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Both increases and decreases in energy density lead to sustained changes in preschool children's energy intake over 5 days

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Abstract

Background and Objective: To investigate preschool children's ability to self-regulate their energy intake, we assessed their response to increases or decreases in dietary energy density (ED) over 5 consecutive days, a period likely long enough for compensatory behavior.

Methods: Using a crossover design, over 3 periods we served the same 5 daily menus to 49 children aged 3-5 y in their childcare centers. During each 5-day period, 3 main dishes and 1 snack per day were systematically varied in ED, from baseline ED to either higher ED (increased by 20%) or lower ED (decreased by 20%). All of the served items were weighed to determine individual intakes.

Results: Modifying the ED of 4 dishes per day had a significant and sustained effect on preschool children's daily energy intake across 5 days. In the baseline condition, children's intakes were similar to daily energy needs (98±2%), but serving higher-ED foods increased energy intake by 84±16 kcal/d (to 105±2% of needs) and serving lower-ED foods decreased energy intake by 72±17 kcal/d (to 89±2% of needs; both $P<0.0001$). The patterns of daily energy intake over the 5 days did not differ across conditions ($P=0.20$), thus there was no evidence that either surfeits or deficits in energy intake led to adjustment over this time period. Furthermore, the response to ED varied, as children with a higher weight status had greater amounts of overconsumption when served higher-ED foods and of underconsumption when served lower-ED foods compared to children with a lower weight status.

Conclusions: These findings counter the suggestion that preschool children's regulatory systems can be relied on to adjust intake in response to energy imbalances. Increasing or

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decreasing the ED of several foods per day leads to sustained changes in the energy intake of preschool children.

Keywords

Energy density; Preschool children; Eating behavior; Energy regulation; Energy intake

1. Introduction

There is a widespread assumption that young children have the ability to regulate their energy intake in response to short-term perturbations in consumption [1-4]. This is based in part on an early study that measured ad libitum consumption of 2- to 5-year-olds and found that although energy intake was variable at individual meals, it was relatively consistent across a day [2]. Subsequent satiation studies, however, demonstrated that changes in food energy density (ED) can lead to substantial changes in energy intake in preschool children [5-8] as well as adults [9,10]. For example, in preschool children the effects of food ED variations on energy intake have been found to persist for up to 2 days [5-8]. Additionally, satiety studies in preschool children have found inconsistent responses to changes in preload ED, some observing accurate compensation in energy intake at the following meal [11-13], and others either over- or underconsumption in response to the preload [14-18]. Thus, it remains unclear how effectively young children adjust energy intake in response to variations in dietary ED. The duration of the previous studies, however, may have been too short to observe compensatory responses, which in adults are suggested to take 3 to 4 days [19, 20]. In the present study, we varied ED over 5 consecutive days, the longest period that we could provide and measure all meals for preschool children in their usual care setting. The objective was to assess energy intake and its regulation in response to both increases and decreases in ED compared to the baseline ED.

It might be assumed that increases and decreases in food energy have a similar influence on preschool children's compensatory response [21]. The literature, however, proposes that there will be a stronger adjustment to energy deficits than to energy surfeits [22, 23], in order to defend against energy depletion and thus promote survival when food is scarce [24]. In the eating environment today, energy surfeits are encouraged by the ready availability of a variety of high-energy-dense foods. The high prevalence of childhood obesity suggests that homeostatic systems may be relatively insensitive to energy surfeits associated with increased dietary ED [6, 8, 25]. Since children's response to energy deficits and surfeits over multiple days is unclear, we examined how preschool children adjust energy intake to both of these types of perturbations in dietary ED.

The aim of this study was to investigate the response of preschool children to variations in ED over time by examining the effects on daily energy intake. We hypothesized that preschool children would show regulation by adjusting intake for changes in ED over 5 days, a period likely long enough for compensatory behavior to occur. We further hypothesized that children would adjust intake closer to baseline in response to energy decreases compared to energy increases. Additionally, children's characteristics were assessed in order to identify any individual differences in the relationship between ED and intake. This study will help inform child feeding recommendations by determining the nature of energy intake regulation in response to changes in dietary ED, as well as individual variability in this response.

2. Methods

2.1. Experimental Design

A within-subjects crossover design was used to investigate the effect on intake of varying the ED of multiple foods served to preschool children across 5 consecutive days. Children were provided with all of their foods and beverages during each 5-day period. The 5-day period (Monday to Friday) allowed children's intake to be accurately measured in the childcare setting, whereas a longer time frame would have included weekends, for which intake at home would be difficult to measure in a controlled manner. In one 5-day period, 3 main dishes (1 served at each breakfast, lunch, and dinner) and 1 afternoon snack on each of the 5 daily menus were served with baseline ED. In the other 2 periods, the ED of the 3 main dishes and 1 snack on each menu were either increased by 20% (higher-ED condition) or decreased by 20% (lower-ED condition). The remainder of the menus consisted of side dishes, snacks, and beverages that were not manipulated in ED. All foods and beverages were consumed *ad libitum* and individual intakes were determined by weighing all items before and after meals and snacks. The orders of presenting the 5 menus across the days (Monday to Friday), as well as the ED conditions across the 3 periods, were counterbalanced across classrooms by the data manager using Latin squares. Thus, a given child received the same order of daily menus across all 3 conditions. The 5-day periods were separated by a 1-week washout period.

2.2. Participants

Children aged 3 to 5 years old were recruited from September 2016 to September 2017 by distributing letters to parents in 10 classrooms at 3 childcare centers in State College, Pennsylvania, USA. Children were eligible to participate if they were available for the study duration and did not have any food allergies, food restrictions, or health issues that precluded their participation. Parents provided written consent for participation of themselves and their child and were financially compensated for their participation. Study procedures were approved by The Pennsylvania State University Office for Research and Protections. The trial is registered at ClinicalTrials.gov (Registration Number: NCT03010501).

The sample size for the study was estimated from a linear mixed model and an approximation technique [26] using exemplary data sets based on prior 1- and 2-day studies in preschool children [6, 27]. We assessed the required sample size for several scenarios with different assumptions about changes in intake on days 3 to 5 that represented quadratic trajectories over time. To detect a clinically significant change of 50 kcal/d over time (movement toward adjustment after 3 to 5 days) with a Type 1 error rate of 5% and power of 90%, it was determined that 45 participants would be required. To allow for withdrawals and non-compliance to the protocol, 56 children were enrolled.

