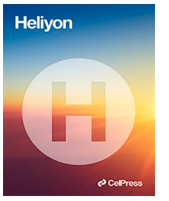
Heliyon 10 (2024) e36943

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Identification of novel anti-obesity saponins from the ovary of sea cucumber (*Stichopus japonicus*)

Hyo-Deok Seo [a,1], Ji-Young Lee [b,1], So-Hyun Park [a,c], Eunyoung Lee [a], Jeong-Hoon Hahm [a], Jiyun Ahn [a,c], A Ra Jang [b], So Hee An [b], Jang Ho Ha [b], Kyoung Tai No [b,**], Chang Hwa Jung [a,c,*]

[a] *Aging and Metabolism Research Group, Korea Food Research Institute, Jeollabuk-do, 55365, Republic of Korea*
[b] *Bioinformatics and Molecular Design Research Center, Yonsei University, Incheon, 21983, Republic of Korea*
[c] *Department of Food Biotechnology, University of Science and Technology, Jeollabuk-do, 55365, Republic of Korea*

ARTICLE INFO

Keywords:
Sea cucumber
Ovary
Adipocyte differentiation
Saponin
Anti-obesity effect

ABSTRACT

The potential anti-obesity effects of sea cucumber extract have been reported. However, the individual saponins responsible for these effects are yet to be isolated and characterized. This study aimed to identify the most effective sea cucumber body part for inhibiting lipid accumulation in adipocytes and to elucidate the compounds responsible for this effect using nuclear magnetic resonance (NMR) techniques. Sea cucumber ovary 80 % ethanol extract (SCOE) demonstrated remarkable efficacy in inhibiting adipocyte differentiation compared to other sea cucumber body parts with 50 % or 80 % ethanol extracts. SCOE anti-obesity effect was evaluated in C57BL/6 mice fed a high-fat diet, which revealed significant reductions in body weight, serum lipids, adipose tissue, and liver weight. Using column chromatography, eight saponins were isolated from the SCOE, four of which exhibited potent inhibitory effects on adipocyte differentiation. Of these, three active saponins, holotoxins A, B, and D1, were newly identified. These findings highlight the potential of SCOE and its saponins as effective anti-obesity agents.

1. Introduction

Sea cucumbers are marine animals belonging to the class *Holothuroidea*, with approximately 1500 reported species worldwide [1]. They are predominantly consumed in East Asian countries such as Korea, Japan, and China [2]. Sea cucumbers can be cooked using various methods, including stir-frying, boiling, steaming, or simmering in soup. They are often dried and rehydrated before use in various dishes and are highly valued for their unique texture and flavor. In addition to their culinary uses, sea cucumbers are used in traditional Asian medicine because of their numerous health benefits, including anti-inflammatory, anticancer, osteoblast differentiation-promoting, and immune-boosting effects [3–7].

Sea cucumbers contain various bioactive compounds, including saponins, polysaccharides, cerebrosides, and fucosylated chondroitins [8]. In particular, polysaccharides and peptides derived from sea cucumbers inhibit oxidative damage by enhancing

* Corresponding author. at: Aging and Metabolism Research Group, Korea Food Research Institute, Jeollabuk-do, 55365, Republic of Korea.
** Corresponding author.
E-mail addresses: ktno@bmdrc.org (K.T. No), chjung@kfri.re.kr (C.H. Jung).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e36943
Received 7 February 2024; Received in revised form 19 August 2024; Accepted 25 August 2024
Available online 27 August 2024

antioxidant activity *in vivo* [9,10]. For example, sea cucumber cerebroside has been reported to exert neuroprotective properties in PC12 cells and SAMP8 mice [11,12], whereas fucosylated chondroitin sulfates have been shown to improve insulin sensitivity and glucose metabolism in sea cucumbers [13]. Among these functional compounds, saponins, which are characterized by their triterpene glycoside (holostane-type) structure, are considered the most prominent [14]. Sea cucumber saponins have attracted attention owing to their abundance, low toxicity, few side effects, and various functionalities, including antitumor, lipid-lowering, non-alcoholic fatty liver improvement, fat accumulation inhibition, and antihypertensive properties [15].

Several studies have reported the potential anti-obesity efficacy of sea cucumber extract. For example, sea cucumber body wall extract has been found to reduce body weight in Kunming mice fed a high-fat diet and to inhibit lipid absorption by inhibiting pancreatic lipase activity [16–18]. Similarly, studies on other sea cucumber body parts, such as sea cucumber gut powder, have demonstrated a reduction in body weight and plasma cholesterol levels in C57BL/6 obese mice [19]. Additionally, sea cucumber ovum has shown promising results in improving fatty liver in a high-fat-fed rat model [20].Despite these positive findings, the specific active components responsible for the anti-obesity effects of sea cucumber saponins are yet to be fully elucidated. It has been suggested that echinoside A and holothurin A may reduce pancreatic lipase activity and weight in C57BL/6 obese mice [21]. However, the saponins responsible for these anti-obesity effects have not been definitively isolated or characterized. Although, based on existing studies, the anti-obesity potential of sea cucumber extract is promising, further research is required to identify and understand the precise active components responsible for these effects. Notably, sea cucumber saponins have demonstrated more favorable outcomes than ginseng saponins in terms of controlling body weight, liver and serum lipid levels, and serum insulin and glucose levels in C57BL/6 mice fed a high-fat diet [22]. These findings indicate the potential application of sea cucumber saponins as functional ingredients for alleviating obesity-associated metabolic disorders.

Although the potential anti-obesity effects of sea cucumber and its saponins have been suggested, no studies have identified the most effective sea cucumber body part and the specific types and quantities of characteristic saponins present in each body part. Therefore, to fill this research gap, the objective of this study was to identify the sea cucumber (*Stichopus japonicus*) body part, such as the dried whole body, ovaries, and gut, that exhibits the highest anti-obesity effect, and to subsequently isolate and identify saponins with anti-obesity properties.

2. Materials and methods

2.1. Sea cucumber extracts

The sea cucumber (*S. japonicus*) samples used in this study were provided by the Boryeong Fishery Corporation (Boryeong, South Korea). Samples consisting of dried whole body, ovary, and gut were extracted with 50 % and 80 % ethanol at a solvent-to-sample ratio of 20:1. Each mixture was stirred and extracted for 24 h, and then filtered using Whatman No. 1 filter paper (11 μm; Thermo Fisher Scientific, MA, USA). The obtained extracts were concentrated under reduced pressure, freeze-dried, and stored at −80 °C for further analysis.

2.2. Cell culture and cell viability assay

The 3T3-L1 murine preadipocytes (ATCC, VA, USA) were cultured in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) containing 10 % calf serum and PSG (100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 2 mmol L^{-1} glutamine) at 37 °C and 5 % CO_2. The cells were seeded at a density of 1×10^4 cells/well (96-well plates) to determine the effects of the extracts and components on cell viability. Various concentrations of the entire body, ovary, and gut using 50 % and 80 % ethanol extract (10–50 μg mL^{-1}) were treated to 3T3-L1 cells for 24 h, after which MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide) solution (0.05 % in phosphate-buffered saline, Sigma-Aldrich, Darmstadt, Germany) was added to each well. After adding 200 μL of dimethyl sulfoxide and dissolving well, the absorbance was measured at 570 nm (Tecan Infinite M200). Cell viability was calculated based on the absorbance of untreated cells.

2.3. Adipocyte differentiation and lipid staining

Adipocyte differentiation was induced according to a previously reported method [23]. First, 3T3-L1 cells were seeded in a six-well plate and incubated for 48 h. Adipocyte differentiation was initiated by replacing the differentiation medium with high-glucose DMEM supplemented with 10 % fetal bovine serum (FBS; Hyclone, UT, USA), 1 μM dexamethasone, PSG, 1 μg mL^{-1} insulin, and 0.5 mmol 3-isobutyl-1-methylxanthine. The cells were treated with the extract for two days, after which the medium was replaced with DMEM supplemented with 10 % FBS and 1 μg mL^{-1} insulin, and the cells were cultured for an additional two days. Finally, the medium was replaced with DMEM supplemented with 10 % FBS and PSG, and the cells were cultured until differentiation was complete. Differentiated 3T3-L1 cells were fixed in 4 % formaldehyde after being washed with phosphate-buffered saline. Subsequently, the fixed cells were washed thrice with 60 % isopropanol and stained with Oil Red O (ORO) solution for 10 min at 25 °C. Images of the stained samples were analyzed using a light microscope (FV3000, Olympus, Tokyo, Japan), ORO stain was dissolved in 100 % isopropanol, and absorbance was measured at 510 nm for quantification.

2.4. Immunoblotting analysis

To prevent degradation of the extracted proteins, protease and phosphatase inhibitors were added to radioimmunoprecipitation assay buffer (RIPA buffer, Thermo Fisher Scientific) to lyse the cells. The lysate was separated by centrifugation and quantified using colorimetric analysis. Electrophoresis was used to separate the proteins by size after loading the protein samples mixed with 5 × sample buffer onto sodium dodecyl sulfate-polyacrylamide gels. Proteins separated on the gel were transferred to polyvinylidene fluoride (PVDF) membranes using a semi-dry transfer apparatus (Bio-Rad, CA, USA). The membrane was incubated with a blocking solution containing 5 % skim milk in Tris-buffered saline with 0.05 % Tween 20 (TBST) for 1 h with gentle shaking. The thrice-washed membrane was reacted overnight with a primary antibody specific for the target protein, and subsequently with a secondary antibody for 1 h. Target protein bands were detected using enhanced chemiluminescence (ECL) solution (Thermo Fisher Scientific) and quantified by Image J software (Bethesda, MD, USA) after imaging on a Syngene™ GBOX Chemi-XX6.

