PARENTING STYLE INFLUENCES ON APPETITE REGULATION IN AFRICAN AMERICAN CHILDREN AND THE EFFECT OF THE FTO GENE

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ABSTRACT

Purpose: Indulgent parent feeding-styles have been associated with higher child body mass index (BMI); more direct influences on children's eating are not well characterized. This study examined whether African American (AA) children exhibited poorer appetite regulation when mothers had an indulgent feeding style relative to other feeding styles. This study also examined whether the *FTO* gene influenced the relationship between feeding style and appetite regulation.

Methods: An observational design was used to evaluate the association of maternal feeding styles with child appetite among 100 obese and non-obese AA children aged 5-6 y. The Child Feeding Styles Questionnaire was used to categorize maternal feeding styles as authoritative, authoritarian, indulgent or uninvolved. Observed child satiation was measured at 4 laboratory-based dinner meals (portion sizes 100%, 150%, 200%, and 250% of those offered in reference condition). Change in energy intake across the 4 meals was estimated using a random slope mixed effects linear model. Parents' reports of child satiety responsiveness were assessed using the Child Eating Behavior Questionnaire. Child BMI percentile and BMI-for-age z-scores were calculated using measured height and weights. Generalized linear models were used to predict child appetite using parental feeding styles (covariates: gender, child BMI, maternal education, and income).

The study center collected DNA and RNA through saliva samples from each child participant. Of the 100 children enrolled, 32 obese children and a random sample of 32 non-obese children were selected for genotyping and expression analysis. This resulted

in the genotyping of three FTO gene SNPs, rs9939609, rs3751812 and rs8050136. FTO mRNA levels were measured using TaqMan Gene Expression Assays.

Results: Children of indulgent feeders showed lower satiation compared to other children by consuming more energy as food portion sizes were systematically increased (p < 0.01). Maternal satiety responsiveness ratings of children of indulgent feeders were also lower compared with other children (p < 0.05).

There were no significant relationships between FTO genotype or mRNA levels and feeding style or appetite regulation.

<u>Conclusions:</u> These findings provide new evidence that indulgent feeding-styles are associated with poorer appetite regulation among AA children.

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CHAPTER 1

INTRODUCTION

Childhood Obesity, on the Increase

According to the most recent National Health and Nutrition Surveys (NHANES) (2007-2010) the prevalence of childhood obesity has more than tripled since 1963, with the largest increases observed during the 1980s and 1990s (Ogden & Carroll, 2010; Ogden, Carroll, Kit & Flegal KM, 2012). Between 1999-2000 and 2009-2010 the obesity prevalence in children 2 to 19 years remained relatively unchanged (Ogden, Carroll, Kit & Flegal KM, 2012) with approximately, 17% of U.S. children, aged 2 to 19 years classified as obese (body mass index [BMI] for age and gender \geq 95th percentile). Additionally, 12.1 % of children 2 to 5 years and 18.0% of children 6 to 11 years are obese. Non-Hispanic African American (AA) children and adolescents have a higher prevalence compared to non-Hispanic white children and adolescents. Figure 1 illustrates the disparity in prevalence of obesity between Non-Hispanic white (14.6%) and AA children (22.1%) between the ages of 2 and 19 years. Racial/ethnic disparities are also apparent when looking at the data separately for preschoolers and school-aged children. Among children between 2 and 5 years of age, Non-Hispanic White children have an obesity prevalence rate of 9% compared to 15% in Non-Hispanic Black children. Among children between 6 and 11 years of age the prevalence rate for obesity is 16.4% in Non-Hispanic White children, compared to 23.9% in Non-Hispanic Black children (CDC/NCHS, NHANES, 2007-2008 and 2009-2010). This research focuses on non-Hispanic black children given the disproportionate risk for obesity in this population.

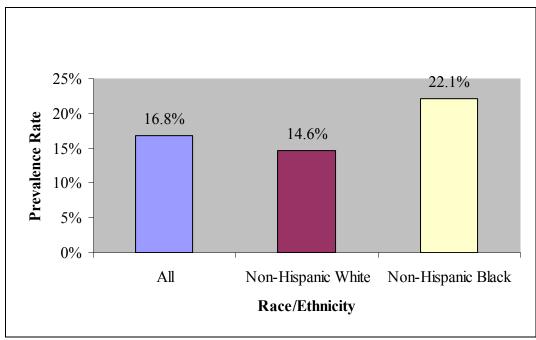


Figure 1: Obesity Rates, ages 2-19: US, 2007-2010

Source: CDC/NCHS, NHANES, 2007-2008 and 2009-2010

NOTE: Obesity is defined as BMI greater than or equal to gender and age specific 95th percentile based on the 2000 CDC Growth Charts.

The rise in childhood obesity rates has given way to the early development of many chronic diseases previously associated with adulthood including type 2 diabetes and cardiovascular disease (Kral & Faith, 2009). Overweight and obese children also suffer from psychosocial issues such as depression, are often victims of bullying and lower school performance (Vos and Welsh, 2010). In addition, being obese in childhood increases the likelihood of being obese in adulthood (Guo, Wu, Chumlea and Roche, 2002). For males, as age increases and BMI remains at or above the 95th percentile the probability for being obese in adulthood increases from <20% at 3-4 y of age to 20-39.9% at 4-12 y of age. For females, the same trend exists except the probability at the 95th percentile is 20–39.9% for 3 to 5 y of age and 40–59.9% for 5 to 12 y of age. (Guo et al, 2002). Given that many obese children will not simply grow out of it, prevention efforts are increasingly focused on early childhood. Because scientific understanding of

obesity determinants among young children is critical to effective prevention, this study focuses on obesity-related behaviors influencing children between 5 and 6 y of age.

CHAPTER 2

BACKGROUND

The cause of childhood obesity is believed to be complex and multi-factorial. A combination of interacting genetic, environmental and psychosocial factors are believed to contribute to obesity risk in children (Davison & Birch, 2001).). At a basic level, weight gain is caused by consuming more energy (calories) than is expended (Vos & Welsh, 2010). In children, obesity is the result of excessive energy intake relative to energy needs (Vos & Welsh, 2010). To understand obesity prevention, it is important to understand the mechanisms that contribute to excessive energy intake. Because parents provide children with genes as well as the environment in which eating habits are formed, the study of familial contributions to appetite in young children may provide important insight on the development of obesity-related behaviors and risk (Davison, & Birch, 2001).

Appetite Regulation in Young Children

Consuming excessive amounts of energy is fundamentally an issue of appetite. Appetite can be described in terms of hunger which involves the drive to eat as well as satiation and satiety. Satiation refers to processes and feelings leading to the termination of eating and influences meal size (Blundell et al, 1993). Satiety involves process related to the suppression of subsequent intake after eating. Early studies of satiety demonstrated that, on average, children have an ability to self-regulate short-term energy intake by adjusting food intake in response to the amount of energy consumed (Birch & Dreysher, 1986; Johnson & Birch, 1994; Johnson, 2000). At the same time, large individual differences in the ability to self-regulate intake have been observed and self-regulation of energy intake has been found to be associated with weight status in children (Carnell &

Wardle, 2007). For instance, Johnson & Birch (1994) found that heavier children were less able to adjust their food intake at a meal in response to covert changes in the energy content of a first-course. Fisher and colleagues (2003, 2008) also observed an association with the amount of energy consumed by children in the absence of hunger when presented with palatable foods with current weight status. More recently, Carnell & Wardle (2008) found that child BMI was positively associated with both parental reports of lower child satiety responsiveness, which reflect the child's sensitivity to fullness, and higher enjoyment of food, which reflect the child's responsiveness to food cues. The basis of individual differences in children's regulation of appetite remains poorly understood. This research considers potential familial genetic contributions to appetite regulation in young non-Hispanic AA children and their interaction with child feeding influences.

Genetic Influences on Child Appetite and Weight

A heritable component of body mass is well-established, with estimates suggesting that 25-75% of population variance in body mass index is attributable to genetic effects (Segal, Feng, McCguire, Allison & Miller, 2009). There is increasing appreciation that genetics do not only influence body mass but behaviors contributing to energy-imbalance. The fat mass and obesity-associated (FTO) gene is perhaps one of the most well-studied genes associated with appetite as well as obesity (Frayling, Timpson, Weedon, Zeggini...& McCarthy, 2007). Genes contain natural variations, called Single Nucleotide Polymorphisms (SNPs), that are often associated with variation in phenotype and disease risk. Three SNPs of the FTO gene, rs9939609, rs8050136, and rs3751812, have been associated with obesity in a variety of populations (Grant, Li, Bradfield, Kim,

Annaiah, Santa,...Hakonarson, 2008; Larder, Cheung, Tung, Yeo, & Coll, 2011). In African American children, the SNPs rs8050136 and rs3751812 were shown to confer a risk for obesity comparable to the obesity risks seen in Caucasian children (Grant, et al, 2008).

