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Novel Approach to Assess Metabolic Flexibility in a Respiratory Chamber "METFLEX"

PROTOCOL

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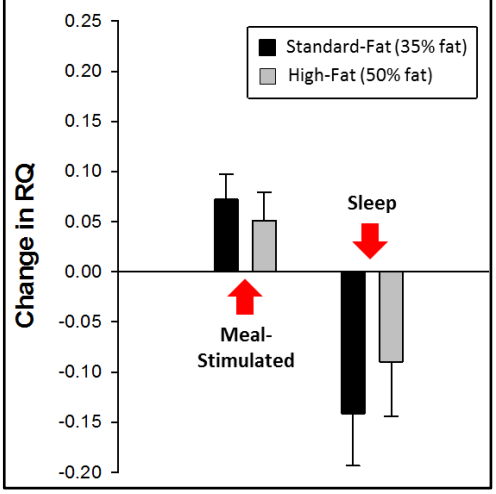
1. BACKGROUND AND SIGNIFICANCE

Metabolic flexibility is the ability of an organism—as a whole, or at the organ, tissue, or cellular level—to adjust fuel oxidation to fuel availability. It was originally described as "the capacity to switch from lipid oxidation during fasting conditions to the suppression of lipid oxidation and subsequent increase glucose uptake, oxidation, and storage under insulin-stimulated conditions" [**1**]. During the non-physiological, highly-controlled conditions of a hyperinsulinemic-euglycemic clamp, individuals with insulin resistance and type 2 diabetes are less metabolically flexible to carbohydrate (CHO), and therefore, have an impaired capacity to increase muscle and whole-body glucose oxidation and storage that is reflected by a blunted increase in respiratory quotient (RQ). However, Galgani et al. pointed out that such impairment in oxidative flexibility was mostly related to the decreased transport of glucose to muscle and liver cells (due to insulin resistance) rather than a true cellular oxidative defect [**2**].

To understand how intramyocellular lipids accumulate and cause insulin resistance, the assessment of metabolic flexibility to a high-fat diet is more relevant than during a hyperinsulinemic clamp. Impaired capacity to upregulate muscle and liver lipid oxidation in face of high-fat availability will lead to increased muscle/hepatic fat accumulation and thus to insulin resistance. Surprisingly, very few studies have investigated the macronutrient oxidized in response to high-fat diets (either single meal or prolonged change in macronutrients composition) [**3-7**]. Understanding the impact of different meal compositions—specifically, high-fat vs. low-fat—on fuel oxidation is important and could lend itself to future dietary prescriptions based on physiologic responses. However, no studies have examined the physiological metabolic flexibility that occurs in response to a single dinner meal and the subsequent drop in RQ that occurs during sleep until the morning awakening.

Using preliminary data that assessed individual adaptation to a high-fat diet in a respiratory chamber [the ADAPT study; **3**], we were able to quantify metabolic flexibility during an overnight stay in 8 subjects following both a standard-fat and high-fat meal (*see **Figure 1***). While both meal-stimulated and sleep changes in RQ were observed, the study was not designed to adequately assess these changes in a very controlled manner since there was no instructions regarding posture and spontaneous physical activity. Therefore, in a more controlled protocol specifically designed to assess metabolic flexibility (measure of baseline and post-meal energy metabolism in sitting position with the least possible motion), ***we will characterize changes in RQ in healthy, young men and women during an overnight stay in a respiratory chamber following a low-fat (high-CHO) or high-fat meal using a randomized, crossover study design***. The study will establish a novel paradigm to investigate true physiological metabolic flexibility in response to meals of different

Fig 1. Metabolic Flexibility in a Respiratory Chamber



macronutrient compositions. The switch from fat to CHO oxidation in response to a standardized meal, as well as the rapidity at which the body starts oxidizing fat during times of fasting (sleep) will be examined. The proposed study will provide preliminary data for future studies measuring metabolic flexibility in individuals with or without obesity/insulin resistance, and pioneer the development of novel physiological approaches of metabolic flexibility assessment and its implications in the development of metabolic diseases.

