Appetite 81 (2014) 295–304

Contents lists available at ScienceDirect

Appetite

journal homepage: www.elsevier.com/locate/appet

Research report

Body weight loss, reduced urge for palatable food and increased release of GLP-1 through daily supplementation with green-plant membranes for three months in overweight women ☆

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ARTICLE INFO

Article history:
Received 30 April 2014
Received in revised form 22 June 2014
Accepted 25 June 2014
Available online 30 June 2014

Keywords:
Craving
Appetite
Reward
Chlorophyll
Thylakoids
VAS

ABSTRACT

The frequency of obesity has risen dramatically in recent years but only few effective and safe drugs are available. We investigated if green-plant membranes, previously shown to reduce subjective hunger and promote satiety signals, could affect body weight when given long-term. 38 women (40–65 years of age, body mass index 25–33 kg/m^2) were randomized to dietary supplementation with either green-plant membranes (5 g) or placebo, consumed once daily before breakfast for 12 weeks. All individuals were instructed to follow a three-meal paradigm without any snacking between the meals and to increase their physical activity. Body weight change was analysed every third week as was blood glucose and various lipid parameters. On days 1 and 90, following intake of a standardized breakfast, glucose, insulin and glucagon-like peptide 1 (GLP-1) in plasma were measured, as well as subjective ratings of hunger, satiety and urge for different palatable foods, using visual analogue scales. Subjects receiving green-plant membranes lost significantly more body weight than did those on placebo ($p < 0.01$). Mean weight loss with green-plant extract was 5.0 ± 2.3 kg compared to 3.5 ± 2.3 kg in the control group. Consumption of green-plant membranes also reduced total and LDL-cholesterol ($p < 0.01$ and $p < 0.05$ respectively) compared to control. Single-meal tests performed on day 1 and day 90 demonstrated an increased postprandial release of GLP-1 and decreased urge for sweet and chocolate on both occasions in individuals supplemented with green-plant membranes compared to control. Waist circumference, body fat and leptin decreased in both groups over the course of the study, however there were no differences between the groups. In conclusion, addition of green-plant membranes as a dietary supplement once daily induces weight loss, improves obesity-related risk-factors, and reduces the urge for palatable food. The mechanism may reside in the observed increased release of GLP-1.

Introduction

Overweight subjects have an increased liking for palatable food (Blundell & MacDiarmid, 1997; Ettinger, Duizer, & Caldwell, 2012), inducing hyperphagia and the obese state. Whether this is a cause or a consequence of obesity is not known (Berthoud & Zheng, 2012). Gut hormones have been demonstrated to regulate the liking and wanting of sweet and fatty foods, ghrelin acting to stimulate wanting (Egecioglu et al., 2010) and GLP-1 to suppress liking (Shin et al., 2008). A common adaptive response upon weight loss by dieting in the obese is an increased hunger and liking for palatable food, in part due to reduced secretion of satiety hormones, like leptin and GLP-1 (Adam, Jocken, & Westerterp-Plantenga, 2005; Blundell & Gillett, 2001). However, bariatric surgery does not trigger the same response. Instead a diminished preference for sweet and fatty foods is observed along with a reduction in ghrelin secretion and an increase in the secretion of GLP-1 and PYY (Miras et al., 2012). The increase in satiety hormone secretion from the distal small intestine following bariatric surgery is partly explained by food digestive products directly reaching the distal part of the small intestine.

We have found that retardation of fat digestion through reversible pancreatic lipase/colipase inhibition by chlorophyll-containing membranes found in green plants, leads to increased satiety and

☆ *Acknowledgements:* This study was made possible through grants from FORMAS, Swedish Medical Research Council, VR, Royal Physiographic Society and Färs and Frosta Foundation. We are thankful for the donation of "laxpytt" (the potato and salmon dinner dish) from Findus AB. Special thanks also to Cecilia Montelius for assisting in performing the study. CM planned and performed all parts of the study, DE and EV assisted in performing the study and in analysing the blood hormones, EE was responsible for the statistical analyses and writing the report, ELS assisted in planning and performing of the study, analysing the results and in writing the report and CEA was responsible for the study and writing the report. *Conflict of interest:* CEA is a scientific advisor for Greenleaf Medical AB, part owner and board member of Thylabisco AB.

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http://dx.doi.org/10.1016/j.appet.2014.06.101
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Table 1
Anthropometric, body composition and fasting serum parameters at baseline and at the end of the study. A decrease in body weight, BMI, FFM (fat free mass), body fat, % body fat, waist and leptin concentrations was found over the course of the study. Alterations in body weight were dependent on treatment. Fasting glucose and insulin did not change during the study.

	Control group			Thylakoid group		
	Baseline (n = 19)	End of study (n = 17)	Change (n = 17) start → end	Baseline (n = 19)	End of study (n = 19)	Change (n = 19) start → end
Age (yrs)	54.6 ± 7.5			50.7 ± 7.0		
Body weight (kg)	80.2 ± 8.2	76.3 ± 8.9[a,b]	−3.5 ± 2.3	79.9 ± 10.8	75.0 ± 10.5[a,b]	−5.0 ± 2.3
BMI (kg/m^2)	28.6 ± 2.3	27.6 ± 2.7[a]	−0.7 ± 0.6	28.9 ± 2.2	27.1 ± 2.2[a]	−1.0 ± 0.5
FFM (kg)	47.6 ± 3.2	46.8 ± 3.3[a]	−0.6 ± 1.1	47.6 ± 4.9	46.3 ± 4.7[a]	−1.3 ± 1.4
Body fat (kg)	32.6 ± 6.2	29.5 ± 6.8[a]	−2.9 ± 1.9	32,3 ± 6,8	28.7 ± 7.0[a]	−3.7 ± 2.5
Body fat (%)	40.4 ± 4.1	38.2 ± 4.9[a]	−2.4 ± 2.1	40.1 ± 4.0	37.8 ± 4.8[a]	−2.3 ± 2.3
Waist (cm)	97.6 ± 9.6	93.3 ± 8.6[a]	−4.4 ± 3.5	93.9 ± 6.7	88.0 ± 7.2[a]	−5.9 ± 3.5
Insulin (mIE/L)	10.6 ± 5.0	9.1 ± 4.5	−0.2 ± 3.4	8.4 ± 4.0	8.3 ± 3.5	−0.1 ± 3.4
Glucose (mmol/L)	5.30 ± 0.50	5.24 ± 0.47	−0.08 ± 0.26	5.34 ± 0.69	5.1 ± 0.56	−0.24 ± 0.46
Leptin (ng/mL)	44.0 ± 22.6	32.3 ± 22.1[a]	−10.5 ± 12.2	42.9 ± 17.7	29.2 ± 17.0[a]	−13.7 ± 17.2

[a] Effect of time, $p < 0.001$, two-way RM ANOVA.
[b] Time and treatment interaction, $p < 0.05$, two-way RM ANOVA.

an elevated release of the gut satiety hormone CCK in response to a high fat diet (Albertsson et al., 2007; Kohnke et al., 2009). In man, the uptake of fatty acids into the circulation is retarded (Kohnke et al., 2009), supporting a delay of fatty acid absorption. In addition to increased satiety following intake of a meal high in fat (Kohnke et al., 2009), green-plant membranes reduce hunger after a carbohydrate-rich meal, with a mechanism suggesting a release of incretin hormones (Stenblom et al., 2013). Long-term studies in rat and mouse have demonstrated that daily consumption of green-plant membranes reduced body weight gain, body fat mass and blood lipid levels (Kohnke et al., 2009) (Montelius et al., 2013). The effects of long-term treatment with green-plant membranes in humans are unknown.

