The effects of Xanthigen™ in the weight management of obese premenopausal women with non-alcoholic fatty liver disease and normal liver fat

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Aim: To investigate the effects of Xanthigen (brown marine algae fucoxanthin + pomegranate seed oil (PSO)) on body weight, body fat, liver lipids, and blood biochemistry; and Xanthigen and its individual components on resting energy expenditure (REE) in obese, non-diabetic female volunteers with non-alcoholic fatty liver disease (NAFLD) and normal liver fat (NLF) content.

Methods: Sixteen-week, double-blind, randomized, placebo-controlled study. Food record data, body composition, REE (only 41 volunteers with NAFLD) and blood sample analysis were assessed weekly for 16 weeks in 151 non-diabetic, obese premenopausal women with liver fat content above 11% (NAFLD) n = 113, and below 6.5% (NLF) n = 38.

Results: Xanthigen-600/2.4 mg (300 mg PSO + 300 mg brown seaweed extract containing 2.4 mg fucoxanthin) resulted in statistically significant reduction of body weight (5.5 ± 1.4 kg NAFLD group and 4.9 ± 1.2 kg NLF group, p < 0.05), waist circumference (NAFLD group only), body (3.5 ± 1.9 kg NAFLD group, p < 0.001; 3.6 ± 0.7 kg NLF group, p < 0.05) and liver fat content, liver enzymes (NAFLD group only), serum triglycerides and C-reactive protein. Weight loss and reduction in body and liver fat content occurred earlier in patients with NLF than in patients with NAFLD. Fucoxanthin (> 2.4 mg) and Xanthigen-400/1.6 mg (200 mg PSO + 200 mg brown seaweed extract containing 1.6 mg fucoxanthin) significantly increased REE in NAFLD subjects compared to placebo.

Conclusions: Xanthigen promoted weight loss, reduced body and liver fat content, and improved liver function tests in obese non-diabetic women. Xanthigen and Fucoxanthin also increased REE. This product may be considered a promising food supplement in the management of obesity.

Keywords: fucoxanthin, liver fat, pomegranate seed oil, resting energy expenditure, triglycerides, weight loss

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Introduction

Overweight and obese individuals often present with elevated liver and serum triglycerides (TGs), which contribute to the development of non-alcoholic fatty liver disease (NAFLD), insulin resistance, metabolic syndrome and ultimately fuel adiposity and obesity [1–3]. The liver enzymes and indices of chronic inflammation [e.g. serum levels of C-reactive protein (CRP)] also tend to be elevated in the NAFLD condition [4]. The term NAFLD, first used by Ludwig in 1980, refers to an accumulation of TGs and resembles alcoholic liver disease, but occurs in individuals with negligible alcohol consumption [5]. The prevalence of NAFLD in the general population is approximately 9% in Western countries [6]. However, among obese individuals the prevalence of NAFLD ranges from 23 to 31% [7]. Gender plays an important role in the development of NAFLD because this condition is more prevalent among women than men. NAFLD is one of the main forms of chronic liver disease and is recognized as the most common pathology behind the hepatic component of metabolic syndrome [8]. The severity of liver fat accumulation positively correlates with visceral fat content and insulin resistance in both obese and non-obese subjects, suggesting that hepatic fat infiltration in NAFLD may be influenced by visceral fat accumulation regardless of body mass index (BMI) [9].

Weight loss often correlates with improvement in NAFLD [10]. Therefore, in addition to the improvement in body composition, liver fat content and liver function tests, healthier lipid blood profiles and reduced biological markers of inflammation should be targeted in any effective weight management programme.

The purpose of the present study was to evaluate the safety and efficacy of a 16-week weight management programme with a standardized botanical food supplement, Xanthigen, and its individual ingredients, brown seaweed extract with...
Fucoxanthin and punicic acids (PA) from pomegranate seed oil (PSO).

Fucoxanthin is a major carotenoid of edible brown seaweeds [11]. Brown seaweeds are also rich in n-3 fatty acids such as ω-3 18:3-α-linolenic acid and ω-3 20:5 eicosapentaenoic acid [12]. Fucoxanthin has shown weight loss properties in animal studies through several mechanisms:

- Induction of the mitochondrial uncoupling protein 1 (UCP1) [13], which leads to an increased resting energy expenditure (REE) by uncoupling a step during cellular metabolism [14]. Fucoxanthin upregulates the expression of UCP1 gene in white adipose tissue (WAT) contributing to the reduction of WAT and a significant reduction of body weight in KK-Ay mice [15].
- Suppression of adipocyte differentiation and lipid accumulation by inhibiting glycerol-3-phosphate dehydrogenase activity. The glycerol-3-phosphate dehydrogenase knockout animals were found to have a lower BMI, a 40% reduction in the mass of WAT and lower fasting blood glucose levels compared to matching controls [16].
- Downregulation of peroxisome proliferator-activated receptor γ (PPARγ) responsible for adipogenic gene expression [16].

