Anti-obesity effect of Microalga, Melosira nummuloides ethanolic extract in high-fat-diet-induced obesity C57BL/6J mice

Injue-Yun¹, Jiwon Choi², Hyeonjeong Choe¹, Ki-ok Kim², Gyung min Go³, Dae-Hee Lee⁴* and Mi-Yeon, Kim ¹,*

¹ Natural FNP Co., Ltd., Cheongju 28118, Korea; ² Jeju Biodiversity Research Institute, Jeju Technopark, Seogwipo 697-943, Korea; ³ JDKBIO Inc., Jeju-si, Jeju-do, 63023, Republic of Korea; ⁴ Department of Wellness Bio Industry, Gangneung-Wonju National University, 120 Gangneung, Gangwon 210-702, Republic of South Korea.

*Corresponding author: Mi-Yeon, Kim, Natural FNP Co., Ltd., Cheongju 28118, Korea

Submission Date: September 7th, 2022; Acceptance Date: November 20th, 2022; Publication Date: December 2nd, 2022


ABSTRACT

Background: Melosira nummuloides is a microalga belonging to the Melosiraceae diatom. The ability of the diatoms to mass-produce essential fatty acids and carotenoid pigments was reported, which has driven research and their industrial application. Melosira nummuloides mass-cultivated with Jeju Lava sea water contains fucoxanthin, which has excellent biological properties such as antioxidant, anti-inflammatory, anti-cancer, and anti-obesity activities. The effect of M. nummuloides ethanolic extract (MEE) on the reduction of fat accumulation was evaluated.

Methods: C57BL/6J mice fed a high-fat diet (HFD) were treated over 8 weeks with Melosira ethanolic extract. Cholesterol in serum and triglyceride in liver after the 8-week were evaluated. The expression of SREBP-1c, FAS, PPAR-γ, SCD-1 and p-AMPKα were measured by western blotting respectively.

Results: The MEE-treated C57BL/6J showed significant body weight and visceral fat loss compared to HFD group. Cholesterol in serum and triglyceride levels in liver showed a significant decrease in MEE groups. Levels of SREBP-1c, FAS, PPAR-γ and SCD-1 were low in mice fed an HFD+MEE.

Conclusion: These results show MEE reduces blood lipid levels by regulating the expression of factors related to lipid synthesis and adipocyte differentiation in adipose tissue and inhibiting new lipid synthesis in the liver. M. nummuloides ethanolic extract confirmed its suitability as an anti-obesity agent.
INTRODUCTION

*Melosira nummuloides* is a microalga belonging to the *Melosiraceae* family of diatoms. These photosynthetic, single-celled microorganisms comprise approximately 200,000-800,000 species distributed worldwide[1]. Microalgae have a high photosynthetic efficiency compared to their cell mass, making them useful resources for biofuels, feed, and food. Their high lipid and photosynthetic pigment content increase their desirability to be used as a functional material[2]. The ability of the diatoms *Phaeodactylum tricornutum*, *Chaetoceros calcitrans*, and *Odontella aurita* to mass-produce essential fatty acids and carotenoid pigments is well known and has driven their research and industrial application[3]. Some of the carotenoid pigments produced by microalgae include xanthine, violaxanthin, neoxanthin, α-carotene, β-carotene, and lutein[4]. The most representative class of carotenoid pigments produced by microalgae is the xanthophylls, which includes fucoxanthin, which exhibits excellent biological properties, such as antioxidant, anti-inflammatory, anticancerous, and anti-obesity activity[4-6], with active research ongoing in each of these areas. In a recent paper, it was found that Fucosxanthin acts on both visceral and subcutaneous fat to reduce body weight, BMI and visceral fat, and consequently, Fucosxanthin can improve moderate overweight status in both men and women[7].