2. STUDY AIM:

The overarching aim of this study: To determine the effect of different meal compositions (high- vs. low-fat) on metabolic flexibility as it relates to meal-stimulated (ΔRQ_1) and sleep (ΔRQ_2) metabolic flexibility, as well as the time course changes in RQ (i.e., peak RQ, time to peak RQ, nadir RQ, time to nadir, slope, area under the curve).

Hypotheses:

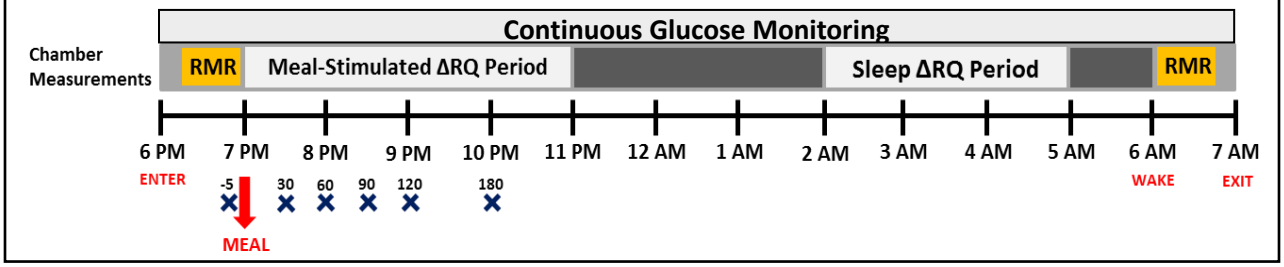
Hypothesis 1: Healthy adults will have greater changes in RQ following a low-fat (high-CHO) meal compared to a high-fat meal.

Hypothesis 2: Healthy adults will have a faster (shorter) time to peak RQ and time to sleep metabolic flexibility following a low-fat (high-CHO) meal compared to a high-fat meal.

3. RESEARCH DESIGN

This is a randomized, crossover study, 8 healthy individuals will complete two overnight stays in a respiratory chamber: once while eating a low-fat (high-CHO) meal (20% fat) and once while eating a high-fat meal (60% fat). Primary endpoints include 2 separate measures of metabolic flexibility—the 4-h meal-stimulated RQ and the subsequent drop in RQ during sleep. Secondary endpoints include time course changes in RQ.

Figure 2. Schedule of Metabolic Chamber Measurements



4. STUDY POPULATION

4.1 Participants

We will enroll up to 10 healthy men or women (goal n=8 completers; 4 male, 4 female). If eligible and enrolled, all individuals will undergo all testing procedures.

4.2 Inclusion Criteria

Eligibility criteria include:

- Healthy male or female

- Ages 18-40 y
- BMI between 20 kg/m^2 and 30 kg/m^2 (±0.5 kg/m^2 will be accepted)
- Are willing to consume pre-prepared meals
- Are willing to maintain current physical activity, sleep schedule, and dietary habits during the study.
- Medically cleared for participation in the study by Medical Investigator

4.3 Exclusion Criteria

Participants are ineligible to participate (or will be excluded from participating in this study) if they meet any of the following criteria:

- Unstable weight in the last 3 months [gain or loss >7 lb (or 3.2 kg)]
- Currently working shift work
- Smoking or use of tobacco products within the last 3 months
- Ammenorrhea (or absence of regular monthly cycles)
- History of clinically diagnosed diabetes or a fasting blood glucose >126 mg/dL
- Average screening blood pressure >140/90 mmHg
- Previous bariatric surgery (or other surgeries) for obesity or weight loss
- Use of medications affecting metabolism or sleep
- History of neurological disease
- History of cardiovascular disease (or other chronic diseases)
- Pregnant, planning to become pregnant, or breastfeeding
- Adherence to special restrained diets (e.g., low-CHO, low-fat, or vegetarian/vegan diets) over the last 3 months.

5. STUDY DESCRIPTION

5.1 Recruitment

Potential participants will be recruited through PBRC via IRB approved recruitment materials (e.g., landing page, listserv, social media). Individuals can either complete the webscreening form directly from the PBRC landing page, call PBRC directly, or e-mail the Recruitment Core. Potentially eligible participants will then undergo a phone screen to answer a series of yes or no questions regarding their age, gender, body weight and height, menopause symptom experience, presence of disease, and smoking status. Eligible individuals will be scheduled for a screening visit at PBRC.

