myIRB#: STUDY00001808

SHORT TITLE: AAAA2

## PROTOCOL TITLE:

**Activity, Adiposity, and Appetite in Adolescents 2 (AAAA2)**

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*04.26.2023*

## REVISION HISTORY

| Revision # | Version Date | Summary of Changes | Consent Change? |
|---|---|---|---|
| 1 | 6.9.2021 | Initial IRB clarifications with revisions | Y |
| 2 | 07.19.2021 | Additional clarifications with revisions | Y |
| 3 | 01.27.2023 | Update insulin sensitivity protocol | Y |
| 4 | 04.26.2023 | Adding KUMC as a site | N |
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# 1.0 Study Summary

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|---|---|
| **Study Title** | **Activity, Adiposity, and Appetite in Adolescents 2 (AAAA2)** |
| **Study Design** | Cross-sectional |
| **Primary Objective** | To examine the association between physical activity and appetite control in adolescents, with metabolic function serving as a mediator. |
| **Secondary Objective(s)** | n/a |
| **Research Intervention(s)/ Investigational Agent(s)** | n/a |
| **IND/IDE #** | n/a |
| **Study Population** | Adolescents age 14-17 (Tanner Stage III-V) |
| **Sample Size** | Approximately 60 (40 completing the entire protocol) |
| **Study Duration for Individual Participants** | Approximately 35 days (this can be extended to accommodate participant's schedules) |
| **Study Specific Abbreviations/ Definitions** | RD – registered dietitian, MS – measurement session, EE – energy expenditure, EI – energy intake, DXA – dual energy X-ray absorptiometer, EGC – Hyperinsulinemic-Euglycemic Clamp, CRF – Cardiorespiratory fitness , RMR – resting metabolic rate, TEF – thermal effect of feeding, CEQ – control of eating questionnaire, VAS – visual analogue scale, FM – fat mass, FFM – free fat mass, CCHLN – Center for Children's Healthy Lifestyles and Nutrition, CRF – cardiorespitory fitness |

# 2.0 Objectives

*2.1* To examine the association between physical activity and appetite control in adolescents, with metabolic function serving as a mediator.

*2.2* Hypothesis 1: Appetite, as measured subjectively, via self-reported hunger and palatability responses, and, objectively, via satiety/appetite hormones (glucose, insulin, ghrelin, and leptin) responses following a standardized meal and calories consumed during ad libitum meal, will be altered in sedentary vs. active adolescents, regardless of body weight status.

Hypothesis 2: Reduced metabolic function, defined as low insulin sensitivity and metabolic flexibility, measured by hyperinsulinemic euglycemic clamp and indirect calorimetry, will be associated with poorer appetite control and physical inactivity as defined in Hypothesis 1.

## 3.0 Background

*3.1* Overall Scientific Premise- Weight gain is determined by energy balance status. Data from the National Health and Nutrition Examination Survey (NHANES) indicate that 16.9% of US children and adolescents are classified as obese (BMI > 95$^{th}$ percentile) and 31.8% of youth are either overweight or obese (BMI > 85$^{th}$ percentile) [1, 2]. This is driven, in part, by declines in physical activity that begin around age 15 years for boys and age 10-13 years for girls [3], with only 27% of 12-17 year olds obtaining 60 minutes of moderate to vigorous physical activity [4]. Healthy eating follows a similar same age-related decline, but at an even lower level with only 10% eating five servings of fruits/vegetables per day [5]. At the most basic level, obesity is the result of a mismatch between the amount of calories consumed and the amount of calories expended over an extended period of time. In reality a variety of known and unknown systems involving complex relationships between biological, physiological, psychosocial, and environmental factors [6, 7] influence the components of energy balance [8, 9]. Clearly, if effective prevention and treatment strategies currently existed based on a complete understanding of energy balance, worldwide levels of obesity would not have reached pandemic proportions [10].

Physical Activity is Associated with Appetite Control. The concept of dysregulation of appetite at low levels of physical activity is not new, with Mayer and others [11, 12] exploring the topic in a series of studies in the 1950s. It was observed in mice, rats, and humans [13-15] that energy intake only increased proportionally with energy expenditure within a certain range of physical activity, described by Mentor Dr. John Blundell [16] as the 'zone of regulation' (Figure 2). In contrast, when activity levels are low (inactivity), the relationship between intake and expenditure is uncoupled and in the "non-regulated zone", resulting in energy imbalance.

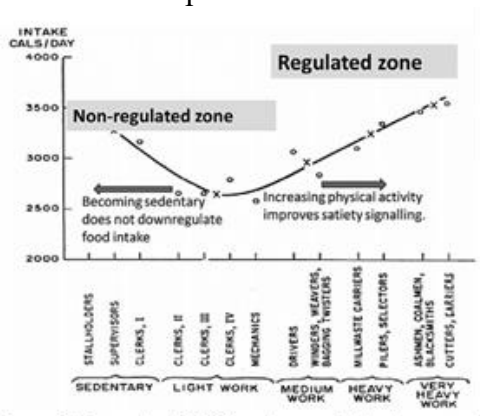



**Figure 2**. Mayer et. al 1956 found energy intake became uncouple at low levels of activity, described by Blundell 2011 as the 'non-regulated zone.'

*3.2* Recent work has confirmed the links between elevated energy intake and appetite dysregulation at low levels of physical activity. We (Shook and Blundell, among others) recently completed an analysis of energy balance among adults (N=421, mean age=28 years) over a 12-month period which included grouping participants by quintiles according to their level of objectively measured physical activity [17]. First, we observed increasing levels of physical activity in quintiles 2-5 corresponded with increases in energy intake, indicating energy balance. However, individuals in the lowest quintile of physical activity had a higher energy intake than groups 2 and 3, despite expending less energy (Figure 1). Second, individuals in this lowest quintile of physical activity had higher levels of disinhibition and preference for savory foods compared to their more active peers. Third, individuals with low levels of physical activity experienced the largest gains in fat mass, resulting in 3.8 higher risk of gaining clinically significant (>3%) amounts of fat

mass over 1-year. Our findings have since been replicated by others [18-20]. Taken together, our results support the hypothesis proposed by Mayer *et al.* [15] and others that a low level of physical activity is a risk factor for weight gain via a link to not only reduced energy expenditure but also dysregulated appetite control which leads to gains in fat mass. However, these findings raise important unanswered questions. What are the biological mechanisms by which physical activity influences appetite? What is the temporal relationship between physical activity and appetite control? Are the links observed in adults also present in adolescents?