The chlorophyll-containing parts of the green plant cell, called thylakoids, contain a hundred different membrane proteins, galactolipids and sulpholipids as well as various vitamins (A, E and K) and antioxidants like carotenoids, lutein, zeaxantin and chlorophyll. They are thus a mixture of bioactive compounds that could be responsible for the retardation of fat digestion previously described (Albertsson et al., 2007). Most importantly, following ingestion of the green-plant membranes, fat digestion is prolonged, but nevertheless complete, and in the end the thylakoids themselves are also digested. Therefore, no steatorrea or rapid excretion of fat is seen following intake of green-plant membranes, in contrast to the effects of irreversible lipase inhibitors (Goedecke, Barsdorf, Beglinger, Levitt, & Lambert, 2003).

In this study we were interested to find out if long-term treatment with green-plant membranes through its satiating effects could affect body weight and metabolic parameters related to obesity. We were also interested in measuring the release of GLP-1, a gut hormone promoting satiety (Flint, Raben, Astrup, & Holst, 1998; Holst, 2007). Based on the fact that GLP-1 regulates reward-induced behaviour for food (Dickson et al., 2012; Egecioglu, Engel, & Jerlhag, 2013a, 2013b; Egecioglu et al., 2013), the urge for sweet, salt and fat was evaluated during one day meal tests in the beginning and the end of the study.

Materials and methods

Subjects

Fifty-three healthy non-smoking women, aged 40–65 years, with a BMI between 25 and 33 were recruited through public advertisement and volunteered for screening, after which 38 women were enrolled in the study (detailed flow chart, Supplementary Fig. S1). The exclusion criteria were diabetes, food allergies, irritable bowel syndrome, food intolerance and recent use of antibiotics. The subjects were not vegetarian and had not followed any diet for the last three months. Baseline characteristics of the 38 women are shown in Table 1.

Experimental study design

The study was conducted at the Overweight and Diabetes Unit and at the Division of Occupational and Environmental Medicine, Skåne University Hospital (SUS), Lund, Sweden, and designed as a single-blinded, single-centered, randomized and placebo-controlled, 12-week diet intervention study. The participants were divided into two groups (n = 19 per group) by a non-algorithmic randomization method (ballot, performed by CM). Normal distribution within and between the groups based on body weight, BMI, blood glucose, insulin, triacylglycerol (TG) and cholesterol (total and LDL) was confirmed after randomization.

Every third week the participants arrived to the laboratory in the morning for anthropometric measurements and blood sampling in the fasted state. Waist circumference was measured with a non-stretchable tape, and body weight, fat-mass and fat free mass (FFM) were measured with a body composition analyser (TANITA-BC 418 MA, Amsterdam, The Netherlands). Fasting blood samples were taken through a venous catheter in the arm.

For meal studies the subjects arrived to the laboratory in the morning in the fasted state on day 1 and day 90 of the supplementation period. After filling in questionnaires, a venous catheter was inserted in the arm for blood sampling. Thereafter, the subjects received a 50 g blueberry drink with or without 5 g of green-plant membranes. Five minutes later, a standardized breakfast was served consisting of vanilla yoghurt, muesli, bread, butter, cheese, juice and coffee or tea (Table 2). The macronutrient composition of the breakfast was 60 E% carbohydrate, 28 E% fat and 12 E% protein. The subjects were instructed to eat all the food within 15 minutes.

Venous blood samples for glucose, insulin, ghrelin and GLP-1 analyses were taken at time point zero, ie prior to breakfast, and thereafter at 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes. Blood samples were collected in chilled 6 mL Vacutainer EDTA-plasma tubes for all analyses, with the addition of 100 μL DPP-IV inhibitor (Cat # DPP4-010; Millipore Corp. Billerica, MA, USA) for GLP-1 analysis. The tubes were centrifuged at 4 °C, and plasma was immediately stored at −80 °C until analysis.

At time point 360 minutes lunch was served consisting of a pizza (Grandiosa Extra Godfather, Procordia AB, Eslöv, Sweden), water and coffee/tea. The subjects were told to eat and drink until satisfied. Thereafter the subjects left the laboratory. At time point 660 minutes

Table 2
Composition of the breakfast.

Ingredients	Amount	Caloric content
Vanilla yoghurt, 2.5% fat	150 g	121,5 kcal
Muesli with tropical fruits	1 dl = 45 g	185 kcal
White bread	1 piece = 40 g	98 kcal
Butter	1 tsp	33 kcal
Cheese, 28% fat	2 pieces = 20 g	77 kcal
Red bell pepper	2 pieces = 20 g	6 kcal
Orange juice	2 dl	86 kcal
Coffee/tea (with 1 tbsp milk 0.5% fat if preferred)	1 cup	~10 kcal
Blueberry drink (± green-plant membrane supplement)	50 g	45 kcal
Total		660 kcal

the participants consumed a third meal, consisting of salmon and potatoes (Laxpytt, Findus AB, Eslöv, Sweden).

Green-plant membranes

The green-plant membranes (thylakoids) used in the present study was provided by Greenleaf Medical AB, Stockholm, Sweden, prepared from baby spinach leaves using the pH-method, as described (Emek et al., 2010), followed by drum drying. 100 g of green-plant membranes contain 23.5 g protein, 11.9 g fat, 41.7 g carbohydrate, 3.5 g salt, 3000 mg chlorophyll, 27.9 mg lutein, 730 ug zeaxantin, 4 760 ug betakaroten, 21 ug vitamin A, 1313 ug vitamin K, 6.07 mg vitamin E and 166 ug folic acid.

The green-plant membranes were mixed with 2.8 g rapeseed oil (Zeta, Di Luca & Di Luca AB, Stockholm, Sweden) and 50 g of blueberry soup (Ekströms original, Procordia Food AB, Eslöv, Sweden) and given to the participants from day 1. The control group received 2.8 g rapeseed oil mixed with 50 g blueberry soup. The blueberry drinks with and without the green-plant membranes contained 209 kJ/50 kcal versus 188 kJ/45 kcal respectively. The blueberry drinks were taken before breakfast every day.

Diet regimen

In the period between the test-days (days 1 and 90) the participants were told, besides taking the daily blueberry drink with or without green-plant membranes, to consume three meals a day containing a large quantity of vegetables and fruit, and to avoid sweet drinks and snacks. They were also told to exercise at low intensity 30 minutes each day.

