



## Decreased Urge for Palatable Food after a Two-month Dietary Intervention with Green-plant Membranes in Overweight Women

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### Abstract

**Background/Aim:** The present study investigates the effect of daily green-plant membrane (thylakoid) supplementation for two months on body weight, body composition, metabolic profile and rating of appetite sensations in overweight women on a restricted diet.

**Methods:** 26 women, Body Mass Index (BMI)  $27.5 \pm 1.9$ , randomized into a thylakoid (n=12) and control group (n=14), followed a 7500 kJ/day diet with or without 5.6 g of thylakoids supplemented in a blueberry drink, and exercised 60 minutes per day. Fasting blood samples were taken with two weeks interval. On the first and last day of the study subjects answered Visual Analogue Scale (VAS) questions regarding hunger and cravings.

**Results:** Both control and thylakoid-treated groups lost body weight and body fat over the course of the study, but no differences were found between the groups. Thylakoid supplementation resulted in decreased hunger ( $p=0.016$ ) and decreased urge for chocolate ( $p=0.052$ ) in contrast to the control group. Leptin levels were significantly reduced at the end of the study in the thylakoid-treated group ( $p=0.012$ ) compared to control, suggesting a decreased fat mass. The overall metabolic profile was also improved in the treated group compared to controls, based on body weight, waist and hip-circumference, trunk and total body fat, p-leptin, p-LDL, p-ApoB1, p-total cholesterol, p-TAG, blood glucose, p-HbA1C and p-insulin ( $p=0.024$ ).

**Conclusions:** Thylakoids added to food in adjunct to lifestyle intervention may be helpful in enabling overweight subjects to lose weight by suppression of hedonic hunger.

**Keywords:** Obesity; Hedonic; VAS; Hunger; Leptin; Palatable food; Thylakoids

### Abbreviations:

ApoB1: Apolipoprotein B1; BMI: Body Mass Index; CCK: Cholecystokinin; E%: Energy Percentage; GLP-1: Glucagon-like Peptide 1; LDL: Low Density Lipoprotein; tAUC: Total Area under the Curve; TAG: Triacylglycerol; VAS: Visual Analogue Scale

### Introduction

An increasing proportion of food consumption in affluent societies is driven by pleasure. This type of hunger has been described as hedonic hunger as opposed to homeostatic hunger caused by energy deficiency [1]. The increased incidence of overweight and obesity since 1970 [2] is suggested to be caused by hedonic eating, i.e. the consumption of food items such as sweets, sweet drinks, chocolate, chips and pizza [3].

One reason for overeating is the inability of palatable food to promote appetite control [4]. Appetite control occurs through the release of various gut hormones [5] including the hunger hormone ghrelin, and the satiety promoting hormones Cholecystokinin (CCK) and Glucagon-like Peptide 1 (GLP-1) [6].

Previously, we have shown that green-plant membranes, thylakoids, have a hunger suppressing, as well as a satiety promoting, effect. In one single-meal study, general hunger was decreased three hours following intake of thylakoids with breakfast [7]. A sustained suppression of hedonic hunger for the whole day has also been shown following intake of thylakoids with breakfast [8]. Suppression of general and hedonic hunger was related to the release of the satiety hormones CCK [7] and GLP-1 [8]. Furthermore, suppression of ghrelin levels in humans has previously also been demonstrated following intake of thylakoids [9]. Thus thylakoids affect hunger and the release of three hormones important for appetite control; ghrelin, CCK and GLP-1.

In a previous study, thylakoids were shown to decrease body weight in human following daily treatment for three months [8]. These effects were achieved without any caloric restriction. However, most weight loss programs are based on a caloric restriction. Hence, we were interested to find out whether treatment with thylakoids would augment body weight loss during a caloric restriction commonly used in weight loss studies [10].

In this study, a daily supplementation of thylakoids for two months in overweight women was used, together with a caloric restriction of 15 Energy % (E%). In addition to following body weight and metabolic

parameters we also studied subjective ratings of hunger, satiety and cravings for palatable food.

## Methods and Procedures

### Subjects

Forty-eight middle-aged (40-65 years) non-smoking overweight women who were not, currently or recently, on a diet were recruited for screening through advertisement in the local newspaper. Exclusion criteria were diabetes, inflammatory bowel disease, thyroid disease, food allergies and a Body Mass Index (BMI) over 33. Thirty individuals were enrolled as participants in the study. All of the 30 enrolled participants finished the study. Compliance was measured through diary entries, interviews and the daily use of pedometers. Four participants (one from the control group and three from the thylakoid group) were excluded from the data analysis due to non-compliance with the diet and exercise regime. Baseline characteristics of the 26 included subjects are listed in Table 1. One participant in the control group was not included in the analysis of subjective ratings of hunger, satiety and urges for specific food items due to non-attendance.

