Double trouble: Portion size and energy density combine to increase preschool children's lunch intake

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Supported by NIH Grant R01-DK082580 and USDA Grant 2011-67001-30117

Running Head: Portion size and energy density affect intake

Abbreviations: ED, energy density

Research Registration: Registration number: NCT02299531 at <http://www.clinicaltrials.gov/>

1 ABSTRACT

2 **Background:** Both portion size and energy density (ED) have substantial effects on intake;
3 however, their combined effects on preschool children's intake have not been examined when
4 multiple foods are varied at a meal.

5 **Objective:** We tested the effects on intake of varying the portion size and ED of lunches served
6 to children in their usual eating environment.

7 **Design:** In a crossover design, lunch was served in 3 childcare centers once a week for 6 weeks
8 to 120 children aged 3 to 5 y. Across the 6 meals, all items were served at 3 levels of portion size
9 (100%, 150%, or 200%) and 2 levels of ED (100% or 142%). The lunch menu had either lower-
10 ED or higher-ED versions of chicken, macaroni and cheese, vegetables, applesauce, ketchup, and
11 milk. Children's ratings of the foods indicated that the lower-ED and higher-ED meals were
12 similarly well liked.

13 **Results:** The weight of food and milk consumed at meals was increased by serving larger
14 portions ($P<0.0001$) but was unaffected by varying the ED ($P=0.22$). Meal energy intake,
15 however, was independently affected by portion size and ED (both $P<0.0001$). Doubling the
16 portions increased energy intake by 24% and increasing meal ED by 42% increased energy
17 intake by 40%. These effects combined to increase intake by 175 ± 12 kcal or 79% at the higher-
18 ED meal with the largest portions compared to the lower-ED meal with the smallest portions.
19 The foods contributing the most to this increase were chicken, macaroni and cheese, and
20 applesauce. The effects of meal portion size and ED on intake were not influenced by child age
21 or body size, but were significantly affected by parental ratings of child eating behavior.

22 **Conclusion:** Strategically moderating the portion size and ED of foods typically consumed by
23 children could substantially reduce their energy intake without affecting acceptability.

24

25 **Keywords:** preschool children, energy intake, portion size, energy density, eating behavior,

26 obesity

27

INTRODUCTION

Both the portion size and energy density (ED) of foods have robust effects on energy intake, and these two factors are often implicated as primary drivers of the obesity epidemic. In preschool children, serving larger portions has been consistently shown to increase energy intake (1-5). Less is known about the effects of ED on children's eating behavior, but the available data suggest that variations in ED have potent effects on energy intake (6-8), and higher-ED diets are associated with increased weight gain and weight status (9, 10). Although children are often exposed to multiple foods that are both large in portion size and high in ED, the potential of these properties acting together to influence consumption is not understood. To determine the combined effects of portion size and ED on energy intake, we systematically varied all the foods in a typical meal served in children's usual preschool environment.

Studies in adults have shown that portion size and ED have strong, independent effects that combine to increase energy consumption, but it is unclear whether young children respond similarly. Only two studies have examined the combined effects of portion size and ED at a meal in children (11, 12). One study found that both portion size and ED affected energy intake independently and that the effects combined (11), but another study found that only ED affected energy intake (12). In addition, these studies modified only the main dish, which does not represent children's current eating environment where multiple foods within a meal vary in both portion size and ED. To date, no research has tested whether children adjust their consumption in response to such variations in an entire meal. Most research has focused on how variations in portion size and ED affect intake of components of a meal such as main or side dishes, and few studies have examined these effects when all foods at a meal are varied (5, 7, 13). When children are served an entire meal of large portions of high-ED foods, they might adjust their intake by

51 eating a smaller proportion of all items or by altering their food selection and eating less of the
52 higher-ED options. If such self-regulation does not occur, however, the powerful effects of
53 portion size and ED have the potential to substantially increase energy intake, especially when
54 everything in a meal is changed simultaneously. Given the prevalence of large portions of high-
55 ED foods (14, 15), it is essential to determine how these factors combine to influence food
56 selection and energy intake in children.

57 Identifying children at risk for overeating when served large portions and higher-ED foods
58 could lead to the development of targeted recommendations, and thus recent studies have
59 focused on relating individual characteristics to variations in children's responses to these
60 factors. The findings, however, have not led to clear and consistent predictors. In some studies,
61 the response to larger portion sizes has been shown to be related to child age, weight status, or
62 two sub-scales on the Child Eating Behaviour Questionnaire: satiety responsiveness and food
63 responsiveness (1, 4, 5, 16, 17). In several other studies, however, these characteristics did not
64 influence the relationship between portion size and intake (2, 3, 6-8, 11, 12, 18, 19).
65 Furthermore, there has been limited research on the influence of individual characteristics on the
66 effects of ED. Since children are exposed to foods that differ in both portion size and ED, it is
67 important to test the effect of individual characteristics when these factors are varied
68 simultaneously.

