Protocol Title: Metabolic, Hormonal, and Physiological Characterization of Isoenergetic High Intensity Interval Training and Moderate Intensity Continuous Training in Adults with Type I Diabetes

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Study Summary

Title	Metabolic, Hormonal, and Physiological Characterization of Isoenergetic High Intensity Interval Training (HIIT) and Moderate Intensity Continuous Training (MICT) in Adults with Type I Diabetes (T1D)
Short Title	HIIT and T1D
Methodology	Randomized Controlled Cross-Over Design
Study Duration	Two years
Study Center(s)	Single site
Objectives	Aim 1: To compare the metabolic responses immediately and 1 hr after one bout of HIIT compared to MICT matched for total energy expenditure, vs no exercise (control; CON) in 14 adults with T1D. Aim 2: To evaluate key counter-regulatory/metabolic hormones during and after HIIT, MICT, and CON sessions and the change in glycemia (using continuous glucose monitoring and glycemic cutpoints [<70 mg/dL, 70-180 mg/dL, >180 mg/dL]) for 48 hrs post exercise. Aim 3: To explore the modulatory effect of physiological variables on metabolism, including biological sex, lean body mass and visceral fat (measured from dual energy x-ray absorptiometry).
Number of Subjects	14 (7 females, 7 males)
Treatment Regimens	All subjects will undergo three conditions, in a randomized order: 1) HIIT 2) MICT 3) CON
Statistical Methodology	Aim 1: Principal Component Analysis for metabolomics data; two-way ANOVA [regimen (HIIT vs. MICT vs. CON) × time (base vs. immediate post vs. 1h post)] Aim 2: Two-way mixed-factorial ANOVA for counterregulatory hormones; Comparison of area under the curve across all trials for CGM, aggregated over 24 hrs Aim 3: Spearman correlations between metabolomic outcome and physiological variables)

Abbreviations and Definition of Terms

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Abbreviation:	**Formal Title:**
HIIT	High Intensity Interval Training
MICT	Moderate Intensity Continuous Training
T1D	Type 1 Diabetes
CON	Control (no exercise)
CGM	Continuous Glucose Monitoring
HbA1c	Hemoglobin A1c
VO_2peak	Peak Oxygen Consumption
PAR-Q+	Physical Activity Readiness Questionnaire
BMI	Body Mass Index
4C Model	Four Compartment Model
FM	Fat Mass
%fat	Percent Body Fat
LM	Lean Mass
DXA	Dual-energy x-ray Absorptiometry Total Body Scan
BIS	Bioelectrical Impedance Spectroscopy
VT	Ventilatory Threshold
HR	Heart Rate
HR_{max}	Maximal Heart Rate
TIR	Time-in-Range
CHO	Carbohydrate
PRO	Protein
FAT	Fat
NDSR	Nutrient Data System for Research
ELISA	Enzyme-Linked Immunosorbent Assay
MVPA	Moderate-Vigorous Physical Activity
EDTA	Ethylenediaminetetraacetic acid
W	Watts
EXSS-IRB SOP	Exercise and Sport Science Institutional Review Board Standard Operating Procedures
DAIDS	Division of AIDS
AE/SAE	Adverse Event/Serious Adverse Event
UPIRSO	Unanticipated Problems Involving Risks to Subjects or Others
DSMB	Data and Safety Monitoring Board
APL	Applied Physiology Laboratory

1 Introduction

This document is a protocol for a human research study. This study will be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

Cardiovascular disease is the most frequent cause of premature death and morbidity in type 1 diabetes (T1D).[1] Overweight and obesity are growing concerns in individuals with T1D, potentially contributing to an increased risk for cardiovascular disease in this already vulnerable population. Exercise is an essential strategy for the management of health and comorbidities in T1D, reducing the severity of cardiovascular risk factors, such as obesity, blood pressure, lipid lipoprotein profile, and systemic inflammation.[2] Regular exercise has been shown to help individuals with T1D reduce hemoglobin A1c (HbA1c) and improve blood lipid profiles, body composition, and endothelial function.[3, 4] People with T1D who exercise more frequently also tend to have lower total daily insulin needs and experience fewer diabetes related complications.[5, 6] Despite the benefits of exercise, less than 20% of patients with T1D report participation in consistent exercise having to overcome a number of barriers, including fear of hypoglycemia, unpredictable glycemic excursions with exercise, inadequate knowledge about exercise management, and lack of time.[5, 7, 8]