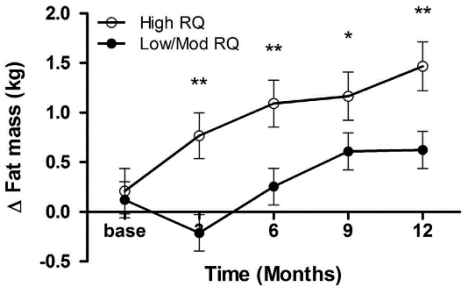



**Figure 4**. High RQ (low fat oxidation) is associated with increases in fat mass after 1 year.

Evidence that Metabolic Function may be a Mediator. As noted by Blundell [21] and by others [22], several clues point to metabolic function as a mediator in the relationship between activity status and appetite control. First, resting substrate metabolism has been shown to partially explain post-exercise eating behavior [23, 24] and be predictive of long-term weight gain [25-27]. We have previously shown that high utilization of stored carbohydrates and low utilization of stored fat (elevated respiratory quotient (RQ)) predicts weight gain 1 year later in healthy adults (Figure 4) [28], and partially explains unexpected weight gain among those who increased their MVPA over 1 year [29]. Second, blunted hepatic and skeletal muscle metabolism has been linked to increased energy intake and subsequent weight gain. Dr. John Thyfault recently observed greater food intake and short-term weight gain following a 3-day western diet in rodents with chronically reduced hepatic fatty acid oxidation (via low aerobic capacity) and mitochondrial respiratory function [30]. His group has recapitulated these findings in mice with liver-specific reductions in mitochondrial FAO due to PGC-1α heterozygozity (Morris et al., *in review*) (Figures 5 & 6). Third, there is evidence that manipulating substrate metabolism has causal effects on energy balance. For example, stimulating liver specific glucose or fat utilization can prevent weight gain [31, 32], while inhibiting glucose or fat oxidation and subsequent declines in hepatic ATP increases food intake via vagal afferent signaling to the central nervous system [33-36]. We believe that sedentary behavior induces negative changes in substrate metabolism that results in the

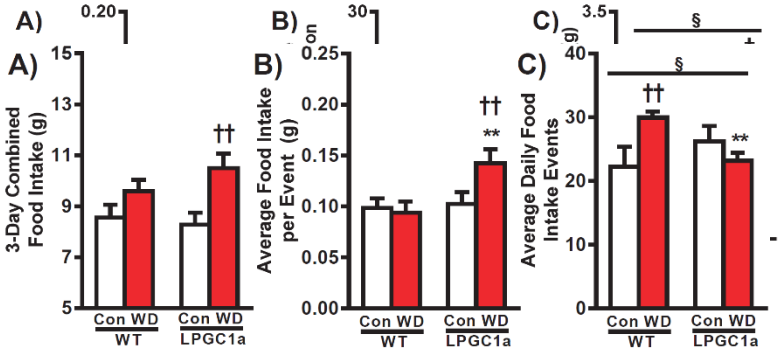



**Figure 6**. LPGC1a+/- mice fed high fat diet for 3-days have A) increased food intake compared to low fat diet. 3-days of WD in LPGC1a+/- mice results in altered feeding patterns: B) food intake per event, C) number of feeding events.

metabolic phenotype described in this section, but it is unknown if this is associated with appetite dysregulation and subsequent weight gain. Conversely, it is well known that increasing physical activity obtained through structured exercise will improve systemic metabolic function [37-39], but it is not clear if this will also improve appetite regulation.

Appetite Control in Adolescents is Unknown. Despite a growing body of evidence in adults that physical activity plays a role in mediating appetite control, little work has been performed in adolescents. Most studies in adolescents consists of appetite measures performed as secondary outcomes in studies with possible confounding factors (e.g. underlying disease risk) [39-41]. Observational data suggests higher levels of obesity are associated with dysregulated appetite as defined by satiety and responses to food cues [42] and higher energy intake despite lower levels of physical activity [43]. A smaller number of clinical trials have found that exercise trials in adolescents improves biomarkers of appetite control [44]. Of particular note is an eight-month aerobic intervention among OW/OB adolescents, with the effects of exercise isolated since there were no changes in body weight (but improvements in body composition) during the study [37]. Hormones peptide YY and resistin decreased following completion of the intervention, suggesting improved regulation of appetite with higher levels of physical activity. However, assessments in each of these studies were limited to fasted measurements, not as part of a more methodologically rigorous and informative meal challenge. Taken together, a small number of studies in adolescents support the scientific premise that physical activity and appetite are associated. What is not clear is strength of the association between the activity and appetite, the role of metabolism in this relationship, nor the effects of a structured exercise regimen in this association.

Challenges to Existing Paradigms. The status quo as it pertains to appetite control emphasizes the role of peripherally derived signals mediating energy intake in the CNS [Figure 7 - e.g. insulin and ghrelin mediate appetite through pathways centered in the hypothalamus [45-47]]. This concept is supported by a strong body of evidence, dating from as far back as 50 years, including the discovery of leptin in 1994. However, it has been argued that this paradigm primarily accounts for the inhibition of eating as opposed to the drive of eating. An alternative approach, centered on the concept of energy balance, postulates that daily energy expenditure regulates appetite (Figure 7). It has been put forth by Mentor Blundell, and adopted by others [20, 22], that overall appetite is regulated through three components: 1) a tonic (i.e. constant) drive for food driven by energy demands; 2) tonic inhibition from signals of energy storage (e.g. leptin); 3) episodic (i.e. response-initiated) signals from the periphery in response to food. This new approach emphasizes the contribution of physical activity and energy expenditure on both the tonic excitatory drive to eat and episodic regulation of fasting hormones (ghrelin) and satiety hormones (GLP-1 and PYY) [19, 48, 49]. Importantly, this new formulation recognizes the role of resting metabolic rate [16] and fat free mass on appetite control. We extend this theory to include metabolic function, as there is evidence that it is also associated with eating behavior (see section A.2.), pointing to a specific biological mechanism by which appetite regulation may occur.

*3.3* New Approaches. The proposed research is innovative because it shifts beyond the tonic inhibition approach, to include the regulation of appetite via energy expenditure and metabolic function. Our preliminary studies (see sections 2.1-2.5) indicate that energy intake and disinhibition become dysregulated at low levels of physical activity, resulting in 3.8 fold greater fat mass 1 year later [50]. We will extend previous findings in adults to adolescents, a previously unexamined population in regard to this concept. Additionally, we have expanded upon previous studies in the proposed project by exploring plausible metabolic mechanisms that underlie appetite dysregulation at low levels of activity. By completing this work, it is our expectation that we will have described the independent effects of physical activity and obesity on appetite and energy metabolism in adolescents.

