Red algae (Gelidium amansii) hot-water extract ameliorates lipid metabolism in hamsters fed a high-fat diet

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Abstract

The purpose of this study was to investigate the effects of Gelidium amansii (GA) hot-water extracts (GHE) on lipid metabolism in hamsters. Six-week-old male Syrian hamsters were used as the experimental animals. Hamsters were divided into four groups: (1) control diet group (CON); (2) high-fat diet group (HF); (3) HF with GHE diet group (HF + GHE); (4) HF with probucol diet group (HF + PO). All groups were fed the experimental diets and drinking water ad libitum for 6 weeks. The results showed that GHE significantly decreased body weight, liver weight, and adipose tissue (perirenal and paraepididymal) weight. The HF diet induced an increase in plasma triacylglycerol (TG), total cholesterol (TC), low-density lipoprotein cholesterol and very-low-density lipoprotein cholesterol levels. However, GHE supplementation reversed the increase of plasma lipids caused by the HF diet. In addition, GHE increased fecal cholesterol, TG and bile acid excretion. Lower hepatic TC and TG levels were found with GHE treatment. GHE reduced hepatic sterol regulatory element-binding proteins (SREBP) including SREBP 1 and SREBP 2 protein expressions. The phosphorylation of adenosine monophosphate (AMP)-activated protein kinase (AMPK) protein expression in hamsters was decreased by the HF diet; however, GHE supplementation increased the phosphorylation of AMPK protein expression. Our results suggest that GHE may ameliorate lipid metabolism in hamsters fed a HF diet.

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1. Introduction

A diet high in fat may cause hyperlipidemia, which is characterized by elevated plasma levels of total cholesterol (TC), triacylglycerol (TG), and low-density lipoprotein cholesterol (LDL-C) [1,2]. All of these symptoms are known to be major risk factors for developing cardiovascular disease [3] and nonalcoholic fatty liver disease [4,5].

Scientists recently demonstrated that algae can play some beneficial effects on the improvement of hyperlipidemia, nonalcoholic fatty liver disease, and obesity. Chlorella pyrenoidosa, a type of green algae, has been shown to lower serum TC, TG, and LDL-C in rats and hamsters [6]. In addition,
Ecklonia cava, an edible brown algae, is effective in reducing adipose tissue, plasma TC concentration, and liver TG content in mice [7]. Moreover, brown seaweed extracts can decrease serum TG levels and liver fat contents in obese nondiabetic women [8]. These results indicate that algae may play a critical role in regulating lipid metabolism in animals and humans.

Gelidium amansii (GA) is an edible red algae that is widely distributed in Japan, Korea, China, and northeast Taiwan. Agar jelly made from hot-water extracts of GA is a traditional desert in Taiwan and Japan. Our previous study demonstrated that supplementation with GA powder in a high-fat (HF) and high-cholesterol diet can reduce plasma and liver lipids in diabetic rats [9]. It was suggested that the abundance of water-soluble fiber in GA was an active component in reducing lipid accumulation in liver and adipose tissue. Other studies have shown that ethanol extracts of GA can inhibit lipid accumulation in 3T3-L1 cells [10], and decrease body weight, epidymal fat weight, and serum TC and TG levels in obese mice [11]. These results indicate that GA, including both water-soluble components (e.g., water-soluble polysaccharides) and water-insoluble components (e.g., flavonoids), may have beneficial effects on the improvement of lipid metabolism. However, the active component and mechanism of action of the lipid-lowering effect of GA is still unclear.

In Taiwan, GA is traditionally processed by exposure to sunlight followed by extraction with hot water. However, this process may result in a loss of phytochemicals and, therefore, the water-soluble fiber in GA is suggested to play a role in regulating lipid metabolism [9]. Cholesterol and bile acid metabolism of hamsters have been reported to closely resemble that of humans, and thus are considered as a suitable animal model for studying lipid metabolism [12,13]. In the present study we further investigated the effect of the hot-water extract of GA on lipid metabolism in hamsters fed a HF diet, and evaluated its possible mechanism of action.

