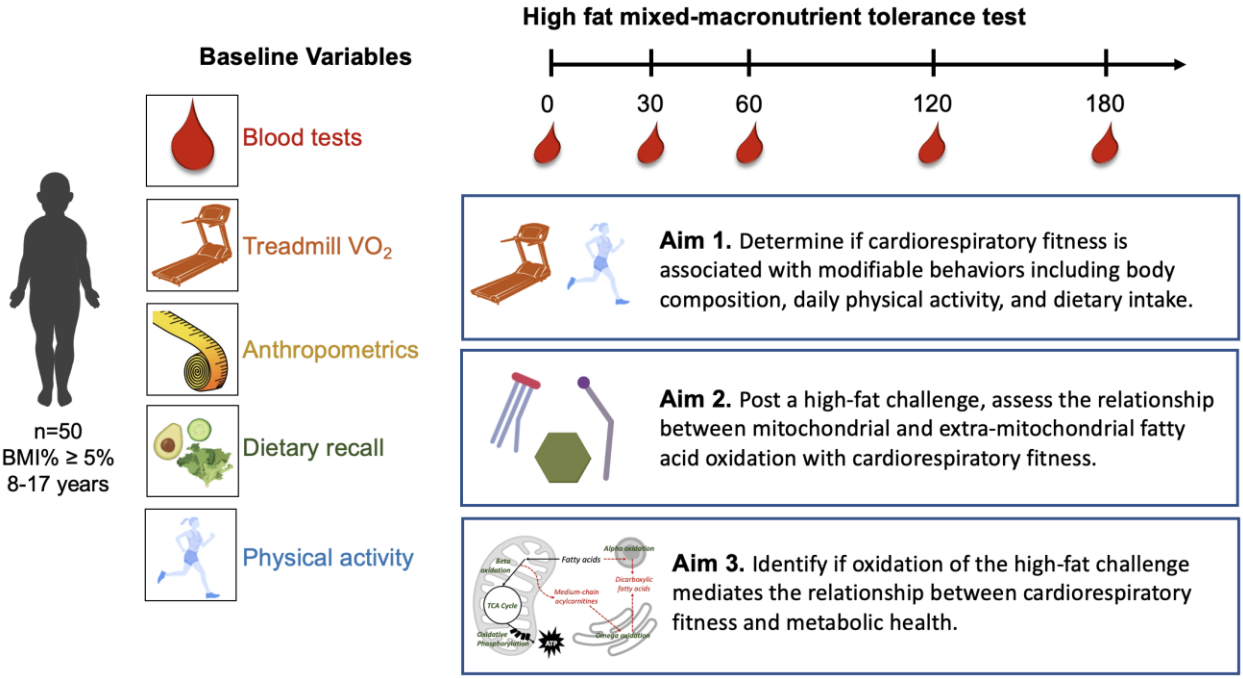

Figure 1. Children (BMI%≥5%) will be recruited (aged 8-17). Assessment of characteristics will include blood sampling (glucose homeostasis), a treadmill maximal aerobic capacity assessment (VO_2 max), anthropometrics (height, weight, bioelectrical impedance, waist circumference), 24-hr dietary recall, and habitual physical activity measured by an accelerometer. Individuals will undergo a high-fat meal challenge (15% carbohydrates, 15% protein, 70% lipid) and blood sampling will be collected on an acute time-course.

3.0 Background*

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Cardiorespiratory fitness is the capacity of respiratory and circulatory systems to supply oxygen to skeletal muscle during exercise for the generation of energy. Beginning in childhood, CRF is predictive IR and T2D (*1*), CVD (*2*), and academic performance (*14*). There is an intrinsic, nonmodifiable aspect of CRF, influenced by genetics (*15, 16*), age (*17*), and sex (*18*). However, in the past three decades, adolescent CRF has declined (*19*), with only 40% of youth in the United States (aged 12-15 years) maintaining a healthy CRF (*4*). Global alterations in behaviors may be influencing the decline in CRF, including increased sedentary time (*5*), decreased moderate-to-vigorous activity (*5*), increased obesity (*6*), and lower dietary quality (*7*). The American Heart Association (AHA) has recently issued a statement (*20*) emphasizing the importance of accurately and reliably measuring CRF "to identify youth who would benefit from lifestyle interventions but may have been missed by subjective PA recall, anthropometric measures, or CVD risk factor testing, which are current standards of care." Profiling maximum oxygen consumption (VO_2max) during intense exercise is the gold standard measure of CRF. The first objective of this grant proposal is to measure VO_2max using a treadmill protocol and to assess the relationship between CRF and body composition, daily PA, and habitual dietary intake (**Specific Aim 1**), with the long-term goal of developing clinical interventions aimed at improving CRF in youth to preserve metabolic health. There have been no studies to date that considered the cross-sectional interaction between PA, measured by Actigraph accelerometers, and habitual dietary intake, measured by the HEI score (*10*), on CRF in youth (*20*). We will consider if the interaction between PA and HEI score modifies the association between percent fat mass and CRF, providing avenues for lifestyle changes in adolescents with overweight and obesity.

To sustain exercise, oxygen is circulated to skeletal muscles to be utilized within the mitochondrial via the electron transport chain, supporting the creation of ATP (reference). Therefore, CRF serves as a measurement of mitochondrial respiratory capacity and has been associated with FA oxidation (*8, 9*). In rats selectively bread for high running capacity (*9*), Overmyer et al. demonstrated that CRF is associated with a more rapid mitochondrial protein deacetylation at the initiation of exercise and a more efficient and prolonged oxidation of FA during exercise. The inability to efficiently metabolize FAs is associated with elevated levels of bioactive lipids, such as ceramides and diacylglycerols (DG), which leads to cellular stress, inflammation, and increased risk of T2D and CVD (*21*). The second objective of this grant proposal is to challenge mitochondrial FA oxidation via administering an acute HF meal, assessing the relationship between FA oxidation and CRF (**Specific Aim 2**). Mitochondria are the central hub of FA oxidation (beta-oxidation); however, the endoplasmic reticulum (omega-oxidation) and peroxisomes (alpha-oxidation) serve as FA oxidation rescue pathways when beta-oxidation is overloaded (*22*). Using plasma metabolomics during the meal challenge, we hypothesize that low CRF will be associated with inefficient mitochondrial beta-oxidation, profiled by AC, and increased extra-mitochondrial oxidation, profiled by FA-COOH (*22, 23*). Beta- and omega-oxidation gene expression will be quantified within PBMC; a cell type frequently used in human challenges due to its accessibility and expression being comparable to skeletal muscle tissue (*24*). There have been no studies to date that have used an acute nutrient challenge to assess the relationship between FA oxidation and CRF. Morris et al. assessed variation in the lipidome in response to an oral lipid tolerance test (OLTT) in healthy adults (n=40) observing differences in production of phosphatidylcholines (PC), sphingomyelins (SM)

and ceramides related to CRF (*25*). Profiling FA oxidation intermediates and PBMC gene expression rather than the lipidome will provide a more direct readout of FA efficiency.

