Anti-obesity effect of eucalyptus leaf extract containing oenothein B in healthy Japanese adults: a randomized, placebo-controlled, double-blind, parallel-group study

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ABSTRACT

Background: Excessive ingestion of fructose can lead to obesity and related diseases. Eucalyptus leaf extract (ELE) contains oenothein B, which inhibits intestinal fructose absorption.

Objective: The antiobesity effects of ELE containing oenothein B were evaluated in healthy Japanese whose body mass index (BMI) was ≥ 23 and < 30 kg/m².

Methods: A randomized, placebo-controlled, double-blind, parallel-group study was performed to evaluate the effect of ELE consumption, for 12 weeks at a 3.38 mg/day dose of oenothein B, on the abdominal visceral fat area (VFA) as the primary outcome. Results were compared to those of a placebo group.

Results: Of the 721 individuals who underwent screening, 198 were randomly allocated into two groups. A total of 95 subjects in the placebo group and 94 in the intervention group were established as the per-protocol set. VFA in the intervention group significantly decreased compared to that in the placebo group 12 weeks after initiating intervention.
This reduction in VFA was considered to have clinical significance. Among the secondary outcomes, VFA, waist circumference, and muscle mass after 8 weeks, as well as body weight and BMI after 12 weeks, were significantly lower in the intervention group compared to the placebo group.

**Conclusion:** ELE containing oenothein B may be effective against obesity and related diseases by reducing VFA levels.

**Keywords:** human trial; *Eucalyptus globulus*; oenothein B; polyphenol; hydrolyzable tannin; ellagittannin; dietary fructose consumption; dietary survey; Calorie and Nutrition Diary; glucose transporter 5

**INTRODUCTION**

Fructose is more lipogenic than glucose in the liver, as it is rapidly metabolized by ketohexokinase (fructokinase) without regulation, subsequently providing substrates for fatty acid synthesis by bypassing the rate-limiting step in glycolysis. Large amounts of ingested fructose led to hepatic *de novo* lipogenesis because fructose is a lipogenic substrate and induces the hepatic master transcription factors that regulate the expression of lipogenic enzymes [1-4]. It facilitates adiposity [2-5,6], fructation (a type of glycation caused by amino-carbonyl reactions with fructose) [7,8], and insulin resistance [2,8,9]. Related chronic states can encompass non-alcoholic fatty liver disease (NAFLD) [4], diabetes mellitus [5,8], hypertension [5,10], and cardiovascular disease [5,11].

Fructose intake has significantly increased over the past five decades due to fructose-containing sweeteners (such as sucrose, high-fructose corn syrups, honey, and fruit juice) in processed foods and beverages [4,5]. Most fructose-containing sweeteners also contain glucose, which causes postprandial glycation stress. Indeed, consumption of sweetened beverages with fructose-containing sweeteners and sugars is positively correlated with mortality [11,12]. As the primary source of sugars, men tended to consume large amounts of sweetened beverages [13,14] while women consumed more snacks [13,15] due to the gender differences in food preferences. Consequently, the incidence of obesity and related metabolic disorders has risen in Japan [13].
The World Health Organization recommends limiting the intake of sugars, such that it accounts for less than 10% of the total energy intake, while promoting a diet with < 5% sugars (roughly 25 g [6 teaspoons] per day) [16]. Recent clinical studies have indicated that restriction of dietary fructose intake ameliorates fatty liver and obesity. A clinical trial in children with obesity reported that hepatic de novo lipogenesis becomes significantly decreased within a short time period, due to isocaloric fructose restriction in diets, thus improving their hepatic and visceral fat levels [17]. Similarly, within obese adults with fatty liver, daily intake of diets supplemented with glucose or fructose for 6 weeks resulted in reduced hepatic fat in the glucose group compared to the fructose group [18]. Moreover, restricting fructose consumption for 24 weeks reduces waist circumference and fasting blood glucose concentration in overweight and obese adults [19].

Since obesity is difficult to control through diet and exercise alone, they are difficult to implement [20]. Some food materials, herbs and nutraceuticals, and their constituents have been shown to have efficacy in preventing chronic diseases like obesity and metabolic syndrome [20-22]. Reducing dietary fructose intake by inhibiting fructose absorption in the intestine may provide a potent pharmacological strategy for diseases caused by excessive consumption of fructose [23].

The evergreen tree Eucalyptus globulus Labill (Myrtaceae) is widely planted worldwide. The leaves are consumed as herbal tea in Europe [24] and are used as a traditional remedy for diabetes mellitus in South America and Africa [25]. Moreover, eucalyptus leaf extract (ELE) has been used as an active ingredient in food products for its function in Japan [26,27]. In fact, we previously reported that ELE, extracted with aqueous ethanol, inhibits intestinal fructose absorption and suppresses the increase in hepatic triacylglycerols (TG) and visceral fat induced by excessive fructose consumption in rats [28]. ELE also suppresses the postprandial increase in peripheral blood fructose concentration compared with placebo following oral administration of sucrose in human subjects [29]. We also found identified oenothein B—classified as an ellagitannin among hydrolyzable tannins—as the primary active component of ELE [30].

In the current study, we evaluated the effect of ELE containing oenothein B on abdominal visceral fat by measuring visceral fat area (VFA) using computed tomography (CT) in healthy Japanese adults who consumed fructose-containing foods and beverages. The primary aim of this study is to confirm whether the intake of ELE containing oenothein B as a food material for health can reverse or manage obesity while consuming fructose-containing foods and beverages.

**METHODS**

The protocols used in this study were modified based on the method described in attachment 2 included in the Consumer Affairs Agency Food Labeling Division Notification No. 259 of October 30th, 2014 [31], and a previously published study [32].