High intensity interval training (HIIT), an exercise strategy where short intense exercise bouts are interspersed with brief recovery periods, requires as little as 10 minutes of exercise per session and results in rapid improvements in cardiorespiratory fitness and mitochondrial function.[9] Our preliminary evidence[10-12] suggests that HIIT is a promising approach for maximizing cardiorespiratory and metabolic adaptations in individuals who are metabolically challenged because of obesity or physical inactivity, including insulin sensitivity and glycemic control.[13, 14] A single bout of HIIT in these individuals activates mitochondrial biogenesis, a key mechanism for regulating cardiometabolic adaptations and fuel utilization before and after exercise. Metabolomics and proteomics have previously been used to understand the acute effects of exercise in healthy populations;[15] but the physiologic mechanisms by which HIIT may induce adaptations in T1D has not previously been evaluated. Carbohydrate oxidation increases with an increase in exercise intensity, whereas fat oxidation is accelerated during more moderate intensity exercise. The extent to which exercise intensity influences amino acid metabolism is unclear.[16] Metabolism of carbohydrate and fats are also regulated by various hormones including insulin, glucagon, cortisol, and catecholamines. These perturbations in metabolism are elevated for several hours following moderate-high intensity exercise; a metabolomics profile in T1D with varied intensity exercise has not previously been explored and could be of great importance with respect to glycemic control both during and in the hours following exercise in individuals with T1D. Importantly, these metabolic responses may also be influenced by obesity, biological sex and the amount and quality of lean mass, an organ with high glucose disposal rates. Studies with HIIT in individuals with T1D are more limited; available evidence suggests HIIT provides similar or superior improvements in cardiorespiratory fitness and endothelial function compared to moderate intensity exercise, without a detrimental decline in blood glucose.[7, 8]

Dynamic Multiparametric Metabolic Response to Exercise, known as metabolomics, is a growing area of research that allows for earlier identification of metabolic dysfunction and creates a better understanding of how lifestyle interventions, such as diet and exercise, influence the metabolic profile (**Figure 1**).[9] Acylcarnitites have been identified as the strongest metabolic biomarker of the exercise trained state.[10, 11] Increased systemic concentrations of plasma acylcarnitines occurs in conditions of dysfunctional fatty acid oxidation, such as insulin resistance and obesity.[10, 12] Systemic acylcarnitine concentrations decrease with exercise training and increased cardiorespiratory fitness, suggesting enhanced mitochondrial oxidative capacity.[11] Succinate, Krebs Cycle intermediates, and amino acids have also been associated with improved insulin sensitivity in response to high-intensity exercise.[11] Acutely, targeted metabolomics approaches have shown concentrations of non-esterified fatty acids to be increased for up to two hours following HIIT, suggesting a shift toward greater lipid metabolism.[16] Chronically, previous results from our lab show promising

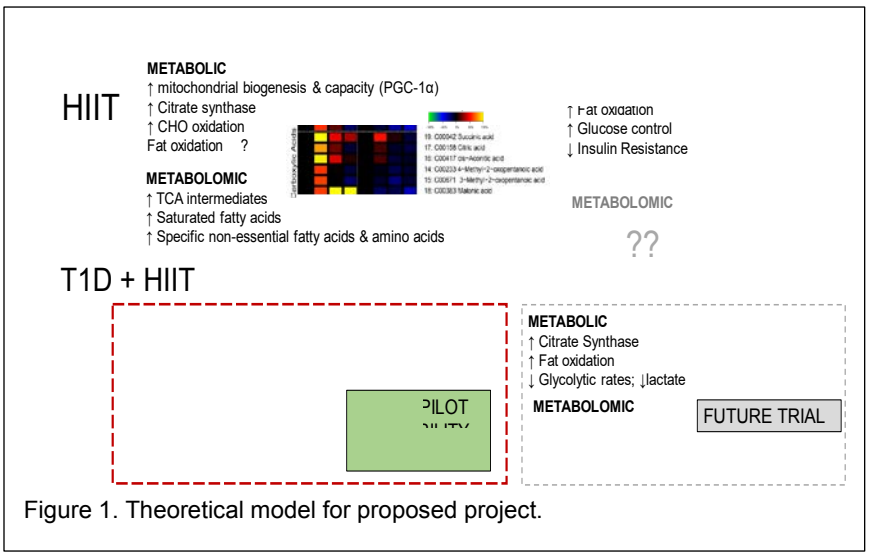

Figure 1. Theoretical model for proposed project.

improvements in skeletal muscle energetics with HIIT, specifically reducing acylcarnitine concentrations after six weeks of HIIT in patients with knee osteoarthritis (NCT03039452; in press).[11]

To date, only a few studies have examined the effects of HIIT in T1D, reporting similar or superior improvements in fitness level and endothelial function from HIIT, compared to traditional MICT, without a detrimental decline in blood glucose.[23, 24] Improving our knowledge of the metabolic and counter-regulatory responses to HIIT in those with T1D is needed to identify effective and feasible exercise strategies to help regulate glycemic control, while also effectively managing T1D associated cardiometabolic comorbidities. As a result of the high prevalence of overweight and obesity, and concomitant exacerbation of cardiovascular and metabolic disease among individuals with T1D, there is a major need to identify effective and feasible behavioral interventions designed to mitigate health risks and optimize glycemic control.