Questionnaires

Questionnaires constructed as Visual Analogue Scale (VAS) (Flint, Raben, Blundell, & Astrup, 2000) were used to measure sensations of hunger, fullness and urge for specific food items during the whole day on the first and last days of the study. The questionnaires were filled in at time points 0, 15, 60, 120, 180, 240, 300 and 360 minutes, when lunch was served, and thereafter at time points 420, 480, 540, 600 and 660 min. The VAS-questionnaires included pictures, to facilitate the evaluation of the urge for specific food items. For high carbohydrate snack a picture of a sandwich was presented; for salt a picture of potato chips; for sweet a picture of candy and for fat and sweet; a picture of chocolate was presented. Written instructions were given on the front page of the questionnaire, and each subject was individually instructed in how to fill out the questionnaire to avoid misinterpretation. Questions were followed by a 100 mm line anchored by descriptors on each side of the line. Subjects were instructed to place a vertical line across the scale, thus rating how strong their sensations were at every time point. Ratings were scored as mm between "not at all" and the individual subjects mark.

Biochemical analyses

Glucose, insulin, TG, cholesterol (total, LDL and HDL) in blood were analysed by standard methods at the Department of Clinical Chemistry at Skåne University Hospital (Lund, Sweden). Leptin was measured with a RIA human/multi species kit using the double antibody/PEG technique (XL-85K, Millipore Corporation, Billerica, MA, USA). Active GLP-1 was measured using EGLP-35K (Millipore, Molsheim, France) and for measures of total ghrelin, EZGRT-89K (Millipore, Molsheim, France) was used.

Ethics

The Ethics Committee in Lund, Sweden approved the study (2006/361). The trial was conducted in accordance with the Declaration of Helsinki. All subjects gave written and oral consent before the study began.

Statistics

Participants were included in each analysis by original assigned groups. All statistical analyses were done using the Prism version 6, statistical software (GraphPad Software, Inc, San Diego, CA, USA). Anthropometric measures and fasting leptin, insulin, glucose and cholesterol concentrations over the course of the study were analysed by two-way repeated measures (RM) ANOVA. Differences at individual time points and changes from baseline at the end of the study were analysed with unpaired t-test. Area under the curve (AUC), time to peak value, increase peak value versus baseline (%) and baseline values for glucose, insulin, ghrelin and GLP-1 during days 1 and 90 were analysed by unpaired t-test or Mann–Whitney U test.

The variations in VAS ratings over time were analysed with a two-way RM ANOVA in order to test time, treatment and time × treatment interaction for the first and the last day respectively. Differences in VAS at individual time points were analysed with unpaired t-tests.

In figures and text data are expressed as mean ± SE or for peak times as median followed by interquartile range. Statistics are based on Mann–Whitney U test if not otherwise stated. p-values <0.05 were considered statistically significant.

Results

All 38 participants are included in the statistical analysis of subjective appetite and hormonal release on day 1. One participant did not follow the dietary and exercise recommendations given and one individual moved abroad during the study and were therefore not included in the statistical analysis at day 90 or in the analysis of longitudinal changes.

Body weight, anthropometric and plasma parameters

Supplementation with green-plant membranes for three months produced an increased weight loss compared to control (Fig. 1). For absolute body weight, an interaction between time and treatment was found over the course of the study ($F(4, 136) = 2.94$, $p < 0.05$, two-way RM ANOVA, Table 1). The ANOVA analysis of weight loss revealed an effect of treatment ($F(1, 34) = 4.18$, $p < 0.05$, two-way RM ANOVA) and an interaction between time and treatment ($F(4, 136) = 2.94$, $p < 0.05$, two-way RM ANOVA, Fig. 1). The weight loss in the treated group was increased at weeks 6, 9 and 12 (Fig. 1).

Anthropometric measures, FFM, body fat and serum concentration of fasting leptin decreased in both groups over the course of

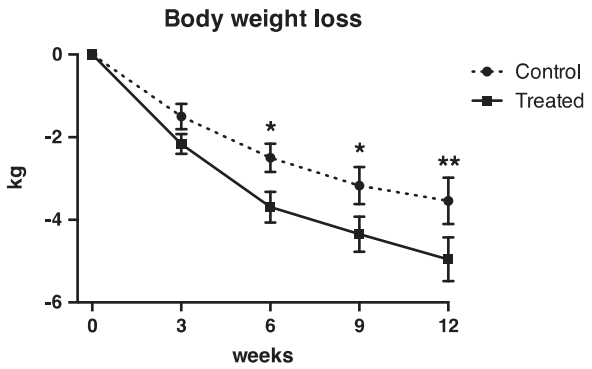

Fig. 1. Body weight loss with and without green-plant membrane supplementation. Dietary green-plant membranes produced a continuous body weight loss that was more marked compared to control at the end of the study. Values are Means ± SE. * $p < 0.05$, ** $p < 0.01$, two-way RM ANOVA followed by unpaired t-test.

the study while fasting glucose and insulin were unaltered (Table 1). No differences between the groups were found (Table 1).

Green-plant membrane supplementation decreased total and LDL-cholesterol compared to control (Fig. 2). An effect of treatment and an interaction between time and treatment were found in total cholesterol ($F(1, 34) = 5.07$, $p < 0.05$ and $F(4, 136) = 3.83$, $p < 0.01$, respectively). Analysis of individual time points revealed that total cholesterol was decreased at 3, 6, 9 and 12 weeks in the thylakoid group compared to control (Fig. 2A). For LDL-cholesterol an effect of treatment was found in the ANOVA analysis ($F(1, 34) = 4.62$, $p < 0.05$) and the levels were decreased at time points 3, 6, 9 and 12 weeks in the treated group (Fig. 2B). HDL-cholesterol and triglycerides were not affected over time or by treatment (Fig. 2C and D).

Glucose, insulin, ghrelin and GLP-1 on day one

In both experimental groups blood glucose increased after breakfast consumption and reached maximal concentration at median time point 15 (15–30) min for control and 30 (15–30) min for the treated group ($p = 0.30$). The glucose concentration was lower at time point 15 min in the treated group compared to control (Fig. 3A).

Plasma insulin increased following breakfast and reached maximum concentrations at median time point 45 (30–45) min for control and 45 (30–45) min for the treated group ($p = 0.33$). Insulin concentrations were lower at time point 15 min in the treated group compared to controls (Fig. 3B).

GLP-1 concentrations increased following breakfast for both experimental groups, reaching a first peak at median time 15 (15.0–33.8) min in the control group and 30 (15.0–30.0) min for the treated group ($p = 0.082$) (Fig. 4A). A second GLP-1 peak occurred at 90 (90.0–120.0) min for both groups respectively ($p = 0.80$). The % maximum increase versus baseline was 2.6 fold higher for the first peak and 1.8 fold higher for the second peak in the treated group compared to control (Fig. 4D). No differences in GLP-1 concentrations were found at baseline, in absolute peak value or at any specific time point between the treated and the control groups (Fig. 4A and C).

