Pilot Data Collection for Activity, Adiposity, and Appetite in Adolescents

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Study Site(s): Children's Mercy Hospital (Don Chisholm Center) and University of Kansas Medical Center (Hoglund Brain Imaging Center)

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1. STUDY OBJECTIVES/HYPOTHESIS

We hypothesize that low levels of fitness and MVPA result in impaired metabolic function in adolescents. For this pilot study, we will quantify variations in metabolic characteristics using a 2 x 2 cross-sectional study design, stratified by bodyweight (normal vs overweight/obese) and physical activity (sedentary vs. active). Our goal is to identify metabolic factors that influence the energy balance system to directly inform interventions designed to prevent or reduce the prevalence of pediatric obesity.

Aim 1: To quantify the independent effects of physical inactivity and obesity on metabolic responses at rest, after a meal, and during exercise using indirect calorimetry.

Aim 2: To determine whether physical activity level impacts appetite control independent of obesity status via metabolic function.

Aim 3: To explore the hormonal and neurocognitive mechanisms of appetite control.

2. RATIONALE

At the most basic level, obesity is the result of a chronic energy surplus within the energy balance system. However, an assortment of physiological, psychological, and environmental factors are related to energy intake and expenditure, resulting in highly variable individual responses to identical intervention strategies [1, 2]. For example, a recent rigorous 10-month weight loss study observed a 5.6% average reduction in body weight, yet individual weight change ranged from a 22% weight loss to a 4% weight gain [3]. Physiologically, differences in energy metabolism likely explain much of this variation [4, 5], and identifying those individuals metabolically at risk for obesity or resistant to traditional weight loss strategies is a 'key challenge' of obesity researchers [6]. Thus, there is a critical need to identify metabolic factors that influence the energy balance system in order to develop effective interventions to treat obesity or prevent its occurrence. This project will clarify the relationship between physical activity, obesity status, and appetite control in adolescents.

3. STUDY DESIGN

The proposed pilot study consists of a 2 x 2 cross-sectional study design (Table 1), which will maximize statistical power despite a small sample size. Inclusion/exclusion criteria for MVPA and body weight are based on established criteria from governmental agencies and clinical practice.

4. TARGET STUDY POPLUATION SPECIFICS

Participants will include 60 adolescent males (aged 14-18 years, Tanner stages III- IV), equally enrolled based on physical activity and body weight status (**Table 1**).

Table 1. Enrollment criteria based on physical activity and body weight status

Adolescents males (age 14-18 years & Tanner stages III- V)	Normal weight ($\geq 5^{th}$ to $<75^{th}$ percentile)	Overweight/obese ($\geq 85^{th}$ to $<99^{th}$ percentile)[41]
Inactive (<30 min/day MVPA)	n= 15	n= 15
Active (>60 min/day MVPA)[42]	n= 15	n= 15

Inclusion Criteria

- Males (Tanner Stage III-IV), ages 14-18
- Weight stable (±5% weight change) over the previous three months as assessed by self-report.
- BMI ≥5th and ≤ 99th percentile
- Physically healthy for exercise as assessed by self report Physical Activity Readiness Questionnaire (PAR-Q) for children.
- For fMRI testing, all participants must be right handed and must have normal or corrected to normal vision (as they will be viewing pictures in the fMRI procedure).

Exclusion Criteria

- History of restrained eating, eating disorders, bariatric surgery, or other significant medical diagnosis that could impact metabolism.
- Participants taking thyroid medications, beta blockers, or other stimulants (medications are known to affect physical activity level and metabolism).
- Due to fMRI procedure requirements, children with a history of Attention Deficit Hyperactivity Disorder (ADHD) or other diagnosed psychiatric issues will be excluded.