In youth, literature widely suggests that insulin regulation and resistance may lead to metabolic alterations inducing the development of CVD (*26*). IR alters lipid metabolism leading to hyperglycemia and dyslipidemia, evidenced by increased in fasting glucose, TGs, and low-density lipoproteins (LDL) and decreases in high-density lipoproteins (HDL). Currently, the American Diabetes Association (ADA) endorses screening for prediabetes and T2D by profiling the response of glucose to an oral glucose tolerance test (OGTT), in which a 75-gram glucose solution is consumed. However, an OGTT has exhibited notable limitations in the pediatric population, including poor reproducibility (*27*), potentially advocating for new screening tools to be developed. In our recent review (*28*), we have assessed how metabolomics can be paired with a nutrient challenge to provide a deeper understanding of the homeostatic ability to adapt to a nutrient load potentially identifying pathways and biomarkers of metabolic health. Previous pediatric metabolomics studies in fasted plasma (*23, 29*) have suggested that mitochondrial and extra-mitochondrial FA oxidation may be an earlier indicator of IR compared to alterations in glucose metabolism. The third objective of this grant proposal is to determine if FA oxidation metabolites and genes during the HF-challenge are associated with measures of IR (e.g. HOMA-IR), glucose homeostasis (e.g. HbA1c), and a continuous metabolic syndrome score (cMetS), composed of WC, MAP, HOMA-IR, HDL-C, and TG (*13*) (**Specific Aim 3**). We will determine if the ability to metabolize the HF-challenge mediates the relationship between CRF and the profiled metabolic markers. There have been no studies to date that have considered using a HF-challenge to understand metabolic health in youth. We propose that a HF challenge may complement or enhance the ability of an OGTT to classify adolescents at risk of T2D and CVD.

4.0 Study Endpoints*

Variable	Instrument / Measurement	Source	Time Series (minutes)				
			0	30	60	120	180
Aim 1: Cardiorespiratory fitness with modifiable behaviors							
Demographics	Questionnaires	Participant self-report	X				
Anthropometrics	Weight, height, BMI percentile, waist circumference, blood pressure, and BIA results	RA	X				
Dietary recall	ASA-24	Participant self-report	X				
Cardiorespiratory fitness	Maximal oxidative capacity treadmill test	Sports cardiology	X				
Physical activity	Actigraph	actigraph	X				
Aim 2: HF-challnege response with cardiorespiratory fitness							
Metabolic response	Untargeted metabolomics and PBMC gene expression	blood	X	X	X	X	X
Cardiorespiratory fitness	Maximal oxidative capacity treadmill test	Sports cardiology	X				
Aim 3: HF-challenge response with metabolic health							
Metabolic response	Untargeted metabolomics and PBMC gene expression	blood	X	X	X	X	X
Fasting laboratory measures	HbA1C, IGF-1, C-peptide, lipid panel	blood	X				
Response laboratory measures	glucose, insulin	blood	X	X	X	X	X

5.0 Study Intervention/Investigational Agent

The shake will be composed of a mixture of BOOST Glucose Control® (Nestlé Products) supplemented with palm oil (**Table 1**). Palm oil contains approximately 50% saturated fatty acids (palmitic acid [FA 16:0] and stearic acid [FA 18:0]) and 40% monounsaturated fatty acids (oleic acid [FA 18:1]). Each participant will consume a volume of liquid equivalent to 25% of their estimated daily caloric needs, calculated by the USDA Dietary Reference Intakes using a moderate activity factor. The investigational agent will only be consumed one time, at the second study visit.

6.0 Procedures Involved*

Assent and Consent: A clinical research coordinator (CRC) or study team member will collect consent from at least one parent/legal guardian and verbal assent (ages 8-12 years) or written assent (ages 13-17 years) from the participant via a telehealth appointment prior to their first study visit.

Protocol – Two Study Visits

Study Visit 1: Participants will arrive at the Clinical Research Unit in Faulkner Building 4M at the Main Campus of Dartmouth Hitchcock Medical Center (DHMC) following a two-hour fast. Consent and assent documents will be available to re-read prior to study visit. A sample of the HF-challenge liquid shake will be available to taste to determine if the adolescents will be willing to participate in Study Visit 2. The CRC or other study team member will collect weight, height, blood pressure, and waist circumference. Each participant will have body composition analyzed using bioelectrical-impedance analyses (BIA).

The CRC or other study team member will transfer the participants and their Parents/Guardians to the Sports Cardiology Unit in Cardiac Services 4A at the Main Campus of DHMC. The CRC or other study team member with CPR and BLS training will acclimate the participant to a treadmill and metabolic cart to undergo a maximal aerobic capacity test, measuring CRF. The maximal aerobic capacity test will use the Bruce Protocol (1), a treadmill multistage incremental protocol. Parents/Guardians will fill out electronic questionnaires about demographics, diabetes family history, and past medical history. Children will fill out electronic questionnaires about puberty status measured by Tanner's Stages.

Participants will be compensated $25 for the completion of the study visit.

Between Study Visit 1 and 2

Between Study Visit 1 and 2, all participants will receive an Actigraph GT9X triaxial accelerometer to objectively assess habitual physical activity for at least seven days. All participants will complete three dietary recalls detailing foods eaten. The timeframe between Study Visit 1 and 2 will be ~7-14 days.

Study Visit 2: Participants will arrive at the Clinical Research Unit in Faulkner Building 4M at the Main Campus of DHMC after an overnight fast (≥12 hours). Upon arrival, the CRC or other study team member will collect accelerometers and data will be exported via ActiLife software. A RN will place an intravenous line to collect blood sampling throughout the acute meal challenge. The CRC or other study team member will mix and provide the HF-challenge, containing approximately 15% calories from carbohydrates, 15% calories from protein, and 70% calories from fat. Participants will receive a survey identifying their taste preference for the HF shake. Blood sampling will occur prior to the consumption of the HF challenge and 30, 60, 120, and 180 minutes after the consumption of the HF challenge. The CRC or other study team member will collect a 24-hour dietary recall of the previous day. After drinking the HF challenge, the CRC or other team member will collect a brief survey about the taste preference of the HF challenge. Metabolomics and assays for gene expression will be conducted across the time-course.

Participants will be compensated $75 for the completion of the study visit.

Protocol – One Study Visit

Participants will receive an Actigraph GT9X triaxial accelerometer to objectively assess habitual physical activity for at least seven days. All participants will complete three dietary recalls detailing foods eaten.

Participants will arrive at the Clinical Research Unit in Faulkner Building 4M at the Main Campus of Dartmouth Hitchcock Medical Center (DHMC) after an overnight fast (≥12 hours). Consent and assent documents will be available to re-read prior to study visit. The CRC or other study team member will collect weight, height, blood pressure, waist circumference, and hip circumference. Each participant will have body composition analyzed using bioelectrical-impedance analyses (BIA). Parents/Guardians will fill out electronic questionnaires about demographics, diabetes family history, and past medical history. Children will fill out electronic questionnaires about puberty status measured by Tanner's Stages.