2 Study Objectives

Specific Aim 1: To compare the metabolic responses immediately and 1 hr after HIIT compared to MICT matched for total energy expenditure, vs no exercise in 14 adults with T1D. Metabolism will be defined as energy expenditure (via indirect calorimetry), carbohydrate metabolism (tricarboxylic acid intermediates), and fat metabolism (acylcarnitines, respiratory exchange ratio).

Specific Aim 2: To evaluate key counter-regulatory/metabolic hormones (i.e. cortisol, lactate, glucagon, and insulin) during and after HIIT, MICT, and control (CON) sessions and the change in glycemia (using continuous glucose monitoring and glycemic cutpoints [<70 mg/dL, 70-180 mg/dL, >180 mg/dL]) for 48 hrs post exercise.

Specific Aim 3: To explore the modulatory effect of physiological variables on metabolism, including biological sex, lean body mass and visceral fat (measured from dual energy x-ray absorptiometry). Body fat percentage, lean mass, and visceral fat will be used to characterize the sample; the relationship of these variables with metabolomic intermediates will be assessed.

3 Study Design

3.1 General Design

In a randomized controlled cross-over design, fourteen adults (7 females, 7 males) with T1D of at least 1-year duration and HbA1c <9% will undergo three conditions, in a randomized order: 1) high intensity interval training exercise session (HIIT) at 90% peak oxygen consumption (VO_2peak); 2) moderate intensity exercise session (MICT; 65% VO_2peak; or 3) a control session (CON; no exercise) (Figure 2).

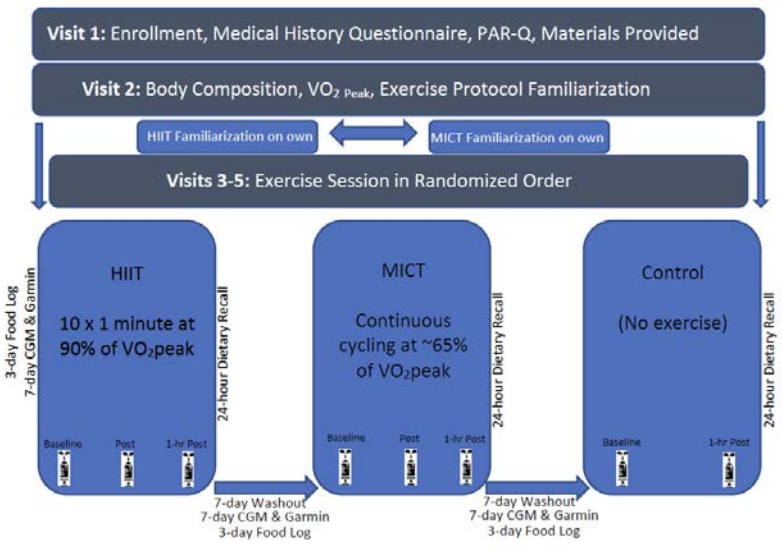

Figure 2. Experimental Design

Subjects will be asked to arrive to the laboratory after an overnight fast (8 hrs) and their usual dose of insulin administered the day prior, with no morning mealtime insulin the day of testing. The lower and higher limit for pre-exercise (i.e. fasted) glucose concentration (i.e. 10 minutes prior to the exercise start time) will be set at 90 and 250 mg/dL, respectively, and the session will be rescheduled if the pre-exercise glucose value is not in range or if hypoglycemia (blood glucose: <70 mg/dL) occurs within an hour of exercise start time. Subjects will also be asked to arrive to testing sessions following a 12 hour fast from caffeine and alcohol and to abstain from physical activity for 24 hours prior to testing. Subjects will complete one electronic contact (phone/email screening) and up to five in-person sessions (enrollment; baseline testing; three conditions [HIIT, MICT, CON]) over the course of 4 weeks

All data collection will be conducted by the Principal Investigator and Research Assistants. All subjects will report to the Applied Physiology Laboratory in Fetzer Hall (Rm 25) for consenting, enrollment, testing, and exercise sessions.

