Acute Effects of Aerobic and Resistance Exercise on Maternal Glucose Metabolism and Vascular Function in Obese Pregnancy

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A Introduction

A1 Study Abstract

Obesity before and during pregnancy is associated with a higher risk for a number of obstetric and metabolic complications in women and their offspring. Of particular importance, obese women have a higher risk of developing gestational diabetes and preeclampsia. In addition, obese women have larger offspring who have a higher risk for the development of obesity and diabetes; both largely attributed to higher maternal glycemia and glucose intolerance during pregnancy. Thus, identifying rehabilitative interventions that improve maternal and offspring metabolic and cardiovascular health in obese pregnancy are critical and have immediate and generational impact. Resistance and aerobic exercise training is a clinical staple for improving musculoskeletal, metabolic and cardiovascular health in non-gravid adolescents and adults with obesity however little is known regarding the effects of exercise during obese pregnancy. This study proposes to collect preliminary data on the independent effects of acute aerobic and resistance rehabilitative exercise on glucose metabolism and vascular function during pregnancy in n=15 obese women in order to inform a large, multisite clinical trial examining the acute and chronic effects of aerobic and resistance exercise on glucose metabolism and vascular function in normal weight, overweight and obese women during pregnancy.

Primary Hypothesis

Acute aerobic and resistance rehabilitative exercise will improve post-exercise glucose metabolism and vascular function in obese women during mid-pregnancy.

A2 Purpose of the Study Protocol

The overall goal is to obtain preliminary proof-of-concept data for a larger study examining acute and chronic physiologic and molecular effects of aerobic and resistance exercise on glucose metabolism and vascular function in normal weight, overweight and obese women during pregnancy. We will also track the number of eligible participants including those who consented and declined for sample size estimation for a larger future study.

B Background

B1 Prior Literature and Studies

Obesity is a metabolic disease that is associated with several comorbidities including cardiovascular, musculoskeletal and neurological conditions. Maternal obesity prevalence is at a historic high with over 1 in 3 women entering pregnancy obese and 1 in 10 extremely obese[1]. Obesity before and during pregnancy is associated with a higher risk for a number of obstetric and metabolic complications in women and their offspring[2] including increased rates of preeclampsia, gestational diabetes, fetal growth disorders, stillbirth, preterm birth and cesarean delivery, which are further exacerbated by excessive gestational weight gain that often occurs in overweight and obese women[3]. In addition, physical inactivity in combination with excess maternal adiposity can alter the intrauterine metabolic environment and contribute to adverse fetal programming leading to unfavorable infant outcomes with long-term health implications for the child[4,5]. Thus, identifying rehabilitative interventions in obese women during pregnancy that improve maternal and offspring metabolic and cardiovascular health are critical and have immediate and generational impact.

Obese women have a higher risk of developing gestational diabetes than normal weight women[6] as obese women have lower pre-gravid insulin sensitivity that is exaggerated during pregnancy[7]. Specifically, obese and morbidly obese pregnant women are 3.5x and 8.5x more likely to develop gestational diabetes, respectively[6]. Gestational diabetes mellitus is defined as glucose intolerance with onset or first recognition during pregnancy. In addition to the adverse risks of obesity during pregnancy, obese women who develop gestational diabetes are at an increased risk of adverse perinatal, maternal and neonatal outcomes, recurrence of gestational diabetes in subsequent pregnancies, and the development of type 2 diabetes postpartum[8]. In addition, obese women, especially those who develop gestational diabetes, have larger offspring with greater adiposity; largely attributed to higher maternal glycemia[9] and glucose intolerance[10] during pregnancy. High birth weight can increase the risk for cesarean delivery[11] and shoulder dystocia[12] and these high birth weight infants are at increased risk of developing obesity[13-15] and diabetes[13,14]. Moreover, increased adiposity in infants is significant because similar to adults[16,17] adiposity is likely a more sensitive and specific risk factor for future obesity, insulin resistance, and cardiovascular disease than weight. This notion is supported by the significant positive relationship between neonatal and childhood adiposity[18] and the finding that body fat, not weight, was associated with cardiovascular disease risk factors later in life[19,20]. Together, these data indicate that obese women, with and without gestational diabetes, often give birth to infants who have high birth weight and adiposity that are at risk of developing adult obesity, diabetes, and cardiovascular disease[21,22] and that these risks appear to be primarily mediated by impaired maternal glucose metabolism.