Ghrelin concentrations decreased following breakfast in both experimental groups (Fig. 4B). Thereafter both curves reverted back to fasting values at time point 360 min. There was no difference in ghrelin concentrations at any time point (Fig. 4B).

Glucose, insulin, ghrelin and GLP-1 on day 90

Glucose concentrations following breakfast on day 90 were similar in the control group and in the treated group (Fig. 5A). Maximum

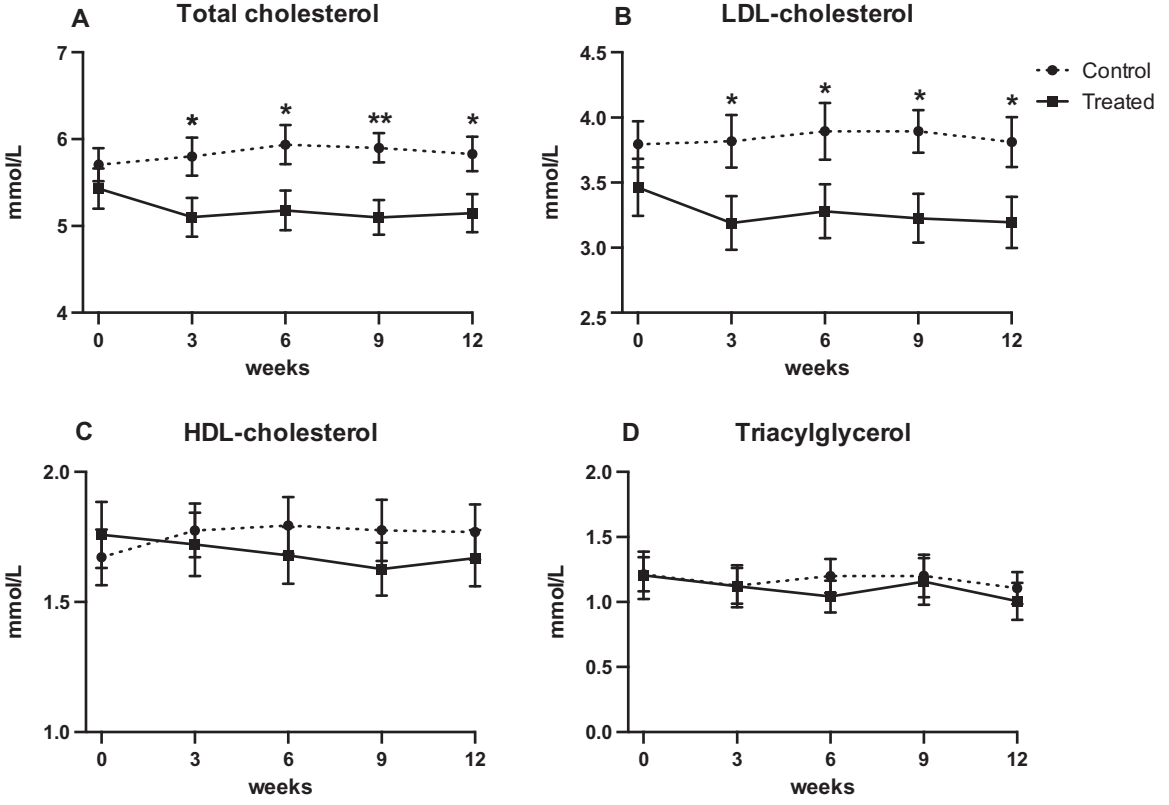

Fig. 2. Blood lipid parameters with and without supplementation with green-plant membranes. Green-plant membranes decreased total and LDL-cholesterol while HDL-cholesterol and TG were unaltered. Values are Means ± SE. * $p < 0.05$, ** $p < 0.01$, two-way RM ANOVA followed by unpaired t-tests.

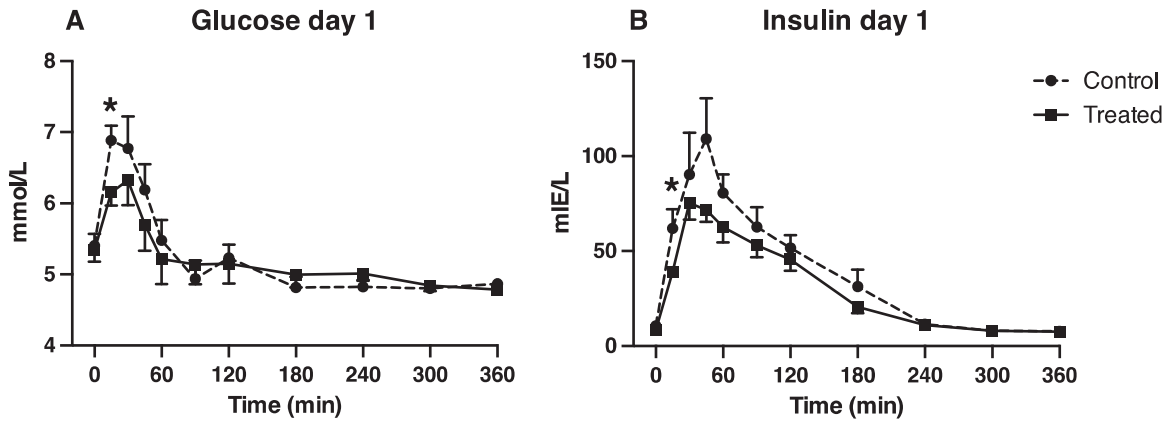

Fig. 3. Postprandial glucose and insulin day 1. Blood glucose (A) and plasma insulin (B) in response to breakfast served with or without green-plant membranes on the first day of the intervention study. Values are Means ± SE. * $p < 0.05$, unpaired t-tests.

concentrations were reached at median time point 15 (15–30) min in the control group and at 30 (15–30) min in the treated group ($p = 0.16$).

Insulin concentrations were increased in a similar way in the two experimental groups after breakfast on the last day (Fig. 5B). No difference in the time to reach maximal concentrations was found between the groups (control: 30, (30–45) min, treated: 30 (30–45) min, $p = 0.69$).

GLP-1 was released after breakfast on day 90 in both experimental groups (Fig. 6A). The concentrations of GLP-1 were similar at baseline and increased at time points 30, 45 and 60 min in the treated group compared to control as was the AUC_{0-60} (control: 393.1; 290.1–595.1, treated: 565.2; 448.37–747.61, $p = 0.039$). Furthermore, the maximum peak value for the initial peak was elevated in the treated group compared to control while no difference in the absolute values for the second peak was seen ($p = 0.14$, Fig. 6C). The initial GLP-1 peak was reached at median time point 15 (15–30) min in the control group and at 30 (15–33.75) min in the treated group ($p = 0.10$). The second GLP-1 peak occurred at median time point 120 (90–120) min for the control group and 90 (60–120) min