Xanthigen is also a source of omega-3 fatty acids derived from brown seaweed which have recognized TG-lowering effects in rats [12]. Suplemental omega-3 fatty acids have been shown to normalize moderately elevated serum TGs in clinical experiments [17].

PA is a 9-cis, 11-trans conjugated linolenic acid (9c, 11t, 13c-CLNA) and constitutes a major (>70%) component of PSO [18]. TG-lowering and anti-obesity properties have also been reported with administration of PA [19,20]. Experimental diet with 5% PA resulted in a significant (27%) reduction of WAT in rats as compared to control animals [19]. PA has also been shown to suppress the production and secretion of TG and apolipoprotein B100, a low density lipoprotein fraction, in human liver cells in vitro [20].

The objective of this 16-week, randomized, placebo-controlled clinical trial was to investigate the effects of orally administered fucuoxanthin, PSO and their combination (Xanthigen™™) in obese, non-diabetic premenopausal women diagnosed with NAFLD or presenting with normal liver fat (NLF). The subgroup of female volunteers with NAFLD was selected to evaluate a possible effect on REE from the active compounds in this study, as increasing energy expenditure by means of physical activity plays an important role in the treatment of this condition.

Materials and Methods

Patients
The population of the study consisted in obese, premenopausal, non-diabetic females with and without NAFLD who were recruited through the National Academy of Natural Sciences in Russia. A total of 151 volunteers agreed to participate in the weight management and the REE trials, comprising 113 volunteers with NAFLD and 38 with NLF. Seventy-two (n = 72) of the 113 obese non-diabetic premenopausal female participants with NAFLD cluster and 38 with NLF cluster agreed to take part in a double-blind, placebo-controlled, randomized clinical trial using body weight, body and liver fat content and blood chemistry as primary efficacy variables. Common inclusion criterion for both clusters was BMI > 30 kg/m².

Individuals in the NAFLD cluster were randomly assigned in equal numbers to Xanthigen-NAFLD (n = 36) and placebo-NAFLD (n = 36) groups, using a simple randomization procedure. Participants in the NLF cluster were randomly assigned in equal numbers to Xanthigen-NLF (n = 19) and placebo-NLF (n = 19) groups, using the same procedure.

A second group of obese non-diabetic female participants (n = 41) diagnosed with NAFLD, from the original 113 NAFLD group were recruited to take part in a double-blind, placebo-controlled trial with a primary objective of measuring the REE.

The criteria for inclusion in the NAFLD cluster were liver fat content above 11%, serum alanine aminotransferase (ALT) not less than 42 U/l, aspartate aminotransferase (AST) not less than 46 U/l, γ-glutamyltransferase (GGT) not less than 44 U/l, and CRP not less than 6.0 mg/l. The main criterion for inclusion in the NLF cluster was liver fat content below 6.5%.

For all groups, individuals with a positive pregnancy test or diabetes were excluded from the study. Exclusion criteria for the latter were fasting glucose above 100 mg/dl and failing results of a standard oral glucose tolerance test with 75 g of glucose [21]. Individuals with a negative serology for hepatitis B or C were included in the trial. Women taking medications known to influence fat metabolism or women with a history of excessive alcohol consumption (i.e. a daily alcohol intake higher than recommended, which would be expected to affect liver function tests) were excluded from the trial. Upon admission, blood samples were tested for the presence of serum desialylated transferrin, a marker of alcohol consumption [22], and subjects testing positive for desialylated transferrin were excluded from the trial. The test was repeated weekly throughout the trial to ensure compliance.

Using the simple randomization procedure, patients in the REE study (n = 41) were assigned to 11 groups as follows: placebo (n = 3), Xanthigen-200/0.8 mg (100 mg PSO + 100 mg brown seaweed extract containing 0.8 mg fucoxanthin, n = 3), Xanthigen-400/1.6 mg (200 mg PSO + 200 mg seaweed extract containing 1.6 mg fucoxanthin, n = 3), Xanthigen-600/2.4 mg (300 mg PSO + 300 mg seaweed extract containing 2.4 mg fucoxanthin, n = 4), Xanthigen-1000/4 mg (500 mg PSO + 500 mg seaweed extract containing 4 mg fucoxanthin, n = 4), fucoxanthin-1.6 mg (n = 4), fucoxanthin-2.4 mg (n = 4), fucoxanthin-4.0 mg (n = 4), fucoxanthin-8.0 mg (n = 4), PSO-1500 mg (n = 4) and PSO-2000 mg (n = 4) (table 2).

The daily dietary intake of individuals was restricted to 1800 kcal, comprised of 50% carbohydrates, 30% protein and 20% fat. All the food and beverages were provided and labelled as B, L and D for breakfast, lunch and dinner, respectively. The women were also instructed to consume only the food and beverages provided and to keep daily records of food intake.
Participants were directed to take their designated dosages of placebo, PSO, fucoxanthin or Xanthigen, three times a day 15–30 min before meals for 16 weeks, and were required to visit the hospital three times a week for anthropometrical, physiological and biochemical analyses.