2.5. Isolation, purification, and identification

To investigate the saponins in sea cucumber ovary 80 % ethanol extract (SCOE), which has the highest efficacy on lipid accumulation, we extracted the dried sea cucumber ovary (5 kg) using 80 % ethanol (25 L) for 24 h. SCOE (333 g) was suspended in distilled water and partitioned with equal volumes of *n*-hexane, ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH). The *n*-BuOH layer was subsequently fractionated by ODS C18 reverse-phase column chromatography (RPCC) and eluted with methanol (MeOH)–H_2O (5:5–10:0, v/v) to obtain five subfractions. Each fraction was further purified by RPCC and silica gel column chromatography using dichloromethane (CH_2Cl_2)–MeOH gradient. Final purification was achieved by semi-preparative high-performance liquid chromatography (HPLC) using a Phenomenex C18 column eluted with acetonitrile (ACN)–H_2O. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Advance II 400 spectrometer using pyridine-d_5, with tetramethylsilane as the internal standard. The HRESIMS values were determined using a Q Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with a Vanquish ultra-high-performance liquid chromatograph (UHPLC; Thermo Fisher Scientific). Semi-preparative HPLC separations were performed on a Waters Prep 150 LC system with a Phenomenex Luna C18 column (250 × 21.2 mm, 5 μm). Fractionation and isolation were carried out using a medium-pressure liquid chromatographic system [Buchi pump Module C-601, silica gel (230–400 mesh, Merck, RP-C18 (40–63 μm)].

2.6. Animal study

In vivo experiments were conducted using SCOE on C57BL/6 mice (male, six-week-old; Orient Bio, Seongnam, South Korea). Mice were housed in a specific pathogen-free state under stable temperature (22 ± 2 °C), lighting (12 h light and dark cycle), and humidity (55 ± 5 %) conditions. After acclimatization for one week, fifty mice were randomly divided into five groups and fed five different diets for eight weeks (Table S1): low-fat diet (LFD, 10 % kcal fat), high-fat diet (HFD, 45 % kcal fat), HFD containing 0.05 % SCOE (HFD + 0.05 % SCOE), HFD containing 0.1 % SCOE (HFD + 0.1 % SCOE), and HFD containing 0.2 % SCOE (HFD + 0.2 % SCOE). Body weight and food intake of the mice in each group were measured once and three times per week, respectively. After 8 weeks, the mice were anesthetized using 4 % isoflurane, and the serum, fat tissue, and liver tissue were collected. The collected fat tissue and liver were immediately frozen in liquid nitrogen and stored at −80 °C until use. All animal experiments were approved by the Animal Care and Use Committee of the Korea Food Research Institute and were performed in accordance with the relevant institutions and national guidelines (approval number: KFRI-M-21018).

2.7. Oral glucose tolerance test

A glucose tolerance test was performed to measure insulin sensitivity in the mice. Briefly, after seven weeks on group-specific diet regimes, the mice were fasted for 4 h, whereafter glucose solution was orally administered (2 g kg^{-1}). Blood samples were collected before (0 min) and after (15, 30, 60, 90, and 120 min) glucose administration. The glucose concentration was measured using a glucometer (Roche Diagnostics, Basel, Switzerland).

2.8. Serum insulin, adiponectin, and leptin level analyses

Serum insulin, adiponectin, and leptin levels were analyzed using different immunoassays. The level of adiponectin secreted into the serum was measured using a Mouse Adiponectin Immunoassay kit (R&D Systems, MN, USA). Mouse leptin and insulin enzyme-linked immunosorbent assay kits (ALPCO, NH, USA) were used to quantify the serum leptin and insulin levels, respectively. All the experiments were performed according to the manufacturer's instructions.

2.9. Analysis of serum and hepatic lipid levels

Serum and liver lipid levels, including high-density lipoprotein (HDL), total cholesterol (TC), and total triglyceride (TG) levels, were measured using assay kits (Shinyang Chemical, Busan, Korea) and spectrophotometry techniques. All the experiments were performed according to the manufacturer's instructions. First, the liver tissue was homogenized in 0.9 % sodium chloride solution and then reacted in a chloroform–methanol mixture (2:1, v/v). The lipid extract was filtered using a chloroform-soaked qualitative filter

paper (Grade 3, Whatman). After evaporation, the lipid extract was stored at −40 °C.

2.10. Histological analysis

Both epididymal white adipose tissue (eWAT) and liver tissue were analyzed histologically. Each tissue sample was washed with phosphate-buffered saline and fixed with 10 % buffered formalin solution for 24 h. Formalin-fixed paraffin-embedded tissues were embedded in paraffin and cut into 5 μm-thick sections. Tissue sections were prepared using hematoxylin and eosin staining for histological analysis under a microscope (Olympus, Tokyo, Japan) at 200 × magnification.

2.11. Reverse transcription - quantitative polymerase chain reaction (RT-qPCR)

Gene expression analysis by RT-qPCR was performed, based on previous experimental methods [24]. RNA was isolated from eWAT and liver tissues using the RNeasy Kit (Qiagen, Hilden, Germany). After quantifying the extracted RNA, 400 ng was used for cDNA synthesis using a qPCR RT Kit (Toyobo, Osaka, Japan). The synthesized cDNA was mixed with the Master Mix (Toyobo) and quantified using ViiA7TM (Applied Biosystems). The relative mRNA levels of peroxisome proliferator-activated receptor γ (PPARγ), fatty acid synthase (FAS), fatty acid-binding protein 4 (FABP4), CCAAT/enhancer-binding protein alpha (C/EBPα), and sterol regulatory element-binding protein 1c (SREBP1c) were measured; the primer sequences are listed in Table S2. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control gene to calibrate the expression levels of the target genes.

2.12. Statistical analysis

Statistical analysis of each dataset was performed using the Prism software (GraphPad Version 9.5.1, CA, USA). One-way analysis of variance (ANOVA) with Tukey's test for multiple comparisons was performed to verify significant differences between groups. Experimental data were expressed as means, and the standard error of the mean (SEM) was used to determine differences between samples. Statistical significance was set at $p < 0.05$.

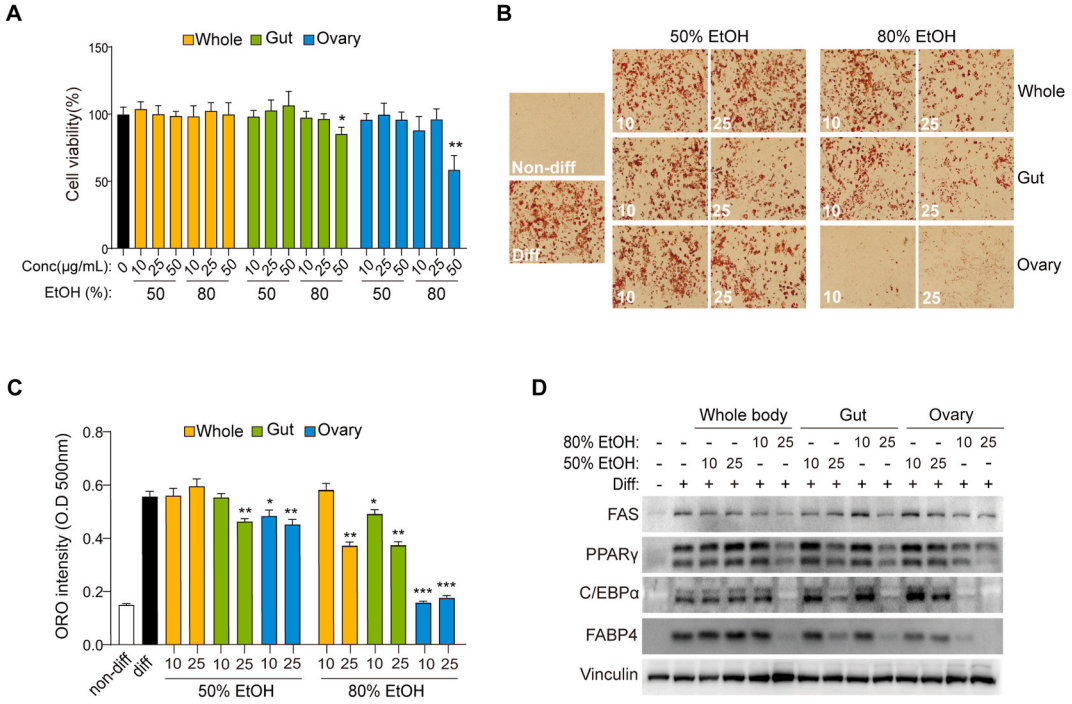


Fig. 1. Anti-adipogenic effects of ethanol extracts from different sea cucumber parts. (**A**) 3T3-L1 cells were treated with ethanol extracts from different sea cucumber body parts and cell viability was measured using MTT assay kit. (**B**) Oil Red O (ORO) staining images and (**C**) intensity of differentiated 3T3-L1 cells treated with the different ethanol extracts. (**D**) Protein levels of FAS, PPARγ, FABP4, and C/EBPα in 3T3-L1 cells treated with the ethanol extracts (non-adjusted images are provided in Supplementary Fig. 1). The derived data and significant differences compared to the veh group were as follows: mean ± SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

