Sucrose compared with artificial sweeteners: a clinical intervention study of effects on energy intake, appetite, and energy expenditure after 10 wk of supplementation in overweight subjects[1–3]

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ABSTRACT

Background: There is a lack of appetite studies in free-living subjects supplying the habitual diet with either sucrose or artificially sweetened beverages and foods. Furthermore, the focus of artificial sweeteners has only been on the energy intake (EI) side of the energy-balance equation. The data are from a subgroup from a 10-wk study, which was previously published.

Objective: The objective was to investigate changes in EI and energy expenditure (EE) as possible reasons for the changes in body weight during 10 wk of supplementation of either sucrose or artificial sweeteners in overweight subjects.

Design: Supplements of sucrose-sweetened beverages and foods (2 g/kg body weight; $n = 12$) or similar amounts containing artificial sweeteners ($n = 10$) were given single-blind in a 10-wk parallel design. Beverages accounted for 80% and solid foods for 20% by weight of the supplements. The rest of the diet was free choice. Indirect 24-h whole-body calorimetry was performed at weeks 0 and 10. At week 0 the diet was a weight-maintaining standardized diet. At week 10 the diet consisted of the supplements and ad libitum choice of foods. Visual analog scales were used to record appetite.

Results: Body weight increased in the sucrose group and decreased in the sweetener group during the intervention. The sucrose group had a 3.3-MJ higher EI but felt less full and had higher ratings of prospective food consumption than did the sweetener group at week 10. Basal metabolic rate was increased in the sucrose group, whereas 24-h EE was increased in both groups at week 10. Energy balance in the sucrose group was more positive than in the sweetener group at the stay at week 10.

Conclusion: The changes in body weight in the 2 groups during the 10-wk intervention seem to be attributable to changes in EI rather than to changes in EE. *Am J Clin Nutr* 2014;100:36–45.

INTRODUCTION

Overweight and obesity are influenced by genetic and environmental factors, including insecurity, stress, lack of sleep, and epigenetics (1, 2). The cause of the worldwide increase in the prevalence of obesity is not known but is most probably a result of 2 major lifestyle factors: an increasingly sedentary lifestyle and the energy content of the modern diet (3). An inappropriate macronutrient composition of the diet can increase energy intake (EI)[4] (4). Both cohort studies and randomized controlled studies have found positive associations between consumption of sugars and body weight, especially when the sugar was added to beverages (5–8). Whether this is the result of an effect by sugar per se or an effect explained by the additional energy from the sugar or of the effects of food forms is controversial. However, it may be relevant to reduce the consumption of sugar as part of a strategy to lose weight or maintain a normal body weight.

Intuitively, using artificial sweeteners as a substitute for sugar could be a way to reduce sugar consumption and thus EI. Whereas some earlier short-term studies have indicated a stimulating effect of artificial sweeteners on appetite, more recent studies have not shown this effect (9). However, these studies have mainly been preload studies (10, 11). There is a lack of appetite studies in free-living subjects supplying the habitual diet with either sugar or artificially sweetened beverages and foods for a longer period of time. Furthermore, until now the focus of artificial sweeteners has only been on the EI side of the energy-balance equation. No studies have compared the effect of sugar and artificial sweeteners on energy expenditure (EE). Diet-induced thermogenesis (DIT) after a meal is proportional to the energy and macronutrient contents of the meal (12). In addition, earlier studies have suggested that both the amount and type of carbohydrate has an influence on EE (13–15); results have shown that consumption of mono- and disaccharides lead to a higher DIT than do polysaccharides, presumably because of an increased sympathetic nervous system activity (13). Because DIT accounts for 10% to 15% of total daily EE, it could be expected that daily EE would be lower with consumption of artificial sweeteners than with consumption of sugars, all else being equal (12).

The current study is a substudy that investigated the effect on energy balance of supplementation of sucrose and artificial sweeteners for 10 wk. Results from the main study showed that during 10 wk, the sucrose group had an increase in body weight of

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[4] Abbreviations used: BMR, basal metabolic rate; DIT, diet-induced thermogenesis; EE, energy expenditure; EI, energy intake; GLP-1, glucagon-like peptide-1; SPA, spontaneous physical activity; VAS, visual analog scale.

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1.6 kg, and the sweetener group had a decrease in body weight of 1.0 kg (16). Considering these results, it could be expected that the sucrose group would be in positive energy balance and the sweetener group in negative energy balance on the test day at week 10. Thus, the objective of the current study was to compare the effects of sucrose and artificial sweeteners on EI, subjective appetite sensations, and EE for 24 h in a respiration chamber.

SUBJECTS AND METHODS

The main part of the study, conducted in 42 subjects, was described in detail elsewhere (16).

Study design

The main study had a single-blind, parallel design with 2 intervention groups. For 10 wk, one group received supplemental drinks and foods containing sucrose and the other group received similar drinks and foods containing artificial sweeteners. In the current substudy, the subjects were tested in respiratory chambers on 2 different test days; the first test day was before the intervention (week 0) and the second test day was the last day of the 10-wk intervention.

Subjects

A total of 24 healthy, overweight subjects were included in this substudy; subjects from the main study volunteered to participate in the substudy. None of the subjects were dieting, and none of the women were pregnant or lactating. Approval was obtained from the Ethical Committee of Copenhagen and Frederiksberg, and the study was performed in accordance with the Helsinki II Declaration. Each subject signed an informed-consent document before the start of the study. The study was conducted in 1995.