A RN will place an intravenous line to collect blood sampling throughout the acute meal challenge. The CRC or other study team member will mix and provide the HF-challenge, containing approximately 15% calories from carbohydrates, 15% calories from protein, and 70% calories from fat. Participants will receive a survey identifying their taste preference for the HF shake. Blood sampling will occur prior to the consumption of the HF challenge and 30, 60, 120, and 180 minutes after the consumption of the HF challenge. The CRC or other study team member will collect a 24-hour dietary recall of the previous day. After drinking the HF challenge, the CRC or other team member will collect a brief survey about the taste preference of the HF challenge. Metabolomics and assays for gene expression will be conducted across the time-course.

Participants will be compensated $75 for the completion of the study visit.

Assessment Details

Physical Assessment

A physical examination will then be conducted, which includes measurement of height, weight, blood pressure, waist circumference, and hip circumference. Measurements will be collected by the CRC or other member of the research team.

Bioelectrical Impedance Analysis

BIA (SECA, Hamburg, Germany) is a non-invasive test providing the measurement of fat and lean mass in each participant. A weak non-detectable electric current will flow through the body and the voltage is measured to calculate impedance (resistance) of the body. Body compartments (fat, bone, lean mass, water) provide differential resistance to the electrical current flow, allowing for an estimation of lean and fat mass.

Maximal Aerobic Capacity Test

The maximal aerobic capacity test will be performed by the expert staff of the Cardiology Unit, including an exercise physiologist, with supervision provided as needed by Dr. Meijer and CRC. Participants will be acclimated to the treadmill prior to procedure.

Stress testing will use the Bruce Protocol (*30*), a treadmill multistage incremental protocol, increasing both the elevation and speed over a duration of time. Stage 1 starts at 10% grade and a 1.7 mph walking pace. In each subsequent stage, the grade is increased by 2% and the speed by either 0.8 or 0.9 mph until the subject is exhausted. Stage levels increase every 3 minutes.

Participants will wear a mouthpiece to allow for the measurement of oxygen consumption and carbon dioxide emission. Participants will wear a heart rate monitor around their chest to continuously monitor heart rate. VO_2 peak is reached when oxygen consumption remains at steady state despite the increased workload. Participants will complete the test until they claim maximum exercise and exhaustion. Staff members will provide the same encouragement to all participants, to help them differentiate between exercise capacity constraints and lack of cooperation in exercise testing. The test will stop immediately if there is an emergency, if the subject fails to conform to the protocol, or if the subject experiences signs of discomfort.

We will adhere to the absolute and relative indications for termination of an exercise test which include the following Absolute Contraindications: moderate to severe angina, increasing nervous system symptoms (e.g. ataxia, dizziness, or near syncope), signs of poor perfusion (cyanosis or pallor), or subject's desire to stop and the following Relative Contraindications: increasing chest pain or fatigue, shortness of breath, wheezing, leg cramps, or claudication.

Previous studies have found success in the incorporation of this protocol in pediatric research (*31*). Additional details on clinical stress testing in the pediatric age group are provided by the AHA (*32*).

When conducting statistical analyses, a respiratory exchange ratio, the ratio between metabolic production of carbon dioxide and the uptake of oxygen, ≥ 1 will serve as an indicator that the participant was nearing exhaustion, rather than stopping prematurely.

Submaximal Aerobic Capacity Test

To estimate submaximal aerobic capacity, participants will undergo a 6-minute walk test (6MWT), where distance walked is measured using a rolling measuring wheel. This test is practical and low-tech, requiring a pre-measured hallway (20-30 meters) and allowing participants to dictate their own intensity of exercise and rest during the test. The information gained from the 6MWT is not considered a replacement for a maximum cardiorespiratory fitness test, however, strong correlations have been observed (*33*). Participants will be instructed to walk as far as possible in 6 minutes, back and forth in a pre-measured hallway. Environmental factors will be standardized in the test facility and all participants will be encouraged to wear comfortable clothing and appropriate shoes for walking. The protocol and encouragement during

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the test will be in accordance with the American Thoracic Society (*34*). Prediction equations to estimate VO_2 max will be used (*33*).

We anticipate funding sources to cover the expenses for a maximal aerobic capacity test, nevertheless, our protocol includes a submaximal aerobic capacity test as a low-cost estimate of VO_2 peak in preparation of lack of funding. The 6MWT can be administered in hallways within DHMC, on Level 5 due to less foot traffic, by the CRC.

Surveys

Parents/Guardians will be asked to fill out electronic questionnaires about demographics, diabetes family history, and past medical history. Children will be asked to fill out surveys about puberty status using the Puberal Development Scale (*35*) and a taste preference survey after the high fat shake.

Actigraph

Participants will be asked to wear an ActiGraph GT9X triaxial accelerometer (ActiGraph, Pensacola, FL) for at least seven days after the first study visit to objectively assess habitual physical activity. This accelerometer was chosen as it has shown to be a reliable and valid measure of habitual physical activity in children with established data acquisition and processing methods (*36*). Participants will wear the monitor on their non-dominant wrist during all waking hours. Participants will be asked to remove the monitor during periods of sleeping, bathing, or swimming, as well as contact sports. Staff will be available by phone or email to answer device usage questions. Participants will complete accelerometer logs, noting the time when the device was put on and taken off. Children will return the device.

Upon collection, data will be exported from the device using ActiLife software, Version 16.3.3 (ActiGraph, Pensacola, FL). The GT9X accelerometer generates a variable output voltage signal, proportional to acceleration in three orthogonal places (vertical, anteroposterior, and mediolateral). Acceleration is converted to activity counts and stored on the device. Physical activity will be classified into sedentary behavior and light, moderate, and vigorous physical activity (*37*).

Habitual Dietary Intake

Three dietary recalls will be administered (two weekdays and one weekend) using Automated Self-Administered 24-h dietary recall (ASA-24). The ASA-24 is based on the validated Multiple Pass Method (*38*) and has been validated against standardized interviewer-administered 24-hr recalls (*39*). Children with Parent/Guardian supervision will report all food and beverage consumed during the previous day (midnight to midnight). The ASA-24 uses the USDA's Food and Nutrient Database for Dietary Studies to calculate dietary intake and HEI scores (*40*), which is composed of a thirteen component scoring system adhering to the 2015-2020 Dietary Guidelines for Americans.
Participants will have a 24-hour dietary recall during the HF challenge to assess food intake the previous day.