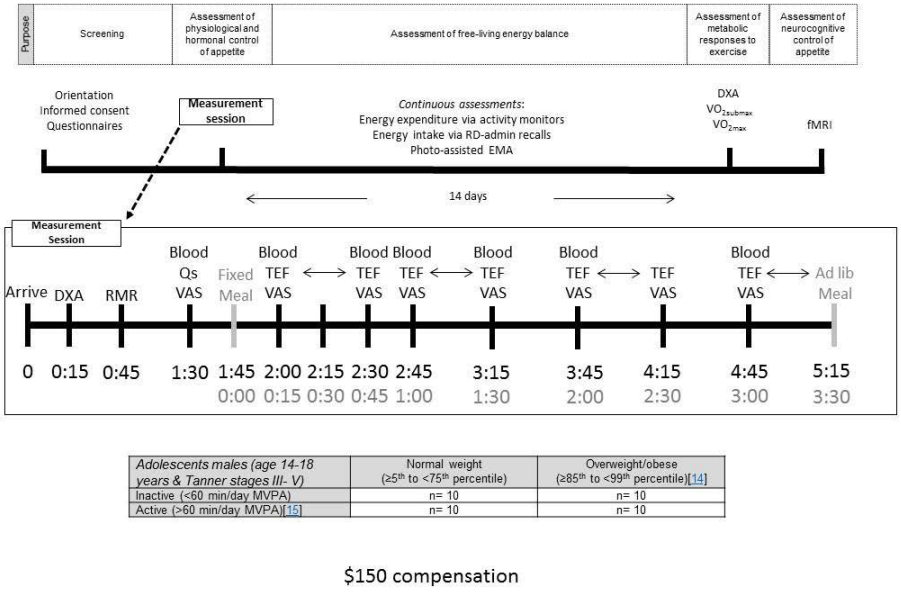
5. DATA COLLECTION

Data Collection Procedures

The study will include 4 visits and 1 home assessment window:

- Visit 1 - Orientation (Consent, Screening Questionnaires) at Don Chisholm
- Visit 2 - Clinic Measurements
    - Body composition, via dual energy X-ray absorptiometery (**DXA**)
    - Resting metabolic rate (**RMR**), via indirect calorimetry;
    - Metabolic responses to a meal, via thermic effect of feeding (**TEF**)
    - Subjective aspects of eating behavior using the Three Factor Eating Questionnaire (**TFEQ**) and Control of Eating Questionnaire (**CEQ**)
    - Appetite, via visual analogue scales (**VAS**) pre- and post a prepared fixed meal and hormones related to satiety and hunger (insulin, leptin, ghrelin, peptide YY, glucagon-like peptide-1) obtained during blood samples (**BLOOD**) from an indwelling catheter.
    - All measurements will occur at the CHLN (<9:00am) following an overnight fast.
- Home assessments - Data collected at home during a 14 day window
- Visit 3 - Fitness Test ($VO2_{submax}$ and VO_{2max}), grip strength, and DXA at Don Chisholm
- Visit 4 - fMRI at University of Kansas Medical Center

Please see Figure 1 below for schedule of measures:



Adolescents males (age 14-18 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

$150 compensation

Figure 1. Description of Measures:

Metabolic function.

- Resting metabolic rate (**RMR**) will be assessed via indirect calorimetry using a ventilated hood following an overnight dietary fast and ≥24-hours since structured exercise (e.g. sports participation).
- Thermic effect of feeding (**TEF**) will measure the energy expenditure resulting from digestion following the fixed meal using the ventilated hood.
- Submaximal oxygen consumption (fitness test) (**$VO2_{submax}$**) and maximal oxygen consumption (**VO_{2max}**) will be assessed with a full-face mask with headgear via standard protocols using a treadmill. Measured during visit 3.

Body composition.

- Assessment of body composition, including fat mass, fat free mass, and visceral adipose tissue, will be completed using dual energy X-ray absorptiometry (DXA). All anthropometric measurements will be performed with the participant dressed in surgical scrubs and in bare feet. BMI (weight [kg]/height [m]2) will be calculated from the average of three height and weight measurements using a wall-mounted stadiometer and electronic scale and recorded to the nearest 0.1 cm and 0.1 kg, respectively.

Fixed/*Ad libitum* meals.

Appetite (see below) will be assessed following a fixed standard breakfast of 500 kcals. The fixed breakfast will be administered while the participant is in a fasted state (≥12 hours since last meal) and at least 24 hours since the last bout of exercise, and they will be asked to consume the meal within 15 minutes. Food intake (total kcals) will be assessed using an ad libitum pizza 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 pizza meal. Meals will be provided in 900 kcal portions, and each

plate of food will be weighed before and after serving, without the subjects' knowledge, and the amount consumed recorded. All meals will be served in a quiet dining room free from distractions located at the NWRC.

Appetite and Eating Behavior.