2. Materials and methods

2.1. GA hot-water extract

GA (Lamouroux) (dry material) was purchased in the market at Keelung, the northeast corner of Taiwan. It was stored at 4°C until used. A 20-g quantity of GA was added to 400 mL of deionized water and autoclaved at 121°C for 20 min. After cooling, the GA hot-water extract (GHE) was filtered through filter paper No. 1 [Advantec. Toyo Roshi Kaisha, Ltd., Tokyo, Japan] and then lyophilized. The harvest weight of hot-water extracts obtained from 20 g GA was 5.71 g (recovery about 28.5%). General compositions of GHE were determined using the methods of the Association of Official Agricultural Chemists (AOAC) [14], including moisture, 6.5%; ash, 4.6%; crude fat, 0.25%; and crude protein, 6.7%. In addition, according to analysis by the Food Industry Research and Development Institute, Hsinchu, Taiwan, GHE contains 68.6% water-soluble dietary fiber and 0% water-insoluble dietary fiber [14]. Moreover, GHE contains 4.1% sulfate (in dry weight). Sulfate content in GHE was determined by the rhodizonate method using sodium sulfate as standard [15].

2.2. Animals and treatments

Six-week-old male Syrian hamsters were purchased from The National Laboratory Animal Center (Taipei, Taiwan). Hamsters were housed in individual stainless steel cages in a room kept at 23°C ± 1°C and 40% to 60% relative humidity, with a 12-h light–dark cycle. Hamsters were fed a standard laboratory diet (5001 rodent diet, Lab Diet, PMI Nutrition International Inc., Brenwood, MO, USA) for 1 week and were then divided into four groups of eight hamsters each. The four groups were as follows: (1) normal diet group [control (CON)]; (2) HF diet group (HF group); (3) HF diet with 1.5% GHE group (HF + GHE group); and (4) HF diet with 1% probucol (Sigma St. Louis, MO, USA) group (HF + PO group). The daily dose of probucol in the HF + PO group was approximately equivalent to 640 mg/kg body weight. Probucol is an antihyperlipidemic drug developed in the treatment of coronary artery disease, which can decrease blood and liver cholesterol in animals and humans [16–18]. The composition of the normal control diet was 5001 rodent diet. Our previous study showed that hamsters fed a diet containing 3% Ching-Shan oil and 0.2% cholesterol can have increased plasma and liver lipid levels [19]. In the present study, the HF diet contained 94.9% (w/w) normal diet, 5% (w/w) Ching-Shan oil, and 0.1% (w/w) cholesterol. The composition of the four diets is shown in Table 1.

The hamsters were fed the experimental diets for 6 weeks. Food and drinking water were available ad libitum. Body weight was measured every week and feces were collected during the final 3 days of the experiment. The feces samples were then dried and weighed. This study was approved by the Animal House Management Committee of the National Taiwan Ocean University. The animals were maintained in accordance with the guidelines for the care and use of laboratory animals as issued by the Animal Center of the National Science Council.

2.3. Collection of blood and tissue samples

At the end of the experiment, hamsters were fasted overnight and then sacrificed by exsanguination via the abdominal aorta while under CO2 anesthesia. Heparin was used as the anticoagulant. Plasma was collected by centrifugation at 1750g for

| Ingredient (%) | CON | HF | HF + GHE | HF + PO |
|---------------|-----|----|----------|---------|
| Chow diet     | 100 | 94.9 | 93.4 | 94.9 |
| Ching-Shan oil | 0   | 5  | 5        | 5       |
| Cholesterol   | 0.1 | 0.1 | 0.1      | 0.1     |
| Gelidium hot extract | 0  | 1.5 | 0       | 0.1     |
| Total         | 100 | 100 | 100      | 100     |
| Probucol      | —   | —  | —        | 1       |
| Total energy Kcal/100 g | 336.0 | 363.9 | 362.1 | 363.9 |

CON, normal diet group; HF, high-fat diet group; HF + GHE, Gelidium amansii hot-water extract diet group; HF + PO, probucol diet group.