## 4.0 Study Endpoints

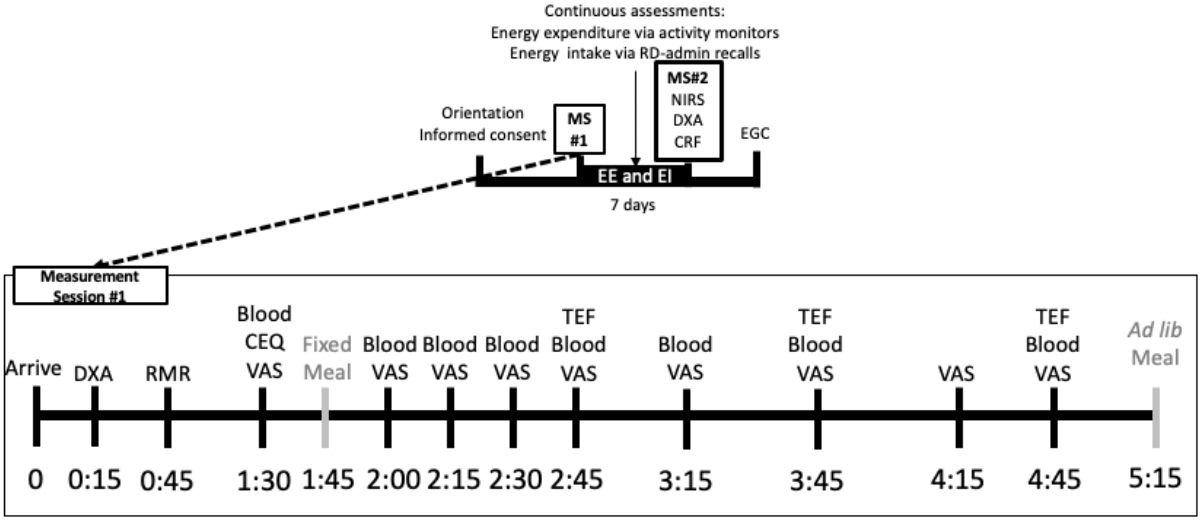
*4.1* The primary study endpoint is the enrollment of 10 participants per study arm (normal weight/active, normal weight/sedentary, overweight, obese/active, and overweight, obese/sedentary).

*4.2* There are no safety endpoints for this study.

## 5.0 Study Intervention/Investigational Agent

N/A – no intervention/investigational agent

## 6.0 Procedures Involved




| *Adolescents (age 14-17 years & Tanner stages III- V)* | Normal weight (≥5th to <75th percentile) | Overweight/obese (≥85th to <99th percentile)[14] |
|---|---|---|
| Inactive (<60 min/day MVPA) | n= 10 | n= 10 |
| Active (>60 min/day MVPA)[15] | n= 10 | n= 10 |

**BLOOD:** Glucose and appetite hormones
**CEQ:** Control of eating questionnaire
**CRF:** Cardiorespiratory fitness
**DXA:** Duel-energy absorptiometry
**EGC:** Euglycemic clamp
**Energy intake:** Dietician-administered recalls
**Energy expenditure:** Actigraph GT9x
**RMR:** Resting metabolic rate
**MS:** Measurement session
**NIRS:** Near-infrared spectroscopy
**VAS:** Visual-analog scales for hunger, fullness, etc.
**TEF:** Thermic effect of feeding

*6.1* This is a cross-sectional study design (see above figure). The study team aims to assign N=10 participants to each arm of the study (normal weight/active, normal weight/sedentary, overweight, obese/active, and overweight, obese/sedentary).

*6.2* Participants who fall into the overweight/obese category (~20) will also be recruited for the AAAA2 – Exercise study (AAAA2 study with an intervention and post intervention tests), which includes the same testing as AAAA2. The AAAA2 - Exercise study will only include those with a BMI percentile from the 85$^{th}$ to 99$^{th}$ percentile. It also has an exercise intervention (same as the BATE study), which 50% of study participants will be randomly assigned to. All 3 visits (measurement session 1, measurement session 2 and insulin sensitivity visit) will be completed before and after the exercise intervention. For those participants that complete testing in the AAAA2 study and are interested in the AAAA2 – Exercise study, their AAAA2 data will be used as baseline data in the AAAA2 - Exercise study. They will then consent for the AAAA2 - Exercise study and start their exercise intervention, or wait until their follow-up visits.

**Orientation Session: 1 hour**. The orientation session will serve as a visit to explain the details of the study, with example images of what tests will be completed. P/A/C documentation will also be completed at this visit if they decide to participate. If a participant and parent opt for in-person orientation, after consent, their height, weight and blood pressure will be collected to classify each participant into an arm of the study. If a virtual orientation is chosen, height and weight will be reported from them, according to their most recent measures. Following P/A/C for both in-person and virtual orientation, the **Contact Information Questionnaire** will be completed. After completion of the orientation session, the parent and subject will be instructed to complete the **Medical History and Demographics Questionnaire** prior to the first measurement session visit. This questionnaire will be sent through a RedCap link.

**Measurement Session 1: 5.0-6.0 hours.** This study visit will take place at the Don Chisholm Center in the morning (<9:00am) following an overnight dietary fast. This study visit will be conducted to assess appetite control and relevant variables under laboratory conditions. The measures conducted in this visit are provided below

- **Surveys**
  - **TFEQ**
  - **CEQ**
  - **VAS Pre-Meal**
  - **VAS Post-Hedonic**
  - **VAS**
- **Anthropometry and Body Composition via DXA.**
  - Fat mass (FM) and fat-free mass (FFM) will be measured using a whole-body dual energy X-ray absorptiometer (DXA, Lunar DPX system, version 3.6).
- **Waist and Hip Measurements.** Waist measured in centimeters, two inches above the umbilicus at skin level, at the end of a normal breath expiration, with arms relaxed at sides. Hip measured in centimeters at the maximum circumference of the buttocks, with arms relaxed at the sides.

- **Fixed/*Ad libitum* Meals.** Appetite (see below) will be assessed following a fixed standard breakfast (40% of RMR, composed of 55% carbohydrate, 15% protein, 30% fat). Food intake (total kcals) will also be assessed using an *ad libitum* meal served three hours after the fixed meal. Participants will be instructed to eat until comfortably full and informed that they can request more of the meal at any point.
- Each plate of food will be weighed before and after serving, without the subjects' knowledge.
- **Appetite and Eating Behavior** Subjective assessment of hunger and palatability will be completed using self-rated visual analogue scales (VAS) [51], with each scale consisting of a 10-cm long line separating statements such as "not at all hungry" and "extremely hungry". **[**VAS tools are widely used to assess health conditions and show good test-retest reliability [52], especially in within-subject repeated measures study designs [53].**]** Perceptions of dietary restraint, disinhibition, and hunger will be measured during each measurement session using the Three Factor Eating Questionnaire (TFEQ) [54]. The Control of Eating Questionnaire (CEQ) will be used to assess hunger, fullness, cravings, the desire to eat certain types of foods, and the ability to resist urges to eat [55]. Objective assessment of appetite will be completed using blood samples taken from an indwelling catheter, and analyzed for episodic regulation of the following fasting and satiety hormones: glucose, insulin, leptin, ghrelin, glucagon-like peptide 1 (GLP-1), and peptide YY (PYY). All samples will be mixed a standard preservative (400 µL of 10,000 KIU aprotinin), centrifuged, the plasma divided and stored in -80°C freezer until later analysis.
- **Resting Metabolic Rate.** Resting metabolic rate (RMR) will be measured via indirect calorimetry using a ventilated hood and open-circuit system metabolic cart (ParvoMedics, Salt Lake City, Utah) following a standard protocol [56].
- Thermic effect of feeding (**TEF**) will measure the energy expenditure resulting from digestion following the fixed meal using the ventilated hood.