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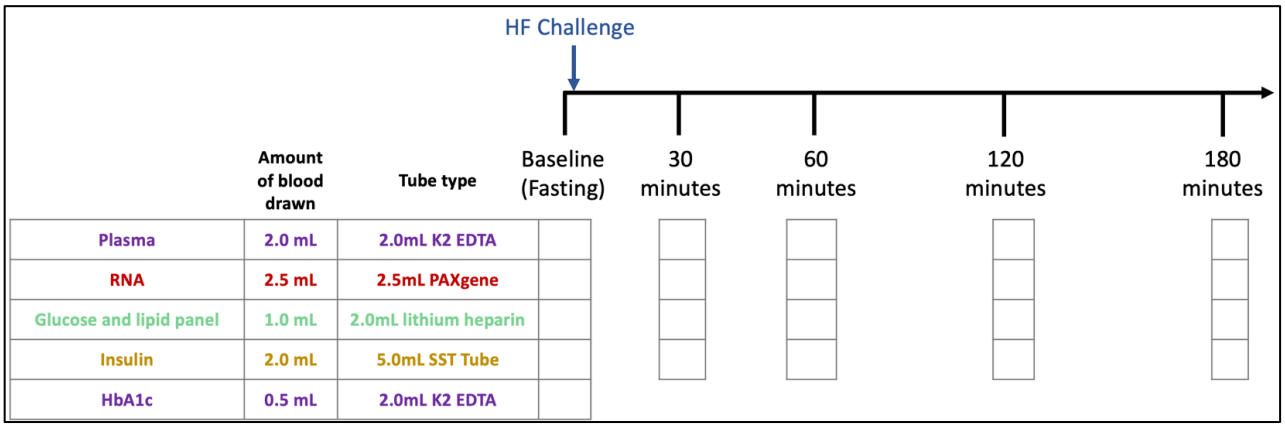
High-fat Challenge

The HF-challenge will be started between 7:00 AM and 10:00 AM. To increase the comfort level of the participants, a topical anesthetic will be applied to numb the location of the needle. An intravenous catheter will be placed, and blood drawn prior the consumption of the shake (baseline). A liquid HF shake will be consumed over a 10-minute period. Participants will be highly encouraged to consume the entire shake; however, pre- and post-weight of the shake will be taken if entire shake isn't consumed. The shake will be composed of a mixture of BOOST Glucose Control® (Nestlé Products) supplemented with palm oil. Palm oil contains approximately 50% saturated fatty acids (palmitic acid [FA 16:0] and stearic acid [FA 18:0]) and 40% monounsaturated fatty acids (oleic acid [FA 18:1]). Each participant will consume a volume of liquid equivalent to 25% of their estimated daily caloric needs, calculated by the USDA Dietary Reference Intakes using a moderate activity factor. Maximum volume of the HF shake will contain 54 grams of fatty acids. Blood samples will be taken at 30, 60, 120, and 180 minutes. A maximum volume of 46 mL of blood (approximately 4 tablespoons) will be collected from each participant during the 3-hour meal challenge. The total blood amount accounts for 1mL of 'flush blood' at each timepoint, to account for the small amount of blood left in the line and flushed out with saline between draws.

	BOOST Glucose Control®	Palm oil	Total
Energy (kcal)	190	240	430
Protein (g)	16	0	16
Carbohydrates (g)	16	0	16
Fat (g)	7	28	35
SFA (g)	1	14	15
MUFA (g)	5	10	15
PUFA (g)	1	4	5

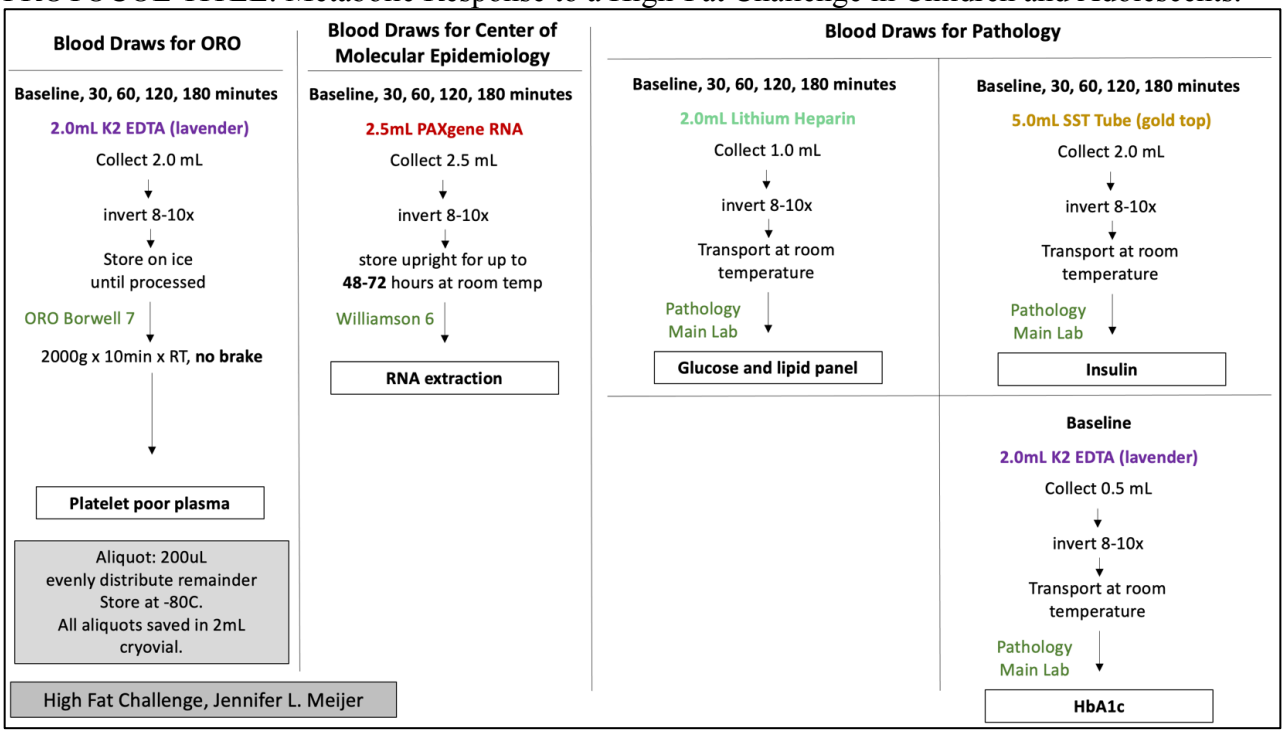
Table 1. Nutrient composition of an 8oz portion of the HF liquid shake composed of a mixture of BOOST Glucose Control® and palm oil.

Figure 1. Sample collection timeline.



	Amount of blood drawn	Tube type
Plasma	2.0 mL	2.0mL K2 EDTA
RNA	2.5 mL	2.5mL PAXgene
Glucose and lipid panel	1.0 mL	2.0mL lithium heparin
Insulin	2.0 mL	5.0mL SST Tube
HbA1c	0.5 mL	2.0mL K2 EDTA

Figure 2. Sample collection details.



Office of Research Operations - Plasma samples (2.0 mL K2 EDTA tubes)

Collection Timepoints: baseline (pre-shake, fasting), 30 minutes, 60 minutes, 120 minutes, and 180 minutes

Sample Collection details:

Amount blood drawn: 2.0 mL

CRU Post-Sample Collection:

1. Following venipuncture, invert the collection tube 10x
2. Transfer collection tube on ice.

CRU Staff will transport sample to ORO Lab (Borwell 7) after each blood draw

ORO Sample Processing:

1. Centrifuge: 2000g for 10 minutes at room temperature. No brake.
2. Extract plasma. Aliquot 200µL. Evenly distribute remainder of plasma.
3. Store at -80C in 2mL screw top cryovials with 0.5mL inserts.

