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Appethyl® and reduction of body weight: evaluation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006

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Abstract

Following an application from Greenleaf Medical AB, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Sweden, the EFSA Panel on Nutrition, Novel Foods and Food allergens (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to Appethyl® and reduction of body weight. Appethyl® is an aqueous extract from spinach leaves standardised by the manufacturing process and its lipase/colipase inhibition capacity *in vitro*. The Panel considers that the food is sufficiently characterised. A reduction in body weight is a beneficial physiological effect for overweight/obese individuals. The applicant identified a total of three human intervention studies that investigated the effects of Appethyl® on body weight as being pertinent to the claim. In weighing the evidence, the Panel took into account that Appethyl® (5 g/day for 12 weeks) had no effect on body weight as compared to placebo under minimal dietary counselling and moderate physical activity, and that no beneficial physiological effects are to be expected for the target population of overweight/obese individuals from the weight loss that could be attributed to the intervention with Appethyl® under predefined energy restriction and moderate physical activity. The Panel also considered that the effect of Appethyl® (5 g/day for 24 weeks) on body weight maintenance after initial weight loss shown in one study has not been replicated in different settings, which questions the external validity of the results, and that no evidence was provided for a plausible mechanism by which daily consumption of Appethyl® could exert a sustained effect on body weight in humans. The Panel concludes that a cause-and-effect relationship has not been established between the consumption of Appethyl® and a reduction of body weight under the conditions of use proposed by the applicant.

Keywords: spinach leaves aqueous extract, thylakoids, energy restriction, weight reduction, health claims

Requestor: Competent Authority of Sweden following an application by Greenleaf Medical AB

Question number: EFSA-Q-2022-00096

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Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1924/2006 harmonises the provisions that relate to nutrition and health claims and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

1.2. Interpretation of the Terms of Reference

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to 'Appethyl®'.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of Appethyl®, a positive assessment of its safety, nor a decision on whether Appethyl® is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim and the conditions of use as proposed by the applicant may be subject to changes, pending on the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

2. Data and methodologies

2.1. Data

Information provided by the applicant

See also the section Steps taken by EFSA at the end of this opinion.

Food/constituent as stated by the applicant

Following the initial application, EFSA requested the applicant to clarify whether the food subject of the claim was thylakoid preparations in general or Appethyl® in particular. In the Additional Data Request, the applicant clarified that the food for which the health claim is made is 'Appethyl®, a standardised product from an industrial and quality-controlled food production process'. It is 'a fine powder made from the green leaves of spinach (*Spinacia oleracea*), which has been enriched for thylakoids through a specific standardised process. Through this process, spinach leaves have been sheared to disrupt cell walls and thylakoids from plant intracellular compartments are enriched by water-based procedures and remain exposed after drying'.

Health relationship as claimed by the applicant

According to the applicant, the health effect relates to body weight management, and specifically to 'body weight reduction during mild caloric restriction'.

'Primary outcome variables in human clinical studies have therefore been body weight loss quantitated by anthropometric and body composition measurement methods'. 'In addition to body weight loss in long-term studies, other outcome variables such as measurements of plasma concentrations of satiety hormones, metabolic parameters and similar biomarkers have also been utilised to establish the relationship between Appethyl® ingestion and weight loss'.

Mechanism by which the food/constituent could exert the claimed effect as proposed by the applicant

The applicant claims that 'the relationship between daily ingestion of Appethyl® and body weight loss over time is based on the capacity of Appethyl® to transiently inhibit lipase/colipase activity during digestion of other foods, without causing steatorrhea. This effect allows undigested lipids to progress in the digestive tract which in turn triggers the release of satiety hormones both acutely and during repeated administration over time which leads to body weight reduction'. The applicant clarifies that 'a reduction in energy intake, due to satiety, is the predominant factor for the loss of body weight.'

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: 'Appethyl® helps your body weight reduction during mild caloric restriction'.

Specific conditions of use as proposed by the applicant

According to the applicant, the target population for the claimed effect is adults 'with overweight (BMI 25–30 kg/m^2) and individuals with obesity (BMI > 30 kg/m^2), but not with morbid obesity (BMI > 40 kg/m^2).'

The following recommendations for use are proposed: 'For powdered product, for daily dosing, mix 5 g powder into a drinking glass of water, fruit juice or similar and drink together with a meal, preferably at lunchtime to influence subsequent food intake during the day. For product in capsules, for daily dosing, take recommended number of capsules together with a drinking glass of water together with a meal, preferably at lunchtime.'

According to the applicant, the product should be avoided if 'you are underweight (BMI < 18.5 kg/m^2), if you are on a weight gaining program or for other reasons should not lose weight' or 'if you have allergy to spinach'.

Data provided by the applicant

The health claim application on Appethyl® pursuant to Article 13(5) of Regulation (EC) No 1924/2006 was presented in a common and structured format as outlined in the Scientific and technical guidance for the preparation and presentation of a health claim application (EFSA NDA Panel, 2021b).

As outlined in the General scientific guidance for stakeholders on health claim applications (EFSA NDA Panel, 2021a), it is the responsibility of the applicant to provide the totality of the available evidence.

