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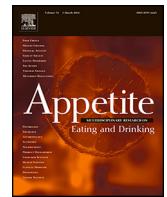
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Research report

Consumption of thylakoid-rich spinach extract reduces hunger, increases satiety and reduces cravings for palatable food in overweight women [☆]

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ABSTRACT

Green-plant membranes, thylakoids, have previously been found to increase postprandial release of the satiety hormone GLP-1, implicated in reward signaling. The purpose of this study was to investigate how treatment with a single dose of thylakoids before breakfast affects homeostatic as well as hedonic hunger, measured as wanting and liking for palatable food (VAS). We also examined whether treatment effects were correlated to scores for eating behavior. Compared to placebo, intake of thylakoids significantly reduced hunger (21% reduction, $p < 0.05$), increased satiety (14% increase, $p < 0.01$), reduced cravings for all snacks and sweets during the day (36% reduction, $p < 0.05$), as well as cravings for salty (30%, $p < 0.01$); sweet (38%, $p < 0.001$); and sweet-and-fat (36%, $p < 0.05$) snacks, respectively, and decreased subjective liking for sweet (28% reduction, $p < 0.01$). The treatment effects on wanting all snacks, sweet-and-fat snacks in particular, were positively correlated to higher emotional eating scores ($p < 0.01$). The treatment effect of thylakoids on scores for wanting and liking were correlated to a reduced intake by treatment ($p < 0.01$ respectively), even though food intake was not affected significantly. In conclusion, thylakoids may be used as a food supplement to reduce homeostatic and hedonic hunger, associated with overeating and obesity. Individuals scoring higher for emotional eating behavior may have enhanced treatment effect on cravings for palatable food.

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Introduction

Eating is a great pleasure in life. However, snacking between meals, as well as ingestion of snacks and fast foods, has been associated with weight gain and obesity in all age groups (Garber & Lustig, 2011; Mesas, Muñoz Pareja, López García, & Rodríguez

Artalejo, 2012). Since life in general has become more sedentary, overeating of palatable, energy dense foods causes a positive energy balance (Blundell & Cooling, 2000; Yeomans, Blundell, & Lessem, 2004) which may result in an increasing incidence of overweight and obesity worldwide (Blundell & Macdiarmid, 1997; Erlanson-Albertsson, 2005; Finkelstein et al., 2012; WHO, 2015).

The reason for overeating is that hunger has a homeostatic component but also a hedonic part, driven by the rewarding values of food (Berridge, 2009; Berthoud, 2011). Hedonic hunger favors energy-dense palatable food, rich in sugar and fat, for example snacks, pastries, desserts, baked confectionery and sweets – foods typically ingested in between meals and preferred by women (Drewnowski, 1997; Montmayeur, le Coutre, Drewnowski, & Almiron-Roig, 2010). While homeostatic eating is due to energy deficiency, hedonic eating is triggered by the anticipation of pleasure, regardless of energy status. The hedonic hunger is constituted by two components, wanting and liking (Finlayson, King, & Blundell, 2007; Mela, 2006). Wanting represents the anticipation phase, the motivation to eat a food item, and is triggered by cues. Liking is the hedonic reaction of the pleasure experienced through a rewarding orosensory stimuli.

Abbreviations: BMC, Biomedical Centre; E%, energy percentage; TFEQR18-V2, The Three Factor Eating Questionnaire, Revised 18-item, Version 2; VAS, visual analog scale.

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Even though the homeostatic and non-homeostatic pathways are separate, they affect each other (Finlayson et al., 2007). When the reward circuit interacts with the appetite-controlling neurons in the hypothalamus, this can result in up-regulated expression of hunger signals and blunting of satiety signals (Erlanson-Albertsson, 2005). Therefore, ingestion of palatable food, instead of terminating food intake, leads to a maintained drive to eat, with continued eating due to reward rather than energy deficit (Finlayson et al., 2007). Over-eating then becomes a possibility (Berthoud, 2006; Blundell & Macdiarmid, 1997). It has been suggested that individuals who are obese, dieting and/or under stress are more susceptible to hedonic eating (Garber & Lustig, 2011). In emotional eaters, who tend to develop overweight due to over-eating, food craving and consumption of food rich in carbohydrates and fat increase in response to stressors or negative affect (Cappelleri, Bushmakin, & Gerber, 2009; Raspopow, Abizaid, Matheson, & Anisman, 2010).

Obviously, there is a need to prevent overeating to avoid weight gain. One way is to attenuate the hedonic drive in those who experience increased cravings for palatable food.

Green-plant membranes, thylakoids, have been shown to reduce feelings of hunger as well as cravings for palatable food in human participants, during diet intervention and simultaneous weight loss in overweight women (Montelius et al., 2014; Stenblom et al., 2013, 2014). We found that these effects are connected to an altered secretion of appetite regulating hormones, including ghrelin, cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), in rodents, pigs and humans (Köhnke, Lindbo et al., 2009; Köhnke, Lindqvist et al., 2009; Montelius, Osman et al., 2013; Montelius, Szwiec et al., 2013; Montelius et al., 2014). Based on these findings of suppressed hedonic hunger and increased levels of GLP-1, we were interested to deepen our knowledge of these effects on overweight middle-aged women in a similar group of participants but in a non-laboratory setting. In addition, since emotional eating is associated with overeating of snacks, we were interested to investigate any potential correlation between scores for eating behavior and the treatment effect of thylakoids.

Aim/primary objectives

The aim of this study was to investigate in a non-laboratory setting 1) how treatment with a thylakoid-rich spinach extract (from now on called thylakoids), served as a meal supplement before breakfast, affects subjective ratings of wanting and liking for snacks and sweets, as well as hunger and satiety, using visual analog scales (VAS), 2) to examine how thylakoid treatment affects intake of palatable food from an *ad libitum* buffet of the same sweets and snacks the participants rated their cravings for during the day, and 3) to investigate if the treatment effect of thylakoids is associated with scores for eating behavior.

Materials and methods

Participants

Thirty-two women were recruited through local advertising. The volunteers were assessed for eligibility through a screening procedure including questionnaires evaluating general health and subjective liking for different food products. During the screening process, 6 women were excluded and 26 women included in the study. Inclusion criteria were: women ages 40–70, normal weight or overweight. Exclusion criteria were: Diabetes, illnesses affecting appetite, food allergies or intolerance to food served in the study, or dieting during the last 3 months. Twenty-two participants completed the study and were included in the final analysis (Table 1).

Table 1
Baseline characteristics.

		Median	Interquartile range	Range (min–max)
Age (years)	(n = 22)	54.5	47.0–59.5	40–66
BMI (kg/m ²)	(n = 22)	25.3	24.5–29.4	22.4–36.2
Body fat (%) ^a	(n = 21)	37.2	31.5–40.4	26.9–47.1

^a Body composition analyzer TANITA-BC 418 MA (Amsterdam, The Netherlands). One participant was excluded from this analysis due to failure of the body composition analyzer.