The mechanism by which the FTO gene influences weight status is not fully understood. The FTO gene is thought to have effects on obesity through regulation of energy intake versus energy expenditure (Wardle, Carnell, Haworth, Farooqi...Plomin, 2008). In other words, instead of affecting the regulation of expenditure, the FTO gene affects food preference and appetite (Cecil, Tavendale, Watt, Hetherington & Palmer, 2008; Wardle, Llewellyn, Sanderson & Plomin, 2009). The influence on appetite is suspected to come from the FTO gene's expression in the hypothalamus (Larder et al, 2011). The hypothalamus, a portion of the brain, is responsible for integrating neural, endocrine and metabolic signals to regulate food intake and energy balance (Spiegelman & Flier, 2001). Disruption in hypothalamic regulation can disrupt satiety, causing increases in energy intake and subsequently increasing the risk for obesity.

There is some evidence linking FTO to appetite in children. Wardle et al (2009), found that FTO SNP rs9939609 was associated with a lower satiety responsiveness. In their study of 131 children aged 4-5 years drawn from the Twins Early Development Study (TEDS) which includes 10,000 pairs of twins born in England & Wales in 1994 & 1995, Wardle and colleagues (2009) followed the eating in the absence of hunger (EAH) protocol to determine if the high risk FTO alleles were associated with increased intake after having eaten to satiety. A significant difference was found among the different alleles within SNP rs9939609, with those children being either homozygous (AA) or

heterozygous (AT) for the risk "A" allele demonstrating higher intake than those who were homozygous for the non-risk allele (TT) (P=0.023 and P=0.027 respectively) (Wardle et al, 2009). There was no statistically significant difference found between the AT genotype and the AA genotype (Wardle, 2009). These findings suggest a link between variation in the FTO gene and responsiveness to satiety cues among children.

This research will extend the work by Wardle et al to consider associations of high-risk alleles of the FTO gene as well as FTO expression levels with parental self-reports of intake and observed child appetite-regulation, specifically children's susceptibility to overeat when presented with large portions. Measuring expression levels as well as FTO gene variation SNPs is of interest because variation in the amount of FTO mRNA may be the mechanism by which FTO gene variation affects appetite regulation in the hypothalamus (Larder et al, 2011). In mice studies, FTO null mice had significant reduction in fat mass and weight (Larder et al, 2011). Mice with over-expressed FTO exhibited increased body and fat mass (Larder et al, 2011). In addition, since it is not entirely clear how the FTO gene affects obesity, the FTO gene is evaluated as a potential moderator to determine if the effects of environment on appetite differ because of genetic risk and whether or not the FTO gene is expressed.

Parenting Style and Socialization Influences on Child Appetite and Weight

Because increases in obesity prevalence have occurred too rapidly to be explained by changes in genes alone, increased attention has been given to environmental and parental contributions to obesity. The family is the first and fundamental context for understanding environmental influences on children's eating (Davison & Birch, 2001). Parents play a key role in socializing their children, which includes helping their children

to regulate their behavior, including their eating habits. Parenting style reflects a broad approach to child socialization and creates the emotional climate that influence children's openness to socialization (Darling & Steinberg, 1993). The influence of general parenting styles on child development has been studied for decades and provides a framework for understanding how parents approach child feeding.

Diana Baumrind (1966 as cited in Darling and Steinberg, 1993) describe the parental role of socialization as "[conforming] to the necessary demands of others while maintaining a sense of personal integrity." Socialization goals include development of specific behaviors such as educational attainment, manners and social skills, as well as gaining independent thinking, and the ability to self-regulate behavior (Darling and Steinberg, 1993). This is accomplished through the parents' style or ideals about parenting and is manifested through their practices. Parenting style is a set of attributes that reflect the parents' goals and values (Darling and Steinberg, 1993). Parenting practices are the behaviors the parents exhibit to achieve a goal. Darling and Steinberg (1993) describe parenting style as having the greatest influence over children's willingness to be socialized and in turn, this willingness then moderates the effect of parenting practice on behavior. Parenting style is conceptualized on the two dimensions of demandingness and responsiveness.

Demandingness refers to the claims parents make on the child to become integrated into the family whole by their maturity demands, supervision, disciplinary efforts and willingness to confront the child who disobeys. Responsiveness refers to actions which intentionally foster individuality, self-regulation and self-assertion by being attuned, supportive and acquiescent to the child's special needs and demands. (199Ia, p. 748, as quoted in Darling and Steinberg, 1993).

The differing degrees of demandingness and responsiveness by parents influence the development of self-efficacy and self-control. Diana Baumrind (as cited in Maccoby and Martin, 1983) describes the following types of parenting style: authoritative, authoritarian and permissive. Maccoby and Martin (1983) further delineated the permissive style into two distinct styles of indulgent and neglectful.

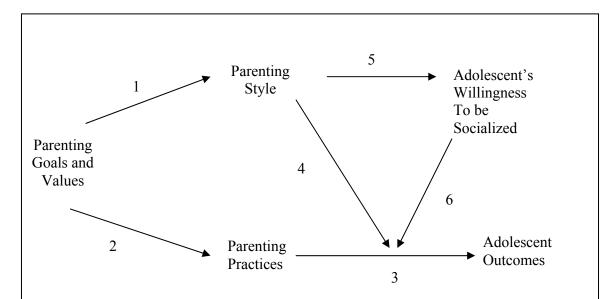
The authoritative style is considered optimal as this includes both responsiveness and affection based on the child's needs balanced with clear requirements of appropriate social behavior and responsibility. These parents tend to be firm yet understanding of the need for the child to learn autonomy (Maccoby and Martin, 1983). Generally, children of authoritative parents have been observed to develop higher levels of self-esteem, selfreliance and to better learn and function independently (Shea & Coyne, 2008). The authoritarian parent relies mainly on restriction and punitive types of behaviors with little responsiveness towards the child's autonomy and need to learn how to regulate their own behavior. Children of authoritarian parents have been observed to be emotionally volatile and sensitive to stressors (Shea & Coyne, 2008). These children have been observed to exhibit external behavior problems throughout childhood and adolescence. The third type is a permissive style, which is attentive to the child's needs but not applying restrictions on behavior. Children of permissive parents have been observed to have poorer development of self-regulation skills. (Shea & Coyne, 2008). They have been observed to be less goal-directed and have poorer self-reliance (Shea & Coyne, 2008). The last type of parenting style is neglectful, which is an uninvolved parent, who sets no rules and has low attentiveness to the child's needs. Table 1 demonstrates the differences between the parenting styles and their levels of demandingness and responsiveness.

Table 1. Baumrind Parenting Typology				
Responsiveness	High Demandingness Low Demandingness			
High Authoritative		Indulgent		
	Respectful of child's	Indulgent without		
opinion, maintains		discipline		
	clear boundaries			
Low Authoritarian		Neglectful		
	Strict disciplinarian	Emotionally uninvolved		
		and does not set rules		

The parenting typologies and associated outcomes outlined by Baumrind were based mainly on middle-class, European Americans. Differences in parenting style and children's adaptions need to be evaluated in a cultural context. In African American parents, the predominant style is authoritarian (Shea & Coyne, 2008). In contrast to European Americans, the AA children of authoritarian parents in low income environments were found to have less aggression and externalizing behavior (Tamis-LeMonda, Briggs, McClowry & Snow, 2008) Children of higher income, middle-class AA populations generally have better outcomes with authoritative parents (Tamis-LeMonda et al, 2008). The authoritarian approach, in low-income AA populations, has been interpreted as having a potentially protective influence on children's development in urban environments (Tamis-LeMonda et al, 2008).

Darling and Steinberg make a distinction between parenting styles and parenting practices. They provide an example using the authoritative parenting style by describing "...the authoritative mother encourages verbal give-and-take and shares with the child the reasoning behind her policies, but her authoritativeness is independent of the content of her socialization." For example, authoritative parents may have different ways of expressing their style such as requiring homework to be completed before going out to

play, whereas another might require the opposite. Furthermore, parenting style expresses the attitudes of the parent towards the child versus the child's behavior. Figure 2 below illustrates Darling and Steinberg's (1993) model of parenting style.