The applicant has submitted a confidential and a non-confidential version of a dossier following the 'General scientific guidance for stakeholders on health claim applications' (EFSA NDA Panel, 2021a) and the 'Scientific and technical guidance for the preparation and presentation of a health claim application' (EFSA NDA Panel, 2021b).

The application contains data claimed as proprietary and confidential by the applicant in relation to the manufacturing process of Appethyl®, and two unpublished study reports of human intervention studies (Holtz et al., 2015 unpublished-GLM-025811; Postrach, 2021, unpublished-GLM-009813).

In accordance with Art. 38 of the Regulation (EC) No 178/20021[1] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[2] the non-confidential version of the dossier has been published in the OpenEFSA portal.[3]

2.2. Methodologies

The approach used by the NDA Panel for the evaluation of health claims is explained in the General scientific guidance for stakeholders on health claim applications (EFSA NDA Panel, 2021a). In assessing each specific food/health relationship, which forms the basis of a health claim, the NDA Panel considers the following key criteria:

[1] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–48.

[2] Decision available at: https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/210111-PAs-pre-submission-phase-and-public-consultations.pdf.

[3] https://open.efsa.europa.eu/questions/EFSA-Q-2022-00096.

i) the food/constituent is defined and characterised;
ii) the claimed effect is based on the essentiality of a nutrient; OR the claimed effect is defined and is a beneficial physiological effect for the target population and can be measured *in vivo* in humans;
iii) a cause-and-effect relationship is established between the consumption of the food/constituent and the claimed effect (for the target group under the proposed conditions of use).

Each of these three criteria needs to be assessed by the NDA Panel with a favourable outcome for a claim to be substantiated. In addition, an unfavourable outcome of the assessment of criterion (i) and/or (ii) precludes the scientific assessment of criterion (iii).

The scientific requirements for health claims related to appetite ratings, weight management and blood glucose concentrations are outlined in a specific EFSA guidance (EFSA NDA Panel, 2012).

2.3. Public consultation

According to Art. 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a Public Consultation on the non-confidential version of the application from 14 June 2023 to 05 July 2023 (PC-0537) for which no comments were received.

3. Assessment

3.1. Characterisation of the food/constituent

The food/constituent proposed by the applicant as the subject of the health claim is Appethyl®.

Appethyl® is an extract from spinach leaves (*Spinacia oleracea*) which has been enriched in thylakoids through a specific, standardised manufacturing process. Appethyl® is described by the applicant as a fine dry powder with a green/brown appearance.

Thylakoids are membrane-bound compartments found within the chloroplasts of plant cells, which are responsible for photosynthesis. Chloroplasts are organelles found in the cells of all green plants, as well as some algae and other photosynthetic organisms. Chloroplasts are composed of an outer membrane, an inner membrane and thylakoid membranes. Thylakoids contain chlorophyll and other pigments in photosystems. These protein complexes capture light energy from the sun and convert it into chemical energy (Pribil et al., 2014).

Confidential and non-confidential summaries of the manufacturing process have been provided. The manufacturing process for Appethyl® is described by the applicant as follows: '(1) Selection of suitable spinach plant leaves of food grade quality for human consumption, (2) Mechanical shearing of dried or fresh green plant leaves, (3) Mixing of plant material into water and citric acid, both of food grade quality for human consumption, to chelate possible heavy metals enriched by the plant, (4) Centrifugation and decanting of thylakoid enriched supernatant to separate and discard fibrous material with low thylakoid content, (5) Drying and grinding of thylakoid enriched supernatant to specified mesh size'.

The specification of the product states that spinaches used originate from Egypt and Hungary.

The applicant provided a compositional analysis for 100 g Appethyl® from a commercial production. The applicant claims that the nutritional composition of Appethyl® may change depending on the starting material.

The applicant used reversed-phase high-performance liquid chromatography (RP-HPLC) to indirectly quantify thylakoids in the final product. The protocol used is thoroughly described in the application. In six batches of Appethyl®, for samples ranging from 72 to 83 mg of product, the content of thylakoid membranes varied from 38.4 mg to 66.3 mg (from 52.6% to 85.9% thylakoid membrane by weight). The Panel notes that the content of thylakoid membranes in Appethyl® can be measured using well-established methods. The Panel also notes, however, that batch-to-batch variability was high.

The applicant notes that RP-HPLC is an expensive and time-consuming method that is not used for routine purposes. Therefore, the standardisation of the manufacturing process is based on the capacity of the final product to inhibit pancreatic lipase/colipase activity *in vitro*, which should be between 50% and 75%. The enzymatic activity of pancreatic lipase is indirectly measured *in vitro* by pH-stat titration method (Albertsson et al., 2007). Lipase/colipase hydrolyses triacylglycerols into monoacylglycerols and free fatty acids and induces a decrease in pH. A buffered solution with glyceryl tributyrate as a fat

source for lipase/colipase is kept at pH 7.0 by titration with 0.1 M NaOH at 25°C. The enzyme activity is monitored by the consumption of NaOH. The applicant claims that the comparison between samples with or without added Appethyl® shows inhibition of lipolysis by thylakoids in Appethyl® and differences in delivered titrant rates form the basis for the calculation of the enzyme inhibition capacity of the preparation. In the batch-to-batch analysis (n = 44 batches), the lipase/colipase inhibition capacity of Appethyl® ranged from 50% to 72.3% compared to control samples.