The CRC for the High-Fat Challenge will label all plasma storage tubes. Nomenclature is: Subject ID_Timepoint_Sample type_date

Eventual shipping for Metabolomics:

Attn: Kari Bonds
University of Michigan
Metabolomics Core
1000 Wall Street
5305 Brehm Tower
Ann Arbor, MI 48105-5714

Shipping conditions:

Samples should be shipped frozen, in a secondary container, on dry ice. Tubes should be clearly labeled with sample identifiers (described in the Sample Submission Form) and include a hard copy of the Sample Submission Form with the shipment

Center of Molecular Epidemiology – RNA extraction (2.5 mL PAXgene tubes)

Collection Timepoints: baseline (pre-shake, fasting), 30 minutes, 60 minutes, 120 minutes, and 180 minutes

Sample Collection details:

Amount blood drawn: 2.5 mL

CRU Post-Sample Collection:

1. Following venipuncture, invert the collection tube 10x
2. Store and transport at room temperature

CRU Staff will transport all samples to Williamson 6 after the visit.

Pathology - HbA1c (2.0 mL K2 EDTA tubes)

Collection Timepoints: baseline (pre-shake, fasting)

Sample Collection details:

Minimum blood drawn: 0.1 mL

Amount blood drawn: 0.5 mL *Please use a syringe to collect this small amount of blood.

CRU Post-Sample Collection:

1. Following venipuncture, invert the collection tube 10x.
2. Transport specimen at room temperature.

CRU Staff will transport sample to Pathology Main Lab after the baseline blood draw

Pathology – glucose and lipid panel (2.0 mL lithium heparin tubes)

Collection Timepoints: baseline (pre-shake, fasting), 30 minutes, 60 minutes, 120 minutes, and 180 minutes

Sample Collection details:

Minimum blood drawn: 0.1 mL

Amount blood drawn: 1.0 mL *Please use a syringe to collect this small amount of blood.

CRU Post-Sample Collection:

1. Following venipuncture, invert the collection tube 10x.
2. Transport specimen at room temperature.

CRU Staff will transport sample to Pathology Main Lab after each blood draw

Pathology – insulin (5.0 mL SST tubes)

Collection Timepoints: baseline (pre-shake, fasting), 30 minutes, 60 minutes, 120 minutes, and 180 minutes

Sample Collection details:

Minimum blood drawn: 1.0 mL

Amount blood drawn: 2.0 mL *Please use a syringe to collect this small amount of blood.

CRU Post-Sample Collection:

1. Following venipuncture, invert the collection tube 10x.
2. Transport specimen at room temperature.

CRU Staff will transport sample to Pathology Main Lab after each blood draw

Metabolomics Analysis

Plasma untargeted metabolomics profiling will be done by the MRC2 (www.mrc2.umich.edu, Directed by Dr. Burant), which has extensive experience in production and analysis of metabolomics data. For untargeted metabolomics, samples are analyzed on an Agilent 1200

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liquid chromatography/6530 quadrupole Time-of-Flight mass spectrometry system (Agilent Technologies, Inc., Santa Clara, CA USA) using the Waters Acquity HSS T3 1.8 µm column (Waters Corporation, Milford, MA). The eluent is analyzed in both positive and negative ion mode electrospray ionization. Each participant will provide five plasma samples for metabolomics after the intake of the high-fat dietary challenge.

Genetic Analysis

The study is looking for an association between a genotype or a biomarker and a specific disease or condition, but at this point it is not clear if the genetic marker has predictive value. RNA will be isolated by the Center of Molecular Epidemiology within Dartmouth College and gene expression will be quantified using PCR for genes related to beta-oxidation, omega-oxidation, and entry into the mitochondria.

7.0 Data and Specimen Banking*

Clinical data / protected health information (PHI) including will be kept under appropriate D-H firewalls through Epic and encrypted institutional computers and on secure REDcap (Research Electronic Data Capture) database. REDCap is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. Datasets with linked identifiers will be stored on institutional servers and password protected. Users of clinical data will have D-H training in privacy protection as well as human participants training, and only aggregate de-identified data will be used in presentation or publication. Separate study identifiers will be assigned to participants at enrollment and will be used for all questionnaires, blood samples, and measurements. Only the primary investigator and research team members who directly contact the participants will have access to the information necessary to link them to their de-identified data. Data will be maintained confidentially and destroyed at the end of the study per NIH policies.

8.0 Sharing of Results with Subjects*

The team will encourage communication with participants' primary care provider if any medical concerns arise from laboratory measures, specifically if the participant has an HbA1c $\geq$ 5.7%. Within network, we will contact the primary care provider via eD-H. Outside of network, we will contact the primate care provider via telephone or fax. Should any participant endorse any symptoms, we will immediately contact the nursing staff of the patient's D-H primary care to coordinate evaluation. During this study, participants who take part will not have access to the study data.

9.0 Study Timelines*

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The study will last from January 2022 through December 2023. We anticipated to start phone recruitment in March 2022. Both study visits will begin at that time. The study team is flexible and can begin human subject interactions once DH-IRB deems appropriate. Primary analyses will be conducted during 2023 with the objective of sharing results by conferences and publications.

10.0 Inclusion and Exclusion Criteria*

Inclusion Criteria

Children ages 8-17 years with a BMI-percentile ≥ 5th.

Inclusion of minorities: Every effort will be made to include minorities in the study population. Recruitment of both boys and girls will occur.

Exclusion Criteria

Previous diagnosis of type 1 or 2 diabetes. Use of concurrent medications known to affect glucose metabolism (metformin, oral steroids, sulfonylureas, insulin). Evidence of inherited disorders of lipid metabolism. Inability to participant in the maximal aerobic capacity test on the treadmill. Allergies to palm oils or protein types within high-fat challenge, such as lactose and soy. Individuals who cannot speak and/or write in English.

11.0 Vulnerable Populations*

The research includes children less than <18 years of age. A clinical research coordinator (CRC) or study team member will collect consent from at least one parent/legal guardian and verbal assent (ages 8-12 years) or written assent (ages 13-17 years) from the participant via a telehealth appointment prior to their first study visit. Permission from one parent is sufficient. We are requesting participant consent for participants ≥ 18 years. See Section 22 for details.

12.0 Local Number of Subjects

For our pilot study in 2022, we anticipate the ability to recruit 25 subjects with overweight and obesity and 25 leans, recruiting from a pool of ~100 pediatric patients seen within the Dartmouth-Hitchcock Weight & Wellness Center (WWC) and ~6000 pediatric patients seen within the Children's Hospital at Dartmouth-Hitchcock (CHaD) (ages 8-17 years).

13.0 Recruitment Methods

Enrollment/Recruitment: Enrollment will begin following D-H IRB approval. Proposed aims will be conducted by recruiting children and adolescents (8-17 years) through the CHaD (assisted by Dr. Susanne Tanski), the WWC (assisted by Dr. Auden McClure), and, as needed, through the community. Flyers have been developed by DHMC marketing.