69 The primary aim of this study was to examine the independent and combined effects on
70 children's intake of changing the portion size and ED of all components of a meal. Using a 3-by-
71 2 crossover design, we systematically varied the portion size and ED of commonly consumed
72 items in a meal served in childcare centers. We hypothesized that increasing the portion size and
73 ED of a meal would have independent effects that combine to increase children's energy intake

(20, 21). To investigate whether the effects on intake varied across child characteristics or might differ for individual foods, we tested a larger sample of children than has been previously tested (1-8, 11-13, 16, 18, 19). Determining how typical variations in the portion size and ED of a meal influence preschool children's energy intake is essential in order to understand the environmental factors that affect children's food intake and to develop practical strategies to counter these effects.

SUBJECTS AND METHODS

Experimental design

A within-subjects crossover design was used to evaluate the effect on intake of increasing the ED and portion size of all foods and milk served to preschool children at lunch. On 1 day a week for 6 weeks, the experimental meal was served to children in their classrooms at 3 childcare centers. Across the 6 meals, all foods and milk were served at 3 levels of portion size (100%, 150%, or 200% of reference amounts) and 2 levels of energy density (100% or 142%) and were consumed ad libitum (**Figure 1**). The order of the six conditions was counterbalanced across classrooms using Latin squares, and classrooms were randomly assigned one of the condition sequences using a random number generator. Since the children were only served one experimental meal per week, they never saw the different meals together. The children's height, weight, and liking of the study foods were assessed after the final experimental meal.

Figure 1: All foods at the experimental meals were served at 3 levels of portion size (100%, 150%, or 200%) and 2 levels of energy density (100% or 142%). Children were served one experimental meal per week for 6 weeks; thus, they never saw the different meals together.

Participant recruitment

Children were recruited by giving letters to parents with 3- to 6-year-old children enrolled at three childcare centers near University Park, PA. Parents provided written consent for the participation of their child in the study as well as their own participation in completing questionnaires. Children with a parentally reported allergy or intolerance to the foods or milk being served were not eligible to participate in the study. Neither parents nor children were informed about the purpose of the study. All procedures were reviewed and approved by The Pennsylvania State University Office for Research Protections.

A power analysis was conducted to determine the number of children needed for the study, based on previous research in a similar population with a similar meal. A clinically significant difference in energy intake was considered to be 48 kcal, which is approximately 15% of typical lunch intake in this population. A power analysis showed that a sample size of 58 children would allow detection of this difference at a significance level of 0.05 and power of 80%. To explore the effect of individual characteristics on the primary outcomes, we enrolled 131 children, which is larger than the sample sizes of previous controlled studies of portion size or ED (1-3, 5-8).

Experimental menu and meal procedures

Experimental menu. The experimental meal consisted of chicken (grilled breast or breaded nuggets), macaroni and cheese, a green vegetable (broccoli or peas), applesauce, ketchup, and milk. This menu was chosen because the foods and milk naturally vary in energy density (ED), are commercially available, and are commonly consumed by preschool children. Across meals, all foods and milk were served at 3 levels of portion size (100%, 150%, or 200%) and 2 levels of

ED (100% or 142%) (Figure 1). In the 100% (reference) portion size condition, the amounts of each food and beverage served were based on preschool children's intake in previous studies (7, 12, 19) and met the minimum recommendations from the Child and Adult Care Food Program (22). The larger portions of each food and milk were chosen to be 150% and 200% the size of the reference portion. As described in **Table 1**, ED was modified primarily by using products and recipes that differed in fat and sugar content. The 42% increase in ED was matched across all items served at the meal; this increase was based on the ED difference between the two types of milk. In addition to milk, children were provided 237 mL of water that was not varied in portion size or ED. The lower-ED meal had 38% of energy as carbohydrates, 34% as protein, and 27% as fat; the higher-ED meal had 41% of energy as carbohydrates, 21% as protein, and 38% as fat.

Table 1: Items served in the experimental meals of lower and higher energy density; amounts of weight and energy served are shown for the 100% portion size conditions.

Lower-energy-dense meal				Higher-energy-dense meal			
Lower-energy-dense items	ED ¹³ (kcal/g)	100% portion size		Higher-energy-dense items	ED (kcal/g)	100% portion size	
		Weight (g)	Energy (kcal)			Weight (g)	Energy (kcal)
Grilled chicken strips ^{1,2}	1.19	100	119	Breaded chicken nuggets ^{2,3}	1.68	100	168
Macaroni and cheese ⁴	1.50	100	150	Macaroni and cheese with extra butter and oil ⁵	2.10	100	210
Broccoli with butter and flavoring ⁶	0.57	75	43	Peas with butter flavoring ⁷	0.81	75	61
Reduced-sugar applesauce ⁸	0.50	100	50	Sweetened applesauce ⁹	0.71	100	71
Reduced-sugar ketchup ¹⁰	0.82	20	16	Regular ketchup ¹¹	1.18	20	24
Low-fat milk (1%) ¹²	0.42	183	77	Whole milk ¹²	0.61	183	112
Total food served	0.96	395	378	Total food served	1.35	395	533
Total food & milk served	0.79	578	455	Total food & milk served	1.12	578	645

¹Fully Cooked Chicken Breast Strips, Tyson Foods Inc., Springdale, AR.

²Chicken strips and nuggets were cut into similar bite-sized pieces.

³Gluten Free Breaded Chicken Breast Nuggets, Bell & Evans, Fredericksburg, PA.

⁴Stouffer's Macaroni and Cheese, Nestle U.S.A. Inc., Solon OH.