**Preparation of Test Samples:** Powdered ELE (commercial name, Eucagrandin®) was prepared via spray-drying method using dextrin as an excipient, according to a previously described procedure [33]. Powdered ELE consists of approximately 80% ELE and 20% dextrin. The capsules colored dark brown with caramel coloring were used to prepare test and placebo capsules. Test capsules (weight: 233 mg/pc) containing 100 mg of powdered ELE and placebo capsules (weight: 283 mg/pc) containing 150 mg of gelatin and 20 mg of dextrin were prepared with 72 mg of gelatin, 38 mg of cellulose, 10 mg of starch, 9 mg of water, 2 mg of calcium stearate, 1 mg of caramel color, and trace amounts of lecithin and talc as common formulation ingredients. The placebo and test capsules could not be distinguished by color, smell, or flavor.
Analysis of oenothein B content: The oenothein B content was determined using the absolute calibration curve method with an HPLC system. Oenothein B (analytical standard, catalog no. 03805) and HPLC-grade solvents were purchased from Sigma-Aldrich (Tokyo, Japan) and Nacalai Tesque, Inc. (Kyoto, Japan), respectively. The capsules were divided into coatings and contents. Each was dissolved in a water/methanol (50:50, v/v) solution and extracted by shaking (100 rpm) for 30 min at 37 °C. After filtration through a TORAST Disc GLCTD-HPTFE1322 hydrophilic PTFE membrane filter (pore size, 0.22 μm; Shimadzu GLC Ltd., Kyoto, Japan), the filtrate was added to a glass vial (TORAST-H Glass Vial; Shimadzu GLC Ltd.) and set on a vial tray in the autosampler at 4 °C. The analysis was conducted using a Shimadzu Nexera X2 (Shimadzu Co., Kyoto, Japan) equipped with SPD-M30A (Shimadzu Co.) as the Diode-Array Detection, according to previously reported methods [30] with slight modifications. The conditions of LC using a Cosmosil 5C18-PAQ packed column (Nacalai Tesque, Inc.; 5 μm, 150 mm × 2.0 mm i.d.) were set according to those described previously [30]. The time programs of the mobile phase were conducted with a linear gradient in the reversed-phase mode using a water/formic acid (1000:1, v/v) solution as solvent A and acetonitrile as solvent B. The gradient program was as follows: 0%–16% B in A for 9 min, 16%–95% B in A for 0.5 min, 95% B in A for 2 min, 95%–0% B in A for 0.5 min, and 0% B in 2 min. Calibration curves were created using the purchased oenothein B standard (Lot. BCCD 7964; purity: 81%) at concentrations between 10 and 200 μg/mL. The $r^2$ value of the calibration curve was 0.9999528. The amount of oenothein B present in the capsules was calculated from the total content of the analytes in the coating and the contents of the capsules.

Study procedures: This was a randomized, placebo-controlled, double-blind, parallel-group study that assigned subjects to groups in a 1:1 ratio. The study was approved by the independent ethics committee of the Takara Clinic, Medical Corporation Seishinkai (Tokyo, Japan) on November 11, 2020 (approval number 2011-2005-NK01-04-TC) and was conducted in accordance with the Declaration of Helsinki (2013) and thoroughly followed medical ethics, including adherence to ethical guidelines for medical and health research involving human subjects in Japan. The protocol labeled as 1st edition was set on November 16, 2020 and subsequently registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000042469; Public title, “Effects of consumption of the test food on the visceral fat area in healthy subjects”). This study was performed by the contract research organization ORTHOMEDICO Inc. (Tokyo, Japan) from November 2020 to July 2021. After initiating the study, the protocol was revised as the 2nd version on April 19, 2021. The change was to extend the recruitment period and the subsequent schedule.

Subjects: The eligibility criteria were set according to a guideline described in attachment 2 included in the Consumer Affairs Agency Food Labeling Division Notification No. 259 of October 30th, 2014 [31]. The eligibility criteria included healthy Japanese adult men and women aged 20 to 65 years with a preference for sweet foods and beverages, a habit of snacking, and a body mass index (BMI) of 23 or higher and less than 30 kg/m$^2$. Among them, a BMI of 25 to < 30 kg/m$^2$ was defined as “obese (level 1)” as stipulated by the Japan Society for the Study of Obesity and as “overweight” by the World Health Organization. Exclusion criteria is described in the next paragraph.

All participants were registered on the website (https://www.go106.jp/) run by ORTHOMEDICO Inc. The study protocols were comprehensively explained to all participants. All participants signed an informed consent
document at the ORTHOMEDICO Inc. office before their participation in the study. No sponsors or funding company members participated in the study. The Takara Clinic, Medical Corporation Seishinkai, the principal clinic for conducting this study, evaluated data obtained and managed the physical condition of the subjects. The examinations were conducted at Takara Clinic, Medical Corporation Seishinkai and Nerima Medical Association Minamimachi Clinic (Tokyo, Japan).

**Exclusion criteria:** The exclusion criteria at recruiting were as follows: 1) any medical history of a malignant tumor, heart failure, or myocardial infarction; 2) implantation of pacemakers and implantable defibrillators; 3) undergoing treatment for any of the following chronic diseases: arrhythmia, hepatic disorder, renal disorder, cerebrovascular disorder, rheumatism, diabetes mellitus, dyslipidemia, hypertension, or any other chronic diseases; 4) daily intake of “Foods with Function Claims,” and/or other foods and beverages with potential physiological functionality including hypoglycemic functions (Foods with Health Claims including “Food for Specified Health Uses” (FOSHU), “Foods with Function Claims” and “Foods with Nutrient Function Claims” are classified as foods that can claim health functions in Japan [34,35]); 5) scheduled surgical procedures during the intake period or within two weeks of the end of the intake period; 6) regular use of medications, including herbal medicines and/or supplements; 7) allergic reactions to medications and/or materials for the test food used in this study; 8) gastrointestinal weakness: possibility of discomfort such as a stomach astringent effect, particularly when consuming food and beverages containing tannin, such as thick green tea, black tea, and coffee; 9) pregnant, lactating, or an expected/planned pregnancy during the study period; 10) extremely irregular diet or irregular rhythm of life; 11) heavy alcohol intake (average weekly intake > 60 g/day); 12) participation in another clinical study within the 3 months prior to signing the study’s informed consent document or participation schedule during the study period; 13) donation of more than the limit amount of blood within the 3 months prior to signing the study’s informed consent document; 14) the sum of the blood collection volume within the 12 months prior to signing the study’s informed consent document and during this study exceeded 1200 mL; and 15) anyone judged as ineligible to participate in this study by the principal doctor.

**Intervention:** The daily dose of powdered ELE was set to an equivalent 3.38 mg dose of oenothein B based on an unpublished pilot study which suggested a reducing effect of daily intake of the powdered ELE containing 3.38 mg of oenothein B on visceral fat. Subjects consumed three pieces of placebo capsules (placebo group) or test capsules (intervention group) with water or lukewarm water just before dinner or a snack (consuming food and beverages containing fructose) taken after 3:00 p.m. The rationale for setting the timing for the test food intake are as follows: diet-induced thermogenesis is greater in the morning than in the evening [36], hepatic glycogen decreases by 40% after a night’s sleep [37]; thus, fructose incorporated into the body is quickly converted into glycogen or energy [38] in the morning, generating less chances of lipogenesis at normal intake levels. The intervention period lasted 12 weeks. The period and other conditions were set based on unpublished pilot studies. The amounts of oenothein B in the placebo and intervention groups were 0 and 3.38 mg/day, respectively. The intervention was conducted from March 2021 to July 2021. The schedule for this study is presented in Appendix 1. The subjects were examined on the screening day, set as a baseline and after 4, 8, and 12 weeks of the start of the intervention. A subject was considered compliant if > 80% of the capsules were
consumed according to the prescribed regimen.