5.2 Location

All clinic visits will occur at Pennington Biomedical Research Center (PBRC).

5.3 Consent Process

The informed consent process will be conducted at PBRC screening visits and will be conducted primarily by the study coordinator, but also on occasion by the study PI (Dr. David McDougal) or by a trained clinic staff. Written consent will be obtained before any procedures are performed. Potential subjects will be given ample time to read the Informed Consent and allowed to ask questions about the study.

5.4 Screening Process

Interested participants will respond to targeted recruitment materials for initial screening by study staff via web, phone and/or email. Following determination of initial eligibility (e.g., age, weight, and height), a fasting screening visit (SV1) will occur at PBRC.

Participants will provide informed consent prior to the initiation of study procedures. Height, weight, and vital signs will be measured. Medical history will be reviewed for exclusionary criteria and a screening health questionnaire will be administered. In addition, participants will have blood drawn for measurement of general health (Chem15 including lipid panel, and CBC). Current (or recent) medication usage will be recorded. If inclusion/exclusion criteria are satisfied, eligible participants will be notified by phone and a future study visit will be scheduled (Visit 1).

6. ASSESSMENT SCHEDULE AND PROCEDURES

An overview of all clinical assessments is presented in **Table 1**. Following confirmation of eligibility via a designated screening visit, three additional study visits including two overnight visits will be administered. Women will be scheduled for the two overnight visits during the follicular phase of their menstrual cycle (based on self-report of the date of their last period or oral contraceptive pack schedule).

Table 1. Schedule of Clinic Assessments

	Screening Visit (SV)	**Visit 1**	**5-14 DAY Interval**	**Chamber 1 (Visit 2)**	**5-7 DAY Interval‡**	**Chamber 2 (Visit 3)**
Visit Type	*Outpatient*	*Outpatient*		*Inpatient (Overnight)*		*Inpatient (Overnight)*
Informed Consent	X					
Randomization *(post-screening)*				X		
Prior/Concomitant Medications	X	X		X		X
Height*, Weight, BMI	X	X		X		X
Vital Signs *(blood pressure, heart rate)*	X					
Fasting Blood Draw *(glucose, lipids, CBC)*	X					
Screening Health Questionnaire	X					
iDXA		X				
Run-In Meals (36-h prior to Visit 2 and 3)			X		X	
12-h Respiratory Chamber				X		X
Meal *(i.e., low-fat, high-fat)*				X *(E)*		X *(E)*
Blood draws†				X (E)		X (E)
Continuous glucose monitoring				X *(E)*		X *(E)*
Urine Collection				X (E)		X (E)

E=evening;

**Height will only be measured during the Screening Visit*

†During both Visit 2 and 3, blood samples will be collected at -5, 30, 60, 90, 120, and 180 mins during the meal (i.e., 6 blood draws total).

‡For female participants the interval between the two chamber visits will be variable to accommodate scheduling both visits during the follicular phase of their menstrual cycle.

7. STUDY VISITS

The study visits are described in this section, while the study procedures that will be performed during these visits are detailed in the latter section "Study Procedures" *(see Section 8)*. There will be 1 screening visit, 1 outpatient visit, and 2 inpatient overnight visits. Interested participants will be asked to abstain from alcohol consumption 24 hours before, and caffeine consumption 12 hours before their Screening Visit. The Screening Visit and Visit 1 will be fasting visits, while Visits 2 and 3 will require participants to only eat pre-prepared meals on the day before and day of the visits and to stop all exercise 24 hours before the visits.

Screening Visit (SV; Outpatient): Participants will undergo consenting and a fasting blood draw to assess glucose, lipid panel, and CBC. Vital signs (blood pressure, heart rate) will be measured.

Visit 1 (Outpatient): Body composition (iDXA) will be measured. Participants will be scheduled for this visit 5-7 days prior to Visit 2.