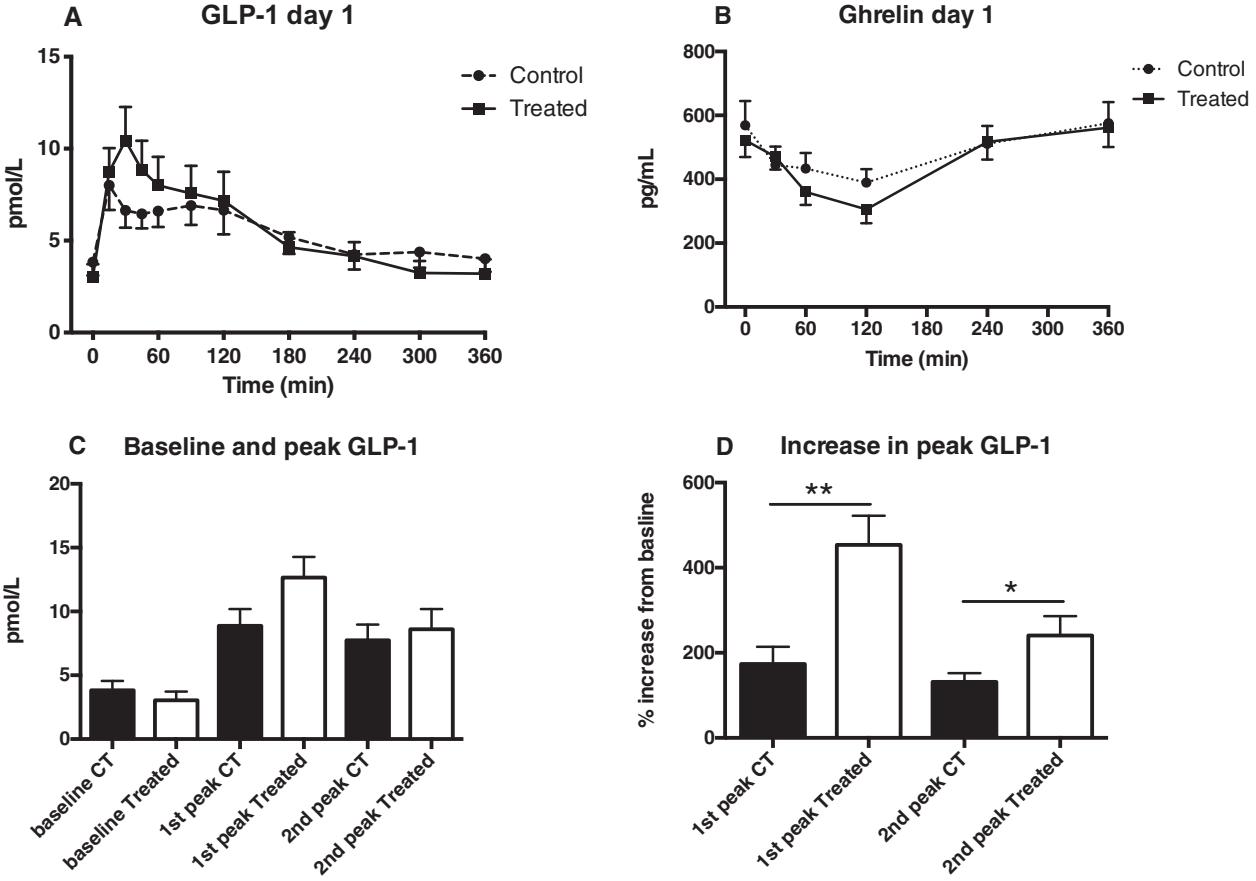

Fig. 4. Postprandial GLP-1 and ghrelin day 1. Plasma concentrations of GLP-1 (A), ghrelin (B), baseline and peak values of GLP-1 (C), percentage increase in peak values of GLP-1 (D) on the first day of the study. Values are Means ± SE. * $p < 0.05$, ** $p < 0.01$, Mann–Whitney U test.

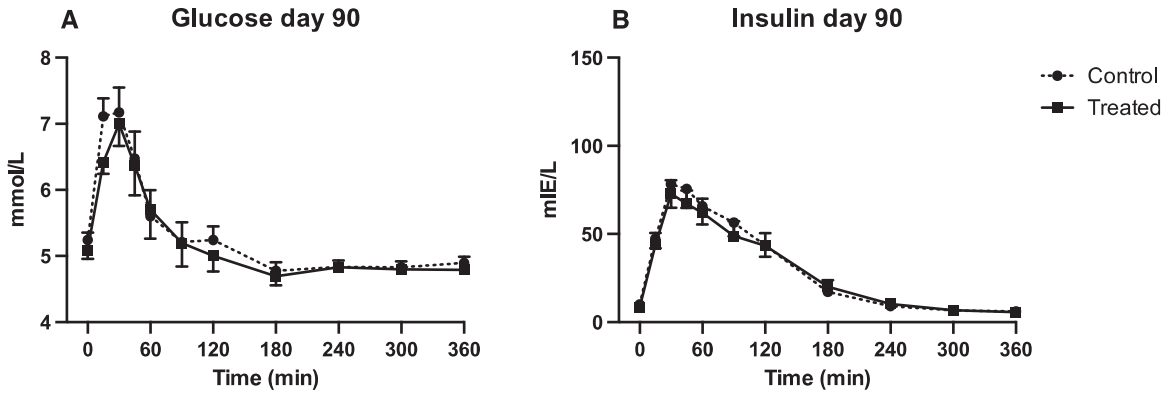

Fig. 5. Postprandial glucose and insulin day 90. Blood glucose (A) and plasma insulin (B) concentrations following breakfast on day 90. Values are Means ± SE.

in the treated group ($p = 0.28$). The % maximum difference versus baseline was not different in the treated group on day 90 compared to control (Fig. 6D).

Ghrelin concentrations were suppressed following breakfast in both the treated group and the control group (Fig. 6B). Thereafter the ghrelin concentrations rose to original values at time point 240 min. There was no difference in response between the two groups (Fig. 6B).

Sensation of hunger, satiety, urge for specific foods and food intake on day 1

Subjective ratings for hunger, satiety and urge for specific food items on day one are presented in Fig. 7. All VAS-ratings were influenced by time ($p < 0.001$ respectively, two-way RM ANOVA) such that hunger and urge for specific food items were increased before mealtime and to a varied extent decreased following meals

