Protocol Title: Break It Up: A study evaluating breaking up daily sedentary behavior in youth.

Abbreviated Title: Evaluation of interrupting daily sedentary behavior

Protocol Number: **17-CH-0130**

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Principal Investigator:

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Study type (check all that apply):

x Archived biological specimens/medical information
Natural history; definition of phenotype, genotype/phenotype correlation
Prospective linkage/gene identification, NOT providing information to participants
Prospective linkage/gene identification, providing information provided to participants
Social science; assessments of knowledge, attitudes and behavior
Genetic counseling
Drugs or devices
Gene transfer
x Other interventions

Estimated Duration of study: 2 years

Human Research Protections Program Investigator and Staff Training:

For this protocol, the following "Just in time" human subjects protection training courses are required for investigators and staff:

- Biomedical- Vulnerable Subjects - Research with Children
- CITI GCP modules

- Unanticipated Problems and Reporting Requirements in Biomedical Research
- Unanticipated Problems and Reporting Requirements in Social and Behavioral Research

Total requested accrual

Subjects of Study:	Number	Sex	Age Range
Healthy Cohort (BMI 5th to < 85th percentile)			
--Screened but not enrolled	15	M, F	7-11.99 y at study entry
--Randomized	60	M, F	7-11.99 y at study entry
Overweight Cohort (BMI ≥ 85th percentile)			
--Screened but not enrolled	15	M, F	7-11.99 y at study entry
--Randomized	60	M, F	7-11.99 y at study entry

Project Uses Ionizing Radiation: ☐ No ☒Yes

☐ Medically-indicated only

☒ Research-related only (*attach RSC/RDRC documentation)*

☐ Both (*attach RSC/RDRC documentation)*

IND/IDE ☒ No ☐Yes *(attach FDA documentation)*

Drug/Device/#_____

Sponsor: _____

Durable Power of Attorney ☒ No ☐ Yes

Multi-institutional Project ☒ No ☐ Yes

Data and Safety Monitoring Board ☒ No ☐ Yes

Technology Transfer Agreement ☐ No ☒ Yes

Samples are being stored ☐ No ☒ Yes

Flesch-Kincaid reading level of consent form: ____9.6_____

Flesch-Kincaid reading level of assent form: _____5.8_____

Précis:

Background: Sedentary behavior is defined as a set of low-intensity activities involving limited body movement (e.g.: TV viewing, prolonged sitting). Some studies have found higher levels of childhood sedentary behavior predict higher body mass index (BMI) and metabolic abnormalities. We and others have found that interrupting sitting with short, 2-3 minute bouts of moderate activity (walking) can improve glucose tolerance during a single session. Thus, interrupting sedentary behavior may be an intervention strategy to reduce health risks. **Objective:** We propose to conduct a randomized pilot study to assess whether interrupting sedentary behavior for 6 consecutive days provides sustained improvement in carbohydrate metabolism without negatively impacting executive function, attention, mood, anxiety, dietary intake or usual physical activity. **Design & Population:** Using a randomized parallel group design, children, ages 7-11.99 years, will complete an assigned randomized condition of either 6 consecutive days of 3 hours of monitored sedentary activity (sitting) or 6 consecutive days of 3 hours of interrupted sitting (in which they will be prompted to walk for 3 minutes every 30 minutes). **Outcome measures:** Twenty-four-hour continuous glucose monitoring and postprandial insulin incremental area under the curve (iAUC) on post-condition oral glucose tolerance testing will be the primary measures. Secondary measures include: postprandial glucose iAUC, executive function, attention, mood, anxiety, dietary intake, and free-living physical activity. **Impact:** This project will investigate if consecutive daily interruption of sitting behaviors improves glucose tolerance, a potential negative health consequences of sedentary behavior in children. If repeatedly interrupting sitting with short bouts has sustained beneficial effects among children, interventions examining the frequency, duration, and intensity of such interruptions could be developed for use in the community setting. Thus, these results have the potential to provide insight into novel behavioral intervention targets in youth.

II. Table of Contents

Appendix G: Questionnaires (Hollingshead, IPAQ, Daily Health History)

Appendix G2: Clinical Trials Survey System Questionnaires

List of Abbreviations

- BMI: Body mass index
- CGM: Continuous Glucose Monitor
- IMCL: Intramyocellular lipid content
- MAGE: Mean amplitude glycemic excursion
- MET: Metabolic equivalent of task
- MRS: Magnetic Resonance Spectroscopy

1. Introduction and Background

Pediatric Obesity and Sedentary Behaviors

Excessive weight gain in children is one of the foremost global health concerns because of its metabolic consequences.[1, 2] In the United States, nearly 1 in 5 children who are 6-11 years old are considered obese (BMI $\geq 95^{th}$ percentile) by the Centers for Disease Control and Prevention standards, almost quadrupling since 1971.[3] Excessive weight gain in childhood is associated with many adverse outcomes. Children with excessive weight have increased incidence of impaired glucose metabolism, dyslipidemia and cardiovascular strain. [4, 5] Long term, such children demonstrate increased incidences of musculoskeletal complications[6-8] and have more difficulty with chronic illnesses such as asthma[9] and depression.[10] These childhood morbidities are reason enough to demand investigation and intervention but childhood obesity is also predictive of adult obesity and with it numerous adult morbidities including cardiovascular disease, metabolic syndrome, cancer, and increased all-cause mortality.[11, 12]

The single most important factor of pathologic weight gain is an unhealthy balance between energy intake and energy expenditure. One component of energy expenditure that has received more attention in the last decade is sedentary behavior.[13, 14] Sedentary behavior is defined as low-intensity [<1.5 metabolic equivalent of task (MET)] activity that involves limited body movement.[14] Sedentary behavior encompasses TV viewing, computer use and prolonged sitting, and is highly prevalent in the modern society of developed countries.

Adult Studies on Sedentary Behavior and Obesity

There is a growing body of work describing the influence of sedentary behavior on health outcomes in adults. Field studies provide support for the role of sedentary behavior in poor health outcomes. In a classic study, Morris et al. observed that sedentary bus drivers had higher rates of cardiovascular disease (CVD) than the more active conductors, independent of waist circumference.[15] Similar increased CVD risk is seen in patients with spinal cord injuries who are forced to spend the majority of the day seated.[16] This is supported by a review of longitudinal studies that suggest a consistent positive relationship between sedentary behavior, mortality and

weight gain from childhood to adulthood.[17] These findings support the negative health effects of sedentary behavior that may begin in childhood and increase the risk for disease in adulthood.

Pediatric Studies on Sedentary Behavior & Obesity

Using data from the National Health and Nutrition Examination Survey (NHANES), Matthews et al. estimated that U.S. 6-11 year olds spend over 6 hours per day in sedentary behaviors.[18] Furthermore, a high amount of time spent in sedentary behaviors is associated with pediatric obesity.[19-21] This is supported by the finding that sedentary behavior in youth predicts greater gain in BMI from 9 to 15 years[22] independent of moderate to vigorous physical activity (MVPA). Summative data from a recent meta-analysis reported that nineteen longitudinal studies found greater time spent watching TV at baseline predicted greater increases in BMI and fat mass over time,[23] and other reviews indicate that the majority of studies report positive associations between reported TV viewing and body fat mass.[24-27]

Sedentary Behavior & Metabolic Dysfunction

In addition to weight gain, sedentary behavior has been associated with metabolic dysfunction. Extensive epidemiology and clinical observational data in adults associate negative metabolic outcomes and sedentary behavior.[28-31] For example, Healy et al. evaluated markers of the cardiometabolic profile and accelerometer-derived activity in 4757 subjects >20 years in the US National Health & Nutrition Examination Survey (NHANES).[31] This study identified persistent negative associations such that, while controlling for moderate to vigorous activity, increased sedentary time in adults was associated with worsening HDL-cholesterol, C-reactive protein, triglycerides, and the homeostatic model of insulin resistance.[31] Additionally, Balkau et al. identified a significant association between sedentary time (also recorded with accelerometry) and insulin resistance as measured by hyperinsulinemic-euglycemic clamps in 801 adults ages 30-60 years.[28] This finding was only partially attenuated when adjusted for body fat.[28]

However, data have been more contradictory in youth. In 308 children ages 9-10 years old, accelerometer-measured sedentary behavior was significantly associated with insulin resistance as measured by the homeostatic model of insulin resistance independent of fat mass.[32] Conversely, using a large pooled convenience sample of 20,871 children ages 4-18 years supplied by the International Children's Accelerometry Database, Ekelund et al. reported that

total sedentary time measured by accelerometry was not associated with cardiometabolic risk (as measured by waist circumference, systolic blood pressure, fasting triglycerides, high-density lipoprotein or insulin) after adjusting for MVPA.[33] The conflicting findings among the studies may be related to age, as sedentary time is higher in adolescence than childhood,[34] and may change the power to detect associations. The conflicting findings in pediatric studies may also be due to how sedentary behavior is measured. The majority of published studies operationalized sedentary behavior as TV viewing time. TV viewing has traditionally been assumed to be a good measure of sedentary behavior in children, yet it is weakly correlated ($r < .20$) with device-based measures (e.g.: accelerometers) of sedentary time,[35, 36] suggesting that they are measuring different behaviors. These screen-based behaviors may be a marker for other behaviors associated with poor health or socioeconomic status that could drive relationships with adiposity.[24] However, other self-report measures designed to capture multiple sedentary behaviors (e.g.: the 3-day physical activity recall) have found that sedentary behavior is associated with increased risk of metabolic disease.[37] In-lab studies have the potential to greatly contribute to this area through the investigation of basic biological pathways between sedentary behavior and metabolic characteristics and clarify the existing literature.

Thus, in summary, recent data suggest that sedentary behavior may be contributing to the perpetuation of the high prevalence of pediatric obesity and metabolic dysfunction. Diet and increasing physical activity have been largely unsuccessful at producing long-term effects on body fatness.[38-40] There is a need for novel behavioral intervention strategies to prevent pediatric obesity and its metabolic complications. Interrupting sedentary behavior is an innovative technique.

Intervention: Limiting Sedentary Behavior

Limiting sedentary behavior has been proposed as a strategy for pediatric obesity prevention and intervention. [1, 41-43] Sedentary behavior has most often been operationalized as TV viewing or screen-based behavior. In a seminal randomized controlled trial, Robinson et al reported that children who received an intervention aimed at reducing TV viewing had significant decreases in BMI, waist circumference, and waist-to-hip ratio.[44] A recent review reported that interventions aimed at reducing TV viewing produce modest improvements in weight parameters and slowing of BMI increases during childhood and adolescence.[45] However,

many of these interventions included other health messages, so it is difficult to distinguish the contributions of the TV-reduction messages alone. Also, continued research on the physiologic mechanisms linking sedentary behavior and obesity may help explain potential behavioral intervention targets that go beyond TV viewing.

Effects of interrupting sedentary behavior

As humans continue to spend the majority of their day sedentary,[46] it is important to investigate how intervening on this behavior influences health outcomes. In adults, epidemiological and cross-sectional evidence suggests that interrupting prolonged sedentary behavior is associated with: lower waist circumference,[31] lower triglyceride levels,[47] and lower postprandial glucose.[48] In children, school-based studies indicate that breaks in sitting increase attention.[49] Reviews of the literature did not find sufficient support for the prospective[50] or cross-sectional[51] effects of sedentary behavior on cardiometabolic health outcomes. However, the findings from the reviews must be interpreted cautiously because sedentary behavior was measured differently across the reviewed studies, and there were no randomized control trials that manipulated the behavior.

Pathophysiologic basis:

There are acute physiologic mechanisms that relate sedentary behavior with increased metabolic risk which support the potential effectiveness of interrupting sedentary behavior.[52] Early research in this area focused on the acute effects of prolonged sedentary behavior on lipoprotein lipase (LPL) activity as a potential link between behavior and health risk. LPL is central in lipid metabolism, and plays a role in free fatty acid (FFA) uptake among different non-hepatic tissues.[53] LPL concentration is high in oxidative (slow twitch, postural) muscle, and is hypothesized to mediate the relationship between sedentary behavior and cardiometabolic risk because prolonged sitting reduces the use of oxidative muscles (such as those used for standing), lowers LPL, and reduces the body's ability to take up fatty acids into tissues after a meal. The result is prolonged exposure of the arterial walls to plasma triglycerides that increases cardiometabolic risk.[54, 55] This mechanistic hypothesis may explain why cross-sectional pediatric studies have found that more time spent in sedentary behavior is associated with increased concentration of circulating cell adhesion molecules that play a role in the development of atherosclerosis and type 2 diabetes.[35] Thus, sedentary behavior may exert a cumulative effect on health risk over the lifespan.