Diets

The diet during the 10-wk intervention

During the 10-wk intervention, the subjects were supplemented with a specific minimum amount of either sucrose-sweetened or artificially sweetened foods and drinks daily. The subjects were assigned to 3 different levels of supplements according to their initial body weight: level 1, 2, or 3 corresponding to 60–75, 75–90, and >90 kg, respectively. The minimum intake of the experimental diet was regulated by the sucrose intake and corresponded to a sucrose intake of 125 g/d (level 1), 150 g/d (level 2), and 175 g/d (level 3). This corresponded to a total EI from sucrose supplements of 2.74, 3.29, and 3.83 MJ/d, respectively. The sweetener group received an equivalent amount (by weight) of foods and drinks, which resulted in an average EI of 694, 832, or 971 kJ/d at levels 1, 2, and 3, respectively. The artificial sweetener content of the intervention diet was 54% aspartame, 23% cyclamate, 22% acesulfame K, and 1% saccharin.

In the sucrose group, ~70% of the sucrose came from drinks (average: ~1.3 L/d), and ~30% came from solids foods. About 80% by weight of the supplements were beverages, and ~20% by weight were solid foods. The beverages consisted of several soft drinks and fruit juices, and the solid foods consisted of yogurt, marmalade, ice cream, and stewed fruit. Some of the artificially sweetened products were low fat, so the subjects in the sweetener group were given additional butter or corn oil to keep the fat intake in the 2 intervention diets as similar as possible.

The subjects were supplied with all the drink and food supplements at the Department of Nutrition, Exercise, and Sports. The content of sucrose and artificial sweeteners in the supplemented products was unknown to the subjects. The subjects were all told that they would receive supplements containing artificial sweeteners, and they were not informed about the real purpose of the study. In addition to consuming the food and drink supplements, the subjects were free to consume their habitual diet ad libitum.

The diets during the chamber stays

During the chamber stay in week 0, the diet was a weight-maintaining standardized diet estimated to meet each subject's individual energy requirement, adjusted to the nearest 0.5 MJ. The subjects' energy requirements were estimated by using the results from the measurements of electrical impedance (17), corresponding to a mean EI of 9.69 MJ in both groups. Carbohydrate provided 50.2% of energy, fat 36.8% of energy, and protein 13.9 % of energy.

During the chamber stay in week 10, the diet consisted of the supplement assigned to each individual; the supplements consisted of the same kind of beverages and foods provided to the subjects during the 10 wk. In addition, the subjects were offered an ad libitum choice of foods served at breakfast, lunch, and dinner. The breakfast buffet contained different types of bread, butter, cheese, fruit juice, cereals, and milk. The lunch buffet consisted of different types of bread, butter, cheese, vegetables, sandwich spread with meat and fish, eggs, and milk. The lasagna served for dinner was made of pasta, minced meat, onion, garlic, oil, carrots, milk, squash, and red pepper. The subjects were allowed to consume all of the meals ad libitum, and the amounts consumed were recorded. The solid supplemental foods were consumed at breakfast and the supplemental beverages were served at all 3 meals. The amount of water, coffee, and tea consumed during the first chamber stay were recorded and reproduced during the second chamber stay.

Measurements during the 10-wk intervention

Body weight, fat mass, and lean body mass were measured at weeks 0, 2, 4, 6, 8, and 10. Subjects completed 7-d weighed dietary records and 24-h urine collections (to validate the protein intake records) at weeks 0, 5, and 10. Waist and hip circumferences, sagittal height, and blood pressure were measured, and blood samples were collected at weeks 0 and 10. After the intervention, subjects also completed a questionnaire about the experimental diet. The questionnaire included questions indicating how much of each of the following substances was in the supplements, in the subjects' opinion: salt, sucrose, protein, vitamin C, artificial sweetener, carbohydrate, fat, or other. The questions were part of a smokescreen to investigate whether all subjects believed that they had been consuming artificial sweeteners. Protocols for carrying out the 7-d weighed dietary records, the collection of urine samples, and the conversion of urinary protein to ingested protein and calculation of dietary protein recovery were reported elsewhere (16). The same applied to the results concerning the effects of the diets on body

weight, sagittal height and blood pressure (16), and blood samples (18, 19).

Experimental protocol for the chamber study

The subjects had been instructed to maintain the same level of physical activity on the day before the test days (weeks 0 and 10) to ensure equally filled glycogen stores (20). On the evening before the test days, the subjects arrived at the respiratory chamber at 2200. Bedtime was set at 2300, but reading was allowed until 2400. The following morning at 0800, body weight was measured after the subjects voided, and body composition was measured after the subjects rested for 10 min in a supine position. The chamber was closed and assessments started at 0900. Urine was subsequently collected throughout the 24-h measurement. Appetite sensations were recorded every hour from 0900 onward. Breakfast was served at 0900, lunch at 1300, dinner at 1800, and coffee or tea at 2000. Palatability ratings were assessed immediately after consumption of the test meals. Bedtime was at 2315; however, reading was allowed until 2400. During the day, physical activity was scheduled; at 0930 and 1430 there were sessions of walking back and forth 25 times in the chamber, and at 1100 and 1600 there was 15 min of cycling on an ergometer bicycle (Monark 814E; Monark AB) (75 W). On the following day, the subjects were wakened at 0730. While the subjects were resting in a supine position, the basal metabolic rate (BMR) was measured from 0800 to 0900; the subjects were awake but lying relaxed in bed.

Appetite ratings

Appetite ratings were recorded on 10-cm visual analog scales (VASs) with words anchored at each end describing the extremes of a unipolar question (eg, for hunger: "I am not hungry at all" and "I have never been more hungry"). VASs were used to assess hunger, satiety, fullness, prospective food consumption, desires for special foods, and palatability of the test meal (taste, smell, visual appeal, aftertaste, and overall palatability) (21).