A methanolic/ethanolic extract of 10 diatom species was...
reported to contain an average of 3.07 mg fucoxanthin/g dry weight (dw)[8]. Fucoxanthin has been isolated from macroalgae and its concentration in these macroalgae ranged from 0.02 to 0.58 mg/g in fresh samples and 0.01 to 1.01 mg/g in dry samples. In contrast, fucoxanthin concentrations in microalgae ranged from 2.24 to 18.23 mg/g in dry samples, which is 1-3 times greater than that found in the macroalgae, demonstrating the potential of diatoms as a promising source of fucoxanthin[9]. Long-term imbalanced diet and modern eating habits alter the lipid metabolism and accumulate visceral fat, leading to overweight, obesity, and related metabolic disorders such as diabetes, high blood pressure, dyslipidemia, and cardiovascular disease. Obesity is caused by the increased differentiation of preadipocytes to adipocytes as a result of fat accumulation, and inhibiting this process can prevent and treat obesity[10]. Thus, it is essential to identify effective strategies to prevent obesity[11].

Melosira also contains functional ingredients such as omega-3 and omega-6 fatty acids, in addition to fucoxanthin[12]. Therefore, the aim of this study was to determine the anti-obesity properties of a M. nummuloides ethanolic extract (MEE). Weight loss and the expression of lipid-related proteins in C57BL/6J mice fed a high-fat diet (HFD) without or with MEE for eight weeks were compared with green tea[13] investigated. Furthermore, the mechanisms of fat synthesis and degradation in the mice's liver were evaluated.

MATERIALS AND METHODS

Melosira nummuloides collection, preparation of ethanolic extracts, and compositional analysis: Melosira nummuloides was isolated from the saline volcanic rock aquifer (lava seawater) at Jeju Island, Republic of Korea. It was cultivated under constant conditions with fresh Jeju lava seawater supplied by JDK Bio., Ltd. (Korea). Morphological identification was performed by Professor Hyungseop Kim of Gunsan University (Figure. 1). A culture medium dominated by M. nummuloides was then inoculated into a concrete open-air tank, equipped with a nylon mesh (0.1–0.3 mm) attachment substrate. It is cultivated in an area that can produce 700–1,000 kg per month. A circulating water tank was used to maintain a constant water temperature between 17 and 22°C, the illuminance was set to 15,000 lx or more using LEDs, and then cultured for 14 days. After 14 days, the collected M. nummuloides were desalted with fresh water and dehydrated until 85% of moisture content. These dehydrated M. nummuloides were extracted 10 times with 95% alcohol (Korea Ethanol Co, Seoul, Korea) at room temperature for 24 h, filtered, and concentrated under reduced pressure at 60°C, and then spray-dried with dextrin (66%).

From the raw M. nummuloides, the ethanolic extract powder was yielded in the range of 2.5 to 5% and as a result of analysis of general components, 2.66% moisture, 15.44% carbohydrates, 9.44% protein, 11.97% lipids, 4.49% ash, and 0.35% fucoxanthin content were issued the by Korea Health Supplement Institute (KHSI) (Table 1). To determine fucoxanthin content, HPLC analysis was performed via a Kromasil 100-5 C18 column (5 μm, 4.6× 250 mm, AkzoNobel, Amsterdam, Nederland) at 35°C with a flow rate of 0.7 mL/min using a gradient mobile phase composed of acetonitrile containing 0.1% formic acid(A) and water containing 0.1% formic acid (B). The mobile phase was 10 : 90 mixture of components A and B as the initial condition of the chromatography; the sample injection volume was 10 μL. The absorption spectrum of fucoxanthin was monitored at 450nm using the photodiode array detector. (Figure. 2). Green tea extract containing approximately 40% catechins about (12% EGC, 15% epigallocatechin-3-gallate (EGCG), 2% EC, and 2% ECG) was supplied by Anhui Redstar Pharmaceutical Corp.
Figure 1. The optical microscope and SEM images of *M. nummuloides*

**Table 1.** Proximate component content of *M. nummuloides* ethanolic extract powder

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>2.66±0.02</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>15.44±0.44</td>
</tr>
<tr>
<td>Protein</td>
<td>9.44±0.22</td>
</tr>
<tr>
<td>Lipid</td>
<td>11.97±0.35</td>
</tr>
<tr>
<td>Ash</td>
<td>4.49±0.35</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>0.35±0.10</td>
</tr>
</tbody>
</table>

The material used for the analysis was a dry matter. Data are presented as the means ± SD of three measurements. The data were the results of analysis by KHSI (Korea Health Supplement Institute). 1: Correction contents= (100 - (composition of moisture, protein, lipid and ash corrected for dextrin))