Sedentary behavior has also been associated with chronic physiologic alterations that may increase the risk for type 2 diabetes. Prolonged sedentary behavior is associated with lower insulin sensitivity and increased postprandial glucose concentrations.[56] Sustained sedentary behavior diminishes the use of skeletal muscle to assist with glucose uptake from the bloodstream after a meal, requiring the pancreas to secrete more insulin that may increase the risk for beta cell dysfunction and type 2 diabetes.[57, 58] Also, once insulin-mediated glucose uptake by skeletal muscle is decreased due to prolonged sitting, the elevated glucose concentrations lead to a longer exposure to high circulating insulin in the blood. This hyperinsulinemia promotes lipogenesis (fat synthesis) in the liver and uptake of fatty acids into adipose tissue.[59] The result is increased adiposity and worsening insulin resistance.[60] These pathways still need to be further explored to explain how the duration and patterns of sedentary behavior may produce negative health effects.

Adult Studies Interrupting Sedentary Behavior

To date, there have been a few published studies evaluating the effect of interrupting sedentary behavior on metabolism. [48, 61-63] Dunstan et al. conducted a randomized crossover study where prolonged sitting was interrupted with 2 minutes of light- and moderate- intensity walking every 20 minutes for five hours in overweight/obese adults (Figure 1).[48] They hypothesized that a lower postprandial glucose response would be observed when interrupting sitting with brief sessions of activity, regardless of the intensity of the

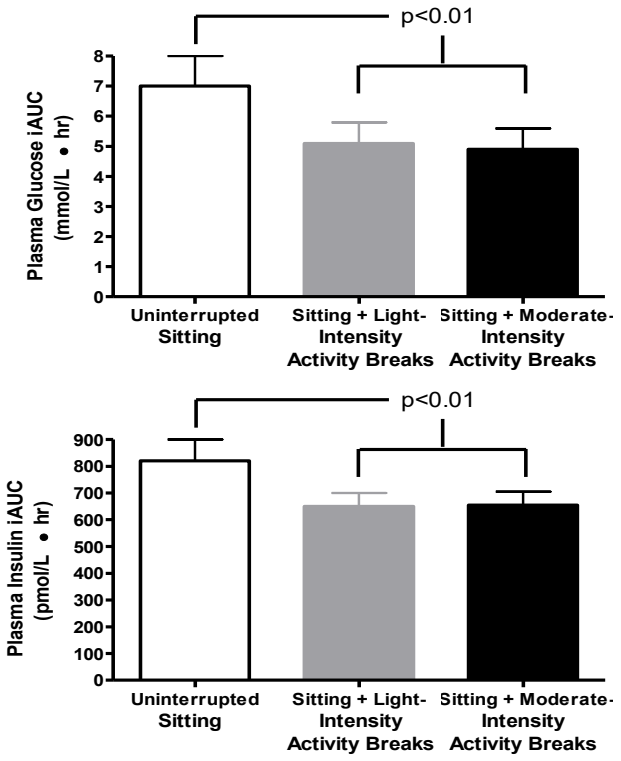

Fig 1. Dunstan et al. Diab Care 2012;35:976-983

activity. Study participants attended three lab visits in random order to complete the three conditions: prolonged sitting, sitting interrupted by light walking, and sitting interrupted by moderate walking. A standard test drink was given at time 0 after which blood was taken every hour. Compared to the prolonged sitting condition, the glucose responses were significantly lower in the light- and moderate- intensity walking conditions – 24.1% and 29.6% respectively – with both conditions statistically significant vs. sitting ($p<0.0001$). Compared to the prolonged sitting condition, the insulin incremental area under the curve (iAUC) was 23% lower in the light- and moderate- intensity walking conditions. There were no significant differences in glucose or insulin responses between the different walking activity conditions, suggesting that intensity of activity during breaks per se did not influence metabolic outcomes, but rather the simple act of interrupting sitting with any activity might be beneficial for metabolic health.

More recently, a separate research group sought to confirm these findings with a similar intervention strategy. Pulsford et al. performed a randomized, cross over intervention of three arms in 25 inactive men (Figure 2).[62] The three arms consisted of: 7 hours of continuous sitting, 7 hours of sitting interrupted by 2 minutes of light intensity walking every 20 minutes, and 7 hours of sitting interrupted by 2 minutes of standing every 20 minutes. Similar to Dunstan et al., the subjects also received a standardized mixed test meal but they also received a fasted OGTT during the beginning of the day. Serum glucose and insulin monitoring every 30 minutes throughout the day revealed significantly decreased glucose and insulin AUC during the walking-interruption arm.[62] They found no significant improvement from the standing-interruption arm.

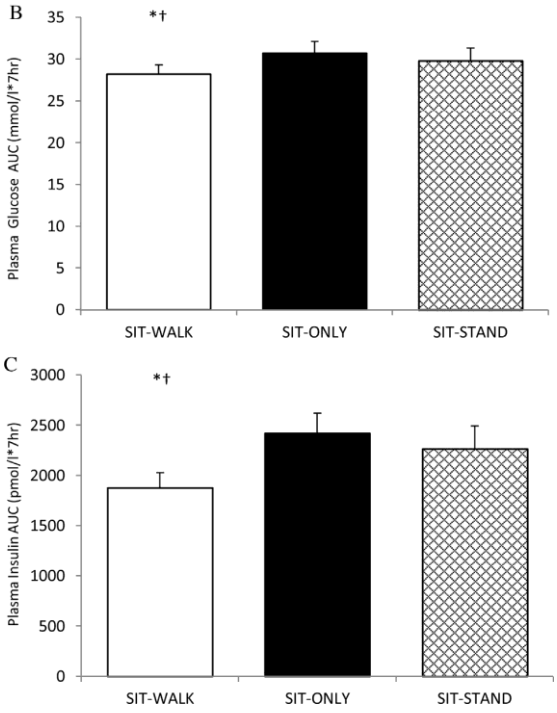

Fig. 2 Pulsford et al. J Sci Med Sport. 2016;16;30155-4

Pediatric Studies Interrupting Sedentary Behavior

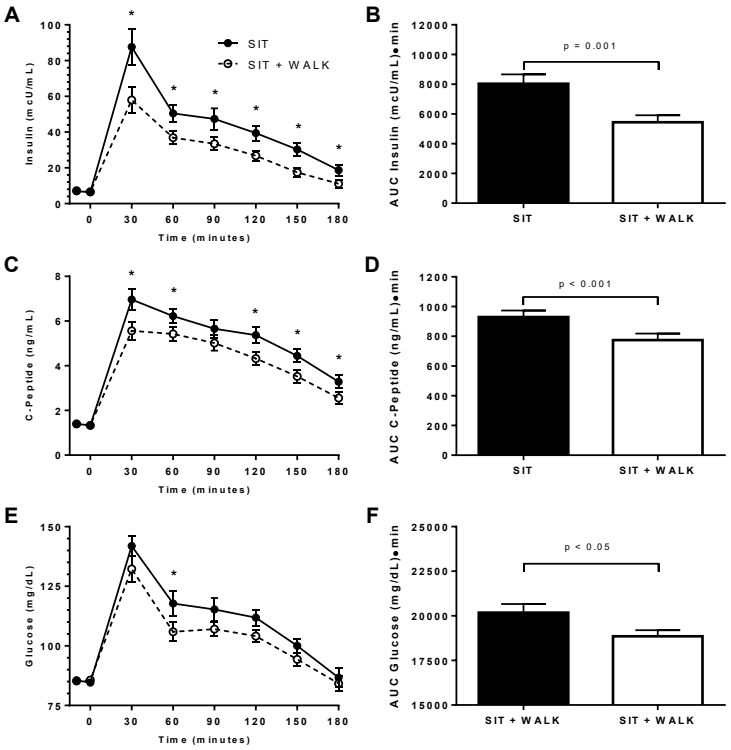

Fig. 3 Belcher et al. JCEM. 2015; 100:3735–3743

In youth, recent data (including our own) suggest that interrupting sedentary behavior has beneficial metabolic effects.[64, 65] In protocol 13-CH-0169 we examined if an intervention to interrupt sedentary behavior in children would provide identifiable improvement in glucose metabolism (Figure 3).[65] Using a randomized cross-over design in 28 healthy weight children, we demonstrated that interrupting prolonged sedentary behavior in non-overweight children by intervening with 3 minutes of moderate intensity walking every 30 minutes for 3hr significantly improved carbohydrate metabolism.[65] In the children studied, interrupting sitting resulted in a 32% lower insulin iAUC (P =.001), 17% lower C-peptide iAUC (P = .001), and 7% lower glucose iAUC (P = .018) vs continuous sitting. Mixed model results indicated that insulin (P = .036) and free fatty acid concentrations (P = .009) were also significantly lower in the interrupted versus the continuous sitting condition.[65] In addition to our published data obtained using a healthy weight cohort, we have obtained confirmatory results from a methodologically similar study carried out in a separate cohort of 37 children whom were overweight or obese. In this cohort, interrupting sitting resulted in a 21% lower insulin iAUC ($p<0.001$) & 18% lower C-peptide iAUC ($p=0.002$). However, no significance difference was identified in glucose iAUC ($p = 0.12$).

In addition to our study, Saunders et al. published a similar randomized cross over study in 2013.[64] This research group applied a similar interrupted activity intervention only with light- (rather than moderate-) intensity walking every 20 minutes for a full day (8 hours).[64] The outcomes measured were also similar: AUC for glucose and insulin. But notably, in addition to

the strength of the intervention being different (light intensity [30% of VO2 peak for Saunders et al] vs moderate intensity [80% of ventilatory threshold for us] walking), the serum sampling was much less frequent occurring only every 90 minutes instead of every 30 minutes as obtained by Dunstan et al. and during our study. Finally, Saunders et al. only evaluated 19 total subjects. With these differences in methods, Saunders et al. failed to identify a difference in glucose metabolism by interrupted activity (i.e. no change in AUC of insulin or glucose).[64] This directly contradicts all previous adult findings and our pediatric studies.[48, 62, 65] As noted above, their null findings may be due to the small sample size and other methodological differences that limited their ability to detect the effects of interrupting sedentary time. Regardless, all the positive data are reported from comparisons of outcome at single sessions of interrupted sedentary behavior and there are no available data testing if the changes observed acutely are sustainable.

Continuous Glucose Monitoring to examine sustained effects on glucose homeostasis

All the data presented above were obtained using indwelling catheters for venous sampling. As a result, only limited data from relatively infrequent sampling during in-lab studies are available. Continuous glucose monitoring (CGM) using transcutaneous sensors that collect interstitial glucose concentrations is a method that can obtain frequent data sampling over prolonged periods. The devices are approximately the size of a quarter and are inserted on the upper arm (Figure 4, Abbott Pharmaceuticals). Adult data indicate that altering physical activity, even minimally, in non-diabetic adults can significantly affect glycemic variation as identified on continuous glucose monitoring.[66-68] Such data specifically include interrupting sedentary behavior, which has been demonstrated to decrease nocturnal glycemia as well as glycemic variability and post-prandial glucose.[66, 67] Blankenship et al. recently published novel CGM data collected on 10 sedentary overweight/obese non-diabetic adults who were given 3 different activity interventions while wearing CGMs.[66] The arms of intervention included: A work day with no activity except one 30 minute block of walking mid-day; A work day with 'frequent long breaks' which varied in length and frequency but totaled the same energy expenditure as one 30 minute block of walking; and A work day with 'frequent short breaks' which varied in length and frequency but

Fig. 4 Continuous Glucose Monitor, Abbott Freestyle Libre

were more frequent and shorter in duration that 'frequent long breaks'. The participants were fitted with a CGM at the beginning of the day and wore the device for 24hrs. During the testing day, they were given a standardized meal tolerance test in order to collect the main outcome data, AUC of glucose and insulin during the meal tolerance test. (Participants were also given standardized fixed meals based on total daily energy expenditure for the remaining meals during each intervention 24hr-day). The mixed meal tolerance test data indicated no difference in AUC of glucose or insulin. However, there was lower glycemic variability in the 'frequent long breaks' compared to the day with one 30-minute block of walking with CGM data. The mean amplitude of glycemic excursion (MAGE) was significantly lower when participants performed 'frequent long breaks' for the day ($p < 0.05$).[66] [Mean amplitude of glycemic excursion is the arithmetic average of all glycemic amplitudes greater than a pre-specified threshold (most commonly set at 1 SD)].[69] Also, nocturnal duration of hyperglycemia (glucose > 7.8 mmol/l or >140 mg/dL) was significantly lower in both 'frequent long breaks' and 'frequent short breaks' compared to the day with one 30 minute block of walking (frequent long breaks = 2.5 ± 2.5 min; frequent short breaks = 45.6 ± 29.6 min; one 30 minute block of walking = 32.7 ± 16.4 min, $p = 0.05$).[66] It is important to note that this study did not include a nonintervention control arm and studied only 10 subjects; nevertheless, the data are highly suggestive the CGM may provide valuable data that cannot be obtained from traditional in-lab sampling during interrupted sedentary activity interventions.