3. Results

3.1. Anti-adipogenic effect of ethanol extracts of different sea cucumber parts

We prepared three different sea cucumber extracts, namely whole body, gut, and ovary extracts, and treated 3T3-L1 cells with different concentrations of the as-prepared extracts (10–50 μg mL^{-1}) for 24 h to investigate their cytotoxic effects. The results indicated no cytotoxicity at concentrations below 25 μg mL^{-1} (Fig. 1A). Therefore, 25 μg mL^{-1} was selected as the maximum extract concentration for further experiments. The effect of each extract on the adipogenic differentiation of 3T3-L1 cells was investigated using the ORO staining method. For differentiated 3T3-L1 adipocytes, an 80 % ethanol extract was more effective at inhibiting lipid accumulation than a 50 % ethanol extract (Fig. 1B and C). Moreover, the 80 % SCOE showed the most potent suppression of intracellular lipid accumulation, even at a relatively low concentration of 10 μg mL^{-1}. Furthermore, we analyzed the effects of each sea cucumber extract on the expression of adipogenesis-related genes. As shown in Fig. 1D, at 25 μg mL^{-1}, all extracts significantly decreased the protein expression of the adipocyte differentiation regulators, including PPARγ, FAS, FABP4, and C/EBPα. Notably, 80 % ethanol SCOE exhibited a more pronounced inhibitory effect on adipogenesis-related protein expression than the other extracts, even at the lowest concentration of 10 μg mL^{-1}. These results indicated that the whole body, gut, and ovary of sea cucumber possess anti-adipogenic effects, with 80 % ethanol SCOE being the most potent in suppressing lipid accumulation in differentiated 3T3-L1 cells.

3.2. Effect of SCOE on weight loss in HFD-fed mice

Next, we examined whether SCOE exhibits anti-obesity effects in animal experiments. To determine the anti-obesity effect of SCOE, body weight changes were evaluated in male C57BL/6J (six-week-old) mice fed an HFD containing 0.05, 0.1, and 0.2 % SCOE for eight weeks. After six weeks, weight changes were observed in the HFD +0.2 % SCOE group compared to those in the HFD group, and there was a significant difference in the final weight at the end of the experiment (Fig. 2A). Notably, a significant weight loss of approximately 30.4 % was observed in the HFD +0.2 % SCOE group compared to that in the HFD group (Fig. 2B). There was no significant difference in food intake between the groups during the experimental period (data not shown). In addition, because obesity affects blood glucose levels, we evaluated whether SCOE improves glucose homeostasis in HFD-fed mice using an oral glucose tolerance test.

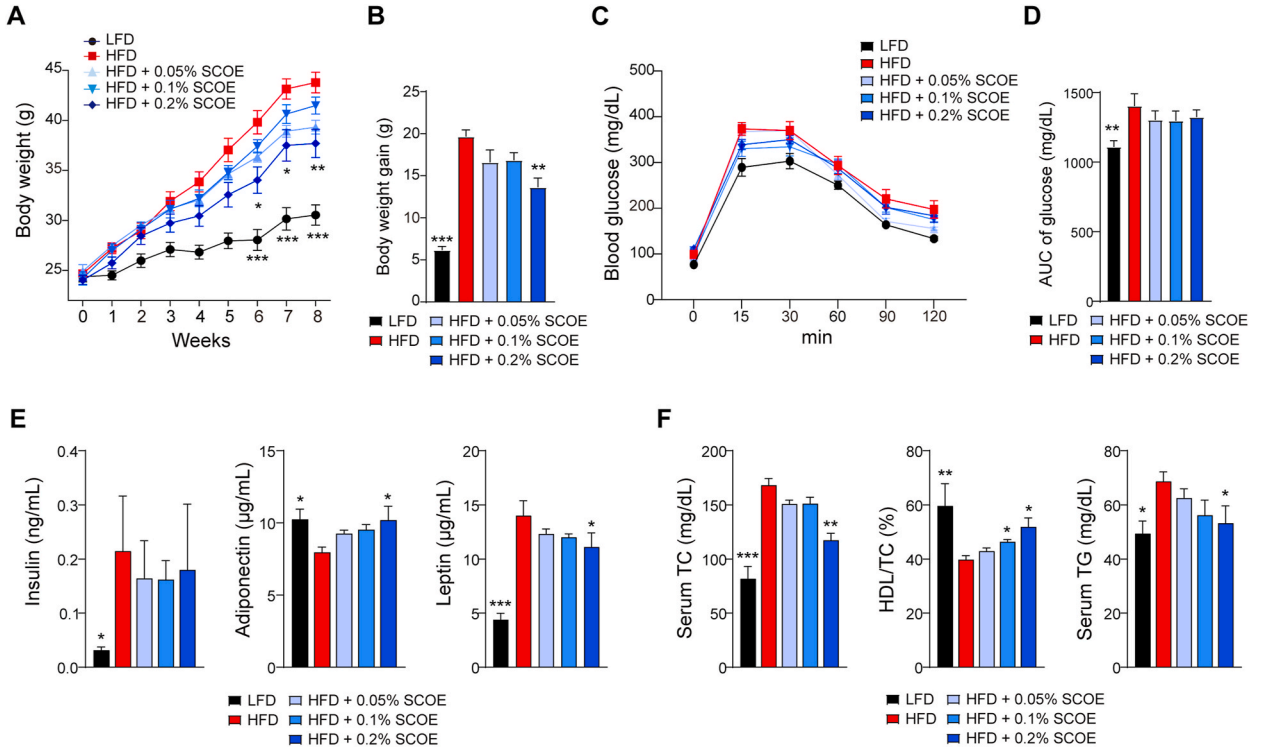


Fig. 2. SCOE significantly reduces body weight gain in obese mice fed a high-fat diet. (**A**) Body weight changes and **(B)** measurement of body weight gain in mice observed after inclusion of 0.05, 0.1, and 0.2 % SCOE in the HFD group for eight weeks. (**C**) Measurement of changes in blood glucose levels using oral glucose tolerance test and (**D**) quantification of data from the area under the curve. (**E**) Serum insulin, adiponectin, and leptin levels. (**F**) Quantification of serum cholesterol and triglyceride concentrations. Derived data and significant differences compared to the HFD + veh group were as follows: mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001. SCOE, sea cucumber ovary extract; LFD, low-fat diet; HFD, high-fat diet.

The blood glucose levels were measured before (0 min) and after (15, 30, 45, 60, 90, and 120 min) glucose administration. A 300 mg/dL increase in blood glucose was detected in the HFD group compared to that in the LFD group; however, the glucose level did not significantly decrease among the SCOE-fed groups (Fig. 2C and D).

Adiponectin is a major adipokine involved in many metabolic activities, and its levels are reduced in obese subjects [25]. In contrast, leptin expression in adipocytes is proportional to the amount of adipose tissue and nutritional status, and its levels tend to be higher in obese subjects [26]. The HFD +0.2 % SCOE group showed increased adiponectin levels and decreased leptin levels in serum compared to the HFD group (Fig. 2E). Moreover, adiponectin levels are closely associated with insulin secretion [27]. Similar to the blood glucose levels, the insulin levels tended to decrease in the SCOE-intake groups, although the difference was not significant. We also analyzed changes in the serum TG, TC, and HDL levels induced by SCOE in HFD mice to confirm changes in the blood lipid concentrations. Our results revealed that HFD-induced increases in serum TG and TC levels significantly decreased only in the HFD +0.2 % SCOE group (Fig. 2F). Furthermore, the HFD decreased HDL levels, which were significantly rescued in the HFD +0.2 % SCOE group.