	Control group (n=14)	Thylakoid group (n=12)	p-values for differences in characteristics baseline
Age (years)	53.0 ± 5.9	51.6 ± 5.2	p=0.54
Weight (kg)	76.6 ± 5.3	73.1 ± 7.4	p=0.24
BMI (kg/m <sup>2</sup> )	27.7 ± 2.2	27.4 ± 1.7	p=0.65
Waist circumference (cm)	88.9 ± 6.6	85.7 ± 8.3	p=0.28
Hip circumference (cm)	103.9 ± 3.9	103.3 ± 4.5	p=0.70
Waist/Hip ratio	0.86 ± 0.07	0.82 ± 0.06	p=0.16

**Table 1:** Baseline characteristics of the 26 women included in the study (average ± SD). There were no differences between the groups (t-test).

Procedures, objectives and requirements of the study were explained in detail to the participants, and written consents were signed both before screening and before the study started. The study was approved by the Ethical Committee of Lund University, and conducted in accordance with the declaration of Helsinki. After completing the study, all participants received a compensation of 2000 Swedish Crowns (SEK) (taxable).

### Experimental study design

The study was conducted at the Overweight and Diabetes Unit, Skånes University Hospital (SUS), Lund, Sweden, and designed as a single-blinded, randomised, diet and exercise intervention study with duration of two months. The participants were randomised into two groups to ensure normal distribution within and between the groups based on body weight, BMI, blood glucose, insulin, Triacylglycerides (TAG) and cholesterol (total and LDL). One of the groups received supplementation by thylakoids in a blueberry drink every day, while the other group served as control, receiving a daily blueberry drink without thylakoids.

Every second week, at the same time in the morning, the participants visited the clinic for measurements of body weight, body composition, waist- and hip circumferences and for blood sampling. To optimise conditions for all measurements, the participants were instructed to have a standardised dinner in the evening before each test day and to abstain from further intake of foods or liquid after 8.00 pm.

The participants had a total of five individually scheduled appointments during the study. On the first and the last day (day 1 and 56), following the anthropometric measurements and blood sampling, an isocaloric breakfast (2114 kJ/505 kcal) consisting of the blueberry drink with or without thylakoids, yoghurt with apple, breakfast cereal, nuts and coffee or tea was served (Table 2). After 240 minutes an isocaloric take-away lunch (2593 kJ/630 kcal) consisting of a frozen thai-curry meal, bean salad and a banana was administered. VAS questionnaires measuring subjective parameters of hunger, satiety and urge for specific foods were filled out at given time points throughout the day on both day 1 and 56.

### Thylakoids

The thylakoids used in the present study were prepared from baby spinach leaves using the pH-method, as described [11]. The thylakoid-slurry was dried to obtain a thylakoid powder, prepared by Swepharm AB (Södra Sandby, Sweden). 100 g thylakoids consists of 41.1 g protein, 14.5 g fat, 36.8 g carbohydrate, 3.5 g salt as well as pigments such as 3640 mg chlorophyll, 28 mg lutein, 730 ug zeaxantin, 4760 mg betakaroten, 21 ug vitamin A, 1330 ug vitamin K, 6.07 mg vitamin E and 166 ug folic acid.

The thylakoid group received 5.6 g of thylakoid powder 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). The control group received 2.8 g rapeseed oil mixed with 50 g blueberry soup. The blueberry drinks with and without thylakoids contained 209 kJ/50 kcal versus 188 kJ/45 kcal respectively. The drinks were taken before breakfast every day.

### Caloric restriction and diet recommendations

Before the study started, average daily energy requirement for the participants was calculated to 8800 kJ (~2100 kcal), with respect to age, weight, height and presumed daily energy-consumption, according to Harris Benedict equation using Dietist XP (Kostdata, Bromma, Sweden). During the study the calculated energy intake was reduced by 15 E% to ~7500 kJ/day (~1800 kcal/day). The participants were provided with a collection of selected recipes (3 breakfasts, 29 lunches/dinners and 4 desserts) to choose from. The recommended energy intake per day during the study was divided into three meals/day: 2100 kJ (~500 kcal) for breakfast; and 2500 kJ (~600 kcal) for lunch and dinner respectively. An additional 400 kJ (~100 kcal) was allowed for milk in coffee/tea and individual adjustments. Water, coffee and tea were allowed between meals, but no additional foods or snacks. The diet did not allow any sweetened drinks.

Subjects were also instructed to accomplish 60 minutes of low/medium intensity exercise each day, such as power walking, swimming, basic aerobics etc.