Parenting goals for socialization influence both parenting style (Arrow 1) and parenting practices (Arrow 2). Parenting practices have a direct effect on specific child developmental outcomes (Arrow 3). In contrast, parenting style influences child development primarily through its moderating influence on the relationship between parenting practices and developmental outcomes (Arrow 4) and through its influence on the child's openness to parental socialization (Arrow 5). The child's openness to socialization also moderates the influence of parenting practice on the child's development (Arrow 6).

Figure 2: Contextual model of Parenting Style

Source: Adapted from Darling, N. & Steinberg, L. (1993). Parenting style as context: An integrative model. *Psychologic Bulletin*, 113(3), 487-96.

Parenting Style and Childhood Obesity

More recently, parenting style and its relationship to parenting practices have been considered in relationship to overweight or obesity in children. Rhee et al (2006) evaluated the relationship between general parenting style and child overweight status in first grade using data from the National Institute of Child Health and Human Development Study of Early Child Care and Youth Development. This study

demonstrated a difference in the BMI of authoritative styles compared to all other parenting styles. The authoritarian style (high demandingness/low responsiveness) had a 4 fold risk of childhood overweight compared to the authoritative style, permissive and neglectful parenting styles demonstrated a 2 fold risk of overweight. However, this study included 82% white children, and was cross-sectional; therefore, a causal relationship could not be determined and this study may not be applicable to other racial/ethnic groups.

Hughes et al (2005) applied the typologies of parenting styles to the feeding domain based on demandingness and responsiveness in child feeding: authoritarian, authoritative, indulgent and uninvolved. Authoritative feeders choose the foods that children eat, but the child decides which foods and how much they eat. Authoritarian feeders use restrictive or power-assertive behaviors to get their child to eat. Indulgent feeders display a lack of control over what the child eats. Uninvolved feeders place no demands or response to the child and also have lack of control over what the child eats. In a study of 130 Hispanic and 101 AA families, Hispanics had a higher level of the indulgent feeding style (Hughes et al, 2005). Hispanics with the indulgent feeding style also had a higher BMI z-score than the authoritarian feeding style. For African Americans in the study, they showed a higher percentage of authoritarian style, and uninvolved style. African-American parents with the authoritarian feeding style had children with lower BMI z-scores, which contradicts previous work suggesting that authoritarian parenting was associated with higher BMI z scores (Hughes et al. 2005). This study suggested more research should be conducted on evaluating cultural

differences in feeding styles, specifically to understand mechanisms through which indulgent feeding styles may increase obesity risk in young children.

More recently, Hughes, Shewchuk, Baskin, Niklas and Haiyan (2008) examined the association of the indulgent feeding style with weight status in low-income preschool families. The participants were African-American, Hispanic and White children enrolled in Head Start. The indulgent feeding style was found to be associated with an increased BMI compared to other feeding styles (Hughes et al, 2008). In contrast to previous studies of middle class families, the authoritarian feeding style showed an association with lower child weight status when compared to the indulgent feeding style in this low-income population (Hughes et al, 2008).

In summary, authoritative approaches to child feeding that balance demands with responsiveness to the child's needs and cues are thought to have protective influence on child obesity risk, but can differ based on race and income. The studies by Hughes and colleagues (2005, 2008, 2009) suggest the indulgent feeding style as conferring the greatest risk of childhood obesity in low income, multi-racial groups compared to the authoritarian feeding style. In a recent systematic review, Hurley, Cross and Hughes (2011) found positive associations between parental feeding control and child weight gain/status. Their review characterized research related to responsive feeding.

"Responsive feeding is characterized by caregiver guidance and recognition of the child's cues of hunger and satiety. Nonresponsive feeding is dominated by a lack of reciprocity between the parent and child, with the caregiver taking excessive control of the feeding situation (forcing/pressuring or restricting food intake), the child completely controlling the feeding situation (indulgent feeding), or the caregiver being completely uninvolved

during meals (uninvolved feeding)." (Hurley et al, 2011, p495) Although, most of the studies reviewed were cross-sectional, many did find a relationship between feeding responsiveness and BMI *z* score. Hurley et al, reported that indulgent feeding showed a positive relationship between BMI and overweight. In particular, indulgent feeding showed decreased intake of fruits and vegetables and a preference for soda and sweets (Hurley, et al, 2011).

The associations of parent feeding styles and children's eating behavior with BMI are clear; however, the mechanisms are not fully understood. The mechanism could hypothetically involve the types of foods offered, the eating experiences offered, and/or the effects of feeding practices on children's eating behaviors and appetite.

This study builds on the current research by evaluating parent feeding style in AA parents to determine if differences in parent feeding style are related to child appetite regulation. More specifically, this study seeks to understand the relationship between indulgent feeding styles and the effects on appetite regulation in AA children, using both parent-reported and observational measures of eating. Most of the research to date has focused on the relationship of parent feeding style and weight status without regard to assessing food intake and regulation (Hughes et al, 2011).

Hypotheses

The primary aim of the study is to evaluate the association between feeding style and appetite regulation in African American children between 5 and 6 years old.

Children of indulgent parents are expected to exhibit poorer appetite regulation than children of non-indulgent parents. Appetite regulation was measured using the CEBQ

and the observed change in energy consumed across meals at which portion sizes were systematically increased.

A secondary aim of the study was to evaluate whether the FTO gene moderated the relationship between parent feeding style and appetite regulation in African American children, aged 5-6 years. The FTO gene was expected to moderate the relationship between parent feeding style and appetite regulation. Children of indulgent feeders were expected to be associated with poorer appetite regulation when children have high-risk FTO alleles. FTO was also expected to be over-expressed in children of indulgent parents thus having decreased satiation.

CHAPTER 3

METHODS

Study Design

An observational design was used to evaluate the association between maternal feeding styles and child appetite among obese and non-obese African-American (AA) children aged 5 to 6 y. Child feeding styles were measured using parental report. FTO SNPs and expression were evaluated using saliva samples among the children. Child appetite was assessed by observation of food intake in a laboratory setting as well as by parental reports. Child heights and weights were measured at baseline.

Study Population

Participants were normal weight and obese 5 to 6 y old AA children and a parent with primary responsibility for feeding the child at home. Convenience sampling was used with participants recruited using flyers posted throughout various North Philadelphia locations including Temple Children's Hospital Outpatient clinics, child-care centers, Head Start Programs, and Clinics of the Special Supplemental Nutrition program for Women, Infants, and Children. Additional recruiting efforts including posting online information through www.craigslist.org and placing ads in METRO magazine. Compensation for participation was as follows: \$30 for each visit and a \$100 bonus for attending the 6 month follow-up. To be eligible for participation, children had to be 5 or 6 y old at the time of the study, be of either normal weight (BMI-for-age percentile 5^{th} - 85^{th}) or obese (BMI-for-age percentile $\geq 95^{th}$) parent reported race/ethnicity as African American or Non-Hispanic Black, and the children had a preference for the food served in the study. Exclusion criteria included: baseline BMI-for-age at less than

the fifth percentile, BMI-for-age between the 85th and 94th percentile, and any medical conditions, medication use, developmental delays, or food allergies known to affect food intake and growth as reported by the caregiver. Children were also excluded if they disliked the main entrée or two more of the other 4 foods provided on the menu used to measure children's observed satiation. Children were screened for all of these criteria upon first visit, and if they did not meet the criteria, they were not enrolled. The study enrolled 100 children. Six individuals did not complete all portions of the study. All data were collected at the Center for Obesity Research and Education at Temple University, Philadelphia, PA. All procedures were reviewed by and carried out in accordance with procedures approved by the Temple University Institutional Review Board.

Measures

Demographic Data

During return visits to the food lab parents were provided time to complete questionnaires while the children participating in the sessions were provided a meal.

Demographic data included parent-reported race, gender and age of the children. Parents also reported their highest level of education and if they used WIC, Food Stamps or Head Start.