3.3. *Effect of SCOE on adipose tissue weight in HFD-induced mice*

To confirm the suppression of body weight gain by SCOE, the weight of WAT isolated from each group was measured. The weights of the subcutaneous, retroperitoneal, and epididymal WAT markedly increased in the HFD group compared to those in the LFD group, whereas they clearly decreased in the HFD +0.2 % SCOE group (Fig. 3A). Moreover, we quantified the lipid droplets in eWAT by analyzing their size and number using histological analysis. Image analysis showed that the size of the lipid droplets was significantly decreased in the HFD + SCOE groups compared to that in the HFD group (Fig. 3B and C). In the quantitative lipid droplet analysis, the

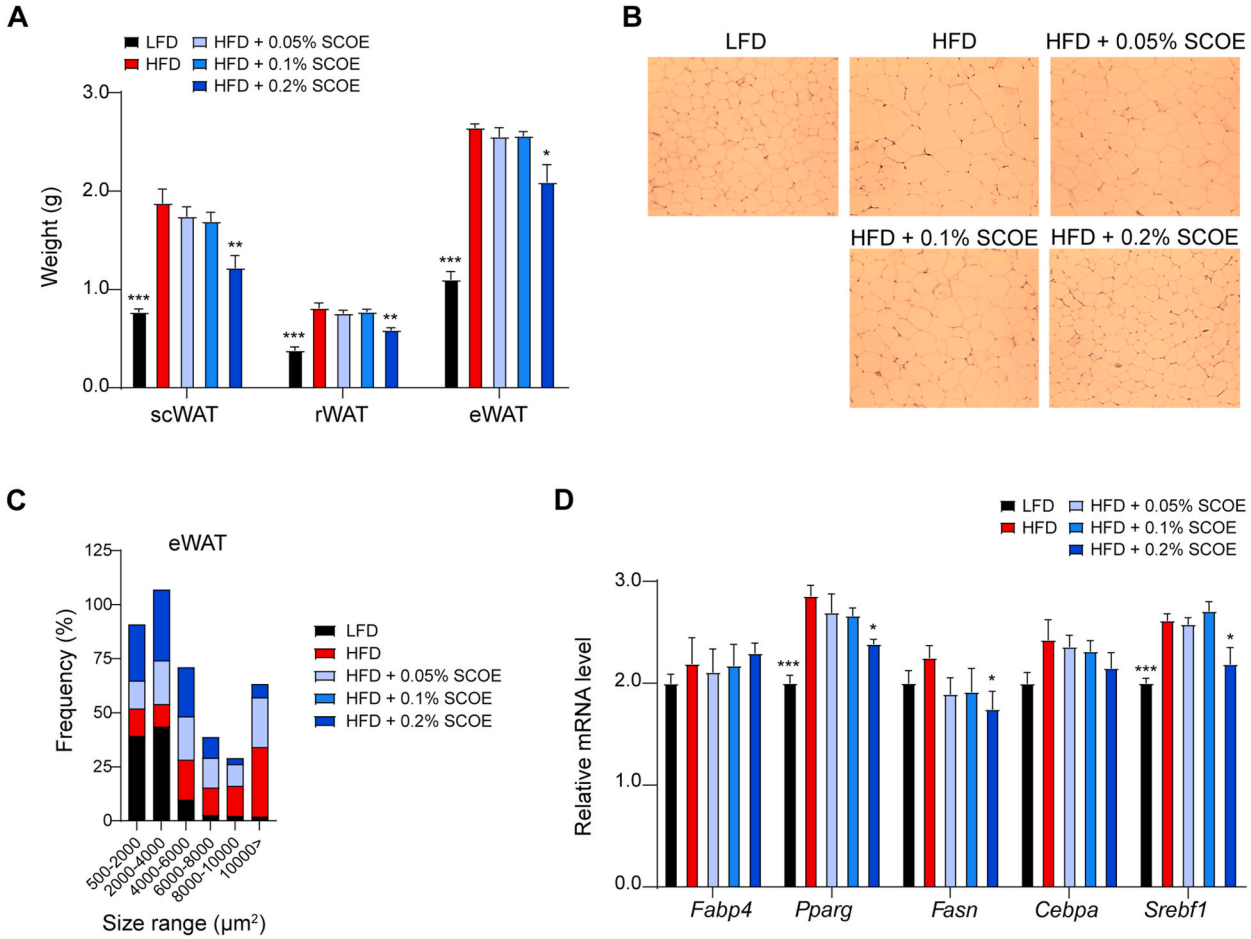


Fig. 3. SCOE reduces the weight of adipose tissues in obese mice. (**A**) Weight measurement of WAT. (**B**) Histological analysis of the size of eWAT and (**C**) measurement of lipid droplet size and frequency in eWAT. (**D**) Measurement of mRNA expression levels of target genes related to adipogenesis compared to GAPDH in eWAT. The derived data and significant differences compared to the HFD + veh group were as follows: mean ± SEM, *p < 0.05, **p < 0.01. WAT, white adipose tissue; scWAT, subcutaneous WAT; rWAT, retroperitoneal WAT; eWAT, epididymal WAT; SCOE, sea cucumber ovary extract; LFD, low-fat diet; HFD, high-fat diet.

frequency of lipid droplets >8000 μm was markedly higher in the HFD group, whereas the frequency of droplets <4000 μm increased in the HFD +0.2 % SCOE group. In addition, we analyzed the changes in adipogenesis-related genes at the mRNA level in the eWAT samples to further analyze the anti-adipogenic effects of SCOE. HFD upregulated the mRNA expression levels of PPARγ and SREBP-1c, which were significantly suppressed in the HFD +0.2 % SCOE group (Fig. 3D). The mRNA expression level of C/EBPα showed a decreasing trend with increasing SCOE concentration; however, the difference was not significant. The mRNA expression levels of FAS, which regulates the terminal differentiation of adipocytes, were significantly decreased in the HFD +0.2 % SCOE group compared to those in the HFD group.

3.4. Effect of SCOE on the fatty liver in HFD-induced mice

A long-term HFD induces abnormal lipid accumulation in hepatocytes and increases the risk of liver disease [28]. Therefore, we subsequently investigated the effects of SCOE on lipid accumulation in the liver. The liver weight was significantly lower in the 0.2 % SCOE group than in the HFD group (Fig. 4A). In addition, significant increases in the liver TG and TC levels were observed in the HFD group, which were notably reduced in the HFD +0.2 % SCOE group (Fig. 4B). SCOE significantly improved the liver lipid levels. These results indicate that SCOE affects the overall liver weight by reducing liver TG, TC, and lipid levels. The livers of the mice in the HFD group were enlarged and light-brown compared to those of the mice in the LFD group, whereas those of the mice in the SCOE groups were relatively small and varied in color. In particular, in the HFD +0.2 % SCOE group, the livers were small and dark red (Fig. 4C). Mice in the HFD group showed significant lipid accumulation in the liver, which was reduced in the HFD +0.2 % SCOE group to an extent similar to that observed in the LFD group. We also investigated the effects of SCOE on the expression levels of genes related to hepatic lipogenesis. Similar to the adipose tissue analysis results, the mRNA expression level of PPARγ was significantly lower in the HFD +0.2 % SCOE group than in the HFD group (Fig. 4D). C/EBPα mRNA levels tended to decrease; however, this change was not statistically significant. The expression levels of the liver transcription factor SREBP-1c and its downstream signaling molecule FAS increased in the HFD group and decreased in the HFD +0.2 % SCOE group.

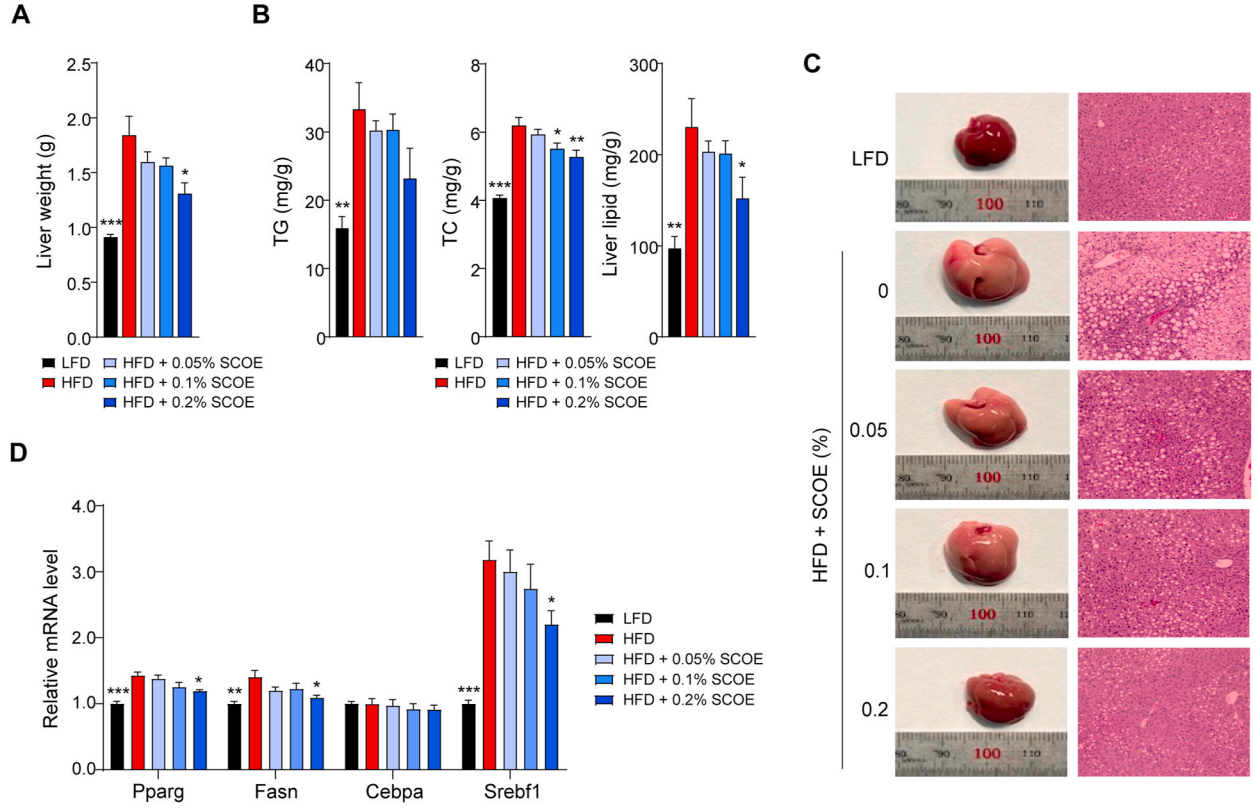


Fig. 4. SCOE improves fatty liver in obese mice. (**A**) Weights of the liver of mice in the HFD + veh and SCOE-intake groups. (**B**) Measurement of triglycerides, total cholesterol, and lipid levels in liver. (**C**) Liver morphology and histological analysis. (**D**) Measurement of mRNA expression levels of target genes related to lipogenesis compared to GAPDH in eWAT. Derived data and significant differences compared to the HFD + veh group were as follows: mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001. SCOE, sea cucumber ovary extract; LFD, low-fat diet; HFD, high-fat diet; TG, triglycerides; TC, total cholesterol.