Each day, the participants answered questions regarding their choice of meals, health-status and exercise in a diary. These data were used to analyse the compliance with the study guidelines.

Meal components	Amount (g)	Calories (kJ/kcal)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)
<b>Breakfast</b>						
Natural yoghurt	300	753/180	10.2	9.0	14.4	0.0
Muesli wt. fruits and nuts	45	775/185	2.9	4.9	30.4	3.4
Apple (Granny Smith)	60	139/34	0.2	0.1	7.4	1.1
Almonds	10	255/61	2.0	5.2	1.3	0.7
Blueberry shot, wt or wth thylakoids	50	192/45	2.7	3.4	5.5	0.5
Total intake	465	2114/505	18.0	22.6	59.1	5.7
<b>Lunch</b>						
Red curry chicken (frozen product)	380	1558/380	15.2	13.3	45.6	1.9
Bean salad (ready product)	100	591/144	6.0	5.0	18.0	Not stated on product
Banana	105	444/106	1.0	0.5	23.1	1.8

**Table 2:** Contents of breakfast and lunch served on first and last days of the study.

### Somatic analyses

Body weight was measured with a digital scale (TANITA WB-100A, class III, Amsterdam, The Netherlands). The composition of body fat mass (kg) and trunk fat (kg) were measured with a Bioelectric Impedance Analyser (TANITA-BC 418 MA). Waist circumference, midway between the lower rib margin and the iliac crest, and hip circumference were measured to the nearest 0.5 cm by using a non-stretchable tape measure.

### Biochemical analyses

Fasting blood samples were taken through a venous catheter in the arm. Blood glucose was measured directly using HemoCue Glucose 201 (HemoCue AB, Ängelholm, Sweden). Plasma (p) insulin, p-HbA1c, p-TAG, p-cholesterol (total and LDL) and p-Apo B1 were analysed by standard methods at the Department of Clinical Chemistry at Skåne University Hospital (Lund, Sweden). P-Leptin was measured with a RIA human/multi species kit using the double antibody/PEG technique (XL-85K, Millipore Corporation, Billerica, MA, USA).

### Questionnaires

Questionnaires constructed as VAS [12] were used to measure sensations of hunger, fullness and urge for specific food items. Pictures assisted the evaluation of the urge for specific food items. For high carbohydrate snack pictures of a sandwich and a sweet cinnamon bun were presented, for salt and fat; pictures of potato chips and salted peanuts, for sweet snack; pictures of candy and a popsicle; and for fat and sweet; pictures of cake and chocolate were presented. First (day 1) and last day (day 56) of the study, subjects answered questions before breakfast (0 min) and at time points 15, 60, 120, 180, 240 (before lunch was served), 270 (after lunch was served), 330, 390, 450 and 630 minutes. 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 (Table 3). 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.

Questions	Anchor: 0 mm	Anchor: 100 mm
How hungry are you right now?	Not hungry at all	Extremely hungry
How full are you right now?	Extremely full	Not at all full
How much would you like to have a sandwich or a cinnamon bun right now?	Not at all	Extremely much
How much would you like to have salted peanuts or chips right now?	Not at all	Extremely much
How much would you like to have sweets/candy right now?	Not at all	Extremely much
How much would you like to have cake or chocolate right now?	Not at all	Extremely much

**Table 3:** Questions in the VAS-questionnaires asked during the first and last days of the study.

### Statistics

Power calculations were based on previous pilot-studies examining thylakoid supplementation in humans with respect to changes in blood-glucose. With a sensitivity of 0.80 and a significance level of 0.05, the power calculations indicated a sample size of 14 in each group, when the clinical difference was set to 0.6 and the within-subject standard deviation of 0.56. Statistical data analyses of all blood samples and body measurements were done using R Development Core Team, version 2.15.3, 2011 (R Foundation for Statistical Computing, Vienna, Austria). The analysis was performed in two steps. First, the simple regression model was fitted for each subject and for each of the 13 measured variables by taking time as explanatory variable (5 time points) and the measured variable as the response

variable. The obtained slope values represented fitted rate of change for a particular individual and variable. Second, multivariate analysis was performed on the so obtained rates of change, using two-sample Hotelling's T2-test with all 13 variables treated together. Deviations from the normality assumption were examined and were determined not to be severe. Additionally, for interpretation purposes, the mean difference in slope variables between the two groups were analysed with the t-test for a univariate two-sample problem for each of the 13 measured variables. The obtained p-values from these individual variable comparisons should be treated with caution due to the effect of multiple testing and thus are only used to discuss and interpret which of the variables contribute most to the significant difference between the groups.