Height and Weight

Height was measured in duplicate to the nearest 0.1 cm and weight to the nearest 0.1 kg using digital scales and stadiometers in the lab. The height and weight of each child was used to generate BMI–for-age percentiles and z-scores using US reference data and growth charts created by the Centers for Disease Control (Kuczmarski, Ogden, Guo et al, 2002)

Feeding Style

Child Feeding Styles Questionnaire. Parental feeding style was measured by selfreport and classified according to Hughes et al (2005) as: authoritative, authoritarian, indulgent and uninvolved. Categorization of these feeding styles was accomplished using the responses from the CFSQ administered at the beginning of the study. The CFSQ was developed for use with low-income AA and Hispanic parents of young children and contains 19 item questionnaire describing verbal and physical feeding strategies used by parents to get their children to eat. Responses included never, rarely, sometimes, most of the time, and always. Ranging from values of 1 to 5. Two scores are developed based on responses to the questions to represent demandingness and responsiveness. All items on the questionnaire are designed to assess demandingness. A subset of the questions (7) represents child-centered strategies to make the child eat. To score demandingness, a total mean score is calculated across all 19 items; to score responsiveness, a ratio of the mean of the 7 child-centered items over the mean of the total score is calculated. Median splits are then calculated and parents categorized based on high/low demandingness and responsiveness as described in Hughes et al., 2005. The CFSQ had a reliability Pearson correlation of .85 on demandingness and .82 for child-centered strategies/responsiveness when measured in Hispanic and Non-Hispanic African American parents (Hughes, 2005). Associations with the parenting dimensions inventory (PDI-S), which assesses overall parenting style (Hughes et al, 2005) provided evidence of convergent validity.

Appetite Regulation

To evaluate appetite regulation, this study used parent self-report and observational assessment of appetite regulation.

Child Eating Behavior Questionnaire. Satiety responsiveness was measured using the 35 item CEBQ which assesses a number of dimensions of appetite; food responsiveness(5 items), enjoyment of food (4 items), emotional over-eating (4 items), desire to drink(3 items), satiety responsiveness(5 items), speed of eating(4 items), emotional under-eating(4 items), and food fussiness(6 items). Carnell & Wardle (2007) showed evidence of validity of the CEBQ as a measure for the risk of obesity. They focused on the three dimensions: Satiety Responsiveness (SR), Food Responsiveness (FR) and Enjoyment of Food (EF). The results of the CEBQ, from a sample of 4 and 5 year olds from the Twins Early Development Study in the United Kingdom, were associated with eating behavior observed over multiple school-based eating sessions. The SR dimension showed the strongest relationship to the observational tests, and a higher SR was associated with lower EAH and better food responsiveness (Carnell & Wardle, 2007). This study focuses on satiety responsiveness as the dimension of interest because it has been related to eating behavior, obesity and the FTO gene in previous research (Carnell & Wardle, 2007 and 2008).

Child susceptibility to portion size. The second assessment of appetite regulation is through direct observation of food intake to determine portion susceptibility. Observed child susceptibility to increasing portion sizes were measured using weighed methods across four dinner conditions in which portion sizes of a standard meal, including macaroni and cheese, cookies, applesauce, and corn, were presented at 100%, 150%,

200%, and 250% of those offered in the reference condition (See Figure 3). Dinner conditions took place once a week, for four weeks. The order of presentation was randomly assigned to small groups of children. Parents were instructed to refrain from offering food or drink to children for 2 hours prior to arrival at the laboratory where dinners occurred. Small groups of 2-4 children ate together, separately from their parent. A trained research assistant was present to ensure that food was not shared, that dropped food and spills were recorded, and that conversations about eating and/or food were avoided. All meals were timed at 20 minutes. Children were instructed to eat as much or as little as desired. Food intake was measured using weighed methods by digital balances. Energy intakes were calculated using manufacturer's information. Portion susceptibility was operationalized as the change in energy intake across conditions of increasing portion size. Susceptibility to portion size was calculated for each child using random slope mixed effects linear models. This new variable representing the slope of the intake in kcal was used as the dependent variable subsequent analyses.



Figure 3: Portion size conditions % of reference portion provided: 1=100%, 2=150%, 3=200% and 4=250%

FTO Genotype and Expression

The study center collected DNA and RNA through saliva samples from each child participant. These data were taken from a larger study evaluating differences in eating patterns and behaviors of young children. Of the 100 children enrolled, 32 obese children and a random sample of 32 non-obese children were selected for genotyping and expression analysis. Three FTO gene SNPs were genotyped: rs9939609, rs3751812 and rs8050136. These SNPs have previously been associated with obesity (Grant et al, 2008; Larder et al, 2011). Of the 62 children genotyped, 60 children had data available for FTO genotype at each SNP and mRNA levels of the FTO gene and also had data available on feeding style, satiety responsiveness, and portion susceptibility. Twenty-nine were

classified as obese (48%) and 31 were classified as non-obese (52%). RNA extraction was performed after RNA purification using Quiagen's (Germantown, MD) RNeasy Micro Kit. DNA was purified and dissolved in TE buffer. Both RNA and DNA were quantified by spectrophotometry using the nanodrop 1000 (Gardner, 2011).

FTO SNPs. DNA sequence analysis was performed for the 3 SNPs associated with FTO and obesity and identified alleles were associated to each subject. Table 2 shows the 3 FTO SNPs of interest and their corresponding risk alleles.

Table 2: FTO Gene SNPs				
SNP	Risk Allele	Alleles		
rs9939609	A	A/T		
rs8050136	A	A/C		
rs3751812	T	T/G		

Oligonucleotide primers adjacent to each SNP were used to amplify the relevant FTO gene segment by polymerase chain reaction (PCR). The purified PCR products were sent to Genewiz (South Plainfield, NJ) for DNA sequencing (Gardner, 2011). Results were downloaded from the Genewiz web site and the accuracy verified with FinchTV trace viewer software (Gardner, 2011).

FTO Expression. Transcript analysis to determine FTO mRNA level was carried out using Applied Biosystem's (ABI) (Carlsbad, CA) Taqmen Gene Expression Assays designed for FTO and TBP(Gardner, 2011). TBP is a housekeeping gene used for comparison of expression of the FTO gene (Gardner, 2011). FTO mRNA levels were easily detectable using quantitative real time reverse transcriptase polymerase chain reaction (qRT-PCR) (Gardner, 2011). FTO expression was determined by calculating the fold-change from the delta cycle threshold (ΔCT) for each individual (Gardner, 2011).

The Δ CT is used to relate the difference in the level of transcript of FTO to the transcript level of the housekeeping gene TBP. Individuals who have similar transcript levels of TBP and the gene of interest will be close to zero. Individuals with greater transcript levels for FTO compared to TBP will have higher Δ CT values (Gardner, 2011).

Procedures

The measures included in this analysis were a subset of those collected for the larger study on individual differences in eating behavior among AA children.

Measurements taken at baseline included: height and weight, a Dual Energy X-ray

Absorptiometry (DEXA) scan to measure total body composition and fat content, a cheek swab for genetic testing, child food preference and a measure of delay of gratification.

During return visits to the food lab, parents were provided time to complete questionnaires while the children participating in the sessions were provided a meal.

Statistical Analysis

Statistical analysis was performed using SAS statistical software (version 9.2; SAS Institute, Cary, NC). Descriptive statistics were performed on the study participant's gender, child BMI at baseline, maternal education level, participation in federal low-income programs and obese status (BMI-for-age $\geq 95^{th}$ percentile or BMI-for-age $< 95^{th}$ percentile). Means and standard deviations were computed for all continuous variables. Frequency distributions were computed for categorical variables. Comparisons of these variables between the parent feeding styles were made using ANOVA for continuous variables. Chi-square tests were used for categorical variables and Fisher's exact tests were used when the expected cell sizes were less than 5.

First, parent feeding style was used as an independent predictor of satiety responsiveness using ANOVA. Then, each parent feeding style was categorized into 4 separate variables (authoritative, authoritarian, indulgent and uninvolved) and used as independent predictors in multivariate linear regression. The indulgent feeding style was operationalized as a dichotomized variable, used as the primary effect of interest, and compared to the other feeding styles. To determine the relationship between parent feeding style and satiety responsiveness, the CEBQ satiety responsiveness scores were treated as continuous variables, using the mean item score for the sub-scale as the outcome measurement. Because lower satiety responsiveness scores indicate a decreased ability to self-regulate intake, satiety responsiveness scores were reverse scored so the results of the analysis would estimate the probability of lowered response scores. To determine whether parent feeding style predicts child susceptibility to portion size, portion susceptibility slope estimates were treated as a continuous outcome variable.