Recruitment via in person. Providers within CHaD and the WWC will have the opportunity to tell patients about the proposed research via in person oral scripts, flyers, and handouts. The eligibility screener will be read verbally for the patient to answer. If the patient desires to participate and they are eligible, we will send their information to the CRC or other study team member to organize a telehealth visit for consent and assent collection.

Recruitment via telephone. The Department of Pediatrics will share a list of eligible patients for the research study. A CRC/member of the research team will telephone patients to screen for eligibility. The eligibility screener will be read verbally for the patient to answer. If the patient desires to participate and they are eligible, we will send their information to the CRC or other study team member to organize a telehealth visit for consent and assent collection.

Recruitment via flyer, intranet, and D-H social media. IRB approved study flyers will be distributed electronically - over social media and over the intranet – and physically - in any public area. Flyers will be distributed by General Ambulatory Services prompting patients to ask their provider for more information and clinical teams will assist in identifying interested participants. If a patient calls the study team directly, the eligibility screener will be read verbally for the patient to answer. If the patient desires to participate and they are eligible, we will send their information to the CRC or other study team member to organize a telehealth visit for consent and assent collection. If a patient emails the study team directly, the eligibility screener will be sent for the patient to answer (RedCap). Upon reception of the survey, if the patient is eligible, we will send their information to the CRC or other study team member to organize a telehealth visit for consent and assent collection.

Eligibility Screener:
Upon expression of interest to participate in the study, participants will receive an eligibility screener (Redcap) either verbally or written. Screener will address child's age, pre-existing conditions influencing ability to walk/run on treadmill, heart conditions limiting physical activity, and diagnosis of any conditions that influence their metabolism or digestion (i.e. diabetes, Crohn's disease, malabsorptive syndrome). Screener will address any food allergies that may be triggered by the high-fat challenge, such as allergies to palm oil, dairy protein, and soy.

Consent and Assent: A clinical research coordinator (CRC) or study team member will collect consent from at least one parent/legal guardian and verbal assent (ages 8-12 years) or written assent (ages 13-17 years) from the participant via a telehealth appointment prior to their first study visit. See Section 22 for details.

Incentives: One Study Visit: Children will be compensated with a $75 gift card to Walmart. Parent will be compensated with a $10 gas card for traveling <20 miles and a $20 gas card for traveling >20 miles. Two Study Visit: Children will be compensated with a $25 gift card to Walmart for the first visit and a $75 gift card to Walmart for the second visit, totaling $100 for

the study. Parent will be compensated with a $10 gas card for traveling <20 miles and a $20 gas card for traveling >20 miles.

The child and their parents are spending at least 5 hours of their time to participant in this study, The fees compensate for their time.

Participants have the option of declining such monetary incentives. All individuals receiving a monetary payment will need to sign a W9 form at that time in line with institutional protocols.

Parking: Participants will be able to park at the parking garage at DHMC.

14.0 Withdrawal of Subjects*

To minimize dropouts, we will provide frequent contact with participants, study incentives, and outreach to primary care providers (to engage partnerships). Reminder and follow up calls/emails/ myDH (as preferred) may be made as needed to provide additional support to help minimize attrition by assessing and responding to potential barriers to regular participation.

If medical issues arise over the course of the study or if the participant endorse any symptoms the obesity medicine specialist who is part of the study team in combination with the patient's primary care physician will be consulted. The study team reserves the right to withdraw subjects if the medical team determines it is necessary.

Data previously collected prior to the point of withdrawal can still be used for research. Participants may be asked to provide a reason for withdrawing from the study. Consent will include language permitting ongoing data collection and participant contact after withdrawal / loss to follow up to compare outcomes and assess barriers to participation.

15.0 Risks to Subjects*

Physical exercise capacity: No more than minimal risk.

During the study, participants will participate in moderate to vigorous activity. As with any moderate to vigorous cardiovascular exercise, there are potential side effects. The most common side effects (occurring in more than 10% of participants) include discomfort during exercise, discomfort while wearing the headgear necessary for this study, and potential muscular soreness the day after the test. Rare side effects (less than 1% of participants) include cardiac complications that could lead to death. The researchers will try to minimize these risks by:

- Participants will be required to answer questions regarding their physical activity level prior to acceptance into this study. This questionnaire is designed to assess the safety of exercise for an individual. Admittance into this study will only be granted to those deemed healthy enough for exercise.
- All study personnel are trained to safely complete a graded exercise test (the test used in this study).

- All study personnel conducting exercise testing and training are CPR/AED certified and trained in emergency procedures specific to our facility. An automated external defibrillator (AED) is located near the exercise room. In case of an emergency, our study team will call 911 and if warranted will use the AED. This device automatically diagnoses and treats life threatening cardiac (heart) problems.
- All participants will wear a heart rate monitor throughout each session, allowing easy assessment of heart rate throughout the exercise tests. In addition, study personnel will monitor participants appearance, form and rating of perceived exertion throughout the entire test to ensure the participant is exercising at a safe level.
- If a participant feels he/she cannot complete the test, study personnel and/or the participant can easily stop the test.

High-fat meal challenge: No more than minimal risk

People on rare occasions may feel nausea or vomit from drinking the high-fat solution, due to the taste of the solution. We will screen for allergies to palm oils or protein types within high-fat challenge, such as lactose and soy, to ensure against allergic reactions.

Blood samples: No more than minimal risk

The known or expected risks are those associated with using a needle to place a plastic catheter (IV) for drawing blood from a vein. These can include discomfort at the site of puncture; possible bruising and swelling around the puncture site; rarely an infection; and, uncommonly, faintness from the procedure. There will be one needle stick to place the plastic IV. The researchers will try to minimize these risks. Sometimes it may require multiple needle pokes (limited to three in this study) to place the plastic IV, but to try and avoid this we will have experienced nurses place the plastic IV.

Breach of confidentiality: No more than minimal risk

As with any study collecting information that can be traced back to the participants, there is a risk that the information we collect may be seen by persons other than the study team. We are taking careful measures to prevent this from happening. For example, we will use coded identifying numbers rather than your name, birthdate, or other identifying information. Further, we will store all data that we collect in password protected files and only the study team will have the passwords to these files. Collaborators both inside and outside Dartmouth-Hitchcock Medical Center may only have access to samples that have been coded but do not contain any of your personal information. Blood samples will be labeled with a unique code rather than your name and will be kept in a freezer in a locked laboratory; no person other than the investigators involved with this study will have access to these samples.

This project will ultimately involve generation of genetic information. Loss of confidentiality of biobank-related information is unlikely, but potential risks are as follows. Information about a person's participation in a genetic study could influence insurance companies and/or employers

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about one's health status. "The Genetic Information Non-Discrimination Act" or GINA protects individuals against employers or insurance companies using genetic information to make decisions. The WWC and IBR do everything possible to safeguard participant information and keep it confidential as described above. Briefly, all samples are coded and locked. The key to the code is kept in a separate, secure location. Access to the code is limited to the IBR Manager as an honest broker. No study information will be released to family members or published in a way that could identify the participant.