Visit 2 and 3 (Inpatient): Each participant will complete two inpatient stays in a respiratory chamber while consuming one meal (dinner). The two visits will only differ on the macronutrient composition of the dinner provided:

- Low-fat (high-CHO) meal – 20% fat, 60% CHO and 20% protein
- High-fat meal – 60% fat, 20% CHO and 20% protein

The designated test dinner will be prepared by the metabolic kitchen and represent 40% of the daily energy requirement calculated on the basis of doubly-labeled water equations developed in a similar population [**8**]. Participants will be randomized to receive either the high-fat or low-fat meal during Visit 2. The participants will then receive the other meal (high-fat or low-fat) during Visit 3. A stratified randomization will be used to insure an equal number of males and females receive the high-fat and low-fat meals during Visits 2 and 3.

Visit 2 and 3 will be separated by a 7-day interval in men and a 5 to 7-day interval in women to accommodate scheduling during the follicular phase of their menstrual cycle.

Participants will be provided run-in meals for 36-h prior to each inpatient stay (i.e., breakfast, lunch, and dinner the day before testing, as well as breakfast and lunch the day of the testing) and to drink only water. Participants will be instructed to eat the provided lunch by 1pm on the day of the visit. Participants will instructed to pick up their meals up to 2 days prior to the run-in period (4 days before the study visit).

Participants will be scheduled to arrive at approximately 4:30pm the day of the visits. Approximately one hours prior to entering the metabolic chamber, participant's will outfitted with a continuous glucose monitoring system (CGM).and an intravenous catheter will be placed in an antecubital vein for blood collection. The participant will enter the chamber at

approximately 5:30 pm and consume the meal at approximately 7pm. A total of six IV blood draws will be made (-5, 30, 60, 90, 120, and 180 mins relative to start of meal) to measure insulin, glucose, and free fatty acid levels (see **Figure 2**). All blood collections (either IV or finger stick) which occur while the participant is in the chamber will be accomplished by having the participant either extend their arm through one of the access ports on the side of the chamber or by having the IPU staff reach into the chamber through the access ports (*See **Figure 3***). A rubber gasket in the port prevents excessive loss of air from the chamber.

Figure 3- Chamber access ports.

8. STUDY PROCEDURES

8.1 Questionnaires

Participants will complete a Screening Health Questionnaire (to assess general health) at Visit 1.

8.2 Blood Draw

A fasting blood sample will be collected at the Screening Visit (SV) to assess overall health (Chem15 including lipid panel, and CBC). These measurement will require approximately **7.5 ml** of blood to be drawn.

8.3 Dual-Energy X-ray Absorptiometry (DXA)

The DXA scans will be performed at the Imaging Facility of PBRC at Visit 1. Total adiposity and regional fat mass will be assessed with DXA using a whole-body scanner (Lunar iDXA; General Electric, Milwaukee, WI). The DXA protocol requires that subjects lie on a table wearing a hospital gown and with no metal objects on them while both legs will be placed together using two Velcro straps. The scanner emitting low energy X-rays, and a detector passes along the body. The scan takes ~10 minutes and the radiation dose is less than 1 mrem, equal to about 12-h of background radiation. The scans will be analyzed with the latest software. The software version is enCORE 13.4. We will run quality control scans on a daily basis, and GE has indicated that accuracy of the data is confirmed with these daily QC scans. A pregnancy test (urine) will be given before DXA scans to confirm absence of pregnancy.