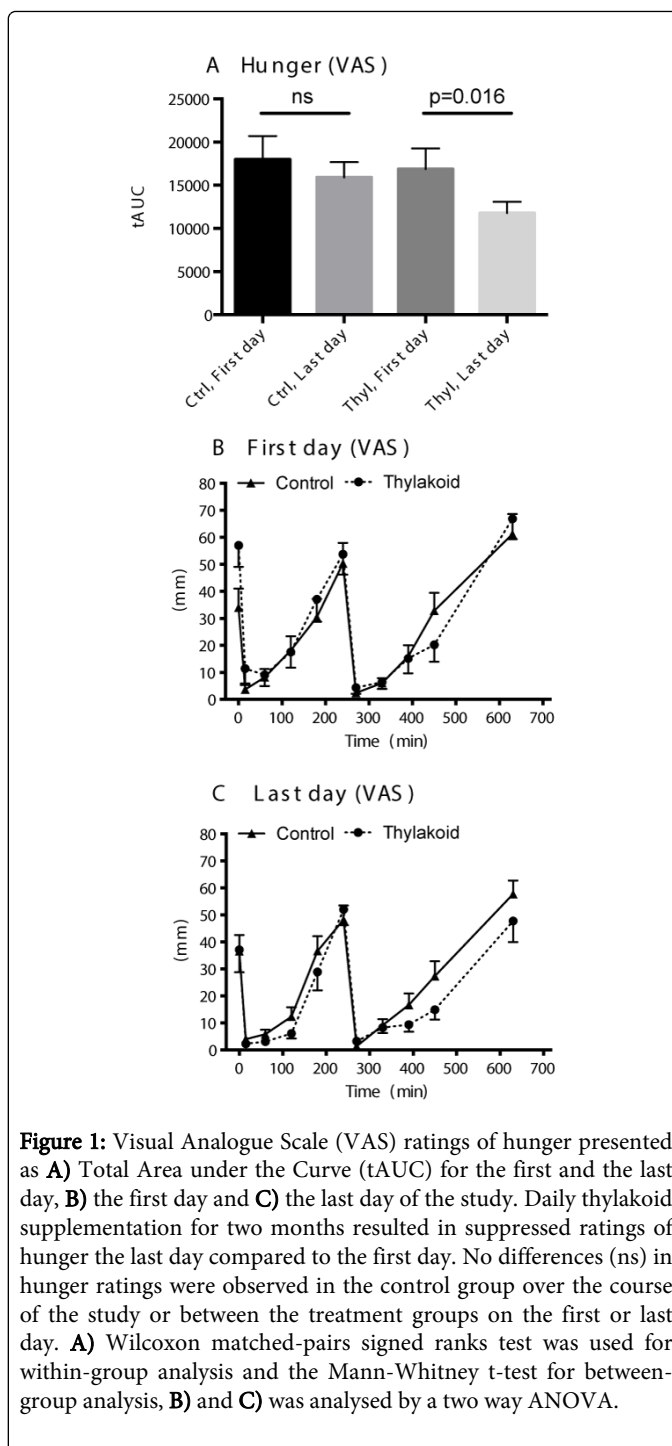
Statistical analyses of the VAS questionnaires were done using Prism, version 6 (GraphPad Software, Inc, San Diego, CA, USA). On the first and last days respectively, the variations in ratings during the day were analysed with a two-way repeated measures ANOVA with treatment and time as fixed factors. Individual time points were further analysed with a multiple comparison test followed by Fisher's LSD test. Numerical calculations of Total Area under the Curve (tAUC) were analysed with Wilcoxon matched-pairs signed ranks test to compare the difference between first and last days of the study within the groups. The Mann-Whitney t-test was also used to compare differences between the groups.

Objective data exhibited normal distribution for most variables and some deviations from normal assumption for certain variables (HbA1c, Apo B and body weight in the thylakoid group), but not critical for the result of the analysis. The latter was assessed by a resampling study. In the figures, data are expressed as mean  $\pm$  SEM, while in the tables data are given as mean  $\pm$  SD. P-values  $<0.05$  were considered to be statistically significant, and p-values  $<0.1$  to be of interest.