Inconvenience: No more than minimal risk

There could be inconvenience to the subject to schedule these procedures. We will try to make every accommodation to them in terms of their schedule.

COVID-19: No more than minimal risk

We anticipate that the risk of contracting the virus because of participating in the study is low. The study team plans to follow all institution guidance on COVID related protocols. This includes but is not limited to current and ongoing recommendations for masks and eye protection for study team members and universal masking of participants as vaccination status cannot be assessed. We will allow for appropriate spacing between participants and study coordinators/RNs.

Participants will have to remove their masks for the maximal oxidative capacity test to use the mouthpiece. Mouthpieces will be discarded after use. Minimal members of the research team will be within the room during the maximal oxidative capacity test.

As with any research study, there may be additional risks that are unknown or unexpected.

16.0 Potential Benefits to Subjects*

The proposed study's main objective is to identify individuals at a young age with a high risk for developing T2D and other metabolic diseases. Classifying the response to a high-fat meal challenge may complement or improve current screening methods.

No direct benefit will be received by patients.

17.0 Data Management* and Confidentiality

Our team is experienced in survey / protocol development, training of study team members, intervention delivery, and data management. Regular research meetings and PI supervision will assure quality and consistency. Questionnaire data anthropometric and laboratory data will be stored on a HIPAA compliant Redcap database. Clinical data / PHI including will be kept under appropriate D-H firewalls through Epic and encrypted institutional computers and databases. Datasets with linked identifiers will be stored on institutional servers and password protected. Users of clinical data will have D-H training in privacy protection as well as human participants

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training, and only aggregate de-identified data will be used in presentation or publication. Separate study identifiers will be assigned to participants at enrollment.

18.0 Provisions to Monitor the Data to Ensure the Safety of Subjects*

The proposed study presents no more than minimal risk to subjects. As children are vulnerable population, our study team want to ensure extra caution for the safety of the research participants. Guidelines are as follows:

Maximal oxidative capacity fitness test: The study team member running the maximal oxidative fitness test will have CPR training and basic life support certification. The maximal oxidation capacity test will occur at DHMC. A cardiologist will be available to interpret the results of VO_2 max test. An adolescent sports medicine pediatrician is a collaborator in the proposed project and helped decide on the appropriate VO_2 max test. In the rare event that a participant has a medical issue and needs assistance, the study team will follow CODE protocols for DHMC to alert medical personnel. In addition, the study team can access the HERT (Hitchcock Early Response Team) team to provide early medical assistance.

Adolescent Sports Pediatrician: Dr. Keith Loud

Pediatric Obesity Medicine Specialist: Dr. Marc Hofley

Blood draw: The clinical research unit has an established protocol for responding to uncommon occurrences (e.g. fainting from blood draw) including contacting the pediatric HERT team.

Lab results of concern: Dr. Hofley will work with Dr. Loud to provide medical oversight including screening of all fasting lab results, including glucose, insulin, HbA1c, and the lipid panel and contacting patients' primary care providers with any abnormal results or other concerns. Dr. Meijer's medical advisors are part of pediatrics and well connected to primary care triage and provider teams which allows for easy communication.

Pediatric Obesity Medicine: Dr. Auden McClure will provide oversight for development of recruitment and clinical protocols

Other Concerns: Other participant concerns can be directed to Dr. Meijer and her research coordinator. Participants will also be provided contact information for IRB as oart of the consent process

Principal Investigator: Jennifer Meijer, PhD, RD, MPH

Email: jennifer.l.meijer@hitchcock.org

Phone 603-650-5250

19.0 Provisions to Protect the Privacy Interests of Subjects

The only people who will have access to the study information are those who are study team members. The PI will endure that the disclosure of PHI obtained during this study complies with the Federal Privacy Regulation. All data will be stored behind institutional or equivalently approved firewalls and password protected.

20.0 Compensation for Research-Related Injury

N/A

21.0 Economic Burden to Subjects

We foresee little economic burden to the subjects outside of mileage to drive to DHMC for the study visits, which our incentives will accommodate.

22.0 Consent Process

To participate in the study, both parent/legal guardian written consent and participant assent will be required. Verbal assent will be required for participants ages 8-12 years and written assent will be required for participants ages 13-17 years. Separate consent and assent forms were created. Signatures will be obtained from at minimum one parent/legal guardian prior to the first study visit via a telehealth appointment, facilitated by the CRC or other study team member. The signature will be obtained via RedCap surveys including the consent and/or assent document. The study team will verbally read the consent form, highlighting details of the study and potential risks. The study team will answer any questions and will use a teach back method of consent to ensure the parent/legal guardian and the participant understand the breath of the study. HIPAA Authorization to review the child participant's medical record is embedded in the consent document. Parent/legal guardians and participants will be provided with a copy of the consent and assent forms. They will be permitted to ask any questions to study personnel. The contact information for study personnel is provided on the consent/assent form.

We will be following *SOP: Informed Consent Process for Research (HRP-090)* and *SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013).*

Non-English-Speaking Subjects

Participants who are non-English speaking will not be enrolled.

23.0 Process to Document Consent in Writing

Consent and assent documents will be emailed/presented via RedCap and signed electronically. We will be following SOP: Written Documentation of Consent (HRP-091).

24.0 Setting

Potential research participants will be recruited through the CHaD and the WWC at DHMC. All study visits will be conducted at DHMC through the Clinical Research Unit or the Cardiology Unit.

25.0 Resources Available

For our pilot study, we anticipate the ability to recruit 25 subjects with overweight and obesity and 25 leans, recruiting from a pool of ~100 pediatric patients seen within WWC and ~6000 pediatric patients seen within CHaD (ages 8-17 years). Dr. Jennifer Meijer will dedicate 0.4 FTE towards conducting and completing the research. The clinical research coordinator will dedicate 0.2 FTE towards conducting and completing the research. We anticipate ~50 hours will be needed for recruitment of 50 participants. The research will utilize the CRU's exception nursing staff and resources. All study team members are required to read the protocol and all related study documents.

References

1. F. B. Ortega, J. R. Ruiz, M. J. Castillo, M. Sjostrom, Physical fitness in childhood and adolescence: a powerful marker of health. *Int J Obes (Lond)* **32**, 1-11 (2008).
2. G. Hogstrom, A. Nordstrom, P. Nordstrom, High aerobic fitness in late adolescence is associated with a reduced risk of myocardial infarction later in life: a nationwide cohort study in men. *Eur Heart J* **35**, 3133-3140 (2014).
3. K. Mandsager *et al.*, Association of Cardiorespiratory Fitness With Long-term Mortality Among Adults Undergoing Exercise Treadmill Testing. *JAMA Netw Open* **1**, e183605 (2018).
4. J. Gahche *et al.*, Cardiorespiratory fitness levels among U.S. youth aged 12-15 years: United States, 1999-2004 and 2012. *NCHS Data Brief*, 1-8 (2014).
5. K. A. McGuire, R. Ross, Incidental physical activity is positively associated with cardiorespiratory fitness. *Med Sci Sports Exerc* **43**, 2189-2194 (2011).
6. C. E. Byrd-Williams *et al.*, Cardiorespiratory fitness predicts changes in adiposity in overweight Hispanic boys. *Obesity (Silver Spring)* **16**, 1072-1077 (2008).
7. M. Seong, Y. Kim, S. Park, H. Kim, O. Kwon, Association Between Diet Quality and Cardiorespiratory Fitness in Korean Adults: The 2014-2015 National Fitness Award Project. *Nutrients* **12**, (2020).
8. J. M. Gaitan *et al.*, Two Weeks of Interval Training Enhances Fat Oxidation during Exercise in Obese Adults with Prediabetes. *J Sports Sci Med* **18**, 636-644 (2019).