**Continuous, Free-living Home Assessments of Energy intake/expenditure: 7 days between Measurement Session 1 and Measurement Session 2.**

- The free-living assessment period is defined as the 7 days between Measurement Session 1 and Measurement Session 2. During these 7 days, subjects will be completing two energy intake/ expenditure assessments while going about everyday life activities at home. These assessments are explained in proceeding bullets.
- Energy intake will be assessed during the free-living 7-day assessment period via standardized 24-hour dietary recalls. Energy intake will be assessed using three dietary recalls occurring on randomly selected non-consecutive days over each 7-day free-living assessment period

(including at least one weekend day) to minimize preparation that could bias recall, by a team of experienced staff employing a multi-pass approach, which utilizes prompts to reduce food omissions and standardizes the interview methodology.

- Physical activity and energy expenditure will be estimated using two physical activity monitors (Actigraph GT9X Link, Actigraph Inc., and ActivPAL, PAL Technologies), which assesses movement using tri-axial accelerometry. The monitors themselves are not being studied as a part of this protocol, and while they do not have 501(k) documents, the devices used in this study are not considered to be more than minimal risk.

**Measurement Session 2: 2.0 hours.** This study visit will take place at the Don Chisholm Center. This visit will include a DXA scan, mitochondrial function testing via NIRS, and cardiorespiratory fitness testing (CRF):

- **Body Composition via DXA:**
  - Fat mass (FM) and fat-free mass (FFM) will be measured using a whole-body dual energy X-ray absorptiometer (iDXA, Lunar DPX system) with the participant dressed in surgical scrubs and in bare feet.
- **Grip Strength Testing:**
  - Grip strength will be assessed using a handheld dynamometer. The participant will hold the device in one hand in line with the forearm at the level of the thigh, away from the body.
  - The participant squeezes the handgrip dynamometer as hard as possible without holding their breath. Neither the hand nor the dynamometer should touch the body. The test is repeated twice in each hand and scored as the sum of the highest measurement from each hand.
- **Mitochondrial Function via Near-Infrared Spectroscopy**
  - Assessment of skeletal muscle oxidative capacity using near-infrared spectroscopy (NIRS) will be performed. The specific protocol used to measure skeletal muscle oxidative capacity was initially described by Ryan et al (2014). An ISS Medical (Champaign IL, USA) Dual-Channel OxiplesTS advanced NIRS device that uses Frequency Domain technology to provide an absolute determination of oxy[heme] and deoxy[heme] in various tissues will be used. This device uses 8 near infrared lasers emitting 690nm to 830nm wavelengths which penetrate the skin without damage and are reflected to a Hamanatsu R928 detector. The instrument is controlled through a laptop computer and specialized software package (both provided by ISS Medical).
  - The NIRS muscle probe will be positioned on the skin longitudinally above the belly of the vastus lateralis muscle of the right leg, halfway between the patella and the hip. The probe will be secured using double-sided adhesive tape and Velcro straps around the thigh. After device calibration and a 30-minute warm-up period, blood pressure cuff designed for the thigh (specific size will be dependent on the patient) will be placed proximal to the NIRS probe as high as anatomically possible. The inflation of the cuff will be

controlled with a rapid-inflation system set to a pressure between >200mmHg, powered with a 15-gallon air compressor.

- Participants will perform short duration (10-15 second) voluntary isometric contractions of the quadriceps to increase muscle oxygen consumption. Following voluntary contraction, recovery kinetics of the muscle will be measured using a series 10 transient arterial occlusions with timing of 5 seconds on – 5 seconds off; followed by a series of another 10 transient arterial occlusions with timing of 10 seconds on – 10 seconds off. Post each occlusion, the rate of change (slope) in the decline in deoxy[heme] will be calculated. The resting procedure will be performed twice with results averaged.

- **Thigh Circumference and Skinfold Measurement**
  - To identify optimal NIRS probe placement, mid-thigh circumference will be measured using the right thigh at the midpoint between the inguinal crease and the proximal border of the patella.
  - Skin and adipose tissue thickness will be measured at the site of the NIRS probe using skinfold calipers. The technician will 'pinch' the skin and adipose tissue with their thumb and fore finger and measure the tissue thickness with a set of calipers.
- **Cardiorespiratory Fitness Testing:**
  - Submaximal oxygen consumption (fitness test) and maximal oxygen consumption will be assessed using an indirect calorimetry system with mouthpiece via standard protocols using a treadmill. Prior to the start of the test, resting blood pressure, heart rate, and oxygen saturation will be assessed using an automated vital signs monitor.
  - The submaximal exercise test will consist of walking at two speeds for five minutes each estimated as 'light' and 'moderate' intensity by the research technician. Following a five-minute break, the maximal test will be performed using the Bruce protocol. The Bruce protocol is the most widely used fitness testing protocol used in the US and consists of a series of three-minute stages that gradually increase the speed and grade of the treadmill. The majority of individuals reach volitional fatigue 9-12 minutes into the test. During both the submaximal and maximal tests, heart rate will be measured via monitor (Polar) and gas exchanged will be measured using indirect calorimetry (TrueOne, ParvoMedics, Parvo, UT, USA). Following completion of the test, blood pressure, heart rate, and oxygen saturation will be assessed using an automated vital signs monitor to ensure an appropriate post-exercise response.
- **Muscular Strength Testing:**
  - Muscular strength will be assessed with a one-repetition maximum (1-RM) test for the chest press and seated leg press using weight stack equipment (Paramount, St. Louis, MO, USA) following the protocol used by Co-I White [57, 58]. Resistance will be progressively increased by 2.5-20 kg until a maximal lift is achieved with a full range of motion within four attempts (3–5 min rest between trials). Muscular strength will be calculated

as the sum of the 1-RM scores for the bench and leg press per kg of body weight.

**Insulin Sensitivity Visit: 5 hours.** This study visit will take place at University of Kansas Medical Center Clinical and Translational Science Unit (KUMC CTSU) located on the Fairway campus. This visit will involve baseline blood samples and blood samples taken throughout the insulin sensitivity test.