In the application, the relationship between the amount of thylakoids (as % weight) and the lipase/colipase inhibition capacity for three batches is provided, but the amount of sample analysed is not given. Upon EFSA's request to clarify the relationship between the lipase/colipase inhibition capacity and the content of thylakoid membranes in Appethyl®, the applicant stated that this information was not available.

EFSA noted that no information had been provided in relation to the lipase/colipase inhibition capacity of the thylakoid preparations used in the intervention studies submitted for the scientific substantiation of the claim, and requested the applicant to clarify whether the claim was made for thylakoid preparations in general or for Appethyl® in particular. The applicant stated that the claim was made for Appethyl® in particular, and that only the thylakoid preparations used in five of the human studies submitted (Montelius et al., 2014a; Holtz et al., 2015; Rebello et al., 2015a; Montelius et al., 2016; Stenblom et al., 2016; Postrach, 2021) with the specifications provided for Appethyl® were therefore pertinent to the claim. The applicant also clarified that, even if not reported in the publications, these studies were conducted with Appethyl® batches for which the lipase/colipase inhibition capacity has been provided in the application.

The applicant claimed that the shelf-life of Appethyl® in cool dry storage conditions is 24 months. Information related to stability and batch-to-batch variability of lipase/colipase inhibition capacity and microbial growth over time was provided.

The Panel considers that the food/constituent Appethyl®, an aqueous extract from spinach leaves standardised by the manufacturing process and its lipase/colipase inhibition capacity *in vitro*, is sufficiently characterised.

3.2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is 'helps your body weight reduction during mild caloric restriction'. The Panel understands that the claimed effect is a reduction of body weight under energy restriction. The proposed target population is adults 'with overweight (BMI 25–30 kg/m^2) and individuals with obesity (BMI > 30 kg/m^2), but not with morbid obesity (BMI > 40 kg/m^2)'.

The scientific evidence for the substantiation of health claims on the reduction of body weight can be obtained from human intervention studies showing a reduction in body weight, which could not be attributed to a reduction in lean body mass/body water. The conditions in which the effect on body fat/body weight is achieved need to be specified (e.g. under energy restriction, eating ad libitum, etc.). Evidence for a sustained effect with continuous consumption of the food/constituent over, e.g. about 12 weeks, should also be provided (EFSA NDA Panel, 2012).

Changes in appetite ratings, energy intake, energy expenditure or fat oxidation have been proposed in the context of claims related to the reduction of body fat/body weight. Evidence for a sustained effect on any of these variables with continuous consumption of the food (in order to exclude adaptation) may be considered in support of the mechanisms by which the food may exert the claimed effect (EFSA NDA Panel, 2012).

The Panel considers that a reduction of body weight is a beneficial physiological effect for overweight and obese individuals.

3.3. Scientific substantiation of the claimed effect

The applicant performed a literature search in PubMed, Embase Cochrane Library and Lund University Research Portal to retrieve human studies published in English using keywords in relation to the food constituent (thylakoid* OR green leave* OR spinach* OR spinacia oleracea OR green-plant membrane*) limited to the [Title/abstract] field and keywords related to the claimed effect (obesity OR overweight OR weight management OR appetite regulation OR appetite control OR body weight OR weight loss OR weight reduction OR body fat OR adipose tissue OR fat reduction OR fat mass OR body mass index OR BMI OR satiety OR satiation OR hunger OR eating behavior OR reward eating). The full search strategy with keywords was provided by the applicant.

The applicant identified 10 publications as being pertinent to the claim. Five publications report on three human intervention studies (Montelius et al., 2014a; Rebello et al., 2015a; Stenblom et al., 2015, 2016; Montelius et al., 2016), two publications report on animal studies (Montelius et al., 2014b; Stenblom et al., 2016), one is a systematic review (Amirinejad et al., 2020) and two are narrative reviews (Rebello et al., 2015b; Foshati and Ekramzadeh, 2020). The applicant also submitted two unpublished human intervention studies as being pertinent to the claim, claimed as proprietary by the applicant (Holtz et al., 2015; Postrach, 2021).

Among the human intervention studies, only three (Montelius et al., 2014a; Holtz et al., 2015; Postrach, 2021) investigated the effect of Appethyl® on body weight for at least 12 weeks. Additional results of the original study by Montelius et al. (2014a) have been presented in two other publications (Montelius et al., 2016; Stenblom et al., 2016). The remaining human intervention studies are single-dose studies which address the effects of Appethyl® on satiety, hunger, food craving and energy intake (Rebello et al., 2015a; Stenblom et al., 2015). The Panel considers that no conclusions can be drawn from these single-dose studies for the scientific substantiation of the claimed effect, but they will be discussed in relation to the mechanism of action proposed by the applicant.