3.5. Isolation and purification of saponins from sea cucumber ovary

The saponin-rich butanol fraction was extracted from the SCOE based on the solvent polarity (Fig. 5). Eight saponins were isolated using a combination of diverse column chromatography techniques and preparative liquid chromatography. These separated and purified saponins were further investigated to assess their potential to inhibit adipocyte differentiation.

3.6. Inhibitory effect of adipogenesis of saponins isolated from sea cucumber ovary

Therefore, we proceeded to determine which of the eight saponins isolated from SCOE inhibited 3T3-L1 adipocyte differentiation. ORO staining revealed that five of the saponins inhibited adipogenesis (Fig. 6A), with S6 and S8 being the most effective, even at the lowest concentrations, followed by S4, S5, and S3. The intensity of the ORO staining supported the same findings (Fig. 6B). Notably, S6 and S8, which exhibited the most potent inhibition of adipogenesis, did not induce significant cytotoxicity at the experimental concentration range (Fig. 6C). Conversely, S3, S4, and S5 displayed cytotoxic effects at a concentration of 5 μg mL^{-1}, suggesting their potential involvement in the partial inhibition of adipogenesis. Furthermore, an investigation of the gene expression related to adipogenesis revealed that S6 and S8 effectively suppressed the expression of all target proteins, including FAS, PPARγ, C/EBPα, and FABP4, even at the lowest concentration of 1 μg mL^{-1} (Fig. 6D). S4 and S5 also inhibited the expression of these proteins. These results suggested that among the eight saponins isolated from the SCOE, S4, S5, S6, and S8 may be anti-obesity substances capable of

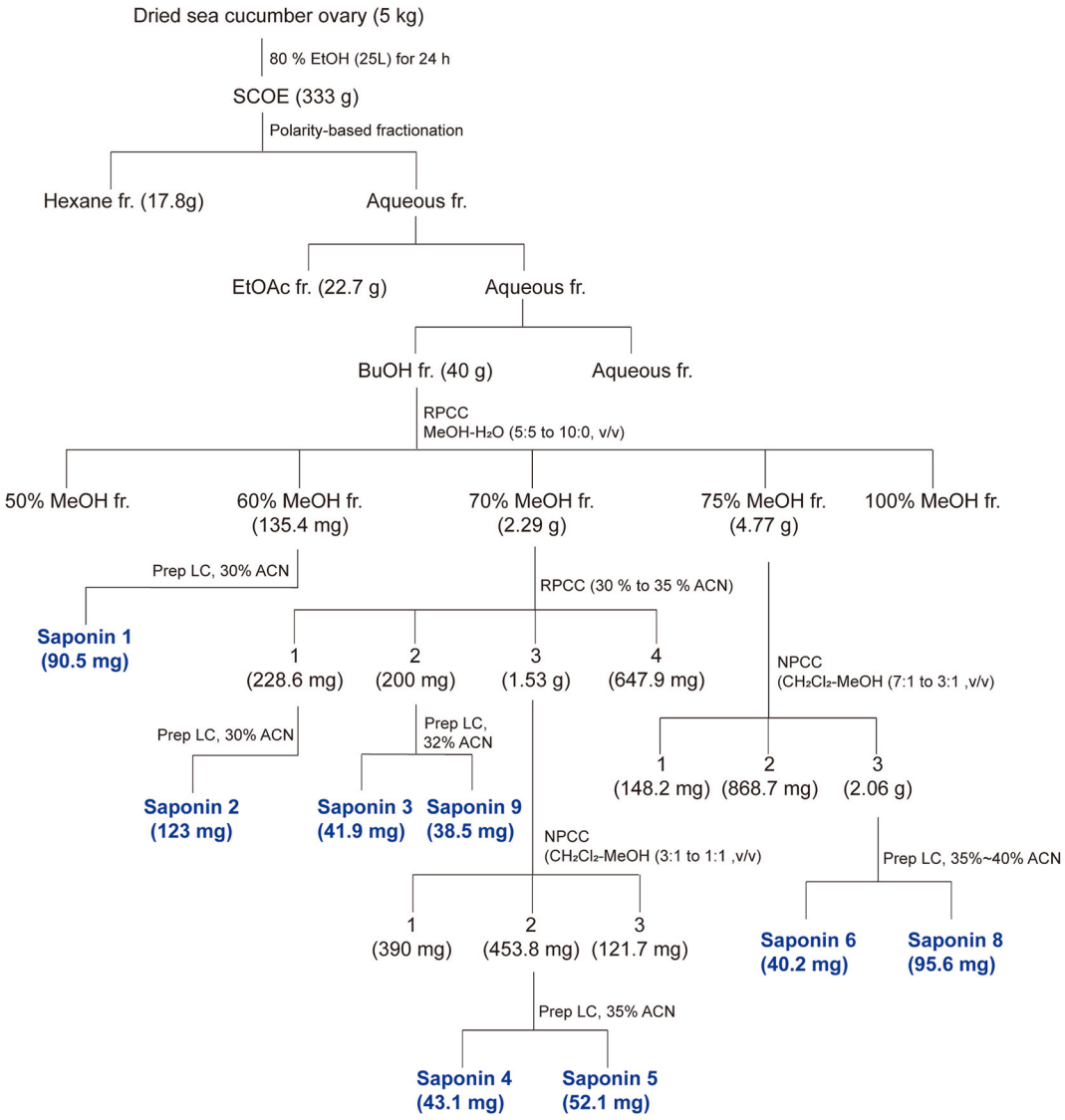


Fig. 5. Flow diagram for the separation and purification of saponins from SCOE. RPCC: reverse-phase column chromatography. NPCC: normal phase column chromatography.

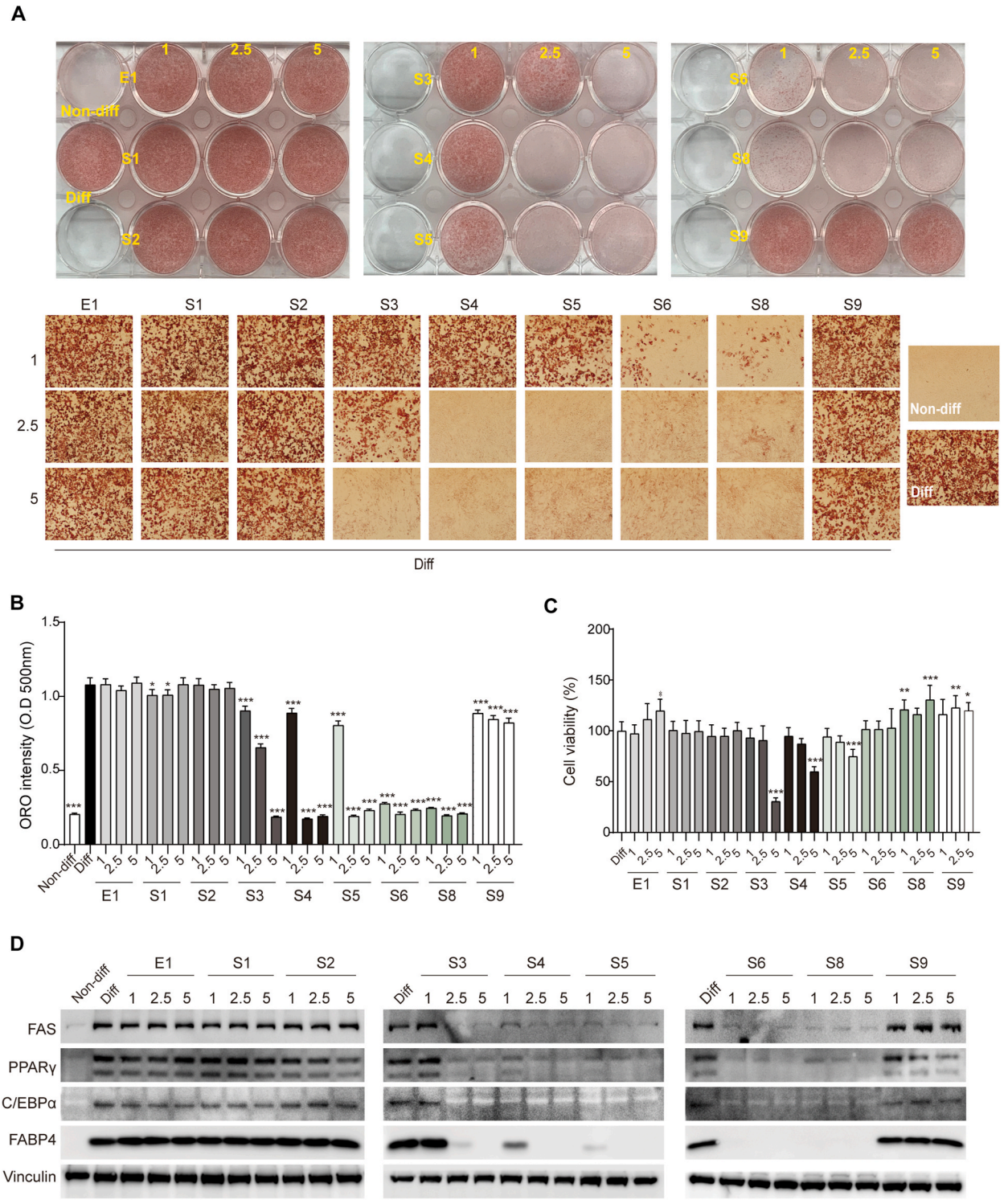


Fig. 6. Inhibitory effect of adipogenesis of saponins isolated from SCOE. (**A**) ORO staining and (**B**) intensity of differentiated 3T3-L1 cells. (**C**) Cell viability. (**D**) Protein levels of FAS, PPARγ, FABP4, and C/EBPα in 3T3-L1 cells (non-adjusted images are provided in Supplementary Figs. 2–4). *p < 0.05, **p < 0.01, ***p < 0.001.