- **Hyperinsulinemic euglycemic clamp.**
  - The hyperinsulinemic-euglycemic clamp is the gold standard for assessing insulin sensitivity as stated by the World Health Organization. It allows the insulin levels to be 'clamped' at a constant state so that insulin stimulated by physiological measures can be assessed. Insulin sensitivity can be determined by the amount of glucose that is infused to maintain euglycemia. The higher the amount of glucose that needs to be infused while insulin is clamped, the more insulin sensitive the subject is.
  - The hyperinsulinemic euglycemic clamp will be performed at the University of Kansas Medical Center Clinical and Translational Science Unit (KUMC CTSU), located at 4350 Shawnee Mission Parkway, Suite 3220 Fairway, Kansas. The CTSU contains 17 clinic rooms, 2 cognitive testing rooms, and open workspaces for up to 5 visiting study coordinators. The CTSU's infusion center has 11 infusion bays supported by two nursing stations and activity in the center has steadily grown. The center also supports research infusions and intensive and complex metabolic research assessments such as hyperinsulinemic-euglycemic clamps (see Morris et al. 2016). There is a CTSU medical monitor available for Adverse Events. The CTSU medical monitor is contacted when there is an urgent, medical concern / AE with the participant; The medical monitors are MD's or NP's / APRN's (aka Advanced Practice Registered Nurse). NOTE: This is in addition to the study-specific DSMB.
  - Participants will arrive fasted (12-hours overnight), and one catheter will be inserted into an antecubital vein of one arm to infuse insulin and 20% dextrose, and another catheter will be inserted into a contralateral vein in the hand/arm for blood sampling. The participant will rest quietly and we will collect 3 baseline blood samples to establish baseline glucose levels. If baseline values are <60 mg/dL the procedure will not continue. Once baseline assessments have been completed, a primed continuous infusion of regular human insulin plus albumin will be administered at a rate of 40 $mU \cdot m^2$ body surface $area^{-1} \cdot min^{-1}$ along with a variable infusion of 20% dextrose to

maintain euglycemia to match baseline levels (expected 90-100 mg/dL). Blood glucose measurements will be completed every 5 minutes for determination of glucose infusion rate using a Nova Statstrip handheld glucometer and YSI analyzer as needed.

- After approximately 90 minutes of acclimatization, a 30-minute data collection period will occur. Insulin sensitivity will be determined as average glucose concentration and glucose infusion rate during this 30-minute period of euglycemia (defined as glucose concentration and glucose infusion rate coefficient of variation <10%).
- After this 30-min period, A 60-minute recovery period begins. Insulin infusion is stopped, and glucose infusion continues at the last rate for 30-min to prevent hypoglycemia. Glucose infusion is stopped 30 minutes into recovery if blood glucose levels remain at acceptable levels.
- Participants will be provided a full lunch meal (e.g., sandwich, potato chips, drink) following the cessation of insulin infusion to minimize the chances of hypoglycemia. Participants will be provided with a 20 mEq potassium pill to take with food. In rare instances hypokalemia can occur resulting from hyperinsulinemia and increased glucose disposal. Potassium supplementation helps prevent this from occurring.
- Following the cessation of glucose, all participants will remain under observation at the CTSU for the final 30 minutes of recovery to monitor for hypoglycemia.
- Participants can be released if participant is asymptomatic and blood glucose levels are acceptable at the end of the observation period.
- Blood is drawn from a hand/arm vein after the hand/arm has been placed under an electric blanket and this technique is called arterialization, as it makes the blood in the vein have similar characteristics to that flowing in the artery by dilating the vessels in the arm. This is a well-accepted technique that allows us to avoid performing an arterial IV placement. The hand/arm will be placed under the blanket for the entire testing procedure.
- Insulin Mixing Procedures: The CTSU Investigational Pharmacy (located onsite) will store the insulin and albumin. The unused insulin and albumin will be returned to the pharmacy after each study. CTSU nurses will mix the insulin, albumin, and saline. For the mixing procedures, 2 mL of human albumin is injected into a bag of 250 mL sterile saline and gently inverted to mix the solution. Using an insulin syringe, 1.25 mL of insulin is added to the albumin/saline mixture and gently inverted to mix the solution.
- The mixing of insulin with albumin is essential to minimize the binding of insulin to the saline bag, as well as the catheter tubing. This procedure for mixing the infusate was described in the initial

report of the hyperinsulinemic-euglycemic technique (DeFronzo et al, 1979) using blood rather than albumin and is still utilized by labs that perform the hyperinsulinemic-euglycemic clamp. We are using albumin in this study to prevent the possibility of clotting associated with the use of blood.

*6.3* Data collected during the study includes self-report survey data (see myIRB local documents) as well as DXA, RMR, VO2, TEF, NIRS, and blood test results. Anthropometric measurements such as height and weight will also be collected. Dietary recall data and activity monitor data will be collected during the free-living assessment period (sample output available on local documents). Finally, insulin sensitivity will be determined through the Hyperinsulinemic-Euglycemic Clamp procedure.

*6.4* There are no plans for long-term follow-up beyond study completion.

*6.5* Standards and Safety of Participants

- All devices used in the study are FDA approved (DXA, RMR hood machine, NIRS, ActiGraph, ActivPAL, etc.).
- This study involves DXA scans –Female participants of child-bearing age are required to complete a pregnancy test before DXA scan.
- A nurse will perform any blood draws and all study staff assigned to perform lab procedures or RQ will be specifically trained to work with that equipment. This is training that is above and beyond the regular research CMH or standard research education.
- Appetite assessments: Blood will be collected via an IV catheter over 8 time points) over a period of 3 hours. Approximately 36mL (two samples at time 0, one sample at all other time points, 9 samples x 4 mL= 36 mL= 1.22 oz) of blood will be collected.
- Euglycemic clamp: Three baseline blood samples will be collected at the onset of the session to determine baseline glucose and insulin levels. The total amount of blood collected at baseline will be approximately 9 mL (3 samples x 3 mL/sample= 9 mL). Blood will be sampled every five minutes during the clamp procedure to monitor glucose levels. The total amount of blood collected to monitor glucose will be approximately 36 mL (36 samples x 1 mL/sample = 36 mL). Every 15 min we will also collect blood samples to store for assessment of insulin at study completion. The total amount of blood to assess insulin will be approximately 24 mL (12 samples x 2 mL/sample= 24 mL). During recovery, blood glucose is assessed every 10 minutes and insulin once. The total amount of blood collected during recovery is 8 mL (6 glucose samples x 1 mL= 6 mL; 1 insulin sample x 2 mL= 2 mL).
- Total amount of blood collected during the entire clamp procedure is approximately 77 mL (9 mL + 36 mL + 24 mL + 8 mL) or 15.6 teaspoons/2.6 ounces.