Obese women also have a 2-3x greater risk of developing preeclampsia[23]. A systematic review that included 1.4 million women revealed that the risk of preeclampsia doubles for every six unit increase in pre-pregnancy BMI[24]. Preeclampsia, the most common hypertensive disorder during pregnancy, can be life-threatening for both pregnant women and their fetuses with 10-15% of all maternal deaths related to preeclampsia and eclampsia[25]. Altered vascular function, caused by inflammatory, metabolic and thrombotic responses, characterizes preeclampsia[26]. Maternal preeclampsia may lead to premature cardiovascular disease, chronic hypertension, ischemic heart disease, and

stroke[27]. In addition, offspring of women who had preeclampsia during their pregnancy have an increased risk of stroke, heart disease, and metabolic syndrome in adulthood[28,29]. Currently, there are no effective treatments for preeclampsia except the delivery of the fetus and placenta.

In non-gravid obese adolescents and adults, moderate-to-high intensity rehabilitative exercise improves metabolic and cardiovascular health[30-32]. Specifically, aerobic[33-36] and resistance[37] exercise training independently improves glucose tolerance and insulin sensitivity in obese adults where combination aerobic and resistance exercise training appears to have the greatest benefit on insulin sensitivity[38]. In addition, previous studies in non-gravid normal weight adults have shown that both resistance and aerobic exercise training reduces systolic and diastolic blood pressure[39] and aerobic exercise training improves blood pressure[34,40] and plasma markers of vascular function[36] in overweight/obese non-gravid adults. However, during obese pregnancy, little is known regarding the effects of acute and chronic rehabilitative exercise training on glucose tolerance and vascular function and nothing is known regarding potential differences between modes (i.e. aerobic vs. resistance) of exercise.

Recent guidelines from the American College of Obstetricians and Gynecologists (ACOG) recommends all women with uncomplicated pregnancies engage in aerobic and resistance exercise throughout their pregnancy[41]. These recommendations are based off of studies that have demonstrated that moderate exercise training during pregnancy is safe and effective in improving several maternal and neonatal outcomes in normal weight, overweight, and obese populations[41,42]. In particular, observational studies suggest that exercise in pregnancy in normal weight women reduces the risk of gestational diabetes[43] and the risk of preeclampsia and other hypertensive disorders[44,45]. These findings might extend to overweight and obese pregnant women as physical activity during pregnancy appears to attenuate the increase in blood pressure and the loss of parasympathetic tone associated with obese pregnancy[46]. In addition, higher maternal physical activity during obese pregnancy improves plasma markers of insulin resistance[47,48] and systemic inflammation compared to their inactive obese counterparts[49]. Despite the observed preliminary benefits of rehabilitative exercise during pregnancy in obese women, several questions remain. These include: 1) “What are the acute and chronic effects of maternal exercise on glucose metabolism and vascular function during pregnancy?”, 2) “Are there different effects of aerobic and resistance type exercise on glucose metabolism and vascular function?”, and 3) “What are the physiologic and molecular transducers of maternal exercise for changes in maternal glucose metabolism and vascular function during pregnancy?” This pilot project aims to collect preliminary data on these questions. In the planned subsequent clinical trial(s), we hope to answer the following additional questions: Are physiologic and molecular responses to AE/RE similar or different between a) gravid and non-gravid women, b) lean and overweight/obese women, and c) different stages of pregnancy? Data from this pilot trial will be used to begin a line of research inquiry aimed to elucidate the optimal mode, intensity, duration (i.e. dose), and timing (i.e. stage of pregnancy) as well as the molecular transducers of rehabilitative exercise training in overweight and obese pregnancy. These data are crucial for an optimal, individualized, and precise exercise prescription in overweight/obese women for the treatment and prevention of maternal and offspring metabolic and cardiovascular disease.

