IMPACT OF BODY COMPOSITION AND EXERCISE ON FOOD INTAKE REGULATION IN CHILDREN

A Dissertation in
Nutritional Sciences

by

Stephanie Nicole Fearnbach

© 2016 Stephanie Nicole Fearnbach

Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

December 2016
The dissertation of Stephanie Nicole Fearnbach has been reviewed and approved* by the following:

Kathleen L. Keller  
Assistant Professor of Nutritional Sciences  
Dissertation Advisor  
Chair of Committee

Barbara J. Rolls  
Helen A. Guthrie Chair and Professor of Nutritional Sciences

Charles Geier  
Assistant Professor of Human Development and Family Studies

Danielle S. Downs  
Associate Professor of Kinesiology, Obstetrics & Gynecology

David Thivel  
Assistant Professor of Sports Sciences, Université Blaise Pascal (Clermont-Ferrand, FR)  
Special Member

Rebecca Corwin  
Professor of Nutritional Sciences  
Graduate Program Chair

*Signatures are on file in the Graduate School.
ABSTRACT

Recent childhood obesity prevalence statistics suggest that 17% of children and adolescents ages 2 to 19 years are living with obesity. Determining alternative methods to decrease daily energy intake and increase energy expenditure is critical for childhood obesity prevention. A “one size fits all” approach to treating obesity has not been effective. This dissertation is based on the Energy Balance Framework from Blundell and colleagues, which is a working model that describes the major influences on appetite control. Emerging research is working to better understand the dysregulation of energy balance, with recent studies in adolescents and adults. However, the effects of the energy balance system and appetite regulation pathways on food intake have not been fully examined in younger, pre-adolescent children. This dissertation extends previous research in these areas and examines the influences of individual differences in body composition and exercise on appetite regulation and food intake in children under the age of 12 years, across three separate cohorts of children. In three of the four papers of this dissertation, the role of fat-free mass as an appetitive driver in children emerged as a common theme, consistent with previous work in adults. There was limited evidence of an association between fat mass and food intake across the three cohorts of children studied. Exercise, compared to a sedentary control, was effective in reducing relative energy intake (i.e., energy intake adjusted for activity-related energy expenditure), but individual differences influenced these relationships. Children who rated the controlled bout of exercise as more difficult had greater ad libitum energy intake than children who rated it as easier. This finding demonstrates the potential for perceived exertion during exercise to influence food intake regulation. In addition to this cognitive factor, there may be individual differences in the brain’s reward response to food-related cues that could also play a role in ingestive behavior. Therefore, the work in this dissertation highlights the role of the brain in the determination of energy balance-related behaviors. Collectively, the findings from these studies provide support for the Energy Balance Framework and extend these findings to pre-adolescent children. These three cross-sectional studies represent a few steps towards understanding the influences of body composition and exercise on food intake regulation in children. Additional longitudinal studies in these areas will aid in the understanding of the reciprocal interactions between energy expenditure, energy intake, and body composition over time. These studies may also inform the development of more effective and sustainable tailored weight management interventions.
# TABLE OF CONTENTS

LIST OF FIGURES vii
LIST OF TABLES ix
Acknowledgements xi

Chapter 1. GENERAL INTRODUCTION 1
   Energy Balance and Obesity in Childhood 1
   Energy Balance Framework 2
   Effects of Body Composition on Energy Intake 4
   Effects of Exercise on Energy Intake 5
   Role of the Brain in Energy Balance 8
      Cognitive Factors 8
      Food-related Neuroimaging Studies 9
         Impact of Exercise within Food-related Neuroimaging Studies 13
   Summary 15
   Dissertation Aims 16
   References 18

Chapter 2. STUDY 1: INTAKE AT A SINGLE, PALATABLE BUFFET TEST MEAL IS ASSOCIATED WITH TOTAL BODY FAT AND REGIONAL FAT DISTRIBUTION IN CHILDREN 29
   Abstract 30
   Introduction 31
   Methods and Procedures 33
   Results 37
   Discussion 42
   References 47

Chapter 3. STUDY 2: BRAIN RESPONSE TO IMAGES OF FOOD VARYING IN ENERGY DENSITY IS ASSOCIATED WITH BODY COMPOSITION IN 7- TO 10-YEAR-OLD CHILDREN: RESULTS OF AN EXPLORATORY STUDY 52
   Abstract 53
   Introduction 54
   Methods 56
Appendix E: Laboratory Test-meal Photos 171
Appendix F: Food Intake Data Sheets 174
Appendix G: “Freddy Fullness” Visual Analog Scale 183
Appendix H: Food Liking and Wanting Visual Analog Scales 186
Appendix I: ActiGraph Accelerometry 188
Appendix J: YMCA Submaximal Cycle Ergometer Fitness Test 194
Appendix K: 70% Intensity Exercise Test 199
Appendix L: Borg Scale for Rating of Perceived Exertion 203
LIST OF FIGURES

Chapter 1. GENERAL INTRODUCTION

Figure 1.1. Energy Balance Framework 3
Figure 1.2. Brain regions of interest with exposure to food-related cues and exercise / physical activity 10

Chapter 2. STUDY 1: INTAKE AT A SINGLE, PALATABLE BUFFET TEST MEAL IS ASSOCIATED WITH TOTAL BODY FAT AND REGIONAL FAT DISTRIBUTION IN CHILDREN

Figure 2.1. (A & B) Scatterplots of associations between body composition and food intake 41

Chapter 3. STUDY 2: BRAIN RESPONSE TO IMAGES OF FOOD VARYING IN ENERGY DENSITY IS ASSOCIATED WITH BODY COMPOSITION IN 7- TO 10-YEAR-OLD CHILDREN: RESULTS OF AN EXPLORATORY STUDY

Figure 3.1. Example of 1 functional run in the fMRI scanning paradigm 59
Figure 3.2. Main effect of energy density in the left thalamus 62
Figure 3.3. Scatterplot of association between fat-free mass and activation for High ED – Low ED foods in the right substantia nigra 63

Chapter 4. STUDY 3A: IMPACT OF IMPOSED EXERCISE ON AD LIBITUM ENERGY INTAKE IN CHILDREN AT RISK FOR OVERWEIGHT

Figure 4.1. Experimental day (Exercise or Sedentary) timeline 77
Figure 4.2. Relative energy intake 85

Chapter 5. STUDY 3B: PERCEIVED EXERTION DURING EXERCISE IS ASSOCIATED WITH CHILDREN’S AD LIBITUM ENERGY INTAKE

Figure 5.1. Scatterplot of association between fat-free mass and energy intake on the Exercise day 105
Figure 5.2. Scatterplot of association between rating of perceived exertion and energy intake on the Exercise day 105
Chapter 6. GENERAL DISCUSSION

Figure 6.1. Summary of the main findings for energy intake, expenditure and balance
LIST OF TABLES

Chapter 2. STUDY 1: INTAKE AT A SINGLE, PALATABLE BUFFET TEST MEAL IS ASSOCIATED WITH TOTAL BODY FAT AND REGIONAL FAT DISTRIBUTION IN CHILDREN

Table 2.1. Serving sizes for foods served at the baseline and palatable buffet test-meals

Table 2.2. Participant characteristics

Table 2.3. Body composition descriptive statistics

Table 2.4. Test-meal intake descriptive statistics

Table 2.5. Partial correlations

Chapter 3. STUDY 2: BRAIN RESPONSE TO IMAGES OF FOOD VARYING IN ENERGY DENSITY IS ASSOCIATED WITH BODY COMPOSITION IN 7- TO 10-YEAR-OLD CHILDREN: RESULTS OF AN EXPLORATORY STUDY

Table 3.1. Participant characteristics

Table 3.2. Talairach atlas coordinates tested in ROI approach

Table 3.3. ANOVA results: main effects of energy density

Table 3.4. Exploratory correlation results for brain activation and body composition

Supplementary Table 3.1. List of foods photographed for fMRI paradigm

Chapter 4. STUDY 3A: IMPACT OF IMPOSED EXERCISE ON AD LIBITUM ENERGY INTAKE IN CHILDREN AT RISK FOR OVERWEIGHT

Table 4.1. Participant characteristics

Table 4.2. YMCA submaximal cycle ergometer test stages

Table 4.3. Food items, serving sizes, and calorie contents for each meal

Table 4.4. Paired t-test results for energy intake and expenditure

Chapter 5. STUDY 3B: PERCEIVED EXERTION DURING EXERCISE IS ASSOCIATED WITH CHILDREN’S AD LIBITUM ENERGY INTAKE

Table 5.1. Participant characteristics
Table 5.2. Descriptive statistics for exercise test variables and intake 104
Table 5.3. Correlations between body composition, exercise test variables, and intake 104
Table 5.4. Linear regression prediction of Exercise day intake using ratings of perceived exertion and fat-free mass index 105
Supplementary Table 5.1. Laboratory test-meal menu 111
ACKNOWLEDGEMENTS

I am very thankful for the opportunity to have worked alongside my advisor, Dr. Kathleen Keller, over the course of the last four years. Her passion for children’s health research has really inspired me to approach science in innovative and thoughtful ways. She has supported the pursuit of my own research questions, beyond the expertise of the lab, and has helped me build a fantastic, collaborative team of mentors along the way. I would also like to thank her for allowing me to briefly run away with the circus after my first year of graduate school and trusting me to come back. I have no regrets.

Several other people have contributed to the work presented in this thesis. I’d like to thank each member of my doctoral committee. Dr. Rebecca Corwin has pushed me to become a better scientist and challenged me to think about things in new ways. Her steady positivity has been so encouraging throughout the roller coaster of graduate school. Dr. Barbara Rolls has provided a great example for what it means to be an independent woman in science. In addition, her attention to detail has helped me grow as a researcher, especially in the areas of study design and scientific communication. Dr. Chuck Geier has been a great mentor in the area of neuroimaging, and provided ample guidance during our lab’s first endeavors into the world of functional MRI. I’d like to thank Dr. Danielle Downs for being a thoughtful collaborator on our Energy Balance Study team, and for helping me combine my interests in exercise science and eating behavior here at Penn State. And of course, I’d like to thank Dr. David Thivel for sparking my interest in the effects of exercise on energy intake. I’ve been very fortunate to build an international collaboration with Dr. Thivel, who has very generously contributed to our combined research efforts over the last four years. This work has significantly enhanced my graduate school experience. I’d also like to thank mentors outside of my doctoral committee, including Dr. John Hayes, Dr. Eric Loken, and Dr. Lynn Panton, for their continued support.

I have had the distinct pleasure of working with the research team in Children’s Eating Behavior Lab. I’d especially like to thank our lab manager, Terri Cravener, and the other graduate students, Travis Masterson, Shana Adise, Laural English, Lizz Carney, Wendy Stein, Catherine Shehan, and Juliana Fritts, for making the lab such a fun place to work and learn. The work we do cannot be done without a solid team. Along those lines, I want to give a shout out to the research assistants who have helped on the various studies, including Haley Schlechter, Amanda Ross, Mike Rykaczewski, and the International Fellows who have visited. In addition,
I’d like to thank the staff at the Clinical Research Center, the Social, Life, and Engineering Sciences Imaging Center, and the Department of Nutritional Sciences for their support of my research and education.

I want to thank my Childhood Obesity Prevention Training Program family and the Nutrition Graduate Student Association for providing long-lasting friendships and enriching my experience as a graduate student at Penn State. In particular, thank you to Heather, Sam, Katie, Mike, and Alissa for your guidance, encouragement, and friendship. Finally, I’d like to thank all of my family members and friends for their love, support, and patience every step of the way. I could not have done this without all of you!
Energy Balance and Obesity in Childhood

Obesity rates have increased substantially over the past forty years, both in the United States and globally [1]. Recent childhood obesity prevalence statistics suggest that 17% of children and adolescents ages 2 to 19 years are living with obesity, defined as weight for length at or above the 95th percentile of sex-specific Centers for Disease Control (CDC) growth charts [2]. With early obesity onset at greater rates in youth, children are at the greatest risk for long-term health complications [3], as obesity is often resistant to treatment [4]. Obesity is often comorbid with other metabolic (e.g., Type 2 Diabetes Mellitus, metabolic syndrome), cardiovascular (e.g., hypertension, hyperlipidemia), and mental (e.g., depression, anxiety) disorders [3]. Consequently, children with obesity are living with these comorbid conditions for longer, resulting in greater physical and financial burden across the life course [5]. The dramatic increase in obesity rates has coincided with increased availability of large portions of high-energy-dense foods, driving a pattern of overeating [6]. This, combined with increases in the amount of time spent doing sedentary activities [7], has led researchers to use the term “obesogenenic” [8, 9] when describing the current environment. Determining alternative methods to decrease daily energy intake and increase energy expenditure is critical for childhood obesity prevention. A “one size fits all” approach to treating obesity has not been effective.

Traditional methods to reduce energy intake typically require explicit caloric restriction. Due to complex physiological, psychological, and cognitive effects that have been reviewed previously [10], dietary restriction is difficult to sustain long-term, and as a result, obesity is often resistant to treatment [4]. It may be particularly difficult to restrict intake of high-energy dense-foods during childhood when preferences for sweet, salty, and fatty foods are high [10-12]. In addition to reducing energy intake, another strategy to manage body weight is to increase spontaneous physical activity. Levels of physical activity both in and out of the everyday school setting have decreased, reducing children’s average daily energy expenditure [13]. Longitudinal research has demonstrated an inverse relationship between physical activity and obesity in youth [14]. However, physical activity in the form of high intensity exercise may not only affect energy expenditure, but also subsequent energy intake [15, 16]. In a meta-analysis, Schubert and
colleagues found that, in adults, high intensity exercise resulted in decreases in subsequent energy intake that were not compensated for over time [16]. However, there may be variability across individuals in compensation for physical activity, due in part to the intensity of the activity [17], the perceived difficulty of the exercise [18], and the extent to which foods are perceived as rewarding [19]. Exploring the mechanisms underlying this variation could improve and more effectively target obesity interventions. Overall, obesity is a multifaceted disease, but understanding the relative contribution to underlying physiological and psychological factors is a step towards improving obesity prevention efforts. The purpose of this dissertation is to examine the effects of body composition and exercise on food intake regulation and energy balance in children.

**Energy Balance Framework**

This work is based on the Energy Balance Framework from Blundell and colleagues, which is a working model that describes the major influences on appetite control [20]. This framework depicts that energy balance is regulated by a complex system of peripheral and central physiological signals ([Figure 1.1](#)). At the center of the framework is the basic Energy Balance = Energy Intake – Energy Expenditure equation that most people are familiar with. The arrows represent different signals or pathways by which Energy Intake and Energy Expenditure may be altered [20]. Tonic signals arise from compartments of adipose and lean tissue, also referred to as fat mass and fat-free mass, which communicate information of longer-term energy balance. These two metabolically active tissues contribute greatly to total energy expenditure, as they are drivers of resting metabolic rate. In addition, fat mass releases leptin and other adipokines as a result of excess energy storage in the form of adipose tissue. There are other episodic signals that arise from the gastrointestinal tract and accessory organs, including both appetite-stimulating and appetite-inhibiting hormones. The brain is then responsible for integrating these signals to determine energy needs and influence subsequent food intake behaviors, either promoting or inhibiting energy intake. This framework is based on a body of literature on appetite regulation in healthy adults [21, 22].
Figure 1.1. “Formulation of the major influences on appetite control using an energy balance framework. There is a distinction between tonic (enduring, relatively stable over days) and episodic (varying in strength during the course of a day) processes. Episodic signals arise as a consequence of food consumption. Tonic signals arise from body tissues and metabolism. The effect of fat mass on energy intake reflects a lipostatic view of appetite control; leptin is a key mediator of the inhibitory influence of fat on brain mechanisms. The metabolic demand for energy arises from energy requirements generated by the major energy using organs of the body (heart, liver, brain, gastrointestinal tract, skeletal muscle) and reflected in resting metabolic rate. The overall strength of the drive for food is the balance between the tonic excitatory and inhibitory processes. It is proposed that, as adipose tissue accumulates in the body, the tonic inhibitory effect of fat on energy intake becomes weaker (due in part to leptin and insulin resistance). Therefore, as people become fatter it becomes more difficult to control appetite. The effect of exercise on appetite control can be understood according to the relative strength of its effects on the tonic and episodic signaling systems.” Reference [20]: Blundell, JE, Gibbons, C, Caudwell, P, Finlayson, G, and Hopkins, M. Appetite control and energy balance: Impact of exercise. Obesity Reviews (2015) 16(Suppl. 1), 67-76. This figure is reproduced with permission from John Wiley and Sons, license no. 3917720636825 © World Obesity

Signaling in this system can be disturbed if any one contributing factor is out of balance. For example, some individuals with obesity also have leptin resistance, which disrupts the downregulation of appetite in times of energy excess. Or for those who are predominantly sedentary, both energy expenditure and body composition can be directly affected, and there may be indirect effects on energy intake. Emerging research is working to better understand the dysregulation of energy balance, with recent studies in adolescents and adults [23-26]. However,
the effects of the energy balance system and appetite regulation pathways on food intake have not been fully examined in younger, pre-adolescent children. While it has been well-documented that sex differences in body composition are pronounced in adolescent and adult populations and are often attributed to pubertal development, previous research has also shown that differences in fat and fat-free mass are present before puberty [27, 28]. From birth, males tend to have a higher average fat-free mass index compared to females, and tend to increase lean mass at a greater rate compared to females after 12 years of age [28]. Females have a higher average fat mass index after 4 years of age, and have a greater rate of increase in adiposity after about 8 years of age [28]. In addition, previous studies have shown that males tend to eat more than females, which is often attributed to the fact that males have greater amounts of fat-free mass [20]. For this reason, it is important to consider biological sex when examining associations between body composition and appetite-related outcome variables of interest, regardless of age. To date, however, no sex differences in the direction or overall magnitude of the correlations between body composition and food intake have been reported [20, 23].

The pieces of this framework that are most relevant to the current thesis are *Tonic Appetite Signals* (fat-free mass and fat mass), *Exercise, Energy Expenditure*, and *Energy Intake*. The role of the brain will also be addressed.

**Effects of Body Composition and Energy Intake**

Emerging evidence, predominantly in adults, suggests that fat-free mass is the best predictor of energy intake due to its influence on resting metabolic rate and total energy expenditure [21, 22, 24, 26, 29]. In controlled laboratory studies with adults, it has been shown that the effects of fat-free mass on objectively-measured intake are mediated almost entirely by resting metabolic rate [25]. Blundell and colleagues found that fat-free mass, but not fat mass or body mass index, was associated with self-determined meal size and daily energy intake in two samples of adults with obesity [29]. It is suggested that a physiological signal associated with fat-free mass may act as an appetitive driver, which may interact with signals related to body fatness (e.g., leptin). This is likely due to the contribution fat-free mass makes in determining resting metabolic rate, which is the largest component of daily energy expenditure [24, 29]. Therefore, the research thus far suggests that the effect of fat-free mass on energy intake is primarily homeostatic. Similar findings have been reported in adolescents with obesity [23], but this has
not been examined in younger populations. It is also unknown whether fat-free mass remains the strongest predictor of energy intake across meals that vary in food variety and palatability.

One of the most objective ways to measure actual short term energy intake is during controlled laboratory test-meal studies, which typically serve a single meal and measure food intake in response to some sort of manipulation or intervention. A criticism of single-meal studies, and laboratory intake studies in general, is that they may not be representative of food consumption under free-living conditions [30]. The laboratory environment may elicit different eating behavior compared to a more naturalistic environment [31]. In addition, intake from meal-to-meal, particularly in children, is thought to vary [32]. For all these reasons, measuring intake at a single test-meal in the laboratory has often not been associated with weight status [33]. The type of foods and/or meal-conditions that would demonstrate an association between intake and body composition are not known. Among children, laboratory test-meal intake has been shown to vary by type of food given [34], portion size [35], palatability [36], and brand packaging [37], all of which are factors that influence energy intake in free-living settings. The associations between child body composition and intake at test-meals of common foods that vary on these attributes have sparsely been examined.

**Effects of Exercise on Energy Intake**

Exercise directly affects energy balance by increasing energy expenditure. However, it may also indirectly affect energy balance by modifying energy intake [15]. The last 25 years have provided a large body of evidence regarding the potential nutritional adaptations (e.g., energy intake, food preferences and appetitive drives) that occur in response to exercise but the range in methodologies used across studies makes direct comparisons difficult. Differences in exercise type, duration and intensity, as well as meal type, and the timing between exercise and meal delivery can all impact the outcomes [38, 39]. More recently, the impact of sedentary behaviors (e.g., screen time, knowledge-based work, etc.) on energy intake has been observed, thus calling into question studies that have used a sedentary wait period prior to meal delivery [40]. Based on a subset of the literature using similar methodology across studies [15, 38, 41, 42], some authors have suggested that the impact of physical activity on energy balance could be primarily through its indirect effect on energy intake rather than its direct effect on energy expenditure [43, 44].
The first real overview of the interaction between physical activity and energy intake stated that a high level of physical activity could aid weight control either by improving the ability to match food intake to energy expenditure or by increasing expenditure to a level at which compensation is difficult [41]. The authors highlighted the uncoupling between the energy expenditure induced by exercise and subsequent energy intake, as previously suggested by Hubert and collaborators [45]. More recently, they underlined that physical activity has the potential to modulate appetite control by three possible mechanisms: 1) by enhancing the sensitivity of the physiological satiety signaling system, 2) by altering macronutrient preferences, and 3) by attenuating the hedonic response to food [46]. The impact of exercise on peripheral satiety signals and their communication with the arcuate nucleus to control energy balance has been reviewed elsewhere [47]. Since the hormonal responses to acute exercise can be categorized as “short-term” signals, recent data highlight the important impact of body composition, specifically fat-free mass, in the control of energy intake.

While exercise, relative to rest, has been shown to be effective in reducing subsequent energy intake and contributing to a negative energy balance in adolescents and adults [39, 42, 44, 45, 48-51], limited research has examined this relationship in children under the age of 12 years [52]. Previous work shows that high intensity exercise can induce a state of lower 24-hour energy balance by reducing subsequent energy intake relative to both low intensity exercise and sedentary activity in obese adolescents [42]. This is commonly referred to as the “transient anorexigenic effect” of exercise. In obese adolescents, the greatest effects of high intensity exercise on energy intake have been seen seven hours post-exercise [42, 53]. These changes were seen without any significant differences in appetite ratings (e.g., hunger, fullness, desire to eat). These findings suggest the use of exercise as a possible alternative strategy to reduce short term energy intake, which could augment attempts to intentionally restrict caloric consumption.

Previous studies have focused on the impact of exercise on intake as a treatment strategy in overweight and obese adolescents. A 2013 meta-analysis of the impact of acute exercise on subsequent energy intake proposed that exercise is effective at producing a short-term energy deficit that tends not to be compensated for with increased food intake [16]. A 2016 systematic review and meta-analysis showed that among 14 studies in lean and obese youth, acute exercise had a significant effect on intake in children and adolescents with obesity [54]. In particular, this decrease in energy intake was only present following high intensity exercise bouts relative to
control. Overall, there are mixed results on the effects of acute exercise on energy intake in lean youth, which are likely due to differences in the methodological approaches used. Based on the meta-analysis, the available evidence points towards no difference between acute exercise and control conditions in healthy weight children and adolescents. Nutritional responses to acute exercise, however, remain highly variable across participants. One limitation of the available literature is that, with smaller sample sizes (range n = 7-22), it is difficult to stratify the group on additional individual characteristics. For example, the “anorexigenic effect” of exercise on intake has not been systematically studied in healthy weight children who are at risk for developing obesity due to family history. Previously studied groups of healthy weight children likely include a mix of some with healthy weight parents, and some with overweight or obese parents. It is possible that children at risk for becoming overweight may behave more similarly to children with obesity in regards to post-exercise food intake, which could contribute to the variability seen in previous studies. Altogether, these results highlight the difficulties in predicting the impact of exercise on energy balance.

Few studies have explored the effect of long term structured exercise interventions on appetite-related hormones, appetite sensations, and subsequent energy intake in obese youth. However, researchers have demonstrated that exercise-induced weight loss was accompanied by an increase in the satiation signal polypeptide YY (PYY\textsubscript{3-36}) concentrations [55-57]. In addition, a 6-week structured exercise intervention has been shown to increase hunger and decrease fullness in obese adolescents [58]. Unfortunately, these studies did not report energy intake, so additional links between the physiological and appetitive responses that link energy expenditure to energy intake cannot be made. The absence of a relationship between basal metabolic rate and energy intake, and between appetite-related hormones and reported perceptions of satiety after long term exercise suggest non-homeostatic pathways may also be involved [43, 59].

In addition to the methodological issues mentioned so far, the inter-individual variability observed in energy intake in response to acute exercise makes firm conclusions difficult [16, 49, 60]. Previous research efforts have focused on the effect of exercise on homeostatic mechanisms of body weight control, such as gastrointestinal hormones and body composition to explain the inter-individual variability. Fewer studies have examined the impact of exercise on food choice, appetitive drives, and other non-homeostatic (e.g., hedonic) mechanisms of feeding. A clearer understanding of the impact of exercise on hedonic feeding and other cognitive factors might
shed light on some of the variations in response to both acute exercise and energy deficits induced by other means (e.g., dieting) [21].

**Role of the Brain in Energy Balance**

*Cognitive Factors*

There are also cognitive factors that influence eating behaviors. Some individuals are predisposed to compensatory responses that render them resistant to the negative energy balance theoretically associated with an exercise-induced increase in energy expenditure [58]. For example, perceived exertion may predict individual responses to a set exercise bout. Studies in adults have shown that increased perceived difficulty of exercise may be associated with caloric compensation and predicts weight regain after successful weight loss [18]. However, there has been limited research examining whether the perceived difficulty experienced during exercise is associated with subsequent behaviors, above and beyond the effects of the physical stress associated with exercise [61, 62]. Researchers have suggested that at submaximal exertion levels, perceived exertion is dominated by cognitive factors and affective responses to exercise, while higher intensity exercise induces heightened sensory attention to the physiological response to the exercise [61, 62]. Such variability in behavioral and metabolic compensatory responses highlights the need for individualized programs to generate effective weight loss [63].

There is a diverse body of evidence on other cognitive factors related to post-exercise eating behavior. According to Finlayson et al., compensatory eating following 50 minutes of 70% intensity exercise was associated with an enhanced implicit wanting for food [19]. Some individuals may not receive the same benefit from imposed exercise due to an increase in the hedonic response to food following exercise-induced energy expenditure. This illustrates that physical activity also affects the hedonic control of energy intake and appetite, and may impact energy intake through non-homeostatic pathways. Other research has suggested that energy compensation following exercise may be related to increased subjective hunger ratings [60] or higher levels of disinhibition [64]. It is important to note that not all behavioral compensatory responses to exercise are deliberate or intentional [63]. Some of these effects may be passive in nature.

There is also a body of literature on the associations between variability in different cognitive factors and weight status. For example, dietary disinhibition (i.e., lack of an inhibitory
response to food-related stimuli) has been shown to have consistent positive associations with body mass index in cross-sectional studies and with weight gain over time in prospective studies [65]. In addition, individuals with obesity tend to give higher ratings of perceived exertion than healthy weight subjects during a standardized exercise bout (i.e., controlled exercise intensity relative to aerobic capacity, ventilatory threshold) [66, 67]. A comprehensive review of cognitive factors related to obesity is beyond the scope of this dissertation. But further investigation into the psychological factors associated with eating and exercise behaviors can help to identify those at risk for positive energy balance and obesity.

**Food-related Neuroimaging Studies**

The brain is the central driver of both energy intake and expenditure, but only recently have advances in brain imaging technology allowed for more rigorous examination of neurobiological differences associated with obesity and eating behaviors [68] (Figure 1.2). Applying this technology to experimental paradigms that include both energy intake and expenditure may help clarify sources of individual variability in susceptibility to obesity. While research has investigated neural responses to food-related cues [68-101] and exercise separately [102, 103], few imaging studies have included both sides of the energy balance equation [104-109]. The majority of these studies have been in adults. For over 50 years, hypotheses related to the hypothalamic control of feeding guided our understanding of how food intake is regulated. More recently, it has become clear that homeostatic regulators can easily be overwhelmed by hedonic or reward-based feeding. Several recent reviews and meta-analyses [110-113] have comprehensively summarized the brain’s proposed role in eating behavior and obesity. Few of these studies have been done in children or adolescents. In this section, the major themes that have arisen from this literature are summarized and several limitations to this work are highlighted.
A challenge of studying eating behavior through the use of neuroimaging paradigms is that the act of eating involves movement through chewing and swallowing, but even small amounts of movement (2-3 mm) can result in serious motion-related artifacts and loss of data. As a result, most studies have used pictures of high- and low-calorie foods [69, 72, 79, 80, 86, 88, 96, 100, 101], food-related words or scripts [78], or small amounts of taste stimuli [89-92, 103] to study the neural mechanisms underlying ingestive behavior. This is one of the most important limitations to making generalizations between functional magnetic resonance imaging (fMRI) and eating behavior under free living conditions. However, a few important brain structures have emerged consistently across studies. The orbitofrontal cortex (OFC) is hypothesized to play an important role in evaluating the pleasantness and reward value of a food-related stimulus (either an image or taste) [82, 113]. In addition, the lateral occipital complex, part of the visual association cortex, is likely thought to be involved in the visual processing of food images.
Within the visual cortex, pictures of food (emotional stimuli) tend to result in greater activation in these regions than non-food pictures (non-emotional stimuli) [81, 113]. Additional brain areas are highlighted in Figure 1.2. In interpreting these findings, it is important to recognize that these regions of the brain have multiple functions in addition to the processing of food-related stimuli, so firm predictions about their role in food intake are premature.

A variety of factors have been shown to affect neural response to food cues, including hunger [76, 77, 83, 87, 97], time of day [84], sex [74, 75, 98, 104], weight status [80, 85, 95, 99], dieting status [73], the energy content of the food [72, 74, 79, 80, 88, 91, 96]. Previous studies have found effects of overall body weight on brain activation in response to high energy density and low energy density food stimuli, noting increased activation for food stimuli in the striatum (caudate and putamen), anterior cingulate gyrus, amygdala, and insula in persons with obesity compared to healthy-weight controls [72, 85, 86, 95, 114-116]. The majority of these regions are thought to be involved in the processing of rewarding and emotional stimuli. It is assumed that this association is driven by higher levels of body fat, since adipose tissue is known to send appetite-regulating signals to the brain [29, 117]. Taken together, the available evidence suggests there may be an association between levels of body fatness and the response to food cues in reward regions of the brain. However, the research thus far has not explicitly examined this association in detail.

In regards to eating behavior, there is substantial research demonstrating that the rewarding aspects of food can drive intake [118, 119]. One food property that is known to increase palatability and drive intake is energy density, defined as the energy content per unit weight (kcal/g) [120-122]. In general, people tend to have higher liking and preference for foods high in energy density (e.g., cookies, pizza) relative to foods low in energy density (e.g., fruits, vegetables) [121]. Increased liking for high energy density foods is thought to partially explain the increases in activation in areas of the brain associated with reward processing when participants are presented with high energy dense food cues [123]. Previous studies in children and adolescents have demonstrated that reward-processing regions, as well as homeostatic regions of the brain, are responsive to food-related cues [69, 77, 80, 124-126]. The stimuli in these studies were generally divided into “high-calorie” or “fattening” versus “low-calorie” or “non-fattening” which correspond approximately to high energy density and low energy density foods, respectively. Regions of the brain that have previously been shown to respond to
rewarding stimuli, like high energy density foods, include the cingulate cortex, insula, caudate, putamen, substantia nigra, and amygdala, among others (Figure 1.2) [127]. All of these regions have been implicated in processing of reward and emotions.

As noted above, most studies have demonstrated differences in neural response to foods that vary in energy density, however, because high energy dense foods are typically more palatable than low energy dense foods [128], energy density and palatability are confounded. Moreover, other issues related to food presentation, like portion size, packaging, and color have received sparse attention [129]. Despite these important gaps, there are several patterns that emerge when comparing brain response to high versus low energy dense foods. First, the OFC tends to show greater activation in response to higher calorie, more palatable foods [82, 95]. In addition to the OFC, other areas of the brain involved with the expectation of food reward, including the insula, striatum, and nucleus accumbens have also demonstrated greater activation in response to high energy dense versus low energy dense foods [112]. Under certain conditions, the prefrontal cortex, an area of the brain thought to play a role in evaluation of food palatability [130] and inhibition of appetitive drives [131], has also shown greater activation in response to high relative to low energy dense foods. Overall, there is substantial variability across studies, most likely due to differences in imaging paradigms, study design, food-cues, and individual subject characteristics.

While there have been fewer neuroimaging studies conducted with children, reports in non-overweight children ages 9 and above have generally shown similar patterns of brain response as found in adults. Using a meta-analytic approach called activation likelihood estimation (ALE), van Meer and colleagues reported that the lateral OFC was the most common region activated in response to visual food cues of different energy content in children [132]. This brain region has been implicated in complex behaviors such as response inhibition, higher level food processing, interpretation of reward value, and learning [82, 133]. Regions of the brain involved with reward processing (e.g., amygdala, insula) showed similar involvement in response to food cues as in adults [132]. In response to food versus non-food pictures, Holsen and colleagues [77] reported greater activation in the amygdala, OFC, and insula in 10-17 year-olds, further supporting that in children and adolescents, food pictures activate regions of the brain associated with reward processing. Killgore and colleagues also reported greater activation in the OFC in response to food vs. non-food images, but they did not find that this activation was
greater for high relative to low energy dense foods. Child age was correlated with greater response in the OFC [80], but follow-up studies are needed to confirm these findings in a broader age-range of children. As in adults, neural responses to food in reward-related areas of the brain seem to be heightened in obese compared to lean children [69, 71].