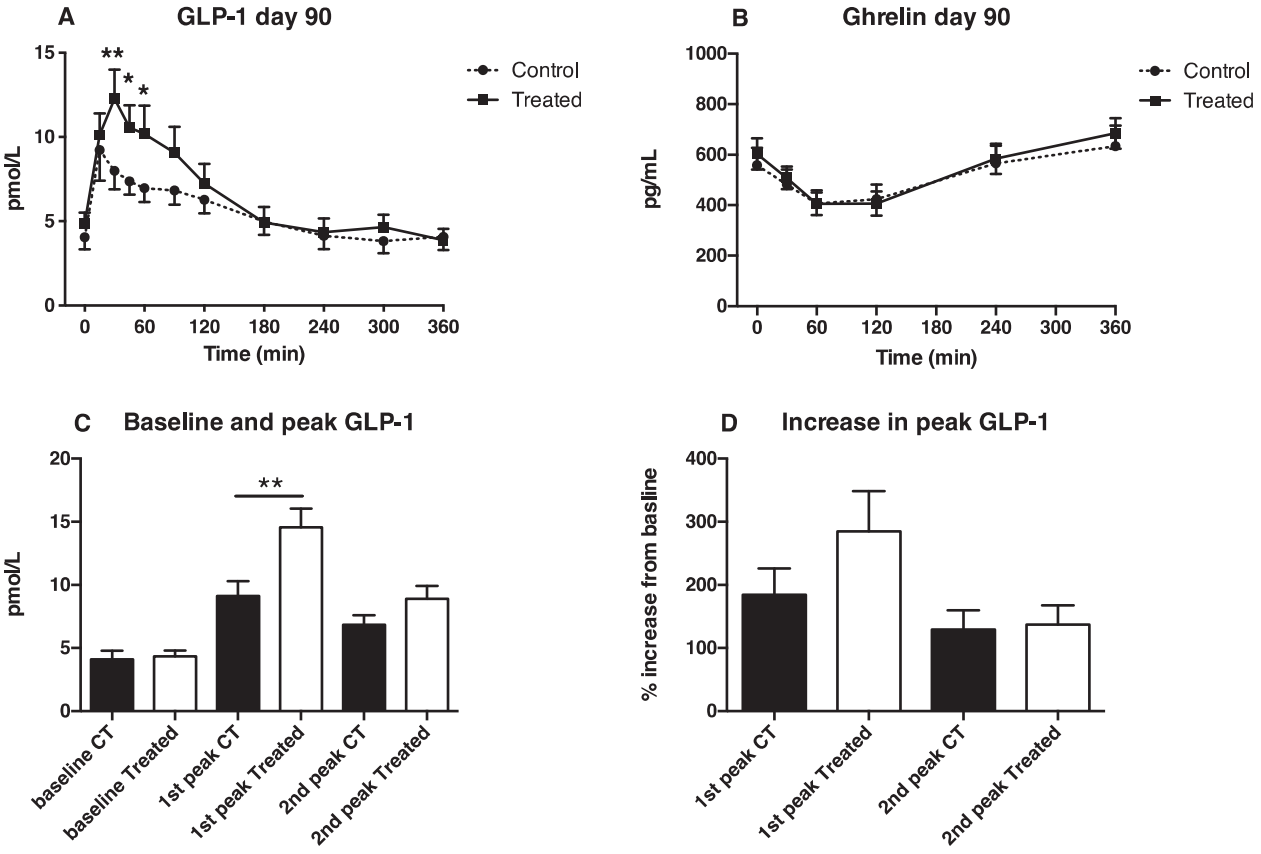

Fig. 6. Postprandial GLP-1 and ghrelin day 90. Plasma concentrations of GLP-1 (A), ghrelin (B), baseline and peak values of GLP-1 (C) and percentage increase in peak values of GLP-1 (D) in response to a breakfast on the last day of the intervention study in overweight women. Values are Mean ± SE. * $p < 0.05$, ** $p < 0.01$, Mann–Whitney U test.

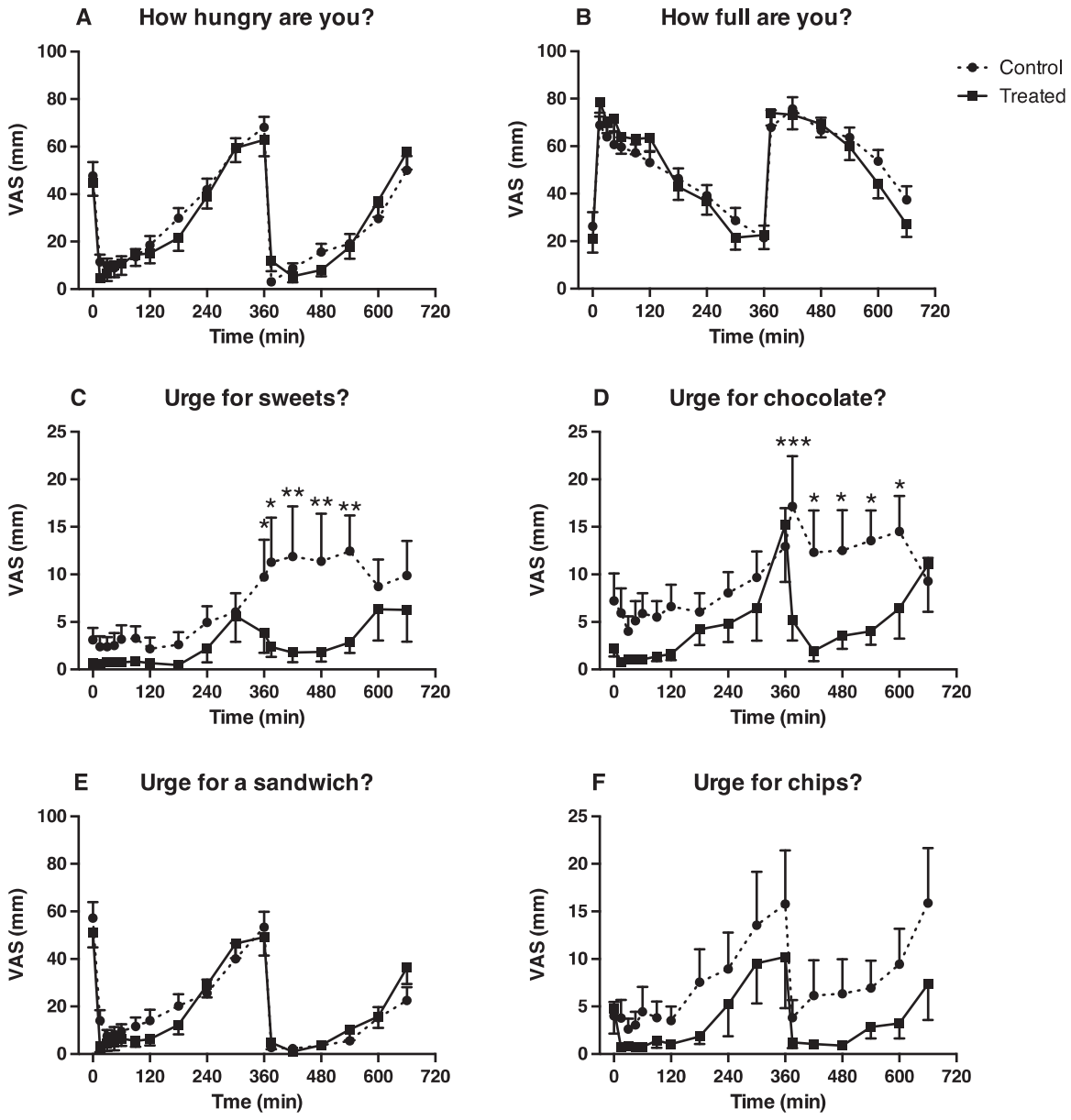

Fig. 7. Hunger, satiety and urge for palatable food day 1. VAS ratings of hunger (A), satiety (B) and urge for sweets (C), chocolate (D), sandwich (E) and chips (F) during day 1. Values are given as Mean ± SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, two-way RM ANOVA followed by unpaired t-tests.