B2 Rationale for this Study

This proposal would be the first study aimed to collect preliminary data on the independent effects of aerobic and resistance rehabilitative exercise in pregnancy, and further, in obese women; a population with high morbidity during gestation. In addition, this proposal would inform a large, multisite clinical trial examining the acute and chronic effects of aerobic and resistance exercise on glucose metabolism and vascular function in obese women during pregnancy. Moreover, this proposal would provide initial evidence of molecular transducers of acute physical activity/rehabilitative exercise necessary for a large, comprehensive clinical trial examining molecular transducers of rehabilitative exercise in normal weight, overweight and obese women during all stages of pregnancy. Physiologic and molecular mediators of physical activity is an area of high clinical and scientific importance, evidenced by a dedicated $170 million NIH funding for the creation of a comprehensive map of the molecular signals that transmit the health effects of physical activity in healthy, non-gravid humans (MoTrPAC trial, https://www.motrpac.org/). Our pilot proposal and subsequent planned clinical trial study design aims to mirror the MoTrPAC trial study design, thereby exponentially increasing the impact of the resulting data through the comparison of the physiologic and molecular effects of aerobic and resistance exercise in gravid and non-gravid humans. Ultimately, these pilot data would set the stage for a multisite clinical trial examining the maternal and offspring physiologic effects of and molecular transducers for rehabilitative aerobic and resistance exercise training in overweight and obese women during pregnancy.

C Study Objectives

C1 Primary Aims

- To characterize the acute effects of aerobic and resistance exercise on glucose metabolism (tolerance, insulin sensitivity and β-cell function) in obese women during mid-pregnancy.
- To characterize the acute effects of aerobic and resistance exercise on vascular function in obese women during mid-pregnancy.

C2 Secondary Aim

- To explore the molecular transducers of physiologic responses in glucose metabolism and vascular function following acute aerobic and resistance exercise in obese women during mid-pregnancy.

C3 Rationale for the Selection of Outcome Measures

The primary outcome variables for this pilot trial are the Matsuda Whole-Body Composite Insulin Sensitivity Index (ISI) following an oral glucose tolerance test (OGTT), and the digital pulse volume response to arterial occlusion (blood pressure cuff) measured by reactive hyperemia peripheral tonometry (EndoPAT 2000, Itamar Medical, Caesarea, Israel). ISI= 10 000/$\sqrt{}$ ($glucose_{fasting}$ × $insulin_{fasting}$) ($glucose_{mean}$ × $insulin_{mean}$) is strongly associated with the glucose-clamp procedure which is considered the gold standard for measuring insulin sensitivity[50,51]. The EndoPAT 2000 has good reproducibility (ICC=0.74) and has been validated as a measure of endothelial function

with an 82% sensitivity and 77% specificity to diagnose coronary endothelial dysfunction[52].

C4 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below:

1. Registering MD's name
2. Patient's race, BMI and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

C5 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

Study Design

C6 Overview or Design Summary

The overall study design is a quasi-randomized, observational, 3-condition comparison of the acute effects of rest, resistance exercise or aerobic exercise on glucose metabolism and vascular function in obese women (n=15) during mid pregnancy.

C7 Subject Selection and Withdrawal

7.a Inclusion Criteria

1. First trimester BMI ≥ 30.0 and <45.0 kg/m^2 (calculated from clinical weight and height)
2. Singleton gestation, between 23 weeks and 0/7 days and 28 weeks and 0/7 days
3. Normal fetal anatomy (no major structural abnormalities identified on standard of care survey before enrollment)
4. Established prenatal care at Women's Health Clinic before 18 weeks of gestation, plans to deliver at Barnes-Jewish Hospital
5. Permision from Obstetrics physician provider to participate in study.

7.b Exclusion Criteria

1. Gestational or pre-gestational diabetes diagnosis
2. Inability to provide voluntary consent
3. Currently using illegal drugs (e.g., cocaine, methamphetamine, opiates) (safety risk and potential confounding)
4. Current smoker who does not agree to stop (confounding)
5. Participation in routine (>1x/week) exercise program (may improve glucose metabolism/vascular function)
6. History of heart disease, orthopedic, metabolic or neurological condition that would contraindicate exercise (safety risk)

Inclusion of Women and Minorities

Women and members of all races and ethnic groups are eligible for this trial. Men are not eligible.

7.c Subject Recruitment Plans and Consent Process

Participants will be recruited from the Center of Women's Health at Washington University School of Medicine/Barnes Jewish Hospital. All obese (BMI ≥ 30) patients with on-going pregnancies will be approached for study participation at the first visit in the the trained clinical research coordinator. Patients will be approached through direct personal recruitment through the clinic and consented.