While fMRI has helped elucidate the role of the mesolimbic dopaminergic system in feeding behavior, it is unknown whether the appetite-regulating centers of the brain are sensitive enough to detect the metabolic fuel shifts that result from exercise. In studies that have investigated neural mechanisms underlying the physiological changes due to food intake, blood-oxygen level-dependent (BOLD) fMRI has been used to detect changes in activation of the limbic system in response to small reductions in blood glucose created with a hyperinsulinemic euglycemic-hypoglycemic clamp [134]. In addition, recent reports comparing isocaloric meals of high versus low glycemic index show alterations in activation in reward regions of the brain detectable with BOLD fMRI [135]. Other studies have used fMRI to detect changes in neural activation in response to covert ingestion of calorie loads as small as 112.5 kcals [136]. These reports suggest that even modest acute shifts in energy balance may produce detectable changes in blood flow to different regions of the brain. Additional food-related imaging studies that incorporate exercise are summarized in the next section.

**Impact of Exercise within Food-related Neuroimaging Studies**

Several studies in adults have used fMRI or electroencephalography (EEG) to investigate how exercise affects neural response to food cues. Across studies, a variety of different exercise and neuroimaging paradigms have been used, and results are highly variable. However, in general, these studies suggest that both acute and chronic levels of exercise can reduce neural activation in reward-related regions in response to food cues compared to control images.

Hanlon and colleagues studied lean and obese, untrained, pre-menopausal women to determine the impact of acute exercise on EEG-measured neural response to food [106]. Results showed that response to food in the limbic system (associated with food reward) was decreased following exercise compared to non-exercise, regardless of weight status. No significant differences were found in 24-hour self-reported energy or macronutrient intake by weight status, exercise condition, or their interaction [106]. Unfortunately, self-reported dietary intake is subject to participant bias [137-140]. In addition, the exercise parameters resulted in different
relative exercise intensities across the participants, and in some women, this might not have been sufficient to alter energy intake. However, this study does suggest a role for the limbic system in the behavioral response to food following exercise.

In another study, Evero and colleagues tested 30 healthy, lean, habitually active young adult men and women [105]. Participants were randomized to either a 60-minute rest or exercise condition performed at approximately 83% maximum heart rate on a cycle ergometer, followed by the other condition one week later. Immediately following each condition, fMRI was conducted to compare neural response to low and high energy foods. Compared to the no exercise condition, exercise reduced response to food vs. control images in the left and right insula, right rolandic operculum, right putamen, left postcentral gyrus, right supramarginal gyrus. The insula and operculum are thought to be involved in the hedonic and reward value of food, while the putamen is involved in dopamine signaling. Further, the left postcentral gyrus is part of the primary somatosensory cortex.

While the studies described above examined the effects of a single bout of exercise, Cornier and colleagues measured the effects of both acute and chronic exercise on neural response to food cues and reported appetite [104]. Overweight and obese adults completed a 6-month exercise intervention, and fMRI scans were performed in the fasted state, at baseline and post-intervention at rest (chronic exercise) and after a bout of acute exercise (chronic + acute exercise). The authors found that chronic exercise vs. baseline reduced response to food cues in bilateral parietal cortices, the left insula and the visual cortex, brain regions associated with memory, taste, hedonic value of food, and visual processing of food images, respectively. Further, changes in activation in the anterior insula were positively correlated with changes in fat mass and body weight. The authors also found that exercise (chronic or acute) did not impact reported hunger, satiety, or appetite [104].

Finally, Killgore and colleagues examined the association between self-reported physical activity and neural responses to food in reward regions of the brain in healthy, adult men and women [108]. Participants reported their typical exercise minutes per week and completed an fMRI scan while viewing images of high and low calorie foods. The authors found that reported weekly exercise was negatively associated with activation in the left insula, as well as the medial OFC, in response to high-calorie, but not low-calorie foods. In other words, those who reported more time spent exercising had reduced responses to high-calorie food images. The magnitude of
activation in these regions was positively correlated with the preference ratings for savory high calorie foods. Kilgore and colleagues concluded that regular exercise was associated with decreased reward response to high calorie foods, which was associated with a lower desire for those foods [108].

These studies together suggest that both acute and regular exercise in healthy participants, in varying forms, may attenuate the neural response to food cues in regions of the brain thought to be involved with reward-evaluation, including the insula, putamen, and operculum. In addition to the studies described in detail, a recent study in lean adult males found that high intensity exercise reduces activation in brain reward regions in response to high calorie food, but increases reward-related activation in response to low-calorie foods [141]. It remains to be seen if these same findings would be observed in obese populations. This could partly explain the decrease in energy intake post-exercise seen in some behavioral studies [39, 42, 48, 51, 142], but not others [50, 143, 144]. These results are supported by recent literature examining the effects of exercise on the neural response to non-food rewarding stimuli, including cigarette smoking and monetary incentives, that showed an attenuation of neural activation in reward regions of the brain following exercise [102, 103]. The evidence suggests a possible neurobiological mechanism to help explain the interaction between exercise and appetite in the control of energy balance.

A limitation of these studies, however, is that the designs have not included objective measures of food intake. Therefore, we cannot make conclusions as to whether the differences in neural activity would correlate with differences in behavior under free living conditions. Additional studies are also needed to better understand the involvement of appetite-related hormones. Furthermore, the studies reviewed were done in highly variable populations, with a range of ages, body weights, and fitness levels. Additional experiments are needed to confirm the results and to explore whether these same relationships exist in younger populations, wherein neural pathways are still developing. For obesity prevention, it is important to identify sensitive periods in development where interventions might be most or least effective.

**Summary**

Clues to the interaction between exercise and energy intake are still emerging, but the two sides of the energy balance equation may share similar neural circuitry. Exercise may be an
effective alternative strategy to reduce energy intake and prevent and/or treat obesity, independent of intentional caloric restriction. However, some individuals might be more responsive than others to weight control strategies that include exercise. In addition, body composition plays an integral role in appetite regulation, within the context of the Energy Balance Framework [20]. This dissertation extends previous research in these areas and examines the influences of individual differences in body composition and exercise on appetite regulation and food intake in children under the age of 12 years.

**Dissertation Aims**

*Study 1. Intake at a single, palatable buffet test meal is associated with total body fat and regional fat distribution in children.*

The first aim of Study 1 was to determine the association between children’s intake at a multi-item test-meal consisting of highly palatable foods and body composition, assessed by dual-energy x-ray absorptiometry (DXA). We hypothesized that children’s intake at the highly palatable buffet would be associated with DXA measures of body fat.

The second aim of Study 1 was to assess the relationship between children’s intake at a baseline test-meal consisting of familiar, moderately palatable foods and body composition. We hypothesized that children’s energy intake at a standard baseline meal, without the presence of highly palatable foods, would be associated with their fat-free mass.

*Study 2. Brain response to images of food varying in energy density is associated with body composition in 7- to 10-year-old children: Results of an exploratory study.*

The aim of Study 2 was to determine the association between children’s body composition, compartmentalized into fat-free mass and fat mass, and brain activation response to images of food that vary by energy density. This particular exploration looked to extend the literature on the associations between body composition and energy intake regulation by providing a potential neural mechanism underlying the effects previously seen in adolescents and adults. The hypotheses for this aim were twofold. First, we hypothesized that fat-free mass would be positively associated with blood-oxygen level-dependent (BOLD) activation in homeostatic regions of the brain, as assessed by functional magnetic resonance imaging (fMRI).
Second, we hypothesized that fat mass would be positively associated with BOLD activation in reward centers in the brain.

*Study 3. The impact of an acute bout of exercise on children’s eating behaviors.*

Study 3 examined the effects of an imposed bout of moderate-intensity exercise on food intake, activity-related energy expenditure, and energy balance in a sample of children at risk for becoming overweight based on parent weight status. The aims from Study 3 are explored in two distinct chapters of this dissertation: Study 3A summarizes the main outcomes of the research project, while Study 3B details the factors related to individual differences in daily energy intake.

*Study 3A. Impact of imposed exercise on ad libitum energy intake in children at risk for overweight.*

The aim of Study 3A was to examine the effects of acute imposed exercise (30 minutes of 70% intensity cycling) versus imposed sedentariness on *ad libitum* daily energy intake in healthy-weight children with at least one overweight or obese biological parent. We hypothesized that total energy intake and relative energy intake (adjusted for activity-related energy expenditure) would be lower on a day with imposed exercise compared to a day where children remained sedentary.

*Study 3B. Perceived exertion during exercise is associated with children’s ad libitum energy intake.*

The aim of Study 3B was to understand individual-level factors that may contribute to differences in daily energy intake after participation in 30 minutes of 70% intensity cycling exercise. In particular, we examined the associations between body composition (i.e., fat-free mass and fat mass), cardiovascular responses to exercise (e.g., heart rate), ratings of perceived exertion, and *ad libitum* energy intake across the experimental day. We hypothesized that children’s fat-free mass would be positively associated with total energy intake, in line with previous research in adolescents and adults. Since the exercise bout was tailored to the same relative intensity of 70% VO$_{2\text{max}}$, we hypothesized that total energy intake would be positively related to children’s ratings of perceived exertion during the exercise, independent of the controlled cardiovascular response to the exercise.
References


84. Masterson, T.D., et al., *Neural reactivity to visual food stimuli is reduced in some areas of the brain during evening hours compared to morning hours: an fMRI study in women*. Brain Imaging Behav, 2015.


CHAPTER 2

Study 1:
Intake at a single, palatable buffet test meal is associated with total body fat and regional fat distribution in children.

Published as:
Abstract:

Previous studies testing the relationship between short-term, ad libitum test-meal intake and body composition in children have shown inconsistent relationships. The objective of this study was to determine whether children’s intake at a palatable, buffet meal was associated with body composition, assessed by dual-energy x-ray absorptiometry (DXA). A sample of 71 children (4-6 years) participated in 4 sessions where ad libitum food intake was measured. Children’s intake at two of the test-meals was retained for the present analysis: a baseline meal consisting of moderately palatable foods and a highly palatable buffet including sweets, sweet-fats, and savory-fats. On the last visit, anthropometrics and DXA were assessed to determine child body composition. Children consumed significantly more calories at the palatable buffet compared to the baseline test-meal. Children’s total fat-free mass was positively associated with intake at both the baseline meal and the palatable buffet meal. Total energy intake at both meals and intake of savory-fats at the palatable buffet were positively associated with children’s total fat mass, total percent body fat, and percent android fat. Intake of sweet-fats was associated with child fat-free mass index. Intake of sweets was not correlated with body composition. Children’s intake at a palatable test-meal, particularly of savory-fat foods, was associated with measures of total and regional body fat.
Introduction:

Recent studies have shown that objectively measured, but not self-reported, energy intake can predict the development of overweight in youth (Stice & Durant, 2014). Objective measures of energy intake, including laboratory test meals, have advantages compared to self-report measures such as food frequency questionnaires, food diaries, and 24-hour recalls because the amount eaten can be measured directly (Goran, 1998). In addition, self-report measures of energy intake have been criticized in the literature due to their inaccuracies, which may contribute to misleading results in nutrition and obesity research (Schoeller, 1995; Schoeller et al., 2013). For example, a majority of self-reported energy intake data from the National Health and Nutrition Examination Survey (NHANES) are not physiologically plausible (Archer, Hand, & Blair, 2013). Moreover, there are examples of both under- and over-reporting across other studies that use self-reported energy intake, particularly in children and adolescents (Fisher, Johnson, Lindquist, Birch, & Goran, 2000; Forrestal, 2011; Santos, Pascoal, Fisberg, Cintra, & Martini, 2010). Heavier youth are more likely to underreport the amount they eat on self-report measures compared to their actual intake (Wolkoff et al., 2011). The lack of accuracy of self-report dietary data demonstrates the need for development of improved measures to estimate energy intake in free-living individuals. Laboratory test-meals offer a practical, objective measure of energy intake that may better correlate with usual eating patterns.

A criticism of single-meal studies is that they may not be representative of consumption under free-living conditions (Acheson, Campbell, Edholm, Miller, & Stock, 1980). The laboratory environment may elicit different eating behavior compared to a more naturalistic environment (Allirot et al., 2012). In addition, intake from meal-to-meal, particularly in children, is thought to vary (Birch, Johnson, Andresen, Peters, & Schulte, 1991). For all these reasons, measuring intake at a single test-meal in the laboratory has often not been associated with weight status (Fisher, Liu, Birch, & Rolls, 2007), despite recent reports of the contrary (Stice and Durant, 2014). Although children’s intake tends to vary across eating occasions, previous studies from our laboratory that tested ad libitum intake at multi-item test-meals, administered across multiple days, showed moderate-to-high within-child correlations ranging from $r = 0.50 – 0.73$ (unpublished data). Similar results have been found in adults (Laessle & Geiermann, 2012). These results suggest that it might be possible to use children’s intake at a single laboratory test-meal to make predictions about usual energy intake and tendency to overeat. However, the type
of foods and/or meal-conditions that would demonstrate an association between intake and body fatness are not known. Among children, laboratory test-meal intake has been shown to vary by type of food given (Epstein et al., 2009), portion size (Kral, Kabay, Roe, & Rolls, 2010), palatability (Keller, Kirzner, Pietrobelli, St-Onge, & Faith, 2009), and brand packaging (Forman, Halford, Summe, MacDougall, & Keller, 2009), all of which are factors that influence energy intake in free-living settings. The associations between child body composition and intake at test-meals of common foods that vary on these attributes have sparsely been examined.

In studies conducted in adolescents and adults with overweight and obesity, evidence suggests that fat free mass may be more closely associated with energy intake than fat mass (Blundell et al., 2012a; Blundell et al., 2012b; Cameron et al., 2014; Caudwell et al., 2013; Weise et al., 2014). Blundell and colleagues found that fat-free mass, but not fat mass or body mass index, was associated with self-determined meal size and daily energy intake in two samples of adults with obesity (Blundell et al., 2012a). It is suggested that a physiological signal associated with fat-free mass may act as an appetitive driver, which may interact with signals related to body fatness (i.e. leptin). This is likely due to the contribution fat-free mass makes in determining resting metabolic rate, which is the largest component of daily energy expenditure (Blundell et al., 2012a; Caudwell et al., 2013). Similar findings have been reported in adolescents with obesity (Cameron et al., 2014), but this has not been examined in younger populations. It is also unknown whether fat-free mass remains the strongest predictor of energy intake across meals that vary in variety and palatability.

The first objective of this study was to determine the association between children’s intake at a multi-item test-meal consisting of highly palatable foods and body composition, assessed by dual-energy x-ray absorptiometry (DXA). We hypothesized that children’s intake at the highly palatable buffet would be associated with DXA measures of body fat. Secondly, we assessed the relationship between children’s intake at a baseline test-meal consisting of familiar, moderately palatable foods, and body composition. We hypothesized that children’s energy intake at a standard baseline meal, without the presence of highly palatable foods, would be associated with fat-free mass. Understanding how the association between energy intake and body composition is affected by the selection and palatability of foods offered at a laboratory test-meal would improve the ability to study the relationship between eating behavior and obesity risk.
Methods and Procedures:

Participants

Participants were recruited from the community by placing advertisements on popular websites and around the hospital. Interested families were screened over the phone to ensure children were healthy, without food allergies, and not taking prescription medications. After receiving information about the study, parents signed informed consent for their children. Children provided voluntary verbal agreement to participate, but written assent was not required. This study was approved by the Institutional Review Board of St. Luke’s Roosevelt Hospital Center.

Design

This study consisted of four 1-hour dinner sessions that took place between 4:00pm and 6:00pm and a fifth session for body composition measurement using DXA. The overall purpose of the study was to examine the relationship between taste genetics and children’s eating behavior. Results from this study have been reported elsewhere (Keller et al., 2014; Keller, Olsen, Kuilema, Meyermann, & Belle, 2013; Keller et al., 2010; Olsen, van Belle, Meyermann, & Keller, 2011). The purpose of this secondary analysis was to investigate the association between children’s body composition and eating behavior at two of the test-meals, a standard baseline meal and a highly palatable buffet designed to elicit excess consumption.

Visit one included a baseline standard ad libitum meal, preceded by child taste and food preference assessments. The test meals served at visits two and three included high- or low-fat versions of the same foods, the details of which have been previously reported (Keller et al., 2014; Keller et al., 2013; Keller et al., 2010; Olsen et al., 2011). Test meal four was a palatable buffet meal consisting of a range of high-energy-dense foods including sweets, sweet-fats, and savory-fats. Children attended the test-meal sessions at least 2 hours fasted. For the purpose of this study, only test meals one and four were considered because the purpose of visits two and three was to compare children’s intake of a limited number of food items that varied in fat content.
Table 2.1. Serving sizes for foods served at the baseline and palatable buffet test-meals

<table>
<thead>
<tr>
<th>Foods at Baseline Test-meal</th>
<th>Serving Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaroni &amp; Cheese</td>
<td>135 grams</td>
</tr>
<tr>
<td>Peanut butter (creamy)</td>
<td>30 grams</td>
</tr>
<tr>
<td>Grape jelly</td>
<td>30 grams</td>
</tr>
<tr>
<td>White bread</td>
<td>2 slices (50 grams)</td>
</tr>
<tr>
<td>Green grapes</td>
<td>110 grams</td>
</tr>
<tr>
<td>Baby carrots</td>
<td>30 grams</td>
</tr>
<tr>
<td>Broccoli spears</td>
<td>85 grams</td>
</tr>
<tr>
<td>Graham crackers</td>
<td>25 grams</td>
</tr>
<tr>
<td>Strawberry yogurt</td>
<td>170 grams</td>
</tr>
<tr>
<td>Whole milk</td>
<td>1 cup (245 grams)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Foods at Palatable Buffet Test-meal</th>
<th>Serving Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Savory-fats</td>
<td></td>
</tr>
<tr>
<td>Cheese bagel bites</td>
<td>4 pieces (~145 grams)</td>
</tr>
<tr>
<td>Cheese pizza rolls</td>
<td>4 pieces (~80 grams)</td>
</tr>
<tr>
<td>Chicken nuggets</td>
<td>5 nuggets (~105 grams)</td>
</tr>
<tr>
<td>Mozzarella sticks</td>
<td>4 sticks (~100 grams)</td>
</tr>
<tr>
<td>Potato chips</td>
<td>28 grams</td>
</tr>
<tr>
<td>Sweet-fats</td>
<td></td>
</tr>
<tr>
<td>Chocolate chip cookies</td>
<td>3 cookies (~35 grams)</td>
</tr>
<tr>
<td>Mini-brownies</td>
<td>4 mini-brownies (~60 grams)</td>
</tr>
<tr>
<td>Chocolate cupcakes</td>
<td>2 mini-cupcakes (~50 grams)</td>
</tr>
<tr>
<td>Donut holes</td>
<td>4 holes (~50 grams)</td>
</tr>
<tr>
<td>Whole-fat chocolate milk</td>
<td>1 cup (245 grams)</td>
</tr>
<tr>
<td>Sweets</td>
<td></td>
</tr>
<tr>
<td>Red licorice</td>
<td>4 pieces (~50 grams)</td>
</tr>
<tr>
<td>Fruit leather</td>
<td>1 package (~20 grams)</td>
</tr>
<tr>
<td>Gummy candies</td>
<td>~45 grams</td>
</tr>
<tr>
<td>Fruit candies</td>
<td>~45 grams</td>
</tr>
<tr>
<td>Fruit punch</td>
<td>1 cup (235 grams)</td>
</tr>
</tbody>
</table>

**Procedures for the baseline test-meal**

The baseline test meal took place on the first test session, and was comprised of standard portions of macaroni and cheese, peanut butter, grape jelly and white bread, grapes, carrots, broccoli, graham crackers, strawberry yogurt, and whole milk. Serving sizes for these foods are listed in Table 2.1. Prior to the meal, children were given an explanation about the foods available at the test-meal and told they could eat as much or as little as they wanted. Children had 30 minutes to eat, but they were not required to eat for this entire time if they finished early. The majority of children consumed their meal within 15-20 minutes. They were also informed that they could ask for additional servings of any of the foods provided. Because children at this age may be too shy to request extra servings, researchers were instructed to ask the children if
they would like more any time they finished a food/beverage. During the meal, a researcher was seated at the table to assist the child if they needed help with any of the foods. The researcher read a nonfood related book to the child as he/she ate. This provided a consistent distraction and avoided the unrealistic situation of the child eating alone in the laboratory. When possible, the same researcher was used for each test visit to increase the child’s level of comfort.

Parents, the majority of whom were mothers (89.7%), were seated in an adjacent waiting room. They could not hear or see what children were eating, but they were allowed to interrupt during the meal if they had any concerns. Further details on the procedures for this test-meal are published elsewhere (Keller et al., 2014; Keller et al., 2013; Keller et al., 2010; Olsen et al., 2011).

To determine total meal intake, the total weight of each food consumed was measured from pre- to post-meal and nutrient values were calculated using nutrition facts label information. For the purposes of this study, total energy intake at the test-meal was retained for the primary analyses. We also examined the proportion of total energy intake from individual macronutrients (i.e. fats, carbohydrates, and proteins).

**Procedures and nutrient analyses for the palatable buffet test-meal**

The purpose of the fourth meal condition was to assess children’s susceptibility to overeating highly palatable foods of different flavor/taste qualities (sweet, sweet-fat, and savory-fat). Foods in the savory-fat or sweet-fat category had either savory or sweet as their predominant flavor characteristic and contained at least 20% of calories from fat. Savory fat foods included items such as chicken nuggets and potato chips. Sweet-fats included baked goods such as cookies, cupcakes, and donuts. Items in the sweet category were primarily sweet tasting and contained less than 1 gram of fat per serving. Food items included in this group were sweet, non-fat candies and sugar-sweetened beverages. The food items were selected because they were highly palatable and familiar to most children (Phillips, 2003). All foods and beverages were served in plain plastic packaging and prepared immediately prior to the visit. A summary of the foods and serving sizes from the buffet test meal is listed in Table 2.1.

Test-meal procedures were similar to the baseline meal. Prior to the meal, children were given an explanation about the foods available at the test-meal and told they could eat as much or as little as they want. Children had 30 minutes to eat, but again, did not have to eat for the entire
They were also informed that they could ask for additional servings of any of the foods provided. During the meal, a researcher was seated at the table to assist the child and read a nonfood related book to the child as he/she ate.

Pre- and post-weights (in grams) for each of the foods were measured and intake was converted to kilocalories (kcal) using the nutrition facts panel. Total energy intake for the entire meal, as well as energy intake from savory-fats, sweet-fats, and sweets was retained for the analyses. We also examined the proportion of energy intake from individual macronutrients.

**Anthropometric measurements and body composition**

Anthropometric measures (height and weight) were performed by a trained researcher. Children were weighed and measured using a standard balance scale (Detecto, Model 437, Webb City, MO) and stadiometer (Seca, Model 202, Chino, CA) in light clothing. Height and weight were converted to BMI (kg/m2), and BMI z-score (BMIZ) and percentile were calculated using the Centers for Disease Control and Prevention (CDC) conversion program (Cole, Bellizzi, Flegal, & Dietz, 2000). CDC cut-offs for child age- and sex-specific BMI percentile were used to define normal weight (<85 %ile), overweight (85-95 %ile), and obese (≥95 %ile) classification. Body composition was assessed using whole body dual-energy X-ray absorptiometry (DXA) (DPX, Lunar Corp., Madison, WI) using Pediatric Software Version 3.8G (Lunar Corp., Madison, WI). The following measures were extracted from the whole body scan and tested in partial correlations: total body fat free mass (FFM), total body fat mass (FM), android percent fat, gynoid percent fat, and total body measures of percent fat (Aucouturier, Meyer, Thivel, Taillardat, & Duche, 2009; Glickman, Marn, Supiano, & Dengel, 2004). To control for differences in body composition as a function of child height, FFM index and FM index (kg/m2) were calculated by dividing the absolute FFM and FM, respectively, by the height squared (VanItallie, Yang, Heymsfield, Funk, & Boileau, 1990).

**Resting energy expenditure**

Sex-specific World Health Organization (WHO) equations for 3- to 10-year-olds were used to calculate estimated resting energy expenditure (REE) from child weight (Balas-Nakash, Villanueva-Quintana, Vadillo-Ortega, & Perichart-Perera, 2008). Resting metabolic rate, which
is largely driven by fat-free mass, has previously been associated with meal size and daily energy intake in adults (Blundell et al., 2012; Caudwell et al., 2013).

Males ages 3-10: \( \text{REE} = 22.7 \times \text{wt(kg)} + 495 \)
Females ages 3-10: \( \text{REE} = 22.5 \times \text{wt(kg)} + 499 \)

Statistical analyses

Descriptive statistics were performed to determine means and standard deviations for continuous variables and frequencies for categorical variables. Independent samples t-tests were used to check for differences in test-meal intake and body composition by child sex. Intakes from the various food categories at the palatable buffet (savory-fats, sweet-fats, sweets) were not normally distributed, and therefore were square root transformed prior to further analyses. The primary study aims were tested using partial correlations, controlling for child age and sex, to determine the association between test-meal intake and body composition. Adjustments were made for child ethnicity, but this did not affect the associations between body composition and intake variables. Therefore, results are reported without these adjustments. Variables that were included in these correlations included: FFM, FFM index, FM, FM index, total percent fat, gynoid percent fat, android percent fat, REE, BMIz, total energy intake at the baseline meal, % of calories from fats, carbohydrates, and proteins at the baseline meal, total energy intake at the palatable buffet meal, intake from savory fats, and intake from sweet fats, intake from sweets, and % of calories from fats, carbohydrates, and proteins at the palatable buffet meal. Analyses were run with and without statistical outliers, which were identified with box plots using a step of 1.5 times the interquartile range. Data were analyzed with SPSS V.21.0. The level of significance was set at \( p < 0.05 \).

Results:

Out of the 79 children initially enrolled in the study, 71 children between the ages of four to six years (mean ± SD = 5.6 ± 0.8 years) had complete data for the test-meals and DXA. Sample characteristics for these 71 children are reported in Table 2.2. Descriptive values for body composition measures obtained from DXA as well as calculated FFM index, FM index, REE and BMIz are listed in Table 2.3. Values are listed for all children and broken down separately by child sex. Boys had significantly greater FFM index (\( t = 3.35, p < 0.01 \)), and girls
had significantly greater gynoid percent fat ($t = -2.78, p < 0.01$). There were no other significant
differences between groups.

Table 2.2. Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>Boys ($n = 28$)</th>
<th>Girls ($n = 43$)</th>
<th>Total ($n = 71$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$ (%)</td>
<td>$n$ (%)</td>
<td>$n$ (%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>11 (39)</td>
<td>19 (44)</td>
<td>30 (42)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (4)</td>
<td>1 (2)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>3 (11)</td>
<td>4 (9)</td>
<td>7 (10)</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>10 (36)</td>
<td>14 (33)</td>
<td>24 (34)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (11)</td>
<td>5 (12)</td>
<td>8 (11)</td>
</tr>
<tr>
<td>Weight Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Weight</td>
<td>12 (43)</td>
<td>23 (53)</td>
<td>35 (49)</td>
</tr>
<tr>
<td>Overweight</td>
<td>7 (25)</td>
<td>11 (26)</td>
<td>18 (25)</td>
</tr>
<tr>
<td>Obese</td>
<td>9 (32)</td>
<td>9 (21)</td>
<td>18 (25)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>5.5 ± 0.8</td>
<td>5.6 ± 0.9</td>
<td>5.6 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>(4 – 6.9)</td>
<td></td>
<td>(4 – 6.9)</td>
</tr>
<tr>
<td>BMIz</td>
<td>1.1 ± 1.1</td>
<td>0.9 ± 0.9</td>
<td>1.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>(-1.7 – 2.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2. Descriptive statistics for the sample

Table 2.3. Body composition descriptive statistics

<table>
<thead>
<tr>
<th></th>
<th>Boys ($n = 28$)</th>
<th>Girls ($n = 43$)</th>
<th>Total ($n = 71$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD (range)</td>
</tr>
<tr>
<td>Total FFM (kg)</td>
<td>16.9 ± 2.5</td>
<td>16.1 ± 3.4</td>
<td>16.4 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>(10.3 – 27.0)</td>
<td></td>
<td>(10.3 – 27.0)</td>
</tr>
<tr>
<td>FFM index (kg/m²) a</td>
<td>13.2 ± 0.9</td>
<td>12.4 ± 1.0</td>
<td>12.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>(10.3 – 15.9)</td>
<td></td>
<td>(10.3 – 15.9)</td>
</tr>
<tr>
<td>Total FM (kg)</td>
<td>4.7 ± 4.4</td>
<td>5.3 ± 3.7</td>
<td>5.1 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>(1.1 – 22.4)</td>
<td></td>
<td>(1.1 – 22.4)</td>
</tr>
<tr>
<td>FM index (kg/m²)</td>
<td>3.5 ± 2.7</td>
<td>3.9 ± 2.1</td>
<td>3.8 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>(0.8 – 13.2)</td>
<td></td>
<td>(0.8 – 13.2)</td>
</tr>
<tr>
<td>Total % Fat</td>
<td>19 ± 10</td>
<td>23 ± 8</td>
<td>22 ± 9</td>
</tr>
<tr>
<td></td>
<td>(7 – 48)</td>
<td></td>
<td>(7 – 48)</td>
</tr>
<tr>
<td>Gynoid % Fat b</td>
<td>32 ± 10</td>
<td>37 ± 7</td>
<td>35 ± 9</td>
</tr>
<tr>
<td>Android % Fat</td>
<td>20 ± 13</td>
<td>23 ± 10</td>
<td>22 ± 11</td>
</tr>
<tr>
<td>REE (kcal)</td>
<td>1021 ± 155</td>
<td>1011 ± 148</td>
<td>1015 ± 150</td>
</tr>
<tr>
<td></td>
<td>(826 – 1622)</td>
<td></td>
<td>(826 – 1622)</td>
</tr>
<tr>
<td>BMIz</td>
<td>1.1 ± 1.1</td>
<td>0.9 ± 0.9</td>
<td>1.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>(-1.7 – 2.9)</td>
<td></td>
<td>(-1.7 – 2.9)</td>
</tr>
</tbody>
</table>
Table 2.3. Descriptive statistics for children’s body composition variables obtained from DXA, broken down by child sex. REE was calculated using World Health Organization (WHO) sex-specific equations for children ages 3-10 years. \(^a\) Boys had significantly higher FFM index (\(p < 0.01\)); \(^b\) Girls had significantly greater gynoid percent fat (\(p < 0.01\))

Descriptive values for the test meal variables are listed in Table 2.4, including total intake at the baseline meal (kcal), total intake at the palatable buffet meal (kcal), and intake from the different food groups at the palatable buffet (savory-fats, sweet-fats, and sweets). Values are listed for all children and broken down separately by child sex. Children’s intake at the palatable buffet (667 ± 274 kcal) was significantly greater than baseline meal (474 ± 250 kcal) (\(t = 3.35, p < 0.001\)). Boys tended to consume more total energy than girls, but this difference did not reach significance (\(t = 1.86, p = 0.07\)). Despite a tendency for boys to consume more, the proportion of intake from each of the food groups (savory-fats, sweet-fats, & sweets) in boys and girls was similar.

Table 2.4. Test-meal intake descriptive statistics

<table>
<thead>
<tr>
<th></th>
<th>Boys ((n = 28))</th>
<th>Girls ((n = 43))</th>
<th>Total ((n = 71))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD (range)</td>
</tr>
<tr>
<td>Total intake at baseline meal (kcal)</td>
<td>449 ± 221</td>
<td>491 ± 268</td>
<td>474 ± 250 (31 – 1312)</td>
</tr>
<tr>
<td>Total intake at palatable buffet (kcal) (^c)</td>
<td>760 ± 357</td>
<td>606 ± 331</td>
<td>667 ± 374 (90 – 1685)</td>
</tr>
<tr>
<td>Intake from savory fats at the palatable buffet (kcal)</td>
<td>279 ± 202</td>
<td>218 ± 197</td>
<td>242 ± 200 (0 – 787)</td>
</tr>
<tr>
<td>Intake from sweet fats at the palatable buffet (kcal)</td>
<td>289 ± 255</td>
<td>231 ± 233</td>
<td>253 ± 242 (0 – 997)</td>
</tr>
<tr>
<td>Intake from sweets at the palatable buffet (kcal)</td>
<td>193 ± 112</td>
<td>157 ± 100</td>
<td>170 ± 105 (0 – 441)</td>
</tr>
</tbody>
</table>

Table 2.4. Descriptive statistics for children’s energy intake (kcal) from the baseline meal, palatable buffet meal, and individual food groups at the palatable buffet (savory-fats, sweet-fats, and sweets), broken down by child sex. \(^c\) Boys tended to consume more calories than girls, but this difference did not reach significance \((p = 0.07)\).