To our knowledge, there are no published data on CGM outcomes from interrupting sedentary behavior in youth. Limited published data from CGM monitoring in non-diabetic youth have demonstrated associations between glycemic variability and vascular stress and show CGM devices are capable of demonstrating differences in glucose outcomes from dietary interventions.[70, 71] For example, Desari et al. evaluated the relationship between CGM parameters including MAGE and markers of oxidative and vascular stress including c-reactive protein, cholesterol, triglycerides, non-esterified fatty acids, intercellular adhesion molecule-1 and vascular cellular adhesion molecule-1, and E-selectin.[71] In 34 children ages 13-21 years (12 healthy weight, 10 healthy obese, 12 with Type 2 Diabetes Mellitus) with 5 consecutive days of CGM data, MAGE was significantly correlated with many markers of vascular stress including but not limited to oxidized low-density lipoprotein, E-selectin and intercellular adhesion

molecule 1.[71] These data indicate that parameters of glycemic variability, as obtained by CGM, in youth can provide additional helpful information about carbohydrate metabolism.

In addition to exploring glycemic variability and vascular health associations with CGM in non-diabetic youth, researchers have also begun to evaluate CGM outcomes for dietary interventions. Bauer et al. evaluated the effects of a high protein breakfast in 28 overweight but non-diabetic teenagers.[70] CGM outcome data were collected on day three of the intervention for a 24hr time period and included: 24hr AUC, morning glucose AUC, overnight glucose AUC, average, SD, maximal response, glucose fluctuation (max-min), and time spent above the glucose threshold of 100 mg/dl.[70] No main effect by intervention was detected but post-hoc analyses revealed reduced 24hr-glucose variability (SD) in the individuals receiving a high protein diet.[70]

The extant data have not evaluated CGM outcomes in sedentary behavior but provide insight and guidance into useful data that become accessible through CGM monitoring in youth. These novel exploratory studies demonstrate successful use of CGM in non-diabetic youth. When such data are combined with the positive CGM outcomes in adult interrupted sedentary behavior interventions, CGM monitoring of sedentary-interruption interventions in children could add important additional information for the effects of this intervention strategy in young cohorts.

In conclusion, given the significant improvements in glucose homeostasis demonstrated in our acute intervention studies, along with the dearth of pediatric studies involving either continuous sampling or sustained interventions to interrupt sedentary behavior, a study that examines how beneficial repeated days of interrupted, versus non-interrupted, sedentary behavior might be to alter children's glucose homeostasis measured with CGM is the logical next step. Such a study would be the first of its kind to assess sustained effects of brief sedentary interruptions in the clinical lab among youth and will provide novel data for within- and between- day effects of the intervention with continuous glucose monitoring. However, it is important to note that metabolism is the not the only outcome that may be affected by interrupting sedentary behavior in youth. Understanding that such an intervention strategy would be ultimately applied in a school or community setting on a routine basis, it is important to

evaluate effects on cognitive function, mood, anxiety, dietary intake, and non-intervention physical activity habits.

Executive function and attention – definitions and alterations associated with obesity

Executive functions are those mental processes that are central to goal planning, decision-making, and choice of behavior.[72] This construct is comprised of several underlying elemental processes such as inhibition, working memory, and cognitive flexibility (e.g.: task switching).[73] Development of executive functions is associated with the prefrontal cortex and emotional systems in the brain.[74] Some data exist that indicate adult-level simple executive functions such as motor inhibition can develop as early as age 10, with gains in functional efficiency extending through early adulthood.[75-77] Cognitive flexibility, goal setting, and information processing develop most rapidly from ages 7 to 9.[78] Thus, executive functions are rapidly evolving during childhood and are necessary for behavioral development, emotional control, and social interactions.[79]

Attention refers to the cognitive process of selectively concentrating on one environmental stimulus while ignoring other stimuli.[80] There are several underlying processes such as sustained attention, selective attention, and divided attention[81] that serve as the foundation for other mental processes. There are rapid changes in attention between the ages of 8 and 10.[82] In a sample of 400 3-12 year old Finnish children, maximum improvement in selective and sustained attention were reached by age 10.[81] Thus, intervening in this age group may be an effective way to support the development of several sub-processes of attention. It is important to support attentional development because it is necessary for academic achievement in children. A longitudinal study in 2595 children reported that focused attention scores in preschool predicted achievement outcomes in childhood, whereas impulsivity scores in preschool predicted behavioral outcomes in childhood.[83]

Relationship to obesity

Executive function and attention are lower in overweight/obese children compared to their lean counterparts.[79, 84] Data from NHANES indicate that higher BMI is associated with decreased cognitive function in children.[85] Other studies also support these findings in children.[86-88] Obese children have lower inhibitory control scores than their normal weight counterparts.[89] Another study found that overweight boys had lower focused attention scores and

more inattention symptoms than their normal weight counterparts.[90] Executive function deficits are also related to low physical activity and high sedentary behavior levels. In a latent class analysis of fourth to sixth graders, children in the "high sedentary, high fat/high sugar snacks, not weight conscious" category had more executive function problems than children in the "active, healthy eating" category.[91] Executive function and attention in early childhood track into adolescence,[79] suggesting that deficits may promote obesogenic behaviors that have long-term consequences.

Obesity risk may be mitigated by the positive relationship between physical activity and executive function and attention in children. A seminal meta-analysis reported a positive association between physical activity level and aspects of executive function in children and adolescents, with a stronger effect in younger age groups (4-13 years). [92, 93] This positive relationship was independent of type of activity. Fourth grade children who were physically active had higher concentration scores,[94] suggesting that attention performance is enhanced after physical activity. Physical activity is of much interest because it has effects on brain activation and development, and children may particularly benefit because their central nervous system is developing.[95] A review of the existing literature on physical activity, fitness, and aspects of cognitive function indicated that physically fit children have greater mobilization of brain resources that allow them to perform cognitive tasks more rapidly than unfit children.[96] Thus, research has focused on the effects of acute and chronic physical activity on executive function tasks in children.

Executive function, attention, and physical activity

Mechanisms

There are two primary mechanisms that have been used to describe the relationship between physical activity and executive function and attention. The first mechanism relates to the physiologic changes produced by physical activity. Physical activity promotes central nervous system development, and the refinement of the prefrontal cortex[97] through the attentional network of the brain that is active in cognitively challenging tasks.[98] Thus, physical movement results in changes in brain activation and function[99] that support the development of executive functions and attention during childhood. The second mechanism relates to the

learning and developmental changes produced by physical activity. Motor and pro-social skills that children learn through physical movement transfer to tasks involving aspects of executive function and attention, such as behavior inhibition and working memory, that relate to academic achievement.[100]

Acute Physical Activity

Executive function and attention are improved by acute physical activity.[101] Researchers hypothesize that short bouts of exercise facilitate transient modulation of neural networks involved with specific cognitive tasks following the exercise.[102] A review found that there was a small to moderate improvement in attention following acute physical activity in a classroom setting.[103] In an early study, Gabbard and Barton found that mathematical scores increased in second grade children following 50-minutes of PE class participation.[104] A more recent crossover study with 20 children (mean age= 9.5 years) reported increased response accuracy and academic achievement test scores following a 20-minute moderate-intensity walking bout.[105] These studies support the view that acute bouts of exercise improve specific aspects of executive function and attention, and may be an intervention target to increase both physical and cognitive health in children.

Chronic Physical Activity

Chronic physical activity is positively associated with executive function and attention in children.[106, 107] Longitudinal repetition of physical activity results in durable structural changes in the brain.[102] A recent randomized control trial used functional MRI to measure brain activity while cognitive tasks were performed by overweight children randomized to either a 13-week exercise or control program. Results demonstrated a dose-response relationship existed: executive function and math achievement were increased in both the low exercise and high exercise groups, but more so in the high exercise group.[108] A review of the longitudinal literature found that participation in school PE was positively associated with academic performance.[109] Donnelly et al. reported that a 3-year clustered randomized control trial on a classroom-based physical activity curriculum that promoted 90 minutes/week of MVPA resulted in a 6% improvement in standardized academic achievement scores and a slowing of BMI increases in

the intervention schools.[107] These studies support the role of chronic physical activity in executive function and academic performance in children.

Sedentary Behavior

Research on the effects of sedentary behavior on executive function and attention is still developing. Longitudinal studies in children have found that more time spent watching TV is associated with greater attention difficulties and worse cognitive function.[110] Results from a randomized control trial indicate that reducing sedentary behavior through 20- or 40- minute bouts of vigorous exercise increased academic performance in 222 sedentary overweight 7-11 year old children.[79, 111] A study conducted in 60 children (age 4) found that executive function was decreased immediately following a 9-minute television show.[112] Because sedentary behavior has traditionally been operationalized as TV viewing in cognitive studies, it is unclear whether other sedentary behaviors influence components of executive function in children.

Psychosocial health

Acute and chronic physical activity produces beneficial effects on mood. Higher levels of physical activity have been associated with lower levels of depression,[113] less perceived anxiety,[114, 115] and better mood,[116, 117] in children. In a sample of 64 children (ages 9-10 years), 15 minutes of exercise increased feelings of positive mood whereas a 15-minute video decreased feelings of positive mood.[118] The research clearly demonstrates that acute physical activity increases positive affect and mood in children.

While there is an extensive literature examining the effect of physical activity on mood and affect, more research is needed to examine the effect of sedentary behavior on psychosocial outcomes in children.[119] High levels of sedentary behavior are associated with increased depressive symptoms.[120, 121] In a sample of 4320 children, sedentary behavior was positively associated with perceived anxiety and emotional symptoms.[122] A recent crossover trial found that after an acute stressor, children spent more time being sedentary (watching TV).[123] Early evidence suggests that sedentary behavior may increase negative mood and depressive symptoms. No previous studies have investigated if interrupting sedentary behavior in a sustained fashion affects psychosocial outcomes in children.

Dietary intake and activity

Dietary intake is related to metabolic risk in children. In overweight children, higher soluble dietary fiber intake was associated with lower waist circumference[124] whereas high sugar intake was associated with poor beta cell function, an early sign of increased risk of type 2 diabetes.[58] Findings from NHANES indicated that youth with higher fruit intake and Healthy Eating Index scores have a lower prevalence of metabolic syndrome.[125] The findings from these studies suggest that balanced dietary macronutrient intake is essential for normal metabolic functioning.

Dietary intake is associated with physical activity in children. Increases in physical activity in are associated with increases in total energy intake.[126, 127] However, few studies have addressed the direct influence of specific dietary macronutrients on physical activity. In a sample of 210 girls, accelerometer-measured moderate-to-vigorous physical activity was negatively associated with percentage of calories from fat and carbohydrate.[128] Other studies have found that diets high in simple carbohydrates have been associated with feelings of fatigue, and low levels of physical activity.[129, 130]

Dietary intake is hypothesized to co-occur with some sedentary behaviors. A recent review found that high amounts of TV viewing was associated with lower fruit and vegetable consumption, higher consumption of energy-dense snack foods, fast food, and drinks.[131] In a sample of 2908 adolescents (ages 14-19 years), screen-based behavior was inversely correlated with fruit and vegetable intake.[132] A cross-sectional study in 1860 children (ages 5-12 years), fast food and snack consumption was positively associated with sedentary behavior.[133] The limited evidence thus far suggests that higher levels of sedentary behavior are associated with unhealthy dietary intake. There are no studies examining the effects of experimentally interrupting sedentary behaviors in a sustained fashion on dietary intake.

Subject Selection

Epidemiologic data indicate that sedentary behavior is associated with cardiovascular disease and all-cause mortality irrespective of adiposity. For example, Thorp et al. report meta-analysis findings on increased all-cause mortality, cancer, and cardiovascular disease in adults after controlling for BMI.[17] Additionally, the effectiveness of this intervention in the preliminary

data described above was not dependent on adiposity.[48, 64, 65] Also, the non-metabolic parameters such as cognitive function are independent of weight. Therefore, our proposed intervention will likely affect healthy weight and overweight children equally within these outcomes. Finally, our intervention is designed to be a preventative strategy intended to prevent dysfunctional metabolism before the onset of pathology such as Impaired Glucose Tolerance or Diabetes Mellitus Type 2 and be feasible for broad spread application into the community setting where the majority of subjects are expected to be healthy. For broad spread application, it is pivotal that preliminary data demonstrate potential benefits and negligible adverse outcomes in all subject populations within which it will be applied. Given this, we will study healthy weight and overweight children who do not have evidence of metabolic disease.

Magnetic Resonance Spectroscopy Arm: Use in Exercise Physiology

A novel technique to explore the underlying physiologic mechanisms of interrupted activity is through skeletal muscle magnetic resonance spectroscopy (MRS). MRS provides a technique to evaluate cellular level fat (specifically intramyocellular lipid, IMCL) content which in turn provides information on FFA storage and usage.[134, 135] IMCL is positively correlated with insulin resistance and thus associated with the diabetes mellitus type 2.[136, 137] Although details of the association (causal or not) have yet to be elucidated, it is readily hypothesized that acutely reducing IMCL improves insulin sensitivity. However, it is also known that repeated, exercise training increases basal or fasting IMCL content in parallel with improved insulin sensitivity.[138, 139] Thus, the generally accepted understanding of IMCL and physical activity is that static, accumulation of IMCL is associated with poor insulin sensitivity but repeated depletion and replenishment of IMCL stores, which leads to increased basal IMCL is associated with improved insulin sensitivity.[140] My collaborator for this proposal, Ahmed Gharib, MD, PhD, Head of Biomedical and Metabolic Imaging Branch within the National Institute of Diabetes and Digestive and Kidney Disease, has used MRS to evaluate skeletal muscle lipid content as a function of insulin resistance.[141] MRS has also been successfully used to identify acute change in intramyocellular fat content surrounding physical activity[142, 143] but has yet to be utilized for evaluating the effects of interrupting sedentary behavior. As such, we propose, as an exploratory analysis, the use of MRS to explore associations of IMCL in children and fat mass and determine

if interrupted sedentary behavior in children acutely alters IMCL content. MRS will be used in accordance with its labeling and not operated in research mode.