while ratings of satiety demonstrated the opposite curve (Fig. 7A and B). There was also an effect of treatment on the ratings for the urge for sweet and chocolate (sweet; $F(1, 36) = 5.79$, $p < 0.05$ and chocolate; $F(1, 36) = 5{,}892$, $p < 0.05$, two-way RM ANOVA, Fig. 7C and D). The urge for sweet was lower in the treated group at the time point prior to lunch and remained lower for the following 3 hours compared to control. Ratings for chocolate were lower immediately following lunch in the treated group and remained lower in the following 3 hours compared to the controls. In contrast, the ratings of hunger, satiety, urge for a high carbohydrate snack or salt were not different between the treated and the control groups (Fig. 7A, B, E and F). No time by treatment interaction was found in any of the VAS ratings on day 1. Total caloric intake during day 1, excluding the standardized breakfast, was similar between the groups (control: 1366 ± 48 kcal, green-plant membranes: 1270 ± 65 kcal, $p = 0.24$, unpaired t-test).

Sensation of hunger, satiety and urge for specific foods on day 90

Subjective ratings for hunger, satiety and urge for specific food items on day 90 are presented in Fig. 8. An effect in ratings over time was found for all VAS measures ($p < 0.001$ respectively, two-way RM ANOVA). Similar to day one there was an effect of treatment on the ratings for the urge for chocolate ($F(1, 34) = 6.2$, $p < 0.01$, two-way RM ANOVA, Fig. 8D). Furthermore, a time by treatment interaction was found for both the urge for sweet ($F(16, 544) = 1.88$, $p = 0.05$, two-way RM ANOVA) and for the urge for chocolate ($F(16, 544) = 1.91$, $p < 0.05$, two-way RM ANOVA). The urge for sweet and fat/sweet (chocolate) was lower in the treated group prior to lunch on day 90 and remained low in the treated group for the rest of the duration of the session compared to control. The ratings of the urge for a high carbohydrate snack or salt were not different between the treated and the control groups (Fig. 8E and F). Caloric intake

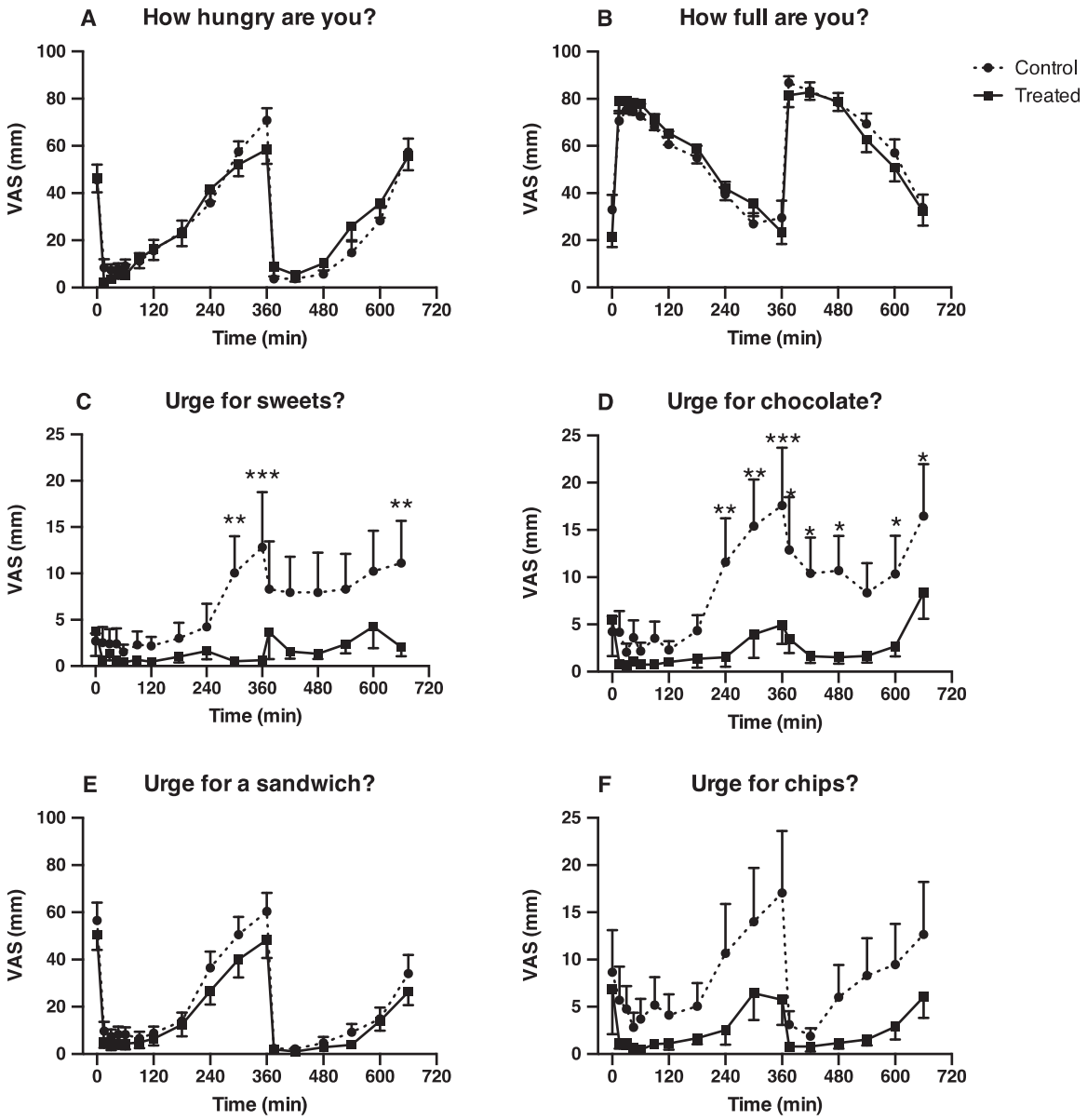

Fig. 8. Hunger, satiety and urge for palatable food day 90. VAS ratings of hunger (A), satiety (B) and urge for sweets (C), chocolate (D), sandwich (E) and chips (F) during the last day of the intervention study. Values are given as Mean ± SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, two-way RM ANOVA followed by unpaired t-tests.

during day 90, excluding the standardized breakfast, was similar between the groups (control: 1241 ± 55 kcal, green-plant membrane: 1297 ± 104 kcal, $p = 0.65$, unpaired t-test).

No adverse events or effects were reported in any of the groups.

Discussion

In this study we demonstrated for the first time that green-plant membranes, when added to the diet daily for three months, markedly reduced body weight in overweight women. In addition, total and LDL-cholesterol levels were reduced compared to control. These findings are supported by animal studies, where body weight loss and blood lipid lowering effects have been observed (Kohnke et al., 2009; Montelius et al., 2013).