Variables considered to potentially confound the relationship between parent feeding style and appetite regulation included child's obese status, mother's education and participation in federal low-income programs programs, including WIC, Head Start or Food Stamps. Child weight status was evaluated as a potential covariate to ensure that associations of feeding style with portion susceptibility were not due to child weight status (Fisher & Kral, 2008, Fisher, Liu, Birch & Rolls, 2007). Maternal education was also included given associations with maternal BMI (Ogden, Lamb, Carroll & Flegal, 2010). Participation in federal low-income programs was used as a surrogate of low-income level. Each covariate was tested for interactions between themselves as well as the indulgent feeding style using backward elimination. All covariates were kept in the

model as main effects regardless of statistical significance on the theoretical grounds that these are all contributory factors influencing parent feeding style and child behavior. The authoritative feeding style was used as the reference group in multivariate regression.

Odds ratios and 95% confidence limits were estimated based on the differences of the mean satiety responsiveness and mean portion susceptibility slope for each independent variable.

The interaction of FTO gene SNPs and feeding style was tested to evaluate a potential moderating influence of the FTO gene on appetite regulation. To examine the relationship between each of the FTO SNPs and parent feeding style Chi-square tests were used and Fisher's exact tests were used when the expected cell sizes were less than 5. A *p*-value < 0.05 was considered statistically significant. Logistic regression was used to evaluate any potential interactions between SNPs and parent feeding style. Because of the potential for low sample size, feeding style was also dichotomized as either indulgent or not indulgent, as this was the feeding style of interest, and demonstrated an association between decreased satiety responsiveness and susceptibility to portion size.

The interaction of FTO gene expression and feeding style was tested to evaluate a potential moderating influence of the FTO gene on appetite regulation. First, logistic regression was used to determine if feeding style was associated with FTO expression. A second model assessed whether parent feeding style predicts the portion susceptibility in those children with genetic data available using linear regression. Finally, a model was constructed evaluating whether FTO expression, parent feeding style and their interaction affect portion susceptibility using multivariate linear regression. Feeding style was again

dichotomized as either indulgent or not indulgent. Odds ratios and 95% confidence limits were estimated based on the differences of the mean satiety responsiveness and mean portion susceptibility slope for each independent variable and the interaction between feeding style and FTO gene expression.

For all analyses, a p-value < 0.05 was considered to be statistically significant. All data are presented as mean (SD) unless otherwise indicated.

CHAPTER 4

RESULTS

Sample Description

Ninety-four individuals had data related to feeding style and were included in the descriptive analysis and further analysis of parent feeding style and appetite regulation. Of these, 43 children (46%) were male, 29 children (31%) were classified as obese, defined as greater than or equal to the 95th percentile. Thirty-eight (40%) of the caregivers had less than a high school education, and 80 (85%) of the caregivers used some type of assistance. Feeding styles were fairly evenly distributed with 28 authoritarian, 19 authoritative, 28 indulgent and 19 uninvolved. Table 3 describes the characteristics of the child participants by feeding style. There were no statistically significant differences across the parent feeding styles in these measurements (no p-value <0.05).

Table 3: Descriptive Statistics by Parent feeding style (n=94)				
	Authoritarian	Authoritative	Indulgent	Uninvolved
N	28	19	28	19
Obese	9 (32%)	6 (32%)	10 (36%)	5 (26%)
Non-obese	19 (68%)	13 (68%)	18 (64%)	14 (74%)
Gender -Male	17 (61%)	8 (42%)	8 (29%)	10 (53%)
Less than HS	13 (46%)	7 (37%)	13 (46%)	5 (26%)
education				
Above HS	15 (54%)	12 (63%)	15 (54%)	14 (74%)
Education				
Use of WIC,	22 (79%)	16 (84%)	25 (89%)	17 (89%)
Head Start or				
Food Stamps				

Feeding Style and Child Appetite

Parent report of satiety responsiveness

On average, parental report of child satiety responsiveness (SR) was 1.43 ± 0.72 . In descriptive terms, children of indulgent parents were reported as having the lowest mean SR of the 4 feeding styles considered at 1.21 ± 0.76 (Figure 4). The ANOVA using feeding style as the independent variable and satiety responsiveness as the dependent variable demonstrated feeding style accounting for 4% of the variance in portion susceptibility with an F Value of 1.34 and p=.2662.

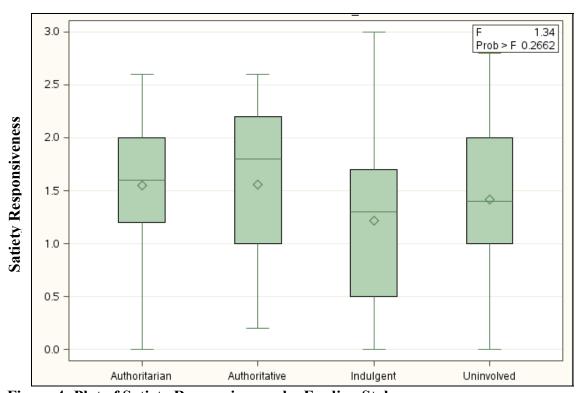


Figure 4: Plot of Satiety Responsiveness by Feeding Style

When tested in an unadjusted generalized linear model, using feeding style as the independent variable and satiety responsiveness as the dependent variable, the indulgent parent feeding style was not statistically significant predictor of parental reports of child SR (p < 0.10, OR 1.41, 95% CI 0.94-2.11) (Table 4).

Table 4: Feeding Style and Satiety Responsiveness (n=94)								
Variable	Parameter Estimate	Standard Error	Wald Chi-Sq	Pr > ChiSq	Wald 95% Confide	nce Limits		
Intercent	-0.56	0.16	12.32	0.0004	-0.87	-0.25		
Intercept								
Indulgent	0.34	0.21	2.78	0.0952	-0.06	0.75		
Uninvolved	0.14	0.23	0.37	0.54	-0.03	0.58		
Authoritarian	0.01	0.21	0.00	0.9694	-0.40	0.41		

Note: Linear regression model with authoritative as the reference category; Confidence Limits that do not include 0 are significant

To determine if child gender, child obesity (at or above the 95th percentile for age and gender) versus not obese, and maternal factors such as education and participation in federal low-income programs explains the variation in satiety responsiveness, these variables were added to the multivariate linear model, including the interaction terms. Of the covariates, child obesity was associated with SR, where obese children were rated by their parents as having lower SR (p <0.05, OR: 1.39, 95% Confidence Limit (CL) 1.03-1.87). A main effect of child gender on SR was not significant; however, there was a statistically significant interaction between gender and indulgent feeding style (p<0.02, OR: 1.64, Wald 95% CL 0.18-1.43). Specifically, sons of indulgent feeders had lower satiety responsiveness than non-indulgent feeders (OR: 2.49, =exp(0.91), CL 1.40 – 4.40), but there was no association between feeding style and satiety responsiveness for daughters (OR: 1.11, =exp(0.11), CL 0.72-1.71) (Figure 5).

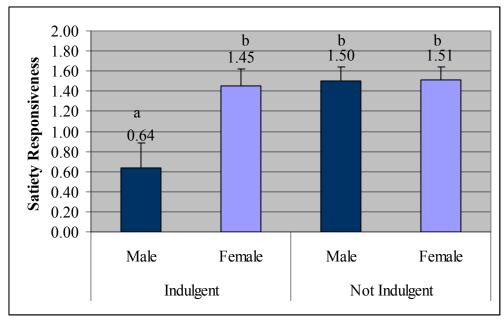


Figure 5: Satiety Responsiveness by Gender Error bars indicate SEs. Different letters denote significance, p < 0.01

Maternal education level and participation in federal low-income programs plans were not associated with satiety responsiveness, and they did not affect the association between the indulgent feeding style and satiety responsiveness. The final model is shown in Table 5. In the adjusted model, the relationship between indulgent and satiety responsiveness was significant, but moderated by gender. Additional interaction terms were removed due to non-significance.

Table 5: Association of Feeding Style and Satiety Responsiveness (n=94)								
Source	Estimate	Standard Error	P> z	Odds	95% CL			
				Ratio				
Indulgent	0.51	0.20	0.01	1.66	1.12-2.47			
Uninvolved	0.14	0.21	0.52	1.15	0.76-1.73			
Authoritarian	0.02	0.20	0.91	1.02	0.70-1.50			
Gender (male)	0.41	0.16	0.01	1.50	1.10-2.05			
Obese	0.33	0.15	0.03	1.39	1.04-1.87			
Gender(male)*Indulgent	0.91	0.29	0.002	2.49	1.40-4.40			
Maternal Education	-0.11	0/15	0.44	0.89	0.67-1.19			
Participation in federal	0.11	0.20	0.58	1.12	0.76-1.63			
low- income programs								

Note: Dependent variable is reverse scored; higher estimates indicate lower satiety responsiveness.