Eating behavior questionnaire

Prior to the trial days, the participants filled out the Three-Factor Eating Questionnaire Revised 18-item, Version 2 (TFEQ-R18V2) (Cappelleri et al., 2009). This is a revised version of the original Three-Factor Eating Questionnaire (TFEQ), a self-assessment scale used in studies of eating behavior. TFEQ-R18V2 contains 18 questions to measure three types of eating behavior: emotional eating, uncontrolled eating and cognitive restraint. Previous results from a large diverse sample of non-obese, as well as obese participants, showed a good reliability, and the domains were reported to be robust and stable (Cappelleri et al., 2009).

Study design

The study was a randomized, placebo-controlled, double blind, single-centered meal intervention study with a cross over design, conducted at the Biomedical Centre (BMC) at Lund University, Sweden. Each participant was tested on two days, separated by a wash out period of at least 1 week, receiving a 5 g supplement of thylakoids (Appethyl, Greenleaf Medical AB, Stockholm, Sweden) on one day and placebo on the other. The allocation to treatment or placebo was randomized and balanced between test days. The participants were not able to identify which drink contained the active component.

Participants arrived in the morning, fasted from 22:00 the night before (schedule, Table 2).

No alcohol or intense physical activity was allowed the days before or during test days.

Before breakfast, the participants filled out a questionnaire constructed as Visual Analog Scales (VAS) (Flint, Raben, Blundell, & Astrup, 2000) with questions regarding hunger, satiety and wanting for specific food products (Table 3). The questionnaire was constructed as a booklet with written instructions on the first page. All questions were followed by a 100-mm horizontal line, on which subjects marked their response. The line was anchored at each end, expressing the minimum value on the left: "Not at all", and the

Table 2
Test day schedule, days 1 and 2.

07:30	Participants arrived at BMC	
07:45	VAS	Baseline; Time point 0
08:00	Blueberry drink, with/without 5 g thylakoids (treatment/placebo)	
	Breakfast, including coffee/tea	
08:15	VAS	Time point 15 min
09:00	VAS	Time point 60 min
10:00	VAS	Time point 120 min
11:00	VAS	Time point 180 min
12:00	VAS	Time point 240 min
12:45	VAS	Time point 285 min
13:00	Lunch	Time point 300 min
13:20	VAS	Time point 320 min
14:00	VAS	Time point 360 min
15:00	VAS	Time point 420 min
16:00	Snack buffet served. VAS	Time point 480 min
16:45	VAS after snack buffet	Time point 525 min

Table 3
VAS-questions (translated from Swedish).

Hunger and satiety	
1.	How hungry are you right now?
2.	How satiated are you right now?
Wanting	
3.	How much do you want potato chips right now?
4.	How much do you want salted assorted nuts right now?
5.	How much do you want milk chocolate right now?
6.	How much do you want dark chocolate right now?
7.	How much do you want sweets right now?
8.	How much do you want "Dumlekola" right now?
9.	How much do you want "Ahlgrens bilar" right now?
10.	How much do you want "Gott & Blandat" right now?
11.	How much do you want "en chokladboll" right now?
12.	How much do you want a cinnamon bun right now?
13.	How much do you want some orange juice right now?
Liking	
1.	How much did you like the potato chips?
2.	How much did you like the salted assorted nuts?
3.	How much did you like the milk chocolate?
4.	How much did you like the dark chocolate?
5.	How much did you like the "Dumlekola"?
6.	How much did you like the "Ahlgrens bilar"?
7.	How much did you like the "Gott & Blandat"?
8.	How much did you like the "chokladboll"?
9.	How much did you like the cinnamon bun?
10.	How much did you like the orange juice?

maximum value on the right: "Extremely". Subsequently, a blueberry drink, with or without supplementation of thylakoids, was served followed by a standardized high carbohydrate Swedish breakfast. The VAS-questionnaire was filled out every hour as well as before and after lunch and the *ad libitum* snack buffet, served at 13:00 and 16:00 respectively.

Between breakfast and lunch, participants left the premises for work or free activities, and were reminded to fill out the VAS-questionnaires every hour via text messages. After lunch, the participants stayed in their allocated seats, sitting side by side with cardboard dividers between them, all facing a window. They were allowed to read or work on laptops until 16:00 when the snack buffet was served at the table on individual trays. A leaflet containing information about the products served accompanied the buffet describing the freshness of the foods. It included names of manufacturers and pictures of the packaging. The same pictures were used for the VAS-questionnaire. Before starting to eat from the snack buffet in front of them, the participants filled out the VAS-questionnaire once more. Then they were allowed to eat freely from the products on their trays, encouraged to follow their urges and ask for more should they run out. They were occupied by the snack buffet for 45 min, not doing anything else during this time. Afterwards, they filled out the VAS-questionnaire again, this time including questions regarding how much they liked the products they tasted that particular day, to enable evaluation of the rewarding properties of the items (Table 3). On the last page the participants were asked about adverse effects: "Have you experienced any new symptom or discomfort during the day, for example headache, rash, or nausea?"

Power and sample size

The number of participants required to achieve a power of 0.8 in this cross-over study was calculated using data from an earlier study (Stenblom et al., 2013). Based on these calculations, 15 participants were needed to detect a 10 mm difference in VAS-ratings of hunger. This number of participants is also sufficient for detecting differences in ratings for wanting palatable food (Flint et al., 2000). According to literature, 17 participants should be sufficient

for detecting a difference in food intake of 500 kJ/120 kcal (Gregersen et al., 2008).

Ethics

The study was approved by the Ethics Committee for Human Studies in Lund (2006/361). The trial was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent before the study began. The participants did not receive any monetary compensation.

Thylakoids

All leafy green vegetables contain thylakoids. Five grams of extracted thylakoids is equivalent to 100 g of spinach. However, humans cannot utilize thylakoids in unprocessed vegetables, since the thylakoids are stacked inside non-digestible plant cell walls. Therefore, the only realistic way to achieve a dose compared to the one used in this study is to consume thylakoids as a supplement.

The thylakoids used in the present study were prepared from baby spinach leaves using the pH-method as previously described (Emek et al., 2010), followed by drum drying. One hundred grams of thylakoids contain 185 kcal, 26.1 g protein, 7.24 g fat, 48.7 g carbohydrate (including non-soluble and soluble fibers), 27.9 mg lutein, 730 µg zeaxanthin, 3.45 mg beta carotene, 21 µg vitamin A, 1330 µg vitamin K₁, 6.07 mg vitamin E and 166 µg folic acid.