Statement of ethics
The authors certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. The final study protocol was approved by the local ethics committee prior to the start of the trial. The women were informed of the trial design both verbally and in writing, and an informed consent for participation was obtained. The study was conducted according to the principles of the declaration of Helsinki, 2000.

Total weight and body fat analysis
Anthropometry, body weight, body fat content and liver fat content were evaluated upon admission and once a week thereafter throughout the trial. Height was measured to the nearest 0.5 cm, body weight to the nearest 25 g with subjects wearing light clothes, and circumferences were taken to the nearest 0.5 cm. A total body scan was performed using dual-energy X-ray absorptiometry (Lunar Radiation, Madison, WI, USA) [23]. Fat-free mass and fat mass were calculated from equations developed in a study using the four-compartment model [24].

Liver fat content analysis
Participants with NAFLD/NLF were screened using magnetic hepatic ultrasound scanning by physicians using an Acuson 128-XP/10 scanner with a 3.5-MHz linear transducer, according to conventional methodology [25]. To quantify liver fat content, image-guided proton magnetic resonance spectroscopy was used. The liver fat percentage was calculated by dividing 100 times Sfat by the sum of Sfat and Swater [25].

Resting energy expenditure measurement
REE was measured by indirect calorimetry, as described previously [26]. Oxygen was measured with an electrochemical oxygen sensor, and carbon dioxide was measured with an infrared carbon dioxide sensor (Ametec Carbon Dioxide Analyzer, Pittsburg, PA, USA). Before each measurement, the instrument was calibrated with a mixture of O2 and CO2 gases. Rates of oxygen consumption (VO2) and carbon dioxide production (VCO2) were calculated and printed out at 1-min intervals. Energy expenditure was derived from VO2 and VCO2 using equations described elsewhere [27]. The reliability was assessed by the coefficient of variation of REE repeated every week. Diastolic and systolic blood pressures were analysed following a method described previously [28].

Blood sample collection and analysis
Venous blood samples were collected in tubes containing sodium EDTA (1 g/l). Blood samples were collected once a week in the morning during the 16-week trial. Plasma samples were prepared within 1 h after blood collection by centrifugation at 600 g for 15 min at 4°C. Blood samples were kept in the dark and on ice until centrifugation. Plasma samples were immediately divided into aliquots and stored under argon at −70°C.

Serum ALT, AST and GGT enzyme levels were assessed upon admission and once a week throughout the trial. Venous blood was drawn on the morning after overnight fasting. Serum levels of AST, ALT and GGT enzymes were analysed using methods, as described previously [29]. CRP was measured using a high-sensitivity two-site enzyme-linked immunosay with a peroxidase-conjugated rabbit antihuman CRP antibody (DK2600, Dako, Glostrup, Denmark) and a polyclonal anti-CRP capture antibody. CRP standard serum was used for calibration. The detection limit of the working range of the assay was established at 0.1 mg/l, as described previously [30]. Serum TG levels were determined using a method described by Bucolo (1973).

Supplements
Each capsule of Xanthigen supplement used in this clinical trial provided a minimum of 100 mg of brown seaweed extract containing 0.8% fucoxanthin, which was suspended in 100 mg of PSO containing a minimum of 70% PA. The same brown seaweed extract with 0.8% fucoxanthin (100 mg) resuspended in olive oil (100 mg) was used to manufacture fucoxanthin soft-gel capsules. Each capsule of placebo and PSO provided 200 mg of olive oil or PSO, respectively. To retain the identical appearance and feel of supplements in the different groups, all soft-gel capsules were made with blue-coloured gelatin. Brown seaweed extract containing a minimum of 0.8% fucoxanthin, PSO and extra-virgin olive oil were provided by Polifenoles Naturales, SL (POLINAT), Spain. All soft-gel supplements were manufactured by the Russian National Center for Professional Sport Performance and Education, Moscow, Russia.

Statistics
Data are presented as mean ± SE. The effect of supplementation on REE was analysed using triplicate measurements. Differences between groups at baseline were examined with one-way ANOVA (Super ANOVA, Abacus Concepts). Differences were considered statistically significant at p < 0.05. The differences between any group and placebo were considered statistically significant at p < 0.05.

Results
Demographics and baseline characteristics
A total of 151 non-diabetic, obese premenopausal women were recruited. One-hundred and thirteen individuals met the NAFLD criteria. Volunteers in this cluster had an average age of 36.7 ± 2.5 years, average body weight of 93.8 ± 2.2 kg, a
body fat content of 42.2 ± 1.9 kg, and plasma TG equal to 193 ± 17 mg/dl. Thirty-eight women had liver fat content below 6.5%. Volunteers in this cluster had an average age of 35.2 ± 3.2 years, an average body weight of 94.2 ± 1.8 kg, a body fat content of 43.0 ± 1.7 kg and plasma TG equal to 176 ± 12 mg/dl. Seventy-two women with NAFLD and 38 women with NLF agreed to participate in the double-blind, placebo controlled, randomized trial evaluating the effect on body weight, body fat and liver fat content. Their body weight ranged between 92 and 96 kg, and their age ranged between 36 ± 2 years. The remainder of individuals in the NAFLD group (n = 41) agreed to take part in a double-blind, placebo-controlled trial with a primary objective to measure the REE. Their average body weight was 91.5 ± 4.4 kg and their average body fat content was 40.4 ± 3.7 kg.