7.d Early Withdrawal of Subjects

Research participation will occur on three separate visits. Participants will be withdrawn if they are unable to complete the procedures during any of the research visits.

7.e Data Collection and Follow-up for Withdrawn Subjects

There will be no follow up of withdrawn participants.

D Study Procedures

D1 Screening for Eligibility

Pregnant women with obesity: Eligibility criteria of patients attending the Center of Women's Health at Washington University School of Medicine/Barnes Jewish Hospital will be reviewed by Dr. Cahill and by the research coordinator.

D2 Visit 1

These procedures will be performed on over 3 visits separated by ≥ 4 days. Participants will be studied within gestation weeks 23-30. The evening before the study visits, participants will consume a standardized meal consisting of 55% carbohydrate, 30% fat, and 15% protein provided by the WUSM Bio-Nutrition Facility. The following morning, fasted participants (10 hrs) will be admitted to the Clinical Translational Research Unit of the Institute of Clinical and Translational Sciences at Washington University School of Medicine (WUSM CTRU) at 8:00 am. Maternal weight (calibrated scale) and height (stadiometer) will be measured.

Body Composition Measurement: Whole-body fat will be quantified using through skin-fold anthropometry (Harpenden, West Sussex, UK).

Assessment of Laboratory Clinical Variables: Venous blood will be collected from the participants to assess 1) CBC, 2) HbA1C, 3) complete metabolic panel, and blood hormone/metabolites including plasma glucose, insulin, c-peptide, free fatty acid, placental growth factor and vascular endothelial growth factor (VEGF).

The participant will quietly lie in bed for 45 minutes.

Immediately following the 45-minute rest period:

Assessment of Laboratory Clinical Variables: Venous blood will be collected from the participants to assess 1) CBC, 2) HbA1C, 3) complete metabolic panel, and blood hormone/metabolites including plasma glucose, insulin, c-peptide, free fatty acid, placental growth factor and vascular endothelial growth factor (VEGF).

Assessment of Vascular Function

Endothelial Function: Reactive hyperemia peripheral tonometry (RHPT, EndoPAT2000, Itamar Medical, Caesarea, Israel) will be measured before, and immediately post-exercise to assess digital pulse volume responses to arterial occlusion (blood pressure cuff). Briefly, the participant is placed in a sitting position and two disposable, inflatable probes are placed on the index fingers. Following 10 min of baseline signal recording, a blood pressure cuff is inflated for 3-min and following release, the hyperemic response is recorded for 5-min. The EndoPAT 2000 has good reproducibility (ICC=0.74) and has been validated as a measure of endothelial function with an 82% sensitivity and 77% specificity to diagnose coronary endothelial dysfunction[52].

Large Artery Function: *Central aortic blood pressure and pressure waveforms.* Pressure waves will be recorded sequentially from different sites, and transit time calculated using registration with a simultaneously recorded ECG by use of the SphygmoCor™ system (AtCor, Sydney, Australia), which has been well-validated and is approved by the FDA for the noninvasive assessment of central blood pressures and central aortic compliance by measurement of pulse wave velocity (PWV) and augmentation index (AIx). In this system, a single high-fidelity applanation tonometer (Millar) is used to obtain a proximal (i.e. carotid artery) and distal pulse (i.e. radial or femoral) recorded sequentially a short time apart; from this, the PWV is calculated from the transit time between the two arterial sites, determined in relation to the R-wave of the ECG. The time between the ECG and the proximal pulse is subtracted from the time between ECG and distal pulse to obtain the pulse transit time. In the case of stiff arteries, PWV rises and the reflected wave arrives back at the central arteries earlier, adding to the forward wave and augmenting the systolic pressure. This phenomenon can be quantified through the augmentation index (AIx), which is defined as the difference between the second and first systolic peaks (P2-P1) expressed as a percentage of the pulse pressure

Central hemodynamics and aortic compliance will be characterized using the SphygmoCor™ system. Pressure waves will be recorded sequentially from different sites using the SphygmoCor™ system (AtCor) using methods described by our group.[53] Derived indices include the central blood pressures and central aortic compliance characterized as the augmentation index (AIx) and pulse-wave velocity (PWV).