Feeding style and susceptibility to portion size

The slope of energy intake overall was 0.66 ± 1.16 . In descriptive terms, children of indulgent parents showed the highest susceptibility to portion size relative to the other feeding styles at 1.08 ± 1.24 (Figure 6). The ANOVA using feeding style as the independent variable and portion susceptibility as the dependent variable demonstrated feeding style accounting for 8% of the variance in portion susceptibility with an F Value of 2.67 and p = .0526.

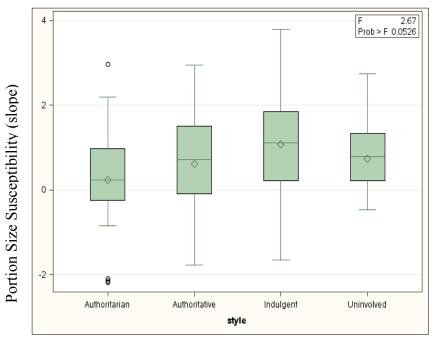


Figure 6: Plot of Slope of Energy Intake (Kcal) across Feeding Styles

As seen in Figure 5, the data for children of authoritarian parents appear to contain potential outliers. Univariate analysis of the slope of kcal demonstrated that all values are within 3 standard deviations of the mean; therefore, all data are retained in the analysis. Analyses were run with and without the outlying cases to examine their influence on susceptibility to portion size, and similar results were obtained. Therefore, all cases were retained in these analyses.

Using authoritative as the reference condition, in multivariate regression, the indulgent feeding style was not associated with greater susceptibility to portion size (estimate 0.45, p <0.17). However, using authoritarian as the reference condition, indulgent feeding style was associated with greater child susceptibility to large portion sizes (p = 0.0041, OR 2.33 95% CL 1.31, 4.15). The uninvolved and authoritative feeding styles were not associated with increased in susceptibility to portion size (p = 0.1182, 95% CL 0.78, 2.18 and 0.2320, 95% CL 0.88, 3.18 respectively). Table 6 demonstrates the regression coefficients for the unadjusted model of parent feeding styles

and portion susceptibility. Evaluation of the model without the potential outliers yielded similar results.

Table 6: Parent Feeding Style and the Association with Portion Susceptibility (slope of kcal) (n=94)							
Variable	Estimate	Standard Error	<i>p</i> -value	OR (95% CL)			
Intercept (Authoritarian)	0.23	0.21	0.28	-			
Indulgent	0.85	0.30	0.004	2.33 (1.31,4.16)			
Authoritative	0.39	0.33	0.232	1.48 (0.78,2.82)			
Uninvolved	0.51	0.33	0.118	1.67 (0.88,3.18)			

Note: Multivariate regression using Maximum Likelihood, authoritarian used as the reference condition.

In contrast to parents' reports of satiety responsiveness, the covariates of gender and obese status did not affect the portion susceptibility. The main effects of maternal education and participation in federal low-income programs also did not change the relationship between the indulgent feeding style and portion susceptibility. Although, those reporting participation in federal low-income programs at less than those without participation in federal low-income programs, the effect of the indulgent feeding style on susceptibility to portion size remained significant (p <0.01) in the adjusted model (Table 7).

Table 7: Association with Portion Susceptibility from indulgent feeding style, adjusted for gender, obesity, education and participation in federal low-income programs (n=94)							
Parameter	Estimate	Standard Error	Pr > ChiSq	OR (95% CL)			
Indulgent	1.01	0.30	< 0.001	2.76 (1.54,4.95)			
Authoritative	0.49	0.33	< 0.125	1.64 (0.87,3.09)			
Uninvolved	0.63	0.33	< 0.053	1.88 (0.99,3.55)			
Obese	0.24	0.25	< 0.33	1.27 (0.78,2.07)			
Gender*	0.33	0.23	< 0.153	1.39 (0.88,2.19)			
Maternal Education†	0.03	0.24	< 0.91	1.03 (0.64, 1.65)			
Participation in federal low-income	-0.65	0.32	< 0.044	0.52 (0.28,0.98)			
programs							

^{*}effect= male

Because there was an association between parent feeding style and portion susceptibility, a separate exploratory analysis was performed to see if parents' reports of satiety might be associated with portion susceptibility. Figure 7 demonstrates that parents' reports of lower satiety responsiveness was associated with portion susceptibility with a Pearson Correlation Coefficient of -0.30, R-Square of 0.08, and a p < 0.01).

[†] effect= HS education or less

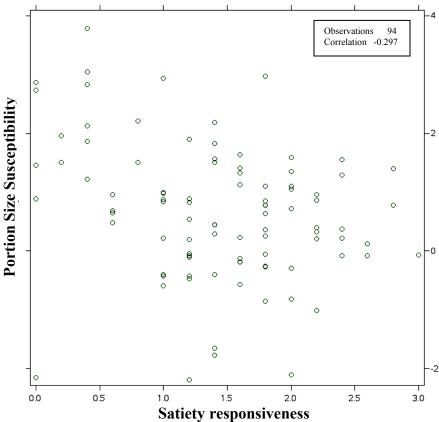


Figure 7: Correlation of Satiety Responsiveness and Portion Susceptibility

FTO Gene and Expression

The distribution of the 3 SNPs associated with the FTO Gene (rs3751812, rs8050136 and rs9939609) and their allele frequencies are illustrated in Table 8.

Table 8: Frequency of FTO SNPs and Associated Alleles* (n=60)							
	•	Feeding St	yle	,			
SNP	Authoritarian	Authoritative	Indulgent	Uninvolved			
FTO_rs3751812							
GG	12	9	14	12			
GT	3	5	4	0			
TT	0	0	1	0			
FTO_rs8050136							
AA	1	3	2	1			
CA	9	8	12	9			
CC	5	3	5	2			
FTO_rs9939609							
AA	1	5	4	2			
AT	9	7	9	8			
TT	5	2	6	2			

^{*} The risk allele is A for both rs8050136 and rs9939609 and T for rs3751812.

Because the distribution across feeding styles produced low sample sizes, a statistical analysis was not completed as both the chi-square tests and Fisher's exact tests would not be interpretable.

The indulgent feeding style was then dichotomized and used in the model as an independent predictor along with each of the FTO SNPs and their interaction with the indulgent feeding style and their effect on portion susceptibility. Each of the FTO SNPs was also dichotomized into those with a high risk allele (homozygous and heterozygous) and low risk allele. To moderate the relationship between the indulgent feeding style and portion susceptibility, there must be a relationship between the indulgent feeding style and portion susceptibility. In this smaller sample, there was no association between the indulgent feeding style and portion susceptibility (p <0.07). In addition, there was no association with any FTO SNP and portion susceptibility (rs8050136 p<0.46, rs3751812 p<0.62, rs9939609 p<0.46). The test of the interactions between the FTO SNPs and the

indulgent feeding style demonstrated no statistically significant relationship with portion susceptibility (data not shown).

Another consideration for the association between the FTO gene and portion susceptibility may be through the expression of FTO. The effect of FTO expression was modeled to determine the relationship between expression and portion susceptibility. There was no significant relationship found (est 0.0567, p = 0.5304 (Table 9). The same model was run for FTO expression and satiety responsiveness, with no significant relationship (est -0.017, p = 0.7583) (data not shown).

Table 9: Effect of FTO expression on Portion Susceptibility							
Variable	DF	Estimate	SE	t Value	Pr > t		
Intercept	1	0.74	0.20	3.7	0.0005		
FTO Expression	1	0.06	0.09	0.63	0.5304		

When the interaction of FTO expression and feeding style, along with obese status as a potential confounder, was added to the model with portion susceptibility as the dependent variable a statistically significant interaction did not exist between feeding style and FTO expression (Table 10), nor did it change the relationship between the indulgent feeding style and portion susceptibility. Therefore, it does not appear that FTO mRNA levels, measured in saliva, moderate the relationship between the indulgent feeding style and portion susceptibility.