The applicant identified two animal studies conducted with Appethyl® as supportive evidence. One was a single-dose study in pigs that investigated blood glucose concentrations and appetite-regulating hormones during an oral glucose tolerance test (OGTT) (Montelius et al., 2014b), and one was a 2-week study in rats that investigated gastric emptying, intestinal transit time and faecal fat content (Stenblom et al., 2016). None of these studies assessed changes in body weight. The Panel considers that no conclusions can be drawn from these animal studies for the scientific substantiation of the claimed effect, but they will be discussed in relation to the mechanisms of action proposed by the applicant.

The systematic review includes eight human intervention studies on the effect of thylakoid powders or boiled spinach on satiety and weight loss (Amirinejad et al., 2020). Of these, only four were conducted with Appethyl® (Montelius et al., 2014a; Rebello et al., 2015a; Stenblom et al., 2015, 2016). The Panel notes that all the studies conducted with Appethyl® have been already submitted in the application and considers that no additional information can be drawn from this systematic review for the scientific substantiation of the claim.

One narrative review (Foshati and Ekramzadeh, 2020) explored the potential effects and mechanism of action of thylakoids in general on overweight and obesity. The other narrative review (Rebello et al., 2015b) evaluated the role of gut fat signalling in appetite control and focused on the potential impact of thylakoids derived from spinach on eating behaviour. These publications do not report additional experimental data on the effects of Appethyl® on body weight. The Panel considers that no conclusions can be drawn from these narrative reviews for the scientific substantiation of the claim.

Human intervention studies

Three human intervention studies have investigated the effect of Appethyl® on body weight under different dietary conditions. Two are studies on body weight loss lasting 12 weeks, of which one relied on minimal dietary counselling to achieve a negative energy balance (Montelius et al., 2014a) and the second had a moderate, predefined energy restriction (Holtz et al., 2015). The third study aimed at body weight maintenance after an initial weight loss achieved before the study start by other means and lasted 24 weeks (Postrach, 2021).

The Panel notes that all these studies have assessed changes in body composition using bioelectrical impedance analysis (BIA), a method with serious limitations to measure small changes in body fat (EFSA NDA Panel, 2012). The Panel also notes that the three studies have been conducted by the same research group in a similar setting, which questions the external validity of the results.

Human intervention studies on body weight loss

In a double-blind, randomised, parallel, three-arm, placebo-controlled, multicentre (three centres) study (Holtz et al. (2015), unpublished), 208 adults of both sexes (18–65 years) with overweight or obesity (BMI of 25–34 kg/m^2) were randomised (block randomisation) to consume either Appethyl® at doses of 3 or 5 g/day, or placebo (maltodextrin) for 12 weeks. The methods used for the recruitment of participants were not described in the study report. Participants were asked to adhere to a 20% energy restriction dietary plan (individual energy requirements were estimated with equations based on sex, BMI and reported activity levels) and to perform up to a maximum of 30 min of daily, moderate intensity physical activity (walking, cycling). Appethyl® and placebo were provided as

cellulose capsules (10 per day, 5 to be consumed at breakfast and 5 at lunch time). Study visits post-randomisation (baseline) were at 4, 8 and 12 weeks.

Sample size was calculated based on the body weight changes observed in a pilot study on the effects of Appethyl® (5 g/day) versus placebo lasting for 4 weeks. Assuming an effect size of 2.0 times and 1.7 times that of the control group for the 5 and 3 g/day Appethyl® groups, respectively (about 4 and 1.5 kg difference in weight loss vs. the control group, respectively), a SD of 2.0, a significance level of 5%, a power of 80% and a dropout rate of 10%, it was calculated that 40 participants per group would be needed. A prespecified interim analysis was conducted when >30% of the participants had completed the 4-week visit. Based on the results showing no effects on body weight of Appethyl® at 3 g/day, it was decided to stop randomisation to that study group and to increase sample size to 84 subjects in the remaining Appethyl® (5 g/day) and placebo groups.

Compliance with the intervention was assessed by counting the number of capsules returned and by checking food diaries. Good compliance was defined as consumption of at least 80% of the capsules and energy intake between −40% and +10% of the target.

Body weight and body composition were measured at each study visit (every 4 weeks) using a BIA device (Tanita BC-420 SMA). The primary outcome was between-group differences in body weight changes through the duration of the study.

A number of secondary outcomes were specified, including between-group differences in mean body weight at baseline and after 4 and 8 weeks of the intervention, difference in relative body weight (as percent change from baseline) after 4, 8 and 12 weeks of the intervention, proportion of subjects who lost at least 3%, 5% and 10% of baseline body weight and changes in waist and hip circumference, BMI, body fat and fat free mass, hunger and food craving (Control of Eating Questionnaire with 21 questions (COEQ21) scores), physical activity (IPAQ scores), appetite (VAS scale), energy intake (food diary), global evaluation of the feeling of satiety (4 point categorical scale), global evaluation of the benefit of thylakoid-rich spinach extract by the subjects and the investigator (4-point categorical scale), blood lipids (triglycerides, total cholesterol, LDL and HDL cholesterol) and HbA1c.