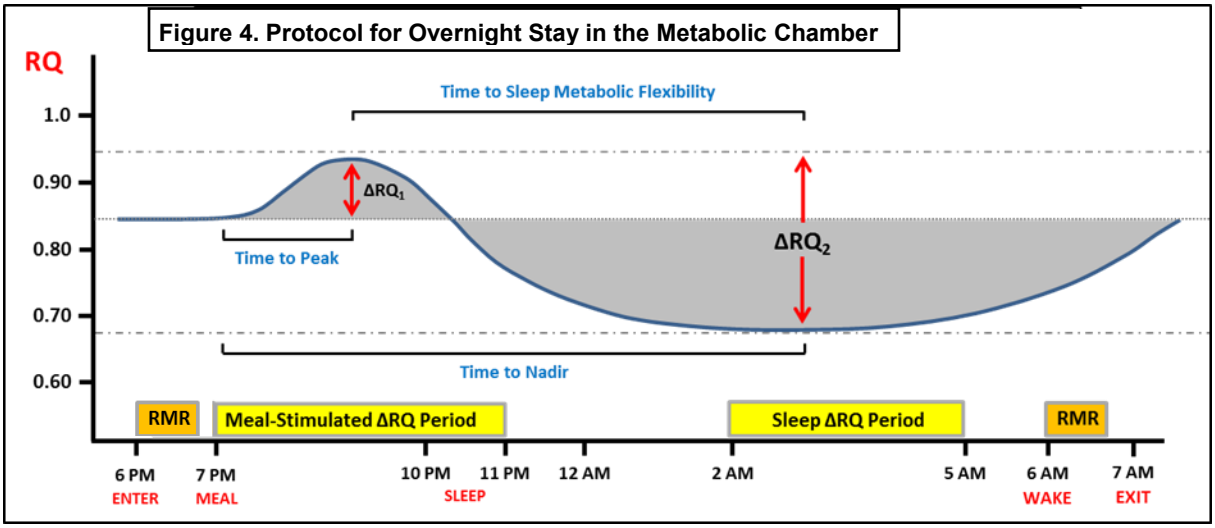
8.4 Continuous Glucose Monitoring

Interstitial glucose will be assessed using continuous glucose monitoring (CGM). Briefly, the abdominal area will be disinfected, and then trained staff from the Inpatient Unit will insert a glucose sensor under the skin in the abdominal area. The sensor has a small needle-like probe that inserts into the subcutaneous fat of the abdomen and that measures blood glucose levels without removing blood from the body. The sensor will then be attached to the recording unit, and the set-up will be secured with adhesive to the participant's body. After an initial period of equilibration with interstitial glucose, the sensor will be calibrated about every 2-12 hours by pricking the participant's finger to measure capillary blood glucose. The CGM device records blood sugar levels every 5 minutes.

8.5 Metabolic Chamber

Subjects will enter the chamber at approximately 5:30 pm and exit the following morning at 7 am. The chamber is a room about 12' x 14' with 2 windows, a bed, a desk and chair, a treadmill, a TV/VCR/DVD, a computer with internet access, a telephone, toilet facilities, motion sensors and a camera. Participants will be able to contact the nurse or chamber personnel by intercom, pager or phone at any time. They will be served 1 meal while they are in the chamber. The sophisticated ventilation system allows their oxygen / carbon dioxide exchange to be measured, thereby showing the number of calories the participant is burning. Energy expenditure and oxidation of CHO, fat, and protein will be calculated based on previous work [**9**] (*See **Figure 4***). Physical activity (% motion) will be assessed by infrared radar. Only minutes where activity is <1% will be used for calculating sleep data (2AM to 5AM). Adjustments will be made to the 4-h meal-stimulated period later if motion is detected. **Baseline RQ** will be defined as the mean RQ calculated during the last 30-min of the controlled, 45-min pre-meal RMR period. **Peak RQ** will be the highest mean, continuous 30-min RQ following the test meal, while **Nadir RQ** will be the lowest mean, continuous 30-min RQ during sleep. The difference between baseline RQ and peak RQ (i.e., **ΔRQ_1**), between peak RQ and nadir RQ (i.e., **ΔRQ_2**), and the time it takes to achieve these changes in RQ (i.e., **Time to Peak**, **Time to Nadir,** and **Time to Sleep Metabolic Flexibility**) will be calculated. While in the chamber, participants will collect all their urine for measurement of urinary nitrogen. Participants will be provided with breakfast after exiting the chamber.

