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Greater resting energy expenditure and lower respiratory quotient after 1 week of supplementation with milk relative to supplementation with a sugar-only beverage in children

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Abstract

Previous studies have linked overweight to lower milk and calcium consumption and have proposed a role of milk consumption on energy expenditure (EE). The goal of this study was to compare EE and food intake after a meal of either mixed-nutrient or single-nutrient beverage and examine whether supplementation with that beverage for 1 week will impact EE. This was a randomized, controlled crossover study testing the effect of 2 beverages, milk or fruit-flavored beverage, before and after a supplementation period of 1 week on EE. Food intake at a meal after a snack intake of each beverage was assessed at the end of each measurement period. Ten children, aged 9 to 10 years, participated in all of the testing sessions in the study. There was a significant beverage by testing day interaction on daily EE and thermic effect of food (TEF), whereby EE was greater with milk consumption relative to the fruit-flavored beverage on day 8 ($P = .0014$) and with fruit-flavored beverage consumption on day 1 vs day 8 ($P = .01$). Similarly, the TEF was greater with milk compared with fruit-flavored beverage consumption on day 8 ($P = .0007$) and with fruit-flavored beverage consumption on day 1 relative to day 8 ($P = .0097$). The TEF declined more rapidly during 6 hours after a fruit-flavored beverage than a milk meal ($P = .0018$). Food intake did not differ after snack consumption of each beverage before and after milk and fruit-flavored beverage supplementation periods. Over the longer term, consumption of milk beverages may have more favorable effects on energy balance in children than consumption of fruit-flavored beverages.

1. Introduction

Consumption of various beverages has shifted in the United States in the past decades. For example, data from the Continuous Survey of Food Intake by Individuals revealed that, between 1977 and 2001, the percentage of energy coming from soft drinks and fruit drinks increased, whereas that coming from milk decreased [1]. Furthermore, the largest reduction in milk consumption occurred among 2- to 18-year-olds, with a reduction in both the proportion of milk consumers among that group and the number of servings consumed. In addition, as children get older, they tend to decrease their intakes of milk at the expense of increased intakes of sweetened beverages. In a longitudinal study of 5-year-old girls, Fiorito et al [2] found that milk intakes decreased between the ages of 5 and 11 years, with fewer girls drinking milk as a beverage at age 11 years compared with age 5 years. Similarly, in the National Heart, Lung, and Blood Institute Growth and Health Study, milk consumption decreased in the early to midadolescent years in black and white girls, whereas regular soda consumption increased [3].

This shift in beverage consumption may have played a role in the increasing prevalence of childhood obesity. Carruth and Skinner [4] found that higher intakes of calcium and servings of dairy products were associated with less body fat at age 6 years. At 8 years of age, there was a negative relationship between dietary calcium and body fat in children [5]. According to this study, 4.5% to 9% of the

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variability in body fat could be explained by changes in dietary calcium. Another recent study that corroborates the above-mentioned findings reported that high dairy consumption at 3 to 6 years of age was related to lower body mass index and sum of 4 skinfolds at age 10 to 13 years in the Framingham Children's Study [6]. Girls consuming <1.25 servings of dairy per day and boys consuming <1.7 servings per day were at higher risk of gaining excessive amounts of body fat by early adolescence. In sum, diverse data suggest that the choice of beverage may impact body composition and weight in children.

We have previously shown in adults that mixed-nutrient beverage consumption, similar in composition to chocolate milk, leads to greater thermic effect of food (TEF) and feelings of satiety than single-nutrient beverage [7]. The first main objective of this study was therefore to measure energy expenditure (EE) after consumption of milk relative to a sugar-sweetened, fruit-flavored beverage in children. The second main objective was to determine whether supplementation, over a period of 6 days, with either milk or a fruit-flavored beverage would alter the thermic response to the beverage. Secondary objectives included analyzing the impact of milk and fruit-flavored beverage supplementation on ad libitum meal intake and overall diet quality.

2. Subjects and methods

2.1. Subjects

Fourteen children, aged 9 to 10 years, were recruited to participate in the study. Subjects were recruited through newspaper advertisements and posters around the St Luke's/Roosevelt Hospital Center and Washington Heights areas in New York, NY. Requirements for inclusion were absence of chronic metabolic disorders, tolerance to lactose, habitual milk intake of <1 serving per day and calcium intake of <600 mg/d as reported by a parent/guardian, and body weight between the 25th and 75th percentile for height and age. Normal-weight children were chosen to participate in this study because we wanted to first obtain data on normal-weight individuals to establish whether there is an effect of beverage type on thermogenesis. A logical follow-up to this study would be to include overweight children. The study was approved by the Institutional Review Board of St. Luke's/Roosevelt Hospital. All parents/guardians and their child signed an informed consent before the child's participation in the trial. Children were recruited and studied between June 2003 and December 2006.