⁵Stouffer's Macaroni and Cheese, Nestle U.S.A. Inc., Solon OH; Unsalted Whipped Butter, Land O'Lakes Inc., Arden Hills, MN; Crisco Pure Canola Oil, The J.M. Smucker Company, Orrville, OH.

⁶Petite Broccoli Florets, Hanover Foods Corporation, Hanover, PA; Unsalted Whipped Butter, Land O'Lakes Inc., Arden Hills, MN; Molly McButter Butter Flavor Sprinkles, B&G Foods Inc., Parsippany, NJ.

⁷Sweet Peas, Foodhold U.S.A LLC, Landover, MD; Molly McButter Butter Flavor Sprinkles, B&G Foods Inc., Parsippany, NJ.

⁸Lucky Leaf Premium Blend Applesauce, Knouse Foods Inc., Peach Glen, PA; Lucky Leaf Unsweetened Applesauce, Knouse Foods Inc., Peach Glen, PA.

⁹Lucky Leaf Premium Blend Applesauce, Knouse Foods Inc., Peach Glen, PA.

¹⁰Tomato Ketchup, H.J. Heinz Co. L.P., Pittsburgh, PA; Reduced Sugar Ketchup with Sucralose, H.J. Heinz Co. L.P., Pittsburgh, PA.

¹¹Tomato Ketchup, H.J. Heinz Co. L.P., Pittsburgh, PA.

¹²Giant Food Stores Milk, Foodhold U.S.A LLC, Landover, MD.

¹³ED, energy density.

Meal procedures. Children consumed the experimental meals *ad libitum* in their usual classrooms in the child care centers and at their regularly scheduled lunchtime. Children ate at tables with the same group of three to six children and one adult, which is standard practice at the child care centers. Foods and beverages were pre-portioned into dishware and then set at each child's place at the table before the children were seated. Before the first experimental meal, researchers explained to the children that they could eat as much or as little as they wanted, but could not request more of any food or beverage. During each meal, adults, including teachers and undergraduate research assistants who did not know the purpose of the study, were instructed to redirect conversations about food-related topics to minimize peer influence on children's lunch intake. After all children had finished lunch, researchers returned any dropped and spilled foods to the correct plate or bowl and recovered any spilled beverage with paper towels. To determine the amount consumed, all foods and beverages were weighed before and after the meal in a separate room out of the children's view. Food weights were recorded to the nearest 0.1 g using digital scales (Mettler-Toledo PR5001 and XS4001S; Mettler-Toledo, Columbus, OH). Energy and nutrient intakes were calculated using information from food manufacturers and a standard food composition database (23). Children in the classrooms who did not participate in the study sat at tables out of the view of participating children and were served the childcare center's scheduled lunch menu.

Assessments

Food liking assessments. Liking for the experimental foods and milk was assessed during an individual session with each child using a 5-point cartoon face scale based on a 3-point version used in previous research (24-26). The child was seated at a table with the 5 cartoon faces and

was instructed on using them to indicate whether a food was “super yummy,” “yummy,” “just okay,” “yucky,” or “super yucky.” After instruction, samples of each food and beverage were presented to the child one at a time; the condiment (ketchup) was not assessed since it was a minor component of the meal and is not usually consumed alone. The order of presenting the samples of the five items was randomized and within each item the low-ED and high-ED versions were also presented in a random order. The children were asked to taste the food or milk and indicate their liking for it by pointing to the appropriate cartoon face. Children’s liking for the foods and milk was assessed within 2 weeks after the final experimental meal. Food liking assessments were completed by 93 (78%) of the children; the remaining children were absent or declined to participate.

Body weight and height. Body weight was measured to the nearest 0.1 kg using a portable digital scale (Seca Onda model 843; Seca Corporation, Hanover, MD). Height was measured in duplicate to the nearest 0.1 cm using a portable stadiometer (model 214; Seca Corporation, Hanover, MD). Body weight and height were used to calculate body size parameters (sex-specific BMI-for age-percentiles and z-scores) using a software program based on nationally representative data (27). Children with sex-specific BMI-for-age percentiles $\geq 85^{\text{th}}$ or $< 95^{\text{th}}$ percentile were classified as overweight and those with percentiles $\geq 95^{\text{th}}$ were classified as obese. Estimated daily energy requirements were calculated using equations for children ages 3 through 5 years with light activity levels (28). Children’s weight and height were assessed within 2 weeks after the final test meal. One hundred and five children (88%) completed the assessments for height and weight; the remaining children were absent or declined to participate.

Parental questionnaires. Parents were asked to complete three questionnaires about parent and child characteristics and behaviors related to the eating environment. The 35-item Child Eating Behaviour Questionnaire (CEBQ) evaluates 8 subscales that relate to eating styles and behaviors of the child (29, 30). Example items include “My child is always asking for food” from the 5-item food responsiveness subscale, and “My child gets full before his/her meal is finished” from the 5-item satiety responsiveness subscale. The 31-item Child Feeding Questionnaire (CFQ) assesses 7 subscales that measure perceived weight concerns and feeding practices (31, 32). An example from the 3-item monitoring subscale is “How much do you keep track of sweets (candy, ice cream, cake, pies, pastries) that your child eats?” For these CFQ and CEBQ subscales, the parent rated each item on a 5-point frequency scale (1=never, 5=always); for analyses, a mean score was calculated across all the items in a given subscale. The 16-item demographic questionnaire assesses family demographics and child health. Parents of 106 children (88%) completed the three questionnaires.