## Results

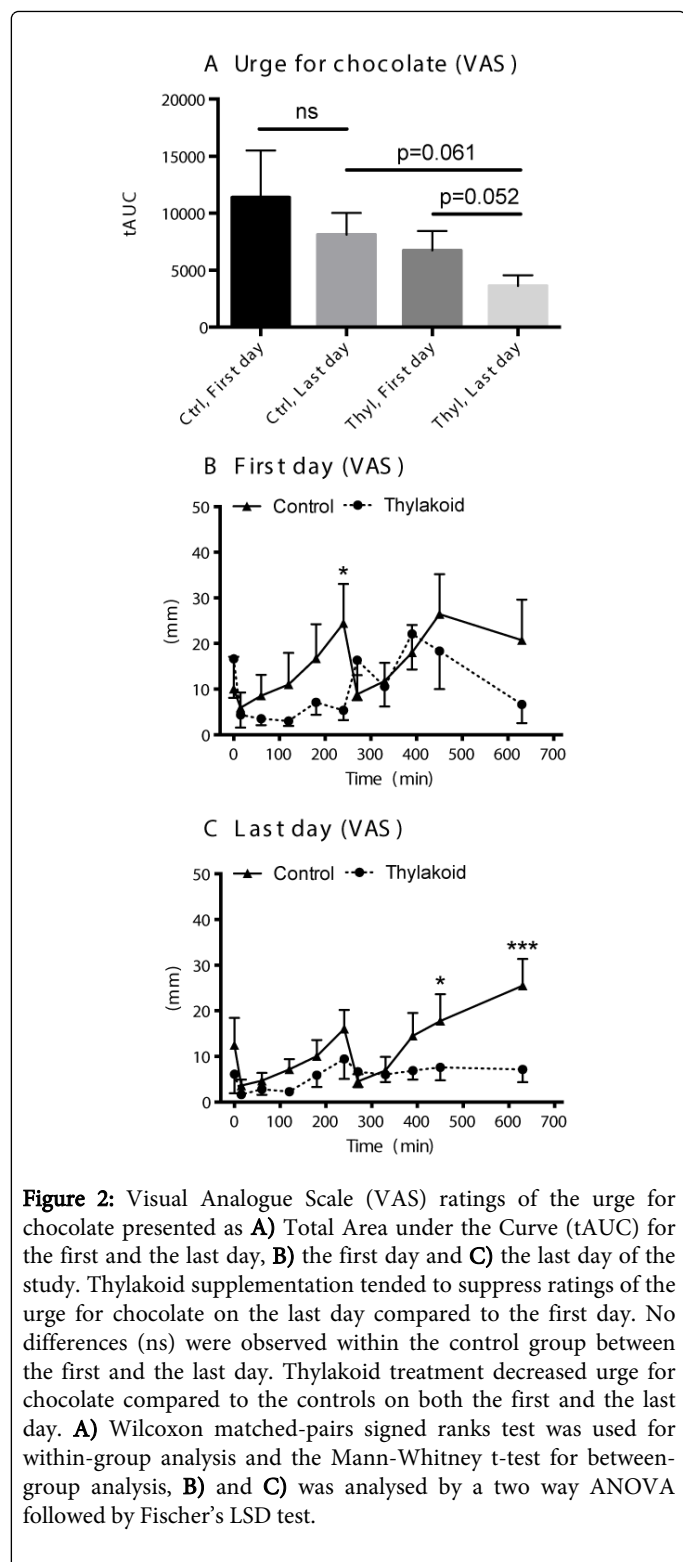
After two months of restricted diet all participants lost body weight and decreased their total body fat with no significant difference between the control and thylakoid treated groups (Table 4).

Analysis of subjective ratings of hunger, using VAS questionnaires, revealed a decreased sensation of hunger within the thylakoid group at the end of the study compared to the first day ( $p=0.016$ , Figure 1A), whereas no change of hunger sensation was found in the control group (Figure 1A). No differences in hunger sensations were found between the thylakoid group and control on the first day ( $F(10,240)=1.6$ , ns) or on the last day of the study ( $F(10,210)=0.83$ , ns) (Figure 1B and 1C).