2.2. Study design

Each child was tested twice (day 1, day 8) in each of 2 treatment conditions (single-nutrient beverage, mixed-nutrient beverage) in a crossover design. Neither the participants nor the clinical coordinator was blinded to treatment condition. However, the metabolic cart readings are automated; and statistical analyses were performed in a blinded fashion. Treatment conditions consisted of 2 different isocaloric and isovolumic beverages. The beverages were either a fruit-flavored beverage (Kool Aid; Kraft, Rye Brook, NY) containing largely water and high-fructose corn syrup (8 oz = 108 kcal and 28.8 g carbohydrates) or 1% fat plain milk (8 oz = 102 kcal, 8 g protein, 11.7 g carbohydrates, 2.6 g fat). After a pretrial period during which parents kept 3-day food records (described later), children were randomized on the first clinic visit to receive either the fruit-flavored beverage/milk or the milk/fruit-flavored beverage sequence. They underwent EE and food intake testing (described below) and were then sent home with a supply of the test beverage to consume as a supplement during the following 6 days. On day 8, they returned to the General Clinical Research Center, where the testing protocol was repeated. After a 2- to 3-week washout period, children returned to the General Clinical Research Center; and the same 8-day testing procedures were repeated with the alternate beverage.

For each testing session, subjects arrived in the laboratory on the morning after a 12-hour overnight fast. Body weight was measured to the nearest 0.1 kg on a standard medical-grade scale (Detecto-3P7044; Detecto, Webb City, MO). Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer. Body fat was measured with a body fat analyzer (Omron HBF-306; Omron Healthcare, Vernon Hills, IL). Subjects were then asked to lie quietly for 30 minutes before the start of EE measurements.

2.3. Energy expenditure measurements

After an initial 30-minute rest period and while in a supine position, a ventilated canopy was placed over the subject's head; and expired gases were measured by a metabolic cart for approximately 30 minutes. This was considered to be the baseline resting energy expenditure (REE) for that session and was calculated as the average of the last 20 minutes of the measurement period. Subjects then consumed the assigned beverage in a maximum time of 15 minutes and were again placed under the ventilated canopy. Energy expenditure measurements were then taken during the final 30 minutes of each hour after the start of beverage ingestion and continued for 6 hours. The beverage provided 33% of the child's daily resting energy requirements, as estimated using the Schofield equation for male and female children aged 3 to 10 years, without taking activity into consideration [8]:

$$\text{Female: REE} = 16.97W + 1.618H + 371.2$$

$$\text{Male: REE} = 19.6W + 1.033H + 414.9,$$

where W is weight in kilograms and H is height in centimeters. With an activity factor entered into the equation, the breakfast allotment was approximately 25% of daily energy requirements, within the range of breakfast energy consumption in US children [9].

Gas exchange indirect calorimetry measurements were made using a ventilated hood (Delta-Trac II and Vmax 29; SensorMedics, Yorba Linda, CA). Two different instruments were used in this study, but each child was consistently tested on the same instrument. To simplify testing for children, each child remained under the ventilated canopy for 30 minutes each hour after beverage consumption. Therefore, each measurement period lasted 30 minutes and was followed by a 30-minute break. The system was calibrated as specified by the manufacturer using standard gases. Readings from the metabolic cart included REE, volume of oxygen consumed, volume of carbon dioxide produced, and respiratory quotient (RQ). Readings were collected at 1-minute intervals. For each postprandial measurement period, the first 5 readings were discarded, leaving approximately 25 minutes of data for each hour. Data from each period were averaged to obtain an hourly rate. Fat and carbohydrate oxidation rates were then determined using the Weir equation [10].

During each measurement period, after the REE measurement, subjects were permitted to watch videotaped programs. The videos were purposely selected so that they would not incite emotions or reactions that would affect the metabolic rate. Subjects were allowed to use the bathroom during the designated breaks.

2.4. Food intake measurement

After completing the EE measurement protocol, children were given a snack consisting of either fruit-flavored beverage or milk, depending on the beverage type they were initially randomized to. The snack provided 10% of the child's estimated resting energy requirements, also determined using the Schofield equation [8], and was followed by the consumption of an ad libitum test meal 20 minutes later. The time between snack consumption and the ad libitum meal was chosen because it has been found that compensation for preloads was more accurate when the meal was presented within 30 minutes after the preload compared with when the meal was presented after longer periods [11]. This interval between preload and meal has also been previously used successfully by other groups [12,13]. The test meal consisted of macaroni and cheese, graham crackers, canned green beans, string cheese, baby carrots, and green grapes. Children were asked to eat as much as they wanted of each food until they felt satiated but were not required to eat from each food item. Food intake was measured by weighing food plates before and after the meal. Energy and macronutrient intakes were then calculated using the nutrient information on the food labels.

Parents/guardians were also instructed how to keep a food diary and were asked to record their children's food intake on 2 weekdays and 1 weekend day at baseline (pretrial), during each supplementation period, and during the washout period. Returned food records were double-checked by the clinical coordinator and reviewed with the parent/legal guardian; and foods were entered in the Diet Analysis Plus software (Version 6.1; Wadsworth Publishing, Salem, OR) for determination of energy, macronutrient, vitamin, and mineral content. These food records were used to determine whether beverage supplementation affected diet quality.

2.5. Supplementation

At the end of the first testing day, children were provided with a supply of either milk or fruit-flavored beverages, depending on the beverage type they were initially randomized to, and were asked to consume 3 servings of milk or 4 servings of fruit-flavored beverage daily for the next 6 days. During the milk supplementation phase, children consumed one serving (240 mL) of 1% chocolate milk and 2 servings (240 mL each) of plain 1% milk per day, whereas during the fruit-flavored beverage supplementation phase, children consumed 4 servings of 200 mL of fruit-flavored beverage (Kool Aid). Supplements were matched on the number of calories.