Figure 4. Protocol for Overnight Stay in the Metabolic Chamber



8.6 Response to a Standardized Meal Test

An indwelling catheter will be inserted into an antecubital vein to obtain venous blood samples. Participants will consume a test meal consisting of 20% fat, 60% CHO and 20% protein (low-fat meal) or 60% fat, 20% CHO and 20% protein (high-fat meal). The meal will be consumed while they are in the metabolic chamber under supervision in the

inpatient unit. Blood will be collected in tubes according to the following schedule: -5, 30, 60, 90, 120, and 180 mins relative to the start of the meal for measuring glucose, insulin, and free fatty acids. These measurement will require approximately **27 ml** of blood to be drawn.

The estimated total blood volume required of each participant in the study is **61.5 ml.**

9. SUBJECT PAYMENT

Participants will receive up to $425 upon completion of the study. If participants do not complete the entire study, they will be compensated $50 for completing Visits 1, $175 for completing Visits 2, and $200 for completing Visit 3. Participants will not be compensated for the Screening Visit. Participant payments will be received via check requested from the PBRC (LSU) payroll department. There is no compensation available for research-related injury.

10. PROTECTION OF HUMAN SUBJECTS

10.1 Risks to Participants

This Human Subjects Research meets the definition of a Clinical Trial. This study does not involve major risk to participants. Efforts to minimize the potential risks of the assessment methods and outcome variables include frequent monitoring by the investigators to assure that no volunteer suffers any adverse effects from participating in the research.

The known risks, inconveniences, or side effects from the proposed procedures in the project are shown:

Potential Risks and Efforts to Minimize the Risks		
Procedure	**Potential Risks**	**Efforts to Minimize the Risks**
Fasting for 10-h	Nausea.	Light snacks will be available to eat once fasting procedures completed.
Questionnaires	Uncomfortable answering questions.	Participants may choose to skip any questions.
Blood Draws (Fasting)	Bruising, bleeding, pain, and infection pose minimal risks.	Trained phlebotomists and personnel will use sterile technique.
Dual-Energy X-ray Absorptiometry (DXA) scan	DXA, using a whole-body scanner, measures the amount of bone, muscle, and fat in the body. The expected dose of radiation is minimal, equivalent to less than 0.0004mSv or ~12 hours of exposure to the sun.	Exposure to X-rays is expected but minimal. A pregnancy test will be performed before the DXA scans on females of child-bearing potential.
IV Procedure	There is a possibility of pain, bruising, or infection at the site of the needle insertion for the IV line.	Trained personnel minimize this risk.

Continuous Glucose Monitoring (CGM)	Because CGM involves the placement of an implantable device below the skin, there is the possibility of discomfort, pain, and bruising at the site where the device is inserted. There is also a small risk of bleeding and a very small risk of infection at the site of the blood draw. Finally, the adhesive may cause redness or irritation of the skin.	Aseptic (sterile) technique and trained personnel minimize these risks.
Metabolic Chamber (Overnight)	Participants may experience some level of claustrophobia or discomfort from staying in the chamber and being continuously monitored by a camera. The camera has been installed for the participant's safety and no one is allowed access to the monitor except chamber personnel.	Participants can open the door of the chamber in case of an emergency. Trained personnel also minimize this risk.
Confidentiality of Data	Taking part in this research may involve providing information that one considers confidential or private. There is a slight risk that data could be revealed inappropriately or accidentally	Study researchers and staff will take steps to protect data that is collected. Efforts, such as coding research records, keeping research records secure and allowing only authorized people to access research records, will be made to keep the data safe.

10.2 Safety Monitoring

In this study, an **adverse event or experience** is defined as any health-related unfavorable or unintended medical occurrence that happens throughout study participation. Examples of adverse events include but are not limited to the following:

- A clinically significant laboratory or clinical test result.
- An event that results in missing a study visit.
- An event that requires a visit to a physician.
- An event that occurs as a result of a study procedure.
- Unanticipated or untoward medical events that may be study related.

Pennington Biomedical Research Center is an AAHRPP accredited institution and, above all else, is committed to ensuring and maintaining the safety of its participants. We will use the provided definitions of Adverse Events and Serious Adverse Events. Events will be reported according to our institutional reporting policy.