All participants were instructed to abide by the following rules during the intervention period: 1) do not alter lifestyle habits and abstain from excessive eating, drinking, or exercise; 2) measure the number of steps using a pedometer every day; 3) do not change daily snacking habits; 4) consume as little “Foods with Health Claims” and/or other foods and beverages with potential physiological functionality as possible; 5) inform the clinic staff and obtain prior permission from the principal doctor of this trial to use any medication, except in emergency situations; and 6) record the consumption time of the capsules, the amount of alcohol intake, sleep time, amount of unusual exercise or physical activity, and menstruation.

The subjects were required to immediately inform the clinical staff about the occurrence or intuition of any adverse events as soon as possible, even if they were tolerable. On the day before visiting the clinic, subjects were prohibited from drinking alcohol or exercising intensely. On the examination day, subjects were asked to abstain from eating, drinking, or smoking for 6 hours prior to the examination and were instructed to visit the clinic without having breakfast, except for water intake (no water or tea with possible physiological effects such as hydrogen water, oxygenated water, and water containing vanadium).

**Dietary record:** Subjects noted their daily food intake for three consecutive days before each examination using the dietary survey method “Calorie and Nutrition Diary (CAND)” Version 1.0 [39]. The ingredients in the capsules were not included in the nutrient intake calculations. The intake of dietary components was defined according to the Standard Tables of Food Composition in Japan 2015 (Seventh Revised Edition) [40] with an available carbohydrate table, [41] and calculated from the dietary survey using the CAND. Each parameter was calculated as the average value per day of the 3-day intake. Subjects recorded the following terms on a daily basis among the classification tables in the CAND as a dietary survey including snacks during the intervention period: no. 1,202 pieces of bread; no. 1,204 pastries and processed pieces of bread; no. 4,102 milk, dairy products, and fermented foods; no. 5,101 fruits; no. 7,101 Japanese sweets; no. 7,102 Western sweets; no. 8,101 soft drinks and carbonated drinks; no. 8,102 fruit juices and beverages; and no. 8,104 other non-alcoholic beverages. The intake of dietary components was calculated from the dietary survey using the CAND. Each parameter was calculated as the average intake per day.

**Measurement:** Subject height and serum nonspecific IgE levels were measured only on the day of screening. At each visit, the subjects underwent physical examinations and physical tests, including body temperature, hematological and biochemical blood tests, and urine tests. Evaluation items were measured on the screening day set as a baseline and after 4, 8, and 12 weeks of initiating the intervention. The fat-containing area of the transverse section of the umbilical region was measured by X-ray CT imaging using a SOMATOM Perspective (Siemens Healthcare GmbH, Erlangen, Germany) to calculate the total fat area (TFA), VFA, and subcutaneous fat area (SFA) of the subjects. Measurements using a body composition analyzer, X-SCAN PLUS (Sowa Medical Co., Fukuoka, Japan), and a multi-frequency segmental body composition analyzer, MC-780A-N (Tanita Co., Tokyo, Japan), were used to measure body weight, BMI, body fat ratio, fat mass, lean mass, and muscle mass. Abdominal circumference was measured with a tape measure placed horizontally at the level of the umbilicus in the standing position. Initially, the height from the ground to the level of the minimum abdominal circumference was recorded with a tape measure fixed to the wall in the standing position [42] on the screening day for the purpose of
measuring waist circumference. Blood pressure and pulse rate were measured using an electronic sphygmomanometer HEM-6022 (Omron Co., Kyoto, Japan). Subjective symptoms related to obesity were evaluated using questionnaires with the Likert scale method, in which a low score indicated lower subjective symptoms. Antecubital venous blood was collected and hematological and biochemical blood parameters, as well as urinalyses were measured at LSI Medience Co. (Tokyo, Japan).

**Enrollment:** A total of 198 eligible subjects (96 men and 102 women) out of 721 (339 men and 382 women) who provided informed consent were included. Subjects were selected by the physician based on the following criteria: 1) subjects who were considered suitable for the study; 2) high fructose intake in the 3-day dietary survey prior to the screening examination; and (3) subjects with a BMI of 23 to < 30 kg/m² and with a VFA of approximately 100 cm².

**Primary outcome:** The primary outcome was the reduction of VFA in the intervention group relative to the placebo group after 12 consecutive weeks of ingestion of the test capsule.

**Secondary outcomes:** The secondary outcomes were as follows: 1) reduction of VFA after eight consecutive weeks of ingesting test capsules. 2) The effect of 8 and 12 consecutive weeks of ingesting the test capsule on the TFA (sum of the AVF and SFA), SFA, body weight, BMI, abdominal circumference, waist circumference, body fat ratio, fat mass, lean mass, muscle mass, and serum biomarkers (total cholesterol [T-Cho], high-density lipoprotein cholesterol [HDL-Cho], low-density lipoprotein cholesterol [LDL-Cho], and TG). (3) Empirical questionnaires related to obesity after 4, 8, and 12 consecutive weeks of ingesting the test capsules.

**Safety evaluation:** Safety evaluations were assessed via physical examinations, physical tests, hematological and biochemical blood analyses, and urinalysis. Biological parameter values outside the normal Japanese range were defined as abnormal, and the possibility that the ingestion of capsules caused the change was assessed. All participants were asked to complete a medical questionnaire to understand their health conditions. Additionally, the subjects were asked to keep a daily journal about their health condition, usage of medication, and lifestyle.

**Sample size:** We did not find any previous studies of consecutive ELE ingestion for 12 weeks in healthy subjects conducted based on those methods. In this study, the required number of subjects was calculated to be 172 (86 subjects in each group) based on the following conditions: differences in VFA between the two groups were assumed to be “moderate”; effect size was set to 0.50 based on Cohen’s d [43]; the significance level α at 5% by an unpaired Student’s t-test and a probability (1-β) at 0.90. Finally, we set the number of subjects required per group to 99, considering the expected dropouts and the possibility of coronavirus disease 2019 (COVID-19) infection in subjects during the study period.

**Randomization and Blinding:**

a) **Sequence Generation:** An allocation manager, who was not directly involved in the study, used a computer to generate random numbers and create an allocation list using a completely randomized design, with the defined variables as factors. Subjects were randomly allocated on the same day to either the intervention or placebo group (each, n = 99) based on a stratified randomized allocation of 1) VFA, 2) BMI, 3) fructose intake in the 3-day dietary survey prior to the screening examination, 4) age, and 5) sex.

b) **Allocation Concealment Mechanism:** This study was double-blinded, and blinding was performed using
indistinguishable capsules. After confirming the indistinguishability between the test and placebo capsules, a code was assigned by the person in charge of shipping from ORTHOMEDICO Inc. to the allocation manager. The allocation manager also created emergency keys along with the allocation list using the code of the capsules provided. The created allocation list was only provided to the person in charge of shipping belonging to the contract research organization ORTHOMEDICO Inc. The emergency key was placed in an envelope marked with the date of sealing for each participant, and the envelope was affixed with a stamp after sealing.

The allocation table and emergency keys were secured by the allocation manager until the key-opening day. No individual related to the trials was aware of the group assignments or involved in the allocation.