2.6. Statistical analyses

Sample size determination was based on the ability to detect a difference of 25 kcal between TEF of the 2 beverages considering a standard deviation of 39 kcal [7] and α and β values of .05 and .8, respectively. Subjects were randomized to each beverage using a randomization table. The statistician (SH) generated the allocation sequence, and the clinical coordinator (NC) enrolled participants and assigned the participants to their initial study beverage. Sealed envelopes were made that contained a beverage sequence. An equal number of envelopes containing each sequence were made. When a child completed screening and was enrolled in the study, an envelope was opened to reveal the beverage sequence.

All statistical analyses were performed using Statistical Analysis Software for Windows (Version 9.1; SAS Institute, Cary, NC). Food record data were analyzed using analysis of variance for repeated measures with phase (pretrial, milk supplementation, fruit-flavored beverage supplementation, and washout) as a factor. Pairwise comparisons were done adjusting for multiple comparisons using the Tukey test for multiple comparisons. Energy and macronutrient consumption at the ad libitum meal was compared between beverage supplementation phases using repeated-measures analysis of variance. Ten children completed the study. Food records indicated that 5 of the 10 completers consumed more than 600 mg of calcium per day before starting the study. Therefore, because habitual calcium intake might affect the results, we adjusted for pretrial calcium intakes in the EE and substrate oxidation data analyses. In addition, food records were analyzed with and without the 5 subjects who did not meet our calcium intake criterion, as determined by their pretrial food records.

Energy expenditure and substrate oxidation data were analyzed using mixed models (SAS PROC MIXED, SAS Institute) with subject, beverage, day, and hour as independent variables. Beverage, day, and hour were treated as fixed effects, whereas subject was treated as a random effect. Daily

Table 1
Characteristics of children who completed the study

Characteristic	Mean ± SEM	Range
Age (y)	9.7 ± 0.48	9-10
Height (cm)	139.6 ± 5.76	132-149
Weight (kg)	33.5 ± 5.92	26-45
Body mass index (kg/m^2)	17.1 ± 1.77	14.9-20.5
Calcium intake (mg/d)[a]	338 ± 179.2	103-581

[a] Data are from the screening questionnaire. Values from pretrial food records are in Table 2.

EE was calculated as the average rate of EE over the entire postprandial period and included baseline REE. Thermic effect of food was calculated as EE minus baseline REE for each hourly measurement. Two- and three-way interactions of beverage, day, and hour were tested in the models. None of the day by hour or phase by day by hour interactions was significant, and they were dropped from the final models. Furthermore, because pretrial calcium intakes may have had an impact on response to beverage supplementation, we repeated the analyses with pretrial calcium as a categorical independent variable. As a categorical variable, 4 subjects were coded as consuming >600 mg of calcium per day. One other subject's pretrial calcium intake was 604 mg/d, and this subject was grouped with those consuming <600 mg/d. The other 4 subjects had pretrial intakes >900 mg/d. These children were screened to have calcium intakes <600 mg/d; but upon analysis of their pretrial food records after the conclusion of the study, higher calcium intakes were found. Data are reported controlling for pretrial calcium as a categorical variable and are presented as means ± SEM. We present analyses of data from 10 of 14 subjects who completed all 4 testing sessions so that subjects may serve as their own control in all comparisons. Of the noncompleters, one subject provided no useable data, another completed only one session, and two completed 2 of 4 sessions. Because this is a mechanistically oriented rather than an efficacy study, exclusion of noncompleters does not introduce bias into tests of our hypotheses. Significance level was set as $P < .05$.

3. Results

3.1. Patients

Fourteen children started the study (10 boys, 4 girls), and 10 (7 boys, 3 girls) completed all 4 measurement periods. Dropouts were mostly lost to follow-up and unavailable for scheduling. Characteristics of the 10 completers are shown in Table 1. Calculated REE using the Schofield equation was 1197.1 ± 31.1 kcal, and the beverages provided 395.0 ± 10.3 kcal at the breakfast meal. There were no apparent differences in baseline characteristics between completers and noncompleters.

3.2. Food records

There was no effect of phase (pretrial, milk, washout, and fruit-flavored beverage) on energy and macronutrient intake, assessed by 3-day food records (Table 2). Of the vitamins, intake of vitamin B2 was greater during milk supplementation relative to pretrial, washout, and fruit-flavored beverage supplementation ($P < .001$ for phase effect). There was a trend for a phase effect on B12 ($P = .0967$), with intakes during milk supplementation being numerically greater than during the other 3 phases. Calcium and phosphorus intakes were greater during the milk supplementation period relative to pretrial, washout, and fruit-flavored beverage supplementation (both phase effect $P < .001$). There were also phase

Table 2
Daily energy and nutrient intakes before, during, and between beverage supplementation phases