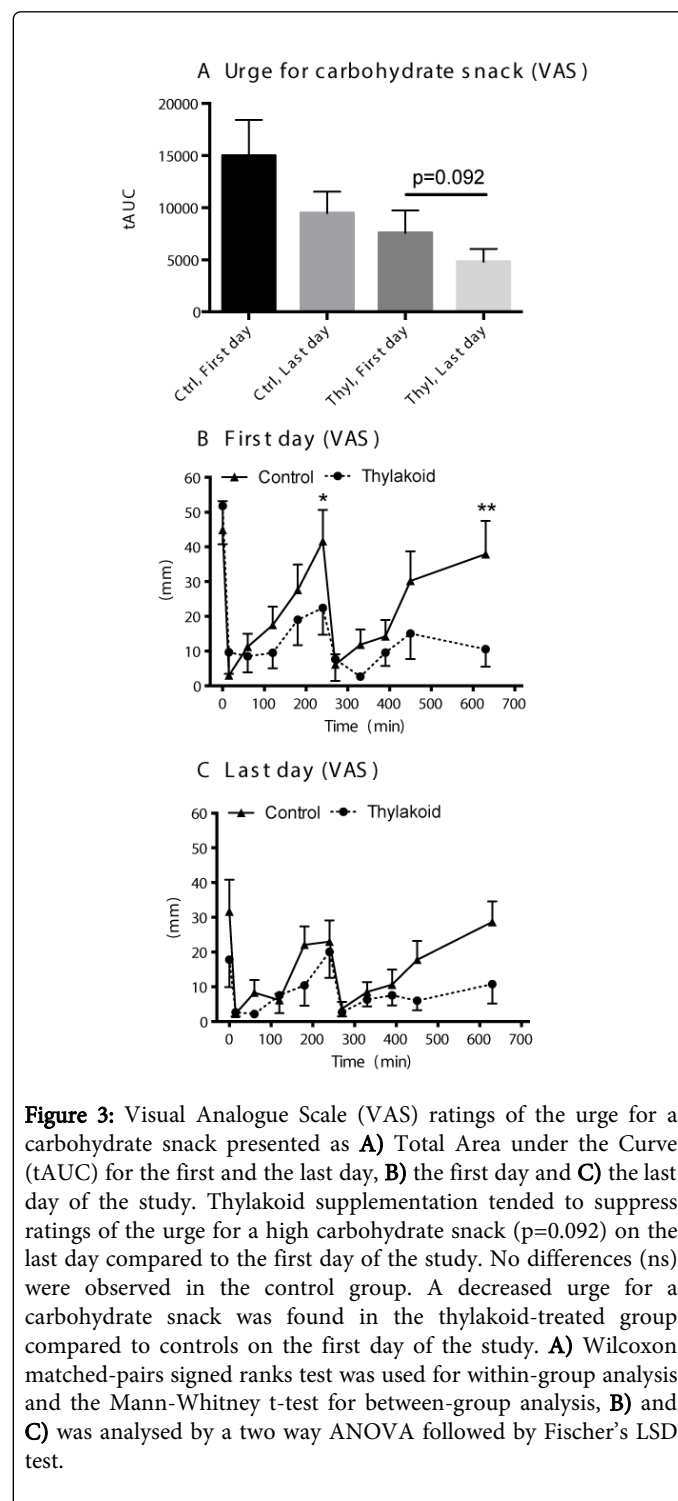
**Figure 1:** Visual Analogue Scale (VAS) ratings of hunger presented as **A**) Total Area under the Curve (tAUC) for the first and the last day, **B**) the first day and **C**) the last day of the study. Daily thylakoid supplementation for two months resulted in suppressed ratings of hunger the last day compared to the first day. No differences (ns) in hunger ratings were observed in the control group over the course of the study or between the treatment groups on the first or last day. **A**) Wilcoxon matched-pairs signed ranks test was used for within-group analysis and the Mann-Whitney t-test for between-group analysis, **B**) and **C**) was analysed by a two way ANOVA.

A strong tendency for a reduction in the urge for chocolate within the thylakoid group was observed at the end of the study compared to the first day ( $p=0.052$ ) but not in the control group ( $p=0.62$ , Figure 2A). The ANOVA analysis of the urge for chocolate between treated and control on the first day and the last day respectively revealed a significant interaction between time and treatment first day; ( $F(10,240)=1.9$ ,  $p<0.05$ ), last day ( $F(10,230)=1.9$ ,  $p<0.05$ ). Analysis of individual time points showed a decreased urge for chocolate in the treatment group prior to lunch on the first day and in the afternoon on the last day (Figure 2B and 2C).



The urge for a carbohydrate snack was not significantly altered over the course of the study in any of the groups (Figure 3A). There was however an interaction between time and treatment in the urge for a carbohydrate snack on the first day of treatment ( $F(10,240)=2.1$ ,  $p<0.05$ ), but not on the last day ( $F(10,230)=1.3$ , ns, Figure 3C). The

urge for a carbohydrate snack was decreased prior to lunch and in the afternoon on the first day (Figure 3B).