Table 10: Effect of Feeding Style and FTO Expression on Portion Susceptibility							
Source DF		Type III SS	F Value	Pr>			
Style	3	9.96	2.31	0.087			
FTO Expression	1	2.63	1.83	0.18			
Obese	1	0.20	0.14	0.71			
Expression*style	3	3.03	0.7	0.55			

CHAPTER 5

DISCUSSION

Child obesity is essentially a problem of energy imbalance and influences on child appetite may be central to understanding obesity risk at an early age. Families provide a central context for understanding the development of children's appetite. Most of the research on child feeding has focused on weight outcomes (Hurley, Cross & Hughes, 2011, Hughes, et al 2008, Ventura & Birch 2008 and Carnell & Wardle 2006-2008). The findings of this research provide new evidence of an environmental influence on appetite regulation among high risk AA, urban children. Children of indulgent feeders were observed to have increased energy intakes as portion sizes were systematically increased, and were rated by their parents as having poorer satiety responsiveness. The findings suggest that indulgent approaches to child feeding may have negative effects on children's regulation of appetite and overall eating patterns. This is important because in contrast to Non-Hispanic White populations, where authoritarian feeding has been associated with higher BMI scores, in AA populations indulgent feeding has been associated with higher BMI, and authoritarian feeding has been associated with lower BMI (Hurley, Cross & Hughes, 2011, Hoerr et al, 2009, Patrick et al, 2005).

Previous studies of food portion size effects on young children's eating have largely failed to explain large individual differences in children's susceptibility to overeat when presented with large portions (Fisher & Kral, 2008). The results of this research provide new evidence that parents' approach to child feeding may influence children's susceptibility to obesogenic aspects of the eating environment. In this research, the association of indulgent feeding styles with portion size susceptibility was observed even

after adjusting for a number of potentially confounding factors including child gender, child obesity, maternal education and participation in federal low-income programs. Previous research has shown that permissive feeding styles in low income families are associated with lower intake of fruits and vegetables (Hoerr et al, 2009). The present findings advance this knowledge by demonstrating that the appetite regulation of children of indulgent feeders may be more susceptible to obesogenic aspects of the environment, including large food portion sizes. Previous research established an association of indulgent feeding patterns among AA parents with increases in BMI (Hughes et al, 2008, 2011). This work builds on these findings through direct observation of food intake based on the portions served thus providing a possible mechanism through which indulgent feeding style may influence weight status—by inhibiting self-regulation of appetite. The reason(s) explaining the role of indulgent feeding styles and susceptibility to large portion sizes among AA children is unclear. Experimental research on portion sizes in children show that children consume more energy at meals when larger portion sizes are offered (Fisher and Kral, 2007). One interpretation of the present findings is that children of indulgent feeders may be routinely offered larger portion sizes and, in turn, routinely consume larger portion sizes than other children. Future research should examine the feeding practices used by indulgent feeders including the presence of large portions in the home and/or offering children large food portion sizes. Alternatively, it may be that children with greater appetites elicit more indulgent approaches to feeding. Additional research is needed to evaluate these interpretations and disentangle the directionality.

In the parent report measure of child satiety responsiveness, children of indulgent feeders were found to have a lower satiety score compared to children with the other parental feeding styles even when gender was applied as a moderator. Sons of indulgent feeders had lower reported satiety responsiveness than non-indulgent feeders; however, this same effect was not seen for daughters. Studies of feeding style have not previously considered associations with child satiety responsiveness using the CEBQ (Hurley et al., 2011). Of those that looked at other aspects of child appetite using the CEBQ in relation to parent feeding style, they did not measure satiety responsiveness. Joyce & Zimmer-Gembeck (2009) measured the roll of restriction with food responsiveness and emotional overeating and found that children with restrictive parents had higher reported food responsiveness and emotional overeating. Powers et al (2006) focused on the parenting dimensions of restriction and control and child eating behaviors of food responsiveness and desire to drink. They found that increases in BMI of children were only associated with restriction when the mother was also obese. Neither of these studies found a moderating effect of gender, nor did they measure satiety responsiveness. This study provides new evidence that the indulgent feeding style, as opposed to the more restrictive authoritarian style is associated with reported lower satiety responsiveness in AA boys. The satiety responsiveness score is based on what parents perceive as the child's behavior with eating and may be associated with a parent's perception of how much a child should eat. This suggests that parents' perception of appetite may differ for sons than daughters. Further studies should test whether gender differences in parent satiety measures of appetite persist.

This study also found that obese children demonstrated lowered satiety responsiveness. These findings support previous research that higher BMI-for-age was association with a lower satiety responsiveness score. (Carnell & Wardle, 2008; Sleddens et al, 2008). The relationship of childhood obesity and the outcome of satiety by parent report and observation also differed. Specifically, observed child susceptibility to portion size was unrelated to weight status. However, this is in line with other research, where child weight status was also not associated with children's intake of large portion sizes in a laboratory environment (Fisher et al, 2007).

A strength of this study was the ability to evaluate both parental reported as well as observed child appetite regulation. This combined the methods from previous research and measured satiety and regulation with the same population. There were different patterns of results based on the different approaches. In the parent reported measurement of satiety, the indulgent feeding style was only associated with satiety responsiveness for boys; however in the lab measure of portion susceptibility a difference by gender did not exist in the relationship between the indulgent feeding style and portion susceptibility. In addition, obese children were reported to have lower satiety responsiveness by their parents; however, in the lab measure of portion susceptibility, child obesity did not have a significant association with portion susceptibility. The measurements themselves may reflect different aspects of children's appetite. The measurement of portion susceptibility showed how much the children ate during each of the meals served. The parents were instructed not to have the children eat before the meal; therefore, the laboratory based measured satiation across dinners at which portion sizes were systematically increased. The satiety responsiveness score may reflect both satiety and satiation. The questions span from "My child gets full before his/her meal is finished" (satiation) to "My child cannot eat a meal if s/he has had a snack just before" which reflects the influence of a meal on subsequent intake or satiety. The exploratory analysis of the relationship between satiety responsiveness and

portion susceptibility (Figure 7) demonstrated that lower reported satiety responsiveness was correlated with portion susceptibility. This association between parent report and observed measured of appetite suggests that children's regulation of satiation is associated with satiety responsiveness. Therefore, using both measurements presents a more complete picture of children's appetite regulation.

We did not find the measurements of the variation in the FTO gene and expression to moderate the relationship between parent feeding style and appetite regulation. The small sample size for the analysis of genetic associations may have limited the findings and result in a null relationship between risk alleles and FTO expression on satiety responsiveness or portion susceptibility. In the studies by Wardle and colleagues (2008, 2009) the sample size for assessing satiety responsiveness using the CEBQ had 3,337 participants and the main effect was FTO genotype's relationship to satiety responsiveness. In the study of observed food intake in a laboratory, there were 131 subjects and there was a statistically significant association between lower-risk FTO alleles and lower intake compared to the higher-risk alleles. Current research has found that FTO genetic variants and expression affect weight status (Larder et al, 2011). However, the mechanism by which FTO expression affects weight status is still unclear. It is also possible that FTO expression in saliva may not be correlated with FTO expression in hypothalamus. Further research is needed in this area.

Because this was a cross-sectional study, the ability to determine causation is not possible. Because children were already obese, it is difficult to determine if children became obese because of lack of satiety responsiveness or current weight status influenced regulation. The results of this study may not have generalizability to children of higher income levels or other race/ethnicities. However, the focus of this work on AA

children is important given the extremely high prevalence of obesity among AA children compared to other race/ethnicities (Ogden et al, 2012).

In conclusion, this study provides the first evidence that indulgent feeding styles are associated with poorer appetite regulation among low-income AA children. This population was limited in size and only included one racial group, thus, further research in this area should continue to evaluate appetite in relationship to parenting styles in different racial/ethnic groups and income levels. Other research that would help understand appetite regulation in children is to observe parent feeding behaviors in the home environment during and between meals. Additional studies could be carried out by comparing actual portions served to the children in the home environment and when eating outside the home.