- The amount of blood is within the allowable amounts to be collected for research purposes.
- No subjects will be less than 16.5 kg

## 7.0 Data and Specimen Banking

*7.1* Samples will be stored in the CCHLN biochemistry lab

*7.2* Study ID, birth year, visit date only

*7.3* CM research only, for the purposes of this study only

*7.4* N/A – samples not retained for long-term research.

## 8.0 Genetic Analysis Information

N/A – no genetic analysis

## 9.0 Sharing of Results with Subjects

*9.1* At the conclusion of the study, a report with the participant's study results will be shared with each participant. Results will include:

- Height and weight
- Blood pressure values
- Fitness percentile
- Resting metabolic rate
- Activity monitor data such as steps per day
- Two body composition scans with bone mineral density
- Grip strength

## 10.0 Study Timelines

*10.1* Describe:

- Individual participation in the study will last approximately 35 days, but this time period can be extended to accommodate participant's schedules.
- Recruitment and data collection will last approximately 18 months.

## 11.0 Inclusion and Exclusion Criteria

*11.1*

<u>Inclusion Criteria</u>

- Males (~50%) and females (~50%)
- Age 14-17 (Tanner Stage III-V, as determined via self-assessment)
- Non-smoking
- Not currently involved in any other research study

- Willing to give informed consent to participate

Exclusion Criteria

- Age <14 or >18 at time of enrollment
- Restrained eater (>13 on the restraint section of the three-factor eating questionnaire; Current/past diagnosis of an eating disorder.
- Self-reported medical conditions (including, but not limited to, diabetes, Crohn's disease, etc.) that may affect adherence to the protocol, exercising safely, or alter metabolism
- Taking medications know to affect metabolism (e.g. thyroid medication, β-blockers, or stimulants).
- Gave birth in the past 12 months or <6 months post-lactation.

Individuals who are not yet adults (infants, children, teenagers) will be included in this study (teenagers age 14-17).

## 12.0 Vulnerable Populations

☐ Children/Minors (under 7 years of age)
☒ Children/Minors (7-17 years of age)
☐ Neonates (infants less than 30 days old)
☐ Neonates of Uncertain Viability (infants less than 30 days old)
☐ Non-Viable Neonates (infants less than 30 days old)
☐ Wards of the State
☐ Fetuses
☐ Pregnant Women
☐ Adults with impaired decision-making capacity
☐ CM Employees
☐ CM Students/Residents/ Fellows
☐ Economically or Educationally Disadvantaged Persons
☐ Prisoners

- This study involves children who are not yet adults. The study will be explained to them at the study visit as well as during the informed consent discussion.
- The research involves no more than minimal risk to the subjects.
- HRP-416 - CHECKLIST - Children has been reviewed.

Local Number of Subjects

*12.1* This study involves four cohorts: normal weight/inactive, normal weight/active, OW,OB/inactive, and OW,OB/active. The goal is to accrue 10 participants in each cohort for a total of 40 study participants. We anticipate the need to enroll about 60 total participants to account for screen fails and dropouts.

*12.2* We expect to make send our study letter to 2,000 potential participants, screen about 900, and enroll around 60, to get our final enrollment goal of 40. This is based off our previous AAAA submission with similar inclusion/exclusion criteria.

*12.3* N/A – no chart review beyond what is required for screening.

## 13.0 Screening and Recruitment Methods

*13.1* Participants will be recruited from previous Energy Balance research studies, from a recruitment list generated by CMH MIT, and a recruitment list generated from KUMC pioneers/frontiers. Recruitment letters and flyers will be mailed to the parents of potential participants from the above recruitment methods.

Additional recruitment methods include social media resources, the CCHLN website, dissemination of the study flyer to partner departments and institutions, and articles posted on the Scope using Daily E-News at Children's Mercy.

*13.2* Participants will be recruited from CMH, KUMC, and the community.

*13.3* When available, study staff will use previous contact with Energy Balance research participants (or potential participants that stated they were interested in other opportunities), CMH MIT generated data or KUMC generated data to pre-screen (or identify) potential participants. When not available, study staff will use a Redcap screener to verify eligibility.

*13.4* Study staff will record, and request (from resources such as CMH MIT) only the minimum necessary identifiable data for the purposes of contacting participants prior to ICF and pre-screening. We are requesting a Waiver of HIPAA Authorization for recruitment and screening purposes only. Minimum necessary PHI on non-participants will only be kept until completion of enrollment.

*13.5* We may disseminate the study flyer to other departments for the purposes of recruitment (Teen clinic, weight management clinic, etc).

*13.6* Individuals will be contacted a maximum of 3 times (including voicemails), when following up after mailing a study letter.

## 14.0 Reimbursement, Payment and Tangible Property provided to subjects

*14.1* Participants will be paid via pre-paid debit card. Participants will be paid when activity monitors are returned at the DXA/CRF visit $175, and another $125 at the insulin sensitivity visit.

*14.2* Participants will be paid $5 for each visit they attend as a travel reimbursement, with a max possible travel compensation of $15 to be paid at the completion of the study.

Payments will be administered as follows:
Measurement Session #2: $175 for study procedures
Insulin Sensitivity Visit: $125 for study procedures, maximum of $15 ($5 per visit) for travel reimbursement
Total Compensation: $300 for study procedures plus a maximum of $15 for travel reimbursement totaling a maximum compensation of $315.

*14.3* N/A – no tangible property given

## 15.0 Withdrawal of Subjects

*15.1* Non-compliance with the protocol.

*15.2* The study team will attempt to contact the participant for the purposes of determining a reason for withdrawal, attempting to reschedule, etc.

*15.3* If a participant decides to withdraw, all data up to the point of their withdrawal will be kept.

## 16.0 Risks to Subjects

*16.1*

- Drawing blood from the participant's arm and hand include discomfort and/or bruising. There is a very low risk of infection, bleeding, clotting, or fainting.
- Wearing activity monitors poses no known risks. However, the participant may feel some discomfort while initially getting used to wearing the monitors. In addition, a small percent of participants have developed a rash associated with the adhesive from the leg activity monitor. If any participant experiences any irritation, itching or redness while wearing either monitor, they will be instructed to stop wearing the monitor immediately and inform the study staff.
- While the radiation used for the body scan (DXA scan) has no observable radiological or biological effect, there is always a risk associated with radiation exposure.
- During the fitness test the participant will experience an increase in heart rate and blood pressure. Additionally, sweating will occur, and they may become fatigued, all of which are to be expected with exercise. It is also possible that abnormal responses may occur such as dizziness, unusually high increases in heart rate and blood pressure, and in rare instances, fainting. Trained personnel will be on hand during the fitness test. Emergency equipment is kept in the exercise testing room and all staff have been trained in its use. Following the fitness test, they may experience muscle soreness for a few days.
- During the study the participant will be asked to eat three meals (breakfast and lunch during the appetite assessment, lunch following the clamp procedure). The study team will inquire about any food allergies the participant may have.
- During and immediately after the clamp procedure there is a risk of hyperglycemia. We will monitor blood glucose every five minutes during the technique and for 1 hour following the technique.
- There is also a slight risk of loss of confidentiality, meaning that someone could know the child participated in this study if they study team's

research records are hacked. The participant's confidentiality will be protected to the greatest extent possible.
- Possible physical discomfort from the pressure from the NIRS muscle cuff

*16.2* Overall, the risks involved in this study are minimal. At this time we do not see any unforeseeable risks, but as information comes available we plan to report it to participants.