**Determination of clinical significance:** Clinical significance was determined on the basis of FOSHU, which is an approval system for the health functions of foods in Japan [31,44]. The tested ELE was considered to have clinically meaningful improvements if it was found to be as effective or more effective than FOSHU. We calculated group differences and their 95% confidence intervals (CI) from the literature based on the effects of FOSHU in the reduction of visceral fat.

A difference of $-0.82 \text{ cm}^2$ between the two groups [45] was the upper limit of the 95%CI in the trials where a significant difference was detected using the amount of change from the baseline as the dependent variable and was closest to zero. The lower limit of the 95%CI was $-23.28 \text{ cm}^2$ [46], which was the smallest value. Therefore, the upper limit of the 95%CI of the difference between the two groups for the primary outcome (VFA) at the final time point (12 weeks after the start) was determined to be equivalent to FOSHU when it was $\leq -0.82 \text{ cm}^2$. It was determined to be more effective than FOSHU when it was $<-23.28 \text{ cm}^2$.

**Statistical Analysis:** All statistical analyses were two-sided. Non-outcome data were expressed as mean ± standard deviation. The baseline data were compared between the two groups using the Student’s t-test. Sex, as well as data from the hematological, biochemical, and urinalysis tests were assigned codes in which “1” was identified as within the normal range (for men), and “0” represented values outside the normal range (for women). These data are expressed as the number of subjects (n) and the ratio of sex; they were compared between groups using the $\chi^2$ test for each sequence. The results for continuous variables used for outcomes are expressed as mean ± standard error (SE), estimated marginal means (EMM), SE based on EMM, and 95% CI. The EMM of the difference was obtained by subtracting the value of the placebo group from that of the intervention group. The data at each time point and the changes from baseline were compared between groups by time in linear mixed models with baseline values as covariates, time, group, the interaction between time and group, the interaction between baseline values and time, and study participants as factors in each sequence. The data at each time point were compared within groups by time in linear mixed models with time and study participants as factors in each sequence. The average intakes of fructose and sucrose per day were compared between the two groups using the Student’s t-test. Subjective symptoms related to obesity, quantified by the questionnaire, were compared between groups at each sequence using Mann–Whitney U test. The significance level was set at 5%. SPSS ver. 23 (IBM Japan, Ltd., Tokyo, Japan) was used for analysis.

**RESULTS**

**Subject background information:** Figure 1 presents the study flow from enrollment to analysis according to CONSORT 2010 Statement guidelines [47]. The subjects were recruited from January 2021 to May 2021. The intervention was completed in July 2021. Of the 721 individuals who underwent screening, 198 were
considered eligible and randomly allocated to the placebo group and intervention group. The full analysis set (FAS) and safety analysis population (SAF) were fixed for 96 subjects in the placebo group and 98 patients in the intervention group because three and one subjects in the placebo and the intervention groups, respectively, dropped out without participation in the 4-week study and subsequent examination. The number of subjects who consumed < 80% of the capsules was one in the placebo group and four in the intervention group. Finally, 95 subjects in the placebo group and 94 subjects in the intervention group completed the trial and were set as the per-protocol set (PPS) shown in Table 1. There were no significant differences in the baseline data between the two groups.

Figure 1. Flowchart for study participants.
Table 1. Baseline characteristics of subjects (PPS).

| Parameter (unit)       | Placebo group (n = 95) | Intervention group (n = 94) | P value |
|------------------------|------------------------|----------------------------|---------|
| Sex\(^1\) (Male)      | 45 (47.4%)             | 45 (47.9%)                 | 1.000   |
| (Female)               | 50 (52.6%)             | 49 (52.1%)                 |         |
| Age\(^2\) (Years)     | 47.7 ± 10.4            | 46.5 ± 10.7                | 0.453   |
| Height\(^2\) (cm)     | 164.6 ± 8.9            | 164.4 ± 8.5                | 0.923   |
| Nonspecific IgE\(^2\) (IU/mL) | 328.3 ± 1373.0       | 352.2 ± 1269.4             | 0.901   |

\(^1\) Data are expressed as the number of subjects (n) and the sex ratio; they were compared between the two groups using the χ\(^2\) test.

\(^2\) Data are presented as the mean ± SD and were compared between the two groups using the Student’s t-test.

**Primary outcome**: Figures 2 and 3 present the VFA results. The intervention group exhibited significantly lower VFA scores after 12 weeks of intervention compared to the placebo group (P = 0.012). The difference between the scores of the two groups after 12 weeks was −5.6 cm\(^2\) (95%CI, −10.0 to −1.3). Comparing the changes in VFA over time (Table 2), we found that VFA after 12 weeks decreased significantly by 6.5 cm\(^2\) in the intervention group compared to baseline (P = 1.5 × 10\(^{-5}\)). However, no significant change was observed in VFA in the placebo group (P = 0.561).

![Graphs showing changes in VFA](image_url)

**Figure 2.** Changes in visceral fat area (VFA). (a) Measured values; (b) Changes in values. Data are presented as the mean ± SE. The supplemental data are presented in Appendix 2. The data of each time and the changes from baseline were compared between groups by time in linear mixed models with baseline values as covariates and time, group, the interaction between time and group, the interaction between baseline values and time, and study participants as factors. (a) VFA at baseline was compared between the two groups using the Student’s t-test. The P-values at baseline and 4 weeks were 0.946 and 0.223, respectively. (b) The P value at 4 weeks was 0.223.
Figure 3. Differences in changes in visceral fat area (VFA) between the placebo and intervention group. Data are presented as the estimated marginal means (EMM) of the difference value and the 95% CI (CI− to CI+). The supplemental data are presented in Appendix 2. EMM of the difference value was obtained by subtracting the value of the placebo group from that of the intervention group. The P value at 4 weeks was 0.223.

Table 2. Intragroup differences in physical examination parameters.

| Parameter (Unit) | Intervention period | Placebo group (n = 95) | P value | Intervention group (n = 94) | P value |
|------------------|---------------------|------------------------|---------|-----------------------------|---------|
|                  |                     | Difference | 95%CI    |                     | Difference | 95%CI    |
| VFA (cm²)        | 4 weeks             | −1.0       | −3.7, 1.8 | 0.482              | −3.7      | −6.6, −0.8 | 0.011*   |
|                  | 8 weeks             | −1.2       | −4.0, 1.5 | 0.384              | −5.7      | −8.6, −2.8 | 1.3 × 10^{-4}* |
|                  | 12 weeks            | −0.8      | −3.6, 1.9 | 0.561              | −6.5      | −9.3, −3.6 | 1.5 × 10^{-5} |
| SFA (cm²)        | 4 weeks             | 0.2        | −3.5, 4.0 | 0.906              | −0.7      | −4.2, 2.8  | 0.705    |
|                  | 8 weeks             | 0.5        | −3.3, 4.2 | 0.801              | 2.3       | −1.2, 5.8  | 0.198    |
|                  | 12 weeks            | 1.5        | −2.3, 5.2 | 0.445              | 2.9       | −0.6, 6.4  | 0.109    |
| TFA (cm²)        | 4 weeks             | −0.8       | −5.6, 4.1 | 0.759              | −4.4      | −9.1, 0.3  | 0.068    |
|                  | 8 weeks             | −0.7       | −5.6, 4.1 | 0.765              | −3.4      | −8.1, 1.4  | 0.161    |
|                  | 12 weeks            | 0.6        | −4.2, 5.5 | 0.793              | −3.6      | −8.3, 1.2  | 0.138    |

Data at each time point were compared within groups by time in linear mixed models with time and study participants as factors. The EMM and the 95%CI are presented in Appendix 3. * P < 0.05.