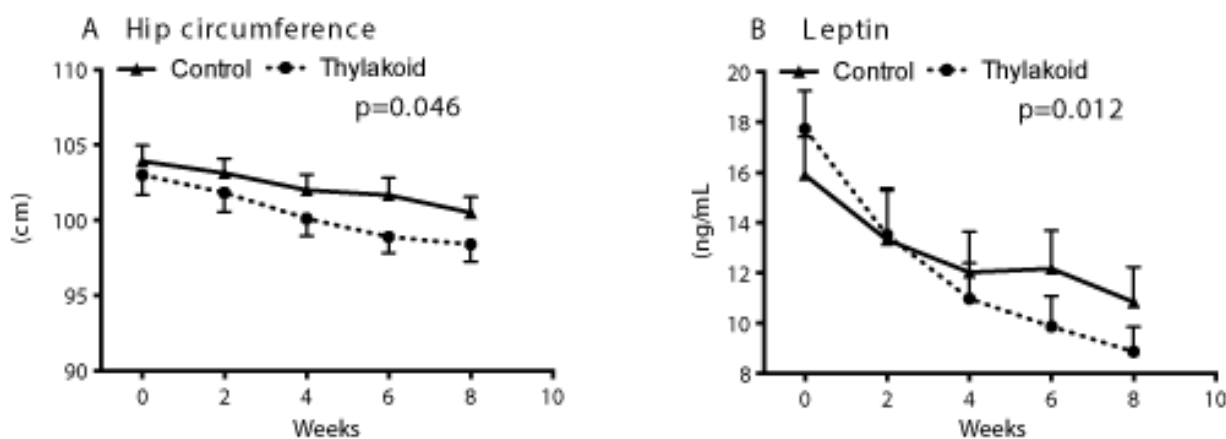


Regarding metabolic parameters, there was a significant difference between the control and treatment groups, when comparing the whole series of weekly changes, using Hotelling's two-sample T2-test (Table 4,  $p=0.024$ ,  $T2=3.28$ ). When analysed individually, a significant difference between control and treatment groups was found in p-



leptin ( $p=0.012$ ) (Figure 4B) and hip circumference ( $p=0.046$ ) (Figure 4A).

There were no differences in baseline values between thylakoid and control groups. No side effects of the thylakoid supplementation were reported.



**Figure 4:** Hip circumference and plasma concentrations of leptin decreased following treatment with thylakoids compared to controls. Weekly change in hip circumference and leptin were analysed using a Univariate t-test.

	Control group (n=14)			Thylakoid group (n=12)			Difference, measured in change per week, between groups
	First day	Last day	Change per week	First day	Last day	Change per week	
Bodyweight (kg)	76.59 ± 5.27	71.72 ± 5.50	-0.59 ± 0.13	73.07 ± 7.49	67.79 ± 8.06	-0.65 ± 0.27	$p=0.448$
Total body fat (kg)	27.83 ± 3.37	23.80 ± 3.50	-0.39 ± 0.22	26.43 ± 5.18	21.85 ± 4.90	-0.50 ± 0.17	$p=0.197$
Trunk fat (kg)	14.20 ± 2.06	11.99 ± 1.89	-0.26 ± 0.13	13.48 ± 3.13	10.89 ± 2.90	-0.32 ± 0.09	$p=0.250$
Waist circumference (cm)	88.89 ± 6.58	82.57 ± 6.67	-0.79 ± 0.28	84.50 ± 7.31	77.93 ± 8.61	-0.84 ± 0.47	$p=0.726$
Hip circumference (cm)	103.9 ± 3.89	100.5 ± 3.96	-0.42 ± 0.22	103.0 ± 4.55	98.40 ± 3.94	-0.61 ± 0.24	$p=0.046$
p-Leptin (ng/mL)	15.9 ± 5.67	10.85 ± 5.23	-0.56 ± 0.43	17.74 ± 5.25	8.88 ± 3.39	-1.07 ± 0.52	$p=0.012$
p-LDL-cholesterol (mmol/L)	3.18 ± 0.50	3.05 ± 0.56	-0.02 ± 0.04	3.73 ± 1.06	3.14 ± 1.06	-0.07 ± 0.08	$p=0.049$
p-Apo B1 (mmol/L)	0.94 ± 0.14	0.94 ± 0.17	0.00 ± 0.01	1.01 ± 0.31	0.95 ± 0.28	-0.01 ± 0.02	$p=0.036$
p-tot-Cholesterol (mmol/L)	5.23 ± 0.61	5.06 ± 0.67	-0.03 ± 0.04	5.83 ± 1.23	5.16 ± 1.19	-0.08 ± 0.10	$p=0.106$
p-TAG (mmol/L)	1.06 ± 0.37	0.88 ± 0.24	-0.02 ± 0.03	0.99 ± 0.45	0.71 ± 0.23	-0.03 ± 0.03	$p=0.613$
p-Insulin (mIE/L)	7.43 ± 3.57	6.29 ± 2.43	-0.17 ± 0.27	8.42 ± 2.47	7.01 ± 2.07	-0.18 ± 0.35	$p=0.925$
Blood glucose (mmol/L)	5.33 ± 0.47	5.74 ± 0.55	0.04 ± 0.06	5.50 ± 0.66	5.31 ± 0.41	-0.02 ± 0.06	$p=0.023$
p-HbA1c (mmol/L)	36.79 ± 2.91	37.29 ± 3.84	0.06 ± 0.23	35.83 ± 3.56	35.67 ± 3.31	-0.2 ± 0.33	$p=0.462$
<b>Hotelling's multivariate T2-test, for all variables: thylakoid vs control groups</b>							$p=0.024$

**Table 4:** First and last day values (average ± SD), and changes per week (± SD) calculated by regression analysis, for the control and thylakoid groups. Statistical differences between thylakoid and control groups are done for changes per week with the univariate t-test, and for the total multivariate analysis for all parameters examined by Hotellings T2-test.