2.3 Experimental menus and meal procedures

2.3.1. Experimental menus—During each 5-day period, the children were served 5 different daily menus, with foods typically consumed by children in the childcare setting (Supplemental Tables 1-5). Each of the 5 daily menus included 4 foods that were manipulated in ED (3 main dishes and 1 snack). The 20 foods that were manipulated were chosen because they were familiar to the children and the ED could be varied while keeping a similar appearance and palatability across conditions. The manipulated foods in the baseline condition were based on standard recipes and the average dietary ED of previous studies in preschool children [27-29]. From the baseline condition, the ED of the 20 foods was increased or decreased by 20%, a difference that can be observed in the current food environment (e.g. cheddar cheese vs. reduced-fat cheddar cheese). In the lower-ED condition, the ED was manipulated by either reducing the fat or sugar content, increasing the fruit or vegetable content, or a combination of these methods. In the higher-ED condition, the ED was manipulated by either increasing the fat or sugar content, decreasing the fruit or vegetable content, or combining these methods. In all 3 conditions, the manipulated foods accounted for 36% of the daily weight of food served and 22% of the daily weight of food plus milk served (Table 1). Of the total daily energy served, the proportion provided by the manipulated foods (averaged across all menus) was 37% in the lower-ED condition, 42% in the baseline-ED condition, and 47% in the higher-ED condition. The remaining foods and milk served on the menus were not manipulated, in order to give children the opportunity to adjust energy intake in response to the ED manipulations by changing the proportions eaten of the non-manipulated foods. As a result of increasing and decreasing the ED of 4 daily items by 20% from baseline, the overall ED of all foods served on the 5 daily menus increased or decreased by about 10% from baseline.

The foods and milk served on the 5 menus met the minimum recommendations of the Child and Adult Care Food Program [30]. Breakfast consisted of a grain-based main dish, fruit, and milk, with a protein food added on 2 of the 5 days; lunch and dinner consisted of a grain and protein-based main dish, vegetable, fruit, and milk; afternoon and evening snacks consisted of 2 of the 5 snack components (milk, grain, protein, fruit, or vegetable); and morning snack consisted of a cereal bar [30]. The portion sizes of all foods and milk served were based on consumption data from other studies conducted in preschool children and the Child and Adult Care Food Program guidelines [4, 29, 30]. Each menu provided more than enough food to meet preschool children's daily energy needs (Table 1).

2.3.2. Meal procedures—All meals and snacks were prepared in the laboratory kitchen from recipes and commercially available products. Just prior to each meal and the afternoon snack, the foods and milk were transported to the childcare centers in warming units or insulated coolers. The children were served in their classrooms at shared tables of 4-8 study participants and 1-2 adult staff. Breakfast, lunch, and afternoon snack were served at the regularly scheduled meal times and dinner was served at the end of the childcare day. The children received a pre-weighed plate of food and a pre-weighed container of milk at each meal and snack. They were told they could eat or drink as much or as little of the food and milk served as they wanted, which is standard practice at the childcare centers. At the tables, research staff diverted any discussion about the food to minimize any influence on intake.

When the children were done eating, any dropped or spilled food was returned to the correct plate by research staff before weighing. Weights were collected on all foods and beverages to determine intake. Morning and evening snacks were weighed and packed in individual containers for home consumption; any uneaten snacks were returned to the childcare center. Parents also completed daily recall forms to report whether their child had consumed any non-study foods or beverages.

2.4. Measures

2.4.1. Food weight and energy—All food and milk weights were assessed to the nearest 0.1 g with digital scales (Mettler-Toledo PR5001 and XS4001S; Mettler-Toledo, Columbus, Ohio, USA). Energy and macronutrient intakes were calculated using information from food manufacturer labels and a standard food composition database [31].

2.4.2. Food liking—Food liking was assessed after the final experimental period during an individual tasting session with each child. It was not practical to have children taste all of the foods provided on the menus, so one manipulated main dish and one manipulated snack were chosen for children to taste in all 3 ED versions. The order of presenting the 6 foods to each child was randomly assigned. The foods were evaluated using a 3-point cartoon face scale to rate the food as “yummy”, “just okay”, or “yucky” [32]. After instruction, the child was presented with a series of food samples, and after tasting each one the child pointed to the cartoon face that represented how much they liked the food.

2.4.3. Physical activity—Each child wore a triaxial accelerometer activity monitor (model wGT3X-BT, ActiGraph LLC, Pensacola, Florida, USA) during all study days from 9:00 am to 5:00 pm (480 min). The activity data was assessed for wear-time validation and scored with Butte Preschoolers cut points using actigraphy data analysis software (ActiLife v6.13.1, ActiGraph LLC, Pensacola, Florida, USA) [33]. Step count measures were used to assign children to physical activity levels in order to calculate estimated energy requirements (EER) [34]. Physical activity data were also used to examine whether any compensatory changes were made across ED conditions in sedentary activity time or moderate-to-vigorous activity time.

2.4.4 Body height and weight—One week after the final study period, anthropometric measurements were collected. The children’s height was measured in duplicate to the nearest 0.1 cm with a portable stadiometer (model 214; Seca Corporation, Hanover, Maryland, USA), and the children’s body weight was measured in duplicate to the nearest 0.1 kg with a portable digital scale (model 843; Seca Corporation, Hanover, Maryland, USA). This was repeated if the 2 measurements differed by >0.2 kg for weight or >0.5 cm for height. The means of the measures were used in the calculation of sex-specific BMI-for-age percentiles and BMI z-scores using a software program based on nationally representative data [35].

2.4.5. Parent questionnaires—Parents were asked to complete questionnaires about parent and child characteristics, child eating behaviors, and parent feeding practices. The demographic questionnaire has 16 items related to family characteristics and child health.

The Children's Eating Behaviour Questionnaire (CEBQ) [36] has 35 items related to children's eating styles. Example subscales on the CEBQ include satiety responsiveness (5 items evaluating how frequently a child becomes full easily and leaves food when finished eating) and food responsiveness (5 items evaluating how frequently a child shows interest in food and wants to spend time eating). The Child Feeding Questionnaire (CFQ) [37] has 31 items related to parent perceptions of their responsibility for child feeding. An example subscale on the CFQ is pressure to eat (4 items evaluating parental tendency to pressure the child to eat more food). Items on both the CEBQ and the CFQ are evaluated on 5-point scales and the items are averaged to give a score for the subscale.

2.5 Statistical analysis

The main outcomes of the study were daily consumption (by weight, ED, and energy) of manipulated foods, non-manipulated foods and milk, and their combined total. Secondary outcomes were cumulative intake for the entire 5 days, daily macronutrient intake, daily intake of non-manipulated items by meal component (fruits and vegetables at main meals, grain-based snacks, dairy snacks, fruit and vegetable snacks, and milk), and physical activity time (daily step counts, sedentary time, and moderate-to-vigorous time). To analyze differences in mean daily outcomes across the 3 experimental conditions, linear mixed models with repeated measures were used. The fixed factors in the models were ED condition (lower-ED, baseline-ED, or higher-ED), study week, study day, menu, classroom, and sex. Interactions between these factors were tested and removed from the model if not significant. Participants were treated as a random effect. The Tukey-Kramer method was used to adjust for multiple pairwise comparisons between means. In order to evaluate the contribution to children's intakes of non-study foods reported by parents, the energy content of the reported items was estimated by research staff. The outcome of daily energy intake was analyzed both with and without the non-study items. This estimated data was not included in further analyses, because these estimates were based on parental recall and were less accurate than measured intake.

Random coefficients models were used to test whether the trajectories of daily intake in the 3 conditions converged over the 5 days, i.e., whether regulation of energy intake occurred over time in response to ED variations. The trajectories of intake over time were modeled separately for each child and allowed to vary randomly. Time (study day) was treated as a continuous covariate in the model and polynomial factors of time (linear and quadratic coefficients) were tested as both fixed and random effects to determine the shape of the intake trajectory. The intercept of the trajectory reflected the magnitude of daily intake. The linear coefficient represented the rate of change in intake (slope) across time after Day 1, and the quadratic coefficient represented the rate of acceleration or deceleration in intake (curvature) across time after Day 1.