3.2 Primary Study Outcomes

The primary study outcomes include circulating metabolites that will be measured using an untargeted mass spectrometry-based platform with fasting concentrations of up to 1500 endogenous metabolites related to mitochondrial function, fatty acid oxidation, insulin resistance, and glucose regulation. Targeted metabolomics analysis for acylcarnitines and amino acids will be completed; non-targeted analyses will also be performed as an exploratory analysis, in order to promote the identification of unknown metabolites in addition to the known biology and physiology. Responses will be collected prior to, immediately post, and 1 hr post exercise.

3.2a. Secondary Study Outcomes

Counterregulatory hormones (cortisol, lactate, glucagon) will be evaluated at the same time points. Glucose will be continuously monitored for 48 h post exercise. , and glycemic control through 48 hrs after HIIT, compared to MICT versus a no exercise CON**.**

3.2b. Tertiary Study Outcomes

Tertiary out comes will evaluate the influence of biological sex and physiological outcomes (i.e. body composition, lean mass, visceral fat) on the metabolomics profile of these subjects.

3.3 Subject Recruitment and Screening

Fourteen patients (7 females, 7 males) with T1D, for at least one year, will be enrolled for this study. Subjects will be primarily recruited from the UNC Endocrinology and Diabetes clinic and UNC Student Health Center. Posters and brochures will be made available in the waiting areas and exam rooms of the diabetes clinics. Additionally, eligible patients will be invited to participate in this study by mailing them an introductory letter that describes the study. These letters will be followed up with telephone calls to answer questions and encourage participation. Individuals interested in the study will be screened for eligibility criteria via a telephone screen to review a health/medical history questionnaire and physical activity readiness questionnaire (PAR-Q+). Patient history will be obtained from self-report from the participant and from available medical records specific to the participant's diabetes history, current diabetes management, other past, and current medial problems. The most recent HbA1c will be recorded (taken within the previous 3 months). Those who meet eligibility criteria and are interested in participating will meet a study team member to complete informed consent, inclusion criteria, and familiarization of the study protocol. Additionally, physician approval will be requested, either from study physician (Dr. Sue Kirkman, Dr. Adam Willson, or personal physician).

3.4 Inclusion Criteria

- Clinical diagnosis of presumed autoimmune T1D, receiving daily insulin
- Last hemoglobin A1c <9%
- 18-51 years
- Duration of T1D: ≥ 1 year
- Body mass index (BMI) <30 kg/m^2
- Generally healthy, with no conditions that could influence the outcome of the trial, and in the judgement of the investigators is a good candidate for the study, based on a review of health history

3.5 Exclusion Criteria

- Physician diagnosis of active diabetic retinopathy that could be worsened by exercise
- Physician diagnosis of peripheral neuropathy with insensate feet
- Physician diagnosis of autonomic neuropathy
- Medications: beta-blockers, agents that affect hepatic glucose production such as beta adrenergic agonists, xanthine derivatives; any hypoglycemic agent other than insulin.
- Severe hypoglycemic event defined as the individual requiring a third party of hospitalization in the last 6 months
- Diabetic ketoacidosis in the last 6 months

- Has a closed-loop pump and not willing to use manual mode
- Physician diagnosis of cardiovascular disease that would affect exercise tolerance
- Currently doing HIIT
- Severely impaired hearing or speech
- Pregnancy

3.6 Early Withdrawal of Subjects

A participant may be withdrawn from the study if they withdraw their consent, if they violate the stipulations of the study inclusion and exclusion criteria, if they are unable to comply with study pre-visit guidelines, or if consistent glucose excursions (more than 4 times) occur that do not align with requirements of the study (blood glucose <70 mL/dL; >300 mg/dL).

3.7 Method for Assigning Subjects to Treatment Regimens

During the second visit to the laboratory, participants will be randomized into exercise order. Exercise order will be randomly assigned by the PI using random allocation software. The allocation sequence will be generated with equal treatment sequence order.

4 Study Procedures

4.1 Procedures:

Pre-screening: *Phone screening (~45 minutes)*
Prior to the enrollment visit, each subject will be sent an electronic version of the informed consent to review the details of participation. Those interested will complete a phone screening for inclusion/exclusion criteria. If the subject qualifies for the study and is interested in participating, verbal consent will be obtained. Following verbal consent, an electronic link for written consent and for a self-reported medical health history questionnaire will be sent, respectively. Patient history will be obtained from self-report from the participant. Each subject will provide electronic written informed consent and complete the health/medical history questionnaire and a physical activity readiness questionnaire (PAR-Q+). Following written electronic consent, health history will be reviewed by the study team; approval for participation will be obtained from the subject's personal physician/endocrinologist or by the study physician.

Visit 1- Baseline Testing: *(~45 minutes)*
Subjects will be asked to arrive to the laboratory after an overnight fast (8 hr) and their usual dose of insulin administered the day prior, with no morning mealtime insulin the day of testing to control for the influence of food intake on metabolic rate, body composition measures, and metabolic markers. Height and weight will be recorded to obtain body mass index.