The mechanism underlying the effect of green-plant membranes to reduce body weight was investigated by two test-meals, one at the start and one at the end of the study. These demonstrated a reduced urge for sweet and fatty foods. The decreased urge following treatment was apparent already on day 1, as well as on day 90, indicating both an immediate and a sustained effect of green-plant membranes. We also observed an increased release in GLP-1 postprandially both on day 1 and day 90, suggesting that green-plant membranes influence pathways that are important for satiety and for the determination of the reward value of food. Furthermore, given that diet-induced weight loss has been shown to reduce postprandial GLP-1 levels (Jarry et al., 2006), it is remarkable that the increased secretion of GLP-1 in the treated group was sustained during weight loss in the present study.

The effect of green-plant membranes on body weight loss (6.3%) after 12 weeks was comparable to the effect of treatment with the GLP-1 analogue liraglutide (Astrup et al., 2009). Thus increasing the endogenous release of GLP-1 may be as efficient for obesity treatment as pharmacological agents. It is very likely however that treatment with green-plant membranes involves other additional mechanisms for body weight reduction.

Palatability and pleasantness are important determinants of what and how much we eat (Erlanson-Albertsson, 2005). Increased intake of energy-dense food high in fat and sugar adds energy but also disrupts functions in the brain controlling appetite and motivation (Berthoud, 2012). In the control group the urge for palatable food increased prior to lunch, was reduced following lunch and thereafter reverted. With consumption of green-plant membranes a decreased urge for palatable food was noted prior to lunch, which persisted following lunch despite no further consumption of green-plant membranes. The mechanism may be related to the increased release of GLP-1.

Release of GLP-1 from enteroendocrine L-cells in the distal intestine occurs through nutrients present in the lumen of the intestine and is dependent on the nutrient composition. The most potent secretagogues for GLP-1 are carbohydrate and fat. The release of GLP-1 following administration of pure glucose results in one peak, occurring at around 15 min after oral glucose load (Steinert et al., 2011). Following consumption of a mixed diet with carbohydrate, fat and protein the release of GLP-1 may be twofold, one early peak at time point 15 minutes and one later peak, at 90 minutes. Rapid release of GLP-1 occurs through neuronal activation and/or endocrine factors of the proximal-distal loop of the intestine and the second peak through direct interaction of nutrients with the enteroendocrine L-cells in the distal ileum.

In the present study the peak concentrations of initial GLP-1 release occurred at time point 30 min in the treated group and at 15 min in the control group, suggesting that the green-plant membranes may interfere with factors that stimulate rapid GLP-1 release. The second peak of GLP-1 was also increased on day 1 in the treated group. A possible mechanism for these effects of the green-plant membranes to increase GLP-1 secretion could be related to CCK. Ingestion of green-plant membranes has been found to release CCK, demonstrated both in rodents and in man (Kohnke et al., 2009) and the importance of CCK for mediating GLP-1 release is supported by studies where blockade of CCK-receptors markedly reduced fatty acid-stimulated GLP-1 secretion (Beglinger et al., 2010). Furthermore, the green-plant membranes retard fat digestion without causing an irreversible lipase inhibition (Albertsson et al., 2007). This is important, since hydrolysis of fat is required for the GLP-1 releasing effects of fat and free fatty acid infusion directly into the intestinal lumen cause a substantial release of GLP-1 (Beglinger et al., 2010). In contrast, orlistat, that irreversibly inhibits fat digestion, leads to a lower release of GLP-1 and CCK (Beglinger & Degen, 2004; Ellrichmann et al., 2008). The mechanism for the green-plant membrane-induced GLP-1 release could thus be a direct luminal effect of fatty acids on enteroendocrine cells via prolonged digestion and absorption of dietary fat and/or an indirect effect mediated through neurohumoral signals such as CCK. In rodents green-plant membranes have been demonstrated to act in a pre-biotic way, changing the intestinal microflora. The release of GLP-1 by these bacterial products via short chain fatty acids (Yadav, Lee, Lloyd, Walter, & Rane, 2013) may be an additional explanation for the sustained effect of green-plant membranes over time.

Various mechanisms influence reward in relation to food, among these are gut appetite hormones, such as ghrelin (Abizaid et al., 2006; Egecioglu et al., 2010; Jerlhag et al., 2006; Merkestein et al., 2012; Skibicka, Hansson, Egecioglu, & Dickson, 2012) and GLP-1 (Alhadeff, Rupprecht, & Hayes, 2012; Dickson et al., 2012). Analogues of GLP-1 have been shown to decrease preference and consumption for palatable foods in both humans and rodents (Inoue et al., 2011; Raun et al., 2007; Zhang et al., 2013). These studies have been extended to demonstrate that GLP-1 analogues suppress sucrose-induced reward and motivation in rats (Dickson et al., 2012). Furthermore, blockade of GLP-1 receptor signalling diminishes food reward-related behaviour in rats indicating that endogenous GLP-1 signalling is involved in the regulation of food reward (Alhadeff et al., 2012). The effect of green-plant membranes to reduce the urge for sweet and fat may thus be linked to the elevation of GLP-1 concentrations and/or alterations of peak GLP-1 levels. Ghrelin is known to stimulate reward-related behaviour and food preference (Egecioglu et al., 2010; Merkestein et al., 2012). Green-plant membranes have previously been demonstrated to decrease ghrelin concentrations in humans and pigs (Kohnke et al., 2009; Montelius et al., 2013) indicating that ghrelin signalling may also be involved in the regulation of urge for palatable food by green-plant membranes. However, in this study we did not find any effects on ghrelin secretion by the green-plant membranes.

Another general mechanism important for reward seeking, particularly related to urge for sweet, is glucose and insulin homeostasis, a lowering of blood glucose that leads to search for sweet food (Figlewicz & Benoit, 2009). In this study we found no pronounced effects on glucose or insulin by the green-plant membranes suggesting that insulin and glucose are not central for the treatment-induced effects on the urge for palatable foods.

The urge for palatable food tends to increase over the course of the day and hence any influence on these urges would be more pronounced in the afternoon. Accordingly, inhibition of the urge for palatable food by green-plant membranes was more evident following lunch in the present study. This long lasting reduction could be due to the relative resistance to hydrolysis of green-plant membranes in the intestine (Emek et al., 2011), thereby influencing hormonal release also following lunch. The possible prolonged effect of green-plant membranes to alter gut hormone secretion after a second meal needs to be further elucidated.

The mechanism for the reduction in blood cholesterol is not known, but may involve an increased production of bile salt, needed for the prolonged intestinal fat digestion and fat absorption (Borgstrom & Erlanson, 1978). The LDL-cholesterol lowering effect of green-plant membranes is in the magnitude of the bile acid sequestering therapeutics (Rosenson & Underberg, 2013).

In conclusion, we demonstrate that consumption of chlorophyll-containing parts of green plants, in overweight patients results in significant weight reduction, and reduction in blood cholesterol together with a decreased urge for palatable food. The mechanism suggests an increased meal-related GLP-1 release that sustained during the intervention period. Green-plant membranes may thus be a new agent for control of appetite and body weight.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.endend.2013.05.004.