The levels of plasma ALT, AST, GGT enzymes and CRP were evaluated as admission criteria. Serum concentration ranges of ALT, AST and GGT enzymes, serum CRP, and diastolic and systolic blood pressure measurements of subjects participating in the trial are summarized in table 1.

Safety

Xanthigen, fucoxanthin, PSO and placebo (olive oil) were well tolerated by all participants who completed the 16-week clinical trial without any subjective or objective adverse effects reported.

Effect of Xanthigen on body weight

At the time of admission, total body weight and body fat content were not significantly different (p = NS) between Xanthigen-600/2.4 mg and placebo groups either in cluster or across the clusters (table 1).

During the first 5 weeks of the trial, there was no significant change in body weight compared to placebo (figure 1a, b). A statistically significant reduction in body weight was noted after 6 and 8 weeks of the trial in the Xanthigen-NLF (figure 1b) and Xanthigen-NAFLD (figure 1a) groups, respectively.

There was a substantial body weight reduction by the end of the trial (week 16) in both NLF and NAFLD groups. The subjects in the Xanthigen-NAFLD had an average body weight loss of 6.9 ± 1.9 kg, from 94.1 ± 2.1 kg to 87.2 ± 3.7 kg (p < 0.05, compared to baseline); in the placebo group average body weight loss was 1.4 ± 0.7 kg, from 93.5 ± 2.4 kg to 92.1 ± 2.8 kg (p = NS, compared to the baseline). The volunteers in the Xanthigen-NAFLD group lost 5.5 ± 1.4 kg (12.1 lb.) body weight more than in the placebo group (p < 0.05) (figure 1).

The body weight of volunteers in the Xanthigen-NLF group was reduced from 94.5 ± 2.1 kg to 88.2 ± 1.9 kg (p < 0.05); the average body weight in the placebo group was 93.9 ± 1.4 at the beginning of the trial to 92.5 ± 1.5 kg (p = NS) by the end of the trial. The participants in the Xanthigen-NLF group lost 4.9 ± 1.2 kg (10.8 lb) more than the placebo-NLF group (p < 0.05). At the end of the trial, there was no statistically significant difference in body weight loss between Xanthigen-NAFLD and Xanthigen-NLF groups (figure 1).

Effect of Xanthigen on body and liver fat content

The reduction in body weight correlated with the reduction in body fat content. In the Xanthigen-NAFLD group, body fat content decreased from 42.3 ± 2.2 to 37.9 ± 2.9 kg at the end of the trial (p < 0.001, compared to placebo). In the placebo group, body fat content did not change from the beginning of the trial (42.1 ± 1.7 kg) to the end of the trial [41.2 ± 2.3 kg (p = NS)]. The Xanthigen-NAFLD group lost 3.5 ± 1.9 kg (7.72 lb.) more body fat than in the placebo group.

In the Xanthigen-NLF group, body fat content decreased from 43.3 ± 2.9 at the beginning of the trial to 38.1 ± 3.2 kg at the end of the trial (p < 0.05). In the placebo group, body fat content did not change from 42.7 ± 2.4 at the beginning of the trial to 41.1 ± 2.9 kg (p = NS) at the end of the trial. The Xanthigen-NLF group lost 3.6 ± 0.7 kg (7.94 lb) more body fat than the placebo group. At the end of the trial, there was no statistically significant difference in body fat content reduction between the Xanthigen-NLF and Xanthigen-NAFLD clusters (p = NS).

Supplementation with Xanthigen-600/2.4 mg (300 mg PSO + 300 mg brown seaweed extract containing 2.4 mg fucoxanthin) did not produce a significant effect on liver fat content until week 8 in the Xanthigen-NAFLD group and week 5 in the Xanthigen-NLF group. At the end of the trial, the liver fat content decreased from 15.3 ± 4.1 to 9.4 ± 3.1% (p < 0.001, compared to placebo) in the Xanthigen-NAFLD group. In the placebo group fat content remained constant from the beginning of the trial (15.1 ± 3.7%) to the end (14.2 ± 3.8%; p = NS) (figure 2).

The liver fat content in the Xanthigen-NLF group decreased from 5.1 ± 1.5 to 3.4 ± 1.8%; in the placebo group fat content did not change (5.3 ± 1.1 to 4.6 ± 1.4%; p = NS). The decrease in the liver fat content in the Xanthigen-NLF group at the end of the trial was statistically significant compared to placebo (p < 0.05).