Statistical analysis

Data analyses were conducted using a mixed linear model with repeated measures (SAS version 9.4; SAS Institute, Inc., Cary, NC) for the outcomes of total meal intake and intake of the individual foods and milk, both by weight (g) and energy (kcal). Fixed factors in the model were meal portion size (100%, 150%, or 200%), meal ED (100% or 142%), study week, and classroom. All interactions were tested and then removed from the model if not significant. Subjects were treated as a random factor. For outcomes with significant effects, the Tukey-Kramer method was used to adjust significance levels for multiple pairwise comparisons between means. Children who were absent for 3 or more conditions were excluded from

analyses. The procedure described by Littell et al. was used to identify children whose intakes were influential on the main outcomes on the basis of extreme values for the restricted likelihood distance (33); no intakes were found to meet the pre-determined criteria.

Analysis of covariance was used to assess the influence of continuous subject characteristics (age, body weight, height, BMI percentile, BMI z-score, CFQ subscales, and CEBQ subscales) on the relationship between ED, portion size, and total meal intake; the children's sex was also tested as a factor in the models. All subject characteristics were tested simultaneously in the final models. Children's ratings of liking for each food were tested as a categorical factor in the model for intake of that food. Ordinal repeated measures logistic regression was used to compare the lower-ED and higher-ED meals as well as individual meal items for differences in the distribution of children's liking ratings; results are reported as odds ratios with 95% confidence levels. *T*-tests were used to evaluate differences between boys and girls in age, body weight, height, BMI percentile, and BMI z-score. Data are reported as means $\pm$ standard errors, and results were considered significant at $P < 0.05$.

RESULTS

Subject characteristics

A total of 131 children from 11 classrooms at the 3 childcare centers were enrolled in the study from May 2013 to July 2014. Eleven children were excluded from the analysis because they were absent for 3 or more of the 6 experimental meals. Thus, intake data was analyzed for 120 children (61 boys and 59 girls). The children had a mean age of 4.4 ± 0.1 years and a mean sex-specific BMI-for-age percentile of 56.8 ± 2.6 (**Table 2**). Fifteen children (14%) were classified as overweight or obese. The sample of children was 69% white, 21% Asian, 3% black

217 or African American, and 7% of mixed or another race; 4% were of Hispanic or Latino origin.
218 Based on the 106 parents (88%) who provided family information, household incomes and
219 education levels were above average: 69% of households had an annual income of above
220 \$50,000 and 92% of mothers and 90% of fathers had a Bachelor's degree or higher.

Table 2: Characteristics of 120 pre.school children participating in a study that tested the effects of increasing portion size and energy density of a meal on lunch intake

Characteristic	Boys (n=61) ¹			Girls (n=59)		
	n	Mean $\pm$ SEM	Range	n	Mean $\pm$ SEM	Range
Age (y)	56	4.4 $\pm$ 0.1	3.2–5.8	56	4.4 $\pm$ 0.1	3.1–5.9
Weight (kg)	52	18.2 $\pm$ 0.5 ^b	11.7– 34.5	55	17.0 $\pm$ 0.4	11.9–24.2
Height (cm)	53	106.5 $\pm$ 1.0	90.4–121.5	55	103.9 $\pm$ 0.9	90.5–115.5
Sex-specific BMI-for-age percentile	51	55.6 $\pm$ 4.1	0.1–99.9	54	57.9 $\pm$ 3.3	1.2–94.4
BMI z-score	51	0.17 $\pm$ 0.15 ^a	-3.27–2.98	54	0.22 $\pm$ 0.10	-2.27–1.59
Daily energy requirements (kcal) ²	52	1163 $\pm$ 13 ^b	988-1539	55	1049 $\pm$ 9	917-1203
Food responsiveness score ³	53	2.5 $\pm$ 0.1	1.2–4.2	51	2.3 $\pm$ 0.1	1.0–3.6
Enjoyment of food score ³	53	3.6 $\pm$ 0.1	2.0–5.0	51	3.6 $\pm$ 0.1	1.8–5.0
Monitoring score ⁴	53	4.0 $\pm$ 0.1	1.7–5.0	50	4.0 $\pm$ 0.1	1.7–5.0

¹ Boys and girls were compared with the use of a *t* test. Significant difference between boys and girls according to a *t* test, ^aP < 0.01, ^b

P < 0.05

²Daily energy requirements were estimated using equations for children ages 3 through 5 years with light activity levels (28)

³Subscale on the Child Eating Behaviour Questionnaire (29, 30)

⁴Subscale on the Child Feeding Questionnaire (31, 32)

221 Food and energy intakes

222 *Weight of food and milk.* There was a significant effect of portion size ($P<0.0001$) but not ED
 223 ($P=0.22$) on the weight of the meal consumed (**Figure 2A**). Compared to the 100% portion size
 224 conditions, meal intake was 21% (60 ± 7 g) greater in the 150% portion size conditions and 26%
 225 (74 ± 7 g) greater in the 200% portion size conditions (both $P<0.0001$). Mean intakes in the
 226 150% and 200% conditions did not differ significantly from each other ($P=0.19$). A similar
 227 weight of food and milk was consumed at the lower-ED and higher-ED meals (328 ± 11 vs. 321
 228 ± 10 g; $P=0.22$).