inhibiting 3T3-L1 adipogenesis. These findings suggest that these saponins may contribute to the anti-obesity effect of SCOE and have great potential as anti-obesity agents.

3.7. Identification of adipogenesis-inhibiting saponins isolated from SCOE

Based on the results of the inhibitory effect of adipogenesis, we investigated the structures of three saponins, S5, S6, and S8. S4, which had an inhibitory effect on adipogenic differentiation, was excluded because its cytotoxicity was relatively higher than that of the remaining three saponins. The chemical shifts of the aglycone and sugar moieties of the three saponins in ^{13}C NMR were confirmed, as shown in Tables 1 and 2. As a result of NMR analysis, it was confirmed that S5 was holotoxin D1, S6 was holotoxin B, and S8 was holotoxin A. The putative structures of these three newly identified saponins are illustrated in Fig. 7.

4. Discussion

The primary underlying cause of obesity is excessive caloric intake resulting from the overconsumption of food and inadequate energy expenditure due to a relative lack of physical activity. This energy imbalance contributes significantly to various complications, including diabetes, hypertension, hyperlipidemia, stroke, and metabolic syndrome [29,30]. Effectively addressing obesity requires comprehensive lifestyle modifications, including the adoption of a balanced and nutritious diet, together with regular and sustained engagement in physical exercise. Concurrently, functional foods have been proposed as a supplementary means of preventing and treating obesity. Recently, the interest in the beneficial biological functions of marine organisms living in environments different from those of terrestrial organisms has increased [31].

Sea cucumbers have gained attention as a representative marine food ingredient because of their various physiological functions. Notably, it has been reported to exhibit anti-obesity effects. For example, in mice fed a HFD, whole sea cucumber extract improved blood lipid levels and reduced liver fat accumulation [17]. Similarly, sea cucumber gut extract effectively reduced body fat and fat mass in obese mice fed a HFD [32]. One key component that has attracted attention in this regard is sea cucumber saponins. Compared with those of terrestrial plants, saponins present in sea cucumbers have a unique chemical structure owing to the marine environment of these echinoderms. Sea cucumber saponins generally consist of triterpenoid oligoglycosides, which is linked to an aglycone (500–1500 Da) and a sugar chain via a β-glycosidic bond. These saponins are classified as holostane and non-holostane types, according to the structure of the aglycone [33]. Most saponins belong to the holostane series; holothurin A and echinoside A, which are known to reduce weight gain and fatty liver in obese mice, are representative holostanes [22]. Although these saponins have received

Table 1
^{13}C NMR (100 MHz) data for aglycones of compounds **S5, S6,** and **S8** in C_5D_5N/D_2O (δ in ppm).

Aglycone			
Position	S5 δ_C	S6 δ_C	S8 δ_C
1	35.3	36.1	38.0
2	26.0	26.9	27.1
3	87.5	88.6	88.8
4	38.8	39.8	39.9
5	48.6	52.7	52.9
6	21.4	20.9	22.2
7	27.5	28.4	28.5
8	37.7	39.6	39.7
9	150.3	151.1	151.3
10	38.7	39.6	39.7
11	110.1	111.0	111.2
12	31.1	31.9	32.1
13	54.7	55.7	55.9
14	41.1	42.0	42.1
15	51.0	51.9	52.1
16	212.1	212.5	213.8
17	60.2	61.2	61.3
18	175.0	176.2	176.5
19	21.1	22.0	22.3
20	82.1	83.0	83.2
21	25.8	26.7	26.9
22	37.5	37.8	38.8
23	21.3	22.2	22.3
24	37.0	38.3	38.5
25	144.6	145.4	145.6
26	109.5	110.4	110.6
27	21.3	22.2	22.3
30	15.7	16.5	16.7
31	27.1	27.9	28.1
32	19.7	20.6	20.7

Table 2
^{13}C NMR (100 MHz) data for glycoside moiety of compounds **S5, S6,** and **S8** in C_5D_5N/D_2O (δ in ppm).

Glycoside			
Position	S5 δ_C	S6 δ_C	S8 δ_C
	Xyl^1(1→C3)	**Xyl^1(1→C3)**	**Xyl^1(1→C3)**
1	104.3	105.1	105.2
2	81.9	83.2	83.4
3	75.6	75.4	75.6
4	76.5	77.7	77.9
5	63.0	63.8	63.9
	Glc^1(1 → 2Xyl^1)	**Quin^1(1 → 2Xyl^1)**	**Quin^1(1 → 2Xyl^1)**
1	104.7	105.0	105.2
2	75.7	75.9	76.0
3	74.7	75.5	75.7
4	79.4	86.9	87.0
5	77.4	71.5	71.7
6 (or Me)	60.3	18.2	18.2
	Xyl^2(1 → 4Glc^1)	**Glc^1(1 → 4Quin^1)**	**Glc^1(1 → 4Quin^1)**
1	103.9	104.5	104.6
2	72.7	73.7	73.5
3	86.9	87.7	87.6
4	68.1	69.5	69.6
5	65.6	77.4	77.6
6	–	61.8	62.0
	MeGlc^1(1 → 3Xyl^2)	**MeGlc^1(1 → 3Xyl^2)**	**MeGlc^1(1 → 3Glc^1)**
1	104.7	105.2	105.2
2	74.6	74.8	75.0
3	87.0	87.5	87.5
4	69.6	70.4	70.6
5	77.2	78.2	77.7
6	61.1	62.2	62.0
Me	59.8	60.7	60.9
	Glc^2(1 → 4Xyl^1)	**Glc^2(1 → 4Xyl^1)**	**Glc^2(1 → 4Xyl^1)**
1	101.8	102.4	102.6
2	72.3	73.3	73.9
3	87.0	87.7	87.6
4	68.7	69.6	69.7
5	77.3	78.0	78.1
6	61.2	61.9	62.1
	Glc^3(1 → 3Glc^2)	**Glc^3(1 → 3Glc^2)**	**MeGlc^2(1 → 3Glc^2)**
1	104.7	105.2	105.3
2	74.2	75.2	74.9
3	77.3	78.3	87.5
4	70.6	71.3	70.6
5	77.6	78.0	78.1
6	61.5	61.9	62.1
Me	–	–	60.9

considerable attention owing to their potential as anti-obesity agents, research on saponins with anti-obesity effects remains limited.

In this study, we conducted an anti-obesity experiment using *S. japonicus*, a Far Eastern sea cucumber favored in East Asian countries including Korea, Japan, and China [34]. First, the possibility of inhibiting adipocyte differentiation was investigated by extracting various parts of the sea cucumber, including the whole body, intestines, and ovaries, with 50 % or 80 % ethanol. Cell-based assays revealed that the 80 % ethanol sea cucumber extract was more effective than the 50 % ethanol extract in inhibiting adipogenesis. Notably, the SCOE exhibited the most potent inhibitory effect on the expression of adipogenesis-related proteins (FAS, PPARγ, FABP4, and C/EBPα), even at lower concentrations than extracts from other sites.

Based on *in vitro* studies using 3T3-L1 cell differentiation experiments, we confirmed the anti-obesity effects of SCOE in HFD-fed obese mice. In a comparative experiment with the HFD only group, body weight gain was significantly reduced in the HFD +0.2 % SCOE group. Serum and liver TC and TG assays showed that 0.2 % SCOE significantly improved the serum lipid profile of HFD-fed mice. Additionally, 0.2 % SCOE significantly reduced the eWAT and hepatic lipid droplet sizes and the mRNA expression of PPARγ, FAS, and SREBP-1c in both eWAT and the liver. PPARγ, FAS, and SREBP-1c are genes characteristically identified in obesity and play key roles in adipogenesis and lipogenesis in eWAT and liver, respectively [35]. PPARγ increases the expression of genes involved in adipocyte differentiation and enzymes involved in lipid metabolism [36]. FAS, an enzyme involved in fatty acid synthesis, increases fat accumulation by increasing fatty acid synthesis [37]. SREBP-1c is a member of the sterol regulatory element-binding protein (SREBP) family and is a transcription factor involved in lipid metabolism [38]. These results suggest that SCOE intake downregulates the mRNA expression of PPARγ, FAS, and SREBP-1c in adipose tissue and the liver, which may help prevent and treat obesity.