The influence of continuous participant characteristics (age, body weight, height, BMI-for-age percentile, BMI z-score, step counts, and questionnaire subscales) on the relationship between the experimental factors and intake was assessed by analysis of covariance with linear mixed models. Because multiple participant characteristics were tested as covariates, the method of Benjamini and Hochberg [38] was used to adjust the significance levels, with the false discovery rate set at 0.05 [38]. In order to standardize for energy needs, models having significant covariance with weight status were adjusted for EER. Differences in subject characteristics between boys and girls, as well as differences in intake between children with and without obesity (sex-specific BMI-for-age percentile ≥85 and <85, respectively), were assessed by independent-samples t-tests. Within conditions, the means of daily energy intakes expressed as a percentage of EER were compared to 100% using a one-sample t-test and controlling the family-wise error rate using the Bonferroni correction.

It was predetermined that children who had incomplete intake measures on ≥ 3 days in all 3 conditions would be excluded from analysis. It was also predetermined that children who ate >95% of the food weight served on at least 1 day would be classified as "plate cleaners", and their influence on the results would be tested. To evaluate whether the distribution of taste ratings (yummy, just okay, yucky) of the lunch and snack dishes differed significantly across ED conditions, ordinal logistic regression was used; results are reported as odds ratios (OR) with 95% confidence intervals. Outcomes from statistical models are reported as mean ± SEM and subject characteristics are reported as mean ± SD. Standardized effect sizes were calculated using Cohen's d statistic, with no adjustment for correlation due to repeated measures. Results were considered significant at $P < 0.05$. All data were analyzed using SAS software (SAS 9.4, SAS Institute, Inc., Cary, North Carolina, USA).

3. Results

3.1. Subject characteristics

Fifty-six children were enrolled in the study, but 6 children withdrew because of scheduling conflicts and 1 child was excluded for incomplete intake measures on ≥ 3 days in all 3 conditions. Thus, 49 children (26 boys and 23 girls) were included in the analysis. Heights and weights were measured for 45 children (Table 2); the other 4 children were absent on the days these measurements were collected. Seven of the children (16%) were classified as having overweight or obesity. The sample of children was 71.4% white, 4.1% black or African American, 8.2% Asian, 10.2% mixed or another race, and 6.1% unreported; 8% were of Hispanic or Latino origin. Parents provided family information for 47 children (96%); 63% reported a household income >$50,000 and 71% of fathers and 78% of mothers had a bachelor's degree or higher.

3.2. Food and energy intakes

3.2.1 Weight of food and milk consumed—Varying the ED of 4 foods per day did not significantly affect the daily weight consumed of these manipulated foods in either the higher-ED (167±11 g/d; P=0.15; d=0.07) or lower-ED (148±11 g/d; P=0.10; d=−0.08) condition compared to the baseline-ED condition (158±11 g/d; Table 3). Furthermore, children consumed a similar total amount of the non-manipulated items (foods and milk) across all conditions (Table 3; P>0.07). No systematic differences in intake were found across conditions by analyzing non-manipulated items according to meal component (Supplemental Table 6). None of the children were identified as plate cleaners according to the predetermined criterion (eating >95% of the food served on at least 1 day).

Analysis of the trajectories of daily intake across the 5 days (for the combined weight of manipulated and non-manipulated items) showed that the linear (P=0.12) and quadratic (P=0.19) coefficients did not differ across conditions(Figure 1a). Thus, the patterns for the weight of food and milk consumed over time were similar for the lower-ED, baseline-ED, and higher-ED conditions, indicating that children showed no significant adjustments in intake by weight in response to ED changes across the 5-day period.

3.2.2 Energy density and energy intake—The mean dietary ED consumed by the children was significantly affected by modifying the ED of 4 daily foods for 5 days (P=0.01; Table 3). Since similar weights were consumed in the higher-ED and lower-ED conditions compared to the baseline condition, the changes in ED had a significant effect on children's daily energy intake. Compared to baseline, increasing the ED led to an increase in energy intake of the manipulated foods (73±10 kcal/d; P<0.0001; d=0.37), while decreasing the ED led to a decrease (78±10 kcal/d; P<0.0001; d=−0.52). For the non-manipulated food and milk, since there was no difference in the weight or ED consumed across conditions, there was also no difference in daily non-manipulated energy intake (P>0.80; Table 3). Consequently, serving higher-ED foods over the 5-day period increased daily energy intake by 9% (84±16 kcal/d; P<0.0001; d=0.27), and serving lower-ED foods decreased daily energy intake by 8% (72±17 kcal/d; P<0.0001; d=−0.33) compared to the baseline-ED condition (Table 3). The increases and decreases in ED from baseline values thus produced parallel changes in daily energy intake, resulting in surfeits (higher-ED) or deficits (lower-ED) compared to intake in the baseline-ED condition. There was no significant difference, however, between the magnitudes of the increase in the higher-ED condition and the decrease in the lower-ED condition (P=0.46). Thus, there was no evidence that children adjusted intake differently in response to energy surfeits compared to energy deficits, contrary to the hypothesis.

Analyzing the trajectories of daily energy intake over time showed that although the magnitude of intake differed across conditions (P<0.0001), there was no difference in the linear (P=0.82) and quadratic (P=0.94) coefficients (the curvature) across conditions. Thus, contrary to the hypothesis, there was no evidence that children demonstrated regulation by adjusting energy intake over the 5 days in response to changes in ED (Figure 1b). Compared to the baseline-ED condition, serving higher- and lower-ED foods led to changes in daily energy intake that were sustained over time. For the entire 5-day period, ED variations resulted in significantly different cumulative intakes: 5549±290 kcal in the higher-ED condition, 4976±221 kcal in the baseline-ED condition, and 4549±180 kcal in the lower-ED condition (all P<0.02; Figure 2).

When estimates of parentally reported non-study foods were included in the analysis, the trajectories of daily intake remained similar, with differences in magnitude across conditions (P<0.0001) but no differences in curvature (both P>0.46). Children consumed similar amounts of energy from non-study foods in both the higher-ED (89±12 kcal/d; P=0.54; d=−0.06) and lower-ED conditions (115±13 kcal/d; P=0.32; d=0.08) compared to baseline (99±12 kcal/d), but consumed more energy from non-study foods in the lower-ED compared to the higher-ED condition (P=0.04; d=−0.14). However, even after accounting for consumption of non-study foods, the effect of changes in ED on energy intake was sustained over time.

3.2.3. Intake in relation to energy requirements—As well as having significant effects on daily energy intake, varying the ED of 4 foods each day led to deficits and surfeits in intake in relation to children's energy needs. Although children consumed close to 100% of their daily energy needs in the baseline-ED condition (98±2 % of EER; adjusted P=0.19), they consumed less than their energy needs when lower-ED foods were served (89±2 % of EER; adjusted P<0.0001; d=−0.33) and more than their needs when higher-ED foods were served (105±2% of EER; adjusted P=0.014; d=0.28).