Nutrient	Pretrial	Milk	Washout	Fruit-flavored beverage
Energy (kcal)	1954.8 ± 89.3	2070.5 ± 192.2	1923.7 ± 146.0	2146.5 ± 103.4
Protein (g)	70.9 ± 5.8	80.6 ± 6.6	75.6 ± 7.2	68.0 ± 7.1
Carbohydrate (g)	275.8 ± 21.4	260.0 ± 15.5	252.1 ± 18.1	317.0 ± 15.8
Fat (g)	70.9 ± 9.9	70.9 ± 10.7	70.0 ± 11.3	71.8 ± 8.9
SFA (g)	24.4 ± 2.6	24.0 ± 2.8	22.9 ± 2.4	21.7 ± 1.8
MUFA (g)	24.0 ± 7.5	23.9 ± 7.1	23.5 ± 7.5	23.2 ± 6.7
PUFA (g)	9.4 ± 1.1	8.6 ± 1.0	8.4 ± 1.3	9.2 ± 1.5
Vitamin A (mg)	593.7 ± 73.7	813.5 ± 84.3	640.3 ± 103.6	540.6 ± 93.7
Vitamin B1 (mg)	1.28 ± 0.12	1.26 ± 0.07	1.19 ± 0.08	1.23 ± 0.20
Vitamin B2 (mg)	1.72 ± 0.16[b]	2.50 ± 0.13[a]	1.60 ± 0.11[b]	1.33 ± 0.13[b]
Vitamin B12 (μg)	3.29 ± 0.48	5.57 ± 1.15	3.19 ± 0.46	3.65 ± 0.66
Vitamin D (mg)	2.43 ± 0.69	30.3 ± 24.9	2.21 ± 0.61	2.18 ± 0.89
Calcium (mg)	720.4 ± 124.5[b]	1381.5 ± 107.1[a]	729.9 ± 91.2[b]	665.0 ± 63.2[b]
Iron (mg)	13.5 ± 0.97	13.9 ± 1.2	14.0 ± 1.2	12.8 ± 1.4
Magnesium (mg)	196.5 ± 17.3[a,b]	241.7 ± 12.0[a]	174.2 ± 11.4[b]	154.4 ± 11.9[b]
Phosphorus (mg)	980.8 ± 106.5[b]	1409.3 ± 80.9[a]	933.6 ± 60.8[b]	862.7 ± 63.3[b]
Potassium (mg)	2175.0 ± 165.5[a,b]	2711.6 ± 192.3[a]	2024.3 ± 197.1[a,b]	1730.7 ± 200.1[b]

Data are means ± SEM. Values with different letters (a and b) within rows are significantly different from each other ($P < .05$). MUFA indicates monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

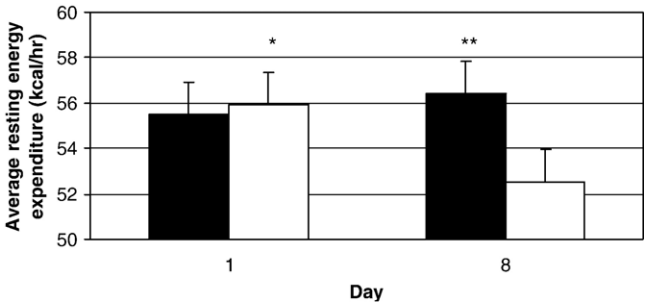

Fig. 1. Average daily EE with milk (black bars) and fruit-flavored beverage (white bars) consumption. There was a significant phase by day interaction ($P = .0058$). *Significantly different from single nutrient day 8 ($P = .004$). **Significantly different from single nutrient day 8 ($P = .0002$).

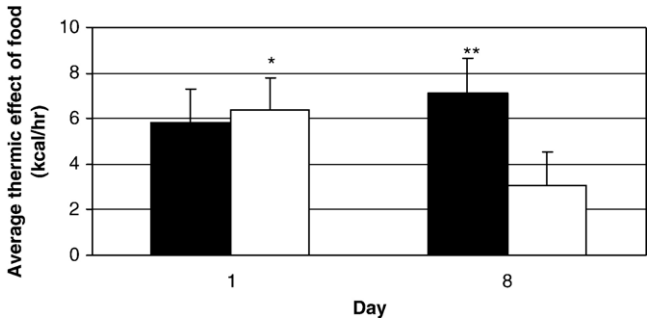

Fig. 3. Average TEF with milk (black bars) and fruit-flavored beverage (white bars) consumption. There was a significant phase by day interaction ($P = .0038$). *Significantly different from fruit-flavored beverage day 8 ($P = .0097$). **Significantly different from fruit-flavored beverage day 8 ($P = .0007$).

effects for magnesium and potassium intakes ($P < .001$). Intakes of magnesium were significantly higher ($P < .05$) during supplementation with milk than with fruit-flavored beverage and washout period. There was no difference in magnesium intake between milk supplementation and pretrial periods. Potassium intakes were significantly greater ($P < .05$) during milk supplementation compared with fruit-flavored beverage supplementation but were not different from pretrial and washout. When the data were analyzed without the 5 subjects whose pretrial calcium intakes were >600 mg/d, all of the nutrients that were significantly affected by phase remained significant. However, we found a trend for a phase effect on vitamin D ($P = .0576$), with values tending to be higher during the milk supplementation phase compared with all of the other phases, and a significant phase effect for sodium ($P = .0287$). Sodium intakes were significantly greater during the fruit-flavored beverage supplementation phase (3054.9 ± 107.4 mg/d) compared with pretrial (2000.5 ± 324.3 mg/d) but were not different from the milk supplementation (2467.7 ± 249.0 mg/d) and washout (2838.0 ± 199.7 mg/d) phases.