Partial correlations between body composition and intake variables, controlling for child age and sex, are reported in Table 2.5. All of the body composition variables were significantly associated with total intake at both test-meals \((p < 0.05)\) and intake from savory-fats at the palatable buffet \((p < 0.05)\). Only FFM index was positively associated with intake of sweet-fats at the palatable buffet \((p < 0.05)\).
Table 2.5. Partial correlations

<table>
<thead>
<tr>
<th></th>
<th>Total intake at baseline meal (kcal)</th>
<th>Total intake at palatable buffet (kcal)</th>
<th>Intake from savory-fats at the palatable buffet (kcal)</th>
<th>Intake from sweet-fats at the palatable buffet (kcal)</th>
<th>Intake from sweets at the palatable buffet (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total FFM (kg)</td>
<td>.42**</td>
<td>.35*</td>
<td>.34**</td>
<td>.10</td>
<td>.13</td>
</tr>
<tr>
<td>FFM index (kg/m²)</td>
<td>.40**</td>
<td>.42**</td>
<td>.27*</td>
<td>.27*</td>
<td>.21</td>
</tr>
<tr>
<td>Total FM (kg)</td>
<td>.49**</td>
<td>.43**</td>
<td>.43**</td>
<td>.13</td>
<td>.13</td>
</tr>
<tr>
<td>FM index (kg/m²)</td>
<td>.48**</td>
<td>.44**</td>
<td>.41**</td>
<td>.16</td>
<td>.13</td>
</tr>
<tr>
<td>Total % Fat</td>
<td>.40**</td>
<td>.38**</td>
<td>.39**</td>
<td>.12</td>
<td>.09</td>
</tr>
<tr>
<td>Gynoid % Fat</td>
<td>.35*</td>
<td>.31*</td>
<td>.35**</td>
<td>.08</td>
<td>.02</td>
</tr>
<tr>
<td>Android % Fat</td>
<td>.41**</td>
<td>.43**</td>
<td>.44**</td>
<td>.14</td>
<td>.07</td>
</tr>
<tr>
<td>REE</td>
<td>.46**</td>
<td>.39**</td>
<td>.41**</td>
<td>.10</td>
<td>.11</td>
</tr>
<tr>
<td>BMIz</td>
<td>.38**</td>
<td>.42**</td>
<td>.36**</td>
<td>.20</td>
<td>.09</td>
</tr>
</tbody>
</table>

Table 2.5. Partial correlations between body composition and intake variables, controlling for child age and sex; Intakes from the food categories (savory-fats, sweet-fats, sweets) were square root transformed prior to analysis; *p < 0.05, ** p < 0.01

In testing the first hypothesis that children’s intake at the highly palatable buffet would be associated with DXA measures of body fat, positive associations were found between total intake and all measures of body fat. The association between total intake at the palatable buffet and total percent body fat is depicted in Figure 2.1A (r = 0.38, p < 0.01). In examining the different categories of foods, only intake of savory fats was associated with child body composition. A positive association was found between intake from savory-fats and android percent fat (r = 0.44, p < 0.01), as shown in Figure 2.1B. This association remained significant even after controlling for total intake at the palatable buffet (p < 0.05). There were no significant associations between the body composition variables and intake from sweets at the palatable buffet. The positive associations between body fat and intake measures remained significant even after controlling for child FFM index (p < 0.05).
In testing the secondary hypothesis that children’s energy intake at a standard baseline meal would be correlated with FFM, we did find a positive association \( r = 0.42, p < 0.01 \). However, total intake at this baseline meal was also positively associated with all measures of body fat \( p < 0.05 \). Finally, the proportion of total calories consumed from fats, carbohydrates, and proteins did not differ between the two test-meals. None of the body composition measures
were significantly associated with intake of fats, carbohydrates, or proteins at either the baseline or the palatable buffet test-meals (all p > 0.05).

**Discussion:**

The primary goal of this study was to determine the relationship between children’s intake at a single, palatable buffet meal and body composition, which was used as a proxy for the tendency to overeat under free-living conditions. In line with our primary hypothesis, we found that intake at a single-session buffet test-meal made up of highly palatable foods was associated with children’s body composition, specifically greater central body fat (android fat percentage). Of the food categories within the buffet, only intake of savory-fat foods was correlated with total body and regional fat measures assessed by DXA. Increased intake from savory-fats, but not sweets or sweet-fats, was associated with greater body fat in children. These findings demonstrate that intake assessed from a single-meal was positively related to children’s total body fat and regional fat deposition, but the causal direction in this pathway is not known. If we assume that body fatness is an indicator of habitual overconsumption, it may be possible to predict the tendency to overeat using a highly palatable test-meal such as the one tested in the present study. Children in this study consumed significantly more calories at the palatable buffet compared to the baseline test meal, indicating that the palatable buffet did promote overconsumption, as intended.

In regards to our second hypothesis, children’s intake at a baseline test-meal consisting of common foods of moderate palatability was positively associated with total body FFM, which has been demonstrated previously in adults by Blundell and colleagues (Blundell et al., 2012). Both FFM and FFM index were positively associated with total intake at the palatable buffet and intake of savory fats, contributing to the literature on FFM as a general appetitive driver (Blundell et al., 2012a; Blundell et al., 2012b; Cameron et al., 2014; Caudwell et al., 2013; Weise et al., 2014). Fat-free mass is a major determinant of basal metabolic rate, which is proposed to influence appetite and eating behavior, and this positive relationship between FFM and energy intake has been seen in adolescents with obesity (Cameron et al., 2014) and adults with overweight and obesity (Blundell et al., 2012).

The current findings, and other studies (Gubbels, Kremers, Goldbohm, Stafleu, & Thijs, 2012; Maffeis et al., 2008), have found that intake of savory-fat foods may be positively
associated with child weight status. In these previous studies (Gubbels et al., 2012; Maffeis et al., 2008), investigators used foods like pizza, French fries, and crackers and described them as “savory,” but many of these foods also contain fat and therefore are in line with the savory-fat category of foods in the present study. Maffeis et al. tested 1837 Italian children and found that when parents’ BMI was included in the analyses, children’s intake of savory-fat foods (servings/week) and participation in sports (hours/week), both assessed by parental report, showed independent associations with obesity. Intake of savory-fat foods was positively associated with obesity, while participation in sports was negatively associated with obesity. In logistic regression analyses, each weekly serving of savory-fat/salty food items reportedly eaten by children increased the likelihood of the child being obese by 2%. In their sample, children with obesity reported consumption of an average of 11 servings of savory-fat snacks per week, compared to 8 servings of sweet snacks per week. Convergent with our analyses, the authors also found that children’s intake of sweet foods was not predictive of child body composition.

Other studies have also implicated intake of savory-fat foods in the development of overweight. The KOALA Longitudinal Study of 2074 Dutch children found that a sedentary-snacking pattern at age 5, derived from parent-reported measures of children’s dietary intake and physical activity, was positively associated with children’s BMI z-score and incidence of overweight at ages 7 and 8 years (Gubbels et al., 2012). It is important to note that the snacking category included both savory-fat (i.e. potato chips, biscuits, fried foods) and sweet snacks (i.e. candy bars, cakes, ice cream), and it is not known whether this association was due to one or both categories of foods. Unfortunately, the authors did not report on individual correlations between intake of different food types and weight gain in children. It would be valuable to investigate the independent contributions of savory-fat foods to weight gain within a longitudinal cohort like the KOALA Study.

It is unclear exactly why we found associations between body fatness and intake of savory-fat food items, but not sweets or sweet-fats. One possibility is that the savory-fat foods make up main meal dishes, or entrées, and that we are simply capturing greater intake from main meal items in children with higher levels of body fatness. These foods also tend to be higher in salt content, and greater intake of salt has been associated with higher BMI z-score and percent body fat in children and adolescents (Libuda, Kersting, & Alexy, 2012). Other studies have attempted to examine underlying mechanisms for this phenomenon. Some insight might come
from recent papers examining the brain’s response to savory food cues (Griffioen-Roose et al., 2014). When adults were in a low-protein dietary state, brain response in the inferior orbitofrontal cortex (implicated in emotion and reward-driven decision-making) was higher for savory food odor and visual cues relative to a control condition, and the participants also exhibited a higher preference for savory foods (Griffioen-Roose et al., 2014). Additionally, another study found a relationship between a polymorphism in the FTO gene, which has been associated with body mass index and obesity risk, and greater total energy and protein intake, but lower carbohydrate intake (Qi et al., 2014). Similar findings were reported in a subsample of children and adolescents (Qi et al., 2015). While the savory-fat foods in our study contained higher amounts of protein than the sweet or sweet-fat foods, we did not find any associations between body composition variables and macronutrient intake.

There were several strengths and limitations in the current study. This study was a cross-sectional, correlational study, which does not allow for prediction in a specific direction. Therefore, we cannot determine whether body fatness is a cause or consequence of increased intake, or both. In addition, the order of the test-meals was not randomized. Differences in intake between the test-meals could possibly be related to familiarity with the laboratory environment, in addition to the differences in palatability between the meals. Children also had 30 minutes to eat, which could be considered a long meal duration for some children. It is possible that the presence of food after the initial cessation of eating may have resulted in additional consumption. However, children could decide to end the meal at any point prior to 30-minute time limit.

Children in the cohort came from diverse ethnic backgrounds, which contributes to the generalizability of results from this study. However, this study was not powered to look at differences in the association between test-meal intake and body composition across ethnic group. It is known that body fat deposition can vary across ethnic group (Deurenberg & Deurenberg-Yap, 2001). In exploratory analyses, no differences in intake or body composition were seen between ethnic groups, but this may be due in part to the small sample size in some subgroups. Controlling for ethnicity in partial correlation analyses did not affect any of the associations between body composition and intake variables. Further studies in larger cohorts should investigate potential differences in the association between food intake and body composition between different ethnic groups.
Our methods also included an accurate measure of body composition that allowed for the analysis of total body fat mass, fat-free mass, and regional body fat, which is a strength compared to conventional anthropometric measures like body mass index and percentile that rely solely on height and weight. A limitation of our study, however, was that our measure of REE was estimated using pediatric-specific equations, which are generalized to all children between the ages of 3-10 years old across all ethnicities. However, the equations used here have been validated (Balas-Nakash et al., 2008), and REE was highly correlated with DXA-measured FFM (p < 0.001). These findings are in line with previous research on the relationship between resting metabolic rate and energy intake (Blundell et al., 2012b; Caudwell et al., 2013).

An additional strength to the study was the inclusion of objective and direct measures of short-term food intake. The direct measure of energy intake at a highly palatable buffet, with a comparison baseline meal, is a strength compared to self- or parent-reported measures typically used (Archer et al., 2013; Champagne et al., 1998). We were also able to demonstrate significantly greater intake at the palatable buffet compared to the baseline test-meal, suggesting that altering the variety and palatability of the foods at the test-meal can elicit overconsumption in young children. For future studies designed to assess the relationship between laboratory test-meal intake and childhood obesity, investigators might design meals that include a selection of savory-fat options.

In conclusion, this study found that children’s ad libitum intake of a palatable test-meal consisting of sweet, sweet-fat, and savory-fat foods was associated with a pattern of body fat deposition associated with greater health risks (Must & Strauss, 1999). Some children exhibited a tendency to overeat in the presence of a variety of highly palatable foods, and this pattern of eating was associated with higher levels of total body and regional fat. Additionally, children’s intake from a single, highly palatable laboratory test-meal was associated with regional body fat deposition, suggesting that future studies might be able to predict obesity-related health risks based on measures of laboratory eating behavior (Must & Strauss, 1999). Additional studies are needed to clarify the role of savory-fat food intake in obesity. Contrary to current scientific understanding (Acheson et al., 1980; Birch et al., 1991), our findings demonstrate robust positive associations between energy intake at an objectively measured, single test-meal and children’s body composition assessed by DXA.
Acknowledgements:
Author Contributions
SNF carried out data analysis, interpretation, and representation in figures/tables. DT assisted in data analysis and interpretation. KM carried out data collection. KLK conceived and carried out experiments, and assisted in data interpretation. All authors were involved in writing and editing the paper and had final approval of the submitted and published versions.

Funding
This work was supported by NIDDK Career Development Award (Keller) K01DK068008 and USDA National Institute for Food and Agriculture Grant #2011-67001-30117 Program A2121 - Childhood Obesity Prevention: Transdisciplinary Graduate Education and Training in Nutrition and Family Sciences. TD is supported by the 2013 Nestlé Foundation Research grant.
References:


CHAPTER 3

Study 2:
Brain response to images of food varying in energy density is associated with body composition in 7- to 10-year-old children: Results of an exploratory study.

Published as:
Abstract:

Energy balance is regulated by a multifaceted system of physiological signals that influence energy intake and expenditure. Therefore, variability in the brain’s response to food may be partially explained by differences in levels of metabolically active tissues throughout the body, including fat-free mass (FFM) and fat mass (FM). The purpose of this study was to test the hypothesis that children’s body composition would be related to their brain response to food images varying in energy density (ED), a measure of energy content per weight of food. Functional magnetic resonance imaging (fMRI) was used to measure brain response to High (> 1.5 kcal/g) and Low (< 1.5 kcal/g) ED food images, and Control images, in 36 children ages 7-10 years. Body composition was measured using bioelectrical impedance analysis. Multi-subject random effects general linear model (GLM) and two-factor repeated measures analysis of variance (ANOVA) were used to test for main effects of ED (High ED vs. Low ED) in a priori defined brain regions of interest previously implicated in energy homeostasis and reward processing. Pearson’s correlations were then calculated between activation in these regions for various contrasts (High ED – Low ED, High ED – Control, Low ED – Control) and child body composition (FFM index, FM index, % body fat). Relative to Low ED foods, High ED foods elicited greater BOLD activation in the left thalamus. In the right substantia nigra, BOLD activation for the contrast of High ED – Low ED foods was positively associated with child FFM. There were no significant results for the High ED – Control or Low ED – Control contrasts. Our findings support literature on FFM as an appetitive driver, such that greater amounts of lean mass were associated with greater activation for High ED foods in an area of the brain associated with dopamine signaling and reward (substantia nigra). These results confirm our hypothesis that brain response to foods varying in energy content is related to measures of child body composition.
1. **Introduction:**

   Energy balance is regulated by a complex system of peripheral and central physiological signals. These signals arise from compartments of adipose and lean tissue, as well as the gastrointestinal tract and accessory organs, to influence energy intake and expenditure [1, 2]. The effects of the energy balance system on appetite regulation pathways have not been fully examined in pre-adolescent children. In addition, it is not known whether the effects of fat mass (FM) and fat-free mass (FFM) on energy balance are mediated by processes in appetite-regulating centers of the brain. Variability in the brain’s response to food could partially be explained by differences in levels of metabolically active tissues (FM and FFM) throughout the body. However, this has not previously been tested using neuroimaging in children or adults, and the physiological factors underlying differences in the brain’s response to food are not known. This exploratory study aims to address some of these gaps by examining the relationship between body composition and children’s brain responses to images of food that vary by energy density (ED).

   Emerging evidence, predominantly in adults, suggests that FFM is the best predictor of meal size and energy intake due to its influence on resting metabolic rate and total energy expenditure [1-5]. In controlled laboratory studies with adults, it has been shown that the effects of FFM on objectively-measured intake are mediated almost entirely by resting metabolic rate [6]. Therefore, the research thus far suggests that the effect of FFM on energy intake is primarily homeostatic. However, the direct effects of these homeostatic signals on areas of interest in the brain, including the hypothalamus (e.g., energy homeostasis, hunger) and the thalamus (e.g., sensory processing), have not been fully explored. In addition, there are several areas of the brain that communicate with the hypothalamus (e.g., limbic system) which have a variety of functions (e.g., reward, motivation, emotion processing, learning, memory). Due to the connections between these regions, it is possible that FFM may also be related to activation in areas of the brain involved with reward processing.

   Previous studies have also found effects of overall body weight on brain activation in response to high ED and low ED food stimuli, noting increased activation for food stimuli in the striatum (caudate and putamen), anterior cingulate gyrus, amygdala, and insula in persons with obesity compared to healthy-weight controls [7-13]. It is assumed that this association is driven by higher levels of body fat, since adipose tissue is known to send appetite-regulating signals to
the brain [3, 14]. However, it is unknown whether food cue-related activation in these brain regions is related to levels of adipose tissue or FFM independently of one another. Examining the independent contribution of FM and FFM to the activation in reward networks will help clarify this relationship.

In addition to body composition, there is substantial research demonstrating that the rewarding aspects of food can also drive intake [15, 16]. One food property that is known to increase palatability and drive intake is ED, defined as the energy content per unit weight (kcal/g) [17-19]. In general, people tend to have higher liking and preference for foods high in ED (e.g., cookies, pizza) relative to foods low in ED (e.g., fruits, vegetables) [18]. This increased liking for high ED foods is thought to be partially related to increases in activation in areas of the brain associated with reward processing [20]. Previous studies in children and adolescents have demonstrated that both reward and homeostatic regions of the brain are responsive to food-related cues [21-26]. The stimuli in these studies were generally divided into “high-calorie” or “fattening” versus “low-calorie” or “non-fattening” which correspond approximately to high ED and low ED foods, respectively. Regions of the brain that have previously been shown to respond to rewarding stimuli, like high ED foods, include the cingulate cortex, insula, caudate, putamen, substantia nigra, and amygdala, among others [27]. All of these regions have been implicated in processing of reward and emotions, but the relationship between body composition and brain activation in these regions has not been fully examined.

The purpose of this exploratory study was to determine the association between children’s body composition, compartmentalized into FFM and FM, and brain activation in response to images of food that vary by ED. To our knowledge, this is one of the first studies to examine the integration of these systems in children. We hypothesized that variability in the brain’s response to food images varying in ED would be partly explained by children’s body composition, such that FFM would be positively associated with blood-oxygen level-dependent (BOLD) activation in homeostatic regions while FM would be positively associated with BOLD activation in reward centers. This hypothesis was based on prior research implicating FFM as a primary determinant of meal size and energy intake [4], while body weight (a proxy for FM) is related to increased brain activation in reward regions in response to food cues [7, 8, 11].
2. Methods:

2.1 Study Design

We conducted a cross-sectional study with a community-based sample of 36 children ages 7-10 years. The overall purpose of the study [28] was to investigate the neural mechanisms underlying the portion size effect, or the tendency to consume greater amounts of food when presented with larger portions [29]. This paper focuses on a secondary aim of the study to explore the relationship between body composition and the brain’s response to food images varying in ED. The study consisted of 5 total visits. For visits 1-4, children reported to the laboratory once per week over four consecutive weeks to eat ad libitum from four randomized test-meals varying in ED and portion size (reported elsewhere). On visits 3 and 4, children completed mock (i.e., practice) fMRI training sessions to increase familiarity with the scanning environment. Children reported for a fifth visit to complete an fMRI scan while passively viewing images of food varying both in ED (high versus low) and portion size (large versus small), although only differences in response to ED will be reported in the present study. Following the scanning session, children completed a fitness test and rated liking and wanting for each of the images shown during the fMRI using visual analog scales. For the main purposes of this paper, only the anthropometric data collected on visit 1 and the fMRI scan collected on visit 5 were considered for analysis. This study was approved by the Institutional Review Board of The Pennsylvania State University.

2.2 Participants

Participants were recruited using flyers and postings on popular websites. Interested families were screened over the phone to ensure children were healthy, right-handed, without metal implants or dental work, without food allergies, and not taking prescription medications. On the first study visit, a parent signed informed consent for their child. Children provided written assent prior to their participation. Out of the 42 children initially enrolled in the study, 2 were lost to follow-up after completion of 2 test-meal visits. Of the children with complete behavioral data (i.e., meal intake, questionnaires; n = 40), 36 children completed a successful fMRI scan, defined as having at least one functional run and corresponding anatomical data. Sample characteristics for these 36 children are listed in Table 3.1.
2.3 Anthropometrics and Body Composition

Anthropometric measures (height and weight) were performed by a trained researcher to the nearest 0.1 cm and 0.1 kg. Children were weighed and measured twice using a standard scale (Detecto model 437, Webb City, MO) and stadiometer (Seca model 202, Chino, CA) in light clothing. Averaged height and weight were converted to BMI z-score (BMIZ), and BMI percentile, calculated using the Centers for Disease Control and Prevention conversion program [30]. Cut-offs for child age- and sex-specific BMI percentiles were used to classify children as normal weight (<85 %ile), overweight (85-95 %ile), or obese (≥95 %ile).

For practical purposes, body composition was measured using bioelectrical impedance analysis (Tanita model BF-350, Arlington Heights, IL). Percent body fat (%BF) was multiplied by body weight (kg) to estimate FM (kg). The difference between body weight and FM was taken to estimate FFM (kg). To control for differences in body composition as a function of child height, FFM index and FM index were calculated by dividing the absolute FFM and FM, respectively, by the height squared (kg/m$^2$) [31].

<table>
<thead>
<tr>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>8.9</td>
</tr>
<tr>
<td>% Body fat</td>
<td>16.4</td>
</tr>
<tr>
<td>Fat mass index (kg/m$^2$)</td>
<td>3.8</td>
</tr>
<tr>
<td>Fat-free mass index (kg/m$^2$)</td>
<td>18.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI percentile class (CDC)</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-overweight</td>
<td>34</td>
<td>94</td>
</tr>
<tr>
<td>Overweight</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Race</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>33</td>
<td>92</td>
</tr>
<tr>
<td>Non-white</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

2.4 Functional Magnetic Resonance Imaging

Scans were performed using a Siemens MAGNETOM Trio 3T whole body MRI scanner (Siemens Medical Solutions, Erlangen, Germany) with a 12-channel head coil. To reduce motion artifacts, children were fitted with headphones and padding around the head, as well as pillows and a blanket to restrict movement of the extremities. Structural scans were collected using a T1-
weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence to acquire 160 slices, TR/TE = 1650/2.03ms, flip angle = 9 degrees, FOV = 256mm, slice thickness = 1mm, sagittal plane, and 1.0 x 1.0 x 1.0mm voxel size. The MPRAGE sequence was approximately 4 minutes in duration. Functional scans were collected using a T2-weighted gradient single-shot blood-oxygen level-dependent (BOLD) echo planar imaging (EPI) sequence to acquire 33 interleaved slices, TR = 2000ms, TE = 25ms, flip angle = 90 degrees, matrix 64x64, FOV = 220mm, AC-PC transverse, oblique plane determined by the mid-sagittal section, and 3.0 x 3.0 x 3.0mm voxel size. In-scan prospective movement correction (PACE) was also used to correct for motion in real time during the acquisition of data [32].

For the functional sequences, participants passively viewed images presented in a pseudo-randomized block design. There were a total of 180 unique images divided into 6 different stimuli categories: 4 food and 2 non-food control categories. Each image was presented only once during the scanning paradigm. The age-appropriate food images included 30 High ED (> 1.5 kcal/g) foods and 30 Low ED (< 1.5 kcal/g) foods depicted in both large (90th percentile of the amount commonly consumed in this age group) and small (10th percentile) portions [33]. The 1.5 kcal/g cut-off was chosen to control for large differences in palatability. Although not always possible, we selected foods of similar palatability levels for both the High and Low ED groups (e.g., Boar’s Head® sliced turkey in the Low ED group is 1.07 kcal/g, while Perdue® chicken nuggets in the High ED group are 2.35 kcal/g). Mean liking ($t_{(35)} = 6.6, p < 0.01$) and wanting ($t_{(35)} = 5.8, p < 0.01$) scores were significantly higher for High ED foods relative to Low ED foods. Average liking ratings for High ED vs. Low ED foods were 113 mm vs. 89 mm out of a possible 150, respectively, while average wanting scores were 106 mm vs. 85 mm, respectively. The full list of High ED and Low ED foods is in Supplementary Table 3.1.

From this point, the 4 food stimulus categories will be referred to with the following tags: High ED Large, High ED Small, Low ED Large, and Low ED Small. The non-food stimuli included 30 household furniture images, and 30 pixelated images (6 images from each of the other 5 stimulus categories, scrambled in Matlab version 8.0 to control for color, brightness, contrast). Activation in response to control images was used as a comparison against activation for the stimuli of interest (High ED and Low ED foods). Only the Scrambled control images were included in the analysis for this paper due to the potentially rewarding nature of some of the Furniture stimuli (i.e. beds, couches), which were highly rated for liking by children on visual
analog scales. Additional details on the development of the images and rationale for the paradigm are reported elsewhere [28].

Each functional sequence consisted of 6 blocks of 5 images in each, including one block from each of the 6 stimulus categories. Within a block, each of the images was presented for 2 seconds, with a fixation for 0.5 seconds between each image. To prevent habituation to the stimuli, we included randomized inter-block fixation times that ranged from 2-11 seconds between each block. The presentation order of blocks was pseudo-randomized so that children did not see more than two food blocks before seeing a non-food control block (ex. Low ED large, Furniture, High ED large, Low ED small, Scrambled, High ED small). There were 6 unique functional sequences, each approximately 3 minutes in duration with a break between each to check on the participants’ comfort level and provide feedback on performance. The scanning paradigm was designed to last approximately 25 minutes, but the total duration of the scan varied from 10-35 minutes, depending on the randomized interval time between blocks and variations in children’s comfort level. An example of the scanning paradigm is depicted in Figure 3.1. Immediately following the scan, children rated how much they liked and wanted to eat each food image on a 150 millimeter visual analog scale. These liking and wanting ratings were used in additional confirmatory analyses, described below.

Figure 3.1. Example of 1 functional run in the fMRI scanning paradigm.

= randomized inter-block fixation (2 – 11 seconds)

Figure 3.1. Example of 1 functional run in the fMRI scanning paradigm.
2.5 Data Preprocessing

Anatomical data for each subject were manually converted to Talairach atlas space [34] using the AC-PC landmark and 6 additional parameters (anterior, posterior, superior, inferior, right-most, and left-most points) on the structural scan. Functional data were preprocessed using temporal filtering with a high-pass filter (GLM-Fourier basis set with 6 cycles) and 3-D motion correction with 6 vectors (3 translations and 3 rotations). Any functional run with greater than 3mm or 3 degrees of movement in any direction relative to the starting position was discarded and excluded from further analysis. Preprocessed functional scans were then coregistered to anatomical data in Talairach atlas space to create a volume time course file for each successful run. A general linear model (GLM) design matrix was also created for each successful run for inclusion in the multi-subject analysis. Only subjects with at least one functional run and corresponding anatomical data were included in the final analysis. These inclusion criteria resulted in a final sample of 36 children with an average of 5.36 successful runs per participant. All 36 children had 3 or more successful functional runs. All fMRI data were preprocessed using BrainVoyager QX (version 2.8, Brain Innovation, Maastricht, The Netherlands).

2.6 Data Analysis

Data were analyzed using a multi-subject random effects GLM. A regions of interest (ROI) approach was used to extract BOLD activation from bilateral brain regions previously implicated in energy homeostasis (hypothalamus, thalamus) and food-related reward (cingulate gyrus, insula, caudate, putamen, substantia nigra, amygdala) [9, 12]. Regions were defined by creating a 5mm radius sphere in BrainVoyager QX (version 2.8) around the Talairach coordinates reported in previous studies [9, 12]. Talairach coordinates for each brain region tested are reported in Table 3.2. We then extracted mean BOLD activation for the defined regions. Two-factor repeated measures ANOVA was used to test for main effects of ED (High vs. Low), collapsed across portion size. Contrast values were then calculated for each individual participant by subtracting BOLD activation for one category of stimuli from the BOLD activation for another category of stimuli (e.g. High ED – Low ED). To illustrate, this contrast value provides the difference in BOLD activation for High ED foods minus Low ED foods within a ROI.
We then calculated Pearson’s correlations in BrainVoyager to determine the relationship between BOLD activation for various contrast values (High ED – Low ED; High ED – Scrambled; Low ED – Scrambled) and child body composition (FFM index, FM index, %BF) with a significance level set at $p < 0.05$. In our predominantly lean sample FM index was not normally distributed; therefore, we opted to retain %BF in our analyses as a secondary measure of adiposity. The Benjamini & Hochberg approach was used to correct for multiple comparisons [35, 36]. The correction was applied to the main effects and correlations separately. In additional confirmatory analyses, partial correlations were calculated in SPSS (version 21.0) to examine whether associations between brain activation and body composition remained significant after controlling for total body weight (BMIz) or children’s rated liking or wanting of High ED and Low ED food images.

Table 3.2. Talairach atlas coordinates tested in ROI approach.

<table>
<thead>
<tr>
<th>Region of interest</th>
<th>Hemisphere</th>
<th>Talairach coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Hypothalamus$^1$</td>
<td>R</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>-4</td>
</tr>
<tr>
<td>Anterior cingulate gyrus$^2$</td>
<td>R</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>-12</td>
</tr>
<tr>
<td>Substantia nigra$^3$</td>
<td>R</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>-8</td>
</tr>
<tr>
<td>Amygdala$^3$</td>
<td>R</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>-22</td>
</tr>
<tr>
<td>Insula$^3$</td>
<td>R</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>-36</td>
</tr>
<tr>
<td>Putamen$^3$</td>
<td>R</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>-18</td>
</tr>
<tr>
<td>Thalamus$^3$</td>
<td>R</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>-18</td>
</tr>
</tbody>
</table>


3. Results:

3.1 ANOVA Results

Results from the GLM ANOVAs are summarized in Table 3.3. Across the whole sample, we found that BOLD activation was greater for High ED foods relative to Low ED foods in the left thalamus ($x, y, z = -18, -22, 8$; $F_{(2,34)} = 6.30, p < 0.05$), which functions in sensory processing (Figure 3.2).
**Table 3.3.** ANOVA results: Main effects of High ED vs. Low ED.

<table>
<thead>
<tr>
<th>Region of interest</th>
<th>Hemisphere</th>
<th>Direction</th>
<th>$F$-value</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalamus</td>
<td>L**</td>
<td>High ED &gt; Low ED</td>
<td>6.30**</td>
<td>0.02**</td>
</tr>
<tr>
<td>Anterior cingulate gyrus</td>
<td>R</td>
<td>High ED &gt; Low ED</td>
<td>4.65</td>
<td>0.04</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>R</td>
<td>Low ED &gt; High ED</td>
<td>5.65</td>
<td>0.02</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>L</td>
<td>Low ED &gt; High ED</td>
<td>3.75</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Note: Computed in BrainVoyager QX ** Survived correction for multiple comparisons.

**Figure 3.2.** Main effect of ED in the left thalamus; Computed in BrainVoyager QX

3.2 Correlation Results

3.2.1 Correlations between body composition and activation for the High ED – Low ED contrast:

BOLD activation for High ED relative to Low ED foods in the right substantia nigra was positively correlated with children’s FFM index (right: $r = 0.42$, $p = 0.01$). In other words, greater amounts of lean body mass were associated with greater BOLD activation for higher ED foods in a region of the brain involved with dopamine signaling and reward (**Figure 3.3**). This association was unaffected after controlling for BMIz ($p < 0.05$, data not shown) and for children’s rated liking or wanting of High ED or Low ED food images ($p < 0.05$, data not shown).

Although not statistically significant after correction for multiple comparisons, the relationship between child FFM index and BOLD activation in the amygdala ($r = 0.42$, $p =$...
0.01), another region known to be involved with the reward and emotional processing of food, was in the same direction as the relationship reported in the substantia nigra.

![Figure 3.3. Positive correlation between fat-free mass index and activation for High ED – Low ED foods in the right substantia nigra; Computed in BrainVoyager QX](image)

**Figure 3.3.** Positive correlation between fat-free mass index and activation for High ED – Low ED foods in the right substantia nigra; Computed in BrainVoyager QX

3.2.2 *Correlations between body composition and brain activation for the High ED – Scrambled contrast:*

There were no significant or trending associations between body composition and BOLD activation for High ED foods relative to Scrambled control images in the ROIs tested (all \( p > 0.10 \) before correction).

3.2.3 *Correlations between body composition and activation for the Low ED – Scrambled contrast:*

The associations between body composition and brain response to Low ED foods relative to Scrambled images did not survive adjustment for multiple comparisons and are reported as exploratory. BOLD activation in the right substantia nigra for Low ED foods relative to Scrambled control images was negatively related to children’s FM index (\( r = -0.38, p = 0.02 \)) and \%BF (\( r = -0.40, p = 0.01 \)). The direction of this relationship suggests that greater amounts of
body fat are associated with decreased BOLD activation in response to lower ED foods in a 
reward-related region of the brain.

3.2.4 Additional correlations that did not surpass statistical correction thresholds, reported for 
exploratory purposes:

For each of the contrasts we examined, we report the results of Pearson’s correlations 
between BOLD activation in ROIs tested and child body composition. The p-values on these 
correlations ranged from \((p = 0.01 – 0.07)\), although none of them survived the Benjamini 
correction [36]. These results are summarized in Table 3.4 for exploratory purposes.

<table>
<thead>
<tr>
<th>Region of interest</th>
<th>Hemisphere</th>
<th>FFM index</th>
<th>FM index</th>
<th>%BF</th>
</tr>
</thead>
<tbody>
<tr>
<td>High ED – Low ED</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>L</td>
<td>0.36</td>
<td>N/A*</td>
<td>N/A*</td>
</tr>
<tr>
<td>Anterior cingulate gyrus</td>
<td>R</td>
<td>N/A*</td>
<td>N/A*</td>
<td>-0.30</td>
</tr>
<tr>
<td>High ED – Scrambled</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>L</td>
<td>N/A*</td>
<td>-0.31</td>
<td>-0.30</td>
</tr>
<tr>
<td>Amygdala</td>
<td>R</td>
<td>-0.36</td>
<td>N/A*</td>
<td>N/A*</td>
</tr>
<tr>
<td>Putamen</td>
<td>R</td>
<td>0.38</td>
<td>N/A*</td>
<td>N/A*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>R</td>
<td>0.30</td>
<td>N/A*</td>
<td>N/A*</td>
</tr>
</tbody>
</table>

Note: Computed in BrainVoyager QX

4. Discussion:

The purpose of this study was to determine the association between children’s body 
composition and brain activation in response to images of food that vary by ED. We found a 
main effect of ED in the thalamus (e.g. sensory processing) such that High ED foods elicited 
greater BOLD activation than Low ED foods. However, there was heterogeneity in children’s 
brain responses to food stimuli, such that not all children responded in the same direction or with 
the same magnitude. We examined whether this variability in BOLD activation could be 
explained by differences in compartmental body composition (FFM and FM). We hypothesized 
that FFM would be associated with BOLD activation in homeostatic regions (hypothalamus,
thalamus) while FM would be associated with activation in reward centers (cingulate gyrus, insula, caudate, putamen, substantia nigra, amygdala). Overall, we found that FFM, but not FM, was positively associated with BOLD activation for High ED foods in a reward region of the brain, the substantia nigra.