Measurements in the respiratory chamber

The 24-h EE, BMR, and substrate oxidation rates were measured in 2 open-circuit respiratory chambers that were described in detail previously (22). The gas exchange of the subjects was calculated from measurements of oxygen and carbon dioxide concentrations at the outlet of the chamber and from measured air flow through the chambers. Protein oxidation was calculated from urea nitrogen content in the 24-h urine collection. The oxygen and carbon dioxide exchanges, including urinary nitrogen measurements, were used to calculate EE, and utilization rates of lipid and carbohydrate were calculated as described by Elia and Livesey (23). The whole unit was regularly calibrated by comparing a known volume of carbon dioxide entering the chamber with the volume of carbon dioxide measured by the unit (22). The within-subject variation for 24-h EE measured in the chambers is 2.3% (22).

DIT was calculated as the incremental area under the curve for EE for 4 h after the dinner meal (1800–2200), with BMR as the baseline measure, and 24-h energy balance was calculated (energy balance = EI − EE). Body weight was measured to the nearest 0.1 kg on a digital scale (Seca model 708; Seca Mess und Wiegetechnik). Body composition was estimated by using bioelectric impedance (Animeter; HTS-Engineering Inc). Fat mass and lean body mass were calculated as described previously (24).

Statistical analyses

On the basis of a previous study (25), power analysis showed that 10 subjects in each group were sufficient to detect a difference of 145 kJ in 24-h EE; 24 subjects were originally recruited in order to allow for dropouts.

Repeated-measures ANOVA was used to test for differences in dietary intake during the 10-wk intervention and during the test day in week 10 and differences in VAS scores between diet groups and time. The MIXED procedure in the SAS software package (version 9.3) was used (SAS Institute). Subjects were included as random factors.

To investigate the effect of diet on EE, carbohydrate and fat oxidation, and spontaneous physical activity (SPA), repeated-measures ANOVA was used to test for interaction between diet groups, week, and time. EE was additionally adjusted for sex, age, 24-h SPA, EI, fat mass, and lean body mass. To investigate the effect of diet on protein oxidation, ANOVA was used to test interactions between diet and week. The effect of diet on body weight, BMI, fat and lean body mass, BMR, DIT, and energy balance was tested with ANCOVA, with week 10 values as response and week 0 values as covariates. BMR was additionally adjusted for sex, age, SPA recorded during the period when BMR was measured, and changes in fat mass and lean body mass at week 10 and EI in the chamber at week 10. Energy balance was additionally adjusted for sex, age, 24-h SPA, fat mass, and lean body mass at week 10. DIT was additionally adjusted for sex, age, SPA recorded during the 4-h measurement, fat mass, and lean body mass at week 10. Tukey's adjustments for multiple testing were applied.

RESULTS

A total of 24 subjects participated, and 22 subjects completed the study; one subject dropped out after the randomization but before the intervention had started, and another subject did not complete the second chamber stay and was excluded. Thus, 12 subjects from the sucrose group and 10 subjects from the sweetener group were included in the data analyses (**Table 1**). No differences were found at baseline (week 0) in food intake, anthropometric characteristics, subjective appetite sensations,

TABLE 1
Characteristics of the subjects at baseline

	Sucrose group (n = 12)	Sweetener group (n = 10)
Age (y)	35.3 ± 9.8[1]	35.2 ± 12.4
Sex (M/F)	2/10	2/8
Body weight (kg)	84.5 ± 8.4	80.5 ± 10.1
Height (m)	1.72 ± 0.69	1.72 ± 0.63
BMI (kg/m^2)	28.7 ± 2.3	27.3 ± 2.5
Fat mass (kg)	31.2 ± 3.9	27.3 ± 4.9[2]
Lean body mass (kg)	53.3 ± 5.8	53.2 ± 8.0
24-h SPA[3] (%)	7.2 ± 0.4	8.7 ± 0.7[2]

[1] Mean ± SD (all such values).
[2] Significantly different from the sucrose group, $P < 0.05$ (unpaired t test).
[3] SPA, spontaneous physical activity.

and respiratory measurements between the 2 groups, except for a higher fat mass and a lower 24-h SPA in the sucrose group than in the sweetener group.

Results from the 10-wk intervention

Dietary intake

The 7-d dietary food records showed that there was a higher intake of carbohydrate and sucrose in the sucrose group compared with the sweetener group during the 10-wk intervention. The intake of energy was higher in the sucrose group than in the sweetener group; the average difference in total EI between the 2 groups was 2.29 MJ (95% CI: 0.56, 4.01 MJ) during the 10 wk. Validation of the protein intake showed no differences between urinary protein and self-reported dietary protein, either between groups or times. Urinary protein correlated with dietary protein at all 3 time points (16).

Body weight

In the sucrose group, mean (±SEM) body weight (1.4 ± 0.6 kg) and fat mass (1.2 ± 0.6 kg) increased and in the sweetener group body weight (−1.2 ± 0.6 kg) and fat mass (−0.9 ± 0.6 kg) decreased during the 10-wk intervention, which resulted in between-group differences amounting to 2.7 kg body weight (95% CI: 0.8, 4.6 kg body weight; $P = 0.007$) and 2.0 kg body fat (95% CI: 0.2, 3.8 kg body fat; $P = 0.007$). No changes in lean body mass was found in the 2 groups during the intervention.