- Subjective assessment of hunger and palatability will be completed using self-rated visual analogue scales (**VAS**). Each scale consists of a 10 cm long line separating statements such as "not at all hungry" and "extremely hungry". Assessments will occur prior to the fixed meal, and every fifteen minutes for the first hour and every thirty minutes for the next two hours.
- Perceptions of dietary restraint, disinhibition, and hunger will be assessed prior to the fixed meal using the Three Factor Eating Questionnaire (**TFEQ**). The Control of Eating Questionnaire (**CEQ**) will be used to assess hunger, fullness, cravings, the desire to eat certain foods, and the ability to resist urges to eat. Assessed at visit 2.
- Blood samples obtained from an (insulin, leptin, ghrelin, peptide YY, glucagon-like peptide-1). Blood will be drawn at the following approximate times: -15 min (x2), 15 min, 45 min, 60 min, 90 min, 120 min, 180 min. Due to the number of blood draws, for participant safety and comfort a flexible, vacutainer accessible catheter will be placed by a registered nurse (who will also perform all blood draws). A total of 32 mL (1.08 oz) of blood will be drawn.

Lifestyle Assessment.

- Energy intake will be assessed using two methods: 1) objectively, as measured by amount of kcals consumed during an ad libitum meal (see above, section Fixed/Ad libitum meals), and 2) subjectively, assessed via interviewer-administered dietary recalls. Three dietary recalls will occur on randomly selected non-consecutive days over each two-week treatment condition (including at least one weekend day) to minimize preparation that could bias recall by the participants. All interviews will be conducted over the telephone, will ask about all foods consumed over the previous 24 hours using a multi-pass approach, and energy intake will be calculated using the Nutrient Data System for Research software (NDSR Version 2014).
- Energy expenditure will be estimated using an arm-based monitor (Sensewear™), which calculates energy expenditure using tri-axial accelerometry and measures of heat flux, galvanic skin response, skin temperature, and near-body ambient temperature. Assessed continually (day and night) over the 14 day home assessment period.
- Sedentary behavior will be assessed using a leg-mounted triaxial accelerometer with inclinometer (ActivPal™) which classifies time spent in sedentary, upright, and stepping activities. Assessed continually (day and night) over the 14 day home assessment period.
  We will assess MVPA using a wrist-worn activity triaxial accelerometer (Actigraph GT9X Link™) which quantifies time spent in light activity and MVPA. Assessed continually (day and night) over the 14 day home assessment period.

SmartPhone prompts to assess cravings, anticipation of eating, and hunger
The SmartIntake™ smartphone app will be used as a photo-based ecological momentary assessment (EMA)**.**

- **Craving**: We will use a single-item craving prompt to assess current food craving level: "Please rate on a scale from 0-100 the extent to which you agree with the statement: I am craving food."
- **Anticipation of eating**: We will use a single item from Nijs et al (2007) General Food Craving-State Questionnaire to assess anticipation of eating: "Eating something tasty would feel wonderful." We have chosen this prompt because it is agnostic as to the rationale for anticipating eating.
- **Hunger**: We will assess pre-meal hunger to account for the temporal relationship between hunger and craving in deprived and non-deprived states (Cepeda-Benito et al., 2000). Although hunger is sometimes conceptualized as an aspect of craving, our purpose is to distinguish physical hunger from craving to avoid confounds. We will use an adapted prompt from Goldschmidt et al (2014) "Please rate the extent to which you agree: I feel physically hungry, on a scale from 0-100, with 0 being not hungry,100 being extremely hungry."

fMRI Procedures.

- The fMRI study visit will take place at visit 4. Children will be met at the Hoglund Brain Imaging Center, part of the University of Kansas Medical Center, for the fMRI portion of the study. This visit to Hoglund will take approximately 1.25 hours. Children will take complete a computized behavioral food-rating task which asks the child to rate sixty food images on how the food tastes, how much the child would like to eat the food, and how healthy is the food. Then the child will complete a food-decision task while in the MRI. The child will see a picture of a food on the screen, and will choose which foods he/she would prefer to eat. Stimuli include items often consumed by children, and were selected to include a wide range of tastiness and healthiness attributes (for example, apple, broccoli, asparagus, glazed donut, French fries and marshmallows). This test will measure how the brain responds to making decisions about food. All food pictures are high-resolution (72 d.p.i.) color images with a size of 300300 pixels. The stimulus presentation and behavioral response collection will be controlled by Presentation software (Neurobehavioral System).
- Additional questionnaires collected at this visit include Monetary choices, Flanker, and Consideration of future consequences.