In a sample of predominantly lean children, we found a main effect of ED in the left thalamus (i.e. sensory perception and processing). The thalamus has been characterized as a sensory hub that relays signals from each of the sensory systems (except olfaction) to an associated primary cortical area. The thalamus has been reported as an area where hunger state and taste sensation are integrated with gustatory network connections to the insula [37]. In our cohort of primarily healthy weight children, results suggest that High ED foods may stimulate this area of the gustatory network to a greater extent than Low ED foods.

In regards to individual differences, we found that FFM index was related to BOLD activation in a reward region, the right substantia nigra. Specifically, FFM index was positively correlated with activation for High ED foods relative to Low ED foods in the right substantia nigra. The substantia nigra is involved in dopamine signaling to the caudate and putamen (i.e., dorsal striatum) as part of the reward system that supports motivated behavior [27]. It has additional functions in learning, motor planning, and GABA inhibitory signaling. Receptors on the dopamine neurons of the substantia nigra have been shown to respond to leptin, insulin, and ghrelin signals, which can influence subsequent dopamine signaling [38, 39]. Therefore, it is possible that traditional energy homeostasis signals also influence areas of the brain involved in reward-seeking behavior [38]. The substantia nigra is functionally related to the limbic system, which controls basic emotions and motivational drives [27]. One area of the limbic system that has been previously implicated in the food-related imaging literature is the amygdala [40, 41]. Though these results did not survive correction, our findings were suggestive of possible associations between FFM and activation in response to High ED foods in the right amygdala. The positive association between FFM and neural response to High ED food images in the substantia nigra, and possible association in the amygdala, supports the hypothesis that FFM is an appetitive driver [2, 4, 6].

Previous studies in adolescents and adults have demonstrated that FFM is the best predictor of meal size and daily energy intake [1-5]. These effects on intake are attributed to the fact that FFM is the largest contributor to resting metabolic rate, and therefore total daily energy
expenditure [4, 6]. However, the underlying mechanism for how FFM affects appetite-regulating centers in the brain is not clear. Our results suggest that increases in FFM are associated with an increased reward response to High ED foods relative to Low ED foods. In sum, children with greater FFM have greater energy requirements, which may partly explain increased responsiveness to higher-calorie foods relative to lower-calorie options.

While we did not find that body fat was associated with activation for High ED foods, our exploratory findings suggest that greater adiposity may be related to a reduced reward response to Low ED food images. There was a trend in the direction of a negative association between the response to Low ED foods in the right substantia nigra and both FMI and %BF, suggesting that as adiposity increases children may be less responsive to healthier, low-calorie foods. However, it is important to note that these findings did not survive correction for multiple comparisons and should be considered within the context of a predominantly lean sample (94% non-overweight). It is important to evaluate this question further across a range of body weights to determine the generalizability of these findings.

We did not find body composition variables to be significantly related to activation in homeostatic regions, including the hypothalamus. The hypothalamus is thought to be a primary site of homeostatic regulation of hunger and food intake. The lateral hypothalamus is known to respond to appetite-inhibiting signals (e.g., leptin, insulin, peptide YY) and appetite-stimulating signals (e.g., ghrelin) which arise from the periphery to influence eating behavior [27]. In this study, task-related activation in the hypothalamus was not related to child body composition.

We tested the possibility that differences in children’s liking or wanting ratings for High ED and Low ED foods would explain the associations between brain activation and body composition. We found that liking and wanting ratings were not correlated with measures of body composition (FFM index, FM index, %BF) or with BOLD activation in ROIs for any of the contrasts (High ED – Low ED, High ED – Scrambled, Low ED – Scrambled). All of our main outcomes analyses remained significant after controlling for children’s rated liking or wanting of the foods. Post-hoc analyses also revealed that the findings for FFM and FM were independent of children’s total body weight (BMIz), and that BMIz was generally not related to activation in ROIs (data not shown). These results, together, suggest that our findings are specific to the compartments of body composition tested, and cannot be attributed to total body weight or liking.
or wanting for the foods used in this study. This warrants further study into the effects of physiological signals that arise from FFM and FM on the brain.

There are several strengths of this study. First, we demonstrated a high scanning success rate for this age range, which can likely be attributed to the use of thorough mock training protocols [28]. Thirty-six out of the 38 children scanned (94.7%) met and exceeded the criteria for inclusion, having 3 or more successful functional runs. Children on average had at least 5 out of 6 functional runs that met the criteria for motion correction. Within this sample of children, we were able to examine associations with compartmental body composition, rather than relying on overall body weight. This approach shed light on the influences of FFM and FM, independently. An additional strength of this study is that the food images used were distributed across a range of ED and well-controlled for age-appropriate portion size. We used a moderate ED cut-off of 1.5 kcal/g in an attempt to control for large differences in palatability between the High ED and Low ED food categories.

A few limitations must also be discussed. The paradigm and food images used for this study were developed in our laboratory and have not previously been validated. However, preliminary test-retest data on a sub-sample of children in this study (n=5) has demonstrated good to excellent reliability for activation in response to High ED vs. Low ED foods (Cronbach’s $\alpha = 0.87 – 0.96$ in the left thalamus; $\alpha = 0.91 – 0.93$ in the right substantia nigra; $\alpha = 0.93 – 0.99$ in the right amygdala). While an ROI approach increases power, one limitation is that we may have excluded activation in additional areas of the brain related to body composition. Due to limitations regarding Small Volume Correction approaches within the BrainVoyager software, the Benjamini and Hochberg approach was used to correct for multiple comparisons across multiple ROI. Replication of the current findings using alternative multiple comparison correction methods would be valuable, and may increase the power to detect significance within an individual ROI. Differences in vascularization depending on localization within the brain may have affected the BOLD signal in our ROI. However, previous research has shown that areas of the midbrain (e.g., substantia nigra, ventral tegmental area) have been successfully imaged with fMRI [47]. There is some evidence to suggest that activation in inhibitory networks may be altered with obesity [42-45], which was not a central hypothesis in this paper. For practical purposes, we used bioelectrical impedance analysis to measure body composition [31], which has good reliability, but for criterion validity relative to gold standards (e.g., three or four
compartment models) the evidence is mixed [46]. Future studies in children could use more accurate methods to quantify compartmental and regional body composition. Finally, this was a cross-sectional analysis in a homogenous sample of predominantly lean children, and it is not known whether our results are generalizable to other populations, or whether activation in these brain areas is a cause or a consequence of differences in body composition. It is possible that the relationship is bidirectional.

In conclusion, these results suggest that the reward response to foods varying in energy content may be influenced by child body composition. Our results highlight the importance of considering compartmental body composition, rather than relying on indices of overall body weight. Fat-free mass and fat mass may differentially relate to brain activation in response to food-related cues, supporting fat-free mass as an appetitive driver in children. In addition, the relationships between body composition and the response to food likely depend on the energy content of the food stimuli. Future research in this area should determine whether these individual differences in brain activity can explain variability in actual eating behavior, and whether they are related to changes in children’s weight status over time.

Acknowledgements:

This study was funded by the Social Science Research Institute and the Clinical and Translational Science Institute at The Pennsylvania State University. We acknowledge the Penn State Social, Life, and Engineering Sciences Imaging Center (SLEIC), 3T MRI Facility. The research personnel involved in this project are supported by the Pennsylvania State University Childhood Obesity Prevention Training Program funded by USDA National Institute for Food and Agriculture Grant #2011-67001-30117 Program A2121.
**Supplementary Table 3.1.** List of foods photographed for fMRI paradigm.

<table>
<thead>
<tr>
<th>High ED Foods</th>
<th>ED (kcal/g)</th>
<th>Low ED Foods</th>
<th>ED (kcal/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheeseburger</td>
<td>3.07</td>
<td>Pork tenderloin</td>
<td>1.47</td>
</tr>
<tr>
<td>Chicken nugget</td>
<td>2.35</td>
<td>Grilled chicken</td>
<td>1.29</td>
</tr>
<tr>
<td>Alfredo pasta</td>
<td>1.58</td>
<td>Vegetable soup</td>
<td>0.27</td>
</tr>
<tr>
<td>Pizza</td>
<td>2.41</td>
<td>Turkey</td>
<td>1.07</td>
</tr>
<tr>
<td>Macaroni &amp; cheese</td>
<td>1.54</td>
<td>Tomato soup</td>
<td>0.30</td>
</tr>
<tr>
<td>Garlic bread</td>
<td>3.60</td>
<td>Broccoli</td>
<td>0.34</td>
</tr>
<tr>
<td>Bacon</td>
<td>4.62</td>
<td>Peas</td>
<td>0.84</td>
</tr>
<tr>
<td>Corn bread</td>
<td>3.13</td>
<td>Corn</td>
<td>0.80</td>
</tr>
<tr>
<td>Cheese cube</td>
<td>4.00</td>
<td>Green beans</td>
<td>0.35</td>
</tr>
<tr>
<td>French fries</td>
<td>3.07</td>
<td>Brown rice</td>
<td>1.03</td>
</tr>
<tr>
<td>Muffin</td>
<td>2.84</td>
<td>Oat breakfast cereal w/ milk</td>
<td>1.11</td>
</tr>
<tr>
<td>Waffle</td>
<td>2.71</td>
<td>Yogurt</td>
<td>0.67</td>
</tr>
<tr>
<td>Cinnamon bun</td>
<td>3.66</td>
<td>Apple sauce</td>
<td>0.80</td>
</tr>
<tr>
<td>Bagel with cream cheese</td>
<td>2.67</td>
<td>Gelatin dessert</td>
<td>0.72</td>
</tr>
<tr>
<td>Biscuit</td>
<td>2.34</td>
<td>Fruit-flavored popsicle</td>
<td>0.60</td>
</tr>
<tr>
<td>Peanuts</td>
<td>5.93</td>
<td>Mixed greens salad</td>
<td>0.24</td>
</tr>
<tr>
<td>Buttered popcorn</td>
<td>1.70</td>
<td>Mushrooms</td>
<td>0.24</td>
</tr>
<tr>
<td>Potato chips</td>
<td>5.56</td>
<td>Cauliflower</td>
<td>0.25</td>
</tr>
<tr>
<td>Peanut butter cracker</td>
<td>5.13</td>
<td>Baby carrots</td>
<td>0.35</td>
</tr>
<tr>
<td>Tortilla chips with nacho cheese</td>
<td>3.06</td>
<td>Sweet gherkin pickles</td>
<td>1.25</td>
</tr>
<tr>
<td>Puffed rice marshmallow treat</td>
<td>4.09</td>
<td>Strawberries</td>
<td>0.32</td>
</tr>
<tr>
<td>Apple pie</td>
<td>2.36</td>
<td>Pineapple</td>
<td>0.50</td>
</tr>
<tr>
<td>Chocolate ice cream</td>
<td>2.16</td>
<td>Watermelon</td>
<td>0.30</td>
</tr>
<tr>
<td>Sugar cookie</td>
<td>4.19</td>
<td>Cherry tomatoes</td>
<td>0.20</td>
</tr>
<tr>
<td>Cake</td>
<td>4.14</td>
<td>Red bell pepper</td>
<td>0.31</td>
</tr>
<tr>
<td>Chocolate candies</td>
<td>5.12</td>
<td>Blueberries</td>
<td>0.57</td>
</tr>
<tr>
<td>Chocolate sandwich cookie</td>
<td>4.74</td>
<td>Peaches</td>
<td>0.54</td>
</tr>
<tr>
<td>Brownie</td>
<td>4.05</td>
<td>Cantaloupe</td>
<td>0.34</td>
</tr>
<tr>
<td>Fruit-flavored chewy candy</td>
<td>2.50</td>
<td>Red grapes</td>
<td>0.67</td>
</tr>
<tr>
<td>Chocolate peanut butter candy</td>
<td>5.24</td>
<td>Apple slices</td>
<td>0.53</td>
</tr>
</tbody>
</table>

**Average** 3.39  **Average** 0.61

Note: 30 High ED foods (> 1.5 kcal/g) and 30 Low ED foods (< 1.5 kcal/g) were presented in pseudo-randomized blocks of 5 similar food images. Each food was photographed in both large and small portions.
References:


CHAPTER 4

Study 3A:
Impact of imposed exercise on *ad libitum* energy intake in children at risk for overweight.

Published as:

Abstract:

**Background:** Exercise not only has a direct effect on energy balance through energy expenditure (EE), but also has an indirect effect through its impact on energy intake (EI). This study examined the effects of acute exercise on daily *ad libitum* EI in children at risk for becoming overweight due to family history.

**Methods:** Twenty healthy-weight children (ages 9-12 years, n = 12 male) with at least one overweight biological parent (body mass index ≥ 25 kg/m²) participated. Children reported to the laboratory for one baseline and two experimental visits (EX = exercise, SED = sedentary) each separated by 1 week in a randomized crossover design. Two hours into the EX day session, children exercised at 70% estimated VO₂max for 30 minutes on a cycle ergometer. Objective EI (kcal) was measured at a standard breakfast (~285 kcal) and *ad libitum* lunch, snack, and dinner. Meals were identical on the EX and SED days. Activity-related EE (kcal) was estimated with accelerometers worn on the non-dominant wrist and ankle. Relative EI (kcal) was computed as the difference between Total EI and Activity-related EE for each testing day. Paired *t*-tests were performed to test differences in Total EI, Activity-related EE, and Relative EI between the EX and SED days.

**Results:** Across all meals, Total EI was not statistically different between the EX and SED days (*t* = 1.8, *p* = 0.09). Activity-related EE was greater on the EX day compared to the SED day (*t* = 10.1, *p* < 0.001). By design, this difference was predominantly driven by activity during the morning (*t* = 20.4, *p* < 0.001). Because children consumed a similar number of kcal on each day, but had greater Activity-related EE on the EX day, Relative EI was lower (*t* = -5.15, *p* < 0.001) for the EX day (1636 ± 456 kcal) relative to the SED day (1862 ± 426 kcal).

**Conclusions:** Imposed exercise was effective in reducing Relative EI compared to being sedentary. Morning exercise may help children at risk for becoming overweight to better regulate their energy balance within the course of a day.
**Introduction:**

Childhood obesity rates have increased substantially over the past forty years, both in the United States and globally [1]. With increasing rates of early onset obesity, children are at the greatest risk for long-term health complications [2] because the disease is often resistant to treatment [3]. The dramatic increase in obesity prevalence has coincided with increased availability of large portions of high energy dense foods, driving a pattern of overeating [4]. In addition, levels of physical activity both in and out of the everyday school setting have decreased, reducing children’s average daily energy expenditure (EE) [5]. Determining alternative methods to decrease daily energy intake (EI) is critical for childhood obesity prevention. Typical methods to reduce EI require intentional energy restriction. These methods may be difficult to sustain as a long-term lifestyle change, particularly during childhood when preferences for sweet, salty, and fatty foods are high [6-8]. Therefore, alternative strategies are needed that impact EI and EE to improve obesity outcomes in children. Incorporating exercise as a regular lifestyle component may provide an alternative means to control appetite and energy balance throughout the life course.

The present study aims to understand the acute effects of morning exercise versus rest on daily *ad libitum* EI in children ages 9-12 years. Exercise not only has a direct effect on energy balance through EE, but has been shown to also have an indirect effect through its impact on EI [9]. Emerging research has implicated the use of exercise as a preventative measure for overeating and subsequent development of overweight and obesity in adolescents and adults [10-31]. While exercise, relative to rest, has been shown to be effective in reducing subsequent EI and contributing to a negative energy balance in adolescents and adults [17-20, 26-29], limited research has examined this relationship in children under the age of 12 years [32]. Previous work shows that high intensity exercise can induce a state of lower 24-hour energy balance by reducing subsequent EI relative to both low intensity exercise and sedentary activity in obese adolescents [27]. This is commonly referred to as the “transient anorexigenic effect” of exercise. In obese adolescents, the greatest effects of high intensity exercise on EI have been seen seven hours post-exercise [27, 33]. These changes were seen without any significant differences in appetite ratings. These findings suggest the use of exercise as a possible strategy to decrease EI, at least in the short term, which could augment attempts to intentionally restrict EI.
Previous studies have focused on the impact of exercise on intake as a treatment strategy in overweight and obese adolescents, but the “anorexigenic effect” of exercise on intake has not been studied in healthy weight children who are at risk for developing obesity due to family history. From a prevention standpoint, it is vital to determine the effectiveness of exercise-related strategies to reduce overeating in children who are predisposed to genetic or environmental factors that promote positive energy balance. This study examined the effects of acute imposed exercise versus imposed sedentariness on \textit{ad libitum} daily EI in healthy-weight children with at least one overweight or obese biological parent. Based on previous research with adolescent populations [27], we hypothesized that Total EI and Relative EI (adjusted for Activity-related EE) would be lower on a day with imposed exercise compared to a day where children remained sedentary.

\textbf{Methods:}

\textit{Study Design:}

A within-subjects crossover design study was conducted with a community-based sample of 20 children ages 9-12 years. Children completed 1 baseline and 2 experimental visits (EX = exercise, SED = sedentary in a pre-assigned randomized order) each separated by exactly 1 week. The baseline visit was a four-hour session in the morning to familiarize children with eating in a laboratory environment (breakfast and lunch) and collect baseline measurements (described below). The EX and SED days consisted of the same four-hour morning session, followed by five hours of free-living time, and then an in-laboratory dinner session (Figure 4.1). Children and their parents received modest financial compensation for their time. This study was approved by the Institutional Review Board of The Pennsylvania State University.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure41.png}
\caption{Experimental day (EX or SED) timeline.}
\end{figure}
Participants:

Children were recruited using flyers posted in local schools and businesses located around the university. Interested parents completed a phone screening to determine eligibility. Children were considered eligible if by parental report they were normal weight (< 85th age- and sex-specific body mass index [BMI] percentile), without food allergies, without medical conditions or contraindications to exercise testing [34], not participating in competitive sports which could skew fitness and exercise test results (year-round or > 3 practice sessions per week), with at least one biological parent who was overweight or obese (BMI > 25 kg/m²). Both child and parent weight status were confirmed by measurement at the baseline visit, described below. One male child was in the overweight category (89th BMI percentile) after baseline measurements, but was retained in the study. This participant did not differ from the group mean in body composition (e.g., % body fat) or on any of the behavioral measures (e.g., food intake, physical activity, exercise test performance). On the first study visit, a parent signed informed consent for their child. Children provided written assent prior to their participation. A total of 20 children were enrolled in the study and completed all three visits. Sample characteristics for these 20 children are listed in Table 4.1.

Table 4.1. Participant characteristics (n = 20). Abbreviations: BMI, body mass index.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>10.3 ± 1.1</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>41.6 ± 21.7</td>
</tr>
<tr>
<td>% body fat</td>
<td>15.6 ± 4.4</td>
</tr>
<tr>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (40)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Non-white</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
Baseline Measurements:

1. Anthropometrics & body composition

Prior to breakfast on the baseline visit, anthropometrics (height and weight) were measured to the nearest 0.1 cm and 0.1 kg by a trained researcher. Children and their parents were each weighed and measured twice using a standard scale (Detecto model 437, Webb City, MO) and stadiometer (Seca model 202, Chino, CA) in light clothing. Height (m) and weight (kg) were converted to body mass index (BMI; kg/m$^2$) for the parent, and age- and sex-specific BMI z-score and BMI percentile for the child using the Centers for Disease Control and Prevention conversion program [35]. Percent body fat was measured using bioelectrical impedance analysis (Tanita model BF-350, Arlington Heights, IL, USA).

2. Fitness testing

Two hours into the baseline visit, children completed the YMCA graded submaximal cycle test to estimate cardiorespiratory fitness [36]. Children were outfitted with a Polar Heart Rate Transmitter chest strap and wrist unit receiver (Polar Electro Inc. model T31-Coded, Lake Success, NY, USA). Participants remained seated for five minutes while a researcher explained the procedure and instructed the child on the use of the Borg Scale for Ratings of Perceived Exertion (RPE) [37]. At the end of the five minute period, a supervising nurse obtained resting heart rate and blood pressure measurements. Children were then familiarized with the cycle ergometer (Lode Corival V2, Lode Holding BV, Groningen, The Netherlands) and completed a three-minute warm-up, followed by the YMCA submaximal cycle test. The YMCA cycle test follows a branching, multi-stage format (Table 4.2) to determine the relationship between heart rate and work rate in order to estimate the individual’s VO$_{2\text{max}}$. Children are required to pedal at a constant rate ($50 \pm 2$ revolutions per minute) while researchers adjust the resistance (i.e., work rate) on the cycle ergometer at each stage. Heart rate values are recorded every minute, while blood pressure and RPE are measured every three minutes. The test requires that each participant completes two separate workload stages that result in steady-state heart rates between 110 and 150 beats per minute. Steady-state is achieved when two consecutive heart rate values are within $\pm 5$ beats per minute. VO$_{2\text{max}}$ was estimated using the graph plot and extrapolation technique [36, 38]. This estimated VO$_{2\text{max}}$ was used to determine the work rate for the 70% intensity cycle test on the EX Day (described below).
Table 4.2. YMCA Submaximal Cycle Ergometer Test protocol; American College of Sports Medicine [36]. Abbreviations: HR, heart rate (beats per minute); kg, kilograms; m, meter; min⁻¹, per minute; W, Watts.

<table>
<thead>
<tr>
<th>Stage 1</th>
<th>HR: &lt; 80</th>
<th>HR: 80-89</th>
<th>HR: 90-100</th>
<th>HR: &gt; 100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>150 kg · m · min⁻¹</td>
<td>0.5 kg</td>
<td>24 W</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>750 kg · m · min⁻¹</td>
<td>2.5 kg</td>
<td>123 W</td>
<td>600 kg · m · min⁻¹</td>
</tr>
<tr>
<td>Stage 3</td>
<td>900 kg · m · min⁻¹</td>
<td>3.0 kg</td>
<td>147 W</td>
<td>750 kg · m · min⁻¹</td>
</tr>
<tr>
<td>Stage 4</td>
<td>1050 kg · m · min⁻¹</td>
<td>3.5 kg</td>
<td>172 W</td>
<td>900 kg · m · min⁻¹</td>
</tr>
</tbody>
</table>

**70% Intensity Exercise Protocol:**

Two hours into the EX day session (Figure 4.1), children completed the cycle ergometer exercise test. Participants were outfitted with the heart rate monitor, and resting heart rate and blood pressure measurements were taken. After a three-minute warm-up, children exercised at their individual 70% estimated VO₂max for 30 minutes. The starting work rate (in Watts) was determined from the linear association between heart rate and work rate established during the submaximal exercise test [36]. The work rate was either confirmed or adjusted throughout the test to maintain a target heart rate between 70-80% age-predicted maximum heart rate (e.g., 10-year-old: 147-168 beats per minute). Children could request water at any point during the test. Researchers and the attending nurse encouraged children throughout the exercise protocol with positive verbal cues, cheering, and clapping. After completion of the exercise test, participants had a five-minute cool-down period on the bike, followed by ten minutes of light stretching.

**Accelerometer Measurements:**

Children wore an ActiGraph GT3X-BT accelerometer on their non-dominant wrist for 10 hours on each testing day (EX, SED). In addition, children wore a second accelerometer on their
non-dominant ankle for the YMCA submaximal cycle test and the 30-minute exercise test (70% individual estimated aerobic capacity) to more accurately measure activity in the seated position on the cycle ergometer. We used this hybrid measure to estimate Activity-related EE [39]. The 4 hours during the morning session were considered in-laboratory time, while the 6 hours in the afternoon were considered free-living time. Activity-related EE was extracted for each child for the entire 10-hour period, and then separately for the morning (in-lab) versus the afternoon (free-living). All data were validated and scored in ActiLife 6 software (ActiGraph, LLC, Pensacola, FL, USA) using Freedson Combination (1998) to calculate Activity-related EE.

Food Intake Measurement:

1. Test-meal procedures:
Children arrived to the laboratory after an overnight fast on all three testing days. Objective EI (kcal) was measured at a standard breakfast and ad libitum lunch, snack, and dinner. Meals were identical on the EX and SED days. Fullness ratings were completed before and after each laboratory meal on a vertical 150 millimeter visual analog scale (VAS) referred to as “Freddy Fullness” (data not shown) [40]. On the first visit, children conducted taste tests to report liking and wanting for each breakfast, lunch, and snack food on VAS. On the second visit, children tasted and rated liking and wanting on VAS for each dinner food (data not shown). Timing for the meals and VAS ratings is depicted in Figure 4.1.

Breakfast: All children were required to consume a standardized breakfast on all three test days consisting of an English muffin toasted with one tablespoon butter, banana, and orange juice (285 kcal total) (Table 4.3). Children’s liking for and willingness to eat the breakfast foods were confirmed at screening, prior to the first visit. Children were considered to have finished the meal if they consumed ≥ 95% of each individual food item within 30 minutes. All 20 children met these requirements at each of the three breakfast meals.

Lunch: Prior to the first visit, children were given the opportunity to select from a pre-set menu of available items for lunch. Children chose a sandwich (peanut butter & jelly or deli meat & cheese), vegetable (carrots or tomatoes) with ranch dip, fruit (apple slices or grapes), and salty snack (pretzels or baked chips). All children also received brownies and a bottle of water with their lunch. All serving sizes were controlled to ensure that any combination of food items provided approximately the same number of total calories (997-1014 kcal), which provided >
50% of children’s caloric needs for the day. Possible food choices and serving sizes are reported in Table 4.3. Children were instructed that they had up to 30 minutes to eat *ad libitum* from the food items provided. If they were finished before the 30 minutes had elapsed, they notified a researcher.

**Snack:** The *ad libitum* snack consisted of a granola bar, fruit cocktail, and juice (~302 kcal) (Table 4.3). All foods were pre-weighed and packed for children to take home during free-living time on the EX and SED days. Parents were given written and verbal instructions to provide the snack at a set time and to return any packaging and uneaten food items to researchers upon arrival for dinner. Additional written and verbal instructions were given to not eat or drink anything except water for the 2 hours prior to dinner. Compliance was checked by sending text message reminders to parents at snack time and requesting a response. These messages asked parents to remind children that they could eat as much as they wanted of any of the snack foods. Upon return, packaging was re-weighed to measure snack intake.

**Dinner:** On the EX and SED days, children reported to the laboratory at least 2 hours fasted for an *ad libitum* dinner test-meal consisting of macaroni and cheese, garlic bread, broccoli, applesauce, and cookies (~1227+ kcal) (Table 4.3). Children were instructed that they had up to 30 minutes to eat as much as they wanted from the available foods. They were also able to request additional servings of the foods at this meal. A researcher was available in the room during the meal and prompted the child if they finished a serving of a particular food. Children could also notify the researcher if they finished eating before the 30 minutes had elapsed.

2. **Nutrient analysis:**

Pre- and post-meal weights for each food item were measured to the nearest 0.1 gram, and used to calculate intake in grams. This was later converted to EI (kcal) by meal (Breakfast, Lunch, Snack, and Dinner) in SPSS Statistics (Version 22; IBM Corporation, Armonk, NY, USA) using nutrition label information. Total EI (kcal) was computed as the sum of EI from each of the individual meals (Breakfast, Lunch, Snack, and Dinner EI). Relative EI (kcal) was computed as the difference between Total EI and Activity-related EE for each testing day.
Table 4.3. Food items, serving sizes, and calorie contents for each meal. Abbreviations: g, grams; fl oz., fluid ounces; kcal, kilocalories.

<table>
<thead>
<tr>
<th>Breakfast Food Item</th>
<th>Serving Size</th>
<th>Kcal per Serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>English muffin (with butter)</td>
<td>1 muffin + 1 tablespoon butter</td>
<td>151</td>
</tr>
<tr>
<td>Banana (without peel)</td>
<td>60 g</td>
<td>51</td>
</tr>
<tr>
<td>Orange juice</td>
<td>178 g (6 fl oz.)</td>
<td>83</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td><strong>285</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lunch Food Item</th>
<th>Serving Size</th>
<th>Kcal per Serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat bread</td>
<td>2 slices</td>
<td>170</td>
</tr>
<tr>
<td>Deli meat (pick one)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ham</td>
<td>114 g</td>
<td>100</td>
</tr>
<tr>
<td>Turkey</td>
<td>95 g</td>
<td>100</td>
</tr>
<tr>
<td>+ Cheese (pick one)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheddar</td>
<td>42 g</td>
<td>157</td>
</tr>
<tr>
<td>Provolone</td>
<td>38 g</td>
<td>140</td>
</tr>
<tr>
<td>American</td>
<td>53 g</td>
<td>151</td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut butter</td>
<td>30 g</td>
<td>190</td>
</tr>
<tr>
<td>+ Jelly</td>
<td>36 g</td>
<td>60</td>
</tr>
<tr>
<td>Pretzels</td>
<td>42 g</td>
<td>165</td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baked chips</td>
<td>39 g</td>
<td>165</td>
</tr>
<tr>
<td>Apple slices</td>
<td>102 g</td>
<td>53</td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grapes</td>
<td>77 g</td>
<td>53</td>
</tr>
<tr>
<td>Carrots</td>
<td>100 g</td>
<td>41</td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomatoes</td>
<td>140 g</td>
<td>41</td>
</tr>
<tr>
<td>Ranch dip</td>
<td>30 g</td>
<td>140</td>
</tr>
<tr>
<td>Brownies (3)</td>
<td>43 g</td>
<td>188</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td><strong>997 – 1014</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Snack Food Item</th>
<th>Serving Size</th>
<th>Kcal per Serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate chip granola bar</td>
<td>1 bar</td>
<td>104</td>
</tr>
<tr>
<td>Mixed fruit cocktail</td>
<td>117 g</td>
<td>62</td>
</tr>
<tr>
<td>Apple juice</td>
<td>250 g (8 fl oz.)</td>
<td>136</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td><strong>302</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dinner Food Item</th>
<th>Serving Size</th>
<th>Kcal per Serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaroni &amp; cheese</td>
<td>400 g</td>
<td>551</td>
</tr>
<tr>
<td>Garlic bread</td>
<td>75 g</td>
<td>270</td>
</tr>
<tr>
<td>Broccoli (with butter)</td>
<td>120 g</td>
<td>53</td>
</tr>
<tr>
<td>Applesauce</td>
<td>128 g</td>
<td>110</td>
</tr>
<tr>
<td>Cookies (3)</td>
<td>46 g</td>
<td>243</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td><strong>1,227</strong></td>
</tr>
</tbody>
</table>
Statistical Analysis:

Descriptive statistics for participant characteristics (i.e., means and standard deviations on continuous variables and frequencies on categorical variables) were calculated on the full sample. Pearson’s correlations were computed to determine the association between Total EI on the EX and SED days. Paired t-tests were performed to test differences in Total EI, EI by meal (standard breakfast, ad lib lunch, ad lib snack, and ad lib dinner EI), Activity-related EE (total, morning, afternoon), and Relative EI between the EX and SED days. Effect sizes (Cohen’s d) were calculated for all paired t-test results. Data were analyzed using SPSS. Test results were considered significant at \( p < 0.05 \).

Results:

Across all meals, Total EI was not statistically different between the EX and SED days (\( t = 1.8, p = 0.09 \)). Total intake on the two days was highly correlated (\( r = 0.93, p < 0.01 \)). By design, EI at the standard breakfast was not different between the two days (\( t = 0.2, p = 0.87 \)). In addition, EI at the ad lib lunch (\( t = 2.0, p = 0.06 \)), ad lib snack (\( t = -1.9, p = 0.08 \)), or ad lib dinner (\( t = 1.2, p = 0.24 \)) did not differ on the EX day versus the SED day. Activity-related EE was greater on the EX day compared to the SED day (\( t = 10.1, p < 0.001 \)). This difference was predominantly driven by in-laboratory activity during the morning (\( t = 20.4, p < 0.001 \)). Afternoon free-living Activity-related EE was not different between the two days (\( t = 1.8, p = 0.09 \)).

Because children ate a similar number of kcal on each day, but had greater Activity-related EE on the EX day, Relative EI was lower (\( t = -5.15, p < 0.001 \)) for the EX day (1636 ± 456 kcal) compared to the SED day (1862 ± 426 kcal) (Figure 4.2). In other words, Total EI adjusted for Activity-related EE was 226 kcal lower on the EX Day than the SED day. Paired t-test results for EI and EE variables and effect sizes for these results are summarized in Table 4.4.
Figure 4.2. Relative EI (kcal) was 226 kcal lower on the EX Day (1636 ± 456 kcal) compared to the SED Day (1862 ± 426 kcal); t = 5.15, p < 0.001.

Table 4.4. Results of paired t-tests for mean comparisons of energy intake (EI) and energy expenditure (EE) variables between the Exercise (EX) and Sedentary (SED) Days. *Significance at p < 0.05.