Body composition: (~15 minutes) (previously approved in IRB#17-0952, #17-0950, #16-1991, and #16-1397 with no adverse events):
A four compartment (4C) model, as previously validated by our laboratory will be used to estimate fat mass (FM; kg), percent body fat (%fat), and lean mass (LM; kg). Components of this equation include: 1) body volume, derived from a dual-energy x-ray absorptiometry total body scan (DXA); 2) total body water, measured using multi-frequency bioelectrical impedance spectroscopy (BIS); and 3) total body bone mineral density, calculated using total body bone mineral content, measured from the DXA.

a. Dual-energy X-ray Absorptiometry (GE Lunar iDXA, GE Medical Systems Ultrasound & Primary Care Diagnostics, Madison, WI, USA) 10-15 min: A trained DXA technician will perform all scans. The subject will be asked to remove all metal, thick clothing, and heavy plastic which could interfere with the DXA scans. The subjects alphanumerical number, age, ethnicity, height and weight will be entered into the computer prior to the scanning. The subject will be asked to lie down on the DXA table in the supine position. The participant will be centered on the table within the scanning area. The subject's shoulders and hips will be centered, and the hands will be placed by the side of the legs in a prone position. (The DXA methodology planned for use has previously been approved with no adverse events: IRB#18-1025, #18-0648, #17-0950, #16-1960, #16-1397, #15-0543).

b. Bioelectrical Impedance Spectroscopy (InBody570, BioSpace, Seoul, South Korea), subjects will be asked to stand on and grasp hold of metal electrodes. While the subject remains standing, a harmless and unperceivable electrical current is then conducted through the body. Both machines are widely-used commercial devices that are FDA approved and pose no risks. Internal to the device, the BIS utilizes 256 frequencies to estimate TBW. The TBW estimate will then be used to estimate Ms using the equation from Wang et al: Ms = TBW x 0.0129. (previously approved in IRB#13-1664, #17-0952, #17-0950, #16-1991, #16-1397, #16-0529, #15-0543 with no adverse events).

Cardiorespiratory fitness (VO_2peak): (~20 minutes)
Peak oxygen consumption (VO_2peak; $L \cdot min^{-1}$ and $ml \cdot kg^{-1} \cdot min^{-1}$) will be used to set an appropriate individualized training load for the start of the HIIT protocol. During this ramp-based cycle ergometer test, respiratory gases will be analyzed over 15 second intervals using indirect calorimetry (Parvo Medics TrueMax 2400®, Salt Lake City, UT) until subjects reach volitional fatigue. The three highest oxygen consumption values will be averaged and recorded as the VO_2peak. Ventilatory threshold (VT) will be determined as the intersection point of two linear regression lines fitted to the upper and lower portion of the ventilation versus VO_2 curve using manufacturer software (True One 2400® Metabolic Measurement System, Parvo-Medics Inc., Provo UT). Heart rate (HR) will be monitored throughout the test (Polar Electro Inc., Lake Success, NY), with the highest HR achieved during the test recorded as the HR_{max}. Interval intensity for the HIIT intervention will be calculated and set at 90% of the maximum wattage achieved. Intensity for the MICT intervention will be calculated and set at 65% of the maximum wattage achieved. Protocol was previously approved in IRB#16-2996, #16-0829, #14-2824, #14-0062 with no adverse events.

Familiarization and Randomization: (~10 minutes)
Subjects will be familiarized with the cycle ergometer for the HIIT and MICT exercise. For familiarization, participants will practice a few intervals and cycle a few minutes at the individualized intensity for each respective exercise. They will also be asked, and provided with instructions, to practice one session (HIIT or MICT) on their own, under a usual fed state, prior to their visit to the lab. Each practice session will occur with CGM and Garmin physical activity monitoring (described below). The sessions should mimic those of the actual exercise session; 5-10 bouts of HIIT will be recommended; MICT time frame will be based on specific calculations (described below), approximately 15-25 min. This is included to provide the research team insight into potential glucose excursions prior to the lab-based exercise session in a fasted state. Our laboratory team has successfully implemented a similar 'home-based' HIIT intervention in healthy and clinical populations, with no adverse events [IRB: 18-2393 (Endometrial Cancer); 16-0829 (Knee osteoarthritis); 15-1150 (Bone marrow transplant patients); 14-2398 (Anterior Cruciate Ligament Reconstructive patients); 13-3515 (patients with cardiovascular disease risk factors).