Figure 2. HPLC chromatogram of fucoxanthin (A) and ethanol-extracted of Melosira nummuloides (B). Detection was carried out at 450 nm.
**Chemicals and reagents:** The fucoxanthin standard were purchased from Sigma-Aldrich (Lot No. MKCK1386,) and the mobile phase was obtained from Thermo-Fisher Scientific (Waltham, MA, USA). Biochemical kits to measure total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, aspartate transaminase (AST), and alanine aminotransferase (ALT) were purchased from Biovision (Milpitas, CA, USA). Antibodies against FAS was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA); against PPAR-γ, SCD-1, and β-Actin were purchased from Cell Signaling Technology (Danvers, MA, USA); and against SREBP-1 and p-AMPKα were purchased from Abcam (Cambridge, UK). Horseradish peroxidase-conjugated secondary antibodies were obtained from Jackson Immuno Research Laboratories, Inc. (West Grove, PA, USA).

**Animal diets and housing conditions:** Animal experiments were conducted after deliberation (approval number GWNU-2018-20-1) by the Animal Experimentation Ethics Committee of Gangneung-Wonju University. Four-week-old male C57BL/6N mice, weighing 15-17 g, were purchased from Orient Bio (Korea), and housed in an animal breeding room at 22±2°C temperature, 50±5% humidity, and 12 h photoperiod (light, 8:00–20:00; dark, 20:00–8:00). After acclimatization to normal rodent solid food and water for a week, the general condition was observed during the adaptation period, and the mice that passed the adaptation period were placed in the following groups: normal diet group (ND, n=10), high-fat diet control group (HFD, n=10), high-fat diet and 100 mg/kg(bw) of green tea extract group (GT, n=10), high-fat diet and 100 mg/kg(bw), *M. nummuloides* ethanolic extract group (MEE-L, n=10), and high-fat diet and 200 mg/kg(bw) *M. nummuloides* ethanolic extract group (MEE-H, n=10). The ND and HFD groups were orally administered with 100 µl saline, and the GT, MEE-L, and MEE-H groups were orally administered 100 µl GT and MEE in the specified concentrations diluted in saline. The HFD group was fed the D12492 feed (Research Diets Inc, New Brunswick, NJ, USA) containing 60% fat, and the ND group was fed the D12450B feed (Research Diets Inc) containing 10% fat. Feed intake and body weight were measured twice a week for eight weeks.

**Blood and tissue collection:** After eight weeks of experimentation, the mice were fasted for 12 h and sacrificed. After laparotomy, the blood was collected through cardiac blood sampling, and the liver and adipose tissue were extracted. After the blood hardened a little at room temperature, it was centrifuged at 4°C, 5000×g for 15 min, and the supernatant obtained was transferred to a new Eppendorf tube and centrifuged again at 4°C and 3000×g for 10 min to collect the supernatant (serum). The liver and fat were separated, washed with PBS, and weighed. The serum and liver samples were stored in a -80°C deep freezer until further analysis.
Biochemical analysis in serum and liver: To assess the lipid content, serum total cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations were measured. The concentration of triglycerides, AST, and ALT in the liver tissue was measured to verify the liver function impairment. The final absorbance values of the biochemical parameters were obtained using the SYNERGY HTX (Biotek, Winooski, Vermont, USA) equipment.

Western blotting: The mouse liver tissue was dissolved in RIPA buffer containing 25mM Tris-HCl (pH 7.6), 150 mM NaCl, Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, and protease inhibitor cocktails (Thermo Fisher Scientific) and centrifuged at 4°C and 5000×g for 20 min to remove insoluble substances, and a lysate was obtained. Protein concentration was measured using a BCA protein assay kit (Pierce™ BCA Protein Assay Kit, Thermo Fisher Scientific), and protein was quantified at 562 nm using a SYNERGY HTX (Biotek) absorbance instrument. The proteins (20 µg) were separated by 8-12% SDS-PAGE and blocked with 5% non-fat milk at 4°C for 2 h. Then, the membrane was incubated with the primary antibody dilution buffer (0.1% sodium azide and 0.5% bovine TBST solution) containing the diluted primary antibodies against SREBP-1 (Cat. No. ab28481, Abcam), FAS (Cat. No. SC-74540, Santa Cruz, USA), PPAR-γ (Cat. No. 2435s, Cell Signaling Technology, USA), SCD-1 (Cat. No.2794s, Cell Signaling Technology, USA), p-AMPKα (Cat. No.2535s, Cell Signaling Technology, USA) and β-actin (Cat. No. A5316-100UL, Sigma-Aldrich) at 4°C overnight. After probing the membrane with a secondary antibody at room temperature for 2 h, protein expression was measured using an ECL solution (EZ-western Lumi Pico; DOGEN) using a Chemidox (Amersham-ImageQuant 800).