Effect of Xanthigen on waist circumference

In the Xanthigen-NAFLD group, waist circumference decreased from 110.6 ± 1.6 to 105.0 ± 5.6 cm (p < 0.05, compared to placebo) at the end of the trial; in the placebo group waist circumference was maintained from 109.2 ± 1.4 to 107.5 ± 1.5 cm (p = NS) (table 1).

In the Xanthigen-NLF group, there was a strong trend towards slimming of the waist circumference, which decreased from 103.1 ± 1.7 to 98.7 ± 2.1 cm (p = NS); in the placebo group the circumference changed from 102.2 ± 1.4 to 100.5 ± 1.6 cm (p = NS) (table 1).

Effect of Xanthigen on serum triglycerides

The Xanthigen-600/2.4 mg induced weight and body fat content loss in obese subjects with NAFLD and NLF were accompanied by changes in serum TG levels (table 1). At the end of the trial in the Xanthigen-NAFLD group, the serum TG levels decreased from 195 ± 19 to 158 ± 21 mg/dl (p < 0.05, compared to placebo); in the placebo group TG levels changed from 191 ± 15 to 180 ± 17 mg/dl (p = NS).
Table 1. Preclinical and postclinical characteristics of obese non-diabetic female volunteers with NAFLD and NLF.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Preclinical Xanthigen-NAFLD n = 36</th>
<th>Postclinical placebo-NAFLD n = 36</th>
<th>Postclinical Xanthigen-NAFLD n = 36</th>
<th>Preclinical placebo-NLF n = 19</th>
<th>Preclinical Xanthigen-NLF n = 19</th>
<th>Postclinical placebo-NLF n = 19</th>
<th>Postclinical Xanthigen-NLF n = 19</th>
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<td>87.2 ± 3.7</td>
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NAFLD, non-alcoholic fatty liver disease; NLF, normal liver fat.

* p < 0.001 compared to placebo.

†p < 0.05 compared to placebo.

‡p < 0.05 compared to the baseline values in the other cluster.

§p = NS compared to placebo.
Figure 1. Effect of Xanthigen™ on body weight in subjects with NAFLD (a) and NLF (b). Arrows indicate the time (weeks) when the first statistically significant differences in weight loss between Xanthigen (△) and placebo (□) groups (p < 0.05) were noted.

In the Xanthigen-NLF group, the serum TG levels decreased from 177 ± 12 mg/dl to 155 ± 14 mg/dl (p = NS, compared to placebo); in the placebo group TG levels changed from 174 ± 12 mg/dl to 168 ± 11 mg/dl (p = NS).

Effect of Xanthigen on levels of plasma aminotransferase enzymes

Xanthigen-600/2.4 mg supplementation in obese subjects with NAFLD produced a significant reduction in the levels of plasma ALT, AST and GGT enzymes (table 1). In the Xanthigen-NAFLD group, ALT levels were reduced from 48 ± 7 to 26 ± 7 U/l, AST levels dropped from 51 ± 5 to 29 ± 6 U/l and GGT levels declined from 47 ± 7 U/l to 31 ± 5 U/l. These changes in plasma aminotransferase levels were significant compared to placebo (p < 0.05). The levels of these enzymes persisted in the normal range 2 weeks after completion of the trial (data not shown).

The baseline levels of plasma aminotransferases in the NLF cluster were statistically significantly lower than the NAFLD cluster (table 1). At the end of the trial, levels of AST, ALT and GGT enzymes were also reduced in the Xanthigen-NLF group, although the effect was not statistically significant.

Figure 2. Effect of Xanthigen™ on liver fat content in obese subjects with non-alcoholic fatty liver disease (NAFLD) and normal liver fat content (NLF). Arrows indicate the time (weeks) when the first significant change in liver fat content was observed compared to placebo (p < 0.05). Symbols: triangles, placebo; squares, Xanthigen™; open symbols, NAFLD; filled symbols, NLF group.

Effect of Xanthigen on plasma C-reactive protein (CRP)

In the Xanthigen-NAFLD group, plasma CRP concentration decreased from 6.2 ± 2.4 to 3.64 ± 2.2 mg/l (p < 0.05, compared to placebo). In the Xanthigen-NLF group, plasma CRP concentration decreased from 5.4 ± 2.6 mg/l to 3.9 ± 1.8 mg/l at the end of the trial (p < 0.05, compared to placebo) (table 1).

Effect of Xanthigen on blood pressure

In the Xanthigen-NAFLD group, systolic blood pressure decreased from 138 ± 6 to 119 ± 6 mmHg, and diastolic blood pressure decreased from 91 ± 4 to 79 ± 3 mmHg. Changes in both systolic and diastolic blood pressure were statistically significant compared to the placebo group (p < 0.05) (table 1).

In the Xanthigen-NLF group, systolic pressure decreased from 128 ± 6 to 112 ± 6 mmHg, and diastolic pressure dropped from 93 ± 2 to 77 ± 3 mmHg. Changes in both systolic and diastolic blood pressure were statistically significant compared to the placebo group (p < 0.05) (table 1).