<table>
<thead>
<tr>
<th></th>
<th>EX Day</th>
<th>SED Day</th>
<th>Paired Diff. (± SD)</th>
<th>t</th>
<th>p</th>
<th>Effect size (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total EI (kcal)</td>
<td>2171 ± 566</td>
<td>2088 ± 497</td>
<td>83 ± 204</td>
<td>1.8</td>
<td>0.09</td>
<td>0.41</td>
</tr>
<tr>
<td>Breakfast EI (kcal)</td>
<td>287 ± 8.2</td>
<td>286 ± 8.5</td>
<td>0 ± 9</td>
<td>0.2</td>
<td>0.87</td>
<td>0.04</td>
</tr>
<tr>
<td>Lunch EI (kcal)</td>
<td>683 ± 186</td>
<td>639 ± 193</td>
<td>45 ± 100</td>
<td>2.0</td>
<td>0.06</td>
<td>0.45</td>
</tr>
<tr>
<td>Snack EI (kcal)</td>
<td>245 ± 72</td>
<td>256 ± 61</td>
<td>-12 ± 28</td>
<td>-1.9</td>
<td>0.08</td>
<td>0.43</td>
</tr>
<tr>
<td>Dinner EI (kcal)</td>
<td>956 ± 362</td>
<td>907 ± 310</td>
<td>50 ± 182</td>
<td>1.2</td>
<td>0.24</td>
<td>0.27</td>
</tr>
<tr>
<td>Total Activity-related EE (kcal)</td>
<td>534 ± 263</td>
<td>226 ± 146</td>
<td>308 ± 137</td>
<td>10.1</td>
<td>0.001 *</td>
<td>2.25</td>
</tr>
<tr>
<td>Morning Activity-related EE (kcal)</td>
<td>394 ± 123</td>
<td>116 ± 77</td>
<td>278 ± 61</td>
<td>20.4</td>
<td>0.001 *</td>
<td>4.56</td>
</tr>
<tr>
<td>Afternoon Activity-related EE (kcal)</td>
<td>144 ± 147</td>
<td>110 ± 74</td>
<td>33 ± 86</td>
<td>1.8</td>
<td>0.09</td>
<td>0.40</td>
</tr>
<tr>
<td>Relative EI (kcal)</td>
<td>1636 ± 456</td>
<td>1862 ± 426</td>
<td>-226 ± 196</td>
<td>-5.15</td>
<td>0.001 *</td>
<td>1.15</td>
</tr>
</tbody>
</table>
Discussion:

The purpose of this pilot study was to examine the effects of acute exercise on ad libitum daily EI in healthy-weight 9-12 year-old children who were at risk for becoming overweight due to family history. Based on previous research on adolescents with obesity [27], we hypothesized that Total EI and Relative EI (Total EI adjusted for Activity-related EE) would be lower on the EX day compared to the SED day. In line with our hypothesis, we did find that Relative EI was 226 kcal lower on the EX day compared to the SED day, indicating a beneficial effect of imposed exercise on children’s energy balance over the course of a day. This finding was a result of the fact that children did not significantly adjust their daily EI to fully compensate for differences in activity-related EE between the two days. Participants consumed essentially the same number of calories (within 85 kcal) on the EX day and the SED day, but had greater Activity-related EE (~310 kcal) on the EX day than the SED day. In sum, we found that imposed exercise had a beneficial impact on daily energy balance in children who are at risk for becoming overweight by increasing Activity-related EE without a significant increase in subsequent ad libitum EI.

In the current study, we looked to extend previous findings in adolescents with obesity regarding the “anorexigenic effect” of exercise on subsequent food intake [27]. We used a similar design to previous studies to examine the effects of exercise in a younger age group of pre-adolescent children who were at risk for becoming overweight. We found that imposed exercise was effective in reducing Relative EI when compared to the SED day. We did not find evidence of a compensatory response to the exercise bout in regards to children’s ad libitum EI at subsequent meals. Children consumed approximately the same number of calories on both days, demonstrating that they did not adjust their EI to match differences in Activity-related EE between the EX day and the SED day. In other words, the short-term benefits of exercise on EE were not immediately offset by compensatory EI in our sample of healthy weight children.

Previous studies in adolescents and adults have shown mixed results for acute post-exercise EI, which can be attributed to differences in participant characteristics (e.g., lean versus obese) or exercise methodologies (e.g., intensity, modality). Generally, subsequent EI is not greater after exercise relative to rest in healthy populations, whether at a single meal or over the course of a day [30, 41]. Once differences in EE from exercise are accounted for, Relative EI is typically lower after exercise versus a control condition [23, 27, 31]. These results, together with ours,
suggest that morning moderate-to-vigorous intensity exercise may be an effective strategy to prevent positive energy balance in children at risk for becoming overweight, at least in the short term. Additional longer-term studies are needed to assess the effectiveness of structured morning exercise for childhood obesity prevention.

It is important to note that the timing of the exercise bout relative to the meals can affect subsequent EI, but previous studies disagree regarding the most effective time interval to reduce EI. A recent study in healthy weight adolescent males demonstrated that exercise immediately prior to lunch was more effective in reducing lunch EI when compared to an identical exercise bout approximately 2 hours prior to lunch [11]. There are several proposed mechanisms for the effects of acute exercise on appetite and EI regulation. Exercise can act indirectly on energy balance through influences on body composition (e.g., fat and lean mass), gut peptide signaling (e.g., polypeptide YY 3-36, glucagon-like peptide-1), and brain responses to food-related cues [9, 42]. These signals, in theory, impact appetite and subsequent food intake [9, 42]. Given the short half-life of many appetite-regulating hormones, it may be expected that a shorter delay between an exercise bout and a meal is more effective in reducing EI than a longer interval. In the current study, the delay between the exercise bout and the lunch test-meal was approximately 45 minutes. We did not find a significant difference in lunch EI between the EX and SED days. Other studies have found lasting effects of acute morning exercise on EI later in the day. For example, Thivel and colleagues found that high intensity exercise was effective in reducing intake at a dinner test-meal 7 hours post-exercise [27]. The mechanism for these more sustained effects is unclear, but important to investigate given that obesity prevention requires chronic regulation of energy balance.

One major methodological difference in our study, versus previous studies [27], is that EE was not matched across the two days (EX versus SED). Activity-related EE was controlled during the four hours of in-laboratory time in the morning, but we also allowed children free-living time in the afternoon. This allowed us to test whether children would compensate for the morning exercise by increasing sedentary time later in the day. We did not find any differences in afternoon Activity-related EE between the EX and SED days. In other words, children were not less active following the exercise bout than they were following imposed sedentary time. The lack of a compensatory response in our sample is suggestive of one promising behavioral attribute that could help children maintain a healthy weight. A recent study in overweight boys
found that after a vigorous exercise session, the participants spontaneously decreased their physical activity EE during the following 24 hours [43]. Participation in structured exercise may be a more effective strategy in healthy weight populations with fewer negative consequences on subsequent leisure-time physical activity. A systematic review in healthy adults found minimal evidence that prescribed exercise affects non-exercise physical activity and EE [44]. Further research in children is warranted to determine which characteristics make an individual more or less likely to compensate for imposed exercise.

This preliminary study represents a novel application of the working model of energy balance in a sample of children at risk for becoming overweight. There are several strengths of this study. First, we have objective measures of daily EI across multiple test-meals during which we were able to assess children’s *ad libitum* energy intake. Objective intake measures are advantageous compared to self-reported EI, a method which introduces misreporting biases [45]. In addition, identical meals across the two experimental days allowed us to look specifically at the within-subjects effect of the exercise bout on Total EI. Another strength is the inclusion of objective measures of Activity-related EE, both in the laboratory and free-living. Our study was designed to facilitate acclimation to the laboratory environment (research personnel, exercise testing, and test-meals) at the baseline visit. This helped to reduce the novelty effect on our outcome variables on the experimental days. Finally, we had a 100% retention rate and full reported and/or observed compliance with instructions and protocols across the three testing days.

Some limitations of the current study should be noted. We were not able to assess intake outside of the 10-hour experimental day and are unsure whether children’s EI differed in the late evening (e.g., dessert) or the following day. In addition, a few of the differences in intake between the two days were non-significant trends (*p* < 0.10), including lunch EI (*p* = 0.06). Post-hoc analyses demonstrated we were underpowered (Power < 0.30) to detect differences in intake of individual meals/snack between the two days. It is possible that these differences would be significant with a larger sample size. Our sample was homogenous in demographic characteristics, which limits the generalizability of our findings. In addition, our classification of “at risk for becoming overweight” was solely based on parent weight status, and not inclusive of additional genetic, family, or environmental-level factors that can impact energy balance-related behaviors [46]. Future studies may be able to identify more specific phenotypes of obesity risk in
the recruitment phase. Finally, this study did not include measures of resting metabolic rate or thermic effect of food. Therefore, we cannot evaluate effects of exercise on total daily EE or overall energy balance.

Conclusions:
Children in this study did not adjust their EI to match differences in Activity-related EE, resulting in relative positive energy balance on the SED compared to the EX day. Imposed exercise may help children at risk for becoming overweight better regulate their food intake within the course of a day. In order to allow for more personalized prevention strategies, future research is necessary to determine the individual-level child characteristics that are likely to impact the effect of exercise on energy intake in this population.

Ethics approval and consent to participate:
This study was approved by the Institutional Review Board of The Pennsylvania State University (#00578). A parent signed informed consent for their child. Children provided written assent prior to their participation.

Funding:
This project was supported by Agriculture and Food Research Initiative Grant #2011-67001-30117 from the USDA National Institute of Food and Agriculture, Childhood Obesity Prevention: Transdisciplinary Graduate Education and Training in Nutrition and Family Sciences, Program A2121.

Acknowledgements:
The project described was also supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1 TR000127. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. We would like to thank the Penn State Clinical and Translational Science Institute, including the Clinical Research Center and the Biostatistics, Epidemiology, and Research Design services. Finally, we would like to acknowledge the Metabolic Kitchen and Children’s Eating Behavior Laboratory at Penn State.
References:


CHAPTER 5

Study 3B:
Perceived exertion during exercise is associated with children’s *ad libitum* energy intake.

Submitted for publication as:
**Fearnbach SN**, Masterson TD, Schlechter HA, Loken E, Downs DS, Thivel D, Keller KL.
Abstract:
PURPOSE: To examine the individual-level factors that predict ad libitum energy intake (EI) following imposed exercise (EX) and sedentary time (SED) in children.

METHODS: Healthy-weight children ages 9-12 years (n = 20) reported to the laboratory for 1 baseline and 2 experimental visits (EX and SED) each separated by 1 week in a randomized crossover design. Percent body fat, weight (kg), and height (m) were used to calculate fat-mass index (FM index) and fat-free mass index (FFM index; kg/m²). On the EX day, children exercised at 70% estimated VO₂peak for 30 minutes on a cycle ergometer while cardiovascular responses and ratings of perceived exertion (RPE) were measured. Objective EI (kcal) was measured at identical meals (standard breakfast, ad libitum lunch, snack, and dinner) on the EX and SED days.

RESULTS: Total EI was not statistically different between the EX and SED days (t = 1.8, p = 0.09). FFM index was positively associated with EI on the EX day (r = 0.54, p < 0.05). RPE was also positively associated with EI on the EX day (r = 0.82, p < 0.001). Together, FFM index and RPE explained 77% of the variability in EX day EI (F(2,17) = 26.4, p < 0.001). For each unit increase in RPE, children consumed ~270 more calories on the EX day. Similar associations were found on the SED day.

CONCLUSION: FFM index was positively associated with EI on the EX day. Despite experiencing the same 70% relative exercise intensity, increased perceived difficulty predicted greater EI on both the EX and SED day. These findings demonstrate a role for both FFM and RPE in explaining EI variability in children.
Introduction:

Previous studies have shown individual differences in the energy intake (EI) response to exercise, but homeostatic or cognitive mechanisms underlying these differences are unclear. In particular, there is limited research in this area in children. In regards to homeostatic mechanisms, there have been studies examining changes in gut peptides, inflammatory markers, and substrate or macronutrient utilization as possible factors that influence post-exercise EI (13, 14, 28). In addition, fat-free mass (FFM) is a known predictor of daily EI in adolescents and adults, predominantly through its effects on resting metabolic rate and total energy expenditure (EE) (4, 8, 9, 23). Furthermore, it has been proposed that cognitive factors also contribute to additional variability in daily EI. For example, studies in adults have shown that increased perceived difficulty of exercise may be associated with caloric compensation and predicts weight regain after successful weight loss (6). This weight regain is assumed to be related to greater EI by participants, but has not been tested.

The majority of studies looking at the effects of exercise on subsequent food intake have focused on homeostatic mechanisms. In particular, there are several pathways by which the body signals information about EE in order to increase EI and properly compensate for the activity-related energy deficit. These include, but are not limited to, gut-brain signaling pathways and markers of glycogen store utilization (13, 14, 26). But homeostatic regulation of energy balance is not limited to metabolic changes associated with acute fluctuations in EE. Body composition, including FFM and fat mass (FM), serves as a longer-term indicator of energy balance. FFM is a large determinant of resting metabolic rate, which contributes greatly to total daily EE (2). In addition, FFM has been shown to be a better predictor of EI than FM in both adolescents and adults (3, 8, 9, 23). However, research in younger children is lacking. Also, the conditions under which FFM is most strongly associated with EI have not been examined. For example, it is not known whether the association between FFM and EI is affected by an exercise-induced energy deficit.

Aside from the homeostatic regulation of EI, there are also cognitive factors that influence eating behaviors. For example, perceived exertion may predict individual responses to a set exercise bout. However, there has been limited research examining whether the perceived difficulty experienced during exercise is associated with subsequent behaviors, above and beyond the effects of the physical stress associated with exercise (22, 31). Of particular interest
to the current study, it is unknown whether the perceived difficulty of exercise is associated with post-exercise EI. It is vital to understand the potential consequences of imposed exercise in a sample of children who are at risk for becoming overweight before implementing prescribed exercise on a broader scale.

The purpose of the current paper was to understand individual-level factors that may contribute to differences in daily EI after participation in 30 minutes of 70% intensity cycling exercise. In particular, we examined the associations between body composition (i.e., FFM and FM), cardiovascular responses to exercise (e.g., heart rate), ratings of perceived exertion, and *ad libitum* EI across the experimental day. We hypothesized that children’s FFM would be positively associated with total EI, in line with previous research in adolescents and adults. Since the exercise bout was tailored to the same relative intensity of 70% VO$_{2\text{max}}$, we hypothesized that total EI would be positively related to children’s ratings of perceived exertion during the exercise, independent of the controlled cardiovascular response to the exercise.

**Methods:**

*Study Design:*

We conducted a within-subjects, crossover design study with a community-based sample of 20 children between the ages of 9-12 years. The overall purpose of the study was to investigate the effects of imposed exercise versus imposed sedentariness on children’s total EI over the course of a day. This paper addresses a secondary aim to determine child characteristics that predict individual differences in post-exercise EI. Children completed 1 baseline and 2 experimental visits (EX = exercise day, SED = sedentary day in a pre-assigned randomized order) each separated by 1 week. For all three visits, children arrived to the laboratory after an overnight fast. The baseline visit consisted of a four-hour session in the morning, which served to familiarize children with eating in a laboratory environment (breakfast and lunch) and collect baseline fitness and anthropometric measurements. The EX and SED days consisted of the same four-hour morning session, followed by five hours of free-living time, and then an in-laboratory dinner session. On the first study visit, a parent signed informed consent for their child and children provided verbal and written assent prior to their participation. Children and their parents received modest financial compensation for their time. This study was approved by the
Institutional Review Board (#00578) and the Clinical Research Center Advisory Committee (#330) of The Pennsylvania State University.

Participants:

Children were recruited using flyers and online media postings in local schools and businesses located around the university. Interested parents completed a phone screening to determine eligibility. Children were considered eligible if by parental report they were normal weight (< 85th age- and sex-specific body mass index [BMI] percentile) with at least one biological parent who was overweight or obese (BMI > 25 kg/m²), without food allergies, medical conditions or contraindications to exercise testing, and were not participating in competitive sports which could skew test results (year-round or more than 3 practice sessions per week). Children’s liking and willingness to eat the test-meal foods was also confirmed at screening, prior to enrollment in the study. Both child and parent weight status were confirmed by measurement at the baseline visit. One male child was retained in the study despite being in the overweight category (89th BMI percentile) after baseline measurements. This participant was not a statistical outlier compared to the group average in regards to body composition (e.g., % body fat) or any of the behavioral measures (e.g., food intake, exercise test performance), and removing him from the analyses did not affect the statistical significance of or conclusions from our findings. All 20 children who were initially enrolled in the study completed all three visits. Sample characteristics for these 20 children are listed in Table 5.1.

Baseline Measurements:

Anthropometrics & body composition:

Prior to breakfast on the baseline visit, anthropometrics (height and weight) were measured to the nearest 0.1 cm and 0.1 kg by a trained researcher. Children and their parents were each weighed and measured twice in light clothing, using a standard scale (Detecto model 437, Webb City, MO) and stadiometer (Seca model 202, Chino, CA). Height (m) and weight (kg) were converted to body mass index (BMI; kg/m²) for the parent, and age- and sex-specific BMI z-score and BMI percentile for the child using the Centers for Disease Control and Prevention conversion program (12).
Body composition was measured using bioelectrical impedance analysis (Tanita model BF-350, Arlington Heights, IL, USA). Percent body fat (%BF) was multiplied by body weight (kg) to calculate fat mass (FM; kg). The difference between body weight and FM was taken to calculate fat-free mass (FFM; kg) (35). To control for differences in body composition as a function of child height, FFM index and FM index were calculated by dividing the absolute FFM and FM, respectively, by the height squared (kg/m²) (36).

**Table 5.1.** Participant characteristics (n = 20). Abbreviations: BMI, body mass index; FM, fat mass; FFM, fat-free mass.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>10.3 ± 1.1 (9 – 12)</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>41.6 ± 21.7 (9 – 89)</td>
</tr>
<tr>
<td>% body fat</td>
<td>15.6 ± 4.4 (6.7 – 26.6)</td>
</tr>
<tr>
<td>FM index (kg/m²)</td>
<td>2.7 ± 0.9 (1.1 – 4.4)</td>
</tr>
<tr>
<td>FFM index (kg/m²)</td>
<td>14.3 ± 1.5 (12.1 – 18.3)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (40)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Non-white</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

**Fitness testing:**

Two hours into the baseline visit, children completed the YMCA graded submaximal cycle test to estimate cardiorespiratory fitness (24). Children were outfitted with a Polar Heart Rate Transmitter chest strap and wrist unit receiver (Polar Electro Inc. model T31-Coded, Lake Success, NY, USA). Participants remained seated for five minutes while a researcher explained the procedure and instructed the child on the use of the Borg Scale for Ratings of Perceived Exertion (RPE) (5, 11, 20, 29). At the end of the five minute period, a supervising nurse obtained resting heart rate (HR) and blood pressure (BP) measurements. Children were then familiarized with the cycle ergometer (Lode Corival V2, Lode Holding BV, Groningen, The Netherlands) and
completed a three-minute warm-up, followed by the YMCA submaximal cycle test. The YMCA cycle test follows a branching, multi-stage format to determine the relationship between heart rate and work rate in order to estimate the individual’s VO$_{2\text{max}}$. Children are required to pedal at a constant rate (50 ± 2 revolutions per minute) while researchers adjust the resistance (i.e., work rate) on the cycle ergometer at each stage. HR values are recorded every minute, while BP and RPE are measured every three minutes. The test ends once a participant has completed two separate workload stages that result in steady-state HR (±5 beats per minute) between 110 and 150 beats per minute. VO$_{2\text{max}}$ was estimated using the graph plot and extrapolation technique (24). This estimated VO$_{2\text{max}}$ was used to determine the work rate for the 70% intensity cycle test on the EX Day (described below).

70% Intensity Exercise Protocol:

Two hours into the EX day session, children completed the cycle ergometer exercise test. Participants were outfitted with the HR monitor, and resting HR and BP measurements were taken. After a three-minute warm-up, children exercised at their individual 70% estimated VO$_{2\text{max}}$ for 30 minutes. The starting work rate (in Watts) was determined from the linear association between HR and work rate established during the submaximal exercise test. The work rate was either confirmed or adjusted throughout the test to maintain a target HR between 70-80% age-predicted maximum HR (e.g., 10-year-old: 147-168 beats per minute). HR values were recorded every minute, while BP and RPE were measured every three minutes. Children could request water at any point during the test. Researchers and the attending nurse encouraged children throughout the exercise protocol with positive verbal cues, cheering, and clapping. After completion of the exercise test, participants had a five-minute cool-down period on the bike, followed by ten minutes of light stretching.

Accelerometer Measurements:

Children wore an ActiGraph GT3X-BT accelerometer on their non-dominant wrist for 10 hours on each testing day (EX, SED). In addition, children wore a second accelerometer on their non-dominant ankle for the YMCA submaximal cycle test and the 30-minute exercise test (70% individual estimated aerobic capacity) to more accurately measure activity in the seated position on the cycle ergometer. We used this hybrid measure to estimate Activity-related EE, extracted
for each child for the entire 10-hour period. All data were validated and scored in ActiLife 6 software (ActiGraph, LLC, Pensacola, FL, USA) using Freedson Combination (1998) to calculate Activity-related EE (34).

Food Intake Measurement:
Test-meal procedures:

Children arrived to the laboratory after an overnight fast on all three testing days. Objective EI (kcal) was measured at a standard compulsory breakfast and *ad libitum* lunch, snack, and dinner. Meals were identical on the EX and SED days. Fullness ratings were completed before and after each laboratory meal on a vertical 150 millimeter visual analog scale (VAS) referred to as “Freddy Fullness” (25). On the first visit, children conducted taste tests to report liking and wanting for each breakfast, lunch, and snack food on a VAS. On the second visit, children tasted and rated liking and wanting on a VAS for each dinner food.

**Breakfast:**

All children were required to consume a standardized breakfast on all three test days consisting of an English muffin toasted with one tablespoon butter, banana, and orange juice (285 kcal total) (Supplementary Table 5.1). Children were considered to have finished the meal if they consumed > 95% of each individual food item within 30 minutes. All 20 children met these requirements at each of the three breakfast meals.

**Lunch:**

Children were offered an identical lunch meal on all three test days. Prior to the first visit, children were given the opportunity to select from a pre-set menu of available items for lunch. Children chose a sandwich (peanut butter & jelly or deli meat & cheese), a vegetable (carrots or tomatoes) with ranch dip, a fruit (apple slices or grapes), and a salty snack (pretzels or baked chips). All children also received brownies and a bottle of water with their lunch. All serving sizes were controlled to ensure that any combination of food items provided approximately the same number of total calories (997-1014 kcal), which provided > 50% of children’s caloric needs for the day. Possible food choices and serving sizes are reported in Supplementary Table 5.1. Children were instructed that they had up to 30 minutes to eat *ad libitum* from the food items provided. If they were finished before the 30 minutes had elapsed, they notified a researcher.

**Snack:**
The *ad libitum* snack (302 kcal) ([Supplementary Table 5.1](#)) was pre-weighed and packed for children to take home during free-living time on the EX and SED days. Parents were given written and verbal instructions to provide the snack at a set time and to return any packaging and uneaten food items to researchers upon arrival for dinner. Compliance was checked by sending text message reminders to parents at snack time and requesting a response. Children were reminded by the researchers and the parent that they could eat as much or as little as they would like of any of the snack foods. Written and verbal instructions were also given to not eat or drink anything except water for the 2 hours prior to dinner. Upon return, packaging was re-weighed.

**Dinner:**

On the EX and SED days, children reported to the laboratory at least 2 hours fasted for an *ad libitum* dinner test-meal (1227+ kcal) ([Supplementary Table 5.1](#)). Children were instructed that they had up to 30 minutes to eat as much or as little as they would like from the available foods. They were also able to request additional servings of the foods at this meal. A researcher was available in the room during the meal and prompted the child if they finished a serving of a particular food. Children could also notify the researcher if they finished eating before the 30 minutes had elapsed.

**Nutrient analysis:**

Pre- and post-meal weights for each food item were measured to the nearest 0.1 gram, and used to calculate intake in grams. This was later converted to EI (kcal) by meal (Breakfast, Lunch, Snack, and Dinner) in SPSS Statistics (Version 22; IBM Corporation, Armonk, NY, USA) using nutrition label information. Total EI (kcal) was computed as the sum of EI from each of the individual meals (Breakfast, Lunch, Snack, and Dinner EI).

**Statistical Analysis:**

Sample size calculations (n = 20) were derived using G*Power software (version 3.1.9.2) for the original aim to compare within-subject differences in EI as a function of condition (EX vs. SED) using paired-samples t-tests (16). The secondary aim of investigating predictors of individual differences in EI was not considered in the sample size calculation. Descriptive statistics for participant characteristics (i.e., means and standard deviations on continuous variables and frequencies on categorical variables) were calculated on the full sample. Paired t-
tests were performed to test differences in Total EI between the EX and SED days. Pearson’s correlations were computed to determine the associations between body composition (FFMI, FMI), exercise test variables (RPE, HR, fitness), and intake variables. Multiple linear regression was performed to predict individual variability in post-exercise EI; dependent variable = EX day EI (kcal), independent variables = RPE, HR, FFMI, FMI. One male child was a statistical outlier on RPE ratings during the 70% exercise test, reporting on the low end of the RPE scale (average rating of 9) compared to the rest of the sample (average rating of 15, range 12 – 18). Results are reported excluding this outlier. Data were analyzed using SPSS Statistics version 22.0 (IBM Corporation, Armonk, NY, USA). Tests were considered significant at $p < 0.05$.

**Results:**

Descriptive statistics for exercise test variables (RPE, HR, fitness), and intake are listed in Table 5.2. Total EI was not statistically different between the EX and SED days ($p > 0.05$), and intake on the two days was highly correlated ($r = 0.93, p < 0.001$). A summary of the correlation results is reported in Table 5.3. FFMI was positively associated with EI on the EX day ($r = 0.53, p < 0.05$) (Figure 5.1), but not the SED day ($p > 0.05$). FMI was negatively associated with baseline fitness ($r = -0.74, p < 0.01$), but was not associated with RPE or HR responses to 70% exercise test. There were no significant associations between FMI and intake.

In addition, RPE was positively associated with EI on the EX day ($r = 0.82, p < 0.001$) (Figure 5.2). This finding was not explained by children’s baseline fitness level, average HR responses to the 70% exercise test, or Activity-related EE (Mean ± SD = 288 ± 72 kcal). The unexpected negative association between HR and RPE ($r = -0.50, p < 0.05$) was driven by a statistical outlier on the HR variables (> 3 SD below the group mean on absolute HR and % age-predicted max HR). Removal of this outlier resulted in non-significant associations between HR and RPE ($p > 0.05$). In regression analyses, FFMI and RPE, together, explained 77% of the variability in EI on the EX day ($F_{(2,17)} = 26.4, p < 0.001$) (Table 5.4). For each unit increase in FFMI, children’s EX Day EI increased by 118 kcal (Table 5.4). For each unit increase in perceived difficulty of exercise on the RPE scale, children’s EX Day EI increased by approximately 270 kcal (Table 5.4).

Similar associations were found between RPE ($r = 0.79, p < 0.001$) and FFM index ($r = 0.41, p = 0.08$) and EI on the SED day, although only the correlation between RPE and EI
reached significance (Table 5.3). Together, RPE and FFM index predicted 66% of the variability in SED Day EI ($F_{(2,17)} = 15.7, p < 0.001$), but only RPE was a significant predictor in the model (RPE, $p < 0.001$; FFM index, $p = 0.16$) (data not shown). For each unit increase in RPE, children’s SED Day EI increased by approximately 230 kcal (data not shown).

**Table 5.2.** Descriptive statistics for exercise test variables and intake on the experimental days. Abbreviations: RPE, rating of perceived exertion; HR, heart rate; bpm, beats per minute; EI, energy intake.

O Results are reported without the RPE outlier.

# Possible range on Borg Scale = 6 – 20.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE avg. during 70% test $^O$#</td>
<td>15 ± 2 (12 – 18)</td>
</tr>
<tr>
<td>HR avg. during 70% test (bpm)</td>
<td>159 ± 7 (140 – 169)</td>
</tr>
<tr>
<td>Baseline fitness (mL/kg/min)</td>
<td>48 ± 6 (36 – 58)</td>
</tr>
<tr>
<td>EX Day EI (kcal)</td>
<td>2171 ± 566 (1285 – 3194)</td>
</tr>
<tr>
<td>SED Day EI (kcal)</td>
<td>2088 ± 497 (1401 – 3085)</td>
</tr>
</tbody>
</table>

**Table 5.3.** Correlations between body composition, exercise test variables, and intake. Abbreviations: FFM, fat-free mass; FM, fat mass; RPE, rating of perceived exertion; HR, heart rate; bpm, beats per minute; EI, energy intake.

O Results are reported without the RPE outlier.

* $p < 0.05$, ** $p < 0.01$.

<table>
<thead>
<tr>
<th></th>
<th>FFMI</th>
<th>FMI</th>
<th>RPE $^O$</th>
<th>HR</th>
<th>Fitness</th>
<th>EX Day EI</th>
<th>SED Day EI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFM index (kg/m$^2$)</td>
<td>1</td>
<td>0.39</td>
<td>0.29</td>
<td>0.44</td>
<td>- 0.30</td>
<td>0.53*</td>
<td>0.41</td>
</tr>
<tr>
<td>FM index (kg/m$^2$)</td>
<td>1</td>
<td>0.25</td>
<td>0.12</td>
<td>- 0.74**</td>
<td>0.38</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>RPE avg. during 70% test $^O$</td>
<td>1</td>
<td>- 0.50*</td>
<td>- 0.24</td>
<td>0.82**</td>
<td>0.79**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR avg. during 70% test (bpm)</td>
<td></td>
<td>1</td>
<td>- 0.01</td>
<td>- 0.29</td>
<td>- 0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline fitness (mL/kg/min)</td>
<td></td>
<td>1</td>
<td>- 0.23</td>
<td></td>
<td>- 0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EX Day EI (kcal)</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>0.93**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SED Day EI (kcal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
**Figure 5.1.** Correlation between fat-free mass index (FFM index) and energy intake (EI) on the Exercise (EX) Day; $r = 0.53, p < 0.05$.

**Figure 5.2.** Correlation between ratings of perceived exertion (RPE) and energy intake (EI) on the Exercise (EX) Day; $r = 0.82, p < 0.001$.

**Table 5.4.** Linear regression prediction of energy intake (EI) on the Exercise (EX) day, using ratings of perceived exertion (RPE) and fat-free mass index (FFM index). Note: $F_{(2,17)} = 26.4, p < 0.001, R^2 = 0.77$.

<table>
<thead>
<tr>
<th>Model</th>
<th>Beta</th>
<th>Std. Error</th>
<th>$t$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>-3648.2</td>
<td>828.5</td>
<td>-4.4</td>
<td>0.001</td>
</tr>
<tr>
<td>RPE</td>
<td>271.1</td>
<td>46.7</td>
<td>5.8</td>
<td>0.001</td>
</tr>
<tr>
<td>FFMI (kg/m²)</td>
<td>118.3</td>
<td>47.8</td>
<td>2.5</td>
<td>0.025</td>
</tr>
</tbody>
</table>
Discussion:

The purpose of the current study was to examine individual differences in *ad libitum* EI in children at risk for becoming overweight according to family history. Our findings demonstrate a role for both FFM and RPE in EI regulation in children. In line with our hypothesis, FFM was positively associated with daily EI. We also confirmed our hypothesis that total EI would be positively related to children’s RPE during the 70% intensity exercise bout, and this was independent of the controlled cardiovascular response to the exercise (e.g., HR). Each unit increase on the Borg Scale was associated with a 270 calorie increase in EI across the EX day. Similar associations were found on the SED day, given that EI was highly correlated between the two testing days.

There is a growing body of literature on the influences of body composition on appetite regulation and energy balance. In particular, FFM has been shown to be positively related to daily EI and meal size (3, 9). FFM may exert its effects on EI through its influence on resting metabolic rate and total EE, as seen in adolescents and adults (3, 8, 9, 23, 37). Our group recently showed in 7- to 10-year-old children that FFM was also positively related to activation in reward areas of the brain (e.g., substantia nigra, amygdala) in response to pictures of high-energy-dense foods (17). These results provide evidence of a potential mechanism for the positive association between FFM and food intake demonstrated previously (3, 8) and in the present cohort. We also found a positive association between FFM and EI on the SED day, but it did not reach significance (*p* = 0.08) in this small cohort. EI and EE have previously been shown to have a stronger linear association at higher levels of EE (30). One possible explanation for our current findings is that children had lower Activity-related EE on the SED day compared to the EX day.

While the association between FM and EI was in the positive direction on both experimental days, these correlations did not reach significance (both *p* > 0.10). However, we recruited only healthy weight children, therefore variation in FM was limited. In a cohort with greater body weight diversity, we recently reported that adiposity was positively related to total EI and intake of savory-fat foods in a laboratory setting (18). However, total EI was also positively related to FFM and estimated resting EE (18). Another recent study in adolescents with obesity demonstrated that even though FM was positively associated with EI assessed by 3-day food records, skeletal muscle mass (lean body tissue) was the strongest predictor of food
intake (8). Collectively, these findings suggest that in youth, lean body mass may be a better predictor of EI than fat mass, consistent with studies in adults (3, 23, 37).

While EI is generally under good homeostatic control in healthy populations, psychological factors can also influence eating behavior. Despite experiencing the same 70% relative exercise intensity, children varied in their perceived difficulty of the exercise. Higher average RPE during the exercise bout predicted greater *ad libitum* EI on the EX day. These findings suggest that greater perceived difficulty of exercise may result in overcompensation for the energy expended through greater EI at subsequent meals, at least in the short term. It is worth noting that because intake was highly correlated across the two experimental days, RPE was also positively associated with EI on the SED day. RPE was also positively associated with EI at each of the *ad libitum* meals (i.e., lunch, snack, dinner) on both the EX and SED days (data not shown). It is possible that individuals with a higher RPE scores on the 70% exercise generally tend to consume more calories day-to-day. Importantly, the association between RPE and intake was significant within the context of a model that controlled for differences in children’s body size (i.e., FFMI). In other words, children who think exercise is more difficult may generally have a tendency to eat more, independent of body weight. Based on the cross-sectional design of the current study, the direction of the more general relationship between RPE and EI across multiple eating occasions cannot be determined.