Secondary outcomes: VFA at 8 weeks after starting intervention in the intervention group significantly decreased compared to that in the placebo group (P = 0.046), as shown in Figures 2 and 3. Comparing the changes in VFA over time (Table 2), we found that VFA after 8 weeks in the intervention group decreased significantly by 5.7 cm² compared to baseline (P = 1.3 × 10^{-4}); in contrast, no significant change was observed in
VFA in the placebo group ($P = 0.384$). The other measurement data for the secondary outcomes are presented in Tables 3 and 4, and Appendix 4. Waist circumference after 8 weeks and body weight, BMI, and muscle mass after 12 weeks in the intervention group decreased significantly compared with that in the placebo group ($P = 0.022, 0.024, 0.020, \text{ and } 0.011$, respectively; Table 3). Data obtained from the empirical questionnaires related to obesity were omitted as there were no significant differences in the results between the two groups. The data at each time point were compared to the baseline within groups by time (Tables 2 and 4). LDL-Cho showed a significant decrease over time in the intervention group only after 8 and 12 weeks ($P = 0.024$ and 0.0029, respectively). The intervention group showed a significant reduction in body weight and BMI after 8 weeks ($P = 0.046$ and 0.041, respectively), and in abdominal circumference, T-Cho, and HDL-Cho ($P = 0.035$, $4.9 \times 10^{-4}$, and 0.046, respectively) after 12 weeks. A significant reduction was observed in body weight and BMI after 12 weeks in both the intervention group ($P = 1.6 \times 10^{-5}$ and $1.8 \times 10^{-5}$, respectively) and the placebo group ($P = 1.0 \times 10^{-4}$ and $1.5 \times 10^{-4}$, respectively). Significant reduction was observed in both intervention group and placebo group after 8 weeks in body fat ratio ($P = 1.5 \times 10^{-8}$ and $1.8 \times 10^{-10}$, respectively) and in fat mass ($P = 6.2 \times 10^{-7}$ and $1.5 \times 10^{-6}$, respectively), and after 12 weeks in body fat ratio ($P = 5.9 \times 10^{-10}$ and $3.3 \times 10^{-13}$, respectively) as well as fat mass ($P = 9.0 \times 10^{-9}$ and $1.2 \times 10^{-11}$, respectively). However, the placebo group showed a significant increase in waist circumference, lean mass, and muscle mass after 8 weeks ($P = 0.044, 3.1 \times 10^{-6}$, and $4.1 \times 10^{-6}$, respectively), and in lean mass and muscle mass after 12 weeks ($P = 5.1 \times 10^{-5}$ and $2.9 \times 10^{-5}$, respectively).
Table 3. Intergroup differences in parameters obtained from physical examination and biochemical analysis between groups.

| Parameter (unit)                  | Baseline¹ | 4 weeks² | 8 weeks² | 12 weeks² |
|-----------------------------------|-----------|----------|----------|-----------|
|                                   | Difference| 95%CI    | P value  | Difference| 95%CI    | P value  | Difference| 95%CI    | P value  |
| Body weight (kg)                  | 0.0       | −2.6, 2.6| 0.983    | −0.2     | −0.8, 0.5| 0.623    | −0.3     | −1.0, 0.3| 0.340    | −0.7     | −1.4, −0.1| 0.024*   |
| ΔBody weight (kg)                 | −         | −        | −        | −0.2     | −0.8, 0.5| 0.623    | −0.3     | −1.0, 0.3| 0.340    | −0.7     | −1.4, −0.1| 0.024*   |
| BMI (kg/m²)                       | 0.1       | −0.5, 0.6| 0.809    | −0.1     | −0.3, 0.2| 0.546    | −0.1     | −0.4, 0.1| 0.287    | −0.3     | −0.5, 0.0| 0.020*   |
| ΔBMI (kg/m²)                      | −         | −        | −        | −0.1     | −0.3, 0.2| 0.546    | −0.1     | −0.4, 0.1| 0.287    | −0.3     | −0.5, 0.0| 0.020*   |
| Abdominal circumference³ (cm)     | −0.1      | −1.9, 1.7| 0.942    | −0.1     | −1.2, 1.0| 0.835    | −0.5     | −1.5, 0.6| 0.412    | −0.1     | −1.2, 1.0| 0.842    |
| ΔAbdominal circumference³ (cm)    | −         | −        | −        | −0.1     | −1.2, 1.0| 0.835    | −0.5     | −1.5, 0.6| 0.412    | −0.1     | −1.2, 1.0| 0.842    |
| Waist circumference⁴ (cm)         | 0.6       | −1.2, 2.5| 0.493    | −0.4     | −1.4, 0.7| 0.492    | −1.2     | −2.2, −0.2| 0.022¹   | −0.5     | −1.5, 0.6| 0.374    |
| ΔWaist circumference⁴ (cm)        | −         | −        | −        | −0.4     | −1.4, 0.7| 0.492    | −1.2     | −2.2, −0.2| 0.022¹   | −0.5     | −1.5, 0.6| 0.374    |
| Body fat ratio (%)                | 0.4       | −1.7, 2.5| 0.685    | −0.3     | −0.9, 0.2| 0.273    | 0.2      | −0.3, 0.8| 0.385    | 0.0      | −0.5, 0.6| 0.939    |
| ΔBody fat ratio (%)               | −         | −        | −        | −0.3     | −0.9, 0.2| 0.273    | 0.2      | −0.3, 0.8| 0.385    | 0.0      | −0.5, 0.6| 0.939    |
| Fat mass (kg)                     | 0.4       | −1.1, 1.8| 0.615    | −0.3     | −0.8, 0.2| 0.288    | 0.0      | −0.5, 0.5| 0.916    | 0.1      | −0.4, 0.6| 0.803    |
| ΔFat mass (kg)                    | −         | −        | −        | −0.3     | −0.8, 0.2| 0.288    | 0.0      | −0.5, 0.5| 0.916    | 0.1      | −0.4, 0.6| 0.803    |
| Lean mass (kg)                    | −0.4      | −3.1, 2.4| 0.797    | 0.5      | −0.1, 1.0| 0.078    | −0.3     | −0.8, 0.2| 0.281    | −0.3     | −0.8, 0.2| 0.197    |
| ΔLean mass (kg)                   | −         | −        | −        | −0.3     | −0.9, 0.2| 0.273    | 0.2      | −0.3, 0.8| 0.385    | 0.0      | −0.5, 0.6| 0.939    |
| Muscle mass (kg)                  | −0.4      | −3.0, 2.2| 0.760    | 0.1      | −0.5, 0.7| 0.699    | −0.2     | −0.9, 0.4| 0.444    | −0.8     | −1.4, −0.2| 0.011*   |
| ΔMuscle mass (kg)                 | −         | −        | −        | 0.1      | −0.5, 0.7| 0.699    | −0.2     | −0.9, 0.4| 0.444    | −0.8     | −1.4, −0.2| 0.011*   |
| T-Cho (mg/dL)                     | 4.6       | −5.8, 14.9| 0.386   | −4.1     | −10.2, 2.1| 0.193    | −2.2     | −8.4, 3.9| 0.475    | −3.2     | −9.4, 2.9| 0.300    |
| ΔT-Cho (mg/dL)                    | −         | −        | −        | −4.1     | −10.2, 2.1| 0.193    | −2.2     | −8.4, 3.9| 0.475    | −3.2     | −9.4, 2.9| 0.300    |
| HDL-Cho (mg/dL)                   | 2.2       | −2.6, 7.1| 0.363    | −0.7     | −2.7, 1.2| 0.458    | −0.8     | −2.7, 1.2| 0.448    | 0.2      | −1.8, 2.1| 0.878    |
| ΔHDL-Cho (mg/dL)                  | −         | −        | −        | −0.7     | −2.7, 1.2| 0.458    | −0.8     | −2.7, 1.2| 0.448    | 0.2      | −1.8, 2.1| 0.878    |
| LDL-Cho (mg/dL)                   | 3.5       | −5.9, 12.8| 0.464  | −6.4     | −12.1, −0.8| 0.026² | −4.0     | −9.7, 1.6| 0.163    | −4.4     | −10.1, 1.3| 0.128    |
| ΔLDL-Cho (mg/dL)                  | −         | −        | −        | −6.4     | −12.1, −0.8| 0.026² | −4.0     | −9.7, 1.6| 0.163    | −4.4     | −10.1, 1.3| 0.128    |
| TG (mg/dL)                        | 0.1       | −23.7, 23.9| 0.991 | 15.8     | −7.9, 39.5| 0.190    | 8.2      | −15.5, 31.9| 0.496   | 3.0      | −20.7, 26.8| 0.801   |
| ΔTG (mg/dL)                       | −         | −        | −        | 15.8     | −7.9, 39.5| 0.190    | 8.2      | −15.5, 31.9| 0.496   | 3.0      | −20.7, 26.8| 0.801   |