Effect of Xanthigen or pomegranate seed oil on resting energy expenditure (REE)

After 2 weeks, there were no changes in REE for any of the groups in the trial (a total of 41 individuals divided in 11 groups, consisting of 3 groups with 3 volunteers and 8 groups with 4 volunteers). Supplementation with either level of PSO, olive oil or Xanthigen-200/0.8 mg (100 mg PSO + 100 mg brown seaweed extract containing 0.8 mg fucoxanthin) did not produce any significant changes in REE throughout the trial compared to both the baseline (p = NS) and placebo values (p = NS).

Supplementation with Xanthigen-400/1.6 mg (200 mg PSO + 200 mg brown seaweed extract containing 1.6 mg fucoxanthin, n = 3) resulted in a statistically significant
increase in REE, but only at the end of the 16-week trial. In this group, the REE increased from 6.02 ± 0.17 kJ/min to 6.43 ± 0.22 kJ/min (p < 0.05) and 591 ± 210 kJ/24 h (p < 0.05) compared to the placebo group at week 16.

In the groups supplemented with Xanthigen-600/2.4 mg (300 mg PSO + 300 mg brown seaweed extract containing 2.4 mg fucoxanthin, n = 4) and Xanthigen-1000/4 mg (500 mg PSO + 500 mg brown seaweed extract containing 4 mg fucoxanthin, n = 4) per day, an increase in REE was recorded only after 5 weeks of supplementation (table 2). The 5-week REE values for the groups receiving Xanthigen-600/2.4 mg and Xanthigen-1000/4 mg increased from 5.87 ± 0.30 to 6.43 ± 0.43 kJ/min, and from 5.92 ± 0.12 to 6.47 ± 0.33 kJ/min respectively, which were statistically significant increases for both groups compared to the baseline (p < 0.05) and placebo values (p < 0.05). At the end of week 16, the REE in the Xanthigen-600/2.4 mg group increased from 5.87 ± 0.30 to 7.03 ± 0.33 kJ/min (p < 0.05), and the net increase was 1612 ± 317 kJ/24 h compared to the placebo group (p < 0.05). In the Xanthigen-1000/4 mg group, REE values increased from 5.92 ± 0.12 to 7.09 ± 0.28 kJ/min and with a net increase of 1628 ± 290 kJ/24 h (p < 0.05) compared to the placebo at week 16. The difference between the two groups (Xanthigen-600/2.4 mg vs. Xanthigen-1000/4 mg) was not statistically significant (p = NS).

The dose response of fucoxanthin alone showed that 1.6 mg of fucoxanthin per day (n = 4) for 16 weeks did not produce statistically significant changes in REE throughout the trial. The minimum effective dose of fucoxanthin alone was determined to be 2.4 mg (n = 4) and resulted in an increase in REE from 5.85 ± 0.27 to 6.39 ± 0.17 kJ/min. (p < 0.05)

The 16-week supplementation with 4.0 mg/day fucoxanthin (n = 4) resulted in an increase in REE from 5.92 ± 0.16 to 6.72 ± 0.22 kJ/min (p < 0.05). The net increase in REE in this group was 1152 ± 290 kJ/24 h compared to the baseline.

Table 2. Effect of Xanthigen, fucoxanthin, pomegranate seed oil and olive oil on resting energy expenditure (REE, kJ/min) in obese non-diabetic female volunteers with NAFLD.