The full analysis set (FAS) comprised all subjects who took the Appethyl® or placebo capsules at least once and for whom outcome data at the 4-week visit were available. The Valid Case Analysis Set (VCAS) comprised all subjects who showed no significant protocol violations. Of the 168 participants randomised to the Appethyl® (5 g/day) and placebo groups, FAS and VCAS populations included 162 (Appethyl® 5 g/day: n = 82, 17 (20.7%) males and 65 (79.3%) females; placebo group: n = 80, 28 (35%) males and 52 (65%) females) and 135 (Appethyl® 5 g/day: n = 71, 14 (19.7%) males and 57 (80.3%) females; placebo group: n = 64, 24 (37.5%) males and 40 (62.5%) females) participants, respectively. Statistical analyses were conducted on the FAS and VCAS data sets. The Panel notes the unbalanced sex distribution between the Appethyl® (5 g/day) and placebo groups in the FAS and VCAS data sets, the difference being statistically significant in the VCAS population.

In the FAS, mean (SD) body weight loss during the study was 2.84 (2.98) kg, 1.76 (2.17) kg and 1.84 (2.90) kg in the 5 g Appethyl®, the 3 g Appethyl® and the placebo groups, respectively. On a two-way repeated measures analysis of variance (RM-ANOVA) using absolute body weight at all time points (baseline, 4, 8 and 12 weeks), the effect of time ($p_{time} < 0.001$) and time × group interaction ($p_{time \times group} = 0.031$) were reported to be statistically significant, whereas the effect of group alone was not reported. No significant differences were reported among the groups in relation to changes in body composition, the blood lipid profile or HbA1c.

In the VCAS, mean (SD) body weight loss during the study was 2.33 (2.61) kg, 1.75 (2.08) kg and 1.80 (2.38) kg in the 5 g Appethyl®, the 3 g Appethyl® and the placebo group, respectively. On a two-way RM-ANOVA using absolute body weight at all time points (baseline, 4, 8 and 12 weeks), the time × group interaction was reported to be statistically significant ($p_{time \times group} = 0.007$). No significant differences were reported among groups in relation to changes in body composition, the blood lipid profile or HbA1c.

The Panel notes that the mean difference in body weight loss between the 5 g Appethyl® and placebo groups is 0.53 kg in the VCAS analysis and 1 kg in the FAS analysis over 12 weeks. Considering that the mean body weight of the study population at baseline was 85 kg, this represents 0.6% and 1.2% of the initial body weight in VCAS and FAS analyses, respectively. The Panel also notes that the initial power calculation was planned for a between-group body weight loss difference of about 4.4 kg (Appethyl® at 5 g/day vs. placebo, corresponding to about 5% of the initial body weight), and that sample size was doubled based on the results of the interim analysis after 4 weeks.

The Panel notes that this study (Holtz et al., 2015) reports a statistically significant effect of Appethyl® on body weight reduction when consumed daily at doses of 5 g for 12 weeks in the context of energy restriction and moderate physical activity as compared to placebo. The Panel considers, however, that no beneficial physiological effects are to be expected for the target population of overweight and obese individuals from the weight loss that could be attributed to the intervention with Appethyl®, corresponding to 0.6–1.2% of the initial body weight.

In a randomised, single-blind and placebo-controlled study (Montelius et al., 2014a), 38 women (40–65 years of age, BMI of 25–33 kg/m^2) were randomised to consume a thylakoid powder (5 g Appethyl®) mixed with 2.8 g rapeseed oil and 50 g of blueberry soup or placebo (same vehicle without Appethyl®) once daily before breakfast for 12 weeks. The participants were divided into two groups (n = 19 per group) by a non-algorithmic randomisation method (ballot). All participants were instructed to consume three meals per day with high amounts of fruit and vegetables, to avoid sweet drinks and snacks and to perform low-intensity exercise 30 min per day. Body weight and body composition were measured at baseline and every 3 weeks using a BIA device (TANITA BC 418 MA). At the same time points, a fasting blood draw was obtained for biochemical analyses. At baseline and at the end of the study, following the intake of a standardised breakfast, glucose, insulin and glucagon-like peptide 1 (GLP-1) were measured in plasma at different time points, as well as subjective ratings of hunger, satiety and urge for different palatable foods using visual analogue scales. The Panel notes that the primary outcome of the study was not identified in the publication, that no power calculations were provided and that multiple comparisons were not considered in the statistical analyses.

Outcome data were analysed for completers only (n = 36), except for the test breakfast-related data at baseline. Two subjects (both in the control group) dropped from the study, one for non-compliance with the intervention and the second changed residence.

Mean (SD) absolute changes in body weight during the study were −5.0 (2.3) kg in the Appethyl® group and −3.5 (2.5) kg in the placebo group. The original publication reports a significant effect of time ($p < 0.001$) and time per treatment interaction ($p < 0.05$) using a two-way repeated measures analysis of variance (RM-ANOVA) on the cumulative body weight changes in the Appethyl® and placebo groups after 3, 6, 9 and 12 weeks of treatment. The Panel notes the misuse of ANOVA with cumulative responses, since data from earlier time points disproportionally contribute to the outcome and can lead to serious errors of inference (Fitts, 2006). Indeed, in a corrigendum to the article published 2 years later (Montelius et al., 2016), the difference in body weight changes between the study groups analysed by two-way RM-ANOVA on interval weight changes (0–3 week, 3–6 week, 6–9 week, 9–12 week) was not statistically significant ($p = 0.076$).