## Discussion

Daily supplementation of thylakoids for two months in combination with a restricted diet resulted in a body weight loss of

similar magnitude in the thylakoid-treated and the control groups. The overall decrease in body weight was 0.65 kg/week in the thylakoid group and 0.59 kg/week in the control group. Feelings of hunger and urge for chocolate were reduced by thylakoid treatment over the

course of the study. In contrast, they were not reduced in the control group. Furthermore, the urge for chocolate and a carbohydrate snack was decreased in the thylakoid treated group compared to controls on the first day of the study and for chocolate also on the last day.

Even though the body weight reduction was similar between the two groups, it would appear that this level of weight loss was reached with less effort in the thylakoid-treated group compared to the control group, based on the reduced subjective ratings of hunger and urge for palatable food in the thylakoid group. This suggests that thylakoids exert their appetite controlling effect even during caloric restriction and that this effect is sustained following weight loss. This is an important property of thylakoid treatment, since hunger is common upon weight loss and often leads to overeating and body weight regain [13].

In the present study, the overweight women treated with thylakoids had a larger decline of p-leptin compared to control, indicating that loss of fat tissue was increased by thylakoid treatment. Low leptin values following body weight and body fat reduction is a natural consequence, which often leads to hunger and overeating, since leptin is a postprandial satiety signal. Low leptin levels are said to constitute an important drive for eating; thus explaining the rapid weight gain that often follows a weight loss program [13]. However, thylakoids appear to have the ability to counteract the drive for hunger associated with low leptin levels.

In addition to the effects of thylakoids on single parameters (Table 4), there was an overall improved metabolic profile in the thylakoid-treated group, based on measures of body weight, trunk and total body fat, waist and hip circumference, p-leptin, p-LDL-cholesterol, p-Apo B1, p-total cholesterol, p-TAG, b-glucose, p-insulin and p-HbA1c, compared to control. Further studies are needed to substantiate these effects, in specific patient cohorts with diabetes and/or dyslipidaemia.

A reduction in the urge for palatable food following thylakoid treatment has been observed earlier, in a study where overweight women were treated for three months with a daily supplement of thylakoids [8]. In contrast to the present study, there was no caloric restriction in the weight loss programme during the 12 weeks of intervention, only a recommendation to eat no more than three meals per day. With such a regimen, the thylakoid-treated women lost significantly more weight than controls (0.41 kg/week in the thylakoid group and 0.29 kg/week in the control group). Hence, thylakoids are more efficient for weight loss in the absence of any caloric restriction. In the 12-week study, there was also a reduced urge for palatable food in the thylakoid treated group that was sustained throughout the treatment period [8]. Thus thylakoids appear to be able to suppress hunger, in particular hedonic hunger, irrespective of caloric restriction.

Previous studies have demonstrated that thylakoids inhibit fat digestion transiently due to a reversible inhibition of lipase/colipase in the intestine [11,14,15]. Through this effect the whole gastrointestinal processing of food is extended. This is likely the explanation for the suppression of ghrelin levels following thylakoid consumption [9,16]. The prolonged gastro-intestinal food digestion also explains the increased release of CCK [7,9] and GLP-1 by thylakoids [8]. Other effects are a prolonged uptake of glucose [16-18] and an improved intestinal microflora [18]. The differences between the thylakoid and the control groups found in the present study may be related to one or several of these effects of thylakoids in the intestine.

The reduced feelings of hunger could thus be due to the suppression of ghrelin and/or an increased release of CCK or GLP-1 by thylakoids. Likewise, the observed suppression of urge for chocolate could be an effect of the above gut hormones. Most importantly, the suppressed hunger and urge for palatable food remained throughout the diet intervention, even following body weight loss.

In conclusion, the present study, even though limited by a short time-period of intervention and a relatively small number of participants, demonstrates that a caloric restriction conceals the effect of thylakoids on body weight loss. However, the effects on homeostatic and hedonic hunger remain. We suggest that thylakoid treatment may alleviate some of the strains coupled to caloric restriction during body weight loss programs.

### Conflict of Interest Statement

CEA is a scientific advisor for Greenleaf Medical AB and a founder of Thylabisco AB.

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