These findings are in line with a previous study that demonstrated that higher perceived difficulty of exercise was associated with greater weight regain following successful weight loss (6). Women in this study were formerly overweight and had completed a weight loss intervention and weight was tracked over the following year. RPE was measured in response to a submaximal walking exercise. In this study, RPE, but not physiological exertion during the submaximal exercise, was positively associated with weight regain (6). The authors suggested that women who have higher RPE during exercise may also have trouble restricting energy intake, which could predispose them towards greater weight regain (6). However, objective intake was not measured and, therefore, conclusions about energy compensation from this study cannot be made. But the hypothesis that weight regain may be attributable to increased EI is in accordance with the results of the current study, wherein we found that RPE was positively associated with short-term EI. The increase in EI was independent of the physiological responses to exercise (i.e., HR), activity-related EE during the exercise bout, or children’s baseline fitness.
levels. We propose that this relationship between RPE and EI is specifically a cognitive phenomenon, and the large effect size warrants further investigation into this particular result. Given the limited sample size and exploratory nature of our study, additional studies should be conducted to confirm this relationship.

This is the first study to our knowledge that attributes individual differences in post-exercise energy compensation to the perceived difficulty of the exercise bout. There is a diverse body of evidence on other cognitive factors related to post-exercise eating behavior. One study in adults demonstrated that compensatory eating following 50 minutes of 70% intensity exercise was associated with an enhanced implicit wanting for food (19). Some individuals may not receive the same benefit from imposed exercise due to an increase in the hedonic response to food following exercise-induced energy expenditure. Other research has suggested that energy compensation following exercise may be related to increased subjective hunger ratings (27) or higher levels of disinhibition (7). Researchers have suggested that at submaximal exertion levels, perceived exertion is dominated by cognitive factors and affective responses to exercise, while higher intensity exercise induces heightened sensory attention to the physiological response to the exercise (22, 31). Based on the design of the current study, we cannot determine whether children had reached an intensity level at which the physiological responses became more apparent (22). It is important to note that not all behavioral compensatory responses to exercise are deliberate or intentional (26). In the current study, we are unsure whether children with higher RPE actively chose to consume more calories on both days, or whether this effect was passive. Further investigation into the psychological factors associated with post-exercise eating behavior would be extremely valuable.

This study represents a novel examination of individual differences in post-exercise energy compensation that may influence eating behaviors. Some strengths of the study are worth noting. In particular, the design included a controlled, individualized exercise bout to maintain 70% relative intensity, which allowed us to better distinguish physiological effort (e.g., cardiovascular responses) from perceived difficulty. In addition, we obtained objective measures of EI at multiple ad libitum test-meals, which limits misreporting biases associated with self-reported food intake (10). The study was designed to facilitate familiarity with the laboratory environment (research personnel, test-meals, and exercise testing) during a baseline visit to the Clinical Research Center, which reduced the novelty effect on the outcome variables of interest.
We also completed the study with 100% retention of enrolled participants, and full compliance with instructions and protocols across all three testing days.

Despite the strengths of the study, there were some limitations. We had a small sample size and we were underpowered to address secondary outcomes. It is important to note that the correlations between RPE and EI ($r = 0.82$, $r = 0.79$) were actually higher than the previously reported test-retest reliability of the instrument ($r = 0.78$) [21], so interpretations regarding this finding should be made with caution. The study design should be replicated before firm conclusions can be made on the effects of RPE and FFM on daily EI in children. In addition, the homogeneity of the sample limits generalizability of our results to other populations. We used a less sensitive measure of “at risk for becoming overweight” based on a single parental factor, and we did not assess additional genetic, family, or environmental influences on child weight status and risk for obesity. Future studies could include a more diverse sample of children characterized by a well-defined obesity risk phenotype. Finally, we did not measure additional homeostatic factors (e.g., gut peptides, inflammatory markers) that might also represent individual differences in appetite regulation.

Pending successful replication of the current findings, future studies looking to incorporate exercise as a strategy for maintaining energy balance may find it valuable to determine strategies to decrease perceived exertion during exercise. A recent study suggested that distraction using virtual reality during treadmill exercise was effective in increasing enjoyment of exercise (1). Other research has shown that listening to music during exercise increases enjoyment (15, 32). Finally, a study in adolescent girls demonstrated that RPE was significantly lower during a self-selected exercise session compared to a prescribed session of matched intensity ($72\% \text{ VO}_{2}\text{peak}$) (21). These are just three examples of many possible strategies (33) to examine in the future to determine whether increasing enjoyment or decreasing perceived exertion of exercise is effective in reducing the likelihood of a compensatory response in post-exercise food intake.

In conclusion, fat-free mass and perceived exertion represent individual-level factors that may contribute to short-term differences in EI and eating behavior, but additional research is needed to confirm these results. These preliminary findings may be useful in future intervention research looking to incorporate exercise.
Acknowledgements:

This project was supported by Agriculture and Food Research Initiative Grant #2011-67001-30117 from the USDA National Institute of Food and Agriculture, Program A2121. The project described was also supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1 TR000127. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. We would like to thank the Penn State Clinical and Translational Science Institute, including the Clinical Research Center and the Biostatistics, Epidemiology, and Research Design services. Finally, we would like to acknowledge the Metabolic Kitchen and Children’s Eating Behavior Laboratory at Penn State.
**Supplementary Table 5.1.** Laboratory test-meal menu. Food items, serving sizes, and calorie contents for each meal. Abbreviations: g, grams; fl oz., fluid ounces; kcal, kilocalories.

<table>
<thead>
<tr>
<th>Breakfast Food Item</th>
<th>Serving Size</th>
<th>Kcal per Serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>English muffin with</td>
<td>1 muffin + 1 tablespoon butter</td>
<td>151</td>
</tr>
<tr>
<td>butter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td>60 g</td>
<td>51</td>
</tr>
<tr>
<td>Orange juice</td>
<td>178 g (6 fl oz.)</td>
<td>83</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>285</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lunch Food Item</th>
<th>Serving Size</th>
<th>Kcal per Serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat bread</td>
<td>2 slices</td>
<td>170</td>
</tr>
<tr>
<td>Deli meat + Cheese</td>
<td>~105 g + ~45 g 30 g + 36g</td>
<td>100 + 150</td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td>190 + 60</td>
</tr>
<tr>
<td>Peanut butter + Jelly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretzels OR</td>
<td>42 g</td>
<td>165</td>
</tr>
<tr>
<td>Baked chips</td>
<td>39 g</td>
<td>165</td>
</tr>
<tr>
<td>Apple slices OR</td>
<td>102 g</td>
<td>53</td>
</tr>
<tr>
<td>Grapes</td>
<td>77g</td>
<td>53</td>
</tr>
<tr>
<td>Carrots OR</td>
<td>100 g</td>
<td>41</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>140 g</td>
<td>41</td>
</tr>
<tr>
<td>Ranch dip</td>
<td>30 g</td>
<td>140</td>
</tr>
<tr>
<td>Brownie bites (3)</td>
<td>43 g</td>
<td>188</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>997 – 1014</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Snack Food Item</th>
<th>Serving Size</th>
<th>Kcal per Serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate chip granola bar</td>
<td>1 bar</td>
<td>104</td>
</tr>
<tr>
<td>Mixed fruit cocktail</td>
<td>117 g</td>
<td>62</td>
</tr>
<tr>
<td>Apple juice</td>
<td>250 g (8 fl oz.)</td>
<td>136</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>302</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dinner Food Item</th>
<th>Serving Size (multiple servings available)</th>
<th>Kcal per Serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaroni &amp; cheese</td>
<td>400 g</td>
<td>551</td>
</tr>
<tr>
<td>Garlic bread</td>
<td>75 g</td>
<td>270</td>
</tr>
<tr>
<td>Broccoli with butter</td>
<td>120 g</td>
<td>53</td>
</tr>
<tr>
<td>Apple sauce</td>
<td>128 g</td>
<td>110</td>
</tr>
<tr>
<td>Cookies (3)</td>
<td>46 g</td>
<td>243</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>1,227</strong></td>
</tr>
</tbody>
</table>
References:


27. King NA, Hopkins M, Caudwell P, Stubbs RJ, Blundell JE. Individual variability following 12 weeks of supervised exercise: identification and characterization of


Chapter 6
GENERAL DISCUSSION

The overall goal of this dissertation was to examine the influences of individual differences in body composition and exercise on appetite regulation and food intake in children under the age of 12 years. Body composition and exercise are integral components of the Energy Balance Framework, as described by Blundell and colleagues [1], but there has been limited work on the integration of these systems in pre-adolescent children. In three of the four papers of this dissertation, the role of fat-free mass as an appetitive driver in children emerged as a common theme, consistent with previous work in adults [2-8]. There was limited evidence of an association between fat mass and food intake across the three cohorts of children studied. Exercise, compared to a sedentary control, was effective in reducing relative energy intake (i.e., energy intake adjusted for activity-related energy expenditure), but individual differences influenced these relationships. Children who rated the controlled bout of exercise as more difficult had greater ad libitum energy intake than children who rated it as easier. This finding demonstrates the potential for perceived exertion during exercise to influence food intake regulation. In addition to this cognitive factor, there may be individual differences in the brain’s reward response to food-related cues that could also play a role in ingestive behavior. Therefore, the work in this dissertation highlights the role of the brain in the determination of energy balance-related behaviors. Collectively, the findings from these studies provide support for the Energy Balance Framework [1] and extend these findings to pre-adolescent children.

The primary aim of Study 1 was to determine the association between children’s intake at a multi-item test-meal consisting of highly palatable foods and body composition, assessed by dual-energy x-ray absorptiometry (DXA). The highly palatable buffet test-meal was designed to elicit overconsumption, and did result in significantly greater intake compared to a baseline meal of familiar, moderately palatable foods. Eating behavior at the palatable buffet was used as a proxy for the tendency to overeat under free-living conditions, since it was positively correlated with measures of adiposity. In line with our primary hypothesis, we found that total and regional fat distribution (android and gynoid fat) were positively associated with energy intake at the palatable buffet. Specifically, these measures were positively associated with intake from the savory-fat category of food items offered. However, body fat was not associated with intake sweets or sweet-fats. Fat-free mass had an independent positive association with intake measures
in Study 1. However, the associations between body fat and intake measures remained significant after controlling for fat-free mass, suggesting that the relationship was unique to the amount of body fat and not driven by overall body size. It is not known whether greater energy intake was a cause or an effect of higher body fat based on the study design. But it is likely that this relationship is bidirectional in nature. The overconsumption of high-calorie foods contributes to positive energy balance and the storage of excess energy as fat [1, 2]. Since abdominal adipose tissue is known to be metabolically active, it is possible that signals from this tissue could contribute to an increased drive to eat [1, 2]. Interestingly, this relationship seemed to be specific to savory-fat foods, and not sweet or sweet-fat foods. Therefore, additional exploration into how children respond to different types of foods is warranted.

Based on the findings from Study 1, it was hypothesized in Study 2 that fat mass and fat-free mass may differentially relate to child brain responses to foods of differing energy content. The aim of Study 2 was to determine the association between children’s body composition and brain activation in response to images of foods that varied by energy density. This exploration looked to extend the literature on the associations between body composition and energy intake regulation by providing a potential neural mechanism underlying the effects previously seen in adolescents and adults [2-8]. We hypothesized that fat-free mass would be related to activation in areas of the brain previously implicated in energy homeostasis, since fat-free mass is a large determinant of resting metabolic rate and total energy expenditure. In addition, we hypothesized that fat mass would be associated with activation in reward areas of the brain, based on previous neuroimaging literature [9-12] and the associations between adiposity and intake of highly palatable foods in Study 1 [13]. We did find associations between body composition and brain activation, but not in the expected areas or directions that we initially expected. The primary finding was that fat-free mass was positively associated with activation in the substantia nigra in response to high energy density foods relative to low energy density foods (i.e., the High ED – Low ED contrast). The substantia nigra is involved in reward signaling, among other functions, and sends dopamine signals to other areas of the reward network such as the striatum [14]. This result supports previous literature that fat-free mass is an appetitive driver [2-8], and suggests that since children with greater lean mass have higher energy needs, they may find high energy density foods to be more rewarding. This finding provides evidence for a potential mechanism for the positive association between fat-free mass and food intake demonstrated previously [2-8].
Although none of the findings for fat mass survived correction for multiple testing, there was a negative association between fat mass and activation in the substantia nigra in response to low energy density foods. To speculate on these findings, it could be that children with greater levels of adiposity find healthier, low energy density foods to be less rewarding, but additional studies are needed to confirm these results. Overall, this study highlights the importance of considering compartmental body composition rather than just overall body weight to inform our mechanistic understanding of appetite regulation.

The second half of this dissertation examined the effects of exercise on short-term energy balance, while still carrying through themes from Studies 1 and 2, to further examine the integration of systems within the Energy Balance Framework [1]. Specifically, Study 3 examined the effects of an imposed bout of moderate-intensity exercise on food intake, activity-related energy expenditure, and energy balance in a within-subjects crossover study of 20 children at risk for becoming overweight based on parent weight status. The influence of the exercise bout was examined at both the group level (Study 3A) and the individual level (Study 3B). Therefore, the primary aim of Study 3A was to examine the effects of acute imposed exercise (30 minutes of 70% intensity cycling) versus imposed sedentariness on ad libitum daily energy intake and energy expenditure. While exercise did not significantly affect total energy intake, it was effective in reducing relative energy intake (a proxy for energy balance). This was a result of the fact that activity-related energy expenditure was greater on the exercise day compared to the sedentary day. Importantly, children’s activity-related energy expenditure during afternoon free-living time did not differ significantly as a result of imposed exercise. Therefore, structured exercise may be an effective strategy for better short-term energy balance regulation in children at risk for becoming overweight.

To follow up on the group level analyses, Study 3B examined individual differences in the response to the exercise bout. The aim of Study 3B was to understand individual level factors that may contribute to differences in daily energy intake after participation in 30 minutes of 70% intensity cycling exercise. In particular, we examined the associations between body composition (fat-free mass and fat mass), cardiovascular responses to exercise (e.g., heart rate), ratings of perceived exertion, and ad libitum energy intake across the experimental day. In line with the first hypothesis, fat-free mass was positively associated with daily energy intake. This study also confirmed the second hypothesis that total energy intake would be related to children’s perceived
difficulty of the controlled exercise bout. While one study had shown an association between perceived exertion and weight regain after successful weight loss [15], no previous studies have reported an association between perceived exertion and objective energy intake, either in the short- or long-term. Collectively, this study demonstrated a role for both homeostatic and cognitive factors in energy intake regulation in children.

**Highlighted Findings and Implications**

Across all three studies in this dissertation, the role for fat-free mass in the regulation of food intake was a common theme. The findings from this work are in line with previous studies in adolescents and adults that fat-free mass is an appetitive driver [2-8]. It is notable that the positive association was present between fat-free mass and both energy intake and the brain’s reward response to food-related cues. For simplicity, energy intake and the fMRI-measured brain response to food images will collectively be referred to as “appetite” in the following paragraphs. The positive association between fat-free mass and “appetite” was robust across three cohorts of children of different ages (ranging from 4-12 years), weight statuses (healthy weight, overweight, obesity), and demographics (i.e., New York City, NY and State College, PA). Sex did not moderate the relationships between body composition and “appetite” in any of the studies. However, we did not have sufficient power to explore the relationships between key study variables in males and females separately to determine whether the relationship between “appetite” and body composition differs by sex. Additional studies are needed to fully investigate these relationships, particularly as they may change with development. This is some of the first work to examine the relationships between fat-free mass and “appetite” in subjects under the age of 12 years, as the majority of the work thus far has been conducted in adolescents and adults [2-8]. This work provides an important extension to the Energy Balance Framework [1]. Children undergo a lot of physical growth, endocrine system maturation, and brain development during these years [16, 17]. One might hypothesize that the dynamic shifts in these systems related to growth and development could alter energy balance regulation systems. But based on the three studies in this dissertation, it seems as though at least parts of the appetite regulation system are functioning similarly to adults even at younger ages.

Along similar lines, past research has attributed positive associations between body weight and “appetite” to greater amounts of adiposity. However, several studies have shown that
fat-free mass is a stronger influence on appetite [2-8], and that fat mass has little additional explanatory value [7]. With greater amounts of body fat, greater muscle mass is needed to support the additional body weight. That accumulation of lean tissue then, theoretically, contributes to increased “appetite.” However, few studies have examined this effect. The independent contributions of fat-free mass and fat mass are seldom disentangled. This is specifically lacking within the context of the neuroimaging literature. Study 2 contains some of the first reports of differential associations for fat-free mass and fat mass with brain responses to food-related stimuli. It is also worth noting that these associations differed as a function of food energy density, as well. So previous studies on the associations between body weight and brain responses to a variety food images lumped together as a single category (e.g., Food versus Non-food) are likely missing complexities in the understanding of appetite regulation. This knowledge can guide future neuroimaging work on the effects of body weight on responsiveness to food, and vice versa.

We found some evidence for an influence of fat mass on food intake in Study 1. Findings from this work suggest that higher levels of adiposity, specifically android adiposity, are associated with greater intakes of savory-fat food items and greater total energy intake in situations designed to promote overeating. Some would argue that the current obesogenic environment is designed to promote overeating [18], with salty, high-fat snacks readily available. In addition, higher body fat may be associated with decreased reward responding to healthier, low energy density food items, as measured with functional neuroimaging. But overall, associations between fat mass and “appetite” were not replicated in Studies 2 or 3. This may be due to the fact that there was greater variability in body composition for the children in Study 1 compared to Studies 2 and 3, the samples for which were comprised of predominantly healthy weight children. The use of DXA in Study 1 provided a more accurate assessment of body composition compared to the bioelectrical impedance analysis measurements in Studies 2 and 3 [19], which could explain some of the discrepancies between studies. The children in Study 1 also differed significantly from Studies 2 and 3 on characteristics previously shown to impact dietary quality and general health, including socioeconomic status, race and ethnicity, and parental education [20]. In addition, the highly palatable buffet in Study 1 was designed to elicit overconsumption, while both the foods and food images used in the other studies had a greater range of palatability. Finally, a major methodological difference was that Study 3 examined
individual differences in energy intake across multiple meals rather than just a single meal. It is possible that children could adjust for overconsumption at one meal through self-regulation at later meals, maintaining energy balance over the longer term [21]. Additional longer term food intake studies would help to clarify this issue.

In regards to fat-free mass and obesity, there could be debate on the implications of recommending the maintenance of lean tissue during weight loss. The goal of some weight loss programs is to conserve fat-free mass, or muscle tissue, in the process of shedding excess body weight in order to maintain physical functionality. This is often achieved by some combination of a diet-induced calorie deficit and an aerobic and/or resistance exercise regimen. However, it’s possible that in the process of maintaining higher levels of lean tissue during an energy deficit, practitioners could be further exacerbating the increased drive to eat in weight loss patients. As an added layer to this argument, however, two studies have recently shown that individuals are less likely to compensate for an exercise-induced energy deficit compared to an isocaloric diet-induced energy deficit [22, 23]. Therefore, incorporating exercise could also be seen as beneficial for maintaining that energy deficit.

To further complicate the issue, exercise has been shown to have independent effects on appetite and subsequent food intake, above and beyond the effects on energy expenditure [1, 2]. A few recent meta-analyses have addressed this phenomenon [24-26]. In terms of the effects of acute exercise on subsequent energy intake, the available literature has shown a beneficial effect in the form of a reduction in total and relative energy intake after exercise compared to a control condition in individuals with obesity. However, there is generally no difference in subsequent energy intake for healthy weight individuals. Notably, acute exercise does not typically result in a compensatory increase in energy intake, regardless of weight status. But the effects of acute exercise on energy intake in those with increased risk of developing obesity have not been systematically studied. Study 3 aimed to extend this work to specifically look at healthy weight children at risk for becoming overweight based on parent weight status. Previous studies have shown that children with at least one overweight biological parent (father or mother) have a 3x greater odds of becoming overweight, and which may be partly attributable to differences in obesogenic behaviors (e.g., sedentary activity, television viewing, poor dietary intake patterns) [27, 28]. However, it’s unknown whether children in this risk category have an energy intake response to imposed exercise more like previously studied populations of healthy-weight
children or obese children. In the examination of the impact of exercise on energy intake and total energy balance, we found results consistent with other healthy-weight populations [26]. Exercise was effective in reducing relative energy intake compared to a sedentary control. This resulted from the fact that children consumed the same number of calories across both days, but had greater activity-related energy expenditure as a result of the exercise bout. A lower relative energy intake on the exercise day represents better short-term energy balance regulation with imposed exercise, which is beneficial for children who are at risk for becoming overweight based on parent weight status.

However, children varied substantially in their total daily energy intake. Seventy-eight percent of this variability was explained by just two factors: fat-free mass and ratings of perceived exertion. While one study has reported that perceived difficulty of exercise was associated with weight regain in previously overweight women, that study did not measure energy intake as a potential explanation for positive energy balance over the course of that year [15]. Study 3B demonstrated that perceived exertion was a strong predictor of total energy intake on the exercise day, but also on the other testing days and across each individual meal. Therefore, children who find exercise to be more difficult may have a tendency to consume more calories on a regular basis. The Rating of Perceived Exertion scale [29, 30] is a simple and practical tool to use in aerobic exercise settings. It would be feasible to use this tool to identify children who may have a negative response to exercise within the context a single, brief test session. From there, practitioners can explore ways to tailor the exercise experience and make it more enjoyable by monitoring changes in perceived exertion over time. More standardized use of this tool could potentially aid in adherence to physical activity guidelines and reductions in compensatory responses to exercise.

While acute moderate-to-vigorous intensity exercise relative to being sedentary was effective in maintaining better short-term energy balance in children at risk for becoming overweight, the effects of this intensity of exercise on energy balance over the longer term could not be examined within the constraints of the study design for Study 3. Previous studies have examined the effects of longer term exercise interventions on food intake and energy balance in children and adolescents who have already developed obesity. In a meta-analysis including only chronic exercise interventions greater than 4 weeks in duration, self-reported daily energy intakes were on average 323 kilocalories lower following the intervention compared to baseline
Half of the studies included in the meta-analysis reported a significant reduction in reported energy intakes, while the other half reported no significant effect of chronic exercise [21]. Similar to the available literature on acute exercise interventions, chronic exercise also does not generally lead to a compensatory increase in absolute energy intake in children and adolescents. There have been similar conclusions regarding the effects of both chronic and acute exercise on self-reported daily energy intakes in adults [31]. Although this has not been systematically studied in individuals at risk for becoming overweight (adults or children), the literature thus far is not supportive of a detrimental effect of exercise or physical activity on energy balance. The available research supports current recommendations for the participation in regular structured exercise for overall health [32].

The cumulative findings from this dissertation are summarized in Figure 6.1. Altogether, the research findings in this dissertation exemplify the importance of investigating individual differences when looking to understand complex systems such as energy balance regulation and the development of overweight and obesity. Understanding the mechanisms for why some children become overweight with time compared to those who are resistant will allow researchers to move away from the “one size fits all” approach and towards personalized, more effective prevention and treatment. Therefore, it is important to determine risk phenotypes and follow-up with tailored strategies for managing that risk.

The four papers in this dissertation are only a small step towards achieving personalized obesity prevention and treatment strategies. Awareness of the fact that lean body mass is a primary driver of energy intake will help practitioners and patients to better understand why fat loss doesn’t always result in reduced appetite. This might mean that, for some patients, additional behavior modifications may be necessary. Shifting attention away from highly palatable, high energy density foods (such as savory-fat items) with the substitution of more healthful, low energy density foods is already a prominent strategy used in weight management. But some individuals who tend to dislike low energy density foods, or find them less rewarding, may need more help integrating them into their daily routines. This could potentially be achieved through simple culinary training with a dietitian or chef, or educational internet tutorials, to make lower energy density foods more appealing while decreasing the overall calorie content of the diet. Regular exercise is also a feasible way to aid in weight management. Screening new participants for perceived difficulty of exercise, whether in schools, gyms, or worksite wellness
programs, can help teachers or trainers design and adapt activities to increase participation and adherence. The perceived exertion scale could also be integrated into popular mobile applications (e.g., Map My Run, My Fitness Pal), prompting users immediately after a workout to answer, “How hard did you feel like you were working?” and following up with personalized feedback. Maintaining a reasonable difficulty level during exercise could help prevent extreme compensatory responses, keeping patients on track to meet their goals. While obesity is, and always will be, a multifaceted disease, these are a few practical applications of the findings from this dissertation, which may help improve prevention and treatment.

Figure 6.1. Summary of the main findings for energy intake, expenditure, and balance. We found positive associations between fat-free mass and either energy intake or the brain’s reward response to food, with more limited evidence of an association between fat mass and energy intake. Imposed exercise was associated with an increase in daily energy expenditure relative to being sedentary. The perceived difficulty of the controlled exercise bout was positively associated with subsequent energy intake, independent of heart rate and fitness level. However, there is a need to further assess the impact of cognitive perceptions of food and exercise on energy intake behaviors.

Strengths and Limitations

There are notable strengths to the work in this dissertation. Across all three studies, the use of highly controlled, objective, laboratory-based measures of ingestive behavior, body composition, brain activation, and exercise test performance allowed for the collection of high quality data. Laboratory-based measures have previously been shown to be valid and reliable assessments of energy and macronutrient intake in children and adolescents [33, 34]. Bioelectrical impedance analysis, while not the gold standard for body composition, has been
shown to be an accurate tool for assessment of fat and fat-free mass in children within the age and body mass index ranges tested, and was previously validated against measures such as DXA [35, 36]. This data allowed for novel applications of the Energy Balance Framework [1] to the understanding of food intake regulation in children. In particular, this work expanded on the role of the brain in the integration of these pathways, including children’s perceptions of food-related stimuli as well as exercise participation. The studies also demonstrated feasibility for using sophisticated measurement tools with children under the ages of 12 years, including both fMRI and DXA. With appropriate training, the large majority of children were successful in completing all study-related tasks.

Several limitations should also be addressed. As noted above, all three studies in this dissertation found associations between fat-free mass and measures of food intake or brain responsiveness to food-related cues. However, one piece missing from the three studies is the measurement of resting metabolic rate. Recent studies in adults have suggested that the association between fat-free mass and “appetite” is mediated almost entirely by resting metabolic rate, since the amount of lean body tissue is a large determinant of daily metabolic needs [2, 4, 7]. Future studies in children should include resting metabolic rate measurement to determine whether the effect of fat-free mass on “appetite” is mediated by this variable, confirming the same pathway determined in adults. In addition, associations between resting metabolic rate and brain activation to food stimuli would provide valuable insight into homeostatic and reward influences on food intake, as these two systems likely work in concert to drive eating behavior [14]. Another missing piece is the measurement of gut-peptides and other hormonal or inflammatory signals known to influence appetite [1, 2]. Collecting a more complete metabolic profile could also clarify some of the relationships reported in these studies. However, the biggest limitation of this work is that all three studies were cross-sectional and short-term. Any associations between body composition and the fMRI brain response to food, body composition and food intake, or perceived exertion and food intake are purely correlational. Therefore, the extent to which the observed relationships would be extended to free living situations is unknown.
Future Directions

The most crucial next steps for extending the work in this dissertation would be longitudinal studies of pediatric energy balance regulation. These studies are particularly difficult to conduct for multiple reasons. First, children and adolescents are growing, which translates into a need for positive energy balance, particularly during periods of rapid growth (i.e., “growth spurts”). Age- and sex-specific longitudinal growth curves for body mass index are available to track overall growth trajectories from ages 2-19 years. However, these types of longitudinal curves are not as accessible for more specific compartments of body composition, such as fat-free mass and fat mass. One accepted means of expressing these variables are as indices (i.e., fat-free mass index, fat mass index) in kilograms over meters squared (similar to body mass index), in order to control for differences in height. However, growth curves and percentiles for these variables have not been firmly established for children and adolescents [37]. Therefore, tracking meaningful changes in these variables over periods of growth would be difficult in longitudinal studies of energy balance. In addition to physical growth, children and adolescents are undergoing endocrine system maturation and puberty, which can have significant effects on hormone levels, body composition, and brain development. All of these factors must be taken into account for studies of energy balance.

A second barrier to the assessment of long term energy balance is the limited availability of research methods to objectively assess energy expenditure and energy intake. The objective measurement of energy intake has been a point of contention in the scientific community [38-42], but advances in technology are attempting to shift the focus away from self-reported measures of food intake which are often subject to bias [40, 42]. The studies in this dissertation predominantly focused on well-controlled, objective, laboratory-based energy intake. However, this may not be representative of eating behavior under free-living conditions. A combination of methods may be the best means to address this issue, including both objective and self-reported measures of energy intake. In addition, it would be beneficial to assess intake patterns in the laboratory but also in a free-living environment, possibly by adapting controlled feeding protocols for ad libitum assessment. Study 3 demonstrated the feasibility of balanced, well-controlled data collection methods that incorporated both in-laboratory and free-living measurement periods. Integrating these methods into the context of a longitudinal study or intervention (e.g., at baseline and follow-up time points) could be optimal for understanding
changes in eating behavior over time. In regards to energy expenditure, vast improvements have been made in accelerometer technology over recent years. But there are still limited resources available for the accurate estimation of energy expenditure from accelerometry data in populations under the age of 18 years. In addition, more accurate methods such as indirect calorimetry or doubly-labeled water are expensive and not feasible for use over the longer term or in large subject populations. Additional work in this area will improve the quantification of exercise in free-living settings.

This work identified individual differences in the response to exercise that may inform future studies. To date, studies have shown that substrate (i.e., macronutrient) oxidation, relative exercise intensity, dietary disinhibition, subjective hunger, and now perceived exertion can all influence post-exercise energy intake [43-46]. Pending successful replication of the findings from Study 3B, future studies looking to incorporate exercise as a strategy for maintaining energy balance may find it valuable to determine ways to decrease perceived exertion during exercise. As noted in Chapter 5, previous studies have used music, virtual reality, non-weight-bearing exercises, or self-selected exercises to decrease perceived exertion [47-51]. One possible extension of this work would be to develop a preference task (e.g., forced choice paradigm) with multiple common modes of exercise that are about the same relative exercise intensity. The most preferred exercise mode could be compared to a non-preferred exercise to determine if: 1) the preferred exercise is perceived as less difficult (i.e., lower perceived exertion); 2) children enjoy the preferred exercise more (i.e., a better affective response); and 3) children consume fewer calories following the preferred exercise. The results would inform the literature on exercise compensation, and provide a potential avenue for intervention. Learning effective ways to decrease perceived exertion could aid in adherence to participation in exercise on a regular basis, improving energy balance over the longer term.

Finally, one promising possibility for tracking change in energy balance regulation over time would be the repeated measurement of neural responses to food- and exercise-related stimuli within a longitudinal cohort of children. Studies of this design would provide insight into the underlying mechanisms for changes in energy balance, as the brain is the ultimate driver for all behaviors. Neuroimaging data on the responsiveness to both food and physical activity may also help to make sense of energy intake and energy expenditure data collected by more conventional means. It may also shed light on physiological changes that occur over time,
including fluctuations in metabolic and endocrine signaling pathways, as well as alterations in body composition. This is an area of research that is constantly and rapidly evolving with advances in technology, and therefore holds promise to help uncover some of the most basic mechanisms of behaviors that have health-related consequences.

Conclusions

In sum, the work presented in this dissertation is a compilation of some of the first data on the integration of the systems within the Energy Balance Framework [1] in children under the age of 12 years. These three cross-sectional studies represent a few steps towards understanding the influences of body composition and exercise on food intake regulation in pre-adolescent children. Additional longitudinal studies in these areas will aid in the understanding of the reciprocal interactions between energy expenditure, energy intake, and body composition over time. These studies may also inform the development of more effective and sustainable tailored weight management interventions.
References


APPENDIX A:

Telephone Screening Questionnaires

Study 2 & Study 3
fMRI PORTION SIZE SUBJECT RECRUITMENT FORM

Parent’s Name: ________________________________________________

Phone: (home) __________________  (work/cell) ____________________

Address:______________________________________________________

Email: _______________________________________________________

Date Call Received: ____________  Date Call Returned: ___________

What is your relationship to the child? _________________________

Child’s Sex:          boy         girl

Child’s Name ____________________________  Age: _______ (if under 7, not eligible, ask permission to record name in database and call back in a few months if child is almost 7)

Child’s DOB: _________     Height:_______     Weight:_______

1. Is your child right-handed?        YES       NO

2. Is your child on any prescription medications?          *YES     NO
   a. If yes, please specify what medications:
       b. How often does your child take this medication?
       c. Please name any over the counter medications your child takes on a regular basis.

3. Does your child have any medical problems?        *YES      NO
   a. If yes, please specify:
4. Does your child have any food allergies?  
   a. If yes, please specify:  
   *YES  NO

5. We will be providing a snack of apple juice and a granola bar to your child.  
   Will your child eat these foods?  
   YES  *NO

6. Is your child reading at or above grade level?  
   YES  NO

7. Does your child have any learning disabilities?  
   YES  NO

8. Does anyone in your child’s immediate family (parents, siblings) have a diagnosed psychiatric illness, such as depression, anxiety, or bipolar disorder?  
   YES  NO

9. Is your child red/green color blind?  
   YES  NO

10. Has your child ever had an MRI before?  
    YES  NO

11. Is English your child’s native language?  
    YES  NO

12. Does your child have any metal in or on his or her body that cannot be removed (like a metal plate or pin, or dental work which may contain metal)?  
    a. If yes, please specify:  
    *YES  NO

13. Does your child have any medical devices that may contain metallic parts (like an insulin pump or pacemaker)?  
    a. If yes, please specify:  
    *YES  NO

14. Has your child ever had an injury to the eye involving a metallic object or fragment?  
    *YES  NO

15. Does your child have any body piercings?  
    a. If yes, would he or she be willing to remove them?  
    YES  *NO

16. Is your child comfortable in small spaces?  
    YES  *NO
Subject is ineligible if any bolded responses are selected. For the starred responses, check with Kathleen and/or Susan Lemieux (SLEIC) to discuss eligibility. If any starred responses are selected, politely inform them you will get back to them shortly with information regarding eligibility.