Visit 3-5: Randomized Treatment Regimen: *(~90 minutes to 120 minutes)*
At baseline testing, subjects will be randomized to one of three treatment regimens: 1) HIIT; 2) MICT; 3) CON (no exercise). Subjects will repeat the following procedures for visits 3-5, except there will be no exercise session on the CON treatment session. Exercise sessions will occur in the Applied Physiology Laboratory on the UNC campus. **There will be a minimum of seven days between each exercise trial.**

Continuous Glucose Monitoring (CGM):
Participants will be given the option to monitor their interstitial glucose levels via their own CGM or through a Freestyle Libre Pro (Abbot Laboratories), which will be provided to them. In the case that they decide to use their own CGM, the researchers will request data only for the dates the participant is involved in the study. If a participant decided to use the Freestyle Libre Pro, sensor will be inserted subcutaneously 7 days before exercise for a period of up to 7 days post exercise. CGM metrics including percent time-in-range (TIR), percent time in hypoglycemia, and percent time in hyperglycemia will be evaluated during and 48 hrs post exercise.

Dietary Controls:
Participants will be asked to complete a diet log on the three days, with at least one day occurring the 24 hrs prior to the conditions (HIIT, MICT, CON) to obtain data on usual total caloric intake (kcals), grams of carbohydrate (CHO), protein (PRO), and fat (FAT). Participants will be asked to consume a similar meal the evening prior to each exercise session.

In addition to the diet logs, to evaluate the influence of dietary intake on glucose excursions, participants' dietary intake will also be obtained for the 24 hrs after each exercise session via 24-hour dietary recall obtained via interview by trained interviewers utilizing the Nutrient Data System for Research (NDSR) software and the multiple pass interviewing method to ensure the capture of food intake, which can impact glucose regulation. Dietary information will be used to anticipate and reduce the risk of hypo/hyperglycemic events. If necessary, it will be used as a statistical covariate during analysis.

Saliva Collection (females only) (~5 minutes): Estrogen concentrations will be determined using a 2.5 mL saliva sample taken at baseline. Estrogen levels will be determined using an ELISA assay for salivary estrogen. To determine if estradiol needs to be included as a covariate in the statistical procedures, it will be measured at baseline. Participants will be asked to avoid brushing their teeth for 45 minutes prior and undergoing dental work for 48 hours prior to sample collection to avoid blood contamination. Immediately upon arrival and prior to sample collection, subjects will be asked to rinse their mouth with water to remove any residues. All samples will be immediately frozen at -20°C until analysis using established enzymatic assays.

Physical Activity:
Daily levels of physical activity will be measured via a Garmin Vivosmart 4 fitness tracker 7 days before exercise for a period of up to 7 days post exercise. The Garmin Vivosmart 4 fitness tracker utilizes a built in Garmin Elevate™ Heart Rate monitor, altimeter, accelerometer, and Bluetooth® Smart and ANT+® technology to measure heart rate (HR), step count, floors climbed, and minutes of moderate-vigorous physical activity (MVPA). The wearable device automatically syncs to the GarminConnect application. This data will be used as a potential covariate when collating CGM data and to help understand any glucose excursions that occur.

Metabolomics (~15 minutes):
Approximately 8 mL of blood sample (~ 1.5 teaspoons) using an EDTA tube will be taken upon arrival, immediately after exercise, and one-hour post exercise. CON intervention visit will consist of a single blood draw. Circulating metabolites will be measured using an untargeted mass spectrometry-based platform with fasting concentrations of up to 1500 endogenous metabolites related to mitochondrial function, fatty acid oxidation, insulin resistance, and glucose regulation. Analysis will be completed at the Duke Molecular and Physiology Institute Metabolomics Core, directed by Dr. Christopher Newgard. Targeted metabolomics analysis for acylcarnitines and amino acids will be completed; non-targeted analyses will also be performed, in order to promote the identification of unknown metabolites in addition to the known biology and physiology. Counterregulatory hormones (cortisol, lactate, glucagon) and insulin will be analyzed. Samples will be centrifuged immediately upon collection, and aliquots of plasma will be immediately frozen at -80° C for batch analysis. All blood draws will be done in the Applied Physiology Lab by an individual trained in phlebotomy. The current lab group has previously received approval for intravenous blood draws from the antecubital region in several IRB#, to name a few: 16-1991, 15-0543, and 14-0777, with no adverse events reported.