Conclusion

In final summary, there are few data for the effects of experimentally interrupting sedentary behaviors on metabolism, cognition, mood, or dietary intake, and no such data for programs that study such interruptions in a sustained fashion. However, what are available suggest that one-session interventions with at least moderate intensity bouts applied 2 or 3 times per hour may be successful at improving glucose homeostasis by decreasing circulating insulin. Therefore, we plan to conduct a pilot study that examines sustained metabolic outcomes from interrupting daily sedentary behavior in children applied with 3-minute interrupting bouts every 30 minutes for a 3-hour duration over 6 days. With this study we will also collect exploratory mechanistic data from liver and muscle spectroscopy. Our chosen intervention mirrors our one-session preliminary trials and is in line with similar published intervention trials.[64, 65] We have chosen the details of this strategy to maintain the potential of translation to the real-world setting. Although some preliminary data have been obtained with an intervention applied every 20 minutes[64] instead of every 30 minutes, this intervention is too frequent to be practical in a school setting. Additionally, the duration of 6 days has been chosen as an ideal intermediary to demonstrate sustained effects prior to broad spread application that will require extensive resources and as of yet are not strongly data-supported. Finally, in addition to glucose homeostasis, we will explore the potential impacts of interrupting prolonged sitting on children's executive function, mood, anxiety, dietary intake, and usual physical activity habits which aim to strengthen the rationale for broad application of this approach in the school setting.

2. Study Objectives

Project Goal: The overall goal of this research is to examine whether providing brief interruptions in prolonged sitting produces long-term salutary changes in metabolism, without adversely impacting executive function, attention, anxiety, mood, or dietary intake. As a bridge between the acute studies that have demonstrated efficacy for this approach and long-term

school- or home- based translational programs, we will examine if brief interruptions of sedentary behavior applied for 6 consecutive days will produce sustained improvements in metabolism and to explore any changes in cognitive function or energy intake in children with intervention.

Objective 1: To investigate if daily interruptions of sedentary behavior (sitting) versus prolonged sitting on 6 consecutive days improve glucose homeostasis. **Hypothesis 1a**: Compared to non-interrupted sedentary behavior, interrupted behavior will result in significantly lower insulin and glucose incremental area under the curve (iAUC) during a 3-hour oral glucose tolerance test. **Hypothesis 1b**: As measured by continuous glucose monitoring, children with daily interrupted sitting will have lower average 24-hr glucose iAUC and lower mean amplitude glycemic excursion (MAGE) than children who engage in non-interrupted sedentary behavior.

Objective 2: To investigate if daily interruption of sedentary behavior adversely influences cognitive and psychological measures, dietary intake, or usual physical activity. **Hypothesis 2:** Executive function, attention, mood, anxiety, dietary intake, and physical activity (at times outside the testing period) will not differ significantly between children randomized to daily interrupted versus daily non-interrupted sedentary behavior.

Objective 3: To investigate if acute or daily interruption of sedentary behavior affects intramyocellular lipid content within the vastus lateralis, tibialis anterior or soleus muscles. **Hypothesis 3a:** Acute interrupted sedentary behavior will decrease intramyocellular lipid content. **Hypothesis 3b:** Daily interrupted sedentary behavior will increase resting/basal intramyocellular lipid content.

3. Subjects

a. Description of study populations

The proposed intervention has demonstrated acute efficacy in the general pediatric population, among both healthy weight[65] and overweight children, ages 7-12 years. Therefore, we will enroll generally healthy child volunteers in a 1:1 ratio of healthy and overweight. We will adhere to a maximum accrual ceiling of 150 participants. Based on previous experience, we anticipate that no more than 20% of screened children will be

ineligible for the study. Thus, we will randomize up to 120 participants and, anticipating that at most 36 children will drop-out of the study before completion or have technical issues limiting data analysis, we expect at least 84 participants to complete the protocol (our target completion sample size is based on power analyses described below). Withdrawals/dropouts will not be replaced.

i. Inclusion criteria

Participants will qualify for the study if they meet the following criteria:

(a) Good general health.

(b) Age ≥ 7 and <12 years.

(c) Fasting plasma glucose < 100 mg/dL

(d) Body mass index (BMI) ≥ the 5th percentile, as determined by the CDC age- and sex- specific growth charts.[144]

ii. Exclusion criteria

Participants will be excluded from the study for:

(a) Significant cardiac or pulmonary disease likely to or resulting in hypoxia or decreased perfusion.

(b) Evidence of impaired glucose tolerance or type 2 diabetes, including fasting plasma glucose ≥ 100 mg/dL.

(c) Presence of other endocrinologic disorders leading to obesity (e.g.: Cushing Syndrome).

(d) Participants who have, or whose parent/guardians have, current substance abuse or a psychiatric disorder or other condition that, in the opinion of the investigators, would impede competence, compliance, or prevent the completion of the study.

(e) Participants who have, or are currently receiving, anti-psychotic drugs that would affect metabolism, cognitive outcomes, and body habitus.

(f) Participants receiving medical treatment other than diet for hypertension or dyslipidemia.

(g) Participants with precocious puberty and/or receiving androgen and estrogen therapy.

(h) Participants currently taking medications for ADHD, or any disorder or use of medications known to affect body composition or weight including but not limited to glucocorticoids or other steroid compounds.

(i) Presence of pre-existing neurocognitive disabilities, or an age-adjusted score below 85 on the Picture Vocabulary Test at the screening visit.

(j) Presence of food allergies, such as peanut/tree nut, dairy, soy or any other food allergy or personal dietary restrictions that would preclude participant from consuming the daily diet or the buffet.

(k) Presence of significant skin disease or allergy to adhesive material prohibiting placement of a continuous glucose monitor.

(l) Participants unable or unwilling to abstain from acetaminophen, ascorbic acid, or salicylic acid during study duration.

(m) Participant does not speak fluent English.

(o) Participant is or becomes pregnant.

(p) Participant has an ambulatory impairment

b. Optional MRS Supplemental Arm

iii. Inclusion criteria

(a) Participants will qualify for the study if they qualify for the primary Break It Up! study.

iv. Exclusion criteria

(a) Cannot have MRI scanning. Some of the reasons a child might not be able to have MRI include:

- Implanted cardiac pacemaker or defibrillator
- Cochlear Implants
- Ocular foreign body (e.g. metal shavings)
- Embedded shrapnel fragments
- Central nervous system aneurysm clips
- Implanted neural stimulator
- Medical infusion pumps
- Any implanted device that is incompatible with MRI.

(b) Is not likely to tolerate an MRI scan. Examples of medical conditions that would make it difficult to undergo MRI include severe anxiety (nervousness) or hyperactivity which make it hard for your child to lay flat for the study.

(c) Requires sedation for MRI studies.

(d) Has a condition that makes entry into the scanner difficult (e.g. weight over 550 lbs, claustrophobia, etc.).

(e) Has severe back-pain or motion disorders that make it hard for a child to lie on his/her back within the MRI scanner and hold still for the scan.

4. Study Design and Methods

a. Study Overview:

i. Trial Design: We will carry out a parallel group randomized controlled trial that compares child carbohydrate metabolism and cognitive function during 5 days of sedentary after-school activities versus after-school activities with structured interruptions provided by walking for 3 minutes on a treadmill every 30 minutes at a moderate activity rate selected according to each participants' level of fitness. A final 6th day will compare results between groups from an oral glucose tolerance test conducted in the fasted state. Subjects will be randomized to one of two conditions:

(a) **<u>Study Intervention: Interrupted sedentary behavior.</u>** This test condition will consist of 6 consecutive daily after-school /afternoon visits for a three-hour experimental session. The first five sessions will be performed mid-afternoon (Monday – Friday, after school or late afternoon); the 6th will be in the morning (Saturday, approximately 8am). Each session will consist of prompted 3-minute moderate-intensity walking bouts performed on a treadmill every 30 minutes for 3 hours. There will be a total of 6 walking bouts (18 minutes total) each day. 'Moderate-intensity walking' speed and grade will be selected to achieve 80% of the heart rate achieved at the ventilatory threshold as determined during a $V0_2$max test.

(b) Study Control: Non-Interrupted sedentary behavior. This control condition will consist of children attending similarly-timed visits for 6 consecutive days providing 3 hours of sedentary behavior/sitting during which the children will not engage in any physical activity. Rather, they will engage in continuous sedentary behaviors (e.g. watching TV or working on homework while seated) for 3 hours each day without interruption.

ii. **Visit Work Flow (Also see Figure 1):** There are no inpatient visits. Participants will be evaluated at a series of day-hospital visits (7 in total) using both the 1NW pediatric day hospital and the 5SWN metabolic units of the *Hatfield* Clinical Research Center. The initial screening evaluation visit will use the 1NW pediatric day hospital and will involve approximately 6 hours of testing. Following screening, the daily test visits will be conducted in the 5SWN Metabolic Unit and involve approximately 3.5 hours each day for the 5 daily visits. The final visit will also take place in the metabolic unit and involve approximately 5 hours of testing. In summary, each participant will complete a screening visit and, if randomized, will complete 6 consecutive days of study visits. The block of daily visits will be initiated no further than 90 days from the date of screening; therefore, each participant should complete study participation within 97 days of screening.

iii. **Relationship with other protocols:** This is an independent protocol with no direct relationship to other study protocols.

b. Recruitment

i. Description of recruitment strategy. Our team has considerable experience in recruiting and retaining child participants in research studies at the Clinical Research Center. Most germane to this study, Drs. Yanovski and Broadney successfully recruited 95 children ages 7-11.99y for the pilot studies of protocol 13-CH-0169 over 3 years to evaluate this sedentary intervention strategy in the acute setting. Dr. Broadney has been in close communication with the NIH Clinical Center Patient Recruitment and Public Liaison (PRPL) Office to develop recruitment materials and strategies to successfully reach the target population. All recruitment efforts will include an email and telephone number for parents to call if interested (See

Appendices A for all media, email, and letters documents drafted for PRPL use). These include flyers, social media content, and mailings to local pediatricians, school listservs and community centers throughout the greater District of Columbia, Maryland, and Virginia metropolis.

ii. **Recruitment Methods:** Participants will be recruited via multiple methods:
   - **(a)** Letters will be mailed to the parents of appropriate-aged children in local areas including Montgomery County, Fairfax County, and Washington, DC.
   - **(b)** Flyers posted at NIH and other local facilities (e.g. elementary schools, community centers, churches, public libraries) with permission.
   - **(c)** Local community groups will be asked to send the flyer to their listservs.
   - **(d)** Schools, churches, pediatricians, and community centers will be contacted regarding the study and fliers will be distributed.
   - **(e)** Potential volunteers that have registered on the NIH Clinical Center ResearchMatch program will be contacted.

iii. **Anticipated Accrual Rate:** We expect approximately 5 interested participants will be screened per month, with increases in accrual during school vacation dates and declines when the surrounding public school districts are in session.

iv. **Initial contact:** Potential participants who have sent their information to the study team will be contacted via telephone and/or email communication upon first inquiry (See Appendix A for telephone script, email template and FAQ's that will be provided). If basic participation criteria are met (i.e. child within age range and without significant medical diagnoses), parents/guardian(s) and participants will receive consent and assent forms (written explanations of the purposes, procedures, and potential hazards of the study) before the screening appointment. If a family wishes to join the study, the participant and a care provider able to give consent for research participation will be invited to the Clinical Research Center for formal screening.

c. Screening

Participants will complete the following during their screening visit.

i. **Vital signs, height, weight.** Blood pressure, height (in cm), weight (in kg), temperature, respiratory rate, and pulse rate will be obtained by NIH nursing staff.