* Ching-Shan oil: a mixture oil of palm oil, lard, and canola oil. In addition, Ching-Shan oil contains saturated fat (32.7%) and cholesterol (162 mg/100 g). The fatty acid composition of Ching-Shan oil is: 14:0 (1.9%); 16:0 (29.3%); 16:1 (2.8%); 18:0 (8.3%); 18:1 (42.5%); 18:2 (14.8%); 18:3 (0.4%).
20 min (4°C) from whole blood. Liver and adipose tissues (perirenal and periadipidymal) were excised from each hamster and weighed. All tissue samples were immediately frozen and were stored at −80°C until further analysis.

2.4. Determinations of plasma lipids, aspartate aminotransferase, and alanine aminotransferase

Plasma TC and TG concentrations were determined by use of a cholesterol enzymatic kit (Audit Diagnostics, Cork, Ireland) and TG enzymatic kit (Audit Diagnostics), respectively. The concentration of lipoprotein in plasma was determined by ultracentrifugation (194,000g for 3 h at 10°C) [20]. The cholesterol levels of the lipoprotein fraction were measured by use of a cholesterol enzymatic kit (Audit Diagnostics). Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined using an enzymatic kit (Randox Ltd., Antrim, UK) and ALT enzymatic kit (Randox Ltd.), respectively.

2.5. Determinations of liver lipids, fecal lipids, and bile acid concentrations

Liver and fecal lipids were extracted with a chloroform/methanol solution (v/v, 2:1) according to the method of Folch et al [21]. TG and TC were determined using the method of Carlson and Goldfarb [22]. Fecal bile acids were extracted using the method of Cheng and Lai [23], and quantified enzymatically according to the procedures provided in the assay kit (Randox Ltd.).

2.6. Liver fatty acid synthase and acetyl-CoA carboxylase enzymes activity

Preparation of liver enzymes was according to the method of Nepokroeff et al [24]. Fatty acid synthase (FAS) activity was determined using a modified version of Goodridge’s method [25]. The reaction mixture contained 2M potassium phosphate buffer, pH 7.1, 20mM dithiothreitol, 0.25mM acetyl-CoA, 60mM EDTA, 0.39mM malonyl-CoA, and 6mM NADPH. FAS activity was determined at 37°C by following the decrease of NADPH at 340 nm. Acetyl-CoA carboxylase (ACC) activity was determined using a modified version of the method of Mohan and Kekwick [26]. The liver enzyme incubation mixture consisted of 50mM Tris–HCl buffer, pH 7.4, 3mM magnesium chloride, 10mM potassium citrate, 3.75mM glutathione, 12.5mM potassium bicarbonate, 0.675mM bovine serum albumin, 0.125mM acetyl-CoA, 3.75mM ATP, and 10mM NADPH. ACC activity was determined at 37°C by following the decrease of NADPH at 340 nm.

2.7. Western blot analysis

Liver proteins were extracted using radioimmunoprecipitation buffer (Cell Signaling Technology, Beverly, MA, USA). Total protein containing 40 μg was separated on 10% sodium dodecyl sulfate polyacrylamide gels and transferred to polyvinylidene fluoride membranes (Bio-Rad, CA, USA). After blocking, the primary antibodies employed were: β-actin (Biovision, Mountain View, USA), adenosine monophosphate (AMP)-activated protein kinase α [(AMPK) Cell Signaling Technology], phosphor-AMPK (Thr172) [(p-AMPK) Cell Signaling Technology], sterol regulatory element binding protein 1 [SREBP1] Novus Biologicals, Littleton, CO, USA] and sterol regulatory element binding protein 2 [SREBP2] Proteintech, Manchester, UK]. The membranes were incubated overnight at 4°C with primary antibodies, followed by incubation with horseradish peroxidase–linked secondary antibodies for 1 h at room temperature. The blots were incubated with appropriate horseradish peroxidase–linked secondary antibodies and then detected by enhanced chemiluminescence (Western Lightning ECL Pro; PerkinElmer, Waltham, MA, USA). Signal intensities were quantitated using the Quantity One Software (BioRad, Hercules, CA, USA).

2.8. Statistical analysis

Results are given as mean ± standard deviation values. The significant difference from the respective controls for each experimental test condition was assessed by one-way analysis of variance and two-tailed Student t test. The statistical software SPSS for Windows version 12.0 (SPSS, Chicago, IL, USA) was used.