Results from the chamber study

Dietary intake

During the test day in week 10, the sucrose group had a higher total EI than did the sweetener group, which resulted in a between-group difference of 3.26 MJ (95% CI: 0.28, 6.58 MJ) (**Table 2**). The mean (±SEM) energy from the supplements was

TABLE 2
Average intake, including the supplements, of energy and macronutrients in the sucrose ($n = 12$) and sweetener ($n = 10$) groups at breakfast, lunch, and dinner on the test day during the chamber stay in week 10[1]

	Breakfast	Lunch	Dinner	P for main diet effect[2]
Energy (MJ)				
Sucrose	4.26 ± 0.42	4.53 ± 0.58	5.77 ± 0.46	0.04
Sweetener	2.91 ± 0.47	3.60 ± 0.45	4.78 ± 0.47	
Carbohydrate (g)				
Sucrose	184 ± 20	126 ± 21	197 ± 14	0.003
Sweetener	110 ± 24	83 ± 8	162 ± 22	
Carbohydrate (% of energy)				
Sucrose	73 ± 3	47 ± 3	58 ± 3	0.05
Sweetener	61 ± 4	41 ± 2	56 ± 3	
Sucrose (g)				
Sucrose	91 ± 13[3]	49 ± 14[3]	28 ± 7	<0.0001[4]
Sweetener	11 ± 8	0.2 ± 0.1	7 ± 4	
Sucrose (% of energy)				
Sucrose	35 ± 3[3]	18 ± 3[3]	12 ± 3[5]	<0.0001[6]
Sweetener	3.6 ± 2.6	0.1 ± 0.0	2.2 ± 1.4	
Fat (g)				
Sucrose	21 ± 4	44 ± 6	42 ± 5	0.3
Sweetener	16 ± 3	39 ± 8	34 ± 4	
Fat (% of energy)				
Sucrose	18 ± 2	37 ± 3	27 ± 2	0.6
Sweetener	21 ± 3	39 ± 3	28 ± 2	
Protein (g)				
Sucrose	31 ± 3	38 ± 6	53 ± 7	0.7
Sweetener	28 ± 3	36 ± 4	46 ± 3	
Protein (% of energy)				
Sucrose	13 ± 1	14 ± 1	15 ± 1	0.003
Sweetener	19 ± 2	17 ± 1	17 ± 1	
Dietary fiber (g)				
Sucrose	7 ± 1	13 ± 1	12 ± 1	0.7
Sweetener	8 ± 1	14 ± 1	11 ± 1	
Energy density (kJ/g)				
Sucrose	3.5 ± 0.2	3.6 ± 0.2	4.5 ± 0.3	0.02
Sweetener	2.7 ± 0.2	3.5 ± 0.4	3.9 ± 0.3	
Weight of food (g)				
Sucrose	1315 ± 174	1296 ± 180	1309 ± 87	0.4
Sweetener	1167 ± 211	1077 ± 89	1267 ± 111	

[1] All values are means ± SEMs.
[2] P values were derived by repeated-measures ANOVA of the interaction between diet groups and meals. Subjects were included as random factors.
[3,5] Significant difference between the sucrose and sweetener groups (Tukey's post hoc tests): [3]$P < 0.0001$, [5]$P < 0.05$.
[4,6] Significant group × time interaction: [4]$P = 0.002$, [6]$P = 0.007$.

3.46 ± 0.09 MJ in the sucrose group and 0.93 ± 0.05 MJ in the sweetener group. No difference in ad libitum EI was found between the groups, which amounted to 11.10 ± 1.13 MJ in the sucrose group and 10.37 ± 1.16 MJ in the sweetener group ($P = 0.7$).

No group × meal interactions were found for any of the variables, except for sucrose (Table 2). The intake of carbohydrate and sucrose and the percentage of energy from protein and the energy density were higher in the sucrose group than in the sweetener group during the test day in week 10 (Table 2). The palatability of the test meals was rated similarly in the 2 groups.

Subjective appetite sensations

On the test day in week 10, a group × time interaction was found in the feeling of fullness ($P = 0.005$) and prospective consumption ($P = 0.003$), and post hoc tests showed that the subjects in the sucrose group felt less full in the periods after lunch and dinner and had higher ratings of prospective food consumption in the period after lunch and dinner, compared with the sweetener group (**Figure 1**). No group × time interactions were found with respect to sensations of satiety and hunger (Figure 1). No differences in the subjective desires for sweet, salty, or fatty foods were found between the 2 groups.

Respiratory measurements

BMR increased in the sucrose group (7.6%) after the 10-wk intervention (between-group mean difference: 24 kJ/h; 95% CI: 0.3, 47.5 kJ/h; $P = 0.02$) (**Table 3**). However, the difference between the 2 groups disappeared after adjustments for sex, age, and SPA recorded during the period when BMR was measured, changes in fat mass and lean body mass, and the energy consumed in the chamber (Table 3). The significant covariates were sex ($P = 0.001$), SPA ($P = 0.01$), and the energy consumed during the chamber stay ($P = 0.008$). No difference in DIT was found between the 2 groups either before or after adjustments for sex, age, SPA recorded during the 4-h measurement, fat mass, and lean body mass.