*16.3* We will screen for pregnancy prior to completing DXA scans on women of child bearing age (all women enrolling in the study), but there are risks associated with radiation exposure.

*16.4* Loss of confidentiality could affect parents/family of participants as well as participants.

## 17.0 Potential Benefits to Subjects

*17.1* N/A – no benefit.

*17.2* Participants may experience no direct benefit.

## 18.0 Investigator Assessment of Risk/Benefits Ratio: **(IRB makes the final determination)** *Based upon your response in Sections 17.0 and 18.0, please provide your assessment of risk and benefits in below table.*

| Select as applicable: | **Pediatric Risk Category:** | |
|---|---|---|
| x | Category 1 | Research not involving greater than minimal risk (45 CFR §46.404 and 21 CFR §50.51) |
| | Category 2 | Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects. (45 CFR §46.405 and 21 CFR §50.52) |
| | Category 3 | Research involving greater than minimal risk and no prospect of direct benefit to individual subjects, but likely to yield generalizable knowledge about the subject's disorder or condition. (45 CFR §46.406 and 21 CFR §50.53) |
| | Category 4 | Research not otherwise approvable which presents an opportunity to understand, prevent, or alleviate a serious problem affecting the health or welfare of children. (45 CFR §46.407 and 21 CFR §50.54) |
| Select if applicable: | **Adult Risk Category:** | |
| x | Not Greater than Minimal Risk | |
| | Greater than Minimal Risk | |

## 19.0 Data Management and Confidentiality

*19.1* **Statistical Analysis Plan.** To address Aim 1, we will compare groups (active vs. sedentary, normal weight vs. overweight/obese) using mixed models (linear or generalized, as appropriate) that adjust

for clustering of time points within person. For VAS appetite measures, which are assessed at multiple time points within a single measurement session, we will conduct analyses in two ways. First, we will conduct between group analyses using each of the seven individual assessments (e.g. 60 min post-meal) to see if the values differ by physical activity or body weight group. Second, we will also calculate area under the curve (AUC) for each variable of interest (e.g. hunger, ghrelin), and test for group differences on that measure using a linear or generalized regression model. To address Aim 1, hypothesis 2, we will repeat the analyses described above and include insulin sensitivity as a covariate.

*19.2* The sample size was determined by a review of similar studies indicating a range of 12-44 participants.

*19.3* All study staff will complete research training, save study documents on drives that require authorization of access, password protection, or encryption. Paper study documents will be protected using physical controls (such as being kept in secured locked offices in locked file cabinets). Study staff will keep identifiers separate from study IDs and data [master list]) during storage, use, and transmission.

*19.4* A Certificate of Confidentiality has been issued for this study. Certificates are automatically issued for NIH funded research per NIH policy.

*19.5* Study surveys will use a study ID for an identifier, study staff will lock documents in secure office locations and keep data collection surveys and instruments with them at all times when in use.

*19.6* Describe how data or specimens will be handled study-wide:

- Study ID and visit date will be included on source documentation
- Study documents will be stored in secure offices in secure file cabinets, Redcap, the W drive, or BOX (the study team has previous approval to use).
- Study source documentation and other data will be kept at least 3 years after submission of the Final Progress Report to the NIH.
- The CMH study team, other approved staff members from approved organizations (KUMC, etc), the sponsor (NIH), the IRB.
- The study lab coordinator, the PI.
- No data will need to be transported, but blood samples will be transported to the analyzing lab through standard biohazard

shipping protocols. All data obtained off of CM premises will be accessed by study staff electronically via approved secure sharing platforms such as OneDrive.

# 20.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

## *20.1* Per NIH requirements:

**Plan for safety monitoring and review**

There will be a chair of the Data Safety Monitoring Board to make decisions on safety of the participants, as well as two other members members to serve in monitoring study progress and unanticipated/adverse events. Data will be reviewed twice a year by the Board Chair. If problems occur during the euglycemic clamp procedure, staff nurses will stop the procedure and notify the CTSU medical monitor, the Board Chair, and Dr. Shook of any issues. The Board Chair will then make a decision on whether the participant should continue with the remaining parts of the study. The PI and other key personnel will be examining research data on a subject to subject basis to ensure it is accurate and to address any potential problems that may arise with the protocols. In addition, Dr. Shook will examine data with KU team at least once per month through in person meetings or by phone to ensure that data collection is occurring in a consistent manner at both locations (KUMC and CMH).

**Plan for adverse event reporting**

Monitoring and reporting adverse events, whether anticipated or unanticipated, is the responsibility of the PI. The reporting requirements of the CMH and KUMC IRB's and for serious and unexpected adverse events will be followed. All adverse events will be shared with Dr. Shook immediately and shared with the Board Chair and the DSMB within 48 hours of PI notification.

**Frequency of monitoring**

In addition to monitoring by the PIs, study coordinators, and safety officer, the IRB monitors all aspects of the project on an annual basis. The DSMB will review study progress (e.g., recruitment, retention, protocol adherence) and every six months at the semi-annual closed door session, per the DSMB charter. At the semi-annual DSMB session, the PI will provide a report detailing:

a. List and summary of adverse events
b. Whether adverse event rates are consistent with pre-study assumptions
c. Summary of recruitment and retention and reason for dropouts
d. Whether the study is on track to be completed and accomplish the stated aims.

**Section 4.1.6. ClinicalTrials.gov Requirements**

In accordance with Public Law 110-85, this clinical trial will be registered in ClinicalTrials.gov.

## 21.0 Provisions to Protect the Privacy Interests of Subjects

*21.1* Study participants will be given a study ID number and all the information they will out for the study will only have that ID number on it. The participant will only be linked to the study with their permission/assent form and a master linking list that connects their ID and name.

*21.2* Participants will be given ample time to think about participation including taking the permission/assent form home to think about it before signing up if they prefer. They will also be told about the study over the phone before coming in for the orientation visit.

*21.3* The study team will be given access to a linking list of participants so they may identify participants in this way to call and remind them of visits, etc. The study team will not leave detailed messages, will not post on social media, and will not share the name or any other PHI with anyone who is not a member of the study team.

*21.4* Identifiers recorded for this project: Name, Date of Service, Date of birth, medical record number, hospital account number, Street address, Telephone number, Email address, Social Security number (for payment purposes only).