Figure 2: Mean ($\pm$ SEM) food and milk intakes (g and kcal) at lunch by meal portion size and energy density (ED) in 120 preschool children. **Figure 2A:** There was a significant effect of portion size ($P<0.0001$) but not energy density ($P=0.22$) on total meal intake by weight. Across the lower- and higher-ED meals, intake was significantly greater when children were served the 150% and 200% portion size conditions compared to the 100% condition. **Figure 2B:** There were significant and independent effects of portion size and energy density (both $P<0.0001$) on total meal energy intake. Across the lower- and higher-ED meals, energy intake was significantly greater when children were served the 150% and 200% portion size conditions compared to the 100% condition (both $P<0.0001$). Across portion size conditions, increasing meal ED by 42% led to an increase in energy intake at the meal ($P<0.0001$). The effects of portion size and energy density combined to increase meal energy intake by 79% or 175 ± 12 kcal when the children were served the higher-ED meal with the largest portions compared to the lower-ED meal with the smallest portions.

229 The effects of portion size and ED on intake differed for each of the individual foods and
230 milk served at the meal. Portion size had significant effects on the intake of macaroni and cheese,
231 applesauce, milk, and ketchup (all $P < 0.02$; **Table 3**), but had no effects on intake of chicken or
232 vegetables (both $P > 0.53$). For example, doubling the meal portion size increased intake of
233 macaroni and cheese by 31%, applesauce by 64%, and ketchup by 49%. Energy density had
234 significant effects on the weight consumed of chicken and macaroni and cheese ($P < 0.0001$;
235 Table 3), but had no significant effects on intake of the other foods or milk (all $P > 0.06$).
236 Compared to the lower-ED standard macaroni and cheese, children consumed 21% less of the
237 macaroni and cheese with added fat; compared to the lower-ED grilled chicken, children
238 consumed 53% more of the breaded chicken.

Table 3: The effects of increasing meal portion size and energy density on the weight, energy, energy density, and macronutrients consumed from a meal served to 120 preschool children¹

	Lower-energy-dense meal			Higher-energy-dense meal			Significant effects ²
	100% Portion Size (n=106)	150% Portion Size (n=107)	200% Portion Size (n=110)	100% Portion Size (n=114)	150% Portion Size (n=114)	200% Portion Size (n=115)	
Total meal intake (g)	280±11	357±14	360±14	283±10	331±13	345±12	PS ³
Chicken (g)	40±3	39±3	37±3	56±3	56±4	63±5	ED ⁴
Macaroni and cheese (g)	58±4	78±5	78±6	49±4	57±5	61±6	PS, ED ⁵
Vegetable (g)	20±2	22±3	18±3	18±2	18±2	20±3	--
Applesauce (g)	71±3	97±5	119±7	70±3	98±5	111±7	PS
Ketchup (g)	7±1	9±1	10±1	7±1	10±1	12±1	PS
Milk (g)	84±7	110±9	98±8	82±7	92±8	83±8	PS
Total meal energy intake (kcal)	222±9	279±11	279±12	321±12	366±14	390±15	PS, ED
Chicken (kcal)	48±4	47±4	44±4	95±5	95±7	106±8	ED
Macaroni and cheese (kcal)	86±6	117±8	116±9	102±8	119±10	127±12	PS, ED
Green vegetable (kcal)	12±1	12±2	11±2	15±2	15±2	16±2	ED

Applesauce (kcal)	36±2	49±3	60±3	50±2	70±4	79±5	PS, ED
Ketchup (kcal)	6±1	8±1	8±1	9±1	12±1	14±1	PS*ED ⁶
Milk (kcal)	35±3	46±4	41±4	50±4	56±5	51±5	PS, ED
Meal energy intake (% total energy requirements) ⁷	20.8±0.9	25.4±1.0	25.7±1.1	29.6±1.1	33.5±1.3	35.9±1.4	PS, ED
Water intake (g)	53±6	42±5	48±5	51±6	46±5	49±5	--
Energy density of food (kcal/g)	0.95±0.02	0.94±0.02	0.92±0.02	1.34±0.02	1.30±0.02	1.30±0.03	PS, ED
Energy density of food and milk (kcal/g)	0.81±0.02	0.80±0.02	0.79±0.02	1.15±0.02	1.13±0.02	1.14±0.02	ED
Carbohydrates (g)	24.2±0.9	32.2±1.3	34.1±1.4	33.5±1.1	40.3±1.4	43.8±1.5	PS, ED
Sugar (g)	12.9±0.5	17.3±0.7	18.6±0.8	17.7±0.6	22.8±0.9	24.6±1.0	PS, ED
Protein (g)	17.1±0.9	19.2±0.9	18.0±1.0	16.3±0.6	17.1±0.8	18.1±0.8	PS
Fat (g)	6.6±0.3	8.4±0.4	8.1±0.5	13.6±0.7	15.1±0.8	15.7±0.9	PS, ED

¹All values are mean ± SEM

²Mixed linear models were used to test the effects of portion size and energy density on all outcomes except for percent energy consumed from carbohydrates, protein, and fat which were analyzed using multivariate analysis of variance.