The measurement values are presented in Appendix 5.

¹ The difference value was obtained by subtracting the value of the placebo group from that of the intervention group. Data represent the different values and 95%CI (95%CI−, 95%CI+) compared between the two groups using the Student’s t-test.

² Data for each time point and the amount of change from baseline were compared between groups by time, in linear mixed models, with baseline values as covariates, and with time, group, interaction between time and group, interaction between baseline values and time, and study participants as factors.

³ The circumference at the level of the umbilicus was recorded.

⁴ The minimum abdominal circumference was recorded.

* P < 0.05
Table 4. Intragroup differences in parameters obtained from physical examination and biochemical analysis.

| Parameter (Unit) | Placebo group (n = 95) | Intervention group (n = 94) |
|------------------|------------------------|-----------------------------|
|                  | 4 weeks | 8 weeks | 12 weeks | 4 weeks | 8 weeks | 12 weeks |
|                  | Difference | 95%CI | P value | Difference | 95%CI | P value | Difference | 95%CI | P value |
| Body weight (kg) | -0.2 | -0.5, 0.1 | 0.198 | -0.3 | -0.6, 0.0 | 0.050 | -0.6 | -0.9, -0.3 | 1.0 × 10^{-4} |
| BMI (kg/m²)      | -0.1 | -0.2, 0.0 | 0.267 | -0.1 | -0.2, 0.0 | 0.056 | -0.2 | -0.3, -0.1 | 1.5 × 10^{-4} |
| Abdominal circumference (cm) | -0.4 | -1.1, 0.3 | 0.304 | -0.1 | -0.8, 0.6 | 0.750 | -0.7 | -1.4, 0.0 | 0.063 |
| Waist circumference (cm) | 0.4 | -0.2, 1.0 | 0.184 | 0.6 | 0.0, 1.2 | 0.044* | 0.0 | -0.6, 0.5 | 0.881 |
| Body fat ratio (%) | -0.6 | -1.0, -0.3 | 1.7 × 10^{-4} | -1.1 | -1.5, -0.8 | 1.8 × 10^{-6} | -1.3 | -1.6, -1.0 | 3.3 × 10^{-11} |
| Fat mass (kg)    | -0.5 | -0.8, -0.2 | 8.4 × 10^{-4} | -0.9 | -1.1, -0.6 | 1.5 × 10^{-5} | -1.0 | -1.3, -0.8 | 1.2 × 10^{-11} |
| Lean mass (kg)   | 0.3 | 0.1, 0.5 | 9.3 × 10^{-3} | 0.5 | 0.3, 0.8 | 3.1 × 10^{-4} | 0.5 | 0.2, 0.7 | 5.1 × 10^{-4} |
| Muscle mass (kg) | 0.3 | 0.1, 0.5 | 9.1 × 10^{-3} | 0.5 | 0.3, 0.7 | 4.1 × 10^{-4} | 0.4 | 0.2, 0.7 | 2.9 × 10^{-4} |
| T-Cho (mg/dL)    | 2.8 | -1.7, 7.3 | 0.218 | -0.8 | -5.2, 3.7 | 0.732 | -3.2 | -7.7, 1.2 | 0.157 |
| HDL-Cho (mg/dL)  | 1.0 | -0.4, 2.4 | 0.144 | 0.1 | -1.3, 1.5 | 0.868 | -1.2 | -2.6, 0.1 | 0.076 |
| LDL-Cho (mg/dL)  | 2.7 | -1.1, 6.6 | 0.166 | -0.2 | -4.0, 3.7 | 0.936 | -0.9 | -4.8, 2.9 | 0.638 |
| TG (mg/dL)       | 1.6 | -9.4, 12.6 | 0.774 | -1.4 | -12.4, 9.6 | 0.806 | -3.5 | -14.5, 7.5 | 0.534 |

The measurement values are presented in Appendix 6. Data at each time point were compared within groups by time, in linear mixed models, with time and study participants as factors in each sequence.

1 The circumference at the level of the umbilicus was recorded.
2 The minimum abdominal circumference was recorded.
* P < 0.05.