Based on a previous dose-response study, a single dose of 5 g thylakoids was used in this study (Stenblom et al., 2013). The thylakoids were served in a cold blueberry drink, mixed with 2.5 g rapeseed oil (Zeta, Di Luca & Di Luca AB, Stockholm, Sweden) and 92.5 g blueberry soup (Ekströms original, Procordia Food AB, Eslöv, Sweden), served immediately before breakfast on treatment test days. On control test days, the participants were served a placebo drink, which consisted of 2.5 g rapeseed oil mixed with 92.5 g blueberry soup. The blueberry drinks contained 73 kcal with thylakoids and 64 kcal without.

Meals

Breakfast on both test days was a standardized Swedish high carbohydrate breakfast with yogurt and muesli, one slice of white bread with butter, cheese and sweet pepper, coffee or tea. Total energy content was 509 kcal. The energy distribution was 31.2 percent of total energy (E%) fat, 15.4 E% protein and 53.4 E% carbohydrates. Sugars constituted 22.6 E%. The lunch served on both test days was a slice of thick crust pizza, made from sourdough, with tomato sauce, ham, onion, mushrooms and cheese, served with boiled broccoli and tap water. Coffee or tea following lunch was optional, but subjects were required to have the same on both test days. Energy distribution of the lunch was 45.9 E% carbohydrates, 32.5 E% fat and 21.6 E% protein. Total energy content was 480 kcal. In addition, participants were allowed one cup of coffee/tea, without sugar or sweetener, between breakfast and lunch, and one cup of coffee/tea after lunch. Calculations of energy distribution were made using Dietist Net Pro (Kost och Näringsdata AB, Bromma, Sweden).

Snack buffet

A product questionnaire was constructed for this study as a tool for choosing well-suited products to measure cravings and snack intake in a Swedish population. It contained specific questions about snacks of the most popular brands in Sweden: potato chips, nuts, chocolate, sweets, baked confectionery, buns, fruit juice and soft drinks. Questions were asked about how often the responders consumed the palatable foods, how much they liked the products on a nine point hedonic scale and which product in each category they

Table 4

Components of the snack buffet, energy content per 100 g.

Products	Calories (kJ/kcal)	Protein (g/E%)	Fat (g/E%)	Carbohydrate (g/E%)	Sugar (g/E%)	Salt (g)
Salty						
Potato chips "Sourcream & Onion" (Estrella, Sweden)	2200/525	6.0/4.6	32.5/56.4	50.5/39	3.6/2.8	1.7
Assorted nuts "Vår klassiska nötmix" (OLW, Sweden)	2450/590	25.0/16.8	48.0/72.5	16.0/10.7	4.2/2.8	1.3
Sweet						
Sweets "Ahlgrens bilar" (Cloetta, Sweden)	1450/350	6.0/6.8	0.3/0.8	81.0/92.4	55.0/62.7	0.25 ^a
Sweets (gummies) "Gott&Blandat" (Malaco, Sweden)	1450/340	0.0/0	0.0/0	85.0/100	61.0/71.8	0.25 ^a
Orange juice "Apelsin juice" (Kiviks Musteri AB, Sweden)	180/40	0.6/5.9	<0.5/11	8.5/83.1	8.5/83.1	0.01 ^a
Sweet-and-fat						
Milk chocolate "Marabou mjölkchoklad" (Marabou, Sweden)	2290/550	4.8/3.5	32.0/53	59.0/43.4	58.0/42.7	0.25 ^a
Dark chocolate "Lindt Excellence 70% dark" (Lindt & Sprüngli, Sweden)	2180/520	8.0/6.1	40.0/68.7	33.0/25.2	28.0/21.4	0.15 ^a
Chocolate covered toffee "Dumlecola original" (Fazer, Sweden)	1980/470	3.6/3.1	21.0/40.4	66.0/56.5	53.0/45.4	0.4 ^a
Chocolate pastry "Delicatoboll" (Delicato, Sweden)	2000/480	5.0/4.2	29.0/54.7	49.0/41.1	30.0/25.2	0.4
Cinnamon bun "Kanelbulle" (Bonjour, VAA SAN Sverige AB, Sweden)	1350/320	6.2/8	9.6/27.8	50/64.3	15/19.3	0.5

^a Salt content initially expressed as sodium content has been converted to amount of salt by multiplying the sodium value by 2.5, as recommended by the Swedish National Food Agency (Livsmedelsverket).

preferred. Based on the responses, a range of snacks, sweets and confectionery was chosen for the snack buffet (Table 4). These products were grouped in three different categories: salty, sweet, and sweet-and-fat snacks. Snacks with salty taste included potato chips and salted assorted nuts; products with sweet taste, containing sugar, included two different kinds of sweets and orange juice; products with sweet taste containing both sugar and fat were: milk chocolate, dark chocolate, chocolate covered toffee, chocolate pastry, and cinnamon bun. Each participant received a tray with the products served in separate containers, weighed before and after consumption. The amount of sweets and snacks was large enough to allow the participants to consume as much as they liked while preventing all of the food from being consumed. Coffee, tea and water were served with the snacks in a limited amount. The drinks were optional, but the participants had to have the same drinks on both test days.

Statistics

All statistical analyses were done using the Prism version 6, statistical software (GraphPad Software, Inc, San Diego, CA, USA). Normal distribution of the paired parameters and the calculated differences between treatment and control conditions was computed by d'Agostino and Pearson omnibus normality test, verified by boxplot and histogram analysis and comparison between mean and median values. Variations in VAS-ratings over time were analyzed with a two-way repeated measures (RM) ANOVA in order to test time, treatment, and time by treatment interaction. Analyses of VAS-ratings at individual time points as well as differences in total area under the curve (tAUC) for VAS-ratings, food intake and ratings of liking were performed using Wilcoxon matched-pairs signed rank test. Reported *p*-values in text and figure legends were analyzed with Wilcoxon matched-pairs signed rank test if not otherwise stated.

Treatment effect of thylakoids on feelings of hunger, satiety and urge for specific food products and liking respectively was calculated by subtracting VAS-ratings for treatment day from control day values, except for satiety, which was calculated the opposite way. Treatment effect on food intake was calculated as difference in caloric intake between control and treatment days. Calculating delta values for the treatment effects was required to account for paired observations. These delta values were then correlated to eating behavior scores, as measured by the TFEQR18-V2 questionnaire, divided into the three domains: emotional eating, uncontrolled eating and cognitive restraint. Correlations were also calculated between eating behavior scores, BMI and body fat percentage (Table 1) as well as

between treatment effects on wanting and liking respectively versus food intake. All correlations were computed by Pearson correlation coefficient. Correlation coefficients (*r*), and coefficients of determination, (*R*²), equal to and above 0.3 were considered fairly strong and have been included in the manuscript. In both figures and text, data are expressed as mean \pm SEM if not otherwise stated. *p*-values <0.05 were considered statistically significant, and *p*-values <0.10 of interest.

