Physiology & Behavior 162 (2016) 3–9

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

# 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

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## HIGHLIGHTS

- Activity in brain reward regions is associated with children's body composition
- Lean mass positively correlated with substantia nigra response to high energy foods
- Findings support fat-free mass as an appetitive driver in children

## ARTICLE INFO

*Article history:*
Received 16 December 2015
Received in revised form 12 February 2016
Accepted 8 March 2016
Available online 10 March 2016

*Keywords:*
Body composition
Fat-free mass
Energy density
Neuroimaging
fMRI
Children

## 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

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http://dx.doi.org/10.1016/j.physbeh.2016.03.007
0031-9384/

of the energy balance system on central 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 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).

**Table 1**
Participant characteristics for 7- to 10-year-old children with complete data ($n = 36$).

| | Mean | S.D. |
|---|---|---|
| Age (years) | 8.9 | 1.2 |
| % body fat | 16.4 | 6.5 |
| Fat mass index (kg/m$^2$) | 3.8 | 2.5 |
| Fat-free mass index (kg/m$^2$) | 18.1 | 2.2 |
| | N | % |
| Sex | | |
| Male | 18 | 50 |
| Female | 18 | 50 |
| BMI percentile class (CDC) | | |
| Non-overweight | 34 | 94 |
| Overweight | 2 | 6 |
| Race | | |
| White | 33 | 92 |
| Non-white | 3 | 8 |

**Table 2**
Talairach atlas coordinates tested in ROI approach.

| Region of interest | Hemisphere | Talairach coordinates | | |
|---|---|---|---|---|
| | | x | y | z |
| Hypothalamus[a] | R | 4 | 0 | −12 |
| | L | −4 | 0 | −12 |
| Anterior cingulate gyrus[b] | R | 12 | 16 | 22 |
| | L | −12 | 16 | 22 |
| Substantia nigra[c] | R | 8 | −22 | −14 |
| | L | −8 | −22 | −14 |
| Amygdala[c] | R | 22 | −10 | −10 |
| | L | −22 | −10 | −10 |
| Insula[c] | R | 36 | −6 | −12 |
| | L | −36 | −6 | −12 |
| Putamen[c] | R | 18 | 12 | −4 |
| | L | −18 | 20 | −6 |
| Thalamus[c] | R | 12 | −16 | 0 |
| | L | −18 | −22 | 8 |

[a] Talairach Client (v 2.4.3), Talairach.org.
[b] Killgore & Yurgelun-Todd, NeuroReport (2005) 16:859–863 [9].
[c] Schur EA, et al., Int. J. Obes. (2009) 33:653–661 [12].

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].

### 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 = 1650/2.03 ms, flip angle = 9°, FOV = 256 mm, slice thickness = 1 mm, sagittal plane, and 1.0 × 1.0 × 1.0 mm voxel size. The MPRAGE sequence was approximately 4 min 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 = 2000 ms, TE = 25 ms, flip angle = 90°, matrix 64 × 64, FOV = 220 mm, AC-PC transverse, oblique plane determined by the mid-sagittal section, and 3.0 × 3.0 × 3.0 mm 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 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].

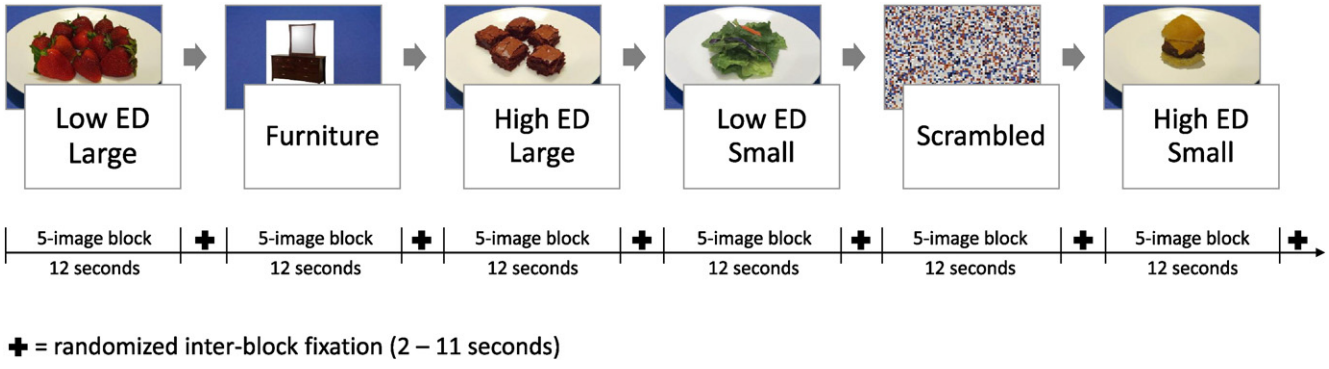


**Fig. 1.** Example of 1 functional run in the fMRI scanning paradigm.

**Table 3**
ANOVA results: main effects of High ED vs. Low ED.

| Region of interest | Hemisphere | Direction | *F*-value | *p*-value |
|---|---|---|---|---|
| Thalamus | L[a] | High ED > Low ED | 6.30[a] | 0.02[a] |
| Anterior cingulate gyrus | R | High ED > Low ED | 4.65 | 0.04 |
| Substantia nigra | R | Low ED > High ED | 5.65 | 0.02 |
| | L | Low ED > High ED | 3.75 | 0.06 |

Note: Computed in BrainVoyager QX.
[a] Survived correction for multiple comparisons.