At 60-minutes following the end of the rest period:

Assessment of Glucose Metabolism

Two-hour oral glucose tolerance test (OGTT): At 60-min following the end of the rest period, a 2-hr oral OGTT will be conducted. Venous samples for glucose, insulin, and C-peptide will be drawn at time 0 and then 30, 60, 90 and 120 minutes after starting

ingestion of glucola (1.75 g/kg of body weight to a maximum of 75 g). Insulin sensitivity and insulin secretion will be estimated by the Matsuda Whole-Body Composite Insulin Sensitivity Index (ISI), HOMA-IR, Insulinogenic Index and C-peptide Index. ISI= 10 000/√ ($glucose_{fasting}$ × $insulin_{fasting}$) ($glucose_{mean}$ × $insulin_{mean}$), HOMA-IR ($Insulin_{t0',\ mU/L}$×$Glucose_{t0',\ mmol/L}$) /22.5), Insulinogenic Index ($\Delta I_{30}/\Delta G_{30}$) and C-peptide index ($\Delta$C-$peptide_{30}/\Delta G_{30}$) will be calculated as previously described[54-56]. Area under the curve for glucose, C-peptide and insulin levels will also be calculated by trapezoidal integration over the entire OGTT for glucose tolerance assessment.
Lunch will be provided.

At 30-minutes following lunch:

Peak Exercise Testing (VO_{2peak})
Participants will perform a graded exercise test on a recumbent cycle ergometer located on the WUSM CTRU. Continuous ECG/BP and oxygen consumption (VO_2) will be recorded during the graded exercise test to determine peak exercise capacity (VO_{2peak}). The graded exercise test protocol consists of 1-min incremental stages, beginning with a 20-watt workload, progressing every minute by 10-20-watts until volitional exhaustion or symptom limitation. Exercise tests will be considered maximal if peak heart rate ≥ 85% predicted maximum (220-age) and/or respiratory exchange ratio ≥ 1.1 according to the American College of Sports Medicine[57]. Peak exercise testing will be supervised by a licensed physical therapist and the CTRU nursing staff.

Ten-Repetition Maximum Strength testing (10RM)
10RM will be determined on 4-upper body and 4-lower body exercises on Universal exercise machines (Body Master®) located on the WUSM CTRU. 10-RM is the maximum amount of weight a participant can lift 10 times using proper form and only primary muscle groups required for the lifting motion. 10RM will be determined on each exercise device by trial and error.

D3 Visit 2

The evening before the study visit #2, participants will consume a standardized meal consisting of 55% carbohydrate, 30% fat, and 15% protein provided by the WUSM Bio-Nutrition Facility. The following morning, fasted participants (10 hrs) will be admitted to the WUSM CTRU) at 7:00 am. Maternal weight (calibrated scale) and height (stadiometer) will be measured.

Acute Aerobic Exercise or Resistance Exercise Session
Visit # 2 will consist of either: A) a 45-minute aerobic exercise (AE) session on a recumbent cycle ergometer at 70% VO_{2peak}, or B): a 45-minute resistance exercise (RE) session. The RE session will consist of 3 sets of 8-10 repetitions at their 10RM load according to the Adult NIH Molecular Transducers of Physical Activity in Humans (MoTrPAC) Study Protocol: https://www.motrpac.org/protocols.cfm. The acute RE session will be individualized, based on the measurements taken from a 10RM (measured without Valsalva maneuver) tests during Visit #1 for 1) knee extension, 2) knee flexion, 3) leg press (i.e. hip and knee extension), 4) ankle plantar flexion, 5) chest press, 6) seated row, 7) bicep curl (elbow flexion), and 8) overhead press. For each exercise, 3 sets of 8-10 repetitions will be performed with a 2-minute rest in between

sets. A 3-second contraction duration for the concentric and a 3-second contraction duration for the eccentric phase of the lift will be targeted. Fasting blood samples will be taken at pre-AE/RE/rest, at 20-min during AE/RE, immediately post-AE/RE/rest, and 60-min post AE/RE/rest as per MoTrPAC protocol. All visits with be separated by 1 week and rest, AE and RE visit order will be randomized through a computerized randomization program. The study scheme is presented in the Figure.