3. Results

3.1. Effects of GHE on body and tissue weight in HF diet fed hamster

Lower body weight was found in hamsters fed a diet with GHE supplementation when compared with the HF group (p < 0.05) (Figure 1). The hamsters fed a HF diet for 6 weeks had increased liver weight (p < 0.05) (Table 2). However, GHE supplementation reversed the increase of adipose tissue weight and liver weights caused by HF diet feeding. GHE had no effect on food intake in hamsters fed with a HF diet (Table 2) (p > 0.05).

3.2. Effects of GHE on plasma, liver, and fecal lipid metabolism in HF diet fed hamster

Hamsters fed the HF diet had elevated plasma TC, TG, low-density lipoprotein cholesterol (LDL-C), very-low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) when compared with the CON group (p < 0.05). GHE supplementation reversed the increase of plasma lipid concentrations caused by HF diet feeding (Figure 1).

Figure 1 – The change of body weight in Syrian hamsters fed a different diet for 6 weeks. Results are expressed as mean + standard deviation. * Significant difference from the HF group at p < 0.05. CON, control; HF, high fat; HF + GHE, high fat + Gelidium amansii hot-water extract; HF + PO, high fat + probucol.
lipoprotein cholesterol (VLDL-C), and TC/high-density-lipoprotein cholesterol (HDL-C) ratio (p < 0.05); however, GHE showed decreased plasma levels of AST, ALT, TC, TG, LDL-C, LDL-C + VLDL-C, and TC/HDL-C ratio (p < 0.05) (Table 3). In this study, animals fed the HF diet also had enhanced TC and TG contents in the liver (p < 0.05). However, GHE supplementation decreased the TC and TG content in liver (p < 0.05) (Table 4). These results indicate that GHE feeding reduced hepatic lipid accumulation in hamsters fed the HF diet.

### Table 3 – Effect of different diet on plasma lipids and glucose in Syrian hamsters for 6 weeks.

|                      | CON     | HF      | HF + GHE | HF + PO |
|----------------------|---------|---------|----------|---------|
| Triacylglycerol      | 56.4 ± 27.0 | 88.4 ± 9.6** | 70.6 ± 19.9* | 95.5 ± 31.6 |
| Total cholesterol    | 90.6 ± 8.2 | 181.3 ± 19.7** | 157.6 ± 23.9** | 146.9 ± 10.6* |
| HDL-C (mg/dL)        | 62.9 ± 5.9 | 103.5 ± 8.0** | 102.4 ± 12.1 | 93.9 ± 14.5 |
| LDL-C (mg/dL)        | 15.1 ± 6.7 | 54.8 ± 19.6** | 36.6 ± 12.8** | 25.5 ± 12.3* |
| VLDL-C (mg/dL)       | 12.6 ± 4.6 | 23.0 ± 5.7** | 18.6 ± 5.6 | 27.5 ± 6.2 |
| LDL-C + VLDL-C       | 27.7 ± 4.1 | 77.8 ± 17.5** | 55.2 ± 16.0** | 53.0 ± 11.5* |
| TC/HDL-C ratio       | 1.4 ± 0.1 | 1.8 ± 0.2** | 1.5 ± 0.1* | 1.6 ± 0.2 |
| AST (U/L)            | 12.4 ± 6.1 | 20.2 ± 7.8** | 12.9 ± 3.2* | 11.8 ± 2.1* |
| ALT (U/L)            | 15.1 ± 3.0 | 21.1 ± 7.2** | 15.0 ± 3.1* | 14.4 ± 3.7* |

Results are expressed as mean ± SD for eight Syrian hamsters.
* Significant difference from the HF group at p < 0.05; ** significant difference from the CON group at p < 0.05.