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 s, with a fixation for 0.5 s between each image. To prevent habituation to the stimuli, we included randomized inter-block fixation times that ranged from 2 to 11 s 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 min 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 min, but the total duration of the scan varied from 10 to 35 min, depending on the randomized interval time between blocks and variations in children's comfort level. An example of the scanning paradigm is depicted in Fig. 1. Immediately following the scan, children rated how much they liked and wanted to eat each food image on a 150 mm visual analog scale. These liking and wanting ratings were used in additional confirmatory analyses, described below.

### 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 >3 mm 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 5 mm 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 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.

## 3. Results

### 3.1. ANOVA results

Results from the GLM ANOVAs are summarized in Table 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 (Fig. 2).

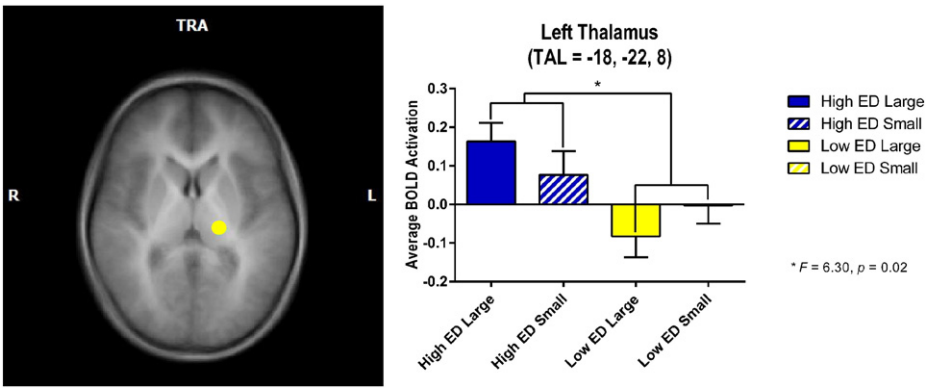


**Fig. 2.** Main effect of ED in the left thalamus; computed in BrainVoyagerQX.

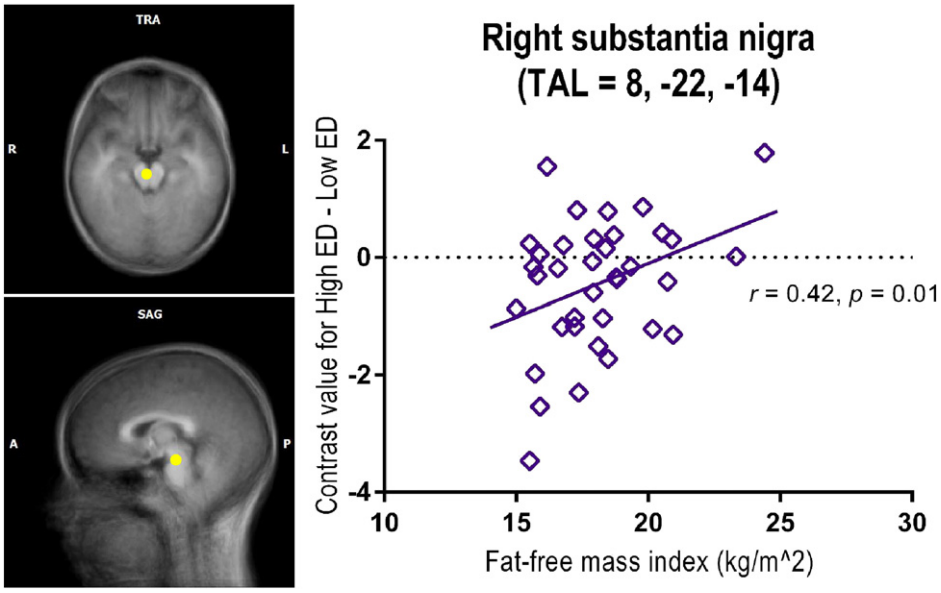


**Fig. 3.** Positive correlation between fat-free mass index and activation for High ED–Low ED foods in the right substantia nigra; Computed in BrainVoyager QX.

### 3.2. 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 (Fig. 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.

#### 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).

**Table 4**
Exploratory correlation results for BOLD activation in ROIs for various contrasts (High ED–Low ED; High ED–Scrambled; Low ED–Scrambled) and body composition (FFM index, FM index, %BF); N/A*: correlation $p > 0.07$.

| Region of interest | Hemisphere | FFM index | FM index | %BF |
|---|---|---|---|---|
| High ED–Low ED | | | | |
| Substantia nigra | L | 0.36 | N/A* | N/A* |
| Anterior cingulate gyrus | R | N/A* | N/A* | −0.30 |
| High ED–Scrambled | | | | |
| N/A* | | | | |
| Low ED–Scrambled | | | | |
| Substantia nigra | L | N/A* | −0.31 | −0.30 |
| Amygdala | R | −0.36 | N/A* | N/A* |
| Putamen | R | 0.38 | N/A* | N/A* |
| Hypothalamus | R | 0.30 | N/A* | N/A* |

Note: Computed in BrainVoyager QX.

#### 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 4 for exploratory purposes.

## 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 regard 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. 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.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.physbeh.2016.03.007.

## 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.

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