BMI will be computed to determine eligibility. Subjects with BMI <5th percentile for age and sex will be excused from the study.

ii. **Protocol review and signing of consent and assent forms**. Parents/guardian(s) and participants will review the consent and assent forms with study personnel designated to do so by the PI. All study procedures will be discussed, and study risks and benefits explained. Communication of this information and of the participant's consent will be documented in the medical record before any study procedures, including other screening procedures, are done. All participants will be informed of their right to withdraw from the study at any point. Participants will be informed of compensation. Parent/guardian(s) and participants will be given signed copies of the consent and assent form for their records.

iii. **Complete medical history and physical exam** will be conducted by a trained practitioner**.** The exam will include Tanner pubertal staging. Waist and hip circumference (cm) will be measured in triplicate by nutrition staff (see below).

iv. **Practice measures.** Participants will be oriented to all procedures that will occur throughout the screening visit day (with the assistance of Child Life services) to reduce any anxiety.

v. **Fasting blood draw.** A fasting blood sample will be drawn to screen for exclusionary conditions and determine metabolic, hematologic, renal, and hepatic function including: acute care panel, insulin, c-peptide, free fatty acids, triglycerides, hemoglobin, creatinine, thyroid function tests (including anti-thyroid antibody panel), mineral, and hepatic panels, thyroid function tests (TSH and Free Thyroxine), C-reactive protein, Hemoglobin A1C, complete blood count, serum beta-human chorionic gonadotropin or urine pregnancy test, and serum and plasma samples to be saved for future research. ELA Max cream will be applied to numb the blood draw site.

vi. **Socioeconomic status (SES).** A demographics questionnaire that assesses race/ethnicity and parent/guardian education level and income will be completed by the parent/guardian so that the Hollingshead two-factor index of social position can be calculated.[145] See Appendix G.

vii. **Receptive vocabulary.** Participants will be given a Picture Vocabulary Test to measure baseline receptive vocabulary, which may serve as a proxy for IQ. The participants are presented with an audio recording of a word and four photographic images on the computer screen and asked to select the picture that most closely matches the meaning of the word. This test was selected from the NIH Toolbox (http://www.nihtoolbox.org). Participants whose age-adjusted score is below 85 (below average function) will be excluded from the study.

viii. **Heart rate monitor.** Participants will be familiarized with the heart rate monitor (Polar Vintage NV, Polar, Port Washington, NY, USA), and will practice wearing the same monitor that will be worn during the test visit(s).

ix. **Cognitive Testing.** Participants will be shown a 2-minute overview video of the Cognitive Brief Battery (Cogstate, New Haven, CT that will be administered to them on Test Day #1 and Test Day #5 (https://cogstate.com/featured-batteries/). CogState Brief Battery (CBB) is an approximately 20-minute computerized battery for children and adults ages 4 - 90 years, which includes tasks tapping processing speed, attention and vigilance, learning and memory, working memory and executive functions.[146] This measure was designed for repeated assessments over a short time period with virtually no practice effects in adults and children due to multiple forms.[147, 148] Age-based standard scores (mean = 100, SD 10) are computed for each task based on a normative sample of several hundred individuals. CogState has strong construct validity, minimal practice effects, and good stability. It was designed to be language independent in order to ensure the validity of the tests in a cross-cultural setting.[149-152] Further, CogState has been used in numerous pediatric, as well as adult, oncology clinical trials.

a. **Detection Task:** This subtest of the CogState takes 2 minutes to administer and measures psychomotor function and processing speed. It uses a well-validated simple reaction time paradigm using playing card stimuli. In this task, the playing cards are all red and black jokers. The subject is asked to press the Yes key as soon as the card in the center of the screen flips over.

b. **Identification Task:** This task takes 2 minutes to administer. It is a measure of visual attention and uses a well-validated choice reaction time paradigm

using playing card stimuli. In this task, the playing cards are all either red or black jokers. The subject is asked whether the card currently being presented in the center of the screen is red. The subject responds by pressing the Yes key when the joker card is red and No when it is black.

c. **<u>One Back Task:</u>** The One Back Memory task takes approximately 2 minutes to administer and is a measure of working memory. It uses a well-validated n-back paradigm using playing card stimuli. In this task, the playing cards are identical to those found in a deck of playing cards except for the joker. The subject is asked whether the card currently being presented is the same as the one presented immediately previously. The subject responds by pressing the Yes or No key.

d. **<u>One Card Learning Task:</u>** This task takes 5 minutes to administer. The One Card Learning task is a measure of visual recognition memory and uses a well-validated pattern separation paradigm using playing card stimuli to measure hippocampal functioning. In this task, the playing cards are identical to those found in a deck of playing cards except for the joker. The subject is asked whether the card currently being presented in the center of the screen was seen previously in this task. The subject responds by pressing the Yes or No key.

x. **Mood.** Participants will complete a practice Positive and Negative Affect Scale-Child (PANAS-C). The test will be explained and any words that the child does not understand will be explained. The 20-item scale consists of a 10-item Positive Affect (PA) scale and a 10-item Negative Affect (NA) and takes approximately 10 minutes to complete.[153] Participants indicate how often they have experienced several states on a 5-point Likert scale (1= very slightly/not at all; 5= extremely). This questionnaire has been validated in children,[153, 154] and demonstrates alphas of .91 and .88 for PA and NA, respectively. Item-total correlations range from .49-.78 on PA and .36-.80 on the NA scales, respectively. The test-retest reliability is high (correlations of .66-.82). Subjects will complete the PANAS-C and the "Feelings Questionnaire" through the NIH Clinical Trials Survey System.

xi. **Anxiety.** Participants will complete a practice State Trait Anxiety Inventory for Children (STAIC).[155] The test will be explained and any words that the child does not

understand will be explained. The scale measures acute (state) anxiety and longer-term (trait) anxiety. It asks the child to rate 20 statements from 'hardly every true' to 'often true' and takes approximately 15 minutes to complete. The scale has been validated in 1551 children ages 6-14 years. Subjects will complete the STAIC through the NIH Clinical Trials Survey System.

xii. **Physical Activity Evaluation.** To determine the average amount of moderate-to-vigorous physical activity each participant has engaged in within the previous week, children will be asked two items from the International Physical Activity Questionnaire (IPAQ) screening form (See Appendix G).[156] This information will be used to:

a. Determine caloric requirements for standardized meal calculation
b. Stratify participants for randomization purposes.

xiii. **Dietary Evaluation & Metabolic Diet Education.** Participants will be interviewed by and receive education instruction from the nutrition staff. This will allow us to obtain each participant's meal preferences and provide metabolic diet education (i.e. goals of the metabolic diet throughout the study period) to improve adherence. Additionally, waist and hip measurements will be obtained in triplicate.

xiv. **Electrocardiogram (EKG).** All children will undergo an EKG. If the EKG or physical examination suggests possible concerns in cardiovascular function, echocardiography &/or formal cardiology consultation will be obtained before the participant performs the maximal exercise test.

xv. **Dual-energy x-ray absorptiometry (DXA) scan.** As part of a whole-body composition and bone mineral density assessment, DXA scan will be performed to identify lean and fat mass. DXA involves passing a collimated X-ray beam of 40 and 70 KeV through the subject and collecting the X-rays that pass through the patient with a standard X-ray detector. Because the delivery and attenuation of the X-rays are measured separately for the two principal energies, the procedure allows the differentiation of substances in their path if the attenuation coefficients of these substances are known. The manufacturer specifies that the array beam delivers an effective dose of 0.003mrem total body irradiation. Participants will undergo DXA scans in the Metabolic Clinical Research Unit (MCRU) using the Lunar iDXA (GE

Healthcare, Madison, WI, USA) machine. The scan takes approximately 20-30 minutes. An external standard, simulating bone, fat, and muscle, is scanned once a week to validate measurements. This standard allows calculation of total body fat, muscle, and bone mass, and percentage of body fat.

xvi. **Treadmill fitness test to determine maximal oxygen uptake ($V0_2max$).** Cardiorespiratory fitness will be measured via a modified Balke continuous ramp treadmill protocol with some individual tailoring if necessary. We will monitor the heart electrical rhythm strip and continuous vital signs as participants exercise, and stop exercise if there are any signs of cardiovascular compromise. A Pediatric Advanced Life Support (PALS) certified physician or nurse practitioner will be present during this test. Each participant will exercise on the treadmill to the limit of their exercise tolerance, according to previously established criteria [157]. Participants will be familiarized with the equipment and procedures prior to any testing. The metabolic cart will be calibrated prior to data collection. Resting heart rate and blood pressure will be determined when the participant has been seated in a chair for at least 5 minutes before testing. EKG lead sites will be prepped and participants will be fitted with the leads, and a facemask, to measure expired gas exchange during exercise. Prior to testing, the participant will be given a 4-minute warm-up at a self-selected pace with 0% grade to orient them to the equipment and prepare them for the first stage of testing. Treadmill speed and grade will then be continuously increased until the subject reaches the limit of their exercise tolerance. Treadmill speed and grade increase will be based on body height, weight and predicted peak VO_2. Participants will be encouraged to exercise to their tolerance limit, but will be allowed to stop the test at any time. EKG, blood pressure, rating of perceived exertion (RPE), and any signs and symptoms of exercise intolerance will be monitored throughout exercise and during recovery. Expired gas exchange will be measured breath by breath by metabolic cart. Lactate threshold (LT estimated by gas exchange, will be determined using the V-slope method and dual criteria graphs[158] Peak oxygen uptake ($peakVO_2$) will be defined as the highest VO_2 achieved at the end of exercise. Treadmill speed and grade equivalent to 80% LT will be used to program walking during the interrupted sitting portion of the study (see text below). Pediatric exercise

testing guidelines, published by the American Heart Association, will be observed regarding termination of exercise:[157]

a. The child requests to stop the test.

b. Ventricular tachycardia.

c. Any significant drop in systolic blood pressure or a failure of systolic blood pressure to rise in the presence of increasing workload.

d. Signs and symptoms indicating potential hazard to the patient such as pain, headache, dizziness, syncope, excessive dyspnea or fatigue.

e. A > 3 mm ST segment depression or elevation.

f. Significant arrhythmia precipitated by exercise such as PVC's with increasing frequency, supraventricular tachycardia, ventricular tachycardia or atrioventricular block.

g. Excessive rise in blood pressure: systolic blood pressure >250 mm Hg.

h. Failure of the monitoring system.

d. Study procedures

All procedures are for research purposes; there is no provision of standard medical care. Participants found eligible after screening will be scheduled for 6 visits (Days #1-6) on consecutive days. Participants will be randomized to one of the two conditions for all of their subsequent visits. Randomization will be done by randomizing each subject to a single treatment by using the method of randomly permuted blocks with average block size 4. Subjects will be stratified by sex (M, F), age (7-9.49 vs. 9.5-11.99y) weight status (BMI<85^{th} percentile vs. ≥85^{th} percentile), and IPAQ result (low, moderate, and high usual physical activity) to promote similar subject characteristics in the two groups.

See **Appendix B** study visit flow table.

i. <u>Test Days #1-6 Daily Procedures:</u>

a. Vital Signs. Blood pressure, height (in cm), weight (in kg), temperature, respiratory rate, and pulse rate will be obtained.

b. **Questionnaires.** Parents and/or participants will answer a brief health questionnaire about participants' medication use, general health, and sleep duration in the previous 24-hours, see Appendix G. Additionally, a 24hr food recall will be administered.

c. **Randomized condition.** Each participant will execute their assigned randomized condition as follows:

(i) Sedentary condition. The participant will execute 3 hours of sedentary (seated or recumbent) activity of their choice. Activity monitoring (described below) will be obtained. Continuous heart rate monitoring (described below) will be obtained on test visit #6.

(ii) Interrupted condition: Throughout the visit, the study team will prompt the participant to walk on the test treadmill for 3-minutes every 30 minutes for 3 hours. There will be a total of 6 walking bouts (total exercise time: 18 minutes). This walking activity will be 'moderate-intensity walking' determined as 80% of maximum heart rate determine during the V02max test described in screening. We will calculate and set the treadmill speed and gradient per the estimated target heart rate prior to beginning the test protocol. Heart rate will be monitored during each walking bout, and maximum heart rate will be manually recorded after each walking bout. Activity monitoring (described below) will be obtained. Continuous heart rate monitoring (described below) will be done on test visit #6. Participants will be required to remain sedentary (seated or recumbent) in between their walking bouts.

d. **Continuous heart rate monitoring:** A heart rate monitor (Polar Vintage NV, Polar, Port Washington, NY, USA) will be used to determine activity state during walking bouts for participants randomized to the "interrupted" condition on all test visit days. Participants randomized to the "sedentary condition will wear a heart rate monitor during test #6 only.. After vital signs are obtained, participants will wear the monitor for the duration of test visit(s) (as specified above), and remove it prior to patient dismissal. Device initiation and data collection will be performed weekly, such that one device will be

initiated prior to the test day(s) of each participant's study week, they will use the same device for the entirety of their visit(s) and data will be collected from the device at the conclusion of the visit(s)(no further than 5 business days post study conclusion).

e. **Continuous glucose monitoring**: Beginning on day #1 of testing/intervention, a continuous glucose monitor (Libre Pro, Abbott products) will be placed on each child to record glucose trends continuously throughout their 6-day study. The Libre Pro device will be evaluated for placement, skin irritation, function, and data collection daily at each testing visit. It is expected that only 1 device will need to be placed to complete the entire 6-day study. On Day #6, the final day of testing, the Libre Pro device will be removed. Continuous glucose monitoring data will allow calculation of average daily glucose iAUC as well as mean amplitude glycemic excursion (MAGE; the arithmetic mean of all increases or decreases in glucose that exceed 1 SD of the total set of glucose values). If a device is lost/falls off between study sessions, a replacement device will be placed on the subject. If a child consumes a listed medication known to interact with glucose readings (acetaminophen, vitamin C, and aspirin), the following 24hrs of CGM data will be admitted from their analyses but the device will otherwise remain on the subject to continue collecting data throughout the remainder of their study block.