3.3. Daily EE

There was a trend toward a significant beverage by calcium category interaction ($P = .1068$) on daily EE, with high-calcium consumers having greater daily EE with milk relative to fruit-flavored beverage consumption (57.44 ± 2.05 vs 54.5 ± 2.07 kcal/h for milk and fruit-flavored beverage, respectively; $P = .0512$). There was also a significant beverage by day interaction ($P = .0019$, Fig. 1) and a beverage by hour interaction ($P = .0047$, Fig. 2) on average EE. Daily EEs during fruit-flavored beverage measurements on day 8 were significantly lower than those on day 1 ($P = .001$) and lower than measurements with milk consumption on day 8 ($P = .0014$, Fig. 1).

3.4. Thermic effect of food

There was no effect of calcium category on TEF. There was a significant beverage by day interaction ($P = .0018$, Fig. 3) and a significant beverage by hour interaction ($P = .0063$, Fig. 4). As with REE, TEF after fruit-flavored beverage consumption on day 8 was lower than that on day 1 ($P = .0099$) and lower than that after milk consumption on day 8 ($P = .0007$, Fig. 3).

3.5. Respiratory quotient and substrate oxidation

Respiratory quotient was significantly affected by a calcium category by beverage interaction ($P = .0217$,

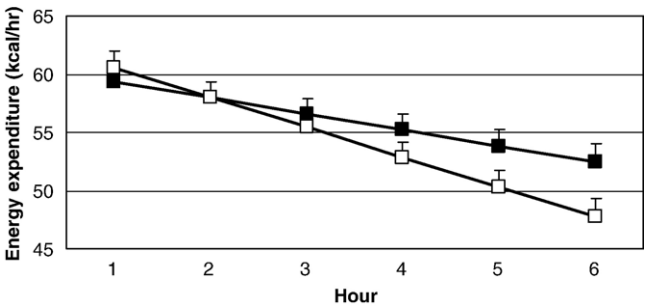

Fig. 2. Average EE with milk (black squares) and fruit-flavored beverage (white squares) consumption by hour over both testing days. There was a significant phase by hour interaction ($P = .0047$).

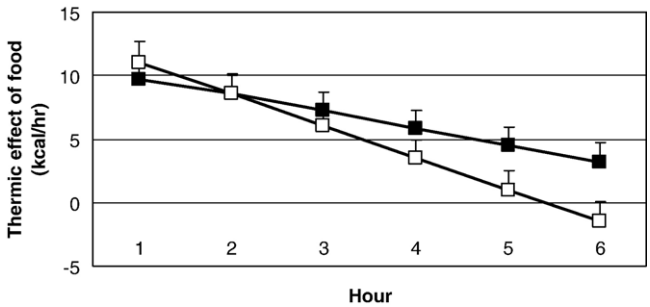

Fig. 4. Average TEF with milk (black squares) and fruit-flavored beverage (white squares) consumption by hour over both testing days. There was a significant phase by hour interaction ($P = .0018$).

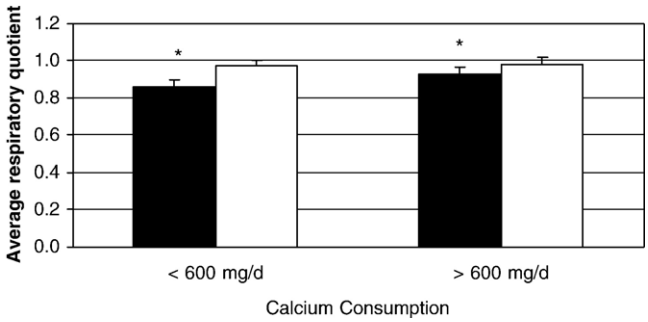

Fig. 5. Average RQ with milk (black bars) and fruit-flavored beverage (white bars) consumption by calcium category over both testing days. There was a significant calcium category by phase interaction ($P = .049$). *Significantly different from fruit-flavored beverage ($P < .01$).

Fig. 5). Respiratory quotient was lower with milk consumption relative to fruit-flavored beverage consumption ($P = .0001$), and the difference was significant in both calcium categories ($P < .0001$ for low-calcium consumers and $P = .0189$ for high-calcium consumers). Lower RQs are generally indicative of greater fat oxidation; and we observed a significant phase effect on fat oxidation, with fat oxidation being greater with milk consumption relative to fruit-flavored beverage ($P = .0019$). This was somewhat counterbalanced by lower carbohydrate oxidation with milk relative to fruit-flavored beverage consumption ($P < .0001$). Calcium category did not influence fat and carbohydrate oxidation rates.

3.6. Ad libitum meal intake

There was no significant effect of beverage, day, or beverage by day interaction on any of the variables assessed at the ad libitum meal (Table 3). One subject ate 775 g of macaroni and cheese at the session after fruit-flavored beverage snack consumption on day 8, although he averaged 319 g at the other 3 meals. Removing his data from the analyses did not significantly alter the results.