3.3. Food liking ratings

The liking assessment for two study foods in all 3 ED versions was completed by 46 children (94%), as 3 children were absent on the day the assessment took place. The foods were well-liked, with 87% of the children (n=40) rating both dishes as "yummy" or "just okay" in all 3 ED versions. Ordinal logistic regression showed that the distribution of liking ratings did not significantly differ between the lower-ED and baseline-ED condition (P=0.27; OR 1.04; 95% confidence interval 0.56 – 1.92) or between the higher-ED and baseline-ED condition (P=0.27; OR 0.76; 95% confidence interval 0.65 – 2.76), as indicated by odds ratios that were not significantly different from 1.0. The food liking assessment showed that children had similar liking for the 3 ED variations of the two dishes that were tested out of the 20 manipulated dishes served.

3.4. Physical activity time

Children's daily step counts did not differ between the baseline-ED condition (4934±140 steps/d) and the lower-ED (4620±142 steps/d; P=0.06; d=−0.34) or higher-ED (4648±144 steps/d; P=0.09; d=−0.21) conditions. The time spent in sedentary physical activity did not differ between the baseline-ED condition (303±7 min/d) and the lower-ED (290±7 min/d; P=0.18; d=−0.21) or higher-ED condition (309±7; P=0.68; d=0.10). Additionally, the time children spent in moderate-to-vigorous physical activity did not differ between the baseline-ED condition (47±2 min/d) and the lower-ED (44±2 min/d; P=0.25; d=−0.22) or higher-ED condition (46±2; P=0.80; d=−0.09). Analysis of covariance found that children's daily step counts did not affect the relationship between ED and daily energy intake (adjusted P=0.44).

3.5. Influence of subject characteristics

Analysis of covariance showed that the relationship between ED condition and daily energy intake was significantly influenced by children's body size, even when intake was standardized to energy needs. Children with a higher sex-specific BMI-for-age percentile had greater changes in response to the ED variations compared to children with a lower sex-specific BMI-for-age percentile (adjusted P=0.047). For example, when ED was varied from the baseline condition, the modeled relationship showed that children with a 90th BMI-for-age percentile had a significant increase in daily energy intake when higher-ED foods were served (104±27 kcal/d; P=0.0001) and a significant decrease when lower-ED foods were served (141±27 kcal/d; P<0.0001). Children with a 50th BMI-for-age percentile also showed an energy increase when ED was increased and a decrease when it was decreased, but the magnitude of the changes was smaller (73±18 kcal/d and 76±28 kcal/d, respectively; $P<0.0001$). In contrast, children with a 10th BMI-for-age percentile had no significant change from the baseline-ED condition when either higher-ED foods (42±47 kcal/d; $P=0.25$) or lower-ED foods (9±37 kcal/d; $P=0.80$) were served. Similar results were found for other measures of body size: BMI z-score, weight, and height (all adjusted $P<0.05$). This suggests that children with a larger body size may respond differently to ED variations compared to children with a smaller body size.

The relationship between ED condition and daily intake by weight or energy was not influenced by the children's age or most of the subscales of the Child Eating Behaviour Questionnaire and the Child Feeding Questionnaire. The pressure to eat subscale on the Child Feeding Questionnaire, however, was found to significantly affect the relationship between ED and energy intake (adjusted $P=0.004$); children whose parents reported higher scores for these practices had smaller changes in response to the ED manipulations than children whose parents reported smaller scores.

4. Discussion

Across 5 days, both increases and decreases in the ED of multiple foods had significant and sustained effects on the energy intake of preschool children. Compared to the baseline condition, serving higher-ED foods increased daily energy intake, whereas serving lower-ED foods decreased intake. Thus, changes in the ED of 3 main dishes and 1 snack each day had a significant influence on children's daily energy intake over time. There were no systematic modifications in the amount of food consumed in response to the ED variations, nor was there stronger adjustment for energy decreases compared to energy increases from baseline. Children with a higher weight status were more responsive to variations in ED than were children with lower weight status, in that they had an even greater amount of overconsumption when served higher-ED foods, even after accounting for energy requirements. These findings demonstrate the robust and sustained effects that dietary ED can have on preschool children's energy intake, and challenge the assumption that energy deficits elicit a stronger regulatory response than energy surfeits.

Despite mixed findings in previous studies related to children's regulation of energy intake, it is generally assumed that young children will adjust intake in response to energy perturbations [1, 2, 11-13]; yet we did not find compensatory changes for the differences in energy intake resulting from ED variations across time. The effect of ED on children's *ad libitum* energy intake over 5 days was similar to that found in previous short-term studies [6-8]. In the longest previous study, which decreased ED over 2 days, preschool children ate a consistent weight of food across conditions and thus consumed less energy when ED was reduced [6]. The present experiment provides an important extension to those results by showing that changes in energy intake persisted over 5 days, a duration thought to be sufficient for regulatory mechanisms to respond to ED variations [20]. When children were served foods either increased or decreased in ED, they consumed a consistent weight of the manipulated foods compared to the baseline condition, giving no evidence of regulatory adjustment. Additionally, in the 2-day study the children only had the opportunity for compensatory eating at the non-manipulated dinner and evening snack, as all the foods and beverages served earlier in the day were manipulated in ED [6]. In the present study, a variety of non-manipulated foods accompanied the manipulated main dishes and snacks to ensure that the children had multiple opportunities to compensate by adjusting food intake. Despite this, no significant adjustment in non-manipulated food intake was detected over the 5 days in response to the ED manipulation. The failure to find compensation suggests that there is not substantial adjustment over the 3- to 5-day time frame suggested by research in adults [20]. At present, there is limited work on the time course of self-regulation of intake. In infants, it is suggested that several weeks are needed to adjust energy intake in response to variations in ED [39, 40], which could be the case for preschool children. Another possibility is that adjustment does occur over the 3- to 5-day time frame, but we failed to observe it because the variations in ED tested were below a level detectable by regulatory mechanisms [41]. Although further research is needed to better understand the amount of time or magnitude of perturbation required to observe compensatory responses, the findings from this study indicate that common variations in foods, such as ED, can override young children's ability to regulate consumption over multiple days.

Preschool children did not show a significant adjustment towards baseline intake as a result of either increases or decreases in the ED of multiple foods served over 5 days. These findings counter the assertion that decreases in energy intake provoke a stronger compensatory response than energy increases [42-44]. In order to better understand children's regulation of intake, we examined the effects of ED on energy intake in relation to daily energy needs and used accelerometry to objectively assess physical activity. We found that across all children, adjustments were not made in response to ED variations in order to meet energy needs, either by eating more or less of the food served or by modifying physical activity. The effect of ED was observed even when energy intakes were standardized for energy needs. These findings extend our knowledge by demonstrating that the effects of ED are sustained over multiple days in preschool children, even when they are consuming more or less than their daily energy needs. Therefore, some consideration is needed when modifying ED, as not all young children need to moderate their energy intake and large modifications in ED may influence children's ability to meet their energy needs, leading to under- or overfeeding.