Treatment Regimens: (~20- 30 minutes)

1) HIIT Intervention: A 2 minute self-selected warm-up, followed by 10 alternating sets of one minute of pedaling at a resistance corresponding to 90% of VO_2peak and one-minute in active recovery at 50 watts (W). The work completed during each interval will be calculated by multiplying the power output for each participant at 90%VO_2peak by the duration of the interval (60 s). The work completed during each recovery period will be added by multiplying the power output (50 W) by the duration of the recovery period (60 s). The work from both bouts will be added together and then multiplied by 10 to calculate the total amount of work that each participant will complete in the HIIT trial.
2) MICT Intervention: Subjects will cycle continuously at a workload corresponding to 65% VO_2peak (W) for a pre-calculated length of time (~20-30 minutes), equivalent to the work that will be completed in the HIIT trials.
3) CON Intervention: No exercise will be performed. Subjects will arrive to the lab following the same pre-testing guidelines followed for HIIT and MICT trials.

HIIT and MICT interventions will be matched for energy expenditure. Heart rate and finger stick capillary glucose will be tracked throughout the duration of the exercise interventions. Indirect calorimetry will be used to evaluate caloric expenditure (kcals) and fuel utilization (respiratory exchange ratio) at the first and last three minutes of exercise. Using the same set-up and device as described for the VO_2peak, indirect calorimetry (Parvo Medics TrueMax 2400®, Salt Lake City, UT) will be used to identify carbon dioxide expiration.

4.2 Training on Procedural Intervention: All procedures performed in the present study will be overseen by the PI (Abbie Smith-Ryan), as well as have formalized training and on ground oversight by experts in the area of T1 diabetes. This will include, Dr. Beth Mayer-Davis, Dr. Sue Kirkman, Dr. Adam Willson, Dr. Klara Klein, Dr. Michael Riddell, and Dr. Dessi Zahareiva. Members of the research team will undergo training to be prepared to handle situations in which glucose excursions occur before, during, and after the exercise protocol.

5 Statistical Plan

5.1 Sample Size Determination

The proposed study was powered based on consideration of multiple effect size calculations from a single bout of HIIT vs MICT in young men (F=0.25-0.99)(16) on markers of carbohydrate oxidation, glucose, citric acid, and succinate acid. To achieve statistical power of 0.80, with a conservative correlation of 0.5 between repeated outcomes, the estimated sample size required was N=12. To account for an 18% dropout/disqualification rate and for equal groups we propose to enroll 14 adults with T1D, with an equal sample of men (n=7) and women (n=7).

5.2 Statistical Methods

For aim 1, changes in the first principal component, as well as any additional components for which the associated eigenvalue is greater than one, will be evaluated using paired t-tests at the 2-sided 0.05 level. Changes in individual metabolic intermediates will be evaluated using paired t-tests at the 2-sided 0.01 level. Differences in metabolomics and counterregulatory hormones markers between treatment regimens will be evaluated using two-way ANOVAs [regimen (HIIT vs. MICT vs. CON) × time (base vs. immediate post vs. 1h post]. Exploratory analysis of the modulatory effects of sex on metabolomics markers will be evaluated using separate regimen-by-sex mixed factorial ANOVAs [regimen × sex], at each time point.

For aim 2, CGM data will be evaluated as previously recommended; data will be aggregated over the 24hrs; area under the curve data will be compared across trials.(25) Counter-regulatory hormones will be evaluated using a two-way mixed factorial ANOVA. Activity data will be captured to potentially be used as a covariate when collating CGM data.

For aim 3, spearman correlations will be completed to understand associations between metabolomics outcomes and physiological variables (i.e. body fat, lean mass, visceral fat).

6 Safety and Adverse Events

Risks associated with participating in this study are **possible**. In-test monitoring by the research team, who are certified fitness professionals trained in CPR/AED, are in place to ensure the safety of all subjects. Personnel trained in phlebotomy and with radiation exposure training will complete the blood draws and DXA scans, respectively.

In accordance with the Exercise and Sport Science Institutional Review Board Standard Operating Procedures (EXSS-IRB SOP), 3 trained research personnel (for safety purposes per IRB procedures) will perform cardiorespiratory fitness testing. Other precautions include: licensed medical professional (MD, ATC) on call, operational AED, and a land line within 10 feet of the testing in order to make emergency call, if required. Additionally, the lab is located within 500 feet of the University Athletic Training facility. If a cardiovascular event does occur, 911 will be called; UNC Hospitals and Emergency room are in very close proximity to the lab.

The risk for any exchange of blood borne pathogens is low. All blood samples will be handled by individuals with blood borne pathogen and laboratory safety training. These procedures follow the rules of universal precautions, and all investigators and research assistants have up to date training. Continuous glucose monitoring (CGM) procedure is routinely used in clinical practice. The main risks of CGM are local irritation from the sensor or tape.