4. Discussion

Our data show for the first time in children that milk consumption induces greater REE and TEF after 6 days of supplementation relative to supplementation with a sugar-only beverage. Furthermore, on both testing occasions, milk led to lower RQ and hence greater fat oxidation than the fruit-flavored beverage. We did not, however, find that beverage type, consumed as a snack, had any impact on food intake 20 minutes later.

Our data showing that milk induces a greater TEF and REE confirm and extend our previous results with adults [7]. In the current study, however, a 6-day supplementation period was necessary to observe differences in REE between phases; and the changes were mostly due to a reduction in REE with fruit-flavored beverage consumption after the period of supplementation. This may be due to the high glycemic load imposed with consumption of the fruit-flavored beverage. Pereira and colleagues [14] have previously found that REE decreases with consumption of a low-fat diet relative to a low–glycemic load diet and attributed this effect to a reduction in blood glucose and free fatty acids after a high–glycemic index meal that would lead to impaired energy metabolism.

Gunther et al [15] showed that long-term (1 year) high–dietary calcium intakes lead to increases in whole-body fat oxidation after a meal high or low in calcium and to greater TEF after a low-calcium meal. They proposed that long-term high–dietary calcium intakes result in long-term adjustments in the ability to oxidize fats and use energy, regardless of the calcium content of the test meal. This is consistent with our data showing that pretrial calcium intakes played a role in modulating RQ with test beverage intakes and tended to differentially affect REE with test beverage consumption. This result was unexpected because others had previously suggested that increasing dairy calcium consumption from low intakes to higher intakes had beneficial effects on weight loss [16] and data seem to indicate a cutpoint of 600 mg of calcium per day as discriminating for body weight in adults [17]. However, the study by Melanson et al [18] included participants with average baseline intakes of 800 mg and found that milk intake enhanced fat oxidation during negative energy balance conditions. In our study, however, we cannot rule out the possibility that the differences in fat content between the 2 beverages would accentuate differences in RQ after their consumption [19,20].

However, the findings of greater REE and TEF and lower RQ with milk relative to fruit-flavored beverage consumption contradict results from Melanson et al [18], who found no effect of high- or low-dairy diets on EE using a 24-hour

Table 3.
Energy and macronutrient intakes at an ad libitum lunch after consumption of a snack of mixed- or single-nutrient beverage before (day 1) and after (day 8) a 6-day supplementation period with mixed- or single-nutrient beverage

Nutrient	Milk day 1	Milk day 8	Fruit-flavored beverage day 1	Fruit-flavored beverage day 8
Energy (kcal)	1053.5 ± 155.0	1094.2 ± 224.9	1088.7 ± 176.3	1306.0 ± 307.8
Protein (g)	34.8 ± 6.3	32.6 ± 6.2	32.6 ± 4.9	40.2 ± 9.3
Carbohydrate (g)	149.9 ± 23.6	163.8 ± 40.9	164.5 ± 33.2	198.4 ± 53.8
Fat (g)	36.2 ± 5.3	33.7 ± 5.1	34.1 ± 3.5	40.3 ± 7.0

Data are means ± SEM.

calorimetry chamber stay when subjects were tested under energy balance conditions. In this study, higher dairy increased fat oxidation and decreased carbohydrate oxidation, hence leading to no effect on overall EE, under conditions of negative energy balance. Furthermore, a recent study in adults found no difference in 24-hour EE, RQ, or fat oxidation with diets high or low in calcium and high or low in dairy products when those were consumed for 5.5 days [21]. Similarly, Jacobsen et al [22] did not find a significant diet effect on 24-hour EE and fat oxidation when comparing low- and high-calcium diets fed for 1 week.

A snack of milk and fruit-flavored beverage did not have different effects on subsequent food intake at an ad libitum meal. Based on our previous data showing lower feelings of hunger and high feelings of satiety after milk consumption [7], we expected a reduction in food intake after the milk snack. Furthermore, previous studies have found that protein-rich preloads lead to lower food intake than carbohydrate-rich preloads [23,24]. Our results are similar, however, to those of Vozzo et al [25], who found similar food intakes after preloads of various macronutrient composition. In contrast to other studies, this study examined preloads containing different mixes of macronutrient, whereas other studies [23,24] examined pure (approximately 100%) macronutrients. Moreover, the high-protein preload of Vozzo et al [25] had a similar macronutrient distribution as milk.

Children ate a large number of calories at the ad libitum meal in this study. This may be due to the long fasting period required during the EE measurement period. In fact, children would often receive their breakfast beverage at 9:00 AM and would not have lunch until close to 3:30 PM. Although the foods chosen may affect how much children eat at one occasion, it is unlikely that the foods included in the test meal would influence the results because each child served as his/her own control and the foods did not vary between test sessions.