Results

VAS-ratings on hunger and satiety

Treatment with thylakoids reduced subjective ratings of hunger compared to control throughout the day ($F(1,21) = 6.237$, $p < 0.05$, Fig. 1A). tAUC for hunger decreased by 21% between control and treated conditions ($p < 0.05$, Fig. 1B). The difference in hunger ratings between control and treatment was most pronounced prior to lunch and before the snack buffet, reaching highest significance at time points 285 min and 480 min after beginning of breakfast (Fig. 1A).

Treatment with thylakoids also increased ratings of satiety compared to control (Fig. 1C, D). Satiety-scores were generally higher during the whole day in the treated condition ($F(1,21) = 8.745$, $p < 0.01$, Fig. 1C), tAUC for satiety being increased by 14% following treatment ($p < 0.01$, Fig. 1D). Significant differences in satiety between control and treatment groups were observed at 60 and 120 min, as well as immediately before the snack buffet at time point 480 min (Fig. 1C).

VAS-ratings on wanting palatable foods

Treatment with thylakoids decreased subjective feelings of wanting all kinds of palatable food compared to control (Fig. 2). VAS-ratings were consistently lower during the treatment test day for all categories of snacks (salty, sweet and sweet-and-fat) as well as total wanting for all snacks.

Ratings for wanting salty snacks in the treated and control conditions are presented in Fig. 2A. Treatment with thylakoids reduced cravings for salty snacks continuously during the day ($F(1,21) = 6.110$, $p < 0.05$). tAUC decreased by 30% between control and treated conditions ($p < 0.01$, Fig. 2B). Specifically, the urge for salty snacks was lower in the treated group at time points 60, 120 and 360 minutes compared to the control (Fig. 2A).

Effect of treatment on ratings for wanting sweet snacks is presented in Fig. 2C ($F(1,21) = 8.412$, $p < 0.01$). Wanting for sweet

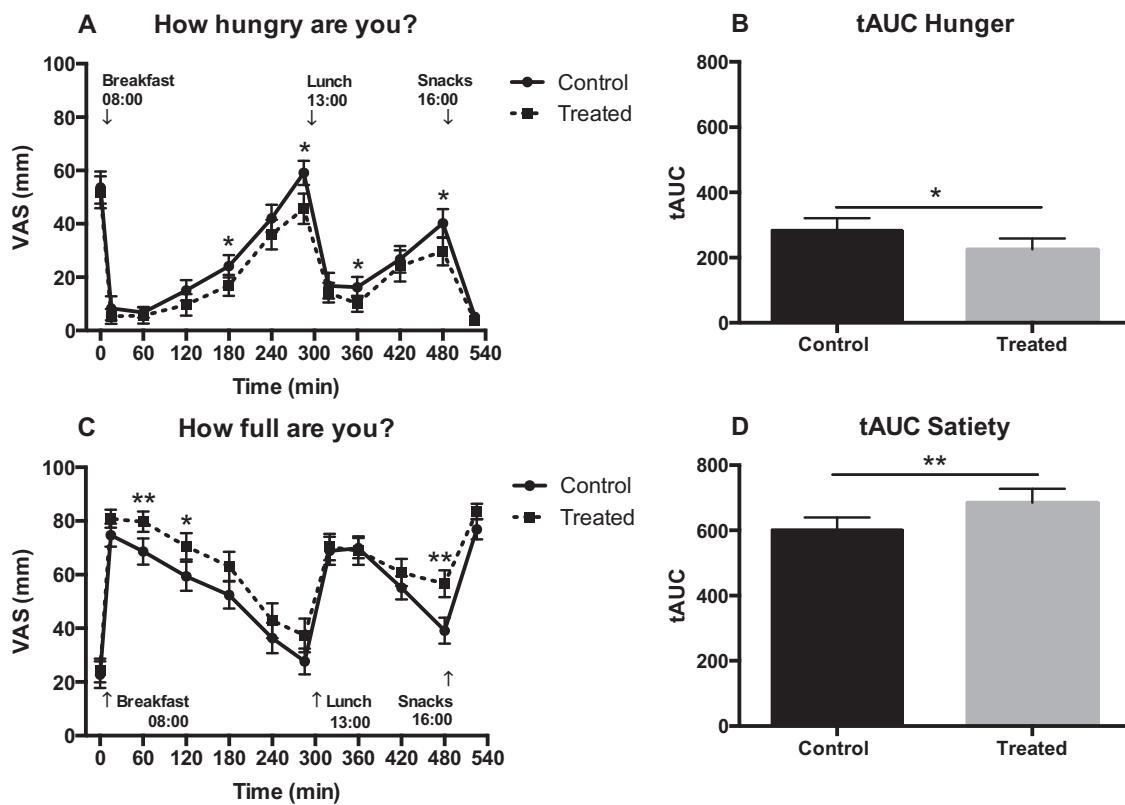


Fig. 1. Hunger- and satiety-ratings following intake of thylakoids or placebo. Treatment with thylakoids decreased ratings of hunger (A, B) and increased ratings of satiety (C, D) compared to the control. * $p < 0.05$, ** $p < 0.01$. A and C: Two-way RM ANOVA followed by Wilcoxon signed rank test for individual time points. B and D: Wilcoxon signed rank test.

decreased 38% between control and treated conditions ($p < 0.001$, tAUC, Fig. 2D). Furthermore, there was a tendency toward a time by treatment interaction in wanting sweet snacks, i.e. the time impact on VAS-ratings depended on whether the participant received treatment or placebo ($F(1,1231) = 1.803$, $p = 0.05$). The urge for sweet snacks was lower in the treated group compared to control at all time points between breakfast and lunch, starting already at 15 minutes after breakfast. It was also lower in the afternoon at time point 420 minutes (Fig. 2C).

Treatment with thylakoids also reduced ratings for wanting sweet-and-fat snacks as shown in Fig. 2E ($F(1,21) = 5.198$, $p < 0.05$). Wanting for sweet-and-fat decreased 36% in the treated group compared to control ($p < 0.05$, tAUC, Fig. 2F). The urge for sweet-and-fat snacks was lower in the treated group compared to control at time points 60, 120, 180 minutes and also right before lunch at 285 minutes (Fig. 2E).

Similar to the effects on the separate categories of snacks respectively, the ANOVA analysis of total wanting of all snacks revealed an effect of treatment ($F(1,21) = 7.364$, $p < 0.05$, Fig. 2G). Subsequent analysis of individual time points showed that treatment decreased total wanting at 15, 60, 120, 180, 285 and 420 minutes after breakfast ($p < 0.05$). Wanting for all snacks combined decreased by 36% in the treated group compared to the control ($p < 0.05$, tAUC, Fig. 2H).

Food intake from ad libitum snack buffet

Of the 22 participants, everyone ate something from the snack buffet on both test days. Median total intake in the control group was 912 kcal (lower and upper quartiles 619–1081 kcal) and median intake in the thylakoid group was 810 kcal (lower and upper quartiles 598–1019 kcal), which corresponds to a reduced intake in the treated

condition by 11% (Fig. 3). This reduction, however, was not statistically significant.