The lack of adjustment for changes in the ED of multiple foods may be partly due to the tendency of children to consume a consistent weight of food when served a similar weight [5,6], which was observed for the higher-ED and lower-ED versions of the manipulated foods compared to baseline. Thus, when ED was varied from baseline, energy intake increased and decreased in parallel to these changes. An additional contribution to the effect may be that the food manipulations provided few visual cues to differentiate energy content [45,46]. It could be the case that the children needed repeated exposure to learn the satiating effects of the various manipulated foods [11, 47, 48] in order to initiate compensation. One study found that over several trials, young children learned about the physiological cues associated with the different levels of ED, and adjusted subsequent food intake accordingly [11]. Additionally, the time period needed for regulatory mechanisms to sense an energy imbalance may differ for an energy deficit compared to an energy surfeit, as human physiology is better able to maintain energy balance when food is scarce, but not in excess

[49]. A further understanding is needed of the mechanisms involved in young children's regulation of energy intake in response to increases and decreases in ED over time.

An important question is whether the weight status of children influences the relationship between ED and energy intake. Cross-sectional studies show a positive association between dietary ED and measures of overweight and obesity [28, 50 ,51], suggesting either that eating higher-ED foods leads to weight gain or that children with a higher weight status consume higher-ED foods. Conversely, experimental studies that have measured children's *ad libitum* intake in response to ED variations have not found weight status to be influential [6-8]. The lack of a relationship between ED and weight status in previous experimental studies could be due to the limited number of intake measurements. The present study, which measured intake at all eating occasions over 5 days, showed a positive effect of weight status on the relationship between ED and energy intake, even after accounting for energy needs. The results suggest that preschool children with a higher weight status may respond more strongly to ED variations, whereas children with a lower weight status may be more resilient to these differences. One reason for this finding could be differences in taste preferences; research suggests that children with overweight and obesity have greater preferences for fat and sweet tastes compared to children with normal weight [52, 53]. In the current study, the ED of the manipulated foods was decreased by reducing the fat or sugar content, incorporating fruits or vegetables, or a combination of these strategies. It is possible that children with higher weight status were more sensitive to these changes; however, the overall effect of weight status and taste ratings on intake could not be tested since only a subset of the manipulated foods was rated by the children. It is unclear why weight status affects the relationship between ED and intake, and the underlying mechanisms remain to be identified.

Investigating individual characteristics can help to identify children who are more or less susceptible to influences in the eating environment. Characteristics of preschool children were assessed both in the present study of ED variations and in a previous study with a parallel design, which measured the response to changes in portion size over 5 days [29]. In both studies, children with a higher weight status were found to be more susceptible to overconsumption, either from higher ED or from larger portions. In contrast, young children's eating behaviors such as satiety responsiveness and food responsiveness, which were previously found to affect the relationship between portion size and intake [29, 54], were not found to affect the relationship between ED and intake. Satiety responsiveness likely had no significant influence because fullness is affected by the weight of food consumed [9], and the weights served and consumed in the manipulated-ED conditions were similar to the baseline condition. Additionally, there was likely no effect of food responsiveness because of the similarity in food appearance and palatability across ED conditions. If the variations in ED had been more obvious, it is possible that the children's degree of food responsiveness would have affected the relationship between ED and intake. Future studies should investigate whether the method of varying ED (covert or overt) influences its effect in different children, as this could provide insight into the cues that determine how food responsiveness relates to changes in energy intake. One characteristic that was found to significantly reduce differences in intake in response to ED changes was parental ratings of their own behavior in pressuring their child to eat more food [55]. This result may be because parents who gave higher ratings for these practices tended to have children who ate smaller amounts, and thus consumed less of the manipulated main dishes. Together, these findings along with the findings from the 5-day portion size study [29] illustrate the importance of investigating individual differences in children's response to factors in the eating environment. It is critical to emphasize both portion size and ED in child feeding guidelines in order to help moderate intake, particularly for children who already have overweight and obesity.

A strength of the current study is that over 5 days we systematically measured preschool children's intake in response to 3 levels of ED. We weighed intake at all meals and snacks over 5 days, to provide one of the most comprehensive assessments of preschool children's consumption in an experimental study. Almost all intake was measured in the childcare setting, the children's typical eating environment. However, during the 5-day period some children consumed non-study food outside of the childcare center, and this consumption was estimated by parental report. Future investigation of children's intake using another paradigm (e.g self-serve) or in other settings (e.g. the home) is necessary, as the environment is a strong determinant of energy intake [56]. Another strength of the study was the use of accelerometry to objectively measure children's physical activity levels, which were used to estimate energy needs with equations specifically designed for preschool children [34]. On average, the children met their daily energy needs during the baseline condition, indicating that this was a reliable assessment. To generalize these findings, investigation of the influence of ED on children's energy intake is needed in samples that are more diverse in age, culture, and socioeconomic status

The present study demonstrates that varying the ED of multiple foods over 5 days leads to sustained changes in preschool children's energy intake. No significant compensation in energy intake was detected in response to either increases or decreases in ED over time. Overall, this resulted in children overconsuming in relation to energy needs when multiple higher-ED foods were served and under-consuming when lower-ED foods were served. Children with higher weight status were found to be more responsive to ED variations than children with lower weight status. Interventions should focus on strategies that improve the ability of susceptible children to regulate intake in the current food environment, in which the available foods offer a wide range of ED. Strategies may include teaching children about proportionality and filling half of their plates with lower-ED foods such as fruits and vegetables. It is important for caregivers to recognize that preschool children's regulatory systems should not be relied on to adjust intake in response to increases or decreases in dietary ED.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Davis CM. (1928). Self-selection of diets by newly weaned infants. American Journal of Clinical Nutrition, 36(4), 651–679.
2. Birch LL, Johnson SL, Andersen MS, Peters JC, Schulte MC. (1991). The variability of young children's energy intake. The New England Journal of Medicine, 324(4), 232–235. [PubMed: 1985244]
3. Shea S, Stein AD, Basch CE, Contento IR, Zybert P. (1992). Variability and self-regulation of energy intake in young children in their everyday environment. Pediatrics, 90(4), 542–546. [PubMed: 1408507]
4. Pearcey SM, de Castro JM. (1997). Food intake and meal patterns of one year old infants. Appetite, 29, 201–212. [PubMed: 9344428]
5. Fisher JO, Liu Y, Birch LL, Rolls BJ. (2007). Effects of portion size and energy density on young children's intake at a meal. American Journal of Clinical Nutrition 2007; 86:174–179.
6. Leahy KE, Birch LL, Rolls BJ. (2008a). Reducing the energy density of multiple meals decreases the energy intake of preschool-age children. American Journal of Clinical Nutrition, 88, 1459–1468. [PubMed: 19064504]
7. Leahy KE, Birch LL, Rolls BJ. (2008b). Reducing the energy density of an entrée decreases children's energy intake at lunch. Journal of the American Dietetic Association, 108, 41–48. [PubMed: 18155988]
8. Kling SMR, Roe LS, Keller KL, Rolls BJ. (2016). Double trouble: Portion size and energy density combine to increase preschool children's lunch intake. Physiology & Behavior, 162, 18–26. [PubMed: 26879105]
9. Bell EA, Castellanos VH, Pelkman CL, Thorwart ML, Rolls BJ. (1998). Energy density of foods affects energy intake in normal-weight women. American Journal of Clinical Nutrition, 67, 412–420. [PubMed: 9497184]
10. Rolls BJ, Roe LS, Meengs JS. (2006). Reductions in portion size and energy density of foods are additive and lead to sustained decreases in energy intake. American Journal of Clinical Nutrition, 83, 11–17. [PubMed: 16400043]
11. Birch LL, Deysher M. (1985). Conditioned and unconditioned caloric compensation: evidence for self-regulation of food intake in young children. Learning and Motivation, 16, 341–355.
12. Birch LL, Deysher M. (1986). Caloric compensation and sensory specific satiety: evidence for self-regulation of food intake by young children. Appetite, 7, 323–331. [PubMed: 3789709]
13. Hetherington MM, Wood C, Lyburn SC. (2000). Response to energy dilution in the short term: Evidence of nutrition wisdom in young children? Nutritional Neruoscience, 3, 321–329.
14. Birch LL, McPhee LS, Bryant JL, Johnson SL. (1993). Children's lunch intake: effects of midmorning snacks varying in energy density and fat content. Appetite, 20, 83–94. [PubMed: 8507070]
15. Cecil JE, Palmer CAN, Wriden W, Murrie I, Bolton-Smith C, Watt P, Wallis DJ, Hetherington MM. (2005). Energy intakes of children after preloads: adjustment, not compensation. The American Journal of Clinical Nutrition, 82(2), 302–308. [PubMed: 16087972]
16. Birch LL, McPhee L, Shoba BC, Steinberg L, Krehbiel R. (1987). "Clean up your plate": effects of child feeding practices on the conditioning of meal size. Learning and Motivation, 18, 301–317.
17. Remy E, Divert C, Rousselot J, Brondel L, Issanchou S, Nicklaus S. (2014). Impact of energy density on liking for sweet beverages and caloric-adjustment conditioning in children, The American Journal of Clinical Nutrition, 100, 1052–1058. [PubMed: 25240072]