**Dietary record:** The average intakes of fructose and sucrose per day for three consecutive days (average over the total for 12 days) were 19.3 ± 10.5 g/day and 46.3 ± 16.9 g/day in the placebo group and 18.3 ± 8.1 g/day and 45.0 ± 15.9 g/day in the intervention group, respectively.

There was no significant difference in each carbohydrate intake between the two groups. Moreover, no significant differences were observed in the average intakes of fructose and sucrose per day recorded daily during the intervention period between the two groups (Data not shown).

**Adverse Events:** Serious adverse events were not observed. The incidence of adverse events was 8.3% and 8.2% in the placebo (n = 96) and intervention (n = 98) groups, respectively (eight events per group; Appendix 7).
These symptoms were considered by the principal doctor to be mild since they were temporary, and reversible by medication. The doctor confirmed that these phenomena were not causally related to the test capsules. As for the results of hematology, blood biochemistry tests, and urinalysis, significant differences in several parameters were observed between the two groups (Appendices 8–11); however, the doctor confirmed that these changes were not related to the test capsules.

**DISCUSSION**

In this randomized, placebo-controlled, double-blind, parallel-group study, we examined the effect of consecutive ELE consumption for 12 weeks at a dose of 3.38 mg/day oenothein B equivalent on VFA as the primary outcome. The parameters were compared between the test group and a placebo group in healthy Japanese adults whose BMI was ≥ 23 and < 30 kg/m². VFA in the intervention group significantly decreased compared to that in the placebo group at 12 weeks after starting the intervention (Figures 2 and 3). A reduction in visceral fat ameliorates the risk of obesity-related metabolic disorders [48]. The clinical significance of the results obtained in this study was determined according to the criteria described in the Section “Determination of clinical significance”. The difference in the change in VFA between the two groups, 12 weeks after intervention initiation was −5.6 cm² (95%CI, −10.0 to −1.3), which is lower than the upper CI limit (−0.82 cm²). Therefore, ELE containing oenothein B was determined to be as effective as FOSHU in reducing visceral fat, and this effect represented a clinically meaningful improvement.

Although dietary fructose is partially metabolized to other substances in the small intestine, it is largely metabolized in the liver. As mentioned above, fructose is metabolized without regulation as it bypasses 6-phosphofructokinase, the major rate-limiting step in glycolysis [1]. Fructose provides carbon atoms for both the glycerol and acyl portions of the TG molecule and serves as a source of acetyl-CoA. Therefore, glucose-6-phosphate dehydrogenase and fatty acid synthase are activated, resulting in a marked increase in TG levels in the liver [2,3,49]. Since TG is transported to adipocytes by very-low-density lipoprotein, elevated TG synthesis leads to visceral fat accumulation [50]. Stanhope et al. [6] reported that consuming fructose-containing beverages for 10 weeks increases visceral fat in obese individuals. Moreover, a 10-week intake of fructose reportedly reduces fat oxidation and lowers resting energy expenditure [51]. It was recently reported that the tendency to gain weight depends on the calorie source, unrelated to calorie intake [52]. Reducing the consumption of fructose, by replacing sucrose with starch, decreases liver fat and visceral fat in children with obesity without reducing calories or losing weight within a short period [17]. Reducing dietary fructose intake by inhibiting intestinal fructose absorption may result in effective visceral fat accumulation, caused by excessive fructose consumption. We previously reported that ELE and its constituent, oenothein B, inhibits fructose absorption in vitro [30]. In addition, we confirmed that ELE lowers ketohexokinase (fructokinase) and glucose-6-phosphate dehydrogenase activity, suppressing the accumulation of fat in the liver and visceral regions in rats [28]. Since fructose intake in the subjects from this study was estimated to be relatively high, some of the visceral fat accumulation was assumed to be due to fructose intake. In this study, ELE containing oenothein B may have reduced visceral fat accumulation by inhibiting intestinal fructose absorption and TG synthesis in the liver. Consecutive intake of fructose reportedly promotes de novo lipogenesis in the liver in lean men with a BMI < 24 kg/m² [3]. Thus, considering that ELE suppresses hepatic lipogenesis [28], ingestion of ELE containing oenothein B may reduce VFA in lean men with a BMI < 24 kg/m².
measuring waist circumference [53,54], herein, it was measured at the level of the umbilicus, as this method is commonly used as a diagnostic criterion in Japan in conjunction with the transverse section area of the umbilical region measured by CT [55]. Since average people perceive “waist” as the slenderest portion of the torso in Japan, we have defined the minimum abdominal circumference, the position of which is defined by Kouchi et al. [56], as waist circumference. Among the secondary outcomes, the intervention group showed significantly lower body weight, BMI, and muscle mass at 12 weeks, and lower VFA and waist circumference at 8 weeks compared to the placebo group. The intragroup comparison over time was conducted for both groups. The VFA after 8 and 12 weeks in the intervention group decreased significantly compared to baseline, while no such change was observed in the placebo group, similar to the intergroup comparison. Moreover, significant reductions in body weight and BMI were observed in the intervention group and occurred more quickly than in the placebo group. LDL-Cho in the intervention group consistently and significantly decreased after 4 to 12 weeks, with no significant changes in the placebo group. In contrast, muscle mass after 12 weeks increased significantly by 0.4 kg in the placebo group compared to the baseline, while it decreased by 0.3 kg, however, with no significant difference, in the intervention group. According to a previous study [57], the muscle mass of the Japanese population fluctuates by approximately 1–3 kg based on the age group. However, a change in muscle mass of ≥ 2 kg is considered clinically significant in daily life. Therefore, the significant difference in muscle mass between the two groups identified in this study was not considered clinically significant. Meanwhile, the values of certain parameters measured after intervention initiation were significantly altered compared to the baseline values in both groups (body fat ratio and fat mass after 8 weeks, and body fat ratio, fat mass, body weight, and BMI after 12 weeks), or only in the placebo group (lean mass and muscle mass after 8 and 12 weeks). The reason for this reduction in the placebo group was unclear; however, a similar phenomenon was observed in other trials [58,59]. One reason for this could be that the subjects diligently followed the rules of this protocol, leading to a more regular lifestyle compared to that before the study. Seasonal variations can also affect the parameters [60,61].

No serious adverse events were observed during or after the intervention. The principal doctor confirmed that the changes in certain physical, hematology, blood biochemistry, and urinalysis parameters were not related to the test capsules. Hence, the intake of ELE containing oenothein B was found to be safe.