A secondary aim of this study was to examine diet quality during milk and fruit-flavored beverage supplementation relative to pretrial and washout periods. Supplementation with the different beverages did not significantly increase energy intake, showing that children compensated for the added calories. However, this compensation was not perfect; and, numerically, children consumed approximately 120 to 150 kcal/d more during milk supplementation and 190 to 220 kcal/d more during fruit-flavored beverage supplementation than during pretrial and washout periods. Over a longer period, the difference in energy intake between beverage supplementation periods, approximately 70 kcal/d, compounded with the difference in EE, also approximately 70 kcal/d, may lead to greater weight gain with fruit-flavored beverage compared with milk consumption. Furthermore, milk supplementation allowed children to meet their dietary requirements for calcium, magnesium, phosphorus, and vitamins A and D, nutrients that were consumed at levels below requirements during the other phases. In addition, potassium requirements were met for all phases except for the fruit-flavored beverage supplementation phase. In this study, supplementation with milk resulted in improvements in the overall diet quality of children.

Our study had several limitations. First, only 10 children completed the 4 testing days of this study. Nevertheless, our small sample size did not prevent us from finding significant differences between beverages on EE and RQ. It did, however, prevent us from examining data from boys and girls separately and may have limited our ability to detect significant effects of pretrial calcium intake category on REE and TEF. Furthermore, all of the children in this study were lean, precluding extrapolation to overweight children.

Differences in pretrial calcium intakes among children are another limitation of this study. We used a dairy and calcium intake questionnaire to screen for children who consumed <1 serving of dairy per day and <600 mg of calcium per day. This level of calcium intake was chosen to reflect previous data that body weight differences are observed between individuals who consume <600 mg of calcium per day relative to those who consume >600 mg of calcium daily [17]. Furthermore, only one serving of milk was provided during the fruit-flavored beverage supplementation period; and we did not want to reduce a child's milk intake during that period. Therefore, we chose to include only children who regularly consumed <1 serving of milk per day. Parents reported, during the phone screening, on their child's dairy and calcium consumption. However, when pretrial 3-day food records were analyzed, 4 children reported an average calcium intake of >900 mg/d and 1 reported an average intake of 604 mg/d. These food records were taken after the child was enrolled and were not analyzed until the child had completed all of the testing sessions. Furthermore, the food records were not intended to be used as a screening method but rather to provide information on the child's baseline food intake. As much as these deviations from the dairy-specific questionnaires were unexpected, it allowed us to examine the impact of pretrial calcium intakes on thermogenic responses to the different beverages and yielded interesting results. A follow-up study with low- and high-calcium consumers would be necessary to further elucidate the role of long-term dairy calcium consumption on EE.

Finally, our study does not provide any information on whether calcium affects thermogenesis. Milk contained a mix of nutrients, including protein and fat, along with calcium, whereas the fruit-flavored beverage contained only carbohydrates and no calcium. Shi et al [26] have observed an increase in core temperature in experimental animals that were fed high-calcium diets. This finding is of particular importance because an increase in core temperature is an indication of a shift from energy storage to EE. They have found that the suppression of 1,25-dihydroxyvitamin D concentration in mice increased thermogenesis as well as adipocyte uncoupling protein 2, supporting a thermogenic effect of calcium. Melanson et al [27] conducted a cross-sectional study and, using indirect calorimetry, showed that a higher calcium intake was associated with a higher rate of

whole-body fat oxidation. There is also an abundance of data showing that protein enhances thermogenesis to a greater extent than carbohydrates [28-31]. It is therefore equally possible that the greater EE with milk consumption relative to the fruit-flavored beverage is due to the macronutrient composition of the beverage. A study with a third group, such as one consuming fruit-flavored beverage plus calcium, would help determine if calcium plays an acute role in thermogenesis.

4.1. Conclusions

In summary, our results show that after 1 week of supplementation with milk and fruit-flavored beverage, EE is greater with milk beverage consumption relative to a fruit-flavored beverage. Although intakes at a test meal did not show differences in energy intakes after a snack of milk or fruit-flavored beverage, our 3-day food records indicated a tendency toward reduced energy intake during milk relative to fruit-flavored beverage supplementation. Although more data are necessary in this area, our study suggests that longer-term milk consumption leads to greater EE and fat oxidation than longer-term fruit-flavored beverage consumption. The total difference in energy retention was approximately 140 kcal/d (70 kcal/d for food intake based on food record data and 70 kcal/d for EE based on extrapolated REE data) in favor of lower energy retention with consumption of milk relative to fruit-flavored beverage. Choice of beverage consumption in children may therefore play a role in their ability to maintain energy balance and control body weight.