18. Remy E, Issanchou S, Chabanet C, Boggio V, Nicklaus S. (2015). Impact of adiposity, age, sex, and maternal feeding practices on eating in the absence of hunger and caloric compensation in preschool children. International Journal of Obesity, 39, 925–930. [PubMed: 25777357]
19. De Castro JM. (1998). Prior day's intake has macronutrient-specific delayed negative feedback effects on the spontaneous food intake of free-living humans. Journal of Nutrition, 128, 61–67. [PubMed: 9430603]
20. Bray GA, Flatt JP, Volufova J, Delany JP, Champagne CM. (2008). Corrective responses in human food intake identified from an analysis of 7-d food-intake records. American Journal of Clinical Nutrition, 88, 1504–1510. [PubMed: 19064509]
21. Flatt JP. What do we most need to learn about food intake regulation? (1998). Obesity Research, 6(4), 307–310. [PubMed: 9688108]
22. Poppitt SD, Prentice AM. (1996). Energy density and its role in the control of food intake: evidence from metabolic and community studies. Appetite, 26, 153–174. [PubMed: 8737167]
23. McCrickerd K, Salleh NB, Forde CG (2016). Removing energy from a beverage influences later food intake more than the same energy addition. Appetite, 105, 549–556. [PubMed: 27356202]
24. Lenard NR, Berthoud HR. (2008). Central and peripheral regulation of food intake and physical activity: pathways and genes, Obesity, 16(3), S11–A22.
25. Blundell J. (2018). Behaviour, energy balance, obesity and capitalism. European Journal of Clinical Nutrition, 72, 1305–1309. [PubMed: 30185852]
26. Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O. (2006). SAS for Mixed Models. 2nd ed. Cary, NC: SAS Institute Inc.
27. Spill MK, Birch LL, Roe LS, Rolls BJ. (2011). Hiding vegetables to reduce energy density: an effective strategy to increase children's vegetable intake and reduce energy density. American Journal of Clinical Nutrition, 94, 735–741. [PubMed: 21775554]
28. Vernarelli JA, Mitchell DC, Hartman TJ, Rolls BJ. (2011). Dietary energy density is associated with body weight status and vegetable intake in U.S. children. Journal of Nutrition, 141, 2204–2210. [PubMed: 22049295]
29. Smethers AD, Keller KL, Meehan CT, Roe LS, Sanchez CE & Rolls BJ. (2017). Can we identify children who are most responsive to large portions? Abstract presented at Obesity Week. Internet: https://2017.obesityweek.com/abstract/can-we-identify-children-who-are-most-responsive-to-large-portions/
30. U.S. Department of Agriculture, Food and Nutrition Service. (2017). Child and adult care food program (CACFP) child care meal pattern. Version current: 29 3 2017. Internet: https://fns-prod.azureedge.net/cacfp/child-and-adult-care-food-program. (accessed 28 June 2018).
31. US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference. Release 28. Version Current: 4 2018 Internet: https://ndb.nal.usda.gov/ndb/ (accessed 15 November 2018).
32. Birch LL. (1979). Dimensions of preschool children's food preferences. Journal of Nutrition Education, 11(2), 77–80.
33. Butte NF, Wong WW, Lee JS, Adolph AL, Puyau MR, Zakeri IF. (2014a). Prediction of energy expenditure and physical activity in preschoolers. Medicine & Science in Sports & Exercise, 46(6), 1216–1226. [PubMed: 24195866]
34. Butte NF, Wong WW, Wilson TA, Adolph AL, Puyau MR, Zakeri IF. (2014b). Revision of Dietary Reference Intakes for energy in preschool-age children. American Journal of Clinical Nutrition, 100, 161– 7. [PubMed: 24808489]
35. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, A SAS program for the 2000 CDC growth charts (ages 0 to < 20 y). Version current: 16 12 2016 Internet: http://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm. (accessed 15 November 2018).
36. Wardle J, Guthrie CA, Sanderson S. Rapoport L. (2001). Development of the Children's eating behavior questionnaire. Journal of Child Psychology & Psychiatry, 48, 104–113.
37. Birch LL, Fisher JO, Grimm-Thomas K, Markey CN, Sawyer R, Johnson SL. (2001). Confirmatory factor analysis of the Child Feeding Questionnaire: a measure of parental attitudes,

beliefs and practices about child feeding and obesity proneness. Appetite, 36, 201–10. [PubMed: 11358344]