Eligibility: YES  NO

May we keep your information to contact you for further studies? YES  NO

Comments:

First visit scheduled:  Date: _______________   Time: ______________

Remind Caller: A Parent or Legal Guardian MUST be at the first visit to sign consent forms.
ENERGY BALANCE - SUBJECT RECRUITMENT FORM

Date Call Received: ___________   Date Call Returned: ___________   Screened by: ___________

Parent’s Name: ______________________________________________________________

Phone: (cell) __________________  (work/home) __________________

Does your cell phone receive text messages? YES *NO

Address:______________________________________________________

Email: _______________________________________________________

Preferred Contact:  ☐ Phone       ☐ E-mail

Child’s Sex:   boy       girl

Child’s Name ____________________________   Age: _______
(if under 9, not eligible, ask permission to record name in database and call back in a few months if child is almost 9)

Child’s DOB: __________   Height:_______   Weight:_______

Child’s BMI %ile: ________  ☐ Normal weight  ☐ Overweight/obese

Mother’s DOB: __________   Height:_______   Weight:_______

Mother’s BMI: __________  ☐ Normal weight  ☐ Overweight/obese

Father’s DOB: __________   Height:_______   Weight:_______

Father’s BMI: __________  ☐ Normal weight  ☐ Overweight/obese

1. Are you [child name]’s biological parent? YES   NO

2. Is English your child’s native language? YES   NO

3. Is your child reading at or above grade level? YES   NO

4. Does your child have any learning disabilities? YES   NO
5. Does your child have a diagnosed psychiatric condition, such as ADD, ADHD, depression, anxiety, or bipolar disorder?  
   YES  NO

6. Is your child on any prescription medications?  
   YES  NO
   a. If yes, please specify medications & purposes:

   b. How often does your child take this medication?

   c. Please name any over the counter medications your child takes on a regular basis.

7. Does your child have any medical conditions?  
   YES  NO
   a. If yes, please specify:

8. Does your child have any chronic conditions such as diabetes, thyroid, or rheumatoid disorders?  
   YES  NO

9. Please say “yes” or “no” to indicate whether your child currently has or has previously had any of the following medical conditions:

   Cancer  YES  NO
   Renal failure  YES  NO
   PKU deficiency  YES  NO
   Acute systemic infection  YES  NO
   HIV / AIDS  YES  NO
   Mononucleosis  YES  NO
   Hepatitis  YES  NO

10. Please say “yes” or “no” to indicate whether your child currently has or has previously had any of the following heart conditions:

    Recent myocardial event  YES  NO
    Unstable angina  YES  NO
Uncontrolled cardiac dysrhythmias, tachydysrhythmia, or bradydysrhythmia

Cardiomyopathy

Myocarditis or pericarditis

Aneurysm

Pulmonary embolus or pulmonary infarction

11. Does your child have asthma? *YES NO
   a. If yes, do they use an inhaler? YES NO

12. Does your child have any other condition that may affect their ability to complete 30 minutes of exercise? *YES NO
   a. If yes, please specify:

13. Does your child participate in regular exercise or physical activity? YES NO
   a. If so, how often?

14. Does your child participate in any competitive sports (i.e. travel soccer)? YES NO
   a. If so, please specify:

15. Does your child have any food allergies? *YES NO
   a. If yes, please specify:

16. Does your child have any dietary restrictions? *YES NO
   a. If yes, please specify:

17. Does your child normally eat breakfast? YES *NO

18. We will be providing a snack of a juice box, a fruit cup, and a granola bar to your child. Will your child eat these foods? YES *NO
19. I am going to list several of the other foods we will serve during this study. Please just say yes or no, whether your child will eat these foods.

<table>
<thead>
<tr>
<th>Food</th>
<th>YES</th>
<th>*NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. English muffin</td>
<td>YES</td>
<td>*NO</td>
</tr>
<tr>
<td>b. Banana</td>
<td>YES</td>
<td>*NO</td>
</tr>
<tr>
<td>c. Orange juice</td>
<td>YES</td>
<td>*NO</td>
</tr>
<tr>
<td>d. Peanut butter &amp; jelly sandwich</td>
<td>YES</td>
<td>*NO</td>
</tr>
<tr>
<td>e. Deli meat &amp; cheese sandwich</td>
<td>YES</td>
<td>*NO</td>
</tr>
<tr>
<td>f. Macaroni &amp; cheese</td>
<td>YES</td>
<td>*NO</td>
</tr>
</tbody>
</table>

20. How did you hear about our study?

21. May we keep your information to contact you for future studies? *YES*  *NO*

Polite[y inform them that you will review their screening form get back to them shortly with information regarding eligibility and scheduling.

Subject is ineligible if any **bolded** responses are selected. For the **starred** responses, check with Nicole and/or Kathleen to discuss eligibility.

Eligibility: *YES*  *NO*

Comments:

First visit scheduled: Date: ___________________  Time: _______________

*Remind Caller: A Parent **MUST** be at the first visit to sign consent forms.*
APPENDIX B:

Consent Forms

Study 2 & Study 3
Title of Project: Neural mechanisms underlying children’s responses to food portion size and energy density.

Principal Investigator: Kathleen Keller, Assistant Professor
Department of Nutritional Sciences and
Department of Food Sciences
110 Chandlee, University Park PA, 16802
814-863-2915, klk37@psu.edu

Please print your name here: ___________________________ so that the person in charge of the research will know that you have had a chance to read the information below. This form may contain words you do not understand. Please ask the researcher to explain any words or information you do not clearly understand.

Please read every page carefully and initial the bottom of each page when you have had all of your questions answered to your satisfaction.

Purpose of the study:

Our study asks “How do children react to viewing images of different foods?” We know that some children are more likely than others to eat more or less of certain foods. We do not know why, but we are studying pictures of children’s brains when they see foods to find out. This study will help us learn more about how the brain works to control what we eat, and this may help us understand how to help children eat a more nutritious diet.

This study will use questionnaires, eating behavior tests, a fitness test, and Magnetic Resonance Imaging (MRI) to take pictures of your child’s brain. MRI is a tool that lets us look at how your child’s brain works. In the MRI scanner your child will look at pictures of foods, as well as other things, like furniture and office equipment.

The MRI scans will assist us in understanding the structure and function of different parts of the body. In this research study, magnetic resonance imaging (MRI) scans of your child will be taken. There are two types of scans that may be done. Anatomy scans are used to image the structure of the body. Scans of function are used to image areas of activity when your child is resting or performing different tasks. In this study we will only be scanning the brain.

NONE of the scans done during this study are designed to detect or evaluate any medical condition your child may have. They are intended solely for research purposes.

Procedures to be followed:

Visits 1-4: You and your child will attend five visits at Chandlee Laboratory. We ask your child to fast for two hours prior to your arrival for each visit. Visits 1-4 will be done at the Children’s
Eating Behavior Laboratory in Chandlee. On the first visit, you will complete the consent form, and both you are your child will do some questionnaires and get height and weight measured. Your child will also eat a meal of common foods that we make in our kitchen. The meal will last for 30 minutes and your child can eat as much or little as they want. On visits 2-4, your child will again eat meals with similar foods.

Visit 5 will take place at the Social, Life, and Engineering Imaging Center in Chandlee for the fMRI scan. We will provide a snack for your child to eat if he or she is hungry. In order to help your child better understand the MRI, we will tell them that they will be helping us explore “Nittany Lion Inner Space.” Inner space is the space inside of your child’s head. Inner space scientists use a special machine—a scanner—to take pictures of the brain’s inner space.

The MRI facility at Penn State has a mock scanner, which is like the real MRI Scanner, only without the magnet. Before going into the MRI room, we will ask your child to lie in the mock scanner in order to see if he or she is comfortable and able to be still. It is very important that your child does not move when we are scanning so that we can get the best pictures of your child’s brain. In the mock scanner we will also show your child some sample pictures and explain to them how to signal to “Mission Control” so they can talk with researchers and/or you if they need to. We will also introduce your child to the sounds that he or she will hear in the scanner. Through all these procedures, our goal is to make your child feel very comfortable while in the MRI scanner.

To date, 150 million MRI studies have been performed around the world. We will be following standard MRI procedures and safety guidelines. MRI has been shown to be extremely safe as long as proper safety precautions are taken. MRI uses strong magnetic fields and radio waves to make pictures of the body. There is no exposure to x-rays or radioactivity during an MRI scan. Levels of energy used are within safety limits established by the U.S. Food & Drug Administration (FDA). This study will use a 3.0 Tesla MRI scanner.

You and your child will be asked to leave metal objects and personal belongings in lockers provided in the prep room of the MRI center. Articles of clothing with metal inserts or clasps must be removed before entering the MRI room. Please ask us if you are unsure about any items.

Next, we will ask your child to complete a set of simple vision screening tests in order to fit your child with special glasses that are safe to use in the scanner. The glasses will partially correct your child’s vision so that he/she can see things we will display in the scanner. If your child wears contacts or has normal vision the special glasses will not be needed.

Your child will be asked to lie on a bed that slides into the long tube of the scanner. Your child will be given earphones and/or earplugs for hearing protection since the MRI scanner makes loud noises during normal operation. Your child will be asked to remain very still at these times. For scans of the head, we may put cushions around your child’s head and we may lightly tape the head to help keep it from moving. Your child will be able to talk to the MRI technologist by an intercom, and you and the technologist will be able to see and hear your child at all times. Your child will also be given a squeeze-ball signaling device. If at any time your child would like to
discontinue the study, he/she can tell the investigators over the intercom or press the squeeze-ball signaling device to be removed immediately from the scanner. You or your child can choose to discontinue the study at any time without penalty.

Following the MRI scan, your child will be asked to look at some pictures of foods and objects like furniture and tell us how much they like and want these items. This test will be done in our Children’s Eating Behavior Laboratory. At the end of the visit, we will ask your child to complete a fitness assessment in the form of a shuttle run. The shuttle run is a very short fitness test, lasting only 10-30 seconds. It is commonly used in physical education classes for children as young as 6 years old, and it is included in the Presidential Physical Fitness assessment. We will fully explain the test to your child so that he or she is familiar with how it works.

For one week during the study, we will ask your child to wear a special device that will keep track of how much he or she moves throughout the day. We would like your child to wear this device every day for a week. During this time, we will also ask that you record how long your child sleeps each night. After your child is done wearing this device, we will ask that you return the activity device and sleep records to our researchers.

**Discomforts and risks:**

Risk of injury is very low during an MRI scan. However, MRI is not safe for everyone. It may not be safe for your child to have an MRI scan if there is any metal containing iron in or on your child's body. This is because metal containing iron can pose a safety risk when in the presence of strong magnetic fields. Radio waves may also heat the body and metallic objects within or on the body, possibly resulting in burns. Before you or your child is allowed in the scanner room, you will be asked a set of questions to determine if it is safe for your child to have an MRI scan at this time. You will also be asked to answer the questions to determine if it is safe for you to enter the scanner room with your child. For you and your child’s safety, it is very important that you answer all questions truthfully.

It is possible that your child may feel uncomfortable or confined once inside the scanner. This feeling usually passes within a few minutes after the study begins. It is possible that your child might experience dizziness, mild nausea, or see tiny flashing lights. These sensations are mostly due to movement while inside the magnet and can be minimized by holding still. All of these sensations should stop shortly after your child leaves the magnet.

The foods used in the meals are all common foods made with ingredients you would find at the market. We make them fresh each day using safe food preparation protocols. However, there is always a chance of food borne illness or of uncovering an allergy in your child due to food exposure.

With the shuttle run test, there is a slight risk that your child will trip and fall. To minimize this risk, we will conduct the test on a level surface, free of obstacles. In addition, we also ask that your child complete the test with his or her maximal effort. This short burst of exercise may leave your child feeling slightly tired or short of breath, but these feelings will pass quickly.
Benefits:

You or your child may enjoy the activities during the study, or feel it is good to contribute to a scientific study. Furthermore, this study may also benefit the community. That is because we know very little about how a child’s brain processes pictures of different foods. Now that we can take pictures of the brain, there is much we can learn from taking images while children look at pictures of food, and comparing it to the foods they eat. What we learn in this study will help future studies of children and help us come up with ways to help children to eat well.

Duration/time of the procedures and study:

This study involves five total visits for you and your child. The first visit will last no more than 2.5 hours from start to finish. You will have up to two hours to complete the questionnaires we give you. Visits 2-4 will last no more than 1 hour. Visit 5 will last no more than 1.5 hours and your child will be asked to lie in the scanner for no more than 25 minutes.

Statement of confidentiality:

Your child’s participation in this research is confidential. All possible steps have been taken to assure your child’s privacy. For the MRI, your child will be assigned a code number that will be used throughout the scan. Only this code (and never your child's name) will be used when analyzing or reporting the data. Any identifying information will be kept in a locked location and password protected electronic files.

This consent and any other identifying information will be kept in a locked file in Dr. Keller’s locked office. All questionnaires that you and your child complete will be identified only by your child’s code and stored separate from any identifying information. Only the PI (Dr. Keller) and study coordinators will have access to your identifying information. All other project staff who are approved by the Penn State IRB will only have access to data files without your name.

Penn State’s Office for Research Protections, the Institutional Review Board, and the Office for Human Research Protections may review records related to this research study.

In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared. The results of the research, including but not limited to your child’s images, may be published and presented at lectures and professional meetings, but your child will not be identified in any such publication or presentation.

Right to ask questions:

Please contact Kathleen Keller at 814-863-2915 with any question, concern or comment about the research. You can also call this number if you feel this study has harmed you or your child in any way. If you have any questions, concerns, or comments about you or your child’s rights as research participants or would like to offer input, please contact Penn State University’s Office for Research Protections (ORP) at (814) 865-1775. The ORP cannot answer questions about
research procedures. All questions about research procedures can be answered by Dr. Keller and the research team.

**Payment for participation:**

The total compensation possible for this study is $160. For visit one, this includes a $30 reimbursement for parent’s time, and $10 for your child. For visits two – five, this includes $30 per visit ($20 reimbursement for parents and $10 for children). If you or your child withdraws before completion of the visit, you will receive compensation for the time you completed in the study, based on a rate of $10 per hour. If we have to release you from the study because you or your child is unable to comply, you will also receive travel costs plus $10 per hour completed. Regardless of whether you complete the study or not, your child will still receive $10.

**Voluntary participation:**

Participation in this research is voluntary. You or your child can choose to stop at any time. To participate in any MRI study, you must answer all Participant Safety and Screening questions accurately for both yourself and your child. However, you do not have to answer any other questions that you do not want to answer. Refusal to take part in or withdrawing from this study will involve no penalty or loss of benefits you would otherwise receive.

Because it is important that your child lies still while being scanned, if your child is unable to lie still in the mock scanner, we will ask that you end the study and you will be compensated for your time.

**Injury Clause:**

In the unlikely event that you or your child is injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

**Incidental findings:**

The investigators for this project are not trained to perform medical diagnosis, and the scans to be performed in the study are not optimized to find abnormalities. On occasion, a member of the research team may notice a finding on a scan that seems abnormal. When a finding is noticed, the investigator or designate may consult a physician specialist, such as a radiologist or neurologist, as to whether the finding merits further investigation. If the specialist recommends further follow-up, the investigator or another designate will contact you within 48 hours of the recommendation and suggest that you contact your private medical provider for follow-up. To facilitate follow-up care, you may be given a copy of your child’s images upon request. Being told about a finding may cause anxiety as well as suggest the need for additional tests and financial costs. Medical insurance may be affected whether or not the finding is ultimately proved to be of clinical significance. Costs for clinical follow-up are not covered in the cost of
research. The decision as to whether to proceed with further examination or treatment lies with you.

**Abnormal test results:**

Please provide contact information so that you can be reached in the event of an incidental finding and/or abnormal test results. You will be notified within 48 hours of an incidental or abnormal finding that is determined to need further investigation. This includes the MRI scan and the language test.

Address__________________________________________________________

Phone____________________________________________________________

**Consent**

By consenting to participate, you agree to:

- Answer the SLEIC 3T MRI Participant Safety & Screening questions accurately for both yourself and your child,
- Tell the investigators about all metallic devices in/on you and your child’s body, and
- Not bring any metal devices (e.g., pens, coins, keys, credit cards) into the scanning room without staff approval.
- Answer questionnaires we provide for you that relate to your household, your child’s physical activity and food behavior habits, your parenting styles, and methods you use, or have used in the past, to feed your child. If you feel uncomfortable with any item on a questionnaire, you may leave it unanswered.

If you agree to take part in this research study and the information outlined above, please sign your name and indicate the date below.

You will be given a copy of this signed and dated consent form for your records.

I consent to participate in this study and to have my child participate in this research study.

<table>
<thead>
<tr>
<th>Printed Name of Child</th>
<th>Date of Birth</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parent’s Printed Name</th>
<th>Parent’s Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Printed Name of Person Obtaining Consent</th>
<th>Signature of Person Obtaining Consent</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
This is a research study that you may choose to join and help. Instead of studying planets and outer space, we want to study Inner Space. Inner Space is in between your ears, inside your head: your brain! We want to know what goes on in kids’ brains when they look at pictures of foods.

We ask that you do not eat anything for two hours before you come to each visit. Today, we will have you eat a meal of common foods. When you come back for visits 2, 3, and 4, you will eat a similar meal. You can eat as much or as little as you like, and you don’t have to eat anything you don’t like.

Today we will also ask you to answer some questions on a computer. You don’t have to answer any questions that make you feel uncomfortable. This may be something that you have done before at school. It will be a short test, and we ask that you try your hardest on it.

For one week during the study, we are going to ask you to wear a little machine that keeps track of how you move during the day. We ask that you wear it every day for a whole week, and then bring it back.

On your last visit, we will use a special camera called an MRI to take pictures of your brain. It’s special because you lie on a bed inside the camera to take the pictures. The MRI is a big tube that kind of looks like a spaceship. It is safe and has been used by many doctors to help see inside your brain.

We take pictures of your brain at Nittany Lion Inner Space Station. We will first ask you to lie in a “pretend” or Mock Scanner. This is so you can feel what the MRI Scanner, or spaceship, is like. You will lie on a comfy bed that moves into the inner spaceship. While you are in the spaceship we will ask you to look at pictures on a screen above you.
In order to get the best pictures of your brain, we will ask you to lie very still. Just like a regular camera, the picture will be fuzzy if you move a lot in the spaceship. Also, like a real spaceship the MRI makes loud sounds. You will wear our special headphones so you can hear us in Mission Control. If you want to stop, you just talk to Mission Control, and we will stop. If you do not want to talk, you can squeeze our special space ball.

We want you to have fun exploring inner space. Any time you have a question, please ask us. We can tell you about the inner spaceship and the pictures it takes.

You choose if you want to help with our Inner Space Mission. If you feel like you want to stop during the study, just let us know. Does this all make sense to you? If not, we can go over it again.

Before beginning the study, the research personnel administering assent should check the appropriate box below after reading the Assent form to the child and answering any questions they have.

- Obtained
- Not obtained because the capability of the child is so limited that the child cannot reasonably be consulted.
CONSENT FOR RESEARCH
The Pennsylvania State University

Title of Project: The impact of an acute bout of exercise on children's eating behaviors.

Principal Investigator: S. Nicole Fearnbach, PhD Candidate

Address: Department of Nutritional Sciences
110 Chandlee Laboratory, University Park, PA 16802
snf129@psu.edu

Telephone Number: (814) 865 4404

Advisor: Kathleen L. Keller, PhD

Advisor Telephone Number: (814) 863-2915

Subject’s Printed Name: _____________________________

We are asking you to be in a research study. This form gives you information about the research.

Whether or not you take part is up to you. You can choose not to take part. You can agree to take part and later change your mind. Your decision will not be held against you.

Please ask questions about anything that is unclear to you and take your time to make your choice.

Some of the people who are eligible to take part in this research study may not be able to give consent because they are less than 18 years of age (a minor). Instead we will ask their parent(s)/guardian(s) to give permission for their participation in the study, and we may ask them to agree (give assent) to take part. Throughout the consent form, “you” always refers to the person who takes part in the research study.

1. Why is this research study being done?

We are asking you to be in this research because your child is within the ages of 9-12 years, a group which has not often been included in nutrition and exercise research studies. This research is being done to find out how children in this age range balance their energy. This study will use questionnaires, eating behavior tests, an activity tracker, a fitness test, and an exercise test to answer this question.

Approximately 25 people will take part in this research study in the State College, PA area.
2. What will happen in this research study?

Visit 1: You and your child will attend three visits on the Penn State University Park Campus. We ask your child to fast overnight prior to your arrival for each visit, and only have water in the morning. All of the visits will take place at the Clinical Research Center in Noll Lab and the Children’s Eating Behavior Laboratory in Chandlee Lab. On the first visit, you will arrive at the Clinical Research Center to complete the consent form. You and your child will have your heights and weights measured, and we will then provide your child with a breakfast. Your child will have 30 minutes to eat all the foods provided at this meal. We will ask your child to rate their fullness and how much they like the foods served at this meal. Both you and your child will also fill out some questionnaires on a computer during this visit.

Your child will then complete the fitness test at the Clinical Research Center. This test will take place on a stationary cycle under the supervision of a certified trainer and a registered nurse or physician. We will measure your child’s heart rate and blood pressure at the beginning and end of the test and will continue to monitor his or her heart rate throughout the fitness test. The test will start with a 5-minute warm-up, and then we will ask your child to maintain a constant pedal rate for the duration of the test. They will cycle at an approximate speed of 11 miles per hour, which is a pretty relaxed pace. We will encourage and remind your child throughout the test to try their best.

Every few minutes, we will ask your child to rate his or her perceived exertion on a scale that is explained to them prior to beginning the test. We will also increase the resistance on the bike every few minutes until the end of the test. The duration of the test will vary for each child, but should take no longer than 20 minutes. Your child will then have a 5-minute cool down. The whole session (warm-up, exercise, and cool-down) will take approximately 30 minutes.

Your child will then be provided a lunch to eat at the Clinical Research Center. Your child will be allowed to select their lunch from a variety of common foods prior to arrival, and will be given this same meal on Visits 2 & 3. Your child can eat as much or as little as he or she would like from this meal, and will have 30 minutes to eat. We will ask your child to rate their fullness and how much they like the foods served at this meal.

Visits 2 & 3: Again, we ask your child to fast overnight prior to your arrival for each visit, and only have water in the morning. Upon your arrival to the Clinical Research Center, we will give your child two special activity tracker devices to track how he or she moves throughout the day. One will be worn on the wrist and the other on the ankle.

Visits 2 & 3 will consist of either an exercise test or a rest day, completed in a random order. Otherwise, the visit procedures will be identical. At the beginning of the day, we will provide your child with breakfast (English muffin, fruit, and juice). He/she will have 30 minutes to eat all the breakfast items. We will ask your child to rate their fullness and how much they like the foods served at this meal. Between breakfast and the test condition (exercise or rest) we will have a TV and DVD player, books, games, and other
activities to keep your child occupied.

Two hours after breakfast, we will begin the test condition. For the rest day, your child will be asked to remain seated for 30 minutes with access to the same activities. The exercise test will occur in a separate room within the Clinical Research Center in Noll Lab. The exercise test will take place on the same stationary cycle as the fitness test. Your child will be given a 5-minute warm-up period, and then will be asked again to cycle at a speed of approximately 11 mph for 30 minutes. We will positively encourage and remind your child throughout the test to try their best. We will monitor his or her heart rate and ask for ratings of perceived exertion throughout the test. If your child’s pedal rate falls below the required range for more than 2 minutes, we will ask him or her to stop. Your child will then be given a 5-minute cool-down period. The entire exercise test will last approximately 45 minutes.

Thirty minutes after the end of the exercise test, your child will then be provided a lunch to eat at the Clinical Research Center. Your child can eat as much or as little as he or she would like from this meal, and will have 30 minutes to eat.

Your child will be given a snack to take with you for the afternoon on Visit Days 2 & 3. We will give you instructions on when to provide this snack to your child, and also send you reminders via text message. We will ask that you respond to these text messages and confirm that you have received them. Your child can eat as much or as little as he or she would like from these foods. We will ask you to return any remaining food items (wrappers, packages, baggies, and any uneaten food) to the researchers. We strongly prefer that you do not provide any additional food items to your child during this time, other than water. We also ask that your child not eat anything for the 2 hours prior to your return for the dinner test meal. With your instructions will be a notes sheet where you can write the timing of the snack, and report any problems that may arise.

You will be asked to report to the Children’s Eating Behavior Laboratory in Chandlee Lab for a dinner meal at a scheduled time. Your child will be given a buffet meal of common foods, and can eat as much or as little as he or she would like. Your child will have 30 minutes to eat. We will ask your child to rate their fullness and how much they like the foods served at this meal.

For one week during the study, between Visits 2 & 3, we will ask your child to wear the same activity tracker device, but just on his or her wrist. We would like your child to wear this device for 24 hours every day for a week. The activity tracker will have to be removed when your child goes in water. On Visit 3, we will ask that you return it to our researchers.

3. What are the risks and possible discomforts from being in this research study?

Risk of injury is very low during a submaximal exercise test. However, exercise testing and moderate-to-vigorous exercise are not safe for everyone. We ask that your child
complete the test to the best of his or her ability. This exercise may leave your child feeling slightly tired or short of breath, but these feelings will pass within a few minutes of completion of the exercise. We will monitor your child's heart rate throughout the exercise tasks, which will be supervised by a certified trainer and registered nurse or physician. There is also a chance that your child may experience muscle soreness for the day or two following both the fitness test and the exercise testing procedure.

The foods used in the meals are all common foods made with ingredients you would find at the market. We make them fresh each day using safe food preparation protocols. However, there is always a chance of food borne illness or of uncovering an allergy in your child due to food exposure.

There is a risk of loss of confidentiality if your information or your identity is obtained by someone other than the investigators, but precautions will be taken to prevent this from happening.

4. **What are the possible benefits from being in this research study?**

There are no immediate benefits to you and your family from the study. However, you or your child may enjoy the activities during the study, or feel it is good to contribute to a scientific study. Furthermore, this study may also benefit the community. That is because we know very little about how children balance their energy. We now have the opportunity to learn how physical activity can influence some children’s eating behaviors. What we learn in this study will help future studies of children and help us come up with ways to help children to adopt healthy lifestyles.

5. **What other options are available instead of being in this research study?**

You may decide not to participate in this research.

6. **How long will you take part in this research study?**

If you agree to take part, it will take you about 3 weeks to complete this research study. This study involves three total visits for you and your child that will occur once a week for three weeks. Visit 1 will last approximately 4 hours. Visits 2 & 3 will last approximately 10 hours, including both the in- and out-of-laboratory tasks. The fitness test will last approximately 30 minutes, and the exercise test will last approximately 45 minutes.

7. **How will your privacy and confidentiality be protected if you decide to take part in this research study?**

Efforts will be made to limit the use and sharing of your personal research information to people who have a need to review this information. Your child’s participation in this research is confidential. All possible steps have been taken to assure your child's privacy. For all of the data we collect, your child will be assigned a code number that will be used throughout the study. Only this code (and never your child's name) will be
used when analyzing or reporting the data. Any identifying information will be kept in a locked location and password protected electronic files. This consent and any other identifying information will be kept in a locked file in Dr. Keller’s locked office. All questionnaires that you and your child complete will be identified only by your child’s code and stored separate from any identifying information. Only the PI (Ms. Fearnbach), Co-I (Dr. Keller), and study coordinators will have access to your identifying information. All other project staff who are approved by the Penn State IRB will only have access to data files without your name.

In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

We will do our best to keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people may find out about your participation in this research study. For example, the following people/groups may check and copy records about this research.

- The Office for Human Research Protections in the U. S. Department of Health and Human Services
- The Institutional Review Board (a committee that reviews and approves research studies) and
- The Office for Research Protections.

Some of these records could contain information that personally identifies you. Reasonable efforts will be made to keep the personal information in your research record private. However, absolute confidentiality cannot be guaranteed.

8. What happens if you are injured as a result of taking part in this research study?

In the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

9. Will you be paid or receive credit to take part in this research study?

The total compensation possible for this study is $150. For each visit, this includes $30 for your time and effort, and $20 for your child’s time and effort, for a total of $50 for each visit. If you or your child withdraws before completion of the visit, you will receive compensation for the time you completed in the study, based on a rate of $10 per hour, up to 5 hours. If we have to release you from the study because you or your child is unable to comply, you will also receive travel costs plus $10 per hour completed. Regardless of whether you complete the study or not, your child will still receive $10.

10. Who is paying for this research study?
11. What are your rights if you take part in this research study?

Taking part in this research study is voluntary.
- You do not have to be in this research.
- If you choose to be in this research, you have the right to stop at any time.
- If you decide not to be in this research or if you decide to stop at a later date, there will be no penalty or loss of benefits to which you are entitled.

12. If you have questions or concerns about this research study, whom should you call?

Please call the head of the research study (principal investigator), Nicole Fearnbach at (814) 865-4404 or Kathleen Keller at (814) 863-2915 if you:
- Have questions, complaints or concerns about the research.
- Believe you may have been harmed by being in the research study.

You may also contact the Office for Research Protections at (814) 865-1775, ORProtections@psu.edu if you:
- Have questions regarding your rights as a person in a research study.
- Have concerns or general questions about the research.
- You may also call this number if you cannot reach the research team or wish to talk to someone else about any concerns related to the research.

INFORMED CONSENT TO TAKE PART IN RESEARCH

Signature of Person Obtaining Informed Consent

Your signature below means that you have explained the research to the subject or subject representative and have answered any questions he/she has about the research.

Signature of person who explained this research Date Printed Name
(Only approved investigators for this research may explain the research and obtain informed consent.)

Signature of Person Giving Informed Consent

Before making the decision about being in this research you should have:
- Discussed this research study with an investigator,
- Read the information in this form, and
- Had the opportunity to ask any questions you may have.

Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been
answered. You will receive a copy of the signed and dated form to keep for future reference.

**Signature of Parent/Subject**

By signing this consent form, you indicate that you voluntarily choose to be in this research and agree to allow your information to be used and shared as described above.

___________________________  __________  __________________
Signature of Subject           Date                        Printed Name

**Signature of Parent(s)/Guardian for Child**

By signing this consent form, you indicate that you permit your child to be in this research and agree to allow his/her information to be used and shared as described above.

___________________________  __________  __________________
Signature of Parent/Guardian  Date                        Printed Name

**ASSENT FOR RESEARCH**

The research study has been explained to you. You have had a chance to ask questions to help you understand what will happen in this research. You **Do Not** have to be in the research study. If you agree to participate and later change your mind, you can tell the researchers, and the research will be stopped.

You have decided:  **(Initial one)**

____ To take part in the research.

____ NOT to take part in the research.

______________________________  __________  __________________
Signature of subject            Date                        Printed Name
This is a research study that you may choose to join and help. We are studying how kids' bodies keep track of how much they eat and move during the day!

We ask that you do not eat anything overnight or in the morning before you come to each visit. This morning, we will have you eat a breakfast of common foods. We will ask you to eat everything we serve at breakfast. This afternoon, we will let you pick the foods you would like for lunch. When you come back for visits 2 and 3, you will eat the same breakfast and lunch meals, and we will also give you a snack and dinner.

On visits 2 and 3, you will take a snack home with you. We will ask you to bring back any food you do not eat. We will also ask you not to eat any other foods that we do not give you. You can eat as much or as little as you like at lunch, the snack, and dinner. You don't have to eat anything you don't like at these meals.

Today and each day you come to the study, we are going to ask you to wear a little “watch” that keeps track of how you move during the day. We will also ask you to wear this watch at home. We ask that you wear it every day for a whole week, and then bring it back to us. Just take it off when you go in water.

Today we will also ask you to answer some questions on a computer. You don't have to answer any questions that make you feel uncomfortable. This may be something that you have done before at school. We ask that you try your hardest on it. There are no right or wrong answers, we just want to know what you think.

Today we will also do a fitness test on a special bicycle. Once you're ready, we will ask you to keep pedaling at the same speed throughout the test. Your speed will be on a little screen on the bicycle. Every few minutes we will ask you to tell us how hard you are working. We will count how many times your heart beats in a minute.
When you come back for visits 2 and 3, you will either rest and watch movies, or ride the bike again during this time. On the day you ride the bike we will have you ride for 30 minutes at the same speed.

Do you have any questions? We want you to have fun helping us with this study. Any time you have a question, please ask us.

You choose if you want to help us. If you feel like you want to stop at any time during the study, just let us know. Does this all make sense to you? If not, we can go over it again.

Before beginning the study, the research personnel administering assent should check the appropriate box below after reading the Assent form to the child and answering any questions they have.