Available evidence suggests HIIT provides similar or superior improvements in cardiorespiratory fitness and endothelial function compared to moderate intensity exercise, without a detrimental decline in blood glucose.(18, 19) However, hypo- or hyperglycemic events are a risk. Study personnel will be trained to identify the signs and symptoms of a blood glucose level that is low or high. They will also be trained to check the blood glucose level, using a glucometer. If low blood glucose occurs during a study visit (< 70 mg/dL), study personnel will be trained to administer 15 grams of an oral carbohydrate, and to repeat as needed every 10 minutes until the blood glucose level is > 70 mg/dL. If the blood glucose level is above 300 mg/dL during a study visit, study personnel will be trained to check for urinary ketones. After the exercise session, subjects will be instructed to take their usual dose of insulin or other diabetes medication as prescribed. If participants experience hypoglycemia or hyperglycemia outside of study visits, they will be advised to treat these situations per their usual diabetes care plan.

Potential adverse events will be reviewed at each visit with each subject, and will be recorded within the subject data collection folder. Adverse events in aggregate will be reviewed by the study team and the nature of adverse events that

would trigger reconsideration of the protocol would be highlighted. If it is clearly found that an adverse event is related to supplements, it will be immediately reported to the sponsor and the IRB and the correspondence will be negotiated.

The 2017 DAIDS Therapeutic Research Program's guide for grading AEs/SAEs during clinical trials will be used to evaluate the severity of the event, and will be categorized as: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (Potentially life-threatening), Grade 5 (death).

6.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research,
- Serious (as defined below) "***Serious" is different than "severe" as reported in the CTC criteria that applies a grade to the AE.***

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

Serious adverse events that meet the following criteria will be reported to the IRB:

Unanticipated problems involving risks to subjects or others" (UPIRSO) refers to any incident, experience, or outcome that:

- is unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- is related or possibly related to a subject's participation in the research; and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study procedures follow-up. For this study, the study procedure follow-up is defined as 30 days following the last administration of study procedures.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

6.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning. Information on all adverse events will be recorded immediately in the subject file. All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

6.3 Reporting of Serious Adverse Events and Unanticipated Problems

Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, must be reported to the clinical members of the team (informal DSMB) and the UNC IRB.

6.4 Stopping Rules

If two serious adverse events, related to the study procedures occur the study would be stopped. In addition, if two or more subjects experience the same or similar Grade 3 adverse event or serious adverse event, the study will be stopped. This study will not involve a formal data and safety monitoring board or committee. Adverse events will be reviewed by clinical members of the study team (Kirkman, Willson, Riddell, Mayer-Davis). The PI will remain in contact with all study subjects and keep track of adverse events.

6.5 Data Handling and Record Keeping

Each subject and their associated information will be identified by a 4-digit alpha-numerical identification code. Subjects will be identified by this code only when labeling any data collection sheets or computer printouts. Code lists linking the subject identification number and their name and email address will be viewed by the research team only, and will be stored in a secure filing cabinet in the Applied Physiology Laboratory (APL) with the consent forms. This list will serve as the only link between a subject's name and ID code and will be destroyed (shredded by the principle investigator immediately after the study has been completed). Email addresses and phone numbers will be obtained solely for the purpose of contacting subjects to schedule testing and data collection times. All data will be stored in electronic format on both the data collection computer and the principle investigator's personal computer. Computer access will be protected via confidential passwords, and backup devices will be stored under lock-and-key in the APL.

6.6 Data Management

Data management will be completed using good clinical practices. Specifically, hard copy data sheets/case report forms will be used to capture initial data. This will then be transferred by a member of the university research team to Excel (Microsoft Office). Once in excel the PI and university research team will review the hard copy and electronic file to ensure correct transfer of the data. Data will be screened for mis-entries by evaluating the range, mean, standard deviation, and median for each outcome variable, in addition to visual inspection of frequency plots. In the case of extreme values, the original source of the data will be double-checked to ensure that the information has been accurately collected and recorded. If the inspection of the data source does not reinforce the accuracy of the data, the data point will be omitted. Electronic date obtained from the study will be stored forever and the data of paper material will be stored for five years since the study is completed. After that, paper material will be shredded by the PI. Participants will not be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law etc. requires the disclosure of such records, including personal information. If disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some

cases, subject's information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies (for example, the FDA) for purposes such as quality control or safety.

7 Study Finances

7. 1 Funding Source

This study is funded by a pilot grant through the North Carolina Diabetes Research Center, awarded to Abbie Smith-Ryan, PhD

7.2 Subject Stipends or Payments

Subjects will receive up to $125 for their participation. Compensation will be prorated, with each visit compensated at $31.25. Free parking will also be provided for all testing and training visits.

8 Publication Plan

Plans for publication are in place following successful completion of study data collection. These plans will be decided upon by the PI and collaborators who will discuss how best to present findings.

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