Assessment of Glucose Metabolism
At 60-min following the completion of AE/RE, a 2-hr oral OGTT will be conducted as described in Visit 1.

Assessment of Vascular Function
Endothelial and large artery function testing will be performed as described in Visit 1.

D3 Visit 3

The evening before the study visit #3, participants will consume a standardized meal consisting of 55% carbohydrate, 30% fat, and 15% protein provided by the WUSM Bio-Nutrition Facility. The following morning, fasted participants (10 hrs) will be admitted to the WUSM CTRU at 7:00 am. Maternal weight (calibrated scale) and height (stadiometer) will be measured.

Acute Aerobic Exercise or Resistance Exercise Session
Visit # 3 will consist of either: A) a 40-minute aerobic exercise (AE) session on a recumbent cycle ergometer at 70% VO_{2peak}, or B): a 40-minute resistance exercise (RE) session as decribed above.

Assessment of Glucose Metabolism
At 60-min following the completion of AE/RE, a 2-hr oral OGTT will be conducted as described in Visit 1.

Assessment of Vascular Function
Endothelial and large artery function testing will be performed as described in Visit 1.

Plasma Hormone and Metabolites
Before, during and immediately following the rest, AE and RE sessions and during the OGTT, maternal blood will be collected for analyses of plasma glucose insulin, C-peptide, free fatty acid, vascular endothelial growth factor (VEGF), and placental growth factor. Additional blood will be collected for future exploratory analyses (e.g. metabolomic, proteomic, transcriptomic). Plasma glucose will be analyzed by an YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH) located on the WUSM CTRU. Plasma hormone and analyte analyses will be performed by the University of Alabama at Birmingham (UAB) Diabetes Research Center Human Physiology Core Laboratory (see letter of support from Dr. Barbara Gower, Director of Core Laboratory).

MicroRNA Expression

Before, immediately following, and 60-minutes following the rest, AE and RE sessions, maternal blood (6 ml) will be collected for analyses of microRNA (miRNA) expression of peripheral blood mononuclear cells. MicroRNAs are small noncoding ~19–24 nucleotide-(nt-) long RNA molecules that play an important role in the modulation of gene expression. Analysis will be performed using a Affymetrix Gene Chip and will be peformed by the Washington University Gene Technology Access Center.

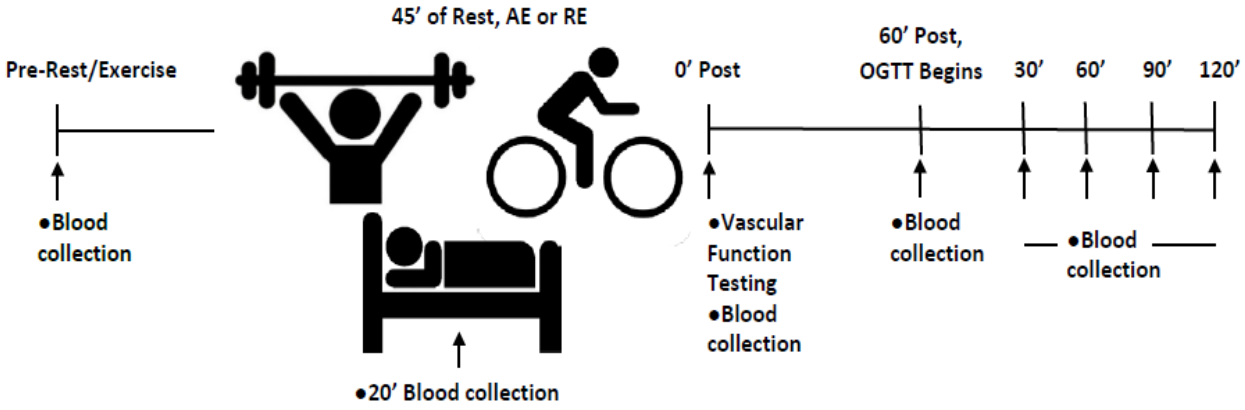

Figure: Acute Rest, AE or RE Session Schematic. AE: aerobic exercise, RE: resistance exercise, OGTT: oral glucose tolerance test.

E Safety and Adverse Events

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below.

The Washington University Human Research Protection Office (HRPO requires all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section G2.