A **serious adverse event** (SAE) is defined as an unanticipated medical occurrence that is deemed associated with study participation by the study Medical Investigator that results in one of the following:

- Death
- Life-threatening event

  - Life threatening events in participants are defined as those that in the view of the research staff and PI put the individual patient at imminent substantial risk of dying, or if continued participation in the study might have resulted in death.
- Hospitalization (initial or prolonged)
  - Hospitalization or acute outpatient evaluation (e.g., in an emergency room) alone is not sufficient to qualify as a serious adverse event.
  - Any medical or surgical procedure performed (e.g., surgery that is not the planned bilateral oophorectomy, transfusion) itself is not the adverse event; instead, the condition that leads to the procedure is the adverse event.
- Disability or permanent damage
  - If the adverse event resulted in a substantial disruption of the participant's ability to conduct normal life functions, i.e., the adverse event resulted in a significant, persistent or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life.
- Medical Intervention to prevent permanent impairment or damage

10.2.1 Surveillance and Reporting Procedures

All AEs from date of consent will be reported. Adverse events will be documented during the scheduled visits or during phone calls during off-weeks. For each sign, symptom or adverse event, the following information will be recorded:

- A brief descriptor of the adverse event
- Start and stop dates
- Intensity (mild / moderate / severe)
- Whether the AE was "serious" or not (as defined below)
- Causal association with the intervention assigned (none / doubtful / possibly / probably / very likely)
- Outcome (resolved / resolved with sequelae / improving / still present and unchanged / death)
- Action taken with respect to the intervention (none / intervention temporarily discontinued / medical therapy required / intervention permanently discontinued / other).

Adverse event data will be collected from the date of consent until the final visit. Adverse event data will be analyzed quarterly, but serious or life-threatening adverse events may require immediate reporting and follow-up. We anticipate most adverse events will be mild and the participant will be able to resume activities within a day or two of reporting the event. Adverse Event reporting will follow the requirements of the IRB of the PBRC specified in the HRPP Policy 8.0 Unanticipated Problems Involving Risks to Subjects or Others. Only adverse events that qualify as unanticipated problems will be reported to the IRB. Unanticipated problems involving risks to participants or others include incidents only if the incident is unexpected, related or possibly related to participation in the research, and indicated that subjects or others are at a greater risk of harm than was previously known or recognized. Any action

resulting in a temporary or permanent suspension of this study (e.g., IRB actions, or actions by the PI and/or co-investigators) will be reported to the appropriate officials.

10.3 Withdrawal of Subjects

There is no risk associated with withdrawal from the study. Participants may be withdrawn from the study if either the PI or MI feels that their continued participation would jeopardize either the subject's health or the results of the study.

10.4 Stopping Rules

This study does not involve major risk for participating. Nevertheless, in addition to monitoring recruitment, we also will monitor the rates of adverse events in our participants. The study investigators will alert the IRB, if a larger than reasonably expected adverse event rate occurs in our participants.

10.5 Data Collection, Storage, and Confidentiality

10.5.1 Protection of Stored Data

Protection of subject privacy will be accomplished by a variety of stringent security measures. All medical records will be stored in locked areas, and access to these areas is limited to the clinic support staff, the Director of the clinical facilities, PIs at PBRC, and authorized designees of the PI. Volunteer medical records will be filed according to an assigned volunteer ID number. All forms on the chart, with the exception of the consent form, will display only the ID number, with no participant-identifiable information. All of these will be kept separate from records with names and other personal information. The only people who will know that these patients are research participants are members of the research team. No information about them, or provided by them during the research, will be disclosed to others without their written permission, except if it is necessary to protect their rights or welfare (for example, in case of injury or emergency care), or if it is required by law. When the results of the research are published or discussed in conferences, no information will be included that would reveal the identity of the individuals. Participants will be identified by codes when the data gathered in this procedure is presented or published. Authorized representatives of the NIH may need to review records of individual participants. As a result, they may see their name; but they are bound by rules of confidentiality not to reveal the participants' identity to others. Electronic data storage is secured with password protection and similarly restricted with only the PI and authorized persons having access to databases containing confidential clinical records (i.e. those containing name, social security number, or other identifying information). Electronic communication will involve only unidentifiable information.