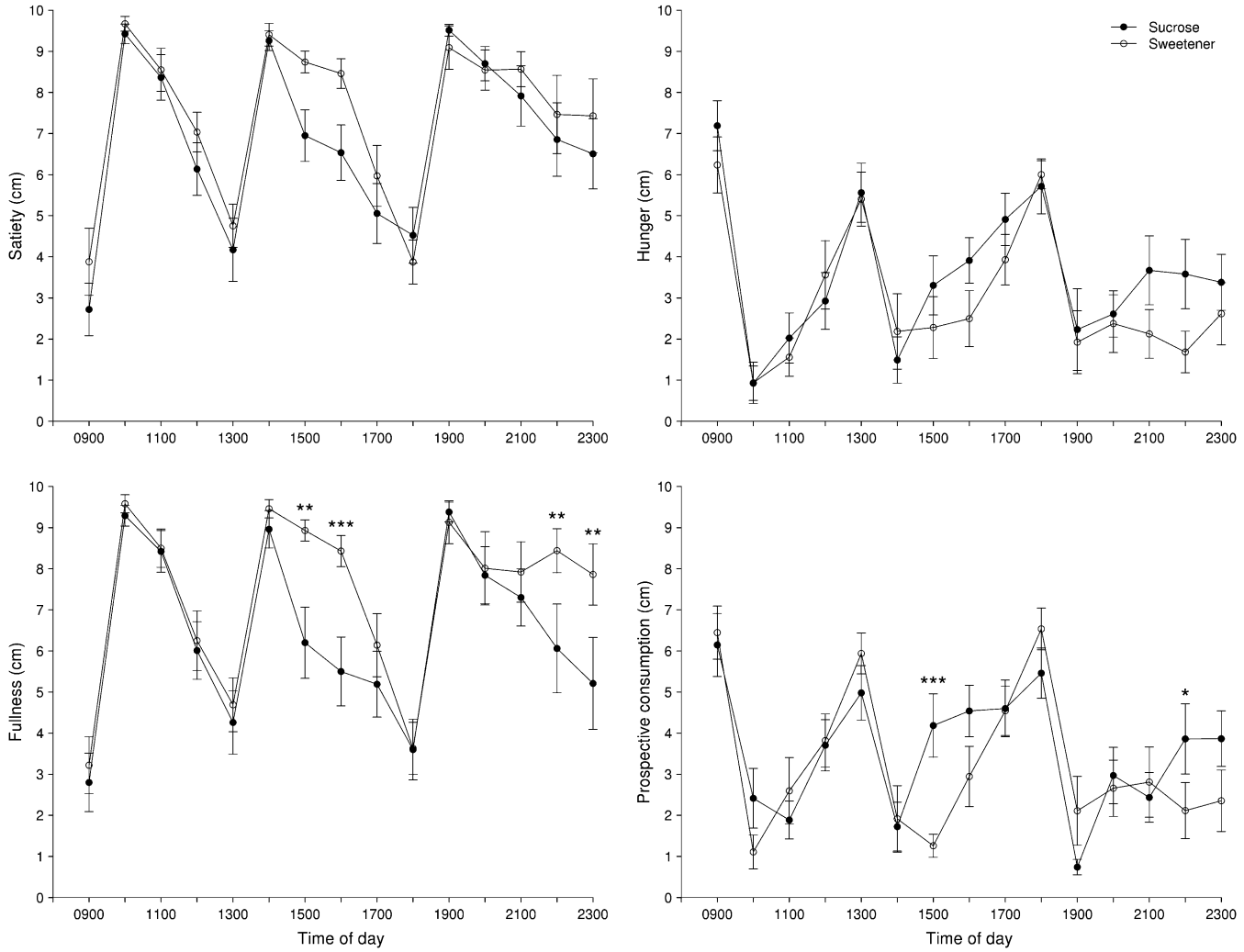

FIGURE 1. Mean (±SEM) appetite scores (hunger, satiety, fullness, and prospective food consumption) recorded during the chamber stay at week 10 in the sucrose group ($n = 12$) and in the sweetener group ($n = 10$). The visual analog scale equal to 10 cm corresponds to "I cannot eat another bite" (satiety), "I have never been more hungry" (hunger), "I am totally full" (fullness), and "I can eat a lot" (prospective food consumption). Repeated-measures ANOVA was used to test differences between appetite scores in the 2 groups. The group × time interactions were significant for the appetite variables: fullness: $P = 0.005$; prospective food consumption: $P = 0.003$. Tukey's post hoc tests: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

TABLE 3
BMR, DIT, and 24-h energy expenditure before (day 0) and after (day 70) the intervention in the sucrose (n = 12) and sweetener (n = 10) groups[1]

	Day 0[2]	Day 70[2]	LS means[3]	P[3]	LS means (adjusted)	P
BMR (kJ/h)						
Sucrose	314 ± 8	338 ± 16	332 (319, 348)	0.02	332 (323, 341)[4]	0.07[4]
Sweetener	310 ± 14	309 ± 18	308 (294, 323)		318 (308, 327)	
DIT (kJ/4 h)						
Sucrose	344 ± 28	377 ± 34	374 (317, 431)	0.4	429 (354, 504)[5]	0.2[5]
Sweetener	332 ± 37	409 ± 28	412 (350, 475)		347 (261, 432)	
24-h EE (kJ/d)						
Sucrose	9611 ± 225	10,298 ± 395				
Sweetener	9402 ± 357	9732 ± 385				

[1] There were no significant differences in baseline values between the 2 groups (unpaired t test). BMR, basal metabolic rate; DIT, diet-induced thermogenesis; EE, energy expenditure; LS, least squares.
[2] Values are means ± SEMs.
[3] Derived by ANCOVA with diet (sucrose and sweetener) as factor and baseline values as covariates. Values in parentheses are 95% CIs (all such values).
[4] P values were derived by ANCOVA with diet as factor and baseline values, age, sex, spontaneous physical activity during measurement of BMR, changes in fat mass and lean body mass, and energy intake during the chamber stay in week 10 as covariates.
[5] P values were derived by ANCOVA with diet as factor and baseline values, age, sex, spontaneous physical activity during the 4-h measurement of DIT, lean body mass, and fat mass at week 10 as covariates.

There was a group × week × time interaction for EE (P = 0.03); however, after Tukey's adjustments for multiple tests, the post hoc tests showed no relevant differences. After adjustment for sex, age, 24-h SPA, fat mass, lean body mass, and EI, the interaction remained and post hoc tests showed a difference between the groups at week 10; EE was higher at 1100 in the sucrose group than in the sweetener group, P = 0.008) (**Figure 2**). A group × week interaction for EE (P = 0.0003) was also found. Post hoc tests showed that 24-h EE increased in the sucrose group by 686 kJ/d (95% CI: 514, 859 kJ/d; $P < 0.0001$) and in the sweetener group by 329 kJ/d (95% CI: 142, 518 kJ/d; $P < 0.0001$) after 10 wk (Figure 2). However, after adjustment for sex, age, 24-h SPA, fat mass, lean body mass, and EI, this interaction disappeared (group × week: P = 0.3). The significant covariates were 24-h SPA ($P < 0.001$), EI ($P < 0.001$), and lean body mass ($P < 0.001$).