The original publication Montelius et al. (2014a) reports a significant decrease in fat free mass and body fat in both groups over the course of the study, and a significant decrease in total and LDL-cholesterol at all time points in the Appethyl® group versus placebo, whereas no significant differences between groups were reported for fat mass, fat-free mass, leptin concentrations, blood glucose, insulin, HDL-cholesterol or triglycerides. In a re-analysis of data on the blood lipid profile using a Bonferroni correction for the post hoc t-tests (Montelius et al., 2016), the effect of treatment was still significant for LDL-cholesterol, but no differences at specific time points during the treatment period were found in the post hoc analysis. Also for total cholesterol, the treatment effect of Appethyl® was still significant, the significant difference being present at time point 9 weeks only.

The Panel notes the inconsistency of the results depending on the strategy used for statistical analysis and considers that this study does not show an effect of Appethyl® (5 g/day) on body weight when consumed for 12 weeks in the context of minimal dietary counselling and moderate physical activity.

Human intervention study on body weight maintenance after initial weight loss

In a randomised, double-blind, parallel placebo-controlled study (Postrach, 2021), 72 adults (18–65 years of age) of both sexes with an initial BMI of 25–35 kg/m^2 but otherwise healthy and, based on participation in trials or diet, with a $\geq$ 3% reduction in body weight during the previous 3–6 months were randomised to consume Appethyl® (5 g/day) or placebo once daily for 24 weeks. Participants were encouraged to maintain a 'nutritionally balanced diet (50% carbohydrates, 30% fat, 20% protein)', and were informed individually about their energy requirements also considering physical activity levels in order to maintain energy balance throughout the study. Subjects were divided into two groups (n = 36 per group) by block randomisation, with sequential random numbers being assigned to them.

Body weight and body composition were measured every 6 weeks using a BIA device (Tanita BC-420 SMA). Hunger and appetite (COEQ21 scores), a global evaluation of feeling of satiety (4-point rating scale), waist and hip circumferences, blood pressure and heart rate were measured at the same time points. At the beginning and end of the study, a fasting blood draw was obtained for biochemical analyses, including blood glucose, blood lipid profile and hepatic enzymes.

The primary outcome was between-group differences in body weight changes during the study. Sample size (n = 72) was calculated based on the assumption that subjects receiving placebo would have reached their original body weight (+1.7 kg) and the test group would decrease their body weight at most by 1% (−0.7 kg), leading to a difference of 2.4 kg in body weight changes between groups, given a significance level of 5%, a power of 80% and a drop-out rate of 20%.

The FAS comprised all subjects that took the Appethyl® or placebo capsules at least once and for whom outcome data for the 6-month visit are available. The VCAS comprised all subjects that showed no significant protocol violations. The FAS consisted of 69 participants (52 female; 34 and 35 subjects in the test and the placebo group, respectively), and VCAS of 66 (31 and 35 subjects in the test group and placebo group, respectively).

In the FAS, mean (SD) body weight changes during the study were −1.03 (2.05) kg in the Appethyl® group and +0.95 (1.88) kg in the placebo group. The difference was statistically significant using ANOVA with baseline body weight as covariate ($p_{group} < 0.001$), and two-way RM-ANOVA with body weight at all time points measured ($p_{time \times group} < 0.001$), with no significant effect of time ($p_{time} = 0.766$).

In the VCAS, mean (SD) body weight changes during the study were −1.18 (2.08) kg in the Appethyl® group and +0.95 (1.88) kg in the placebo group. The difference was statistically significant using ANOVA with baseline body weight as covariate ($p_{group} < 0.001$), and two-way RM-ANOVA with body weight at all time points measured ($p_{time \times group} < 0.001$) with no significant effect of time ($p_{time} = 0.743$).

Changes in body fat, fat-free mass and waist circumference followed those on body weight. No significant differences in feelings of satiety, appetite, hunger or food craving, the biochemical parameters assessed or blood pressure were found between groups.

The Panel considers that this study shows an effect of Appethyl® on body weight maintenance after initial weight loss when consumed at 5 g/day for 24 weeks.

The Panel notes that, in the three human intervention studies provided which investigated the effect on Appethyl® on body weight, Appethyl® consumed daily at doses of 5 g for 12 weeks had no effect on body weight as compared to placebo under minimal dietary counselling and moderate physical activity (Montelius et al., 2014a – corrigendum in Montelius et al., 2016), and that no beneficial physiological effects are to be expected for the target population of overweight and obese individuals from the weight loss that could be attributed to the intervention with Appethyl® under predefined energy restriction and moderate physical activity (Holtz et al., 2015, unpublished). The Panel also notes that the effect of Appethyl® (5 g/day) consumed daily for 24 weeks on body weight maintenance after initial weight loss (Postrach, 2021, unpublished) has not been replicated by other research groups in different settings, which questions the external validity of the results.