☐ Obtained
☐ Not obtained because the capability of the child is so limited that the child cannot reasonably be consulted.
APPENDIX C:

Demographics Questionnaire

Study 2 & Study 3
Demographic Questionnaire

Please answer the following questions about your child:

1. Date of birth (MM/DD/YYYY): ________________
2. Birth weight: ________________
3. Birth length: ________________
4. Was your child born premature? YES NO
   a. If yes, by how many weeks? ______
5. Was your child primarily breast-fed or primarily formula-fed?
   a. Breast-fed
   b. Formula-fed
6. If your child was breast-fed, for how many months was he/she exclusively (only) fed breast milk? ______
7. What ethnicity is your child (please check only one)?
   a. Hispanic or Latino
   b. Not Hispanic or Latino
8. What ethnicity is your child (please check only one)?
   a. American Indian/Alaskan Native
   b. Asian
   c. Black or African American
   d. White
   e. Hawaiian/Pacific Islander
Please answer the following questions about yourself and your family:

1. What is your relationship to the child?
   a. Mother
   b. Father
   c. Other (please specify): ____________________

2. What is your date of birth (MM/ DD/YYYY)? ____________________

3. What is your ethnicity (please check only one)?
   a. Hispanic or Latino
   b. Not Hispanic or Latino

4. What is your ethnicity (please check only one)?
   a. American Indian/Alaskan Native
   b. Asian
   c. Black or African American
   d. White
   e. Hawaiian/Pacific Islander

5. Please indicate who lives in your household, and if applicable how many (i.e. Sibling 2).
   a. Mother ______
   b. Father ______
   c. Sibling ______
   d. Uncle ______
   e. Aunt ______
   f. Grandmother ______
   g. Grandfather ______
   h. Cousin ______
   i. Others, describe __________________________

6. What is your marital status?
   a. Married
   b. Single (never married)
   c. Widowed
   d. Divorced
   e. Separated
   f. Remarried
7. What is your total or combined family income, before taxes?
   a. Less than $20,000
   b. $21,000 - $35,000
   c. $36,000 - $50,000
   d. $51,000 - $75,000
   e. $76,000 - $100,000
   f. $100,000 +

8. What is your highest level of formal education?
   a. High school (12 years)
   b. Associates (14 years)
   c. Technical/Vocational School (14 years)
   d. Bachelor’s Degree (16 years)
   e. Master’s Degree (16 years)
   f. PhD (20 years)
   g. MD (20 years)
   h. JD (20 years)
   i. Other, describe _____________________

9. If applicable, what is your partner’s highest level of formal education?
   a. High school (12 years)
   b. Associates (14 years)
   c. Technical/Vocational School (14 years)
   d. Bachelor’s Degree (16 years)
   e. Master’s Degree (16 years)
   f. PhD (20 years)
   g. MD (20 years)
   h. JD (20 years)
   i. Other, describe _____________________

10. Are you currently employed? YES  NO

11. Are you currently retired? YES  NO

12. How many hours per week are you at work (not traveling to & from)? __________

13. Is your partner currently employed? YES  NO
14. Is your partner currently retired? YES  NO
15. How many hours per week is your partner at work (not traveling to & from)? __________
16. Who is primarily responsible for feeding your child?
   a. You
   b. Your partner
   c. Both
   d. School
   e. Other, please specify: _______________
17. Who is primarily responsible for buying food in your household?
   a. You
   b. Your partner
   c. Both
   d. Other, please specify: _______________
18. On average, how frequently does your family eat out or get delivery/take-out for dinner?
   a. Once a month or less
   b. Twice a month
   c. Once a week
   d. Two times a week
   e. Three times a week
   f. Four or more times a week
19. On average, how many nights a week does your family eat dinner together as a group (with
    most family members present)?
    a. 1
    b. 2
    c. 3
    d. 4
    e. 5
    f. 6
    g. 7
APPENDIX D:

Anthropometrics Data Sheets

Study 2 & Study 3
Participant ID ______________
Date ________ Time ________

Child:
Height #1 ________  Weight #1 ________
Height #2 ________  Weight #2 ________
Average Height ________  Average Weight ________
% Body fat ________
Shuttle run time ____________

Parent:

Parent 1: □ Mother  □ Father
Height #1 ________  Weight #1 ________
Height #2 ________  Weight #2 ________
□ Measured □ Self-reported  □ Measured □ Self-reported
Average Height ________  Average Weight ________
% Body fat ________

Parent 2: □ Mother  □ Father
Height #1 ________  Weight #1 ________
Height #2 ________  Weight #2 ________
□ Measured □ Self-reported  □ Measured □ Self-reported
Average Height ________  Average Weight ________
% Body fat ________
Participant ID __________________
Date ___________ Time ___________

Child:
DOB ______________
Sex □ Male   □ Female
Height #1 ____________  Weight #1 ____________
Height #2 ____________  Weight #2 ____________
Average Height ____________  Average Weight ____________
% Body fat ____________

Parents:
Parent 1: □ Mother   □ Father  
Height #1 ____________  Weight #1 ____________
Height #2 ____________  Weight #2 ____________
□ Measured □ Self-reported  □ Measured □ Self-reported
Average Height ____________  Average Weight ____________
% Body fat ____________

Parent 2: □ Mother   □ Father  
Height #1 ____________  Weight #1 ____________
Height #2 ____________  Weight #2 ____________
□ Measured □ Self-reported  □ Measured □ Self-reported
Average Height ____________  Average Weight ____________
% Body fat ____________
**ActiGraph Data:**

**Visit 1:**

<table>
<thead>
<tr>
<th>Serial Number</th>
<th>Wear Site</th>
<th>Initialized Date</th>
<th>RA Initials</th>
<th>Collected Date</th>
<th>RA Initials</th>
<th>Data Downloaded Date</th>
<th>RA Initials</th>
<th>Data Validated Date</th>
<th>RA Initials</th>
<th>Data Scored Date</th>
<th>RA Initials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial Number 1</td>
<td>Wear Site</td>
<td>Initialized Date</td>
<td>RA Initials</td>
<td>Collected Date</td>
<td>RA Initials</td>
<td>Data Downloaded Date</td>
<td>RA Initials</td>
<td>Data Validated Date</td>
<td>RA Initials</td>
<td>Data Scored Date</td>
<td>RA Initials</td>
</tr>
<tr>
<td>Serial Number 2</td>
<td>Wear Site</td>
<td>Initialized Date</td>
<td>RA Initials</td>
<td>Collected Date</td>
<td>RA Initials</td>
<td>Data Downloaded Date</td>
<td>RA Initials</td>
<td>Data Validated Date</td>
<td>RA Initials</td>
<td>Data Scored Date</td>
<td>RA Initials</td>
</tr>
</tbody>
</table>

**Visit 2:**  **Condition □ Exercise □ Rest**

<table>
<thead>
<tr>
<th>Serial Number</th>
<th>Wear Site</th>
<th>Initialized Date</th>
<th>RA Initials</th>
<th>Collected Date</th>
<th>RA Initials</th>
<th>Data Downloaded Date</th>
<th>RA Initials</th>
<th>Data Validated Date</th>
<th>RA Initials</th>
<th>Data Scored Date</th>
<th>RA Initials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial Number 1</td>
<td>Wear Site</td>
<td>Initialized Date</td>
<td>RA Initials</td>
<td>Collected Date</td>
<td>RA Initials</td>
<td>Data Downloaded Date</td>
<td>RA Initials</td>
<td>Data Validated Date</td>
<td>RA Initials</td>
<td>Data Scored Date</td>
<td>RA Initials</td>
</tr>
<tr>
<td>Serial Number 2</td>
<td>Wear Site</td>
<td>Initialized Date</td>
<td>RA Initials</td>
<td>Collected Date</td>
<td>RA Initials</td>
<td>Data Downloaded Date</td>
<td>RA Initials</td>
<td>Data Validated Date</td>
<td>RA Initials</td>
<td>Data Scored Date</td>
<td>RA Initials</td>
</tr>
</tbody>
</table>

**Between Visits:**

<table>
<thead>
<tr>
<th>Serial Number</th>
<th>Wear Site</th>
<th>Initialized Date</th>
<th>RA Initials</th>
<th>Collected Date</th>
<th>RA Initials</th>
<th>Data Downloaded Date</th>
<th>RA Initials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial Number</td>
<td>Wear Site</td>
<td>Initialized Date</td>
<td>RA Initials</td>
<td>Collected Date</td>
<td>RA Initials</td>
<td>Data Downloaded Date</td>
<td>RA Initials</td>
</tr>
</tbody>
</table>
Visit 3:  

**Condition**  □ Exercise  □ Rest  

Serial Number 1 ________________________  Wear Site ________________________  
Serial Number 2 ________________________  Wear Site ________________________  
Initialized Date _________________  RA Initials _____________  
Collected Date _________________  RA Initials _____________  
Data Downloaded Date _________________  RA Initials _____________  
Data Validated Date _________________  RA Initials _____________  
Data Scored Date _________________  RA Initials _____________
APPENDIX E:

Laboratory Test-meal Photos

Study 1, Study 2, & Study 3
Study 1:

Palatable Buffet

Study 2:

Dinner Meals Varying in Portion Size

Note: Intake data from these meals was not presented in the current dissertation
Study 3:

Standard Breakfast

Ad libitum Lunch

Ad libitum Dinner (Multiple servings available)
APPENDIX F:

Food Intake Data Sheets

Study 2 & Study 3
# Intake Sheets: Portion Size Study

## Condition 1: 100% (Std.)

Check 1: __________

Check 2: __________

Subject ID: _____________

Date: _________________

Week: _________________

<table>
<thead>
<tr>
<th>Lunch Food</th>
<th>Pre-Weight</th>
<th>Post-Weight</th>
<th>Amount Consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaroni &amp; Cheese (300g)</td>
<td>(w/o plate)</td>
<td>(w/plate)</td>
<td></td>
</tr>
<tr>
<td>Garlic bread (2” piece)</td>
<td>(w/o plate)</td>
<td>(w/plate)</td>
<td></td>
</tr>
<tr>
<td>Broccoli (90g)</td>
<td>(w/o bowl)</td>
<td>(w/bowl)</td>
<td></td>
</tr>
<tr>
<td>Cherry Tomatoes (50g)</td>
<td>(w/o plate)</td>
<td>(w/plate)</td>
<td></td>
</tr>
<tr>
<td>Red grapes (100g)</td>
<td>(w/o bowl)</td>
<td>(w/bowl)</td>
<td></td>
</tr>
<tr>
<td>Angel Food Cake (40g)</td>
<td>(w/o plate)</td>
<td>(w/plate)</td>
<td></td>
</tr>
<tr>
<td>Water (1L)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Time Meal: ________________

**Freddy Fullness pre-meal:** ________________

**Freddy Fullness post-meal:** ________________
Participant ID __________
Date __________
Visit # __________
Condition: Exercise    Rest

Energy Balance Study
Breakfast Data Sheet

<table>
<thead>
<tr>
<th>Food</th>
<th>Pre-weight w/ container (g)</th>
<th>Pre-weight w/ container (g)</th>
<th>Post-weight w/ container (g)</th>
<th>Amount eaten (g)</th>
<th>% of food eaten</th>
</tr>
</thead>
<tbody>
<tr>
<td>English muffin (sliced, UNTOASTED)</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>English muffin (sliced, toasted)</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Butter spread 1TBSP</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>English muffin w/ butter spread (total)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>½ Banana ~60g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 oz. juice ~177.5g (circle one)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Time of meal ______________
Duration of meal ______________

Freddy Fullness
Pre-meal ____________mm
Post-meal ____________mm

RA's Present
1) _______________
2) _______________
3) _______________
Participant ID __________
Date ____________
Visit # ___________
Condition: Exercise Rest

**Energy Balance Study**  
**Lunch Data Sheet**

<table>
<thead>
<tr>
<th>Food</th>
<th>Pre-weight w/out container (g)</th>
<th>Pre-weight w/ container (g)</th>
<th>Post-weight w/ container (g)</th>
<th>Amount eaten (g)</th>
<th>% of food eaten</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 slices of bread</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deli meat (circle one)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ham</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese (circle one)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>American</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheddar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Provolone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut butter 2TBSP / 30g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jelly 36g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sandwich (total)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit side (circle one)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple slices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grapes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable side (circle one)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baby carrots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomatoes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carb side (circle one)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretzels</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baked chips</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brownie bites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>Ranch dip</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water bottle</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td></td>
</tr>
</tbody>
</table>

Time of meal ______________
Duration of meal ______________

Freddy Fullness
Pre-meal _____________ mm
Post-meal _____________ mm

RAs Present
1) ______________
2) ______________
3) ______________
Participant ID __________
Date ____________
Visit # ____________
Condition: Exercise Rest

Energy Balance Study
Snack Data Sheet

<table>
<thead>
<tr>
<th>Food</th>
<th>Pre-weight w/out container (g)</th>
<th>Pre-weight w/ container (g)</th>
<th>Post-weight w/ container (g)</th>
<th>Amount eaten (g)</th>
<th>% of food eaten</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granola bar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit cocktail</td>
<td>---------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 oz. juice bottle</td>
<td>---------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Time of meal ______________
Duration of meal ______________

Freddy Fullness
Pre-meal ______________mm
Post-meal ______________mm

RAs Present
1) ______________
2) ______________
3) ______________
We have provided a snack for your child to eat at home this afternoon. Please see the following instructions.

1) The snack should be served at the following time:

2) We will send you a text message reminder at the time we would like you to serve your child the snack. Please respond to confirm you have received these reminders.

3) **Please return all packaging** (wrappers, bags, containers) and **any remaining uneaten food** items to researchers when you return for the dinner meal. It is **very important** to us that these items come back to us.

4) We strongly prefer that you do not provide any additional food items to your child during this time.

5) Please be sure that your child does not eat anything for the 2 hours before the dinner meal. Tonight’s dinner visit is scheduled at:

   Dinner _____________

6) You may serve your child water at any time.

Please **write notes below** to report any problems that may arise. You can also call our lab at 814-826-4497 if you have any concerns.
Participant ID __________
Date ____________
Visit # ___________
Condition: Exercise Rest

---

**Energy Balance Study**
**Dinner Data Sheet**

<table>
<thead>
<tr>
<th>Food</th>
<th>Pre-weight w/out container (g)</th>
<th>Pre-weight w/ container (g)</th>
<th>Post-weight w/ container (g)</th>
<th>Amount eaten (g)</th>
<th>% of food eaten</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaroni &amp; Cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving #1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macaroni &amp; Cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving #2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macaroni &amp; Cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving #3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garlic bread</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving #1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garlic bread</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving #2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garlic bread</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving #3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broccoli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving #1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broccoli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving #2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broccoli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving #3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Applesauce</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving #1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Applesauce</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving #2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Applesauce</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving #3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate chip</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---
|                                     |   |   |   |   |
|                                     |   |   |   |   |
| cookies                             |   |   |   |   |
| Serving #1                          |   |   |   |   |
| Chocolate chip cookies              |   |   |   |   |
| Serving #2                          |   |   |   |   |
| Chocolate chip cookies              |   |   |   |   |
| Serving #3                          |   |   |   |   |
| Water                               |   |   |   |   |

Time of meal ____________
Duration of meal ____________

**Freddy Fullness**
Pre-meal ____________mm
Post-meal ____________mm

**RAs Present**
1) ____________
2) ____________
3) ____________
APPENDIX G:

“Freddy Fullness” Visual Analog Scale

Study 2 & Study 3

Reference:

Introduction: “I have a doll here whose name is Freddy. You can use Freddy to tell how full your stomach feels after eating food or a meal, like breakfast, lunch, or dinner. For example, if you hadn’t eaten anything and your stomach felt empty, you’d put the slider at the very bottom. If you ate so much and were so full that you felt like you could burst and you couldn’t possibly eat anymore, you’d put the slider at the top. Why don’t you try to move the slider to get a feel for it? “

(Allow child to demonstrate how the slider can move up and down Freddy’s tummy.)

“Now, I have a few questions for you, and I want you to move the slider up and down Freddy’s tummy to tell me how full you think you would be. Okay?”

“Imagine if you ate just a little bit, like one cookie, how full do you think your stomach would feel?”
(If child moves the slider more than 25%, ask whether he/she is sure that’s really a little bit. – Moving the slider too far for a little may indicate the child doesn’t understand).

“Now, imagine that you ate a few more cookies, how full do you think your stomach would feel?”
(Any response between the last one and the top is acceptable.)

“Now imagine that you ate so many cookies that you didn’t want any more, but you could still eat something else, how full do you think your stomach would feel?”
(Child should put the slider between 60 and 80% of the distance.)

“Now, if you ate so much that you couldn’t possibly eat anymore of ANY food, how full do you think your stomach would feel?”
(Child should use the maximum.)

“Do you understand how Freddy works?”
(Allow child to respond. If child is still unclear, go through explanation again.)

Fullness Determination and Study Meal

“Can you use Freddy to show me how full your stomach feels right now? Remember, if your stomach feels empty, you push the rectangle to the bottom of the page like this.”
(Push the slider to the bottom of the page).

“If you have eaten a lot and you can’t possibly eat anymore, you push the rectangle all the way to the top like this.”
(Push the slider to the top of the page).

“Do you understand? Great! Move the rectangle to show me how you feel right now.”
(Let child use Freddy to rate how full he/she feels. Mark it on the Pre-Meal Freddy sheet.)
APPENDIX H:

Food Liking and Wanting Visual Analog Scales

Study 2 & Study 3
1. How much do you like this (insert food name)?

Not at all  

Like very much

2. How much do you want this (insert food name)?

Not at all  

Want very much
APPENDIX I:

ActiGraph Accelerometry

Study 3
ActiGraph Accelerometry Procedures

Initialization:
- Be sure battery is charged prior to initialization.
- Plug in the ActiGraph device into the computer with USB cable. It should appear in the grid under the “Devices” tab.
- Click “Initialize”, and select “Regular Initialization”.
- Enter the following information:
  - Start date and time, Stop date and time
  - Keep default Sampling Rate and device options
  - Enter subject info: ID, age (date of birth), height, weight, race, sex
  - Wear site: non-dominant wrist (or ankle for exercise testing)
- Select “Initialize 1 Device.” A progress bar in the devices grid will indicate when the initialization process is complete.
- Eject the device, unplug, and replace the cap over the USB port.

Data Download:
- Open ActiLife software, plug in ActiGraph, and select device.
- Click “Download”
- Select location where files will be saved.
- Select naming convention, Check box for “Create AGD File” (10 second epochs, 3 axes)
- Check boxes for “Steps,” “Inclinometer,” and “Lux”
- Click “Download all devices”
- Data will load into 2 files: .AGT and .GT3X files. Be sure to save both of these.
- Upload 2 files to ActiGraph’s Vault and into Box.com for sharing.
- Remove identifying information from device and recharge if needed.

Wear procedures for participants:
Wrist worn devices:
- From above the device, insert the thin end of the wear time wrist strap into one of the GT3X wings with the velcro side facing out. Pull until the thick end of the wrist strap catches the wing.
- Insert the thin end into the opposite wing to create a loop. The strap should not cover the back of the device.
- Secure and adjust the wrist strap by folding the excess length outward and fastening to the velcro.
- Subjects should be instructed to wear the device strapped securely to the non-dominant wrist with the ActiGraph logo facing up when viewed like a wrist watch.
- Instruct subjects to take off the device during water activities (swimming, bathing).
- Exposure to rain and sweat is okay.
Scoring for weekly data:

Data Validation:
- Once you have all of the data downloaded you can validate it. On the top of the screen select "Wear Time Validation".
- Using the parameters below, set up the Wear Time Validation.
- Select "Add Dataset(s)" from middle of screen and click "Add Files"
- Download datasets from ActiLife Data Vault or Box.com where you stored them.
- Select the data sets that you would like to validate and select calculate.

**Validation Parameters (see screenshot below):**
- Wear time Validation - Floating window
- Choi (2011) - Default setting
- Ignore wear period less than - Unchecked
- Minimum wear time per day - 8 hours (480 minutes)
- Minimum Number of days - 3
- Sleep Period options - Mark as wear time
- Evaluate Wear Sensor data (if available) - Checked

Data Scoring:
- Select "Scoring" from top of the screen and enter in scoring parameters below.
- Click on Add Dataset(s) and select "Add Files"
- Choose the datasets you would like to work with from ActiLife Data Vault or Box.com
- Be sure to select the datasets you would like to work with once they are on the ActiLife 6 screen list and then press calculate at bottom of screen.
- Export scored data as .CSV files and save to Box.com
Scoring Parameters (see screenshot below):
EE - Use Freedson Combination (1998)
METs - Use Feedson Children (2005)
Cut points - Evenson Children (2008)
Bouts - Checked
Sedentary Analysis - Checked
NO HREE
Exclude Non-wear time from analysis - Checked
Use subject log diaries – Unchecked

Scoring for daily data:
Data Validation:
- Once you have all of your information downloaded you can validate it. On the top of the screen select "Wear Time Validation".
- Using the parameters below set up the Wear Time Validation.
- Select "Add Dataset(s)" from middle of screen and click "Add Files"
- Download datasets from Actilife Data Vault and Box.com were you stored them.
- Select the data sets that you would like to validate and select calculate.

Validation Parameters (see screenshot below):
Wear time Validation - Floating window
Choi (2011) - Default setting
Ignore wear period less than - Unchecked
Minimum wear time per day - 3 hours (180 minutes)
Minimum Number of days - Unchecked
Sleep Period options: Mark as wear time
Evaluate Wear Sensor data (if available) - Checked
Data Scoring

- Select "Scoring" from top of the screen and enter in scoring parameters below.
- Click on Add Dataset(s) and select "Add Files"
- Choice the data sets you would like to work with from Actilife vault or Box.com
- Be sure to select the datasets you would like to work with once they are on the Actilife 6 screen list and then press calculate at bottom of screen.
- Export scored data as .CSV files and save to Box.com

**Scoring Parameters:**

- EE - Use Freedson Combination (1998)
- METs - Use Feedson Children (2005)
- Cut points - Evenson Children (2008)
- Bouts - Checked
- Sedentary Analysis - Checked
- NO HREE
- Exclude Non-wear time from analysis - Checked
- Use subject log diaries - Unchecked
Scoring for ankle data (exercise test):
Data Validation:
Do not do wear time validation (does not go below 3 hours of wear time)

Data Scoring:
- Select "Scoring" from top of the screen and enter in scoring parameters below.
- Click on Add Dataset(s) and select "Add Files"
- Choice the data sets you would like to work with from ActiLife Data Vault or Box.com
- Be sure to select the datasets you would like to work with once they are on the ActiLife 6 screen list and then press calculate at bottom of screen.
- Export scored data as .CSV files and save to Box.com

Scoring Parameters:
- EE - Use Freedson Combination (1998)
- METs - Use Freedson Children (2005)
- Cut points - Evenson Children (2008)
- Bouts - Checked
- Sedentary Analysis - Checked
- NO HREE
- Exclude Non-wear time from analysis - Checked
- Use subject log diaries - Unchecked
APPENDIX J:

YMCA Submaximal Cycle Ergometer Fitness Test

Study 3

Reference:

Instructions for Administering the YMCA Submax Cycle Protocol

1. Pre Test
   a. Write down 85% of max heart rate on test form
   b. Resting blood pressure, resting heart rate
   c. Adjust seat height and record for future tests

2. Test
   a. Record blood pressure, RPE (6-20) and workload for each stage. Palpate heart rates each minute.
   b. If the last two heart rates are greater than 6 bpm apart the stage should be continued and the heart rate taken at the end of each minute until a steady state is achieved. Then proceed to the next stage.

3. Post test
   a. Cool down at work rate equivalent to first stage (0.5 kp) or lower
   b. Test administrator continues to monitor heart rate, blood pressure, signs and symptoms

Workloads for submaximal exercise test:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Workload</th>
<th>HR: &lt; 80</th>
<th>HR: 80-89</th>
<th>HR: 90-100</th>
<th>HR: &gt;100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>150 kg · m · min⁻¹</td>
<td>0.5 kg</td>
<td>24.5 W</td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>750 kg · m · min⁻¹</td>
<td>600 kg · m · min⁻¹</td>
<td>450 kg · m · min⁻¹</td>
<td>300 kg · m · min⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 kg</td>
<td>2.0 kg</td>
<td>1.5 kg</td>
<td>1.0 kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>122.6 W</td>
<td>98.1 W</td>
<td>73.5 W</td>
<td>49 W</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>900 kg · m · min⁻¹</td>
<td>750 kg · m · min⁻¹</td>
<td>600 kg · m · min⁻¹</td>
<td>450 kg · m · min⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0 kg</td>
<td>2.5 kg</td>
<td>2.0 kg</td>
<td>1.5 kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>147.1 W</td>
<td>122.6 W</td>
<td>98.1 W</td>
<td>73.5 W</td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>1050 kg · m · min⁻¹</td>
<td>900 kg · m · min⁻¹</td>
<td>700 kg · m · min⁻¹</td>
<td>600 kg · m · min⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.5 kg</td>
<td>3.0 kg</td>
<td>2.5 kg</td>
<td>2.0 kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>171.6 W</td>
<td>147.1 W</td>
<td>114.4 W</td>
<td>98.1 W</td>
<td></td>
</tr>
<tr>
<td>Stage 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

work rate in kg*m/min (will be a # in the hundreds)
- Multiply by 0.164 to get Watts for cycle
The objective of the YMCA submaximal bicycle test is to obtain two separate workloads resulting in steady state heart rate values between 110 bpm and the subject’s 85% APMHR. Steady state is determined by the last two heart rates being within 6 bpm apart. This establishes linearity between heart rate and workload for the person being tested. To establish the line of best fit on the graph, at least two points are needed.

Once the test is completed, the heart rates should be plotted against the respective workload in the graph provided. A straight line should be drawn through the points and extended to the subject’s age predicted max heart rate (220-age). The point where the diagonal line intersects the horizontal predicted max heart rate line will represent the maximal working capacity.

A perpendicular line will then be drawn from this point to the base where the maximal physical workload capacity can be read in kgm/min. This can then be used to predict a person’s maximal oxygen uptake.

Perform YMCA test and graph your data and provide an estimated VO2max by $\text{VO}_2 (\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = [(1.8 \times \text{work rate}) / \text{Body wt in kg}] + 7$. 

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:00–0:45</td>
<td>Monitor your client’s work output (cadence and resistance)</td>
</tr>
<tr>
<td>0:45–1:00</td>
<td>Pulse count for 15 seconds (for practice)</td>
</tr>
<tr>
<td>1:00–1:45</td>
<td>Monitor your client’s work output (cadence and resistance)</td>
</tr>
<tr>
<td>1:45–2:00</td>
<td>Pulse count for 15 seconds (2 min HR)</td>
</tr>
<tr>
<td>2:00–2:30</td>
<td>Stage BP check</td>
</tr>
<tr>
<td>2:30–2:45</td>
<td>Stage RPE check</td>
</tr>
<tr>
<td>2:45–3:00</td>
<td>Pulse count for 15 seconds (3 min HR)</td>
</tr>
</tbody>
</table>
Palpated heart rate taken every minute. BP completed at the end of every third minute. Speed should be 50 rpm.

Participant ID:_______________

Age: _______  Age pred. max HR: _______  85% age-pred max HR: _______

Ht: _______ (ins)  Wt: _______ (kgs)

HR (sitting): ___________________  BP (sitting): ___________________

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Workload (kp)</th>
<th>HR (bpm)</th>
<th>BP (mm Hg)</th>
<th>RPE (6-20)</th>
<th>Signs/Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1 1</td>
<td>0.5 – 150 kg m/min</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Stage 2 4</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Stage 3 7</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Stage 4 10</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>1 Rec</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>2 Rec</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>3 Rec</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX K:

70% Intensity Exercise Test

Study 3
Instructions for Administering the 70% VO2max Cycle Protocol

1. **Pre Test**
   a. Write down 70% VO2max workload equivalent on test form
   b. Resting blood pressure, resting heart rate
   c. Adjust seat height
   d. Warm up for 5 minutes at 0.5 kp

2. **Test (30 minutes)**
   a. Adjust resistance to 70% VO2max workload equivalent
   b. Record blood pressure and RPE (6-20) every 3 minutes. Palpate heart rate each minute and record workload/rpm every minute.
   c. If participant falls below 50 rpm for more than two consecutive minutes, end the test and record duration

3. **Post test**
   a. Cool down at workload of 0.5 kp or lower
   b. Test administrator continues to monitor heart rate, blood pressure, signs and symptoms
# 70% VO2max Exercise Testing Data Sheet

Palpated heart rate taken every minute. BP completed at the end of every third minute. Speed should be 50 rpm.

Participant ID:_____________________

Age: _________ Estimated VO2max workload: _________ 70% VO2max workload: _________

Ht: ___________ (ins) Wt: ___________ (kgs)

HR (sitting): ______________________ BP (sitting): ___________________

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Workload (kp/rpm)</th>
<th>HR (bpm)</th>
<th>BP (mm Hg)</th>
<th>RPE (6-20)</th>
<th>Signs/Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX L:

Borg Scale for Rating of Perceived Exertion

Study 3

Reference:

Rating of Perceived Exertion

Around the world in health clubs on the walls beside treadmills, stationary bikes and step machines, one often sees a scale going from 6-20. This is called an RPE Scale, which stands for “Rate of Perceived Exertion.” It is a psychophysiological scale, meaning it calls on the mind and body to rate one’s perception of effort. Understanding the meaning and use of this chart will benefit the average fitness enthusiast.

The RPE scale measures feelings of effort, strain, discomfort, and/or fatigue experienced during both aerobic and resistance training. One’s perception of physical exertion is a subjective assessment that incorporates information from the internal and external environment of the body. The greater the frequency of these signals, the more intense are the perceptions of physical exertion. In addition, response from muscles and joints helps to scale and calibrate central motor outflow commands. The resulting integration of feedforward-feedback pathways provides fine-tuning of the exertional responses.

Perceived exertion reflects the interaction between the mind and body. That is, this psychological parameter has been linked to many physiological events that occur during physical exercise. These physiological events can be divided into respiratory/metabolic (such as ventilation and oxygen uptake) and peripheral (such as cellular metabolism and energy substrate utilization.) Previous studies have demonstrated that an increase in ventilation, an increase in oxygen uptake, an increase metabolic acidosis or a decrease in muscle carbohydrate stores are associated with more intense perceptions of exertion. The scale is valid in that it generally evidences a linear relation with both heart rate and oxygen uptake during aerobic exercise.

How is perceived exertion measured?
The level of perceived exertion is often measured with a 15 category scale that was developed by the Swedish psychologist Gunnar Borg. The Borg scale is shown below:

6 No exertion at all
7 Extremely light
8
9 Very light
10
11 Light
12
13 Somewhat hard
14
15 Hard (heavy)
16
19 Extremely hard
20 Maximal Exertion
© Gunnar Borg 1985

The Borg scale is simple to understand and very user-friendly. However, to use it effectively, it is necessary to adhere to the standard guidelines in measuring perceived exertion. These guidelines are:
1) It should be clear to either the client, patient, or athlete that perceived exertion is a method to determine the intensity of effort, strain, and/or discomfort that is felt during exercise;
2) The range of sensations must correspond to the scale. For example, number 6 should be made in reference to the feelings during rest, whereas number 20 should refer to the maximal level of exertion;
3) Either the RPE should be made specific to the overall body perception or the perception derived from a certain anatomical region of the body such as chest, arms and/or legs. Typically, individuals interested in monitoring the stress of a workout use RPE ratings.
4) It is important to know that when rating one’s perception of exertion there is no right or wrong answer for the rating. However, the individual must clearly understand the meaning of the descriptors, so careful explanation of the scale is necessary before using.

How can ratings of perceived exertion be used?
Due to its reasonably linear relation with oxygen uptake and heart rate, RPE can be used to guide the progression of a graded exercise test. This is accomplished by providing subjective confirmation that end-points of the test have been achieved once the terminal rating is reported or by signaling the relative metabolic stress at a given time during the test. Based upon the fact that RPE’s positively correlate to power output over a wide range of intensities, they can also be used to predict aerobic power in a manner analogous to the way that heart rate is employed in submaximal testing.

Ratings of perceived exertion can also be used to prescribe and monitor exercise intensity during a workout. A common approach is to periodically ask a person to rate his or her perceived exertion for a given exercise intensity during a stress test and then match it to an appropriate exercise intensity prescription. Attempting to keep the RPE within a training range similar to heart rate training ranges can be effective. Using this procedure, the target RPE ratings are based upon prior test results, and the person is requested to produce intensity perceived to be similar to the target rating during a workout. The key is close approximation to heart rate in aerobic exercise, where the RPE scale is most often used.

A question is sometimes raised as to whether the intensity produced based on perceptual ratings is actually what it is supposed to be. Several recent studies have attempted to answer this question. These studies have used oxygen uptake as an objective variable and found no difference between the oxygen uptake that was estimated from the prior test results and oxygen uptake that was produced during a subsequent workout. This finding suggests that using a “target RPE” as a guide to regulate exercise intensity is valid.

It is important to note that using the RPE can be especially important in two situations. If heart-rate measurement is difficult for some reason, or if the individual is on medication that alters normal heart rate response to physical stress, RPE can be an excellent tool to regulate and monitor intensity. The RPE scale continues to be a useful tool, offering subjective reflection of physiological responses during physical exercise, and enabling the individual to regulate effort to gain maximum benefit.

Written for the American College of Sports Medicine by Alan C. Utter, Ph.D., M.P.H., FACSM, Jie Kang, Ph.D., FACSM, Robert J. Robertson, Ph.D., FACSM
VITA
Stephanie Nicole Fearnbach

EDUCATION
The Pennsylvania State University, Ph.D. in Nutritional Sciences 2012 – 2016
Florida State University, B.S. in Exercise Science 2007 – 2012

PUBLICATIONS


SELECTED ORAL PRESENTATIONS


FELLOWSHIPS AND AWARDS
USDA Childhood Obesity Prevention Training Fellowship 2012 – 2016
Graham Endowed Fellowship 2012 – 2013
Grace M. Henderson Graduate Scholarship 2013 – 2014
2nd Place Health and Life Sciences, PSU Graduate Research Exhibition 2014
Graduate Travel Grant, PSU Office of Global Programs 2015
Professional Development Award, College of H&HD 2015
New Investigator Travel Award, Society for the Study of Ingestive Behavior 2015
Travel Award, Energy & Macronutrient Metabolism RIS, American Society for Nutrition 2016
Student Research Award, Nutrition Interest Group, American College of Sports Medicine 2016
Student Travel Award, World Congress on Energy Balance, American College of Sports Medicine 2016