³PS, There was a significant, independent effect of portion size (all P<0.02) on the outcome.

⁴ED, There was a significant, independent effect of energy density (all P<0.01) on the outcome.

⁵PS, ED, There was a significant, independent effects of portion size (all P<0.03) and energy density (all P<0.009) on the outcome.

⁶PS*ED, Portion size and energy density significantly interacted to affect energy consumed from ketchup (P=0.02).

⁷Daily energy requirements were estimated using equations for children ages 3 through 5 years with light activity levels (28)

Energy intake. The portion size and energy density of the meals had significant and independent effects on preschool children's energy intakes at lunch (**Figure 2B**; both $P < 0.0001$). Compared to the 100% portion size conditions, meal energy intake increased by 18% (49 ± 7 kcal) and 24% (66 ± 8 kcal) when children were served the 150% and 200% portion conditions, respectively (both $P < 0.0001$). Meal energy intake at the 150% and 200% conditions did not differ ($P = 0.09$). Increasing meal ED by 42% led to a 103 ± 7 kcal or 40% increase in energy intake at the meal ($P < 0.0001$). The effects of portion size and energy density combined to increase meal energy intake by 79% or 175 ± 12 kcal when the children were served the higher-ED meal with the largest portions compared with the lower-ED meal with the smallest portions (Table 3). Children consumed $20.8 \pm 0.9\%$ of their estimated daily energy requirements from the smallest portion of the lower-ED meal compared to $35.9 \pm 1.4\%$ of their requirements from the largest portion of the higher-ED meal.

The effects of portion size and ED on energy intake differed for each of the individual foods and milk (Table 3). Serving larger portions led to increased energy intake from macaroni and cheese, applesauce, milk, and ketchup (all $P < 0.03$), but had no effect on the amount of energy consumed from chicken or vegetables (both $P > 0.44$). For example, doubling the meal portion size increased energy intake from macaroni and cheese by 31% and applesauce by 63%. Varying the ED of the foods and milk significantly affected the amount of energy consumed from all of these items (all $P < 0.02$). The increased energy intake from the largest portion of the higher-ED meal compared to the smallest portion of the lower-ED meal (175 kcal) was primarily attributable to greater energy intake from the breaded chicken nuggets (60 ± 9 kcal; 34% of the increase), macaroni and cheese with added fat (42 ± 5 kcal; 24%), and sugar-sweetened applesauce (46 ± 4 kcal; 26%).

Energy density. The ED of the food and milk consumed at the meal did not differ by meal portion size ($P=0.57$), but it was significantly affected by the meal ED served ($P<0.0001$). ED of food and milk averaged 0.80 ± 0.01 kcal/g at the lower-ED meals and 1.14 ± 0.02 kcal/g at the higher-ED meals (Table 3). Thus, when the ED of the foods and milk served was increased by 42%, the ED consumed at the meal increased by 42%. This indicates that children did not shift their overall ED of the meal by changing their selection or intake of the individual items to adjust for increases in meal ED.

Macronutrient intake and composition. The portion size and energy density of the meals had significant and independent effects on preschool children's intake of carbohydrate, sugar, and fat (all $P<0.0001$; Table 3), but only portion size significantly affected protein intake ($P=0.01$). Compared to the 100% portion size conditions, carbohydrate intake (including sugar) increased by 36%, sugar increased by 42%, fat increased by 19%, and protein increased by 7% when children were served the 200% conditions (all $P<0.0001$). Increasing meal ED led to a 32% increase in carbohydrate intake (including sugar), a 35% increase in sugar intake and a 94% increase in fat intake (all $P<0.0001$), but did not affect protein intake ($P=0.07$).

Food liking ratings

Figure 3 shows the distribution of food liking ratings for the 93 children who completed this assessment. When all the items were considered together, ordinal logistic regression indicated that there was no significant difference in the distribution of liking ratings between the lower-ED and higher-ED meals ($P=0.15$); the odds ratio was not significantly different from 1.0 (mean 1.22

[95% confidence interval 0.93-1.60]). Likewise, there were no significant differences in the distribution of liking ratings between the lower-ED and higher-ED versions of the individual foods and milk (all $P>0.05$). Thus, even though children's intake of the chicken and macaroni and cheese differed by ED, their liking ratings did not. All the foods and milk were well-liked; children chose "super yummy" or "yummy" ratings in 73% of the assessments for chicken, 70% for macaroni and cheese, 70% for vegetables, 83% for applesauce, and 76% for milk. Applesauce was the most likely to be rated as "super yummy" and vegetables were the least likely ($P=0.02$). The children's liking ratings of the foods did not influence the effects of portion size and ED on intake of the individual foods.

Figure 3: Frequency distribution of liking ratings (faces from left to right represent "super yucky," "yucky," "just okay," "yummy," and "super yummy") for the lower- and higher-energy dense versions of a meal consisting of chicken, macaroni and cheese, green vegetable, applesauce, and milk. There was no significant difference in distribution of the ratings between the two versions of the meal, according to ordinal repeated measures logistic regression ($p=0.15$). ED, energy density.