There was a tendency toward a reduced caloric intake of salty food items following treatment with thylakoids compared to control, with a median difference of 65 kcal ($p = 0.0547$, Fig. 3). This corresponds to a reduction in intake of salty snacks by 26% between control and treated conditions, since median intake of salty snacks in the control group was 248 kcal (lower and upper quartiles 111–322 kcal) and median intake in the thylakoid group was 183 kcal (lower and upper quartiles 80–294 kcal).

There were no significant differences in the intake of sweet or sweet-and-fat snacks between treatment and control conditions.

Liking for palatable food, measured after consumption from the snack buffet

Only the participants who tasted an item were allowed to rate their liking for it. For each specific food product to be included in the analysis of liking scores, participants had to taste and rate their liking for the product at both control and treatment days. One participant was excluded from the analysis due to failure to fill out the form correctly. Treatment with thylakoids produced a lower liking of sweet products after consumption, compared to control days ($p < 0.01$, Fig. 4). Median rating for liking sweet was 32 in the control group (lower and upper quartiles 21–52) and 23 in the thylakoid group (lower and upper quartiles 9–40). This corresponds to a reduction in liking sweet by 28%. Median liking for all snacks together was 27 in the control group (lower and upper quartiles 16–37) and 17 in the treated group (lower and upper quartiles 15–34). This however was not significant ($p = 0.15$, Fig. 4). Liking for salty and sweet-and-fat products was not significantly changed between treatment and control days.

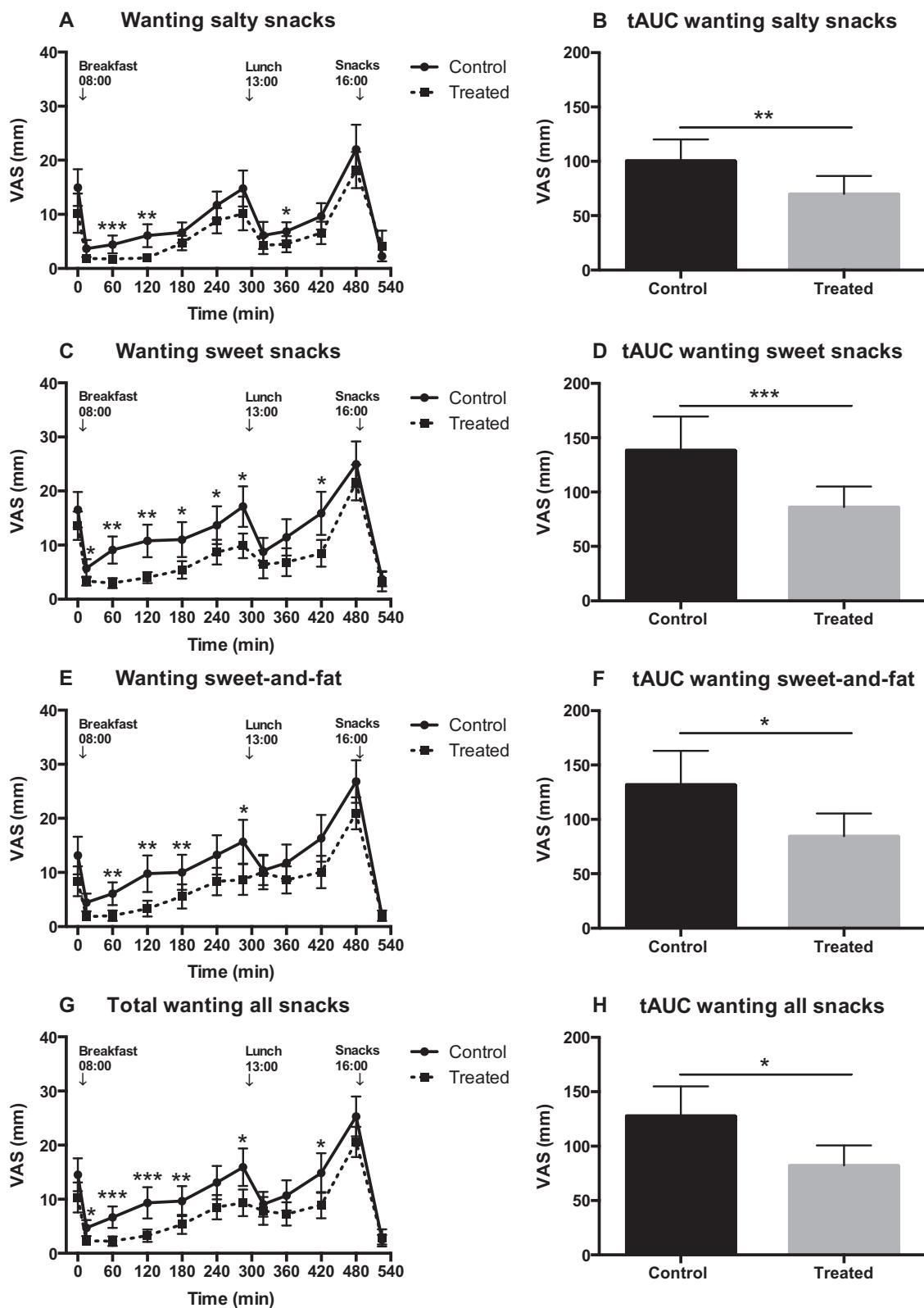


Fig. 2. VAS-ratings of wanting salty, sweet, sweet-and-fat snacks and all snacks combined, comparing treatment and placebo conditions (control). Treatment with thylakoids reduced wanting for all categories of snacks compared to the control (A–H). Presented data are an average of the ratings for the individual items of palatable food, presented per category (Table 3). For all parameters, effect of treatment was analyzed by tAUC and by two-way RM ANOVA followed by analysis of differences in individual time points using Wilcoxon matched-pairs signed rank test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

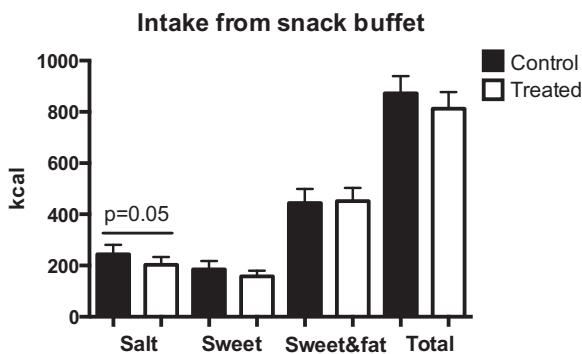


Fig. 3. Energy intake from the afternoon snack buffet. Supplementation with thylakoids produced a tendency toward reduced caloric intake of salty snacks on treatment day compared to control ($p = 0.0547$, Wilcoxon matched-pairs signed rank test, $n = 22$).