38. Benjamini Y, Hochberg Y. (1995). Controlling for the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B, Statistical Methodology, 57, 289–300.
39. Fomon SJ, Filer LJ, Thomas LN, Rogers RR, Proksch AM. (1969). Relationship Between Formula Concentration and Rate of Growth of Normal Infants. Journal of Nutrition, 98: 241–254. [PubMed: 5783305]
40. Fomon SJ, Filer LJ, Thomas LN, Anderson TA, Nelson SE. (1975). Influence of formula concentration on caloric intake and growth of normal infants. Acta Paediatrica Scandinavica, 64: 172–181. [PubMed: 1168981]
41. Forde CG. (2018). From perception to ingestion; the role of sensory properties in energy selection, eating behavior and food intake. Food Quality and Preference, 66, 171–177.
42. Weingarten HP. (1985). Stimulus control of eating: implications for a two-factor theory of hunger. Appetite, 6, 387–401. [PubMed: 3911890]
43. De Castro JM. (1996). How can eating behavior be regulated in the complex environments of free-living humans? Neuroscience and Biobehavioral Reviews, 20(1), 119–131. [PubMed: 8622818]
44. Blundell JE, Gillett A. (2001). Control of food intake in the obese. Obesity Research, 9(4), 263S–270S. [PubMed: 11707552]
45. Maus N, Pudel V. (1988). Psychological determinants of food intake In Thomson DMH (Ed.), Food acceptability (pp.181–192). New York, NY: Elsevier Science Publishers LTD.
46. Herman CP, Polivy J. (2005). Normative influences on food intake. Physiology & Behavior, 86, 762–772. [PubMed: 16243366]
47. Birch LL, Anzman SL. (2010). Learning to eat in an obsesogenic environment: a developmental systems perspective on childhood obesity. Child Development Perspectives, 4(2), 138–143.
48. Rolls BJ, Leahy KE. (2010). Reductions in dietary energy density to moderate children's energy intake In Dube L, Bechara A, Drewnowski A, Lebel J, James P, Yada RY, Laflamme-Sanders M (Eds.). Obesity prevention: the role of brain and society on individual behavior. 543–554. Burlington, MA: Elsevier.
49. Rogers P. (2018). Combating excessive eating: a role for four evidenced-based remedies. Obesity, 26, S18–S24. [PubMed: 30290075]
50. Aburto TC, Cantoral A, Hernandez-Barrera L, Carriquiry AL, Rivera JA. (2015). Usual dietary energy density is positively associated with excess body weight in Mexican children. Journal of Nutrition, 145(7), 1524–1530. [PubMed: 25926409]
51. Zhou X, Xue H, Duan R, Liu Y, Zhang L, Harvey L, Cheng G. (2015). The cross-sectional association of energy intake and dietary energy density with body composition of children in southwest china. Nutrients, 7, 5396–5412. [PubMed: 26151177]
52. Lanfer A, Knof K, Barba G, Veidebaum T, Papoutsou S, Henauw S, Soos T, Moreno L, Ahrens W, Lissner L. (2012). Taste preferences in association with dietary habits and weight status in European children: results from the IDEFICS study. International Journal of Obesity, 36, 27–34. [PubMed: 21844876]
53. Wardle J, Guthrie C, Sanderson S, Birch L, Plomin R. (2001). Food and activity preferences in children of lean and obese parents. International Journal of Obesity, 25, 971–977. [PubMed: 11443494]
54. Mooreville M, Davey A, Orloski A, Hannah EL, Mathias KC, Birch LL, Kral TVE, Zakeri IF, Fisher JO. (2015). Individual differences in susceptibility to large portion sizes among obese and normal-weight children. Obesity, 23, 808–814. https://doi:10.1002/oby.21014. [PubMed: 25683105]
55. Galloway AT, Fiorito L, Lee Y, & Birch LL. (2005). Parental pressure, dietary patterns, and weight status among girls who are "picky eaters". Journal of the American Dietetic Association, 105, 541–548. [PubMed: 15800554]
56. Mrdjenovic G, Levitsky DA. (2005). Children eat what they are served: the imprecise regulation of energy intake. Appetite, 44(3), 273–282. [PubMed: 15927729]

Highlights

- Modifying energy density had a sustained effect on children's intake over 5 days
- Energy intake was not adjusted over 5 days in response to changes in energy density
- Energy deficits did not elicit a stronger regulatory response than energy surfeits
- The effects of energy density were greater for children with a higher weight status

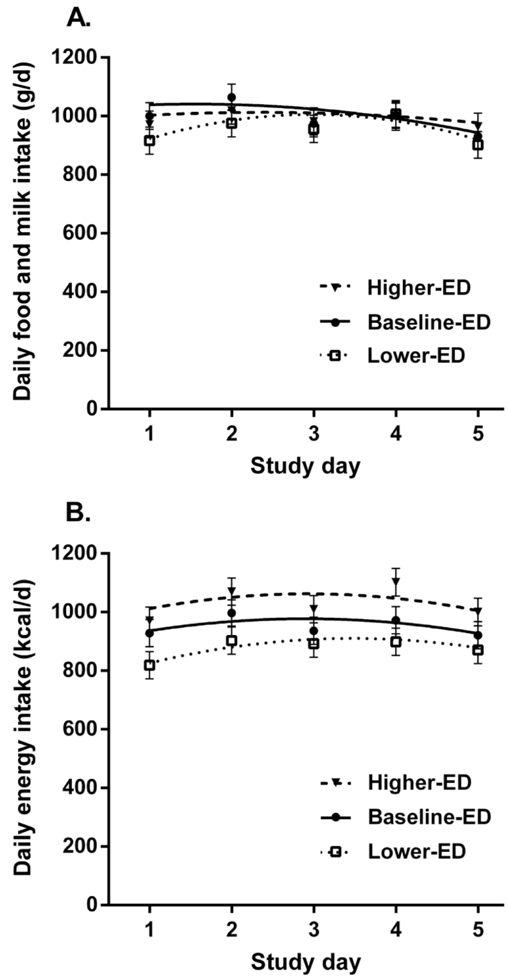

FIGURE 1.

Mean (±SEM) daily intake across time according to the (A) weight and (B) energy consumed by 49 preschool children who over 3 different 5-day periods were served multiple lower-energy density (ED), baseline-ED, and higher-ED foods. Mean daily intakes and trajectories are from mixed models that were adjusted for study week, menu, classroom, and sex. A random coefficients model showed that the pattern of daily intake over 5 days did not differ between conditions for weight (P=0.19) or energy (P=0.94), indicating intake was not adjusted in response to changes in ED. Thus, increases and decreases in energy intake from the baseline-ED condition were sustained over time.

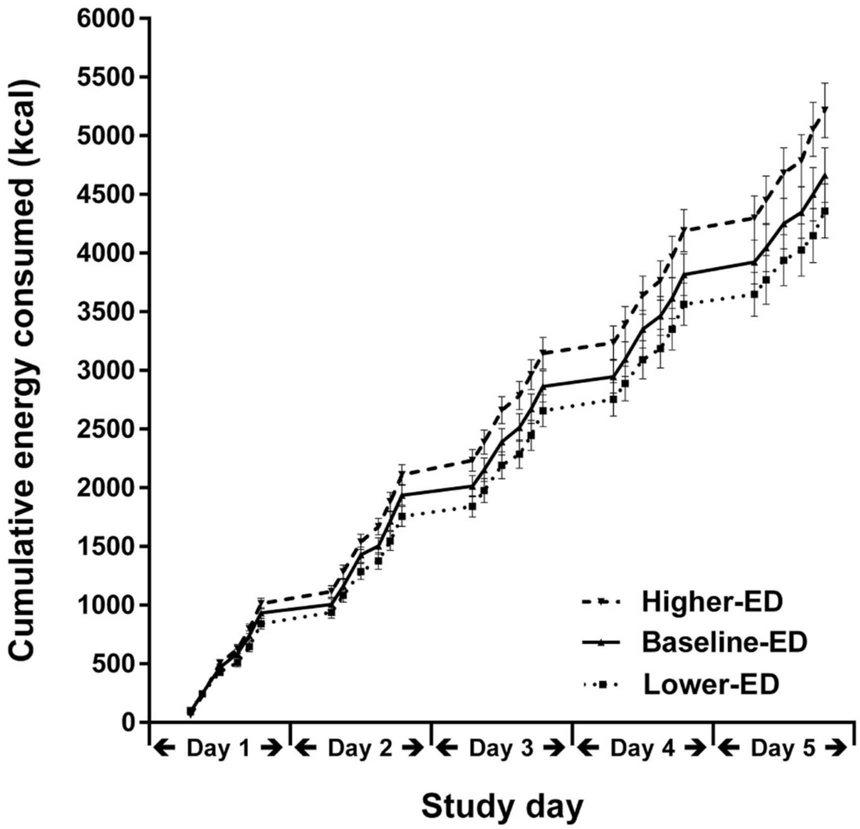

FIGURE 2.