Influence of subject characteristics

Analysis of covariance showed that the relationship between the portion size or ED and meal intake by weight and energy was not influenced by children's sex, age, height, body weight, sex-specific BMI-for-age percentile, or BMI z-score. Most of the subscales of the Child Eating Behaviour Questionnaire (CEBQ), including satiety responsiveness, and the Child Feeding

Questionnaire (CFQ) did not influence the relationship between the experimental variables and meal intake; however, a few of the subscales did affect these relationships as described below.

Parental ratings of the children's food responsiveness (a 5-item subscale on the CEBQ) significantly affected the relationship between portion size and meal intake by weight (**Figure 4**; $P < 0.01$). Children who were rated as being more responsive to food had larger increases in intake when served the 150% and 200% portions of the meal than children who were less responsive. When portion sizes were doubled, children with a food responsiveness score of 1 (frequency of responsive behavior = never) increased their meal intake by a non-significant mean of 29 grams, whereas children with a score of 4 (frequency = often) increased their intake by 124 grams. Thus, although most children had increased intake when served larger portions, children whose parents rated them as the most responsive to food showed an even stronger portion size effect. In addition, ratings of use of parental monitoring on the CFQ significantly affected the relationship between portion size and meal intake; children with the least parental monitoring did not have increased intake when served the 200% portion compared to the 100% and 150% portions ($P < 0.02$). For the relationship between meal ED and intake, only ratings of child enjoyment of food on the CEBQ significantly affected the relationship ($P < 0.01$); children with highest scores ate more of the lower-ED meal than the higher-ED meal.

Figure 4: Effect of parental ratings of child food responsiveness on the relationship between meal portion size and weight of the meal consumed. Scores on the food responsiveness scale indicate the mean frequency of 5 responsive behaviors and range from 1 (never) to 5 (always). Analysis of covariance showed that the slopes of the regression lines for the 150% and 200% portion conditions were significantly greater than the slope for the 100% condition (both

P<0.01). Thus, children who were rated as being more responsive to food had larger increases in intake when served the 150% and 200% portions of the meal (compared to the 100% condition) than children who were less responsive.

316 **DISCUSSION**

317 Variations in the portion size and ED of all foods served at a meal in childcare centers had
318 substantial effects on energy intake of preschool children. The weight consumed of the meal was
319 affected by portion size but not by ED; in contrast, energy intake was independently affected by
320 both properties. When meal portion size and ED were increased simultaneously, the two factors
321 combined to increase children's meal intake by 175 kcal or 79%. Both the lower-ED and higher-
322 ED meals were well accepted, as indicated by the children's ratings of liking and the
323 consumption of a similar weight of the same portions. These results show that changes in meal
324 portion size and ED have potent effects on children's energy intake and demonstrate that
325 reductions in ED and portion size can moderate these effects without a loss of acceptability.

326 The combined effects of portion size and ED on intake of multiple items at a meal have not
327 previously been investigated in preschool children. Two studies varied both these factors in a
328 single dish served to children and found inconsistent effects on consumption (11,12). One study
329 found independent effects of doubling the portion of the main dish while also increasing its ED
330 (11), and the other found no effect of a 25% change in the portion of the main dish but a
331 significant effect of changing the ED (12). The lack of a portion size effect in the latter study
332 may have been due to the modest difference that was tested or because even the smallest portion
333 was overly large for the children (34). The results of the present study indicate that when all
334 foods in a meal are varied, children show little evidence of compensatory adjustment to changes
335 in portion size and ED, and these food properties have independent effects that combine to affect

energy intake. This is of concern because children are often exposed to large portions of multiple higher-ED foods at a meal; in the longer term, such exposure has the potential to promote overconsumption and lead to excess weight gain.

Strategies to moderate the energy intake of preschool children need to address both meal portion size and ED, since these two powerful factors combine to increase intake. The existing data, however, suggest that reducing ED may be the more effective strategy. The present study found that a 42% increase in ED of all foods increased energy intake by 40%. Likewise, within the range of ED tested, researchers generally find that a given percentage change in ED leads to an equivalent percentage change in energy intake of the varied foods (6-8, 35-37). For example, a 27% decrease in the ED of multiple meals served to preschool children reduced energy consumption at those meals by 25%, which was sustained over 2 days (7). In contrast, the relationship between portion size and intake is curvilinear (34, 38); thus, changes in portion size have proportionally smaller effects on intake than changes in ED. A 100% increase in portion size increases intake by substantially less than 100%, ranging from 21% to 53% in controlled studies (39) and averaging 24% in the present study. These results suggest that reducing the ED of foods and beverages would lead to more robust reductions in energy intake than similar percentage reductions in portion size, which may be more noticeable and difficult to implement and maintain (40-43).