<table>
<thead>
<tr>
<th>Dosage per day</th>
<th>Baseline</th>
<th>2 weeks</th>
<th>5 weeks</th>
<th>10 weeks</th>
<th>16 weeks</th>
<th>Total EE/kJ/24 h; p-value compared to baseline</th>
<th>p-value compared to placebo at week 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>600 mg Placebo, n = 3 (olive oil)</td>
<td>5.91 ± 0.32</td>
<td>5.95 ± 0.26</td>
<td>5.55 ± 0.24</td>
<td>5.59 ± 0.32</td>
<td>5.95 ± 0.19</td>
<td>From 8510 to 8568, Net 58 ± 40, p &lt; NS</td>
<td>p = NS</td>
</tr>
<tr>
<td>Xanthigen-200/0.8 mg n = 3; (100 mg PSO + 100 mg BSE with 0.8 mg fucoxanthin)</td>
<td>5.72 ± 0.22</td>
<td>5.54 ± 0.32</td>
<td>5.59 ± 0.29</td>
<td>5.67 ± 0.36</td>
<td>5.88 ± 0.31</td>
<td>From 8237 to 8467, Net 230 ± 125, p &lt; NS</td>
<td>p = NS</td>
</tr>
<tr>
<td>Xanthigen-400/1.6 mg n = 3; (200 mg PSO + 200 mg BSE with 1.6 mg fucoxanthin)</td>
<td>6.02 ± 0.17</td>
<td>5.98 ± 0.29</td>
<td>6.12 ± 0.31</td>
<td>6.53 ± 0.15</td>
<td>6.43 ± 0.22</td>
<td>From 8668 to 9259, Net 591 ± 210, p &lt; 0.05</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Xanthigen-600/2.4 mg n = 4; (300 mg PSO + 300 mg BSE with 2.4 mg fucoxanthin)</td>
<td>5.87 ± 0.30</td>
<td>5.68 ± 0.52</td>
<td>6.43 ± 0.43</td>
<td>6.88 ± 0.27</td>
<td>7.03 ± 0.33</td>
<td>From 8453 to 10123, Net 1670 ± 310, p &lt; 0.05</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Xanthigen-1000/4 mg n = 4; (500 mg PSO + 500 mg BSE with 4.0 mg fucoxanthin)</td>
<td>5.92 ± 0.12</td>
<td>6.11 ± 0.30</td>
<td>6.47 ± 0.33</td>
<td>6.79 ± 0.21</td>
<td>7.09 ± 0.28</td>
<td>From 8524 to 10210, Net 1686 ± 290, p &lt; 0.05</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Fucoxanthin-1.6 mg n = 4;</td>
<td>5.82 ± 0.38</td>
<td>5.69 ± 0.21</td>
<td>5.93 ± 0.15</td>
<td>5.69 ± 0.21</td>
<td>5.98 ± 0.18</td>
<td>From 8381 to 8611, Net 230 ± 147, p &lt; NS</td>
<td>p = NS</td>
</tr>
<tr>
<td>Fucoxanthin-2.4 mg n = 4;</td>
<td>5.85 ± 0.27</td>
<td>5.89 ± 0.14</td>
<td>5.98 ± 0.17</td>
<td>6.11 ± 0.21</td>
<td>6.39 ± 0.17</td>
<td>From 8424 to 9202, Net 778 ± 260, p &lt; 0.05</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Fucoxanthin-4 mg n = 4;</td>
<td>5.92 ± 0.16</td>
<td>6.02 ± 0.23</td>
<td>5.92 ± 0.27</td>
<td>6.29 ± 0.31</td>
<td>6.72 ± 0.22</td>
<td>From 8525 to 9677, Net 1152 ± 290, p &lt; 0.05</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Fucoxanthin-8 mg n = 4;</td>
<td>6.04 ± 0.24</td>
<td>5.91 ± 0.31</td>
<td>6.32 ± 0.22</td>
<td>6.92 ± 0.31</td>
<td>7.37 ± 0.35</td>
<td>From 8698 to 10613, Net 1915 ± 246, p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Pomegranate seed oil n = 4; 1500 mg</td>
<td>6.01 ± 0.19</td>
<td>5.89 ± 0.32</td>
<td>5.92 ± 0.27</td>
<td>6.02 ± 0.19</td>
<td>6.12 ± 0.24</td>
<td>From 8654 to 8813, Net 159 ± 65, p &lt; NS</td>
<td>p = NS</td>
</tr>
<tr>
<td>Pomegranate seed oil n = 4; 2000 mg</td>
<td>5.95 ± 0.24</td>
<td>6.00 ± 0.30</td>
<td>6.10 ± 0.26</td>
<td>6.02 ± 0.25</td>
<td>6.07 ± 0.19</td>
<td>From 8568 to 8741, Net 173 ± 92, p &lt; NS</td>
<td>p = NS</td>
</tr>
</tbody>
</table>

PSO, pomegranate seed oil; BSE, brown seaweed extract; NAFLD, non-alcoholic fatty liver disease.
Figure 3. Effect of Xanthigen™ and its individual components in REE over time (baseline to 16 weeks). The graph displays only those supplements which showed a statistically significant difference compared with placebo. Y-axis scale is 5.00–7.50 kJ/min for ease of display. For further details the reader is referred to table 2.

(p < 0.05), and 1100 ± 295 kJ/24 h compared to the placebo group (p < 0.05).

The most significant increase in REE in subjects receiving fucoxanthin alone was observed in the 8.0 mg group (n = 4). The REE increased from 6.04 ± 0.24 to 7.37 ± 0.35 kJ/min, with a net increase of 1915 ± 246 kJ/24 h compared to the baseline (p < 0.001) and 1857 ± 260 kJ/24 h compared to the placebo group (p < 0.001). There was also a statistically significant increase in REE in the fucoxanthin-8.0 mg group as compared with the fucoxanthin-4.0 mg group (p < 0.05). Results are summarized in table 2 and figure 3.

Discussion

The results of Xanthigen-600/2.4 mg trial indicate that the formula (300 mg PSO + 300 mg brown seaweed extract containing 2.4 mg fucoxanthin) and its individual components have clinically relevant anti-obesity properties in reducing the body weight, body fat and liver fat content in non-diabetic obese female volunteers. Xanthigen-600/2.4 mg was particularly effective in reducing liver fat content in participants diagnosed with NAFLD. We could not determine whether this effect is mediated by a direct effect of Xantigen-600/2.4 mg on liver fat content or whether this effect is mediated by promoting weight reduction. Interestingly, obese subjects with NLF content responded to Xanthigen-600/2.4 mg supplementation significantly earlier than those with higher liver fat content. However, at the end of the trial, there was no statistically significant difference in body weight or fat content between Xanthigen-NAFLD and Xanthigen-NLF groups. Notably, both groups (NAFLD and NLF) were analysed separately rather than together. We decided to analyse the results this way because these groups had different metabolic profiles (i.e. liver enzymes and TG levels). As a result, we found statistically significant results and a combined analysis became unnecessary.