In the present study, the HFD-induced increase in blood glucose levels was not significant but was alleviated by SCOE. Moreover,

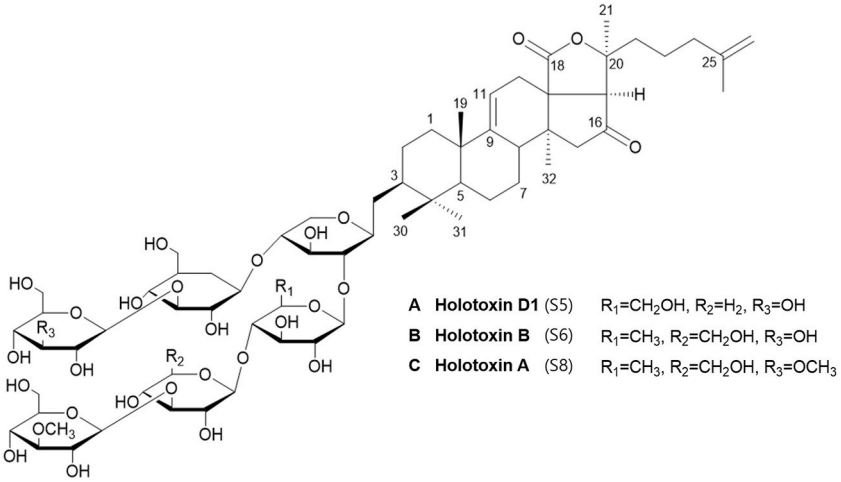


Fig. 7. Putative structures of saponins with anti-adipogenesis effect. (**A**) Holotoxin D1, (**B**) Holotoxin B, and (**C**) Holotoxin A.

SCOE decreased insulin levels; however, this effect was also not significant. Conversely, SCOE affected the adipokine levels. Adiponectin, a protein secreted by adipose tissue and vascular endothelial cells, plays an essential role in preventing metabolic diseases such as diabetes and obesity by increasing insulin sensitivity [39]. Leptin is a hormone secreted by adipose tissue that promotes weight loss by suppressing appetite [40]. SCOE significantly increased adiponectin and decreased leptin levels. These results suggest that an increase in adiponectin levels and decrease in leptin levels can generally be considered favorable for metabolic health, especially in the context of obesity and related metabolic disorders.

Next, we aimed to identify saponins in SCOE that exhibited inhibitory effects on adipogenesis. We obtained the butanol fraction of SCOE through polarity-based fractionation, and subsequently isolated eight saponins using various column chromatography techniques. Among these saponins, we identified four that effectively inhibited adipogenesis in 3T3-L1 cells. Through structural analysis, three of these compounds were characterized as novel saponins, namely holotoxins A, B, and D1, whose anti-adipogenic properties have not been previously reported. Holothurin A and echinoside A, which have been previously reported as saponins with anti-obesity properties found in sea cucumbers, are mainly extracted from the body walls of sea cucumbers [41,42]. Notably, sea cucumbers contain a plethora of saponins that have not yet been fully characterized. Therefore, the process of verifying the anti-obesity efficacy of various sea cucumber body parts and the discovery of new saponins provides an interesting perspective for identifying unknown saponins and compounds.

5. Conclusions

SCOE demonstrated the highest efficacy in inhibiting 3T3-L1 adipocyte differentiation among the various sea cucumber body parts investigated. Furthermore, in animal experiments involving obese mice, SCOE effectively reduced weight gain by inhibiting lipid accumulation in white adipose and liver tissues. Notably, this study identified three novel saponins (holotoxins A, B, and D1) in SCOE, demonstrating potent inhibition of adipocyte differentiation. There are some limitations in that preclinical research on newly isolated saponins has not been conducted, and the safety of SCOE and its saponins has not been verified. Nevertheless, our findings provide a promising foundation for the development of SCOE and novel saponin-based treatments for obesity. Follow-up research on safety verification and preclinical evaluation of newly isolated saponins is needed, which may contribute to the development of novel anti-obesity agents and functional foods using sea cucumber ovaries.

Ethics statement

All animal experiments were approved by the Animal Care and Use Committee of the Korea Food Research Institute and were performed in accordance with the relevant institutions and national guidelines (approval number: KFRI-M-21018).

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Hyo-Deok Seo: Writing – original draft, Methodology, Formal analysis, Data curation. **Ji-Young Lee:** Writing – original draft, Methodology, Investigation, Data curation. **So-Hyun Park:** Investigation, Data curation. **Eunyoung Lee:** Investigation, Data curation.

Jeong-Hoon Hahm: Methodology. **Jiyun Ahn:** Supervision, Methodology. **A Ra Jang:** Formal analysis. **So Hee An:** Methodology. **Jang Ho Ha:** Methodology. **Kyoung Tai No:** Funding acquisition, Conceptualization. **Chang Hwa Jung:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the Ministry of Trade, Industry, and Energy (P114000049), and the Korea Food Research Institute's Basic Research Project (E0210103).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36943.