Statistical analysis: Results are expressed as the mean ± standard error of the mean (SEM). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Duncan’s multiply comparison test or a two-tailed, unpaired Student’s t-test. ANOVA with the Duncan’s post hoc test was used when analyzing the means of more than two groups. The Student’s t-test was used when analyzing the means of two specific groups. IBM SPSS Statistics version 21 was used to perform statistical analysis. Statistical significance was set at p < 0.05.

RESULTS

Changes in body weight, visceral fat, and liver weight in obesity-induced mice: Changes in the body weight of MEE-treated mice were compared with those in the HFD-fed mice every week for eight weeks (Figure. 3A). Compared to the HFD group, the weight of the visceral fat, according to the weight change, after eight weeks was significantly higher, up to 42% in the GT group, 47% in the MEE-L group, and 37% in the MEE-H group. However, there was no difference in liver weights in any group (Figure. 3B).
Figure 3. Change in mouse weight according to high-fat diet (HFD) intake. (A) Change in body weight of C57BL/6J mice fed a HFD for eight weeks. (B) Effect of *M. nummuloides* ethanolic extract (MEE) on visceral fat mass and liver weight in HFD-fed mice. Treatment groups: normal diet (ND); high-fat diet (HFD); HFD + 100 mg/kg green tea extract (GT); HFD + 100 mg/kg MEE (MEE-L), and HFD + 200 mg/kg MEE (MEE-H). Data are presented as the mean ± SD, n = 9–10. *: Significant difference compared with the HFD group (*p < 0.05, **p < 0.01, ***p < 0.001). #: Significant difference in comparison between the ND group and the HFD group (#p < 0.05, ##p < 0.01, ###p < 0.001).

Biochemical changes in the serum and tissues according to changes in food intake and body weight: After one week of treatment, the HFD group showed a significant weight gain compared to the ND group. After two weeks, all treatment groups showed significant weight loss compared to the HFD group. After eight weeks, the body weight of the mice in the HFD, GT, MEE-L, and MEE-H was 69.5%, 51.8%, 55.7%, and 54.8%, and that of the ND group increased by 30.1%. Green tea extract and EGCG have been reported to reduce daily food intake compared to animals fed HFD[14]. The ND group showed a slight increase in the daily intake compared to the HFD group, while the GT group showed a decrease. Similar results were obtained in this study as well; the GT group showed a significant decrease in dietary intake compared to that of the HFD group. This decrease in dietary intake could be due to the caffeine component of green tea, as tested in previous studies. The MEE-L and MEE-H groups did not show a significant difference (p < 0.05) compared to the dietary intake of the HFD group. The total serum cholesterol level of the HFD group increased by 58% over the 8-week treatment period compared to that of the ND group, while the GT, MEE-L, and MEE-H groups showed a significant decrease of 22%, 28%, and 19%, respectively, compared to that of the HFD group. The serum LDL-cholesterol level of the HFD group significantly increased...
by 73% compared to that of the ND group, whereas that of the GT, MEE-L, and MEE-H groups decreased by 8% ($p < 0.05$), 22%, and 12% respectively, compared with that of the HFD group. The HDL-cholesterol levels showed an increasing trend in the HFD intake group; however, it was decreased in the MEE-L and MEE-H groups compared to the HFD group, but no significant difference was observed. The triglyceride levels in the mouse liver of the HFD group increased by 69% compared to that of the ND group. In contrast, the triglyceride levels in the livers of GT, MEE-L, and MEE-H groups were significantly lower than that of the HFD group, but not significantly different from that of the ND group. Compared to the HFD group, it was confirmed that the total cholesterol level in the blood and that of triglyceride in the liver tissue decreased in the group that consumed MEE along with a HFD. AST and ALT levels in the MEE-L, MEE-H, and GT groups were reduced, indicating the hepatoprotective effects of MEE (Table 2). Liver lipid accumulation is most commonly observed in obesity, and hepatic lipid accumulation is highly associated with obesity[15].