f. **Continuous physical activity monitoring:** On day #1 of testing/intervention participants will be given a wrist accelerometer (Actigraph GT3X+) to wear continuously throughout their 6-day intervention to assess in-study as well as free-living activity, sedentary behavior, and sleep efficiency. This device will also be removed on day #6 and data will be downloaded. These data will be used to determine if physical activity outside of the test condition changes as a result of intervention. Additionally, the participant will be fitted with a hip accelerometer on test visit #6, which will be worn for the entirety of test visit #6. These monitors will provide added data to evaluate fidelity of the interventions.

g. **Standardized meal intake:** Beginning on day #1 of intervention, the participants will be given daily standardized meals to consume throughout the duration of the intervention. Specifically, they will be given a standardized snack (10% of daily caloric intake) upon arrival for their daily test visit (days 1-5 of intervention) which will be consumed during their 3 hours of intervention. Then they will be sent home with 3 packed meals and one snack (dinner, mid-morning snack, breakfast and lunch) each day to be consumed as their sole caloric intake until they return for their next daily clinic visit. (Meals with consist of the following caloric proportions for daily total intake: breakfast 20%, lunch & dinner 30% each, snacks 20% of total daily requirement). This cycle will continue until they return for their final fasting visit on day #6 (see below). Nutrition staff will verify intake of provided foods and collect any leftover foods to be weighed back. Participants will also be asked to report any additional food (not from the standardized meals) that was consumed. The energy content of the standardized meals and snacks will be calculated based on individual weight & caloric expenditure assessed from weekly physical activity ascertained from questionnaire data (International Physical Activity Questionnaire screening form).[156]

Food handling details: All foods are weighed on electronic balance scales in gram weights to the nearest gram; foods consumed are determined by subtracting the food weights after the participant's meals from initial weights. Nutrition staff preparing and measuring food have yearly HACCP food safety training that focuses upon safe food handling, proper temperatures, hand washing, and using gloves for handling foods. All nutrition staff has additionally received training in weighing/processing foods for controlled feeding studies to ensure consistency in the method and presentation.

ii. **Test Day #1:** Cognitive function, mood and anxiety questionnaires. (See details under *Screening visit).* On the first day of testing, participants will perform the same anxiety and mood questionnaires as performed during the *Initial Screening* and be administered the Cogstate Brief Battery (both practice and baseline) as follows:

a. *Cogstate Brief Battery practice battery. Administered 10 minutes prior to beginning of 3-hour session.

b. Cogstate Brief Battery. Administered at time 0 and again at time 120 minutes of study visit.

c. PANAS-C. Administered at time 0 and again at time 180 minutes of study visit.

d. STAIC. Administered at time 0 and again at time 180 minutes of study visit.

*

iii. **<u>Test Day #5:</u>** Cognitive function, mood and anxiety questionnaires. (See details under *Screening visit).* On the second to last day of testing, the participants will perform the same cognitive function, anxiety, & attention questionnaires as performed during *Test Day#1* as follows:

a. Cogstate Brief Battery. Administered at time 0 and again at time 120 minutes of study visit.

b. PANAS-C. Administered at time 0 and again at time 180 minutes of study visit.

c. STAIC. Administered at time 0 and again at time 180 minutes of study visit.

iv. **<u>Test Day#6:</u>** Participants will be asked to fast overnight in preparation for this morning visit. In addition to the daily procedures described above for *Test Days #1-6* (including engaging in their randomized assignment to sitting-only or sitting interrupted with walking), the participant will perform the following final study procedures:

a. **Placement of IV catheter.** ELA Max cream will be applied to numb the IV site. The NIH Vascular Access Device service will be consulted as needed to place the IV catheter for this test.

i. **Fasting oral glucose tolerance test (OGTT).** A standardized dextrose oral solution (75 g per 300mL; dose 1.75g per kg of body weight up to a maximum dose of 75g) will be given at time 0. The participants will be asked to execute their randomized condition (seated or interrupted seated as described above). Blood will be

sampled at times -5, 0, +20, +30, +60, +90, +120, +150, and +180 minutes for measurements of insulin, C-peptide, glucose, cortisol, FFA, and triglycerides. Research specimens (10ml) will be obtained for future analyses at time 0, 30 and 120 minutes. During the 180 minutes of prolonged sitting, participants will be permitted to use the restroom if necessary and drink water. After completion of this sampling, the intravenous line will be removed.

b. **Cognitive function, mood and anxiety questionnaires.** (See details under *Screening visit).* On the last day of testing, the participants will perform the same cognitive function, anxiety, & attention questionnaires as performed during *Test Day#1 & #5* as follows:

   i. Cogstate Brief Battery. Administered at time 0 and again at time 120 minutes of study visit.
   ii. PANAS-C. Administered at time 0 and again at time 180 minutes of study visit.
   iii. STAIC. Administered at time 0 and again at time 180 minutes of study visit.

c. **Hunger ratings.** Hunger will be assessed using a visual analog scale (VAS), through Clinical Trials Survey System (CTSS). The VAS is a simple category scale of satiety with anchors at the end-points and at five equally spaced intervals between the anchors.[159] The VAS assesses satiety using a 0mm to 100mm range, with "Not at all hungry" at 0mm and "Greatest imaginable hunger" at 100mm. The VAS will be administered at pre-test, +30, +60, +90, +120, +150, and +180 minutes after the walking and blood draws. They will take approximately 5 minutes to complete. VAS scale scores are reproducible across multiple time points and predict subsequent food intake in children.[160-162] A comparison between different VAS scales found the bipolar hunger/fullness scale to have the best correlation (r= 0.64) and reliability.[163]

d. **Buffet test meal.** A standardized multiple-item buffet test meal (approximately 10,000 kcal) will be given at the end of each condition block, day #6 of testing. The meal has an array of macronutrients (approximately 12% protein, 51%

carbohydrate, and 37% fat) from foods frequently consumed by children.[65] Food energy and macronutrient content are calculated using the USDA National Nutrient Database for Standard Reference. Participants are instructed to 'eat as much as you want' and the amounts of foods and beverages consumed are calculated by measuring the differences in weights (g) of each item before and after the meal. Duration of eating is measured from the time the experimenter leaves the room until the participant indicates eating is completed. Energy and macronutrient consumption will be calculated.

Food handling details: See *Standardized Meal Intake above*

e. CGM device removal.

f. Accelerometer device removal.

Optional Supplemental MRS Arm Study Design:

a. We will enroll up to 75 children from the Break It Up protocol for this supplemental arm. Participants qualifying for the Break It Up parent protocol will be offered the opportunity to participate. A separate consent and assent form have been prepared and will be signed before these studies are done.

b. Baseline MRS scan: Participants will attend a visit 0-21 days prior to beginning their Break It Up! test week to perform a baseline 1H-MRS scan of liver and up to 3 skeletal muscles (vastus lateralis, tibialis anterior and soleus muscles) using 3T Magnetic Resonance scanner and a multi-channel flexible receiver coils. Magnetic Resonance Spectroscopy (MRS) will be performed to quantify liver fat and intra- and extramyocellular lipid content. This pre-intervention, baseline scan will be performed in a standard fashion after participants consume a standard meal shake (calculated to be approximate 30% of daily energy needs). In effort to minimize external factors, subjects will be asked to refrain from strenuous activity, such as aerobic exercise 30minutes consecutively or more, on the day prior and the morning of this scan. Additionally, a 24hr dietary recall will be performed.

c. Post Intervention MRS scans: On day # 4 or #5 of the main study (based on equipment availability), participants will repeat the same MRS scan of their

skeletal muscle as their pre-intervention baseline scan two more times. The first will be performed at approximately 12pm under the same conditions of the baseline scan (including consuming the same breakfast shake and refraining from strenuous activity, such as aerobic exercise 30minutes consecutively or more, on the day prior and the morning of this scan). After this scan, the subject will be transferred to the metabolic unit (via wheelchair) for their main study intervention session. The subject will then carry out their randomized intervention session and consume their standard diet (per the Break It Up! protocol). The third and final MRS scan will be performed immediately after the subject has completed their 3-hour intervention session at approximately 4pm.

*Of note, on this day the original CGM device will be removed prior to the MRS scan and new device placed after the final MRS given that the CGM device is not compatible with MRI scanning.

End of participation

a. Participants will complete study participation at the end of the *Test Day #6* visit.

b. Participant families will be told they should return for routine medical care to their usual pediatrician.

c. The guardians of each participant will receive the following information. Additionally, this information will be shared with a participant's health care provider upon request:

1) Abnormal fasting blood test results, such as high fasting blood sugar, from the outpatient screening visit.

2) Abnormal findings from the physical exam or EKG at the outpatient screening visit.

3) Questionnaire results from the outpatient screening visit if there is any evidence of significant emotional concerns on the anxiety or mood questionnaires.

d. Formal medical records will be available to all participants through the NIH Medical Records Department:

1) Clinical center laboratory results
2) Physical exam documentation
3) Electrocardiogram

5. Management of Data and Samples

a. Storage

i. Human biospecimens will be collected and stored using procedures appropriate for the type of biospecimen being collected and will be handled in accordance with the U.S. Occupational Safety and Health Administration's Bloodborne Pathogens Standard for Samples. Sample freezers will be operated using facility environments that include ambient temperature controls, good air circulation, lighting, and security. Systems are in place to allow for local and remote temperature monitoring of freezers. The NIH has an emergency preparedness plan that covers equipment failures and power interruption, including back-up storage capacity and back-up power generators.

ii. The principal investigator and the co-investigators will have access to identifiable participant records. We will follow guidelines developed by the NIH for projects funded by the Federal Government for managing such samples.

iii. Samples and data will be coded. Data will be coded by subject number, but will be readily associated with identifiable participant records. Records containing personal identifiers will be maintained consistent with the security measures required by the NIH. Data will either be transferred directly from the electronic medical record into the NICHD Clinical Trials Data Base (CTDB), or for data where direct transfer is unavailable, will be double-entered by hand into CTDB.

iv. All data will be kept on secure computers in the SGO office, whose doors will be locked when staff is not present. All paper data will also be kept in locked areas in the SGO office.

- Serum and plasma samples not analyzed immediately (as described above) will be stored in locked freezers by the NICHD sample management team supervised by the NICHD Clinical Director and tracked/managed through CTDB. Human

biospecimens in storage will have a unique identifier, will be labeled with a printed label and will contain a barcode. Samples will be tracked using a computer-based inventory system that records the location and detailed information of every specimen in the repository.

- Data will be stored in a password-protected computer system and in paper files in locked file cabinets and office areas to which only the NIH research team will have access.
- Samples and data collected from participants in this research study will be saved and used for future biochemical testing related to body weight and metabolism. This testing may be done by research laboratories that are not at the NIH. Additional consent will be obtained to use these samples if the consent form indicates that such consent is required. Prior to transfer of samples and data to non-NIH collaborators, a material transfer agreement (MTA) with the outside institution will be established. We will provide the NICHD IRB with a listing of MTA's that have been established under these provisions at the time of each Continuing Review.
- If unauthorized disclosure of identifying information linked with samples or data occurs, the IRB will be notified by the Principal Investigator within 7 days of learning of the occurrence.
- Samples and data will be stored for a minimum of 3 years after completion of analyses for this study, but may be kept indefinitely. Furthermore, in compliance with federal law, records will be maintained until all subjects enrolled in the study have reached a minimum age of 23 years. Subjects may choose to withdraw from the study at any time and may request for their stored samples to be destroyed. All withdrawals from the study will be documented and included in the annual report for continuing review by the IRB.
- The PI will report to the IRB loss or destruction of data or samples when substantial numbers of samples/data records (more than 10) are unintentionally destroyed (for instance, by a freezer failure). Loss or destruction of samples saved only for tertiary outcomes or future unspecified research will not be routinely reported, unless such loss also entails a breach of the Privacy Act.

b. Data and sample sharing plan (See Appendix F for Scientific Director Approval)

- This protocol is not subject to the Genomic Data Sharing policy as no genomic data will be collected.
- Data will be submitted to the Clinical Trial Database (CTDB) for storage throughout data collection and to BRRIS at the conclusion of data collection.
- A Data Sharing Plan for this study has been approved by the NICHD Scientific Director. See Appendix F, Data Sharing Plan for complete details.
- Additionally:

  Data will be shared with Cogstate LTD and Britni Belcher, PhD, MPH (see **Collaborating Researchers and Affiliated Institutions** below).

  Data and samples may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to additional NIH-designated repositories and databases if consent for sharing is obtained in the future with protocol amendment.

  Samples and data will be stripped of identifiers and will be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH to permit the NIH research team to link clinically significant findings with a subject so that the subject can be informed and appropriate follow-up arranged if medically indicated. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

c. **Collaborating Researchers and Affiliated Institutions:** This paragraph may be amended in the future to describe any MTAs that are established to use samples from this study.