*21.5* The research team will be obtaining HIPAA Authorization from subjects/parents to access and/or record PHI for this research study.

- the HIPAA Authorization will be wrapped in with the permission/assent/consent form(s)

## 22.0 Compensation for Research-Related Injury

*22.1* N/A – not more than minimal risk

*22.2* N/A – will use standard NIH contract language

## 23.0 Economic Burden to Subjects

*23.1* N/A

## 24.0 Permission/Assent/Consent Process

*24.1* Indicate whether you will you be obtaining permission/assent/consent, and if so describe:

- P/A/C will be obtained electronically via eConsent over the phone or in person at the Don Chisholm building via eConsent.
- Participants will be invited to ask questions and if they are uncomfortable enrolling at the time of the PAC conversation they will be told they can take the form home to think about it.

- As procedures are happening through the study they will be explained to the participant (and their parent/guardian).
- We will be following CM research policies on informed permission/assent/consent.

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

- Individuals <18 years
- Parental permission will be obtained from a singular parent.
  - Permission may be obtained from individuals other than parents. These people will be determined to be Legally Authorized Representatives via court documentation either provided to the study team or already in the CMH HER.
- Assent will be obtained from all children (our inclusion criteria includes only children of the age of assent).
- When assent of children is obtained describe how it will be documented – children will sign and date the Permission Assent document.

***Consent at 18 years of age, when minor subjects become adults***

- We will re-consent any participants that turn 18 during the study with an adult addendum.

***Non-English Speaking Subjects***

- We plan to enroll only English speaking participants, due to the limitations of coordinating study visits with the assistance of an in-person interpreter.
  - We will utilize translation services or Cyracom to obtain permission/assent for any Spanish speaking parents of participants. We would request ORI to provide translations of our uploaded surveys and P/A forms.
-

## 25.0 Process to Document Permission/Assent/Consent

### *25.1* The study team will obtain permission/assent/consent via eConsent Lite over the phone or in-person at the Don Chisholm building at CM

- The p/a/c process will take place via telephone or in-person at the Don Chisholm building on CM campus

- The potential participant will have a chance to ask questions and decide if they'd like to participate.
- The only ongoing P/A/C process will include an adult addendum, if necessary for a participant that turns 18 during the duration of their study participation
- The Study team will be following CM research policies on informed permission/assent/consent.
- The study team may obtain permission/assent/consent via telephone, and will follow CM research policy (10.05 Telephone Process).
- The study staff listed on the IRB, as well as the study coordinator, and PI's, may complete consent
- Participants and their families will be given plenty of time to consider participation (they can decline to participate during the consent appointment and request follow up) as well as ask any questions they have.
- Study staff will explain each main section of the p/a/c form as well as provide a summary. Checking for understanding while going through the form.
- The study team will follow CM research policy for phone consent, if necessary (not in-person). This policy as well as other helpful guidance on the use of alternative communication methods to obtain permission/assent/consent are available in 10.05 Telephone Process.

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

- The study team will consider participants in the sample age 14 - 17 not yet adults.
- One parent will provide permission, even if the other parent is alive, known, competent, reasonably available, and shares legal responsibility for the care and custody of the child.
- Permission may be obtained from individuals other than parents. These people will be determined to be Legally Authorized Representatives via court documentation either provided to the study team or already in the CMH records.

## 26.0 Setting

*26.1* Describe the sites or locations where your research team will conduct the research.

- Participants will be identified and recruited from CMH, KUMC, and the community.
- Research procedures will be performed at CMH and KUMC.
- We will not be conducting research at any institutions outside the organization that have site-specific regulations or customs affecting the research or local scientific and ethical review structures outside the organization.

## 27.0 Resources Available

*27.1* Describe the resources available to conduct the research: For example, as appropriate:

- See the Power Analysis for more information about the sample size determination. All Study Personnel will complete research training required by their institution.
- The following resources are available for the Energy Balance lab at Don Chisholm: DXA, laboratory equipped with freezer and hood, blood draw capabilities, exam rooms, fitness test equipment (treadmill, mask, monitors), thermic effect of feeding equipment. Study staff and graduate student support to complete the study measures as well as a kitchen on site to prepare the fixed/ad lib meals is also available.
- There is an established partnership with KUMC and Dr. Shook. fMRI procedures are being utilized for another study that Dr. Shook is leading at the center currently. Dr. Shook is a volunteer faculty in the KUMC Department of Pediatrics.

## 28.0 Multi-Site Research

***Choose ALL relationship types that apply:***

☐ ***Multi-Site Research:*** *Multiple sites will be engaged in this human research project. Sites will use the* ***same*** *protocol to conduct the* ***same*** *human research activities (except for minor variations due to local context considerations).*

☒ ***Collaborative Research:*** *Multiple sites will be engaged in this human research project. Sites will* ***not*** *be performing the* ***same*** *research activities. The Site submission will specify the specific research activities each site will perform.*

***REQUIRED****: Enter summary of site-specific activities that differ from the overall protocol: The insulin clamp procedures will be happening at the*

*KUMC CTSU. Also, we will have KUMC study personnel assisting with study procedures, data analysis, and manuscript preparation.*

☐ ***Student(s)*:** Student(s) will help with this project and will be engaging their home institution.

☐ **Visiting *Resident(s) /* Visiting *Fellow(s)*:** Visiting Resident(s) / Visiting Fellow(s) will help with this project and will be engaging their home institution.

**Is Children's Mercy (CM) acting as the single IRB of Record (sIRB)?**

☐ ***No, each site is getting their own IRB approval.***

☒ **Yes, some or all sites will rely on the CM as the sIRB**.

- ***Reliance is required*** *for non-Exempt* ***NIH*** *or other* ***Federally Funded*** *research where:*
  - *The institution's employees or agents intervene or interact with human subjects for research purposes;*
  - *The institution's employees or agents obtain individually identifiable private information or identifiable biospecimens about human subjects for research purposes; or*
  - *The institution receives a direct HHS award to conduct human subjects research, even where all activities involving human subjects are carried out by a subcontractor or collaborator.*

**If CM is sIRB for another site, complete the chart for that site(s) (***Add a new row for each site relying on the CM IRB, delete chart if not acting as sIRB):*

| Site Name | Enrollment Goal for Site(s) *Choose One* | Relying on CM IRB? |
|---|---|---|
| University of Kansas Medical Center | ☐ **Site Enrollment Goal:** *Insert #*<br><br>☒ **Site will not enroll** | ☒ External Site will rely on the CM IRB as the IRB of Record using a reliance agreement. ()<br>☐ **Not Applicable**. Site will **not** interact or intervene with human participants or their identifiable data / identifiable biospecimens. Site is also not a primary NIH or federal grant recipient. |

| | | |
|---|---|---|

## 29.0 International Research

29.1. N/A

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