<u>Records to be kept</u>
Data collected will be deidentified with a subject ID number. A linking list will used, which will contain the subject ID, subject's name, gender, and date of birth. This log will only be accessible be the PI and the study team.

<u>Secure Storage of Data</u>

Consent statements and participant data in separate locked file cabinets so that individuals are not easily connected to the study results. All digital data will be stored on a firewall and password-protected project server at Children's Mercy Hospital. Data collected at the CMH Don Chisholm Center CMH will be analyzed and stored in secure shared drive at the Don Chisholm Center. Blood samples will be collected at Don Chisholm and shipped to Quest for processing. fMRI data will be collected at Hoglund Brain Imaging Center at KUMC and will be analyzed by KUMC study personnel at KUMC. This data will be deidentified, and will be sent back to the PI at CMH using secure file transfer.

SmartIntake App Data Security:

The app was developed and validated by Dr. Corby Martin and colleagues at the Pennington Biomedical Research Center (PBRC) at Louisiana State University. The company Rapid Development Productions (RapDevPro) worked with Dr. Martin and colleages to develop the app. By design, Dr. Martin and colleagues set up this app and secure data hosting platform to be available to researchers nationally and his team provides researchers data analysis services for the dietary intake data. Reseachers can sign a contractagreement to utilize the app for research purposes. No identifying information will be collected through the SmartIntake photo-EMA app.

SmartIntake is used to capture images of food and beverage intake before and after consumption. Photos are sent directly to a secured data server (see details of server security below) for analysis of dietary intake. Participants initiate each photo by opening the app, taking the picture, and then submitting the picture through the app. The app sends a reminder email 15 minutes after submission of the first photo to remind participants to submit their post-consumption photo. The app sends photos to the secured server by email, thus an email address is required to send the photos. Participants will be provided a secured email address that does not contain or link to any identifying formation, such as their names, etc, that the app an use to send the images. When images are sent to the server, the email address is stripped from the images before they are stored; thus all data stored on the PBRC server is deidentified. The app does not utilize, store, or transfer info from other mobile phone data sources including contacts or geolocation.

To minimize the risk of caputring identified data in the photos, participants will recieve a training. Participants will be told that they should never include pictures of other people or their surroundings (bar signs, etc) in their photos. We will emphasize that we have a responsibility to collect strong research data while protecting those around them from inadvertently being included in photos without their consent. Participants will be shown that once the photo is taken, it will be stored on their phone's photo app. We will show participants how to go into their photo app and delete the picture taken with SmartIntake. We will tell them they should do this immediately after sending the photo in SmartIntake.

Photo data stored on the secured PBRC server: The only data stored with photo images is the unique participant ID. Email address is stripped from the data before storage to the server. Thus, only non-identifying info is stored on the PBRC server for analysis. The server that the data is stored on is in the PBRC domain and requires PBRC user credentials

to access the files stored on that server. Only Dr. Martin and his staff who directly manage or analyze the app data have access to the files where the images are stored. All PBRC logins are required to have a password of 15 or more characters and must be changed on a regular basis.

NOTE: The photo image data collected by the SmartIntake app will NOT be transferred to CMH at any point. The study PI will receive the deidentified dietary intake data that will be transferred through the PBRC secured data transfer system.

6. STUDY DURATION/STUDY TIMELINE

Target start date is May 2017
Stage 2, recruitment and data collection, June- November 2017
Stage 3, data analysis and grant/manuscript development, December 2017- May 2018
Total study duration will be 1 year.

We anticipate 4-6 waves of enrollment, with each participant enrolled for 4 weeks. Following an orientation (visit 1) during which informed consent will be obtained, individuals will participate in a clinic measurement session (visit 2), a 14-day free-living lifestyle assessment period, a 2nd laboratory visit (visit 3) for an exercise testing session, and conclude with a fMRI assessment (visit 4) All laboratory activities will occur at the CCHLN research facility, and the fMRI will occur at KUMC Hoglund Brain Imaging Center.

7. STATISTICAL CONSIDERATIONS

Measures

- Primary measures: Metabolic function and body composition
- Secondary measure(s): Demographics, appetite, food cravings, and eating behaviors, lifestyle assessment, fMRI, energy intake

General Design Issues

The proposed pilot study consists of a 2 x 2 cross-sectional study design, which will maximize statistical power despite a small sample size. This pilot study will generate data needed for a larger study.