In this study, CAND was used as a dietary survey method, which was simplified into three intake levels: high, normal, and low, for easy understanding and reporting by the recorders. CAND showed a statistically significant positive correlation with 45 nutrients, including calories, compared to the photographic dietary assessment [39] and exhibited a positive correlation with the photographic method in saccharides (unpublished data). The subjects in this study had an average energy intake of 2,808.9 ± 682.0 g/day in the placebo group and 2,867.0 ± 798 g/day in the intervention group for three days prior to the screening examination. As the daily energy requirement for both groups was approximately 1,930 kcal/day, as calculated by the Long method [62] (data not shown), the subjects in this study were an over-energetic group. The average intake of total fructose for three days was estimated to exceed 40 g/day in both two groups. Sweetened beverages and/or sugars consumption has been found to be correlated with obesity and related diseases in Japan [14,63-65]. Kobayashi et al. [66] reported that Japanese patients with NAFLD preferentially consume fruit and confectionery. Acute onset diabetic ketosis (‘soft-drink ketosis’), caused
by long-term consumption of large amounts of sweetened beverages (>2 L/day over several months) is occurring among young men, namely “soft-drink ketosis” [67]. Hence, it is speculated that a percentage of the Japanese population should limit their intake of fructose.

ELE containing oenothein B reduced VFA on consecutive ingestion for 12 weeks in a randomized, placebo-controlled, double-blind, parallel-group study with many subjects. This study indicated that ELE containing oenothein B lowers the risk of obesity while consumption of fructose-containing food and beverages. Many anti-obesity agents are described by mechanisms such as energy expenditure, α-glucosidase inhibitory activity, and lipase inhibitory activity [20,21,68]. To the best of our knowledge, this is the first report of the anti-obesity effects of a fructose absorption inhibitor in humans. Inhibiting the fructose absorption by the body via ingestion of ELE is clinically significant for people who should limit their fructose intake for the following reasons. 1) It is desirable to consume an appropriate amount of fructose but difficult to control the amount of its intake in the age where people eat until they are full and satisfied. 2) Eating is one of the greatest pleasures for humans, and severe restriction of diet will cause dissatisfaction and stress from improper intake of tasty foods. As such, ELE containing oenothein B, which suppresses fructose absorption, is expected to reduce the risk of fructose-induced obesity and maintain quality of life. ELE containing oenothein B can be supplied as capsules and tablets, as well as in foods containing fructose and sucrose, such as nutrition bars, cereals, chocolate, and soft drinks. It may also be used in combination with α-glucosidase inhibitors. However, because oenothein B has a strong astringency as a hydrolyzable tannin, masking techniques are important for its inclusion in such food and beverage products. ELE containing oenothein B could be submitted to a health food certification system such as the Foods with Function Claims system in Japan [31,32] because this study was conducted in accordance with the guidelines of the FOSHU system [27]. In addition, this product could also be approved for a functional food certification program in the United States. The Functional Food Center (FFC; Dallas, TX) suggests the definitions, classification, and regulation of Functional Foods to solve the problem of the lack of a defined definition of Functional Foods, and to ensure that consumers are supplied with safe and effective products [44]. With the publication of this study, ELE containing oenothein B will fulfill up to Step 8 of the proposed functional food development process. If the FFC’s proposed regulations are enforced, ELE oenothein B will be a reliable functional food ingredient that can help maintain the health of those in the United States who consume excessive amounts of fructose.

This study has certain limitations. First, consecutive ELE consumption at a dose of 3.38 mg/day oenothein B equivalent, decreased VFA at 8 and 12 weeks in healthy Japanese adults whose BMI was ≥23 and <30 kg/m²; therefore, the effects of doses lower than those used in this study, or intakes longer than 12 weeks, remain unclear. Second, intake of only one dose of ELE containing oenothein B was implemented. Therefore, the dose-dependent manner of ELE containing oenothein B intake in humans was not assessed. Third, the clinical significance of the effects was determined based on criteria applied to Japanese patients. It is, therefore, necessary to conduct comprehensive clinical research on ELE containing oenothein B by expanding the scope of various conditions.

CONCLUSION

Overall, this study showed that ELE containing oenothein B reduces VFA by consecutive ingestion for 12 weeks in healthy Japanese adults with a BMI ≥ 23 and < 30 kg/m². The effect was determined to be as effective as FOSHU and was considered a clinically meaningful improvement.
More specifically, ELE containing oenothein B is considered to reduce fructose intake-induced visceral fat. ELE was safe for use in this study.

**List of Abbreviations:** ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMY, serum amylase; AST, aspartate aminotransferase; BASO, basophil; BMI, Body mass index; BUN, blood urea nitrogen; CAND, Calorie and Nutrition Diary; ChE, cholinesterase; CI, confidence interval; CK, creatine kinase; COVID-19, coronavirus disease 2019; Cre, creatinine; CT, computed tomography; D-BIL, direct bilirubin; ELE, Eucalyptus leaf extract; EMM, estimated marginal means; EOS, eosinophil; FAS, full analysis set; Fe, serum iron; FFC, the Functional Food Center; FOSHU, Food for Specified Health Uses; GA, glycoalbumin; γ-GT, γ-glutamyl transpeptidase; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-Cho, high-density lipoprotein cholesterol; HPLC, high-performance liquid chromatography; Ht, hematocrit; I-BIL, indirect bilirubin; IgE, immunoglobulin E; IP, inorganic phosphorus; LAP, leucine aminopeptidase; LD, lactate dehydrogenase; LDL-Cho, low-density lipoprotein cholesterol; LYMP, lymphocyte; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; NGSP, The National Glycohemoglobin Standardization Program; MCV, mean corpuscular volume; MONO, monocyte; NAFLD, nonalcoholic fatty liver disease; NEUT, neutrophil; PLT, platelets; PPS, per-protocol set; PTFE, polytetrafluoroethylene; RBC, red blood cells; SAF, safety analysis population; SD, standard division; SE, standard error; SFA, subcutaneous fat area; T-BIL, total bilirubin; T-Chol, total cholesterol; TFA, total fat area; TG, triacylglycerols; TP, total proteins; UA, uric acid; VFA, visceral fat area; WBC, white blood cells.

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**Competing Interests:** KS, HF, HT, and KN are employees of Nagaoka Co. Ltd. The sponsor of this study, Nagaoka Co., Ltd., entrusted ORTHOMEDICO Inc. with conducting the study. KY is the head of ORTHOMEDICO Inc. NS, SY, YT, TK, and AB are employees of ORTHOMEDICO Inc. TT (MD), the principal investigator of this study, is the director of Medical Corporation Seishinkai, Takara Clinic, and he monitored all the conditions of the subjects. TY has no conflicts of interest to declare.

**Authors’ Contributions:** Conceptualization, KS, HF, SY, YT, TK, AB, and TT; data curation, KS and HT (HPLC analyses), and TK and TT (Clinical study); formal analysis, KS and HT (HPLC analyses) and TK (Clinical study); investigation, KS, HT, and TT; methodology, KS, HT, SY, YT, AB, and TT; project administration, KN, KY, and TT; supervision, KN, JY, and NS; writing—original draft preparation, KS; writing—review and editing, KS, HF, SY, YT, TK, AB, TT, and TY All authors have read and agreed to the published version of the manuscript

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