Interventions to modify portion size and ED should take into account the individual meal components, which can have differential effects on both energy intake and diet quality. In particular, alterations in main dishes should be a primary target of these strategies, since they usually contribute the largest proportion of meal energy (1, 5, 16, 40, 44). Side dishes, however, also play a role (8, 17, 45-47). In this study, larger portions of applesauce promoted energy

intake, but if equally palatable, lower-ED versions were served instead of higher-ED, sweetened versions, fruit intake and nutrient density of the diet could be improved with smaller increases in energy consumption. In the present study, serving larger portions of vegetables did not increase vegetable intake or energy intake. Since vegetables were the least likely food to be rated as “yummy” or “super yummy”, increasing the palatability or preference for the vegetables might improve intake (34, 47). Within a complex meal, serving larger portions of fruit and vegetables can be an effective strategy to increase intake; however, consideration needs to be given to the portions of main dishes, the other options in the meal, and the palatability of the foods (47).

Most of the children’s characteristics, including sex, age, body size, and satiety responsiveness, did not influence the effects of portion size and ED on intake, in contrast to some findings from previous studies (1, 4, 5, 16, 17). This disparity may be due to differences in study design; for example, in studies that found an effect of age, the portion sizes were tailored to different age groups (1, 4), whereas in the present study, the same portions were served to all children. Differences in the distribution of child characteristics may also explain their differential influence across studies. In particular, studies that reported an influence of weight status or satiety responsiveness on the portion size effect had a larger proportion of children who were overweight or obese or of lower-socioeconomic status than the sample in the current study (5, 16, 17). However, similar to a previous study (5), we found that children with higher scores for food responsiveness increased their intake of large portions to a greater extent than children with lower scores. The comparable findings across studies suggest that responsiveness to food cues may predict the degree of susceptibility to portion size. The present study suggested that low parental monitoring may lead children to be less responsive to very large portions; however, this effect was not consistent in the two larger portion conditions (150% and 200%) and thus the

practical implication of this effect is not clear. In addition, individual characteristics have not been found to substantially influence the effect of ED on intake, but future research should explore how children's enjoyment of food influences their intake of foods that vary in ED (6-8). Despite differences in response, most of the children tested were susceptible to the effects of both portion size and ED, which indicates that recommendations focusing on these factors should be widely disseminated.

A strength of the present study is the large sample of children whose eating behavior was assessed. Previous studies investigating the effects of portion size or ED with a crossover design have included an average of 55 children (1-8, 11-13, 16, 18, 19), and only one study of 172 children (4) had a sample size larger than the present study (120 children). A further strength was that the lunch was served in the children's usual environment, and included popular, commonly consumed foods that had typical variations in portion size and ED. In addition, the lower-ED and higher-ED versions of the meals were similar in palatability. Thus, the setting and the meal were generalizable to a wide range of children. However, the meal was pre-plated instead of served family style, a typical practice of childcare centers. To further assess the generalizability of the findings, the effects of portion size and ED should be investigated using family style meals. In addition, intake was measured at a single meal and the effect of meal portion size and ED on later intake was not investigated. Future research should be conducted for longer periods of time on different populations to extend these findings and determine if children adjust for variations in meal portion size and ED at later meals. Previous research suggests the effects of portion size persist up to 24 hours and those of ED up to 2 days (3, 7), but the combination of these factors has not been investigated beyond a single meal in children.

Varying the portion size and ED of multiple items at a meal had a greater effect on energy intake than previously found in studies that manipulated only one of these factors or one food; these changes in all foods led children to consume a sizeable proportion (about 36%) of their estimated daily energy needs at a single meal. This demonstrates that strategically changing portion size and ED of a variety of foods and beverages should be a focus of obesity prevention initiatives at both individual and public levels. In particular, reducing the ED of foods and beverages should be a priority in developing strategies, since modest changes in ED have a large impact on intake and are unlikely to be noticed by most children or adults (6-8, 20, 21, 17).

There are several effective strategies to reduce the ED of meals while maintaining palatability; for example, recipes can be modified by reducing the fat or sugar content or adding water-rich ingredients, including fruits and vegetables (6-8, 37). Another option is choosing palatable lower-ED, commercially available products (such as the unbreaded, grilled chicken pieces and reduced-sugar applesauce used in this study), which may be a more practical strategy for caregivers. With acceptable recipes and products, strategies to reduce ED can be implemented in homes, restaurants, and childcare settings, and can be strategically combined with the beneficial effects of reductions in portion size, for example by serving larger portions of lower-ED foods with smaller portions of higher-ED foods (48). These strategies to moderate the effects of portion size and ED are practical and effective in reducing energy intake; however, caregivers need clear guidance and acceptable products to implement such strategies and counter these pervasive environmental influences.

Acknowledgements

We thank Charlotte Dupont, Brittany James, Alicia Leitch, Cara Meehan, Christine Sanchez, Anne Vos, Faris Zuraikat, and undergraduate students in the Laboratory for the Study of Human Ingestive Behavior for assistance with food preparation and data collection. In addition, the support and participation of staff, parents, and students at the Bennett Family Center, The Child Care Center at Hort Woods, and Step by Step School for Early Learning is greatly appreciated.

The authors' responsibilities were as follows – SMRK: the design of the experiment, collection and analysis of data, and writing of manuscript; LSR: the design of the experiment, analysis of data, and writing of the manuscript; and KLK and BJR: the design of the experiment and the writing of the manuscript. None of the authors had a personal or financial conflict of interest.

Supported by NIH Grant R01-DK082580 and USDA National Institute for Food and Agriculture Grant 2011-67001-30117 Program A2121.

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