Mechanism of action

The applicant claims that Appethyl® acts through a transient inhibition of the pancreatic lipase/colipase activity *in vivo*, which does not result in steatorrhea. Such transient inhibition of the enzyme activity would increase the secretion of appetite-suppressing hormones, inducing a reduction in energy intake due to increased satiety and resulting in body weight loss.

Human studies

Five human intervention studies assessed the impact of Appethyl® on appetite, hunger, satiety and energy intake, three of which have been previously described in relation to body weight (Montelius et al., 2014a; Holtz et al., 2015; Postrach, 2021) and two others are single meal studies (Rebello et al., 2015a; Stenblom et al., 2015). Of these, only one (Montelius et al., 2014a) assessed the impact of long-term Appethyl® intake on serum leptin, ghrelin and glucagon-like peptide 1 (GLP-1), and on faecal fat content (results reported in Stenblom et al., 2016).

Holtz et al. (2015) measured hunger and food craving (COEQ21), appetite (4 VAS), feelings of satiety (4-point categorical scale from 0 = no satiety to 3 = strong satiety) and energy intake (weekly 3-day food diary) at baseline and 4, 8 and 12 weeks. There were no significant differences between groups in COEQ21 scores from baseline to week 12, except for question 9 ('how often did you have

craving in the last seven days?') between the 5 g/day Appethyl® and placebo groups in the FAS population. No correction for multiple testing was applied. No significant differences between groups were reported for either feelings of satiety or energy intake.

Montelius et al. (2014a) included single-meal tests performed on days 1 and 90 of the study among 38 overweight women. At different time points before and after a standardised breakfast, leptin, ghrelin and GLP-1 were measured in blood and subjective sensations of hunger, fullness and urge for specific food items were assessed through questionnaires constructed on VAS. No significant differences were observed between the Appethyl® and control groups for any of the variables measured except for a higher GLP-1 area under the curve (AUC) and lower urge for sweets and chocolate in the Appethyl® group versus placebo on day 90. No correction for multiple comparisons was applied. No differences in faecal fat content were reported (Stenblom et al., 2016).

Postrach (2021) measured appetite, hunger and food craving using the COEQ21 and feelings of satiety with a 4-point categorical scale at baseline and at 24 weeks of the intervention. Energy intake was not reported. No significant differences were observed between the Appethyl® and control groups for any of the variables measured except for question 7 ('how alert have you felt?') of the COEQ21 (results reported for 19 questions only).

In a double-blind randomised crossover study (Rebello et al., 2015a), 60 overweight and obese adults (18–65 years of age) of both sexes with an initial BMI of 25–35 kg/m^2 consumed a single dose of 5 g Appethyl® and placebo (in random order) at least a week apart. A standard breakfast, a standard lunch (4 h later) and an ad libitum pizza dinner were offered. Feelings of hunger, fullness and desire to eat, prospective intake, satisfaction, thirst and appetite for sweet, salty and savoury foods were evaluated using VAS questions. Liking and wanting were evaluated using VAS questions and 16 food image stimuli. Appethyl® or placebo was consumed before the standardised lunch. Then, VAS were administered at 30, 60, 120 and 240 min after the lunch meal. Three participants dropped from the study. Appethyl® was reported to increase fullness ($p = 0.04$) and reduce hunger ($p < 0.01$), longing for food ($p < 0.01$) and prospective intake ($p = 0.01$) over the 2-h period following lunch. There was no difference between the study groups in energy intake at dinner, liking or wanting of specific food products. The primary outcome of the study is not indicated in the publication and no correction for multiple comparisons was made.

In another human intervention study with a similar design which included 26 women (40–60 years of age), 5 g Appethyl® per day was reported to significantly reduce feelings of hunger ($p_{RM\ ANOVA} < 0.05$) and increase satiety ($p_{RM\ ANOVA} < 0.01$). Appethyl® was also reported to decrease subjective feelings of wanting palatable food, whereas it did not affect food intake in an ad libitum snack buffet (Stenblom et al., 2015).

The Panel notes that, whereas two single-dose studies (Rebello et al., 2015a; Stenblom et al., 2015) reported an effect of Appethyl® (5 g/day) on feelings of hunger, satiety and food craving after a standard lunch, this did not result in a reduction of energy intake in subsequent ad libitum meals. The effect on hunger, satiety and food craving was not observed in studies lasting 12 or 24 weeks (Montelius et al., 2014a; Holtz et al., 2015; Postrach, 2021). No effect of Appethyl® on energy intake was reported in these studies either. The Panel also notes that the acute effects of Appethyl® on leptin, adiponectin and GLP-1 were only measured in one study, in which only an effect on GLP-1 was reported (secondary outcome, no corrections for multiple testing) (Montelius et al., 2014a). Consumption of Appethyl® (5 g/day) did not lead to steatorrhea in the only human study which measured faecal fat content, and no evidence has been provided by the applicant that Appethyl® can inhibit lipase/colipase activity *in vivo* in humans.

Animal studies

Two animal studies conducted with Appethyl® were provided in support of a mechanism by which Appethyl® could exert the claimed effect (Montelius et al., 2014b; Stenblom et al., 2016).