10.5.2 Confidentiality

PBRC complies with the federal 1996 Health Insurance Portability and Accountability Act (HIPAA). Specifically, PBRC protects the privacy and confidentiality of medical records and information contained in medical records of persons who are subjects of research projects, including all protected health information (PHI) as defined by the HIPAA privacy Regulations. PHI of research subjects and the use or disclosure of

such information is governed by PBRC research policies as well as Common Rule, FDA regulations, and other applicable laws.

PBRC and study PI (the person chiefly responsible for the record) protect the privacy of research subjects and their PHI collected during a research project. PBRC will not use or disclose existing PHI or PHI created during a research project, unless the:

- Subject signs both (a) a HIPAA Authorization for use and disclosure of PHI using an approved Authorization Form or other form containing all the elements of legally effective HIPAA authorization; and (b) the informed consent to participate in research form approved by IRB; or
- IRB grants a waiver to the requirement of obtaining a signed HIPAA authorization Form, or
- IRB approved protocol uses properly de-identified PHI

All volunteers are assured of their anonymity and confidentiality both verbally and in the informed consent.

10.5.3 ClinicalTrials.gov Requirements

Upon successful funding of the study, the clinical study will be registered at the *ClinicalTrials.gov* website, in accordance with NIH recommendations. The unique NCT identifier will be included in all future Progress Reports and publications.

11. DATA MANAGEMENT

11.1 Power Calculations

Assuming a 10% dropout rate, this study is powered at the 83% level to detect a significant (α=0.05) difference between the high- and low-fat diet for the primary endpoint, sleep metabolic flexibility (see Fig 2). The analysis, based on data from the ADAPT study, assumed a sleep metabolic flexibility of 0.14 for the standard-fat meal and 0.09 for the high-fat meal.

11.2 Statistical Analyses

A linear mixed model accounting for both within-subject repeated measures and crossover effect will be used to estimate the effect of meal composition over time. Results will be based on t-tests using the least squared means from the mixed models.

12. BENEFITS, RISKS, AND SAFETY

12.1 Potential Benefits

We cannot promise any benefits from participants being in the study. However, possible benefits include gaining information about your overall health and body composition. The results of this study could also provide important information about how an individual's metabolic flexibility changes following ingestion of meals with differing fat content.

12.2 Risks

This study involves the following risks to subjects:

- **Fasting for 10 hours:** There is a possibility that fasting for 10 hours may make you feel nauseous. Light snacks such as granola bars and juice are available for you to eat once the fasting procedures are completed.
- **Body weight:** There is no known risk associated with recording body weights.
- **Questionnaire**: Due to the sensitive nature of the questionnaire, you may skip any questions that you do not wish to answer.
- **Urine collection:** There are no known risks of collecting urine into a container.
- **Blood draws:** There is the possibility of infection and/or pain and bruising at the vein on your arm where the needle is inserted. Aseptic (sterile) technique and trained personnel minimize these risks.
- **Body composition by DXA scan:** The amount of radiation used for this procedure is very small. The radiation dose for this scan is equivalent to the radiation you are naturally exposed to in the environment in less than one day. Scans will not be performed on any subject who is pregnant. A pregnancy test will be performed within 72 hours before the scan on females of child-bearing potential.
- **Continuous Glucose Monitoring (CGM):** Because CGM involves the placement of an implantable device below the skin, there is the possibility of discomfort, pain, and bruising at the site where the device is inserted. There is also a small risk of bleeding and a very small risk of infection at the site of the blood draw. Aseptic (sterile) technique and trained personnel minimize these risks. Finally, the adhesive may cause redness or irritation of the skin.
- **IV Procedure:** There is a possibility of pain, bruising, or infection at the site of the needle insertion for the IV line. Trained personnel minimize this risk.
- **Metabolic Chamber (Overnight):** Participants may experience some level of claustrophobia or discomfort from staying in the chamber and being continuously monitored by a camera. The camera has been installed for the participant's safety and no one is allowed access to the monitor except chamber personnel. Participants can open the door of the chamber in case of an emergency. Trained personnel also minimize this risk.

In addition to the risks listed above, participants may experience a previously unknown risk or side effect.

13. REFERENCES

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