Hepatic lipid accumulation occurs when the amount of fatty acids accumulated in the liver exceeds the consumption of fatty acids. In this study, the hepatoprotective effect of MEE was shown to prevent obesity by preventing the accumulation of fat in the body.

Table 2. Effect of *M. nummuloides* ethanolic extract (MEE) supplementation on body weight gain and food intake of mice fed a high-fat diet (HFD) and lipid parameters and liver index in HFD-induced obese mice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ND</th>
<th>HFD</th>
<th>GT</th>
<th>MEE-L</th>
<th>MEE-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>18.3±1.2</td>
<td>19.0±0.6</td>
<td>19.1±1.2</td>
<td>18.3±1.2</td>
<td>18.6±1.3</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>23.8±1.7c</td>
<td>32.2±2.3a</td>
<td>29.0±2.4b</td>
<td>28.5±2.3b</td>
<td>28.8±2.6b</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>0.7±0.2c</td>
<td>1.7±0.3a</td>
<td>1.2±0.3b</td>
<td>1.3±0.3b</td>
<td>1.3±0.3b</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>2.7±0.3a</td>
<td>2.5±0.3ab</td>
<td>2.2±0.3b</td>
<td>2.4±0.2ab</td>
<td>2.5±0.3ab</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>138.7±23.7c</td>
<td>219.0±14.3a</td>
<td>170.7±13.4b</td>
<td>158.0±2.47bc</td>
<td>178.4±21.1b</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>36.0±3.5b</td>
<td>24.6±6.0ab</td>
<td>22.1±9.4b</td>
<td>26.5±3.9ab</td>
<td>24.8±7.0ab</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>88.0±24.1c</td>
<td>152.1±14.7a</td>
<td>139.3±5.1ab</td>
<td>118.5±7.5b</td>
<td>134.6±13.6ab</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>46.5±5.8b</td>
<td>78.7±7.2a</td>
<td>48.8±11.4b</td>
<td>42.7±9.6b</td>
<td>47.2±15.7b</td>
</tr>
<tr>
<td>AST(U/L)</td>
<td>18.3±1.1b</td>
<td>28.7±2.9a</td>
<td>22.4±3.0ab</td>
<td>23.7±2.5ab</td>
<td>24.4±1.3ab</td>
</tr>
<tr>
<td>ALT(U/L)</td>
<td>13.2±2.9b</td>
<td>37.7±6.7a</td>
<td>28.2±7.3ab</td>
<td>26.0±5.6ab</td>
<td>26.8±3.9ab</td>
</tr>
</tbody>
</table>

Treatment groups: normal diet (ND); high-fat diet (HFD); HFD + 100 mg/kg green tea extract (GT); HFD + 100 mg/kg MEE (MEE-L), and HFD + 200 mg/kg MEE (MEE-H). Data are presented as the mean ± SD, n = 9-10 (changes in food intake and weight) and n=5(serum and liver experiments). Superscript letters a,b,c: Values without a common superscript letter are significantly different at $p < 0.05$. Statistical comparisons between groups were analyzed by Duncan's multiple comparison test.

**Analysis of proteins related to fat synthesis and metabolism in C57BL/6J mice:** Adipogenic factors were also analyzed in the livers of C57BL/6J mice. The protein expression levels of fat synthesis-related enzymes FAS and SCD-1, which are involved in the synthesis of new fatty acids, were significantly lower in the MEE-H group.
than in the HFD group. In addition, the expression of fat synthesis-related proteins PPAR-γ and SREBP-1c and fat metabolism-related protein AMP-activated protein kinase (AMPKα) was measured (Figure 4). The suppression of the expression of these fat synthesis- and lysis-related enzymes in the liver of MEE-treated mice lowered the triglyceride content in the liver as well as the mass of visceral fat tissue. Inhibition of triglyceride synthesis from sugar and the lack of a supply of new fatty acids from the diet due to fat lysis would aid in the breakdown of accumulated body fat, thereby reducing body fat. The expression of PPAR-γ and SREBP-1 proteins was effectively suppressed in all treatment groups compared to that of the HFD group. The protein expression level of FAS and SCD-1, which are involved in the synthesis of new fatty acids, was significantly lower in the MEE-H group relative to the HFD group. In addition, AMPKα suppresses the activation of PPAR-γ and SREBP-1 and the inhibition of ACC, thereby playing an important role in body fat regulation. Herein, the AMPKα levels were higher in the MEE-L, MEE-H, and GT groups than in the HFD group.