References

[1] A. Hossain, D. Dave, F. Shahidi, Northern Sea cucumber (Cucumaria frondosa): a potential candidate for functional food, nutraceutical, and pharmaceutical sector, Mar. Drugs 18 (2020), https://doi.org/10.3390/md18050274.
[2] F. Yang, C. Zhou, N.T. Tran, Z. Sun, J. Wu, H. Ge, et al., Comparison of the complete mitochondrial genome of Phyllophorus liuwutiensis (Echinodermata: Holothuroidea: Phyllophoridae) to that of other sea cucumbers, FEBS Open Bio 10 (2020) 1587–1600, https://doi.org/10.1002/2211-5463.12914.
[3] N.B. Janakiram, A. Mohammed, C.V. Rao, Sea cucumbers metabolites as potent anti-cancer agents, Mar. Drugs 13 (2015) 2909–2923, https://doi.org/10.3390/md13052909.
[4] R. Ru, Y. Guo, J. Mao, Z. Yu, W. Huang, X. Cao, et al., Cancer cell inhibiting sea cucumber (Holothuria leucospilota) protein as a novel anti-cancer drug, Nutrients 14 (2022), https://doi.org/10.3390/nu14040786.
[5] J. Song, T. Li, X. Cheng, X. Ji, D. Gao, M. Du, et al., Sea cucumber peptides exert anti-inflammatory activity through suppressing NF-kappaB and MAPK and inducing HO-1 in RAW264.7 macrophages, Food Funct. 7 (2016) 2773–2779, https://doi.org/10.1039/c5fo01622c.
[6] Z. Li, Y. Tian, H. Ma, M. Wang, Z. Yan, C. Xue, et al., Saponins from the sea cucumber promote the osteoblast differentiation in mc3t3-E1 cells through the activation of the BMP2/smads pathway, Curr. Pharmaceut. Biotechnol. 22 (2021) 1942–1952, https://doi.org/10.2174/1389201021666200519135446.
[7] E. Garcia-Candela, C. Pariona-Velarde, A. Mondragon-Martinez, V. Chumpitaz-Cerrate, Antiviral activity of the sea cucumber tegument extract (Pattalus mollis) on human rotavirus A (RVA), Nat. Prod. Res. 35 (2021) 1014–1018, https://doi.org/10.1080/14786419.2019.1611807.
[8] C. Xu, R. Zhang, Z. Wen, Bioactive compounds and biological functions of sea cucumbers as potential functional foods, J. Funct.Foods 49 (2018) 73–84, https://doi.org/10.1016/j.jff.2018.08.009.
[9] T.Y. Zhou, X.W. Xiang, M. Du, L.F. Zhang, N.X. Cheng, X.L. Liu, et al., Protective effect of polysaccharides of sea cucumber Acaudina leucoprocta on hydrogen peroxide-induced oxidative injury in RAW264.7 cells, Int. J. Biol. Macromol. 139 (2019) 1133–1140, https://doi.org/10.1016/j.ijbiomac.2019.08.092.
[10] M. Lu, A. Mishra, C. Boschetti, J. Lin, Y. Liu, H. Huang, et al., Sea cucumber-derived peptides alleviate oxidative stress in neuroblastoma cells and improve survival in C. elegans exposed to neurotoxic paraquat, Oxid. Med. Cell. Longev. 2021 (2021) 8842926, https://doi.org/10.1155/2021/8842926.
[11] H. Che, L. Du, P. Cong, S. Tao, N. Ding, F. Wu, et al., Cerebrosides from sea cucumber protect against oxidative stress in SAMP8 mice and PC12 cells, J. Med. Food 20 (2017) 392–402, https://doi.org/10.1089/jmf.2016.3789.
[12] B. Zhang, C.H. Xue, T.Y. Feng, X.Q. Hu, B.X. Ren, J. Xu, et al., [Effect of sea cucumber cerebroside on lipid metabolism in fatty liver rats], Zhejiang Da Xue Xue Bao Yi Xue Ban 39 (2010) 493–498, https://doi.org/10.3785/j.issn.1008-9292.2010.05.009.
[13] S. Hu, Y. Chang, M. He, J. Wang, Y. Wang, C. Xue, Fucosylated chondroitin sulfate from sea cucumber improves insulin sensitivity via activation of PI3K/PKB pathway, J. Food Sci. 79 (2014) H1424–H1429, https://doi.org/10.1111/1750-3841.12465.
[14] Y. Bahrami, W. Zhang, M.M.F. C, Distribution of saponins in the sea cucumber Holothuria lessoni; the body wall versus the viscera, and their biological activities, Mar. Drugs 16 (2018), https://doi.org/10.3390/md16110423.
[15] Y.-C. Zhao, C.-H. Xue, T.-T. Zhang, Y.-M. Wang, Saponins from sea cucumber and their biological activities, J. Agric. Food Chem. 66 (2018) 7222–7237, https://doi.org/10.1021/acs.jafc.8b01770.
[16] L. Wen, R. Li, Y.C. Zhao, J.Y. Yang, X.Y. Li, C.H. Xue, et al., A comparative study of the anti-obesity effects of dietary sea cucumber saponins and energy restriction in response to weight loss and weight regain in mice, Mar. Drugs 20 (2022) doi:ARTN 62910.3390/md20100629.
[17] L. Guo, Z.Y. Gao, L.Q. Zhang, F.J. Guo, Y. Chen, Y.M. Li, et al., Saponin-enriched sea cucumber extracts exhibit an antiobesity effect through inhibition of pancreatic lipase activity and upregulation of LXR-beta signaling, Pharm. Biol. 54 (2016) 1312–1325, https://doi.org/10.3109/13880209.2015.1075047.
[18] X. Hu, N. Tao, X. Wang, J. Xiao, M. Wang, Marine-derived bioactive compounds with anti-obesity effect: a review, J. Funct.Foods 21 (2016) 372–387, https://doi.org/10.1016/j.jff.2015.12.006.
[19] S. Gangadaran, S.K. Cheema, A high fat diet enriched with sea cucumber gut powder provides cardio-protective and anti-obesity effects in C57BL/6 mice, Food Res. Int. 99 (2017) 799–806, https://doi.org/10.1016/j.foodres.2017.06.066.
[20] B. Han, Y. Jian, X. Xia, W. Hu, L. Zhang, P. Zhou, Studying the effects of sea cucumber ovum powder on nonalcoholic fatty liver disease by proteomics techniques in a rat model, Food Funct. 11 (2020) 6139–6147, https://doi.org/10.1039/d0fo00741b.
[21] Y.M. Wang, J.H. Wang, R.C. Yanagita, C.H. Liu, X.Q. Hu, P. Dong, et al., Effects of two sulfated triterpene saponins echinoside A and holothurin A on the inhibition of dietary fat absorption and obesity reduction, Biosci., Biotechnol., Biochem. 78 (2014) 139–146, https://doi.org/10.1080/09168451.2014.877830.
[22] J. Meng, X. Hu, T. Zhang, P. Dong, Z. Li, C. Xue, et al., Saponin from sea cucumber exhibited more significant effects than ginsenoside on ameliorating high fat diet-induced obesity in C57BL/6 mice, Medchemcomm 9 (2018) 725–734, https://doi.org/10.1039/c7md00653e.
[23] S.H. Park, D.H. Lee, M.J. Kim, J. Ahn, Y.J. Jang, T.Y. Ha, et al., Inula japonica thunb. Flower ethanol extract improves obesity and exercise endurance in mice fed A high-fat diet, Nutrients 11 (2018), https://doi.org/10.3390/nu11010017.
[24] H.D. Seo, E. Lee, J. Ahn, J.H. Hahm, T.Y. Ha, D.H. Lee, et al., Codium fragile reduces adipose tissue expansion and fatty liver incidence by downregulating adipo- and lipogenesis, J. Food Biochem. 46 (2022). ARTN e1439510.1111/jfbc.14395.
[25] V. Francisco, C. Ruiz-Fernandez, J. Pino, A. Mera, M.A. Gonzalez-Gay, R. Gomez, et al., Adipokines: linking metabolic syndrome, the immune system, and arthritic diseases, Biochem. Pharmacol. 165 (2019) 196–206, https://doi.org/10.1016/j.bcp.2019.03.030.

[26] C. Pico, M. Palou, C.A. Pomar, A.M. Rodriguez, A. Palou, Leptin as a key regulator of the adipose organ, Rev. Endocr. Metab. Disord. 23 (2022) 13–30, https://doi.org/10.1007/s11154-021-09687-5.
[27] H.W. Liu, Y.C. Chang, Y.C. Chan, S.H. Hu, M.Y. Liu, S.J. Chang, Dysregulations of mitochondrial quality control and autophagic flux at an early age lead to progression of sarcopenia in SAMP8 mice, Biogerontology 21 (2020) 367–380, https://doi.org/10.1007/s10522-020-09867-x.
[28] C.Y. Lian, Z.Z. Zhai, Z.F. Li, L. Wang, High fat diet-triggered non-alcoholic fatty liver disease: a review of proposed mechanisms, Chem. Biol. Interact. 330 (2020) 109199, https://doi.org/10.1016/j.cbi.2020.109199.
[29] T.C. Dinh, T.N. Thi Phuong, L.B. Minh, V.T. Minh Thuc, N.D. Bac, N. Van Tien, et al., The effects of green tea on lipid metabolism and its potential applications for obesity and related metabolic disorders - an existing update, Diabetes Metabol. Syndr. 13 (2019) 1667–1673, https://doi.org/10.1016/j.dsx.2019.03.021.
[30] E. Nisoli, M.O. Carruba, Emerging aspects of pharmacotherapy for obesity and metabolic syndrome, Pharmacol. Res. 50 (2004) 453–469, https://doi.org/10.1016/j.phrs.2004.02.004.
[31] S.F. Hosseini, M. Rezaei, D.J. McClements, Bioactive functional ingredients from aquatic origin: a review of recent progress in marine-derived nutraceuticals, Crit. Rev. Food Sci. Nutr. 62 (2022) 1242–1269, https://doi.org/10.1080/10408398.2020.1839855.
[32] S. Gangadaran, S.K. Cheema, A high fat diet enriched with sea cucumber gut powder provides cardio-protective and anti-obesity effects in C57BL/6 mice, Food Res. Int. 99 (2017) 799–806, https://doi.org/10.1016/j.foodres.2017.06.066.
[33] Y.C. Zhao, C.H. Xue, T.T. Zhang, Y.M. Wang, Saponins from sea cucumber and their biological activities, J. Agric. Food Chem. 66 (2018) 7222–7237, https://doi.org/10.1021/acs.jafc.8b01770.
[34] Y.Q. Chang, Z.G. Feng, J.P. Yu, J. Ding, Genetic variability analysis in five populations of the sea cucumber Stichopus (Apostichopus) japonicus from China, Russia, South Korea and Japan as revealed by microsatellite markers, Mar Ecol-Evol Persp. 30 (2009) 455–461, https://doi.org/10.1111/j.1439-0485.2009.00292.x.
[35] B. Ahmad, C.J. Serpell, I.L. Fong, E.H. Wong, Molecular mechanisms of adipogenesis: the anti-adipogenic role of AMP-activated protein kinase, Front. Mol. Biosci. 7 (2020). ARTN 7610.3389/fmolb.2020.00076.
[36] R. Siersbæk, R. Nielsen, S. Mandrup, PPARγ in adipocyte differentiation and metabolism – novel insights from genome-wide studies, FEBS (Fed. Eur. Biochem. Soc.) Lett. 584 (2010) 3242–3249, https://doi.org/10.1016/j.febslet.2010.06.010.
[37] A.P. Jensen-Urstad, C.F. Semenkovich, Fatty acid synthase and liver triglyceride metabolism: housekeeper or messenger? Biochim. Biophys. Acta 1821 (2012) 747–753, https://doi.org/10.1016/j.bbalip.2011.09.017.
[38] D. Eberle, B. Hegarty, P. Bossard, P. Ferre, F. Foufelle, SREBP transcription factors: master regulators of lipid homeostasis, Biochimie 86 (2004) 839–848, https://doi.org/10.1016/j.biochi.2004.09.018.
[39] A.E. Achari, S.K. Jain, Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction, Int. J. Mol. Sci. 18 (2017) doi:ARTN 132110.3390/ijms18061321.
[40] M. Obradovic, E. Sudar-Milovanovic, S. Soskic, M. Essack, S. Arya, A.J. Stewart, et al., Leptin and obesity: role and clinical implication, Front. Endocrinol. 12 (2021). ARTN 58588710.3389/fendo.2021.585887.
[41] X.Q. Hu, Z.J. Li, Y. Xue, J. Xu, C.H. Xue, J.F. Wang, et al., Dietary saponins of sea cucumber ameliorate obesity, hepatic steatosis, and glucose intolerance in high-fat diet-fed mice, J. Med. Food 15 (2012) 909–916, https://doi.org/10.1089/jmf.2011.2042.
[42] X.Q. Han, L.Y. Zhang, L. Ding, H.H. Shi, C.H. Xue, T.T. Zhang, et al., Synergistic effect of sea cucumber saponins and EPA-enriched phospholipids on insulin resistance in high-fat diet-induced obese mice, Food Funct. 10 (2019) 3955–3964, https://doi.org/10.1039/c9fo01147a.