E1 Definitions

1.a Adverse Events

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease. For purposes of this research project, AEs associated with peak exercise testing, 1-repetition maximum testing, aerobic or resistance exercise sessions, blood draw/OGTT, and vascular function testing will be tracked.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

1.b Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

1.c Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

1.d Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

1.e Unanticipated Problems

Definition:

- 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;
- related or possibly related to participation in the research ("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); 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.

1.f Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

1.g Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

1.h Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

E2 Reporting to the Human Research Protection Office (HRPO) at Washington University

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

E3 Timeframe for Reporting Required Events

Adverse events will be tracked for 24 hours after date of participation. The event need not be causally related to 31P-MRS, blood draw, and echocardiogram to qualify as an adverse event to be collected.

F Statistical Plan

F1 Sample Size Determination and Power

The primary objective of this pilot grant is to obtain preliminary data necessary for proof-of-concept and for sample size estimation for a large, multisite clinical trial. However, we anticipate having enough power to demonstrate independent acute effects of AE and RE on glucose metabolism and vascular function necessary for potential publication of these pilot data. Using data from non-gravid adults with obesity, Newsom et al.[58] demonstrated an increase in next day insulin sensitivity following a low-intensity AE session. Rynders et al.[59] found lower 2-hr OGTT glucose value following acute AE in prediabetic non-gravid adults. Further, Breen et al.[60] demonstrated a 17% improvement in glucose AUC following acute RE in normoglycemic non-gravid adults. Using these data, a sample size of n=14 would be adequate to see a statistical difference with either acute AE or RE on glucose metabolism. For vascular function, Haynes et al.[61] demonstrated increased (~4-12%) reactive hyperemic responses following acute RE and AE in older men. Also, Kingsley and Figueroa[62] found increased reactive hyperemic responses following acute RE in 24 obese older women. These data suggest that a sample size of n=11 will be necessary to see statistically significant differences in vascular function pre/post acute AE or RE, assuming similar responses.

F2 Statistical Methods

We plan to assess differences in glucose metabolism and vascular function between the three different conditions (i.e. acute, AE, RE) using a repeated measures ANOVA with post-hoc testing. We anticipate detecting differences between AE/RE and rest but likely will not have the power to detect potential differences between AE and RE. The two primary outcomes will be the Matsuda ISI and endothelial hyperemic response (digital pulse volume). Other glucose metabolism and plasma analyte variables will be secondary outcomes.

G Study Monitoring, Auditing, and Inspecting

During monthly group meetings, the research team (Dr.'s Cade, Cahill) and the research coordinator will monitor and review: (a) Recruitment, screening and enrollment progress, goals and strategies; (b) Preliminary findings that might affect the risk/benefit ratio or alter the procedures described in the protocol; (c) Any out of range laboratory value and a course of action; (d) Any adverse or serious adverse events that might result from the research procedures. In monthly meetings, Drs. Cade and Cahill will monitor and review: (a) research-related risks that might alter the risk/benefit ratio, (b) any laboratory or blood pressure findings that are out of range and require follow-up with the participant, (c) any new information/findings about obese pregnancy that might facilitate this study, reduce risk or the burden of testing for participants. All serious adverse events will be reported to the WUSM Human Research Protection Office (HRPO), NIH and the Research Subject Advocate on the WUSM Clinical Research Unit (CRU) within 15 days of the event. There are standard reporting forms for the WUSM CRU and the HRPO. Should there be a serious adverse event that increases the risks to participants, the study will be stopped, an investigation conducted, and findings generated before the study is resumed. These findings are shared with the HRPO, NIH and the Research Subject Advocate. If necessary, the research procedures are modified and appropriate revisions to the consent document and consent procedures are made. Each individual adverse event is reported to the WUSM HRPO which has the option of stopping or discontinuing this project if they deem that the risk/benefit ratio becomes too high. The HRPO may rule to continue the study once the research procedures are modified and appropriate revisions to the consent document and consent procedures are made. This Data Safety Monitor Committee plan is in compliance with and will be approved by The Research Advisory Committee of the CRU.

H Study Administration

H1 Organization and Participating Centers

Washington University is the only participating site.

H2 Funding Source and Conflicts of Interest

Departmental funding. No conflicts of interest.

H3 Subject Stipends or Payments

Participants will receive a total of $150 for completion of the study.

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