No difference in SPA was found between the sucrose (7.7%; 95% CI: 7.0, 8.4%) and sweetener (8.4%; 95% CI: 7.7, 9.2%) groups during the chamber stay in week 10. A group × week interaction was found for carbohydrate oxidation ($P < 0.0001$); post hoc tests showed that carbohydrate oxidation was 44% higher in the sucrose group than in the sweetener group during the chamber stay in week 10 ($P < 0.0001$) (**Figure 3**). A group × week interaction was also found for fat oxidation ($P < 0.0001$). Post hoc tests showed that fat oxidation was 37% lower in the sucrose group than

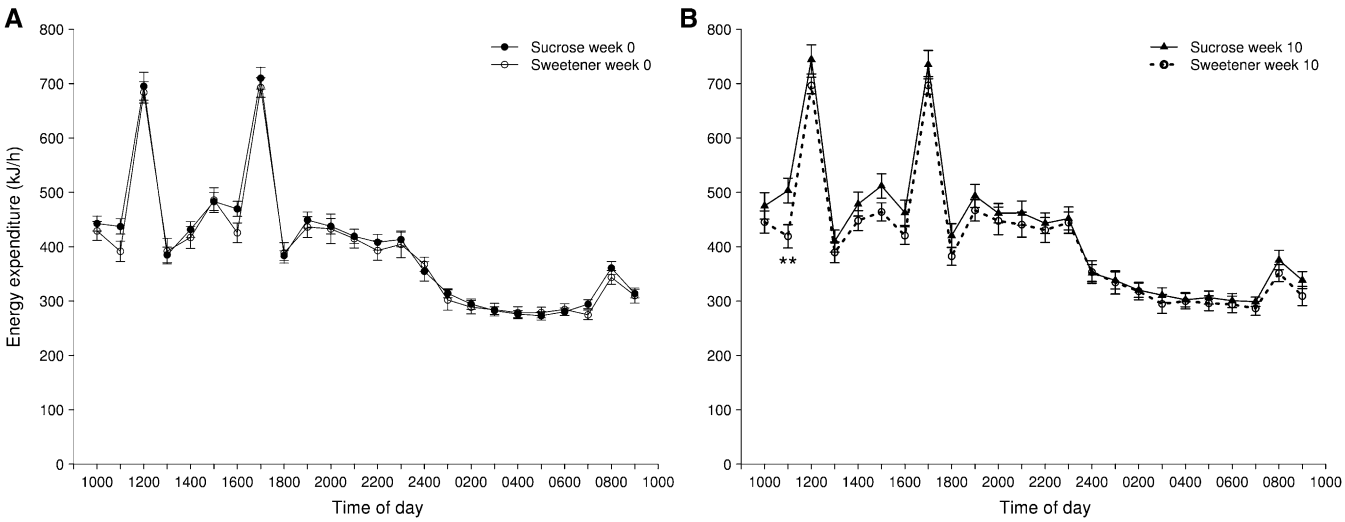

FIGURE 2. Mean (±SEM) energy expenditure during 24 h measured at week 0 (A) and after 10 wk of intervention (B) in the sucrose group (n = 12) and in the sweetener group (n = 10). Breakfast was consumed at 0900, lunch at 1300, and dinner at 1800. Physical activity was scheduled: walking back and forth 25 times in the chamber at 0930 and 1430 and 15 min of cycling on an ergometer bicycle at 1100 and 1600. Repeated-measures ANOVA was used to test for differences between energy expenditure in the 2 groups. The group × week × time interaction was significant (P = 0.03). Tukey's post hoc tests after adjustment for sex, age, 24-h spontaneous physical activity, fat mass and lean body mass, and energy intake during the chamber stay: $^{**}P<0.01$.