### Table 4 – Effect of different diets on liver and fecal lipid concentrations in Syrian hamsters for 6 weeks.

|                      | CON HF | HF + GHE | HF + PO |
|----------------------|---------|----------|---------|
| Liver Total cholesterol (mg/Liver) | 20.5 ± 6.0 | 159.3 ± 27.4** | 122.0 ± 24.1* | 104.4 ± 19.5* |
| Triglyceride (mg/Liver) | 23.7 ± 7.0 | 51.0 ± 7.0** | 40.3 ± 5.5* | 40.9 ± 4.3* |
| Feces Total cholesterol (mg/day) | 4.3 ± 1.8 | 5.1 ± 1.5 | 7.5 ± 2.4* | 8.1 ± 2.2* |
| Triglyceride (mg/day) | 7.1 ± 2.9 | 6.7 ± 2.4 | 9.0 ± 1.5* | 7.5 ± 2.5 |
| Bile acid (mol/L) | 2.6 ± 0.7 | 2.7 ± 0.7 | 4.0 ± 0.7* | 4.6 ± 0.6* |

Results are expressed as mean ± SD for eight Syrian hamsters.
* Significant difference from the HF group at p < 0.05; ** significant difference from the CON group at p < 0.05.

However, GHE supplementation increased fecal TC, TG, and bile acids in hamsters (p < 0.05) (Table 4).

#### 3.3. Effects of GHE on lipogenic enzyme activities in liver

Hamsters fed the HF diet showed increased liver FAS and ACC activities (Figures 2A and 2B, p < 0.05), but GHE reduced the hepatic FAS and ACC activities (p < 0.05). Lower FAS activity was observed in animals after probucol treatment (p < 0.05).

#### 3.4. Expression of lipid metabolism-related proteins

After 6 weeks of the feeding study, the HF diet significantly decreased the phosphorylation of AMPK protein expression in the liver of hamsters (Figure 3, p < 0.05). However, GHE and probucol treatments induced an increase in hepatic phosphorylation of AMPK protein expression (p < 0.05). Figure 4 shows the hepatic SREBP1 and SREBP2 protein expressions. Hamsters fed the HF diet had elevated protein expressions of SREBP1 and SREBP2 (p < 0.05). GHE or probucol supplementation reduced the SREBP1 and SREBP2 protein expressions in hamsters fed a HF diet (p < 0.05).

#### 4. Discussion

In this study, we found that GHE reduced plasma and liver lipids in hamsters fed with the HF diet. GHE reduced not only plasma TC, TG, LDL-C, and LDL-C + VLDL-C concentrations but also lowered hepatic TG and TC levels. These results might be due to the ability of GHE to increase fecal excretions of cholesterol and bile acids.

Scientists have reported that algae possess hypolipidemic effects in animal models [6,27]. Algae are rich in dietary fiber and the total dietary fiber contents of the main edible algae ranged from 25% to 75% (on a dry weight basis). About 51% to 85% of total dietary fiber was found as water-soluble fiber [28].
In this study, GHE contained abundant water-soluble fiber (about 68.6%). It has been demonstrated that consuming water-soluble dietary fiber can reduce plasma and liver lipids [29,30]. The cholesterol-lowering effects of water-soluble fibers are believed to be a result of the interference with the enterohepatic circulation of bile acids [31]. It has been shown that animals fed a high-cholesterol diet had an increased hepatic cholesterol content, resulting in increased TG biosynthesis and reduced fatty acid oxidation [32,33]. Indeed, our data showed that increased hepatic ACC and FAS activities as a result of the HF diet supported the hypothesis of these previous studies. In this study, fecal bile acid excretion, the major degradation of endogenous cholesterol, was enhanced after GHE feeding in hamsters and this might stimulate the biosynthesis of bile acid using cholesterol as the precursor. Thus, these results suggest that the hepatic lipid-lowering effect of GHE in cholesterol-fed hamsters may be primarily related to lower absorption of bile acids, resulting in an increase in hepatic cholesterol catabolism and thus leading to a reduction in hepatic cholesterol and thereafter TG accumulation. Furthermore, due to lowering the cholesterol pool by GHE supplementation, the hepatic LDL receptor might be induced to enhance the uptake of LDL-C from the circulation, thus lowering blood cholesterol concentration [34]. Numerous studies have demonstrated that the lipid-lowering effects of dietary water-soluble fibers are primarily related to the increased fecal excretions of cholesterol and bile acids. Therefore, it is suggested that the reduction of plasma and liver lipids after GHE treatment is probably due to its abundance of dietary water-soluble fiber (68.6%), which may increase fecal lipid excretion. Moreover, the sulfated polysaccharides from green algae showed a high anti-hyperlipidemia activity in mice [35]. Ginzberg et al [36] reported that chickens fed sulfated polysaccharides from red algae had reduced blood cholesterol. Some water extracts of red algae have sulfated polysaccharides [37], which contain around 3% to 10% of sulfate in their structures [38]. Similar to the results of previous studies, our data also showed that GHE contained sulfated polysaccharides (4.1% sulfate). Therefore, we suggest that sulfated polysaccharides in GHE, at least in part, may play an important role in improving lipid metabolism in hamsters.