9. K. A. Overmyer *et al.*, Maximal oxidative capacity during exercise is associated with skeletal muscle fuel selection and dynamic changes in mitochondrial protein acetylation. *Cell Metab* **21**, 468-478 (2015).
10. S. M. Krebs-Smith *et al.*, Update of the Healthy Eating Index: HEI-2015. *J Acad Nutr Diet* **118**, 1591-1602 (2018).
11. J. Xia, I. V. Sinelnikov, D. S. Wishart, MetATT: a web-based metabolomics tool for analyzing time-series and two-factor datasets. *Bioinformatics* **27**, 2455-2456 (2011).
12. S. Krug *et al.*, The dynamic range of the human metabolome revealed by challenges. *FASEB J* **26**, 2607-2619 (2012).
13. J. C. Eisenmann, K. R. Laurson, K. D. DuBose, B. K. Smith, J. E. Donnelly, Construct validity of a continuous metabolic syndrome score in children. *Diabetol Metab Syndr* **2**, 8 (2010).
14. C. C. A. Santana *et al.*, Physical fitness and academic performance in youth: A systematic review. *Scand J Med Sci Sports* **27**, 579-603 (2017).
15. C. Bouchard *et al.*, Familial resemblance for VO2max in the sedentary state: the HERITAGE family study. *Med Sci Sports Exerc* **30**, 252-258 (1998).
16. M. H. De Moor *et al.*, Genome-wide linkage scan for athlete status in 700 British female DZ twin pairs. *Twin Res Hum Genet* **10**, 812-820 (2007).
17. N. Armstrong, J. Welsman, Development of peak oxygen uptake from 11-16 years determined using both treadmill and cycle ergometry. *Eur J Appl Physiol* **119**, 801-812 (2019).
18. R. J. Winsley, J. Fulford, A. C. Roberts, J. R. Welsman, N. Armstrong, Sex difference in peak oxygen uptake in prepubertal children. *J Sci Med Sport* **12**, 647-651 (2009).
19. G. R. Tomkinson, J. J. Lang, M. S. Tremblay, Temporal trends in the cardiorespiratory fitness of children and adolescents representing 19 high-income and upper middle-income countries between 1981 and 2014. *Br J Sports Med* **53**, 478-486 (2019).
20. G. Raghuveer *et al.*, Cardiorespiratory Fitness in Youth: An Important Marker of Health: A Scientific Statement From the American Heart Association. *Circulation* **142**, e101-e118 (2020).
21. E. Sokolowska, A. Blachnio-Zabielska, The Role of Ceramides in Insulin Resistance. *Front Endocrinol (Lausanne)* **10**, 577 (2019).
22. R. J. Wanders, J. Komen, S. Kemp, Fatty acid omega-oxidation as a rescue pathway for fatty acid oxidation disorders in humans. *FEBS J* **278**, 182-194 (2011).
23. J. L. LaBarre *et al.*, Mitochondrial Nutrient Utilization Underlying the Association Between Metabolites and Insulin Resistance in Adolescents. *J Clin Endocrinol Metab* **105**, (2020).
24. I. Rudkowska *et al.*, Validation of the use of peripheral blood mononuclear cells as surrogate model for skeletal muscle tissue in nutrigenomic studies. *OMICS* **15**, 1-7 (2011).
25. C. Morris *et al.*, Modulation of the lipidomic profile due to a lipid challenge and fitness level: a postprandial study. *Lipids Health Dis* **14**, 65 (2015).
26. V. Ormazabal *et al.*, Association between insulin resistance and the development of cardiovascular disease. *Cardiovasc Diabetol* **17**, 122 (2018).

27. I. M. Libman, E. Barinas-Mitchell, A. Bartucci, R. Robertson, S. Arslanian, Reproducibility of the oral glucose tolerance test in overweight children. *J Clin Endocrinol Metab* **93**, 4231-4237 (2008).
28. J. L. LaBarre, K. Singer, C. F. Burant, Advantages of Studying the Metabolome in Response to Mixed-Macronutrient Challenges and Suggestions for Future Research Designs. *J Nutr*, (2021).
29. S. J. Mihalik *et al.*, Metabolomic profiling of fatty acid and amino acid metabolism in youth with obesity and type 2 diabetes: evidence for enhanced mitochondrial oxidation. *Diabetes Care* **35**, 605-611 (2012).
30. W. F. Mead, Maximal exercise testing--Bruce protocol. *J Fam Pract* **9**, 479-490 (1979).
31. B. Marinov, S. Kostianev, T. Turnovska, Modified treadmill protocol for evaluation of physical fitness in pediatric age group--comparison with Bruce and Balke protocols. *Acta Physiol Pharmacol Bulg* **27**, 47-51 (2003).
32. S. M. Paridon *et al.*, Clinical stress testing in the pediatric age group: a statement from the American Heart Association Council on Cardiovascular Disease in the Young, Committee on Atherosclerosis, Hypertension, and Obesity in Youth. *Circulation* **113**, 1905-1920 (2006).
33. J. Vanhelst, P. S. Fardy, J. Salleron, L. Beghin, The six-minute walk test in obese youth: reproducibility, validity, and prediction equation to assess aerobic power. *Disabil Rehabil* **35**, 479-482 (2013).
34. A. T. S. C. o. P. S. f. C. P. F. Laboratories, ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* **166**, 111-117 (2002).
35. A. C. Petersen, L. Crockett, M. Richards, A. Boxer, A self-report measure of pubertal status: Reliability, validity, and initial norms. *J Youth Adolesc* **17**, 117-133 (1988).
36. A. Santos-Lozano *et al.*, Actigraph GT3X: validation and determination of physical activity intensity cut points. *Int J Sports Med* **34**, 975-982 (2013).
37. A. H. K. Montoye *et al.*, Development of cut-points for determining activity intensity from a wrist-worn ActiGraph accelerometer in free-living adults. *J Sports Sci* **38**, 2569-2578 (2020).
38. Y. Park *et al.*, Comparison of self-reported dietary intakes from the Automated Self-Administered 24-h recall, 4-d food records, and food-frequency questionnaires against recovery biomarkers. *Am J Clin Nutr* **107**, 80-93 (2018).
39. A. F. Subar *et al.*, The Automated Self-Administered 24-hour dietary recall (ASA24): a resource for researchers, clinicians, and educators from the National Cancer Institute. *J Acad Nutr Diet* **112**, 1134-1137 (2012).
40. N. C. Institute. (2020).