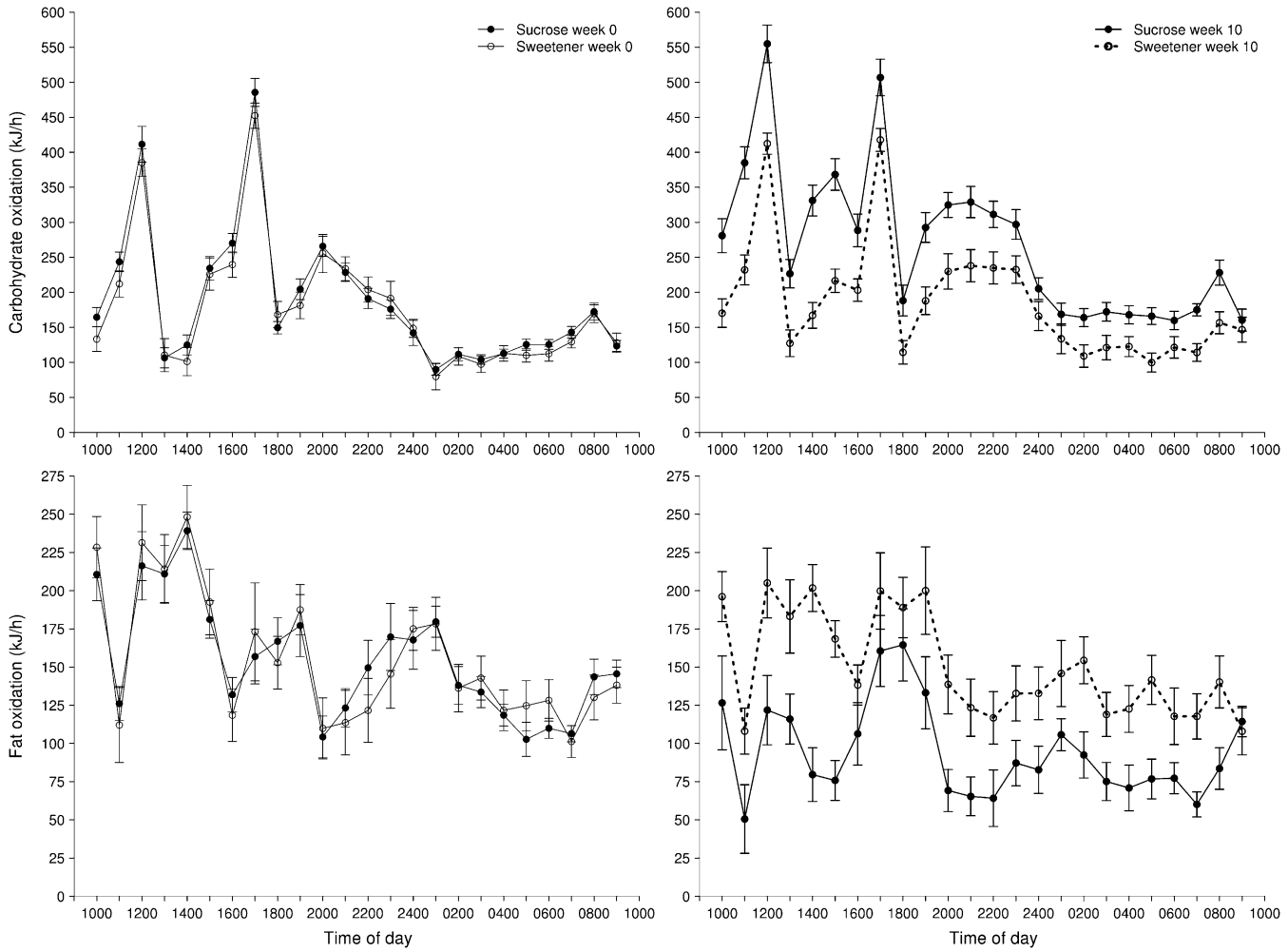

FIGURE 3. Mean (±SEM) carbohydrate oxidation and fat oxidation during 24 h measured at week 0 and after 10 wk of intervention in the sucrose group (n = 12) and in the sweetener group (n = 10). Breakfast was consumed at 0900, lunch at 1300, and dinner at 1800. Physical activity was scheduled: walking back and forth 25 times in the chamber at 0930 and 1430 and 15 min of cycling on an ergometer bicycle at 1100 and 1600. Repeated-measures ANOVA was used to test for differences between carbohydrate oxidation and fat oxidation in the 2 groups. The group × week interaction was significant for both carbohydrate and fat oxidation ($P < 0.0001$). Tukey's post hoc tests: difference between weeks 0 and 10 for the sucrose group ($P < 0.0001$) and difference between the sucrose and the sweetener groups for substrate oxidation ($P < 0.0001$).

in the sweetener group during the chamber stay in week 10 ($P < 0.0001$) (Figure 3). No difference in 24-h protein oxidation was found between the 2 groups during the chamber stay in week 10. The 24-h respiratory quotient was higher in the sucrose group (0.87; 95% CI: 0.85, 0.88) than in the sweetener group (0.92; 95% CI: 0.90, 0.94; $P < 0.0001$) during the chamber stay at week 10.

Energy balance

Mean (±SEM) energy balance was more positive in the sucrose group (4264 ± 889 kJ) than in the sweetener group (1565 ± 997 kJ); the mean difference was 3321 kJ (95% CI: 997, 5645 kJ; $P = 0.008$). After adjustments for sex, age, 24-h SPA, fat mass, and lean body mass, the difference disappeared ($P = 0.2$). The energy balance correlated with 24-h respiratory quotient at the chamber stay in week 10 ($r = 0.64$, P=0.001).

DISCUSSION

The main findings of the current study were that the sucrose group had a higher EI during the chamber stay in week 10 than did the sweetener group. Despite this, there was a strong trend for the subjects in the sucrose group to have a higher appetite between lunch and dinner and after dinner compared with the sweetener group. BMR increased in the sucrose group, whereas no difference in DIT or 24-h EE was found between the 2 groups after the 10-wk intervention. Both groups were in positive energy balance during the chamber stay in week 10, although energy balance in the sucrose group was more positive than that in the sweetener group.

Nearly 3.5 MJ of the higher EI in the sucrose group was from the supplements; 70% of the sucrose in the supplements came from sucrose-sweetened beverages. Already in the 1990s, it was hypothesized that liquid calories fail to trigger appetite regulation (26, 27); at present, several studies support this. Recently, Maersk et al (11) conducted a test meal crossover study that served a sucrose-sweetened soft drink, semiskim milk, an artificially sweetened soft drink, or water. An ad libitum meal was served after 4 h. Total EI was higher after the energy-containing beverages than after the artificial sweetened beverage and water, as were insulin and glucagon-like peptide-1 (GLP-1) concentrations (11). In a crossover design, Anton et al (28) served preloads

containing stevia (290 kcal), aspartame (290 kcal), or sucrose (493 kcal) before an ad libitum lunch and an ad libitum dinner. Despite differences in energy in the preloads and a difference in glucose and insulin concentrations, no differences in ad libitum food intake and hunger or satiety scores were observed (28). This phenomenon could be a result of insufficient sensory signaling (29) because liquids are easier to consume and therefore can be consumed more rapidly (30).