Correlations between treatment effects on wanting versus treatment effects on food intake

The treatment effect on wanting sweet-and-fat snacks was positively correlated with the treatment effect on intake of sweet-and-fat snacks, so that greater reduction in wanting sweet-and-fat snacks during the course of the day correlated with a reduced intake of

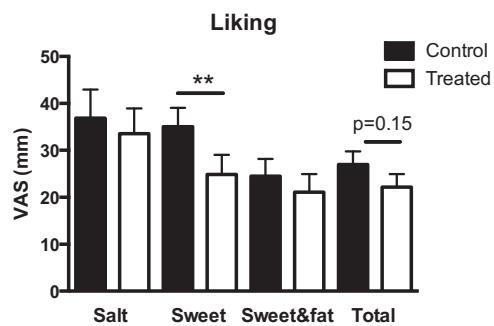
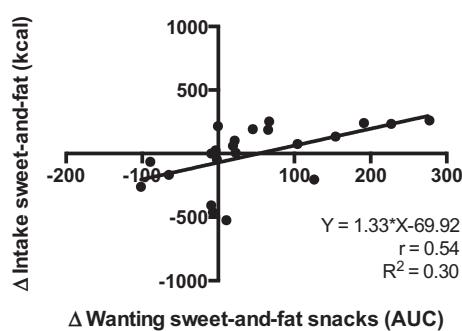


Fig. 4. Liking for specific food products after consumption, with and without treatment with thylakoids. Liking of products (VAS) scored immediately after the *ad libitum* snack buffet, presented in categories and as total liking for all products, divided by the number of products in each category to produce an average score. Treatment with thylakoids decreased the liking of sweet products between control and treatment days ($p < 0.05$, Wilcoxon matched-pairs signed rank test, $n = 18$ for liking salt, 18 for sweet, 20 for sweet-and-fat, and 21 for total liking).

A Treatment effects on wanting vs intake sweet-and-fat (ctrl-treated)



sweet-and-fat snacks at the *ad libitum* snack buffet in the afternoon ($p < 0.01$, $r = 0.54$, Fig. 5A). Similarly, there was a positive correlation between the treatment effect on wanting all kinds of snacks and the treatment effect on intake of all kinds of snacks ($p < 0.01$, $r = 0.60$, Fig. 5B). The correlation plots show that participants with higher treatment effect on wanting, i.e. the greatest reduction in ratings for wanting in the treated condition compared to control, also have greatest reduction in food intake between control and thylakoid trial days. There was also a positive correlation between the reduction in wanting sweet-and-fat and the difference in total intake between control and treated conditions ($p < 0.01$, $r = 0.64$).

Correlations between treatment effects on liking versus treatment effects on food intake

The treatment effect on liking for sweet was positively correlated with the treatment effect on intake of sweet, so that participants with greatest reduction in intake of sweet snacks between control and treated conditions rated lower liking for sweet after consumption from the *ad libitum* snack buffet ($p < 0.05$, $r = 0.58$). Similarly, there was a positive correlation between the treatment effect on liking sweet-and-fat snacks and the treatment effect on intake of sweet-and-fat snacks ($p < 0.01$, $r = 0.58$, Fig. 6A), liking all kinds of snacks versus total snack intake ($p < 0.01$, $r = 0.65$, Fig. 6B), liking all kinds of snacks versus intake of sweet-and-fat snacks ($p < 0.01$, $r = 0.57$), and liking sweet-and-fat snacks versus total intake of all snacks, control minus treated conditions ($p < 0.01$, $r = 0.58$).

Eating behavior measured by the three-factor eating questionnaire (TFEQ-R18V2)

Participants responded to each of the 18 questions about statements characterizing eating-related behaviors on a four-point Likert scale (Likert, 1932). Responses were coded on a four-point scale (1–4) with higher values indicating more of the behavior. Mean values for each of the three behavioral categories were: emotional eating domain 2.27 (+/– 0.15), cognitive restraint domain 2.52 (+/– 0.15) and uncontrolled eating domain 2.26 (+/– 0.10).

Correlations between treatment effect on wanting and scores for eating behavior

The treatment effect on wanting sweet-and-fat snacks as well as all kinds of snacks was positively correlated with emotional eating

B Treatment effects on wanting vs intake all snacks (ctrl-treated)

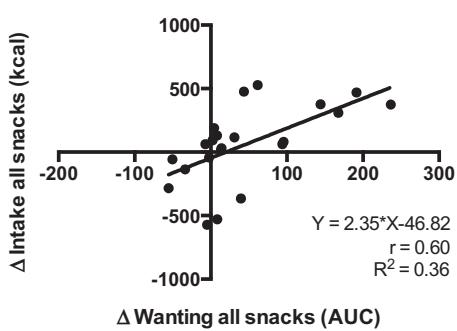


Fig. 5. Correlations between treatment effects on wanting and treatment effects on food intake. Graphs show correlation plots of the treatment effect on VAS-ratings for wanting sweet-and-fat snacks versus the treatment effect on intake of sweet-and-fat (A) and all kinds of snacks together (B). VAS-ratings and caloric intake are presented as delta values, i.e. control day minus treatment day values in each category, the difference representing the treatment effect of thylakoids. The treatment effect on wanting sweet-and-fat snacks was positively correlated to a reduction in food intake ($p < 0.01$, A), so was the treatment effect on all categories of snacks together ($p < 0.01$, B).

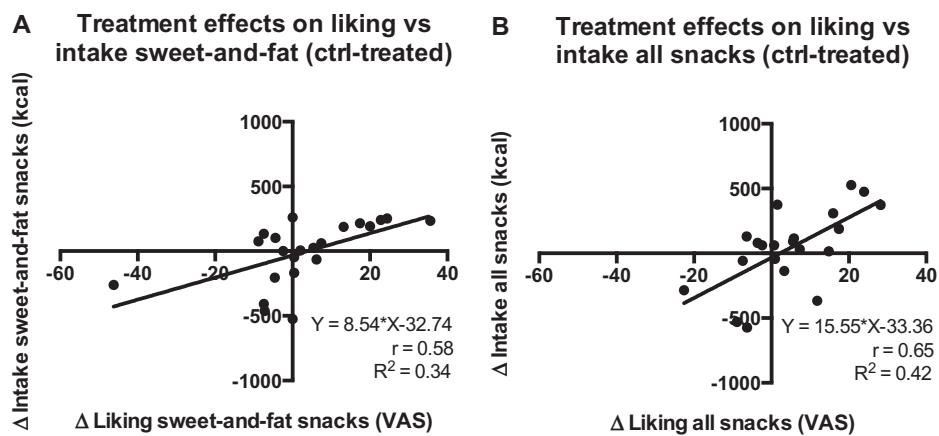


Fig. 6. Correlations between treatment effects on liking and treatment effects on food intake. Graphs show correlation plots of the treatment effect on VAS-ratings for liking sweet-and-fat (A), and all snacks (B), versus treatment effect on intake of the corresponding categories of snacks. VAS-ratings and caloric intake are presented as delta values, i.e. control day minus treatment day values in each category, the difference representing the treatment effect of thylakoids. The treatment effect on liking sweet-and-fat snacks was positively correlated to a reduction in intake of sweet products ($p < 0.01$, A), so was the treatment effect on all categories of snacks together ($p < 0.01$, B).

scores, so that higher scores for emotional eating behavior was correlated to a greater treatment effect of thylakoids on ratings for wanting sweet-and-fat foods ($p < 0.01$, $r = 0.62$, Fig. 7A) and all categories of palatable foods ($p < 0.01$, $r = 0.55$, Fig. 7B). Scores for cognitive restraint and uncontrolled eating behavior were not correlated with treatment effects on wanting.