The first study (Montelius et al., 2014b) was conducted in six pigs. After 1 month of feeding with a high-fat diet (36% energy from fat), glucose, insulin, ghrelin and cholecystokinin (CCK) responses were assessed during a 2-h OGTT following a single dose of Appethyl® (1 g/kg bw) or placebo (cross-over design) after 1 month of feeding with a high-fat diet. Blood glucose concentrations during the first hour and plasma ghrelin concentrations after 2 h were significantly lower with Appethyl® than with placebo, whereas plasma cholecystokinin concentrations were significantly higher with Appethyl® compared to placebo. No differences in serum insulin concentrations were observed.

The second study (Stenblom et al., 2016) was conducted in rats, which were fed a high-fat diet with Appethyl® (0.33 g per gram) or without Appethyl® either once (1.3 g) for the acute, one-dose

study (n = 14) or ad libitum for 2 weeks for the longer term study (n = 16). Gastric emptying and intestinal transit time were measured by providing Evans blue before the tests. Gastric emptying was significantly decreased in the Appethyl® group versus controls in the acute but not in the longer term test, whereas transit time and CCK (the latter measured in the single-dose test only) were not significantly different between groups.

The Panel notes that these two animal studies do not provide evidence in support of a mechanism by which continuous consumption of Appethyl® could exert the claimed effect in humans.

Summary

The Panel considers that no evidence has been provided for a plausible mechanism by which daily consumption of Appethyl® for at least 12 weeks could exert an effect on body weight *in vivo* in humans.

Weighing of the evidence

In weighing the evidence, the Panel took into account that Appethyl® consumed daily at doses of 5 g for 12 weeks had no effect on body weight as compared to placebo under minimal dietary counselling and moderate physical activity (Montelius et al., 2014a, 2016), and that no beneficial physiological effects are to be expected for the target population of overweight and obese individuals from the weight loss that could be attributed to the intervention with Appethyl® under predefined energy restriction and moderate physical activity (Holtz et al., 2015, unpublished). The Panel also took into account that the effect of Appethyl® (5 g/day) on body weight maintenance after initial weight loss (Postrach, 2021, unpublished) has not been replicated by other research groups in different settings, which questions the external validity of the results, and that no evidence has been provided for a plausible mechanism by which daily consumption of Appethyl® could exert a sustained effect on body weight with continuous consumption of the food in humans.

The Panel concludes that a cause-and-effect relationship has not been established between the consumption of Appethyl®, an aqueous extract from spinach leaves standardised by the manufacturing process and its lipase/colipase inhibition capacity *in vitro* and a reduction of body weight under the conditions of use proposed by the applicant.

4. Conclusions

On the basis of the data presented, the Panel concludes that:

- The food/constituent Appethyl®, an aqueous extract from spinach leaves standardised by the manufacturing process and its lipase/colipase inhibition capacity *in vitro*, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect is 'helps your body weight reduction during mild caloric restriction'. The target population proposed by the applicant is 'adults with overweight (BMI 25–30 kg/m^2) and individuals with obesity (BMI > 30 kg/m^2), but not with morbid obesity (BMI > 40 kg/m^2)'. A reduction of body weight is a beneficial physiological effect for overweight and obese individuals.
- A cause-and-effect relationship has not been established between the consumption of Appethyl® and a reduction of body weight under the conditions of use proposed by the applicant.

Documentation as provided to EFSA

Health claim application on pursuant to Article 13.5 of Regulation (EC) No 1924/2006 (Appian number: HC-2022-3072). Submitted by Greenleaf Medical AB.

Steps taken by EFSA

1) This application was received by EFSA on 14/02/2023. The application was validated on 20/02/2023 and the scientific evaluation started.
2) The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence and which included a request for the protection of proprietary data.
3) The Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. EFSA sent a first

Additional Data Request (ADR1) letter to the Applicant on 31/05/2023. The clock was stopped on 31/05/2023. The clock restarted on 15/06/2023.

4) Following the reply of the applicant, the Working Group on Claims of the NDA Panel agreed on a new list of questions for the applicant to provide additional information to accompany the application. EFSA sent a second Additional Data Request (ADR2) letter to the Applicant on 14/07/2023. The clock was stopped on 14/07/2023. The clock restarted on 28/07/2023.
5) During its meeting on 31/08/2023, the NDA Panel, having evaluated the data, adopted an opinion on the scientific substantiation of a health claim related to the consumption of Appethyl® and reduction of body weight.

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Abbreviations

ADR	Additional Data Request
ANOVA	analysis of variance
AUC	area under the curve
BIA	bioelectrical impedance analysis
BMI	body mass index
bw	body weight
CCK	cholecystokinin
COEQ21	Control of Eating Questionnaire including 21 questions
FAS	full analysis set
GLP-1	glucagon-like peptide 1
HbA1C	glycated haemoglobin
HDL	high density lipoprotein
IPAQ	International Physical Activity Questionnaire
LDL	low density lipoprotein
NDA	Panel on Nutrition, Novel Foods and Food Allergens
OGTT	oral glucose tolerance test
RM-ANOVA	repeated measures analysis of variance
RP-HPLC	reverse-phased high-performance liquid chromatography
SD	standard deviation
VAS	visual analogue scale
VCAS	valid case analysis set