The liver accumulation of TGs and central adiposity are closely related findings [31]. Xanthigen may play a role in the normalization of indices of inflammation such as CRP that positively correlate with central adiposity [32]. This normalization may be associated with the weight loss induced by Xanthigen in our study.

The TG-lowering, anti-inflammatory and weight-promoting mechanisms of Xanthigen seem to be based on the individual actions of its constituents: brown marine algae extract standardized for 0.8% fucoxanthin, marine algae omega 3-fatty acids and PSO containing 70% PA.

The literature data indicate that dietary PSO can significantly reduce serum TG levels [19,20]. PA of pomegranates has been shown to suppress delta-9 desaturase (an enzyme in fat metabolism), a possible mechanism to explain the effects of PSO lowering hepatic TG accumulation [19,20]. The brown algae omega-3 fatty acids may provide an additional mechanism to decrease serum and liver TG concentrations because of the reported omega-3 promotion of hepatic fatty acid β-oxidation [33]. This hypolipidemic mechanism may be potentiated further by co-administration of fucoxanthin with omega-3 fatty acids, which can increase the amounts of dietary omega-3 fatty acids in the liver [34].

Obese patients with NAFLD commonly present with elevated markers of liver inflammation and injury, including CRP, AST, ALT and GGT [4]. The elevated activity of GGT is linked with arterial hypertension, accumulation of the liver fat and central obesity [1]. High plasma ALT levels are associated with decreased hepatic insulin sensitivity and an increased risk for the adult onset diabetes. Individuals with high visceral fat often have elevated inflammatory processes in the body, increased plasma oxidized LDL [35] and increased CRP levels, which predict the development of insulin resistance, metabolic syndrome and diabetes type 2 [36]. On the other hand, a significant reduction in body weight and fat in obese individuals results in the downregulation of inflammation and inflammatory markers [8], a finding similar to our Xanthigen-600/2.4 mg study.

The normotensive effect observed in the Xanthigen group is likely as a result of a significant reduction in the body weight, body and liver fat content, serum TG, markers of inflammation and liver enzymes. The broad mechanism of the formula could be because of the unique fatty acid composition of fucoxanthin. The presence of specific fatty acids in fasting plasma could have a significant impact on the level of inflammatory markers. For example, lower α-linolenic acid content is associated with higher CRP, whereas a high plasma n-3 fatty acid content is associated with lower levels of pro-inflammatory and higher levels of anti-inflammatory markers [37–39]. As previously discussed, although PA is structurally related to linolenic and linoleic acids, it has a distinct mechanism of action. For example, animals on a diet consisting of conjugated linoleic acid (CLA) or a mixture of conjugated linoleic acid isomers other than PA developed insulin resistance and fatty liver [33,34] despite a significant decrease in body weight [33].

An important component of the weight loss mechanism of Xanthigen may depend on its effect on REE. The results of this part of the trial should be reviewed with caution, as the number of patients per group was low (41 subjects divided into 11 experimental groups). Nevertheless, we found encouraging results because the statistical analysis showed significant differences between some of the groups. Supplementation with...
both Fucoxanthin and Xanthigen, but not PSO alone, increased REE in obese women with NAFLD. The minimum effective dose of fucoxanthin alone was 2.4 mg in our study. A lower dose of 1.6 mg fucoxanthin, although not effective per se, when supplemented with 200 mg of PSO (Xanthigen-400/1.6 mg per day) showed a significant increase in REE (p < 0.05) by week 16 of the study. The REE was further increased with higher doses of the PSO. These results suggest that PSO may have a synergistic and dose-dependent effect on fucoxanthin-induced increases in REE. This synergistic effect of PSO on fucoxanthin-induced stimulation of REE was specific to PSO, and its component PA, because substitution with other oils including CLA, did not produce a similar clinical result (data not shown). The low number of recruited volunteers makes this part of the trial exploratory in nature. These results would require confirmation with a larger number of individuals.

To the best of our knowledge, this trial is the first clinical evidence reporting the anti-obesity effect of Xanthigen, with special relevance to obese patients with NAFLD and elevated indices of chronic inflammation. Xanthigen may reduce body weight and body fat in part due to the stimulation of REE, but also due to the broad anti-inflammatory and metabolism normalizing actions. This study suggests that pomegranate oil may aid in promoting the REE-stimulating action of fucoxanthin. Although the effect of fucoxanthin and Xanthigen on the expression and induction of the UCP protein was not the objective of this clinical trial, REE increases may be related to the induction of UCP proteins by fucoxanthin as previously demonstrated in rodents [13].

References