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APPENDIX A Caregiver's Feeding Styles Questionnaire

	These questions deal with <u>YOUR</u> interactions with your preschool child during the dinner meal. Circle the best answer that describes how often these things happen. If you are not certain, make your best guess. How often during the dinner meal do YOU	Never	Rarely	Some times	Most of the Time	Always
1.	Physically struggle with the child to get him or her to eat (for example, physically putting the child in the chair so he or she will eat).	1	2	3	4	5
2.	Promise the child something other than food if he or she eats (for example, "If you eat your beans, we can play ball after dinner").	1	2	3	4	5
3.	Encourage the child to eat by arranging the food to make it more interesting (for example, making smiley faces on the pancakes).	1	2	3	4	5
4.	Ask the child questions about the food during dinner.	1	2	3	4	5
5.	Tell the child to eat at least a little bit of food on his or her plate.	1	2	3	4	5
6.	Reason with the child to get him or her to eat (for example, "Milk is good for your health because it will make you strong").	1	2	3	4	5
7.	Say something to show your disapproval of the child for not eating dinner.	1	2	3	4	5
8.	Allow the child to choose the foods he or she wants to eat for dinner from foods already prepared.	1	2	3	4	5
9.	Compliment the child for eating food (for example, "What a good boy! You're eating your beans").	1	2	3	4	5
10.	Suggest to the child that he or she eats dinner, for example by saying, "Your dinner is getting cold".	1	2	3	4	5
11.	Say to the child "Hurry up and eat your food".	1	2	3	4	5

12.	Warn the child that you will take away something other than food if he or she doesn't eat (for example, "If you don't finish your meat, there will be no play time after dinner").	1	2	3	4	5
13.	Tell the child to eat something on the plate (for example, "Eat your beans").	1	2	3	4	5
14.	Warn the child that you will take a food away if the child doesn't eat (for example, "If you don't finish your vegetables, you won't get fruit").	1	2	3	4	5
15.	Say something positive about the food the child is eating during dinner.	1	2	3	4	5
16.	Spoon-feed the child to get him or her to eat dinner.	1	2	3	4	5
17.	Help the child to eat dinner (for example, cutting the food into smaller pieces).	1	2	3	4	5
18.	Encourage the child to eat something by using food as a reward (for example, "If you finish your vegetables, you will get some fruit").	1	2	3	4	5
19.	Beg the child to eat dinner.	1	2	3	4	5

NOTE: Questions 3, 4, 6, 8, 9, 15 and 17 are considered child-centered and used to calculate the Responsiveness score.

APPENDIX B

Child Eating Behaviour Questionnaire (CEBQ)

Please read the following statements and tick the boxes most appropriate to your child's eating behaviour.

	Never	Rarely	Some -times	Often	Always	
My child loves food						EF
My child eats more when worried						EOE
My child has a big appetite						SR*
My child finishes his/her meal quickly						SE*
My child is interested in food						EF
My child is always asking for a drink						DD
My child refuses new foods at first						FF
My child eats slowly						SE
My child eats less when angry						EUE
My child enjoys tasting new foods						FF*
My child eats less when s/he is tired						EUE
My child is always asking for food						FR
My child eats more when annoyed						EOE
If allowed to, my child would eat too much						FR
My child eats more when anxious						EOE
My child enjoys a wide variety of foods						FF*
My child leaves food on his/her plate at the end of a meal						SR
My child takes more than 30 minutes to finish a meal						SE

	Never	Rarely	Some -times	Often	Always	
Given the choice, my child would eat most of the time						FR
My child looks forward to mealtimes						EF
My child gets full before his/her meal is finished						SR
My child enjoys eating						EF
My child eats more when she is happy						EUE
My child is difficult to please with meals						FF
My child eats less when upset						EUE
My child gets full up easily						SR
My child eats more when s/he has nothing else to do						EOE
Even if my child is full up s/he finds room to eat his/her favourite food						FR
If given the chance, my child would drink continuously throughout the day						DD
My child cannot eat a meal if s/he has had a snack just before						SR
If given the chance, my child would always be having a drink						DD
My child is interested in tasting food s/he hasn't tasted before						FF*
My child decides that s/he doesn't like a food, even without tasting it						FF
If given the chance, my child would always have food in his/her mouth						FR
My child eats more and more slowly during the course of a meal						SE

SCORING OF THE CEBQ

(Never=1, Rarely=2, Sometimes=3, Often=4, Always=5)

Food responsiveness = item mean FR

Emotional over-eating = item mean EOE

Enjoyment of food = item mean EF

Desire to drink = item mean DD

Satiety responsiveness = item mean SR

Slowness in eating = item mean SE

Emotional under-eating = item mean EUE

Food fussiness = item mean FF

Wardle, J, Guthrie CA, Sanderson, S and Rapoport, L. Development of the Children's Eating Behaviour Questionnaire. *Journal of Child Psychology and Psychiatry.* **42**, 2001, 963-970.

^{*}Reversed items

APPENDIX C IRB APPROVAL LETTER



Office for Human Subjects Protections Student Faculty Conference Center **Institutional Review Board**

Medical Intervention Committees A1 & A2 Social and Behavioral Committee B

3340 N Broad Street - Suite 304 Philadelphia, Pennsylvania 19140 Phone: (215) 707-3390

Fax: (215) 707-8387 e-mail: irb@temple.edu

Certification of Approval for a Project Involving Human Subjects

Protocol Number: 20334

Fisher, Jennifer O.

Approved On: 17-Jan-2012 Review Date: 17-Jan-2012

Committee: B BEHAVIORAL AND SOCIAL SCIENCES

School/College: Health Professions (0900) Department: CHP:Public Health (09100)

Project Title: The relationship between parenting style and appetite regulation in African American school age

children and the effect of the FTO Gene

In accordance with the policy of the Department of Health and Human Services on protection of human subjects in research, it is hereby certified that protocol number 20334, having received preliminary review and approval by the department of CHP:Public Health (09100) was subsequently reviewed by the Institutional Review Board in its present form and approved on 17-Jan-2012 with respect to the rights and welfare of the subjects involved; appropriateness and adequacy of the methods used to obtain informed consent; and risks to the individual and potential benefits of the project.

In conforming with the criteria set forth in the DHHS regulations for the protection of human research subjects, and in exercise of the power granted to the Committee, and subject to execution of the consent form(s), if required, and such other requirements as the Committee may have ordered, such orders, if any, being stated hereon or appended hereto.

It is understood that it is the investigator's responsibility to notify the Committee immediately of any untoward results of this study to permit review of the matter. In such case, the investigator should call the IRB at (215) 707-3390.

This is the Certificate of Approval, Supplemental documentation will follow under separate cover. Enrollment may not begin until all documents have been reviewed and processed by the IRB and received by the study team.

Board determined conditions of approval applied to this protocol:

Name (Fulfilled Date) Description

> ZEBULON KENDRICK, Ph.D. CHAIRMAN, IRB

Lellen VIK

CITI Collaborative Institutional Training Initiative Human Research Curriculum Completion Report Printed on 8/7/2010

Learner: Meredith Borine (username: tuc35185)

Institution: Temple University

Contact 172 Cardinal Rd

Information Chalfont, PA 18914 USA Phone: 267-308-0211

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Social/Behavioral Research Course: Choose this group to satisfy CITI training requirements for Investigators and staff involved primarily in Social/Behavioral Research with human subjects.

Stage 1. Basic Course Passed on 08/07/10 (Ref # 4720237)

	Date	
Required Modules	Completed	Score
Belmont Report and CITI Course Introduction	08/06/10	3/3 (100%)
Students in Research - SBR	03/30/10	10/10 (100%)
History and Ethical Principles - SBR	08/06/10	4/4 (100%)
Defining Research with Human Subjects - SBR	08/06/10	5/5 (100%)
The Regulations and The Social and Behavioral Sciences - SBR	08/06/10	5/5 (100%)
Assessing Risk in Social and Behavioral Sciences - SBR	08/06/10	5/5 (100%)
Informed Consent - SBR	08/06/10	4/5 (80%)
Privacy and Confidentiality - SBR	08/06/10	3/3 (100%)
Research with Prisoners - SBR	08/06/10	4/4 (100%)
Research with Children - SBR	08/06/10	4/4 (100%)
Research in Public Elementary and Secondary Schools - SBR	08/06/10	4/4 (100%)
International Research - SBR	08/06/10	3/3 (100%)
Internet Research - SBR	08/07/10	4/4 (100%)
HIPAA and Human Subjects Research	08/07/10	2/2 (100%)
Workers as Research Subjects-A Vulnerable Population	08/07/10	4/4 (100%)
Conflicts of Interest in Research Involving Human Subjects	08/07/10	1/2 (50%)
Temple University	03/30/10	no quiz

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI participating institution. Falsified information and unauthorized use of the CITI course site is unethical, and may be considered scientific misconduct by your institution.

Paul Braunschweiger Ph.D. Professor, University of Miami