In connection with the current study, another substudy was conducted to measure, among others, fasting and postprandial glucose, insulin, leptin, and GLP-1 concentrations (18). Blood sampling was started after the subjects had ended the stay in the chambers. Breakfast and lunch were precise reproductions of the meals on the day before in the chamber. Analyses showed that fasting insulin and leptin concentrations and postprandial glucose, insulin, GLP-1, and leptin concentrations were higher in the sucrose group than in the sweetener group. Regardless of this, the sucrose group consumed 3 MJ more than did the sweetener group. Furthermore, the sucrose group reported a periodically higher appetite. So, it seems that the physiological satiety mechanisms were being put out of action in the current study. This was also the case with the studies mentioned above by Maersk et al (11) and Anton et al (28). Karl et al (31) also showed that higher-energy-density meals were associated with higher concentrations of insulin, peptide YY, and GLP-1; however, there was no energy compensation at the following meal. The authors suggest that an explanation for the lack of energy compensation observed in their study may be that the effects of gut hormones on appetite are masked by the numerous environmental, sensory, and cognitive cues that can affect appetite and EI (31). The current study provides possible support for this suggestion. Results from the main study showed that the subjects in the sucrose group were partly unaware of the caloric manipulation (16). Consequently, they did not acknowledge the need to compensate for the extra energy. The sweetener group, on the other hand, thought that they received no sucrose and medium to much artificial sweetener. In another study conducted by Maersk et al (32), participants consumed 1 L/d of sugar-sweetened cola, diet cola, semiskim milk, or water for 6 mo. That study was not blind, which gave the participants the opportunity to acknowledge the additional energy they consumed. After the 6-mo intervention, there were only small numerical differences in body weight between the 4 groups. The sugar-sweetened beverage group had managed to compensate for most of the extra energy during the 6 mo (32).

The sweetener group in the current study had a reduced EI during the 10-wk intervention. However, in the chamber, the subjects increased the ad libitum EI equivalent to that in the sucrose group. The unexpected higher ad libitum intake in both groups could partly be a result of boredom or because of the awareness of the time before they had access to food again. In a preload-test meal design, De Graaf et al (33) showed that subjects who had 90 min between an ad libitum soup and an ad libitum meal consumed more soup than when they only had 15 min between the 2 meals. Giving the subjects in the current study access to 1 or 2 more meals and also instructing the subjects to eat only until comfortably satisfied could probably have avoided some of the overeating.

BMR increased in the sucrose group by 7.6% after 10 wk and body weight increased by 1.7%. Most of the weight gain in the sucrose group consisted of fat mass, and neither fat mass nor lean body mass was associated with changes in BMR. So, the increase in BMR can probably not be explained by the increase in body weight. It is likely that the increase is related to the "overfeeding situation." Dauncey et al (34) measured resting metabolic rate after just 1 d of overfeeding and found that resting metabolic rate remained elevated by 12% 14 h after the last meal. Both Diaz et al (35) and Ravussin et al (36) observed increases in BMR after long-term overfeeding. However, both research groups stated that the length of time from the last meal to the measurement of BMR was possibly inadequate to ensure that the thermic effect of the evening meal had completely disappeared. Diaz et al performed the measurements after 12.5 h and Ravussin et al after 14 h. Harris et al (37) recorded weekly changes in BMR during 8 wk of overfeeding. BMR increased over the first 2 wk, decreased in the third week, and increased and reached a plateau after 5 wk (37). In that study, BMR was measured $<$10 h from the last meal. When the current study was planned, it was not predicted that the sucrose group would overeat in that way. Therefore, the 12.5 h between the last meal and the measurements of BMR may have been inadequate. This makes it difficult to distinguish between the short-term effect of the diet and the long-term effect of the intervention. Statistical adjustments including the EI during the chamber stay made the difference between groups disappear, which indicates that most of the increase in BMR was a result of overeating during the chamber stay.

The lack of difference in DIT between the groups was unexpected because the difference in EI at the dinner meal was almost 1 MJ. A study by Weststrate (38) showed that meal size differences of $<$1 MJ result in differences in DIT. An explanation could be that the measured BMR in the sucrose group was high because it was influenced by the last meal. This would lead to a lower estimated DIT in the sucrose group and mask the difference between the groups.

DIT was only estimated after the dinner meal. It is possible that if DIT had been estimated after every meal, a difference between groups and/or between weeks 0 and 10 would have been shown, especially because of the breakfast meal, which was 1.35 MJ larger in the sucrose group. In addition, the sucrose intake was higher in the sucrose group than in the sweetener group during breakfast and lunch. The design of the test day made it impossible, however, to estimate DIT for the breakfast and lunch meals because of physical activity during the day.

The objective of the current substudy was to investigate the effect of the sweetener supplements on energy balance, including some of the components of total EE. To detect small effects requires a high accuracy of measurements and control of day-to-day variation in physical activity to ensure any effects to be detected. Therefore, the respiratory chamber was chosen because that was the only way to measure all of the variables of interest. With the use of doubly labeled water, only 24-h EE would be measured and the ventilated-hood system could measure some of the components but not total EE. The effect of the sweetener supplements on 24-h EE and components of total EE could have been measured in the chamber by serving the subjects meals with fixed energy instead of ad libitum meals. This could, however, not have been done within the 10-wk intervention period.

In conclusion, body weight increased in the sucrose group and decreased in the artificial-sweetener group during the 10-wk intervention. BMR increased in the sucrose group, but no

difference in 24-h EE was found between the 2 groups in week 10. The increase in BMR was probably caused by the very high EI in the sucrose group during the chamber stay in week 10. Thus, the changes in body weight in the 2 groups during the 10-wk intervention seemed to be a result of changes in EI rather than of changes in EE.

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