- A technology transfer agreement with Cogstate LTD has been executed for this study. This study will use Cogstate software as detailed throughout the protocol and in turn Cogstate LTD will be provided the following de-identified data: questionnaire performance, medical status (specifically any neurologic or psychologic diagnoses), ethnicity, level of education, yearly household income, and randomization assignment.
- Extramural collaborator Britni Belcher, PhD, MPH will have continuous access to deidentified data (coded by subject number and otherwise without name, date of birth or other unique identifiers) throughout study to assist with data analyses.

6. Additional Considerations

a. Research with investigational drugs or devices

Not Applicable.

b. Gene therapy

Not Applicable.

7. Risks and Discomforts

As with any research study, there may be risks, inconveniences, and discomforts that occur during the course of the study. There will be an effort make to minimize the risk and discomfort associated with the study.

a. The **physical examination** by a health care provider will be conducted with the participant unclothed. While all efforts will be made to protect privacy, some participants may find this embarrassing.

b. Non-invasive **body composition methods** such as waist circumference measurements will be done with a flexible tape measure; these measurements involve no risk and are not painful. However, some participants may find it embarrassing to stand in their underwear.

c. **Total blood draw** amount will remain within the NIH guideline of 5 mL/kg in a single day and 9.5 mL/kg every 8 weeks. Blood collection by venipuncture is associated with mild discomfort (which will be attenuated by offering to administer ELA max cream before venipuncture), and the possibility of localized bruising or extravasation. The risk of infection, phlebitis (a blood clot), or fainting is extremely small. An area of white or a red rash that usually goes away within a few hours may be seen after use of ELA max cream.

d. The **DXA scan** may cause inconvenience of the time required and results in minor risk from radiation. DXA scanning involves radiation exposure for research purposes only. According to the manufacturer's specifications, DXA scanning for body composition using the array beam delivers an effective dose of 0.003mrem total body radiation. This calculated value is known as the "effective dose" and is used to relate the dose received by each organ to a single value. This amount is well below the recommended radiation exposure for child volunteers (< 500 mrem per year). For comparison, the average person in the U.S. receives a radiation exposure of 0.3 rem (300 mrem)/yr from natural background sources, such as from the sun, outer space, and from radioactive materials that are found naturally in the earth's air and soil.

e. The **exercise tests** conducted in the study are low risk for participants without known heart disease. The major risk of exercise is that unsuspected heart disease could be uncovered. Even though participants will be examined before the study to try to rule out the presence of heart disease, it is possible to have undetected heart disease leading to exercise-related chest pain, an unusual heart beat rhythm (arrhythmia), or myocardial infarction (heart attack) with heart damage or death. All participants will undergo EKG and, if indicated by EKG results or physical examination, echocardiography before performing the maximal exercise test. We will monitor heart function as participants exercise, and stop the exercise if there are any signs of heart problems. A physician or Pediatric Advanced Life Support (PALS) (PALS) certified nurse practitioner will be readily available during these tests. In settings where apparently healthy adults undergo maximal exercise tests, the risk of death has been estimated to be between 0 and 3 deaths/100,000 tests. Risk for death in otherwise healthy children will be lower than the rate cited for adults. Other risks of exercise include the possibility of slipping and falling

while exercising, muscle or joint soreness, and muscle fatigue caused by exercising that could last several days. Some individuals may exhibit mild skin irritation of the areas required for EKG lead placement.

f. The **Cognitive Tests and Mood Questionnaires** involve no known risk. Participants may feel embarrassment or discomfort about answering questions about mood, anxiety, and performing cognitive tests. Although we expect this embarrassment or discomfort to be minimal, these tests and questionnaires do take time and may cause inconvenience. If there is slight discomfort due to the wording of some questions, the participant is not required to answer those particular questions.

g. Using **continuous glucose monitor** (CGM) devices may cause some mild discomfort, as this device includes a subcutaneous insertion site through a minimally invasive needle stick. The most common cited complication of CGM use is pain during insertion and skin irritation from adhesive material. These concerns are infrequently reported. For example, in randomizing approximately 100 adults and children to a CGM device, Diess et al. reported that 4 individuals withdrew CGM use citing pain, adhesive complication, or alarm complications.[164] We will minimize these complications by using: 1. Child Life services for education and distraction techniques in preparation for and during the insertion procedure, and 2. Multiple adhesive techniques for skin application including skin preparation and various covering materials including skin tape and Tegaderm. Other than these concerns, CGM devices have never been reported to directly cause medical harm. Zero significant adverse events such as infection were reported by Diess et al. (n = 108 randomized to CGM use).[164] However, given that the insertion procedure requires breaking the dermis, there is always potential for introduction of infectious material into the body. The procedure requires appropriate skin preparation and clean technique to prevent this. For our study, only proficiently trained licensed providers will insert CGM devices to ensure proper technique. Furthermore, given the superficial placement, if the skin and device preparation fail to prevent inoculation, prompt identification and removal of the device will allow for healing and clearing of a potential infection. Our study will be providing close monitoring with daily evaluation of the CGM site by research study staff, making note of any signs or symptoms of infection or allergic reaction. If any signs or symptoms develop, such as redness, swelling, skin

pealing, or drainage, or the subject reports irritation or discomfort to the study staff, a licensed provider will be notified, and the site will be removed if deemed appropriate. Replacement of a new device in a separate location will only be performed if removal was indicated due to skin irritation and will be executed by using new adhesive mechanisms. If removal is indicated due to concern for infection, no additional CGM monitoring will be obtained on the participant in question.

h. The study as a whole requires a significant **time commitment** on the part of study participants and their families. The screening visit will take approximately 6 hours. Each test visit will take approximately 3.5 hours.

i. **False Positives**. Abnormal test results may cause anxiety and could lead to additional medical evaluations.

j. **MRS Scan**. This machine requires laying supine for up to approximately 120 minutes; therefore, there may be some discomfort or restlessness. The child will be able to be in communication with their guardian and study team throughout the entire imaging in order to communicate significant distress or request to terminate the study.

8. Subject Safety Monitoring

Study participants will be examined at the screening visit and both test visits. At the beginning of each visit, participants will be asked whether they are experiencing any unusual symptoms, and measurements will be made of their vital signs (oral temperature and arm blood pressure). Stopping procedures for the VO2max test are outlined above. Heart rate will be monitored during test visit #6 and during walking bouts for participants randomized to "interrupted" condition (see above).. If unanticipated serious adverse events occur during the course of the study, subjects will be withdrawn and we will promptly report them to the IRB.

Criteria for withdrawal of subjects from study:

Participants will be informed that they can withdraw from the study at any time. Noncompliance with study procedures may lead to subjects being withdrawn by Investigators. Other events that will result in study discontinuation include:

a. Inability to complete treadmill fitness test.

b. Inability to tolerate sitting (or sitting + walking) protocol.

c. The development of a severe psychiatric disorder.

d. Any verified abnormal laboratory values or physical status that is not allowed by the study.

9. Outcome Measures

a. Primary outcome measure. The primary outcome is insulin iAUC obtained during the oral glucose tolerance test at the final experimental visit. A difference in insulin demand, demonstrated by a difference in iAUC between groups, is hypothesized to occur with this intervention through increased non-insulin mediated glucose uptake.[165, 166] A decrease in insulin demand is clinically relevant as it is a marker of improved carbohydrate metabolism and decreased risk of dysglycemia or the development of diabetes mellitus.

b. Secondary outcomes are glucose iAUC obtained during the oral glucose tolerance test; mean iAUC as obtained from 24hr- continuous glucose monitoring, and Mean Amplitude Glycemic Excursion (MAGE) as obtained from 24hr- continuous glucose monitoring. Tertiary outcomes are cognitive function (working memory, inhibition, attention, and processing speed), mood, anxiety, daily physical activity (steps counts/day), and dietary intake at the final test meal. Improved circulating glucose values as demonstrated by lower glucose iAUC would directly support our primary insulin outcome and would be clinically relevant as this result would indicate improved metabolism. Additionally, MAGE has been demonstrated to be associated with metabolic control such that elevated MAGE is associated with worsening cardiometabolic outcomes in individuals with diabetes.[167]

The executive function, mood and anxiety outcomes are being collected for independent objectives. This group of measures is an important objective to provide information that will be necessary to know for feasibility of this intervention. This intervention is poised to community application such as school class rooms. Therefore, it is important to understand if executive function is adversely affected by the intervention as this would limit its feasibility for implementation. Finally, all analyses will be performed within the subgroups of healthy weight and overweight subjects to evaluate for differential effectiveness based on weight status.

10. Statistical Analysis

a. Analysis of data. Prior to conducting the main model analysis, descriptive analyses will be conducted on all outcome and covariate to assess potential outliers and calculate summary statistics. If significant deviations from the assumed distribution (e.g.: normal) occur, then appropriate transformations will be applied to fulfill model assumptions. If transformations are not sufficient to meet model assumptions, then nonparametric tests will be used. Descriptive statistics will investigate correlations between variables at pre-test and post-test to assess potential confounders prior to analyses.

The parallel study design allows for the examination of between-individual comparisons of test effects and within-individual longitudinal test effects (i.e. repeated daily CGM metrics). Significance level will be set at $\alpha = 0.05$. Covariates will include: age, sex, race/ethnicity, baseline cognitive ability (for cognitive outcomes only), baseline homeostatic model of insulin resistance (HOMA-IR), body fat mass (except for subgroup analysis by weight status), and Tanner stage. We will also evaluate for a weight status interaction (intervention x healthy weight / overweight).

The following analyses will be performed:

i. Participant characteristics will be summarized using means (standard deviations) and frequencies.

ii. Baseline (time 0) values for participant characteristics, insulin, glucose, heart rate, and blood pressure between the groups will be compared using t-tests.

iii. The primary analysis will be ANCOVA comparing children randomized to the two groups for insulin iAUC at the end-study oral glucose tolerance test, with age, sex, race/ethnicity, HOMA-IR, body fat mass, and Tanner stage as variables in the model. If all covariates are nonsignificant, unpaired t-tests will be reported for the differences in OGTT insulin iAUC by condition (prolonged vs interrupted sitting). Similar analyses will be used to examine glucose iAUC, 5- or 6-day average 24hr CGM iAUC and 24hr MAGE. (Subjects may only have 5 days of CGM data if a device is

accidentally removed and replaced at the next visit or subject uses a medication that interacts with glucose readings such as acetaminophen-after which 24hrs of data will be omitted from analysis). Mixed models will also be used to assess the influence of prolonged vs. interrupted sitting on insulin and glucose iAUC over the 3 hours of observation. To evaluate differences in the dependent variables at each time point, planned comparisons of model-based mean differences between each time points will be calculated. Additionally, mixed models will be used to assess condition influence on mean 24hr CGM glucose iAUC and 24hr MAGE.

iv. ANCOVA or (as indicated above) t-tests will investigate differences in working memory, inhibition, attention, processing speed, mood, anxiety, and kcal intake between prolonged sitting and interrupted sitting at the post- test.

v. Mixed models will also be used to assess the influence of prolonged vs. interrupted sitting on change in cognitive function, mood and anxiety (pre-post test scores) controlled for significant covariates. Covariates to be considered include those listed above in addition to free living activity and sleep.

vi. As a secondary analysis, all the analyses described above will be performed on the subgroups: Healthy weight and overweight subjects.

vii. Supplemental MRS Study: Repeated measures ANOVA will investigate the change in IMCL content between baseline & pre-session (thus, evaluating for daily intervention effect) as well as immediately pre- and post-intervention session (thus, evaluating the immediate effect of intervention) by condition. ANCOVA analyses will compare the change in IMCL content by condition with age, sex, race/ethnicity, HOMA-IR, body fat mass, and Tanner stage as variables in the model.

b. Power Analysis

Based on our preliminary published data,[65] and our unpublished data in overweight youth, we expect a moderate to large (Cohen's d = 0.8) effect size. With an 80% power, α=0.05, our sample size requires evaluable data from 21 in each group; with our goal of subgroup analysis within healthy weight and overweight groups, we will require 84 participants total (42 overweight and 42 healthy weight). This is supported by previously reported adult data published by Dunstan et al. who identified the mean (standard

deviation) difference between the two groups was 191.0 (334.9) pmol/L yielding a moderate to large effect size.[48] Taking into consideration more variability due to the parallel design (versus cross over designs used previously), we will randomize up to 120 participants and estimate that at most 36 children will drop-out of the study before completion. We expect at least 84 participants to complete the protocol which is our target completion based on these analyses.