Sample size determination

As a pilot study, we will have resources to collect data from N=60 participants. We will use this data to determine power requirements for a larger study.

Data Analyses

Statistical significance for comparison between groups will be tested using analysis of variance (ANOVA) for continuous variables and chi-square tests for categorical variables. Statistical significance will be set at $P<.05$ (two-sided) for all analyses.

fMRI Analysis. We will estimate several general linear models (GLMs). All of the models allow for first-order auto regression and included six motion parameters,

constants, and linear time trends for each run as regressors-of-non-interest. A two-stage mixed-effects analysis will be performed in which the regression coefficients for each condition of interest will be tested across participants via t-tests. Two-tailed tests are used for all statistical analyses. We will perform multiple comparison corrections at the cluster level using Monte Carlo simulations with the AlphaSim program.

EMA-Food Craving Analysis. A regression model will test whether mean EMA ratings of anticipation (craving) and reward (pleasantness rating, ate more than intended, ate beyond satiety) are correlated with postprandial glucose surge and area under the curve for glucose following the standardized lab meal.

We will use a hierarchical mixed model to examine the nested association between EMA-measured craving, food pleasantness, and overeating *within* eating episodes in free-living conditions. Pre-meal craving and within-meal pleasantness ratings will be used as predictors in models with overconsumption rating post-meal. Food photos will be used to distinguish HF from non-HF (fat/carb: 25%+/25%+; sweet/fat: 15-35%/30%+; salt-fat: 0.5-2.5%/10-35%) using the detailed NDSR nutrition data output we will receive from the app.

We will conduct a series of Pearson correlations to test whether craving and reward symptoms measured in the standardized meal and free-living conditions are significantly correlated.

We will use a linear regression model to test the degree to which anticipation (craving) and reward (food enjoyment, eating despite satiety) as measured using photo-EMA, and intensity of glucose surge during the standardized lab meal will be associated with % fat mass and visceral adipose tissue as measured using DXA.

8. HUMAN SUBJECTS

Institutional Review Board (IRB) Review and Informed Consent

The IRB of record is Children's Mercy Hospital.

This protocol, and any subsequent modifications, will be reviewed and approved by the Pediatric IRB at The Children's Mercy Hospital & Clinics.

Potential participants will be recruited from our various weight management programs at the Center for Children's Healthy Lifestyles & Nutrition (CHLN), from our electronic medical records at Children's Mercy Hospital and the University of Kansas Medical Center, and from the community. We plan to use the available patient registries at CMH and KUMC to mail our recruitment materials to a random number of patients who meet the inclusion/exclusion criteria. We will also place out recruitment materials in the Primary Care clinics at CMH and KUMC. Recruitment materials will be distributed in the community through our partners via the Weighing In and Healthy Lifestyle Initiative listserves. Based on past experience recruiting for similar studies, we anticipate needing

174 initial contacts and 82 screens + dropouts to achieve our goal of 60 completed participants.

Parents of potential subjects will contact the study team using the phone number or email address on the recruitment flyer. The study team will do a 10 minute phone screening with the parent to determine eligibility, and will schedule a time for the parent and child to come in for an orientation. The parent and child will them come to the Don Chisholm center at CMH for an orientation. The consent form will be reviewed and ample time will be given for the parent and child to consider participation. Prior to drawing any blood or performing any other procedures related to this study, the permission/assent form or consent form will be reviewed carefully with the participant and parent in person. If the parent and child agree to participate, further screening questions will take place. The family will then schedule a time to come in for the measurement session (Visit 2).

Subject Confidentiality

All records will be kept in a locked file cabinet. Human subject's names will be kept on a password protected database and will be linked with a study identification number for this research. All computer entry and networking programs will be done using study identification only. All data will be entered into a computer that is password protected. Data will be stored in a locked office of the investigators and maintained for a minimum of three years after the completion of the study.

Subject Payment

Subjects will be paid via ClinCard $75 when the activity monitors are returned at visit 3 and upon completion of the study at visit 4. Total payment per subject is $150. SSN is required for payment, and will be entered directly into the Greenphire secure website. SSN will not be retained in the subject's study file.

9. PUBLICATION OF RESEARCH FINDINGS

- Results are intended to be presented at pediatrics or physical activity-related conferences by the study team within the next year.
- Results are intended to be published in a physical activity or public health journals.

10. REFERENCES

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