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References

[1] Nielsen SJ, Popkin BM. Changes in beverage intake between 1977 and 2001. Am J Prev Med 2004;27:205-10.
[2] Fiorito LM, Mitchell DC, Smiciklas-Wright H, Birch LL. Dairy and dairy-related nutrient intake during middle childhood. J Am Diet Assoc 2006;106:534-42.
[3] Striegel-Moore RH, Thompson D, Affenito SG, et al. Correlates of beverage intake in adolescent girls: the National Heart, Lung, and Blood Institute Growth and Health Study. J Pediatr 2006;148: 183-7.
[4] Carruth BR, Skinner JD. The role of dietary calcium and other nutrients in moderating body fat in preschool children. Int J Obes Relat Metab Disord 2001;25:559-66.
[5] Skinner JD, Bounds W, Carruth BR, Ziegler P. Longitudinal calcium intake is negatively related to children's body fat indexes. J Am Diet Assoc 2003;103:1626-31.
[6] Moore LL, Bradlee ML, Gao D, Singer MR. Low dairy intake in early childhood predicts excess body fat gain. Obesity (Silver Spring) 2006;14:1010-8.
[7] St-Onge MP, Rubiano F, DeNino WF, et al. Added thermogenic and satiety effects of a mixed nutrient vs a sugar-only beverage. Int J Obes Relat Metab Disord 2004;28:248-53.
[8] Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985;39(Suppl 1):5-41.
[9] Rampersaud GC, Pereira MA, Girard BL, Adams J, Metzl JD. Breakfast habits, nutritional status, body weight, and academic performance in children and adolescents. J Am Diet Assoc 2005; 105:743-60.
[10] Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 1949;109:1-9.
[11] Rolls BJ, Kim S, McNelis AL, Fischman MW, Foltin RW, Moran TH. Time of effects of preloads high in fat or carbohydrate on food intake and hunger ratings in humans. Am J Physiol 1991;260:R756-63.
[12] Rolls BJ, Roe LS, Meengs JS. Salad and satiety: energy density and portion size of a first-course salad affect energy intake at lunch. J Am Diet Assoc 2004;104:1570-6.
[13] Williamson DA, Geiselman PJ, Lovejoy J, et al. Effects of consuming mycoprotein, tofu or chicken upon subsequent eating behaviour, hunger and safety. Appetite 2006;46:41-8.
[14] Pereira MA, Swain J, Goldfine AB, Rifai N, Ludwig DS. Effects of a low-glycemic load diet on resting energy expenditure and heart disease risk factors during weight loss. JAMA 2004;292:2482-90.
[15] Gunther CW, Lyle RM, Legowski PA, et al. Fat oxidation and its relation to serum parathyroid hormone in young women enrolled in a 1-y dairy calcium intervention. Am J Clin Nutr 2005;82: 1228-34.
[16] Zemel MB. Role of calcium and dairy products in energy partitioning and weight management. Am J Clin Nutr 2004;79:907S-12S.
[17] Jacqmain M, Doucet E, Despres JP, Bouchard C, Tremblay A. Calcium intake, body composition, and lipoprotein-lipid concentrations in adults. Am J Clin Nutr 2003;77:1448-52.
[18] Melanson EL, Donahoo WT, Dong F, Ida T, Zemel MB. Effect of low- and high-calcium dairy-based diets on macronutrient oxidation in humans. Obes Res 2005;13:2102-12.
[19] Smith SR, de Jonge L, Zachwieja JJ, et al. Fat and carbohydrate balances during adaptation to a high-fat. Am J Clin Nutr 2000;71: 450-7.
[20] Schrauwen P, van Marken Lichtenbelt WD, Saris WH, Westerterp KR. Changes in fat oxidation in response to a high-fat diet. Am J Clin Nutr 1997;66:276-82.
[21] Boon N, Hul GB, Viguerie N, Sicard A, Langin D, Saris WH. Effects of 3 diets with various calcium contents on 24-h energy expenditure, fat oxidation, and adipose tissue message RNA expression of lipid metabolism-related proteins. Am J Clin Nutr 2005;82:1244-52.
[22] Jacobsen R, Lorenzen JK, Toubro S, Krog-Mikkelsen I, Astrup A. Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion. Int J Obes (Lond) 2005;29:292-301.
[23] Bowen J, Noakes M, Trenerry C, Clifton PM. Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. J Clin Endocrinol Metab 2006;91:1477-83.
[24] Vander J, Wal S, Marth JM, Khosla P, Jen KL, Dhurandhar NV. Short-term effect of eggs on satiety in overweight and obese subjects. J Am Coll Nutr 2005;24:510-5.
[25] Vozzo R, Wittert G, Cocchiaro C, et al. Similar effects of foods high in protein, carbohydrate and fat on subsequent spontaneous food intake in healthy individuals. Appetite 2003;40:101-7.
[26] Shi H, Dirienzo D, Zemel MB. Effects of dietary calcium on adipocyte lipid metabolism and body weight regulation in energy-restricted aP2-agouti transgenic mice. FASEB J 2001;15:291-3.
[27] Melanson EL, Sharp TA, Schneider J, Donahoo WT, Grunwald GK, Hill JO. Relation between calcium intake and fat oxidation in adult humans. Int J Obes Relat Metab Disord 2003;27:196-203.

[28] Eisenstein J, Roberts SB, Dallal G, Saltzman E. High-protein weight-loss diets: are they safe and do they work? A review of the experimental and epidemiologic data. Nutr Rev 2002;60:189-200.

[29] Johnston CS, Day CS, Swan PD. Postprandial thermogenesis is increased 100% on a high-protein, low-fat diet versus a high-carbohydrate, low-fat diet in healthy, young women. J Am Coll Nutr 2002;21:55-61.

[30] Luscombe ND, Clifton PM, Noakes M, Parker B, Wittert G. Effects of energy-restricted diets containing increased protein on weight loss, resting energy expenditure, and the thermic effect of feeding in type 2 diabetes. Diabetes Care 2002;25:652-7.

[31] Mikkelsen PB, Toubro S, Astrup A. Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate. Am J Clin Nutr 2000;72:1135-41.