Additional correlations analyzed

There were no statistically significant correlations between the different eating behavior scores and the treatment effect of thylakoids on hunger, satiety, food intake or liking. Neither were there any correlations between BMI or body fat percentage and scores for eating behavior, nor any correlations between the different eating behavior scores.

No serious adverse events or effects were reported. Mild cases of nausea during or after the snack buffet occurred at three occasions in the placebo group and at two occasions in the treated group. Headaches were reported at two occasions in the placebo group and at one occasion in the treated group.

Discussion

The present study demonstrates that a single dose of thylakoids prior to breakfast increased subjective ratings of satiety and

decreased ratings of hunger as well as cravings for snacks and sweets during the day. Intake of thylakoids also decreased subjective liking for sweet, scored directly following consumption. There are strong correlations between the treatment effect on wanting and actual food intake between control and thylakoid conditions, as well as between the treatment effect on liking and food intake, even though there were no significant differences in food intake per se. When correlated to eating behavior scores, treatment effect of thylakoids on wanting was positively correlated to emotional eating for all snacks, sweet-and-fat foods being specifically targeted.

The promotion of satiety by thylakoids found in this study is an entirely novel finding. This started 60 minutes after breakfast, suggesting enhanced early satiety signaling. In addition, ratings for satiety in the treated group were also higher several hours after lunch, compared to placebo. Both the acute and late effects of thylakoids to enhance satiety may be explained by increased secretion of satiety hormones CCK and GLP-1 (Köhne, Lindbo et al., 2009; Montelius et al., 2014; Stenblom et al., 2013).

Both CCK and GLP-1 are known to have a biphasic pattern of secretion. Through gastric distension, as well as by calories entering the small intestine, CCK is released, first in the hypothalamus, and later peripherally, which induces satiety (Pappas, 1992). GLP-1 secretion has a similar pattern, the early phase being mediated by the vagus nerve and the second phase by direct contact of luminal nutrients with the L-cells in the distal small intestine (Baggio & Drucker,

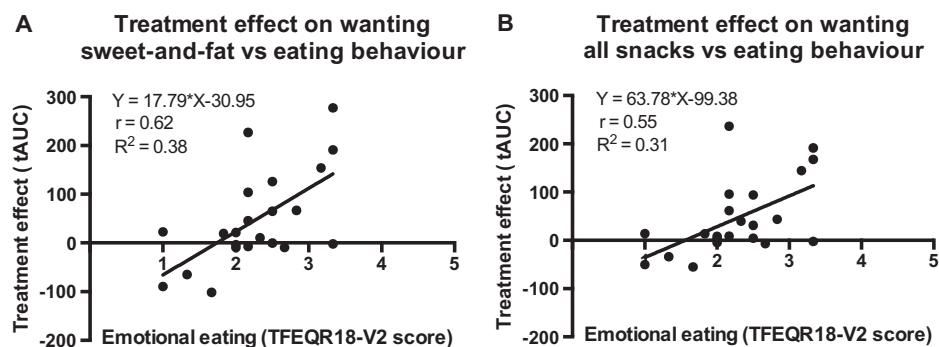


Fig. 7. Correlation between treatment effect of thylakoids on wanting palatable food and emotional eating behavior scores. Graphs show correlation plots of the treatment effect on VAS-ratings for wanting versus scores for emotional eating measured by the TFEQR18-V2 (A and B). VAS-ratings are presented as delta values, i.e. control day minus treatment day values for the tAUC in each category, the difference representing the treatment effect of thylakoids. The treatment effect on wanting sweet-and-fat snacks was positively correlated to emotional eating ($p < 0.01$, A). So was the treatment effect on all categories of snacks together ($p < 0.01$, B).

2007; Flint, Raben, Ersbøll, Holst, & Astrup, 2001; Näslund & Hellström, 2007; Wishart, Horowitz, Morris, Jones, & Nauck, 1998).

Treatment also had an attenuating effect on hunger ratings throughout the day. In comparison to ratings for satiety, the difference in hunger ratings reached significance later, at 180 minutes after breakfast in comparison to 60 minutes for satiety. The present findings on hunger verify previous reports on decreased hunger ratings following treatment with thylakoids (Stenblom et al., 2013, 2014). The suppressed hunger may both be related to reduced ghrelin-levels (Köhnke, Lindbo et al., 2009) and an indirect effect of increased circulating levels of the satiety hormones CCK and GLP-1. Indeed, the late appearance of hunger suppression suggests an indirect effect by thylakoids on promotion of satiety hormones. GLP-1 in physiological levels has been shown to inhibit gastric emptying and reduce ratings of hunger (Flint et al., 2001). A reduction in ghrelin levels would cause a similar effect, since ghrelin accelerates gastric emptying and stimulates eating (Cummings, Frayo, Marmonier, Aubert, & Chapelot, 2004).

Besides increasing satiety and reducing hunger, in this study, treatment with thylakoids also reduced wanting for all categories of palatable snacks: salty, sweet, sweet-and-fat and all snacks together, compared to control. This is a novel finding. In comparison, decreased urge for sweet and sweet-and-fat foods has previously been reported in long-term studies (Montelius et al., 2014; Stenblom et al., 2014), but never in a single dose meal study using within-subject comparisons, which is more sensitive and accurate in measuring appetite scores (Flint et al., 2000). Interestingly, in the three-month study (Montelius et al., 2014), on the first day of treatment, ratings for wanting sweets and wanting chocolate were significantly lower in the treated group compared to control in the afternoon. In contrast, in the present study, treatment suppressed wanting for sweets and snacks already before lunch and this effect lasted throughout the day. The difference between the two studies lies in the experimental setting. In the three-month study, participants stayed in the laboratory for blood sampling between breakfast and lunch. During this time, they did not score high for wanting. In the afternoon however, in their natural environment, the control group scored higher, and the differences in wanting appeared. In the present study, participants went back to their everyday circumstances between breakfast and lunch. Consequently, they were exposed to food cues such as colleagues having coffee and cake at the midmorning break. In comparison, on day 90 of the three-month study, cravings for sweets and chocolate were significantly lower both before lunch and after lunch in the treated group, showing the potentiation of the effect due to the repeated administration of thylakoids on wanting.