11. Human Participant Protections

a. **Rationale for subject selection**

This is a study to examine the effects of interrupting sitting on metabolic characteristics, executive function and attention, mood, anxiety, and dietary intake in children. Both healthy weight and overweight children will be studied because our pilot data suggest similar effects on insulin iAUC are seen. Pre-adolescent children were selected because the approach being studied is most suitable as a preventive measure that could be implemented in elementary or middle schools. This pilot study therefore has the potential to inform future intervention studies aimed at intervening on behaviors that promote pediatric obesity. Recruitment efforts will aim to enroll 50% girls, 50% boys, age 7 to <12. Participants from all socioeconomic backgrounds in the metropolitan Washington D.C. area will be invited to participate in the study. Children of all race/ethnic groups will be included.

b. Equitability

This study will provide equal opportunity to participants. We will screen and enroll interested participants without concern of race, ethnicity, sex, disability, nationality, religion, handedness, or age (within inclusion criteria).

c. Justifications for exclusions:

All exclusions are either based on identification of problems in the participant's physical or mental health, based on the ability of the participant to complete protocol studies, or are required for the goals of the study (e.g., age 7-11.99y). Individuals will be excluded if they are not proficient in English due to the availability of our mood and

anxiety testing questionnaires which are only available in English). Identification of exclusions will lead to referral to an appropriate physician or mental health professional.

d. Justification for sensitive procedures: N/A

e. Safeguards for vulnerable populations: **Children**

This study involves many minimal risk procedures: outpatient visits, physical examination, phlebotomy, anthropometric measurements, cognitive testing, exercise testing, and DXA scanning for child participants. Efforts will be made to schedule Clinical Center visits during school holidays and breaks to minimize absences. The protocol considers psychological as well as physical discomfort, and provision has been made for soliciting the written assent of children and the written permission of parents.

Study staff will emphasize to participants that they are able to withdraw from the study at any time without penalty. To protect against the risk of embarrassment or discomfort, participants will be allowed to skip questionnaire items they do not wish to answer, or stop any study procedures. Written, active informed consent will be obtained from at least one parent/guardian (both will need to sign if otherwise required because, for example of joint custody), and assent will be obtained from children.

Every effort will be made to maintain the confidentiality of participant medical records for this study. Only group information without personal identifiers will be included when submitting manuscripts for publication. All information gathered for the purposes of this study will be treated as a medical record with the same degree of confidentiality.

12. Risks/Benefits Analysis

a. Known Potential Benefits:

There are no expected benefits to participants.

b. Known Potential Risks

Potential risks and discomforts are described in the consent form and in Section 7. These include discomfort or bruising during intravenous catheter placement and blood draws,

nausea during glucose tolerance testing, and the risks of walking on a treadmill (such as spraining an ankle) and of undergoing MRI (claustrophobia), In the literature, there are reports of healthy children who have undergone each of these tests in IRB-approved studies. Individually, all the procedures proposed as part of this protocol have been considered to represent minimal risk. For example, a panel convened in 1993 by the Secretary of Health found that placement of two intravenous catheters, administration of insulin and dextrose for the purpose of inducing mild hypoglycemia, and monitoring of blood glucose measurements every 5 minutes for many hours were of "minimal risk." The investigators have further taken many steps to minimize potential discomfort, for instance by administration of ELA max cream before blood is drawn. Children also routinely undergo venipuncture, which in children not infrequently requires multiple sticks to obtain samples. Furthermore, children are routinely required to complete cognitive tests during school. We believe the studies outlined in this protocol are reasonably commensurate with those children may be exposed to in their actual or expected medical, dental, psychological, social, or educational experiences. We therefore believe that the minimal risks of this study are reasonable in relation to anticipated benefit.

13. Consent Documents and Process

a. Designation of those obtaining consent

Study investigators designated as able to obtain consent in this protocol document will obtain informed consent from parents or guardians of study subjects. Written assent will be also obtained from the minor subjects of this study. See **Appendices C & D** for consent and assent documents.

b. Consent procedures

Each participant will receive a written explanation of the purposes, procedures, and potential hazards of the study. Communication of this information and of the participant's assent as well as the consent of a parent or guardian will be documented in the medical record. Written, active informed consent will be obtained in-person during

the screening visit from a parent/guardian, and assent will be obtained from youth by the PI or designated associate investigators. All participants will be informed of their right to withdraw from the study. The purpose of the project, all testing procedures, and study components, and possible risks and inconveniences will be described in detail in language understandable to the parent(s)/guardian(s). The investigator will also explain the study to the minor who is of a younger age and level of understanding. Sufficient time and opportunity will be given for discussion of the research as well as to answer any questions they may have, taking care to minimize or eliminate the perception of coercion or undue influence. When the NIH informed consent and assent forms are signed, parents or guardians will be given a signed copy of the consent and assent form for their records. In the unlikely event that the parents/guardians of protocol-eligible subjects cannot travel to the NIH Clinical Center for a considerable period of time, the PI or other protocol staff designated to obtain consent will obtain informed consent by a telephone conversation. Consent may be obtained via telephone and/or another electronic process, rather than in person. In such cases, the written signed consent will be faxed and/or mailed and made part of the patients' NIH records. No research procedures will commence until the investigator has confirmed that written, legally effective consent has been obtained. In general, Investigators obtaining consent (whether in person or over the telephone) will be encouraged to have a second person (who is not obtaining informed consent) present during the entire informed consent discussion and, if applicable, the signature process. Investigators will also document the consent process in a note placed in the research record for all consent discussions.

As noted above, given the minimal risk of the study, the consent of one parent will be considered sufficient for protocol participation except in cases where two signatures are required (for instance because of shared medical decision-making explicated in a divorce decree).

c. Non-English–Speaking Participants

If a child who speaks English has a parent who is found not to be English-speaking is eligible for enrollment, the parent will be provided with the CC Short Written Consent Form for Non-English Speaking Research Participants and a verbal explanation of the

purpose, procedures and risks of the study as described in MAS Policy M77-2, NIH HRPP SOP 12 and 45 CFR 46.117(b)(2). The IRB-approved English consent form will serve as basis for the verbal explanation of the study. All NIH policies on utilizing the "short form" consent process, including having a witness during the "short form" consent process, will be followed.

d. Consent documents

The submitted consent form contains all required elements for consent. One consent form, for parents/guardians to sign for their minor children, and one assent form, for minor children, are submitted with this protocol. See **Appendices C & D.**

14. Data and Safety Monitoring

a. Data and safety monitor

For this un-blinded, minimal risk trial, we propose that the PI will be sufficient to monitor the study.

b. Data and safety monitoring plan for Non-IND

The following monitoring plan will be implemented to ensure the protocol data are in compliance with Good Clinical Practice (GCP), NICHD Institutional Review Board (IRB) and Federal regulations. This will ensure the validity, accuracy and integrity of the data.

1. Study Staff Responsibilities

Dr. Jack Yanovski, Principal Investigator, will be responsible for all aspects of the study. Some responsibilities may be delegated to other associate investigators as detailed in the *Study Personnel Sheet & Delegation of Responsibility Log.*

2. Source Documentation and Case Report Forms

Jack A. Yanovski, MD, PhD is responsible for coordinating data collection and will review the data for accuracy and completeness within seven days of each subject visit.

The PI along with study associate investigators will conduct the monitoring. Patient consent documents, primary outcome and safety laboratory results, and diagnostic test results will be monitored for accuracy, correct dating, and agreement between case report forms and source documents. As case report forms are entered electronically into the NICHD Clinical Trials Database, the computer system contains logs indicating changes made and the circumstances leading to these changes.

The medical records of active subjects (defined as subjects undergoing study procedures in the current year) will be monitored quarterly or more frequently as required. Any major findings will be summarized in writing and reported to the NICHD Institutional Review Board, if indicated. Investigator credentials, training records, and the delegation of responsibility log will also be reviewed on an annual basis.

3. IRB and DSMC Documentation

All IRB documentation can be found in iRIS. The Principal Investigator, *Jack Yanovski, MD, PHD*, is responsible for maintaining IRB correspondence related to this protocol, including records of all reviews of the study and submissions to the IRB. This protocol does not meet the criteria as outlined in the NIH HRPP SOP 17 for data to be submitted to a data safety monitoring committee. The PI and IRB can provide adequate oversight for this open trial.

Clinical Trial Studies (non IND/IDE) will have random audits performed by the NICHD Office of the Clinical Director: https://science.nichd.nih.gov/confluence/display/ocd/Protocol+Navigation).

4. FDA Documentation

Not Applicable – no IND.

5. Study Completion

Upon completion of the study *Jack A. Yanovski, MD, PhD* will retain possession of the electronic Protocol Binder in a secure location. HHS regulations require that subjects' records be maintained for at least three years after completion of a study. Since subjects of this investigation include children age 7-11.9 years, even longer record retention is required. All records will be retained until all participants have reached age 23 years.

6. Data safety and monitoring board (DSMB)

Because there is no blinded component to these studies, the studies do not involve administration of any pharmaceutical agents, and the procedures are of minimal risk, we propose

that a formal DSMB is not required, and that the investigators and IRB can adequately supervise this study. All adverse events will be documented and reported to the NICHD IRB as described above in 'Protocol Monitoring Plan.' We expect that the risk for an adverse event will be within the "Rare but serious (Event Rate < 1%)" category.

15. Quality Assurance

a. Quality assurance monitor

Jack A. Yanovski, MD, PhD is responsible for coordinating data collection and will review the data for accuracy and completeness within seven days of each subject visit. The NICHD Standard Operating Procedure for Intramural Clinical Protocol Monitoring (rev. 5.20.2015) will be followed.

b. Quality assurance plan

Quality assurance will be executed in accordance with the NICHD quality assurance plan. Adverse events and protocol deviations will be reviewed and recorded on a weekly basis at the SGO protocols review meeting.

Protocol-specific monitoring will include:

Review of biochemical results from OGTT testing within 72 hours of test.

Daily data retrieval from continuous glucose monitoring devices.

Weekly data retrieval from continuous actigraph and heart rate monitoring devices.

Weekly data retrieval from the NIH Toolbox Assessment center for cognitive measure outcomes.

16. Reporting of Unanticipated Problems, Adverse Events and Protocol Deviations

Event Characterization and Reporting to the IRB and Clinical Director (CD): Unanticipated problems, non-compliance, and other reportable events will be reported to the NIH IRB as per Policy 801 ("Reporting Research Events."). All reportable events occurring during

the study, including those observed by or reported to the research team, will be recorded. Reportable Events will be reported by the PI verbally and in writing as soon as possible. but not more than 7 days after the PI first learns of the event. Deaths will be reported to the Clinical Director within 24 hours after the PI first learns of the event. All adverse events (serious and non-serious, expected and unexpected) will be reported annually to the IRB for review. Electronic data sheets or summary language will be supplied for IRB review of adverse event reports at continuing reviews.

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems, adverse events (AEs), including serious adverse events (SAEs), and deviations in accordance with NIH policy, IRB requirements, and federal regulations.

17. Alternatives to Participation

The current proposal is a physiological study that does not treat any known condition; as a result, there are no alternatives other than non-participation. Participants and their families will be informed that they will not receive treatment as a result of study participation. For participants found to have medical conditions, treatment referrals will be provided.

18. Privacy

All research activities will be conducted in as private a setting as possible. This will be most noteworthy during the history and physical examination will be conducted in a private room in the pediatric day hospital.

19. Confidentiality

- Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers and on the secured data server hosted by the Section on Growth and Obesity as described above. Samples will be kept in locked storage. Only study investigators will have access to the samples and data.
- De-identified results will be posted on clinicaltrials.gov

20. Conflict of Interest

a. Distribution of NIH guidelines

- NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of interest

This is a covered protocol because of the research agreement with Cogstate LTD**.** There are no conflicts-of-interest to report for NIH investigators. Non-NIH investigators will abide by the conflict-of-interest policies of their own institutions.

c. Role of a commercial company or sponsor

Cogstate supplies software for collection of cognitive data during the study and will have access to some data under the terms of the signed agreement. The company had no role in the study design and will have no role in the decision to publish.

21. Technology Transfer

There is a technical transfer agreement negotiated with Cogstate LTD. See Collaborating Researchers and Affiliated Institutions under Management of Data and Samples above.

22. Research and Travel Compensation

Volunteers will be compensated for time and research-related inconveniences as follows:

1. Complete screening visit: $100
2. Complete outpatient test visits days #1-5: $100/visit (5 visits; $500 total)
3. Complete final testing visit, day #6: $150
4. Complete CGM wear: $20/wear (6 days; $120 total)
5. Complete wrist activity monitor wear: $10/day (6 days; $60 total)

(Study total $930)

Volunteers taking part in the MRS Supplemental arm of the study will be compensated for time and research-related inconveniences as follows:

1. Complete baseline MRS scan: $100
2. Complete follow up scan #1 (immediately before testing session): $75
3. Complete follow up scan #2 (immediately after testing session): $75

(Study Total $250)

Compensation will be prorated for parts completed if subjects do not complete the study as guided by the reimbursement plan above.
No additional incentives will be provided.
Payment will be provided in the name of child in check form.

Payment will be issues within 7 days after completion of last study visit. Travel/lodging compensation. Local travel may be covered to facilitate the daily visits required.

- No escort fee will be provided.
- NIH employees or staff who wish to have their child participate during work hours must have permission from their supervisor. NIH employees or staff must either participate outside of work hours or take leave in order to receive compensation.

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