AMPK and SREBP played critical roles in the regulation of lipid metabolism [39]. They were considered as the important factors of hyperlipidemia [40] and liver lipid accumulation [41]. AMPK activation has been proposed as a major therapeutic target for obesity [42], fatty liver [41], and hyperlipidemia [43]. AMPK phosphorylation suppresses the activity of the key proteins involved in lipogenesis, such as SREBP 1 and SREBP 2 [44]. SREBP1 primarily regulates the lipogenic process by activating the FAS and ACC involved in TG synthesis [45], and SREBP 2 mainly controls cholesterol homeostasis by activating the genes required for cholesterol synthesis [44,46]. In the present study, hamsters fed the HF diet showed a
decrease in the expression of the phosphorylation of AMPK, and an increase in both SREBP 1 and SREBP 2 protein expressions, indicating that lipogenesis is increased in liver. In this study, GHE supplementation increased hepatic AMPK phosphorylation and reduced SREBP 1 and SREBP 2 protein expressions. These alternations by GHE treatment might explain a lower lipogenesis in liver. Galisteo et al. [47] reported that water-soluble fiber can increase the phosphorylation of AMPK, consequently inhibiting ACC protein expression. In addition, water-soluble fiber displayed a lower hepatic SREBP2 mRNA expression, thereby reducing the serum TC [48]. Moreover, water-soluble fiber has been shown to down-regulate SREBP1 and FAS mRNA expression [49] and thus reduced TG synthesis in liver [50]. Because GHE is rich in water-soluble fiber, it is possible that the reduction of liver lipids by GHE may be related to the activation of AMPK phosphorylation and inhibition of SREBP pathways in hamsters fed the HF diet. Studies have shown that water-soluble fiber can enhance the amount of AMPK phosphorylation in HepG2 cells [51] and mouse liver [52]. However, the reason why water-soluble fiber can activate AMPK is still unknown. Yamauchi et al. [53] showed that adiponectin may be an important factor in the activation of AMPK. We have reported that feeding a dry powder of GA can increase plasma adiponectin levels in rats with diabetes [9]. Therefore, it is proposed that the increased AMPK activation by GHE may be due to the increased plasma adiponectin.

Our previous study demonstrated that GA feeding increased adipose tissue hormone-sensitive lipase activity, thereby reducing the adipose tissue weight [9]. In the present study, GHE reduced body weight, liver weight, and adipose tissue weight in hamsters fed a HF diet. The reduced body weight might be due to the decreased liver weight, adipose tissue weight, and their lipid accumulation in these tissues. Therefore, GHE might have a beneficial effect on reducing lipid accumulation in liver and adipose tissue in hamsters fed a HF diet. Again, the lipid-lowering effects of GHE may have been due to the increase in fecal lipid excretion. Similar actions were shown in other dietary fiber materials, such as GA powder [9] or chitosan [54].

In conclusion, the present study demonstrated the lipid-lowering potential of GHE in hamsters, and this effect may be possibly be a result of enhanced fecal excretions of cholesterol, TG, and bile acids. Our results also show that GHE is able to activate AMPK phosphorylation and reduce SREBP 1 and SREBP 2 protein expressions in liver, thereby decreasing the hepatic lipogenesis which may result in lipid accumulation in liver. Further studies are needed to elucidate the different Molecular Weight (MW) of water-soluble fiber or sulfated polysaccharides content in GA, underlying the lipid-lowering effects.

Conflicts of interest

There is no potential conflict of interest.

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