Mean cumulative energy intake consumed by 49 preschool children who over 3 different 5-day periods were served lower-energy density (ED), baseline-ED, and higher-ED foods. A mixed linear model demonstrated that ED variations resulted in significantly different cumulative intakes: 5549±290 kcal in the higher-ED, 4976±221 in the baseline-ED condition, and 4549±180 in the lower-ED condition (all $P<0.02$).

Table 1

Mean daily amounts served to 49 preschool children in the lower-energy density (ED), baseline-ED, and higher-ED conditions over 3 different 5-day periods [1]

	Experimental Conditions		
	Lower-ED	**Baseline-ED**	**Higher-ED**
Weight served (g)			
Manipulated foods[2]	550 ± 0	550 ± 0	550 ± 0
Non-manipulated food and milk[3]	1998 ± 29	1998 ± 29	1998 ± 29
Total[4]	2548 ± 29	2548 ± 29	2548 ± 29
Energy density (kcal/g)			
Manipulated foods	1.47 ± 0.05	1.82 ± 0.06	2.19 ± 0.07
Non-manipulated food and milk	0.69 ± 0.01	0.69 ± 0.01	0.69 ± 0.01
Total	0.86 ± 0.01	0.93 ± 0.01	1.01 ± 0.01
Energy content (kcal)			
Manipulated foods	809 ± 28	1001 ± 32	1205 ± 38
Non-manipulated food and milk	1370 ± 31	1370 ± 31	1370 ± 31
Total	2179 ± 57	2371 ± 60	2575 ± 65

[1]All values are mean ± SEM.

[2]Manipulated foods on each daily menu were varied in ED and consisted of one main dish at each meal of breakfast, lunch, and dinner plus one dish at afternoon snack. Recipes for the manipulated foods can be obtained by contacting the corresponding author.

[3]Non-manipulated foods on each daily menu were not varied in ED and consisted of side dishes served with breakfast, lunch, afternoon snack, and dinner plus foods served at morning and evening snacks.

[4]Total food and milk on each menu consisted of manipulated foods, non-manipulated foods, and milk.

Table 2

Characteristics of 49 preschool children

Characteristic.	Boys (N= 26)		Girls (N=23)		Significance of difference[1]
	Mean ± SD	**Range**	**Mean ± SD**	**Range**	**(*P*-value)**
Age (y)[2]	4.3 ± 0.7	3.2 – 5.5	4.3 ± 0.7	3.2 - 5.5	0.98
Weight (kg)[3]	17.5 ± 2.5	13.6 – 22.8	18.1 ± 5.3	13.6 – 39.3	0.60
Height (cm)[3]	104.3 ± 6.4	92.1 – 117.7	105.1 ± 7.3	95.2 – 127.9	0.71
Sex-specific BMI-for-age percentile[3,4]	57.9 ± 26.7	9.3 – 95.5	61.7 ± 23.3	11.5 – 99.6	0.62
BMI z-score[3,4]	0.26 ±0.83	−1.32 – 1.70	0.39 ± 0.83	−1.20 – 2.66	0.60
Estimated energy requirements (kcal/d)[3,5]	1029 ± 61	924 - 1148	947 ± 86	868 - 1272	0.0006
Satiety responsiveness[2,6]	2.9 ± 0.5	2.2 – 3.6	3.1 ± 0.4	2.0 – 3.8	0.16
Food responsiveness[2,6]	2.6 ± 0.9	1.4 – 4.4	2.5 ± 0.6	1.4 – 3.6	0.68
Pressure to eat[2,7]	2.5 ± 1.1	1.0 – 5.0	2.6 ± 0.9	1.0 – 4.0	0.71

[1]Differences between means of boys and girls were evaluated by independent-samples t-tests.

[2]2 children (1 boy; 1 girl) did not have the questionnaires filled out by a parent.

[3]4 children (1 boy; 3 girls) were absent on the days heights and weights were collected.

[4]BMI percentiles and z-scores were calculated from body weight, height, age, and sex using a software program based on nationally representative data [29].

[5]Estimated energy requirements were calculated from body weight, height, sex, and physical activity level using equations for preschool children [28].

[6]Subscale on the Children's Eating Behaviour Questionnaire [30].

[7]Subscale on the Child Feeding Questionnaire [31].

Table 3

Mean daily amounts consumed by 49 preschool children who over 3 different 5-day periods were served lower-energy density (ED), baseline-ED, and higher-ED foods[1].

	Experimental Condition		
	Lower-ED	Baseline-ED	Higher-ED
Food and milk intake (g/d)			
Manipulated dishes[2]	148±11[a]	158±11[ab]	167±11[b]
Non-manipulated foods and milk[3]	803±36 [a]	838±36 [a]	822±36 [a]
Total[4]	952±41[a]	997±41[b]	988±41[ab]
Energy density (kcal/g)			
Manipulated dishes	1.48±0.02[a]	1.87±0.02[b]	2.23±0.03[c]
Non-manipulated foods and milk	1.13±0.03[a]	1.12±0.03[a]	1.13±0.03[a]
Total	0.93±0.02[a]	0.97±0.02[b]	1.06±0.03[c]
Energy intake (kcal/d)			
Manipulated dishes	219±22 [a]	297±22 [b]	370±22 [c]
Non-manipulated foods and milk	659±26 [a]	657±26 [a]	664±26 [a]
Total	884±38 [a]	956±37 [b]	1041±3 [c]
Macronutrients (% energy)			
Fat	19.1±0.6[a]	22.5±0.6[b]	26.6±0.6[c]
Protein	14.5±0.4[a]	14.5±0.4[a]	13.2±0.4[b]
Carbohydrate	66.2±0.8[a]	62.7±0.8[b]	60.0±0.8[c]

[1]All values are mean ± SEM from mixed linear models with repeated measures; values in the same row with different superscript letters are significantly different ($P < 0.05$).

[2]Manipulated foods on each daily menu were varied in ED by decreasing or increasing by 20% from the baseline ED, and consisted of one main dish at each meal of breakfast, lunch, and dinner plus one dish at afternoon snack.

[3]Non-manipulated foods on each daily menu were not varied in ED and consisted of side dishes served with breakfast, lunch, afternoon snack, and dinner plus foods served at morning and evening snacks.

[4]Total food and milk on each menu consisted of manipulated foods, non-manipulated foods, and milk.