**Figure 4.** Regulatory effect of *M. nummuloides* ethanolic extract (MEE) on lipogenesis-related proteins levels in the liver. Western blotting analysis of lipogenic SREBP-1c, PPAR-γ, FAS, SCD-1 and p-AMPK, protein levels. Treatment groups: normal diet (ND); high-fat diet (HFD); HFD + 100 mg/kg green tea extract (GT); HFD + 100 mg/kg MEE (MEE-L), and HFD + 200 mg/kg MEE (MEE-H). Data are presented as the mean ± SD; n = 3, *: Significant difference compared with the HFD group (*p < 0.05, **p < 0.01, ***p < 0.001). #: Significant difference in comparison between the ND group and the HFD group (#p < 0.05, ##p < 0.01, ###p < 0.001).

**DISCUSSION AND CONCLUSIONS**

In this study, *Melosira* extract was evaluated for its putative effects on weight loss, obesity-related lipid parameters, and regulation of protein expression levels of fat synthesis-related enzymes in high-fat diet-induced obese mice. Our data clearly demonstrated that when C57BL/6J mice were fed an HFD containing 60% fat, the weight gain per week in the HFD group was 30% higher than that in the MEE-L and MEE-H groups. Visceral fat mass decreased by 50% in the MEE-L group and by 38% in the MEE-H group compared to that in the HFD group.
Remarkably, the MEE-L and MEE-H groups showed significant reductions in serum total cholesterol levels and liver triglyceride levels compared with those of HFD treated mice. This indicated that the synthesis of de novo triglycerides and their accumulation in the liver are suppressed by MEE in mice fed an HFD.

In this study, when 100 mg/kg of Melosira extract was administered to HFD-fed mice, it showed an anti-obesity effect equivalent to that of 100 mg/kg of green tea extract, which already had an anti-obesity effect. There was no difference in the daily feed intake between mice fed the HFD and the HFD+MEE (MEE-L, MEE-H). In contrast, the green tea extract group showed a significant decrease in dietary intake compared to that of the HFD group. This can be seen as a decrease in dietary intake due to the caffeine component of green tea, as tested in previous studies. Simultaneously, serum AST and ALT levels in the MEE-L and MEE-H groups were reduced, indicating the liver protective effects of MEE. Lipid accumulation in the liver is highly associated with obesity (Rinella, 2015).

Adipogenic factors were also analyzed in C57BL/6J mice livers. The protein expression levels of fat synthesis-related enzymes FAS and SCD-1, which are involved in the synthesis of new fatty acids, were significantly lower in the MEE-H group than the HFD group. The suppression of the expression of these fat synthesis and lysis-related enzymes in the liver of C57BL/6J mice treated with MEE lowered the triglyceride content in the liver as well as the mass of visceral fat tissue. Inhibition of triglyceride synthesis from sugar and the lack of a supply of new fatty acids from the diet due to fat lysis would aid in the breakdown of accumulated body fat, thereby reducing body fat.

When pyruvate is metabolized further in the TCA cycle, the citrate can be converted to oxaloacetate and acetyl-CoA by ATP-citrate lyase (ACL). In turn, the acetyl-CoA can be converted to malonyl-CoA by acetyl-CoA carboxylase (ACC). Thereafter, malonyl-CoA is used in the synthesis of fatty acids by the FAS complex enzyme. The fatty acids ingested from the diet are metabolized forming fatty acyl-CoA, which are then stored by synthesizing triglycerides and sterols together with glycerol. SCD-1 is a key regulator of monounsaturated fatty acid synthesis. The expression of SCD-1 and FAS, key enzymes involved in fatty acid synthesis, is regulated by SREBP-1c, a transcriptional regulator[16]. In addition, AMPKα suppresses the activation of PPAR-γ and SREBP-1, thereby playing an important role in body fat regulation. Here, AMPKα levels were higher in the MEE-L, MEE-H, and GT groups than in the HFD group[17].