The present results demonstrate that treatment with thylakoids have immediate effects on wanting. This may be an effect of altered levels of appetite regulating hormones, since both GLP-1 and ghrelin have been implicated in food reward (Egecioglu et al., 2010; Skibicka, 2013; Skibicka, Hansson, Egecioglu, & Dickson, 2012).

The suggested mechanism, according to previous studies in humans, may be the increased release of GLP-1 following thylakoid supplementation (Montelius et al., 2014). GLP-1 analogs have been shown to decrease the rewarding value of sweet (sucrose) and sweet-and-fat (chocolate) food, decrease food intake and shift food preference from candy to chow in rat (Dickson, Shirazi, Hansson, Bergquist, & Nissbrandt, 2012; Raun et al., 2007).

In the present study, participants who scored higher for emotional eating behavior experienced greater effect of thylakoids on reducing wanting for palatable food, particularly sweet-and-fat foods. Emotional eating behavior is associated with increased consumption of sweet-and-fat foods, especially in response to stressors and negative emotions (Cappelleri et al., 2009; Epel, Lapidus, McEwen, & Brownell, 2001; Keskitalo et al., 2008). Therefore, the participants in this study scoring high for emotional eating behavior were

more susceptible to the effects of thylakoids to reduce the rewarding properties of sweet-and-fat foods. Since the correlation between eating behavior types and BMI is strongest for emotional eating behavior, it is important to target overeating in emotional eaters specifically to avoid weight gain in this group (Karlsson, Persson, Sjöström, & Sullivan, 2000).

Measuring food intake is difficult due to the complex nature of eating behavior (Blundell et al., 2010). Accordingly, despite the increased satiety and decreased hunger and wanting during the day, food intake was not significantly affected in the experimental setting of the present study. There was however a tendency toward decreased intake of salty snacks by treatment, which is a new finding. Measuring food intake alone is not reliable, since measurement of food is constrained by the experimental design. Instead, food intake is best used in conjunction with measurements of motivation to eat (Stubbs et al., 2000). Visual analog scales (VAS) are reliable tools for this assessment (Drapeau et al., 2007; Flint et al., 2000). In the present study, correlation analyses showed that treatment effects on VAS-scores for wanting and liking correlated positively with a reduction in food intake between control and thylakoid conditions. These findings indicate that individuals who experience the greatest reduction of VAS-ratings for wanting and liking also have the greatest reduction in food intake of the corresponding products. Though measurements of consumption of the foods tested did not show statistically significant differences between treated and controls in this single dose study design, the correlation analyses reveals treatment effects of thylakoids that are likely to influence food consumption in repeated dose situations as shown in a previous three-month study with daily supplementation of thylakoids (Montelius et al., 2014). Thus, ratings of wanting may be more accurate in predicting eating behavior in a real-life situation compared to food intake alone. This emphasizes the importance of the reported findings that thylakoids suppress hunger and wanting. However, although we demonstrated a depressed hunger and wanting by thylakoids in this study we cannot assure that this guarantees a decreased food intake.

After the afternoon snack buffet, ratings of liking for sweet food were suppressed in the treated group compared to placebo. Liking is generally considered to be related to opioid signaling (Berridge, 2009). The preference for sweet taste is regulated by endogenous opiates (endorphins), which are also implicated in food cravings and drug reward. Consistently, opiate antagonists have been shown to reduce rated pleasure for sweet (Fantino, Hosotte, & Apfelbaum, 1986; Yoshida et al., 2010). Enterostatin, released during fat digestion, acts like an opioid antagonist in animal models, in particular when the fat digestion is retarded (Berger, Winzell, Mei, & Erlanson-Albertsson, 2004; Ookuma, Barton, York, & Bray, 1997; Takenaka et al., 2008). Consequently, one could argue that thylakoids through its retardation of fat digestion, as previously demonstrated (Albertsson et al., 2007), may cause an increased release of enterostatin and thus induce an opiate-blocking effect, which affects liking for sweet in this study. Leptin has also been demonstrated to stop the liking of sweet food (Yoshida et al., 2010). In a previous publication (Köhnke, Lindbo et al., 2009), there was an increased release of leptin at time point 360 min after intake of breakfast with thylakoids compared to control. Consequently, leptin may also assist in the suppression of liking sweet caused by thylakoids.

Findings on reduced liking are important. Indeed, overweight women have been shown to have an increased liking for sweet (Ettinger, Duizer, & Caldwell, 2012). Furthermore, liking of sweet-ness influences wanting of sweet-tasting products (Drewnowski, Mennella, Johnson, & Bellisle, 2012). Thus, a reduced liking may, over time, cause a reduction in wanting for sweet. As a consequence, this may reduce snacking in between meals and hence overeating. In this study, besides reducing liking for sweet after consumption,

treatment reduced wanting for sweet to a greater extent compared to other kinds of snacks. There was also an earlier onset; wanting for sweet was reduced from 15 minutes following breakfast and onwards, in comparison to wanting for salty and sweet-and-fat snacks, which were reduced from 60 minutes respectively. This implies that treatment with thylakoids has a particular effect on liking and wanting sweet. The mechanism for this targeting is not known. Further studies are needed to understand the effects of thylakoids to suppress the liking for sweet taste, diminish the urge for palatable food and its particular effect in emotional eaters, including measurement of gut hormones.

Conclusion

Supplementation with thylakoids in the morning affects subjective ratings of appetite during the rest of the day. It reduces feelings of hunger and increases feelings of satiety. It also reduces wanting for palatable food, and this effect is enhanced in emotional eaters. Furthermore, the treatment effect on wanting and liking is correlated to reduction in food intake. In addition, liking for sweet is reduced after consumption. We suggest that these effects are due to altered secretion of appetite regulating hormones, induced by the thylakoids, affecting reward-related areas in the brain.

Even though analysis of appetite regulating hormones could have made our study stronger, we deliberately did not take any blood samples in this study. Since laboratory settings have been shown to interfere with thoughts about food and food intake, a natural surrounding was preferred in order to achieve more accurate results on VAS-ratings and food consumption (Blundell et al., 2010). Another possible limitation to this study was the small number of participants even though the cross over design increased the power.

With these limitations in mind, we have shown that treatment with thylakoids attenuate hunger, homeostatic and hedonic, including wanting for palatable food and liking for sweet. Reducing wanting is important, since wanting is a major cause of hedonic eating, which contributes to overconsumption and obesity (Blundell & Gillett, 2001; Koenders & van Strien, 2011; Yeomans et al., 2004). In addition, individuals who are obese and/or dieting are even more susceptible to hedonic hunger (Garber & Lustig, 2011). Therefore, reducing cravings for palatable food is necessary, both to control appetite, prevent weight gain and to facilitate a permanent weight loss. Supplementation by thylakoids may help prevent eating between meals, hence over-eating and in the long run, weight gain.

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