From the above results, it was demonstrated that MEE reduces blood lipid levels by regulating the expression of factors related to lipid synthesis and adipocyte differentiation in adipose tissue and inhibiting new lipid synthesis in the liver.

Obesity increases the risk of cardiovascular diseases, diabetes, musculoskeletal disorders, and cancer. Obesity is caused by increased differentiation of preadipocytes to adipocytes as a result of fat accumulation. To prevent and treat obesity, it is necessary to inhibit this differentiation process. In this study, 1. Adipose tissue growth was inhibited by regulating the expression of factors related to adipocyte differentiation; 2. Controls the breakdown of adipose tissue and reduces the production of triglycerides in the liver by reducing the fatty acids carried to the liver through the bloodstream; 3. Lipid synthesis would have been inhibited by inhibiting the secretion of fatty acid synthase. Based on three hypotheses, we demonstrated the suitability of M. nummuloides ethanol extract as an anti-obesity agent in C57BL/6J mice. Although further clinical studies are needed, these studies support the potential of MEE as a lipid metabolism improver. Thus,
**M. nummuloides** can be considered a valuable marine resource for the future.

**List of abbreviations:** MEE: *Melosira nummuloides* ethanolic extract, ND: normal diet, HFD: high-fat diet, MEE-L: HFD + 100 mg/kg MEE, MEE-H: HFD + 200 mg/kg MEE, and HFD + 100 mg/kg, GT: green tea extract, PPAR-γ: Peroxisome proliferator activated receptor γ, SREBP-1c: Sterol regulatory element binding protein 1c, FAS: Fatty acid synthase, SCD-1: Sterol CoA desaturase-1, MD: mean difference, SD: standard deviation.

**Authors’ contributions:** I.J.Y was the general manager of the mouse experiment. J.W.C. experimented with mice. H.J.K experimented with mice. K.-O.K mouse experimental design. G.M.G produced *Melosira* raw material. D.-H.L managed the mouse experiments and completed all experiments. M.Y.K supervised, managed and finalized all experiments.

**Competing interests:** All authors have no financial interests or conflict of interests.

**Funding details:** This research was a part of the project titled ‘Mass production process development and anti-obesity function evaluation of standardized fucoxanthin containing extracts using *Melosira* originated from Jeju Lava Seas’ (No. 200332), funded by the Ministry of Oceans and Fisheries, Korea with the JDK BIO organization.

**REFERENCES**

1. IPET. The Latest Trends in Microalgae and Implications for Agro-Food Fields. Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry, IPET i ISSUE Report, Naju, Korea (2021)


5. SEELY, G. R.; DUNCAN, M. J.; VIDAVER, W. E. Preparative and analytical extraction of pigments from brown algae with dimethyl sulfoxide. Marine Biology, 1972, 12.2: 184-188. DOI: [https://doi.org/10.1007/BF00350754](https://doi.org/10.1007/BF00350754)


7. HITOE, Shoketsu; SHIMODA, Hiroshi. Seaweed fucoxanthin supplementation improves obesity parameters in mild obese Japanese subjects. Functional Foods in Health and Disease, 2017, 7.4: 246-262. DOI: [https://doi.org/10.31989/ffhd.v7i4.333](https://doi.org/10.31989/ffhd.v7i4.333)


11. GAMMONE, Maria Alessandra; D’ORAZIO, Nicola Antonio. Anti-obesity activity of the marine carotenoid fucoxanthin. Marine drugs, 2015, 13.4: 2196-2214. DOI: [https://doi.org/10.3390/md13042196](https://doi.org/10.3390/md13042196)


17. JEONG, Hyeon Ju; PARK, Ju Hee; KIM, Myong-Jo. Ethanol extract of Hippophae rhamnoides L. leaves inhibits adipogenesis through AMP-activated protein kinase (AMPK) activation in 3T3-L1 preadipocytes. Korean Journal of Plant Resources, 2015, 28.5: 582-590. DOI: https://doi.org/10.7732/kjpr.2015.28.5.582