Microalgal Oil Supplementation Has an Anti-Obesity Effect in C57BL/6J Mice Fed a High Fat Diet.

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ABSTRACT: This study investigated the impact of microalgal oil (MO) on body weight management in C57BL/6J mice. Obesity was induced for 8 weeks and animals were orally supplemented with the following for 8 additional weeks: beef tallow (BT), corn oil, fish oil (FO), microalgal oil (MO), or none, as a high fat diet control group (HD). A normal control group was fed with a normal diet. After completing the experiment, the FO and MO groups showed significant decreases in body weight gain, epididymal fat pad weights, serum triglycerides, and total cholesterol levels compared to the HD and BT groups. A lower mRNA expression level of lipid anabolic gene and higher levels of lipid catabolic genes were observed in both FO and MO groups. Serum insulin and leptin concentrations were lower in the MO group. These results indicated that microalgal oil has an anti-obesity effect that can combat high fat diet-induced obesity in mice.

Keywords: microalgae, omega-3, polyunsaturated fatty acids, lipid profile, obesity

INTRODUCTION

Omega-3 fatty acids, especially eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), are long-chain polyunsaturated fatty acids (PUFAs) that are used as health promoting supplements. Many studies have demonstrated protective effects of omega-3s against cardiovascular disease (1), cancer (2), atherosclerosis (3), and obesity (4). However, the omega-6/omega-3 ratio plays a critical role in the outcome; high amounts of omega-6 fatty acids have been shown to be associated with several diseases such as cardiovascular disease, cancer, inflammatory disease, and autoimmune disorders, while higher levels of omega-3 averts such negative effects (5).

Transitions in global dietary patterns are frequently observed and have been well documented. The preference for food preparation using vegetable oil has increased, leading to a higher portion of omega-6 fatty acids in the lipid pool (5,6).

The major sources for omega-3 PUFAs are marine fish and fish-based products. However, increased awareness of the benefits of omega-3 PUFAs has increased, along with the demand for fish and its oil. However, humans cannot depend only on fish-based products, as intensive fishing to meet the demand of fish oil will result in the threat of extinction for many fish species. Moreover, fish oil also have disadvantages such as an unpleasant odor and danger of contaminants (e.g., methyl-mercury, dioxins, and poly-chlorinated biphenyls) (6,7). Hence, there is a need for an alternative source of omega-3 PUFAs.

Several alternative omega-3 PUFA products have been investigated. For example, transgenic oilseed crops and animals can produce an increase in the omega-6/omega-3 ratio of their fat. However, the use of genetically modified foods is highly controversial (6,8-11). Other alternative sources are microalgae, krill, and seal oil. Among these, microalgal oil is an attractive alternative for a substantial number of reasons. Microalgae, the common name for multiple single-cell, photogenic, heterotrophic, and mixotrophic organisms, have a high growth rate, short life cycle, and are less affected by environmental conditions such as season, location, and climate (6,12). Several species of microalgae can be stimulated for overproduction of a specific fatty acid, including EPA or DHA, through relative simple manipulations of their culture medium (13). Moreover, microalgal oil has better oxidative stability than fish oil (14) and thus some of the problems of fish oil can be avoided.

In this current study, microalgal oil was extracted from *Aurantiochytrium* sp. KRS101 (12). *Aurantiochytrium*

sp. KRS101 is an oleaginous heterotrophic microalga found in the Malaysian mangrove ecosystem like soil, leaf and pneumatophore samples. These microalgae are capable of producing high quantity of lipid with DHA content over 45% of total fatty acid (12) which is safe for use in food and feed industries (15).

Many studies indicate that microalgal oils improve lipoprotein profiles; however these studies did not show any beneficial effect in terms of obesity (16-18). Therefore, this study was designed to investigate whether microalgal oil has beneficial effects against the development of high fat diet-induced obesity in rodents. Studies using C57BL/6J mice show that a high fat diet for 8 weeks will lead to significantly increased fat mass and plasma leptin levels (19). Therefore, after inducing obesity in C57BL/6J mice with a high fat diet, we then treated the mice orally with microalgal oil using an oral gavage and compared its effects to supplementation with other oils.

MATERIALS AND METHODS

Animals and diets

Male C57BL/6J mice, aged 6 weeks, were purchased from Charles River Laboratories (Tokyo, Japan). The animals were maintained on a pellet diet (Research Diets, New Brunswick, NJ, USA) for 1 week, and then randomly divided into 6 groups: normal diet with distilled water treatment group (ND), high fat diet with distilled water treatment group (HD), high fat diet with beef tallow treatment group (BT), high fat diet with corn oil treatment group (CO), high fat diet with fish oil treatment group (FO), and high fat diet with microalgal oil treatment group (MO). Before initiating the experiment, animals were fed with either a normal diet or a high fat diet for 8 weeks in order to induce obesity. After completion of the 8 week obesity-induction period, the animals were supplemented with distilled water, beef tallow, corn oil, fish oil, or microalgal oil for 8 weeks more. Beef tallow and corn oil were purchased from the Ottogi Company (Seoul, Korea). Microalgal oil was obtained from Korea Research Institute of Bioscience and Biotechnology (KRIBB, Jeongeup, Korea); the oil from the microalgae was extracted by using a previously reported method (12,15). The animals were housed in a temperature-controlled environment with a 12-h light/dark cycle and free access to food and water during the entire experimental period. The food intake was measured daily and body weight was measured weekly. The experimental protocol was approved by the Animal Care and Use Committee of Chonbuk National University (CBU 2012-0007).

Collection of serum and tissue samples

After a 12 h overnight fast, each mouse was deeply anesthetized using di-ethyl ether and blood was collected by orbital vein puncture. Blood samples were left on ice for 1 h, and then serum was collected from the clotted blood by centrifugation at 1,100 g for 15 min at 4°C (Micro 17R, Vision Scientific Co., Seoul, Korea). Serum samples were stored at −80°C until analysis. Tissues were surgically removed, weighed, and stored at −80°C until analysis.

Analysis of lipids

Hepatic lipids were extracted following the method previously described (20). Briefly, chloroform/methanol solution (2:1, v/v) was added to the homogenized liver tissues and the solution was vortexed and centrifuged. The lower phase was collected and evaporated at room temperature under a fume hood (Daihan Labtech Co., Ltd., Namyangju, Korea). The remaining semi-dried pellet was dissolved in 10 mL/L Triton X-100 (Yakuri Pure Chemicals Co., Ltd., Kyoto, Japan). The resulting solution was used to estimate hepatic triglyceride (TG) and total cholesterol (TC). Serum and hepatic TC and TG along with high density lipoprotein-cholesterol (HDL-c) were measured by an enzymatic method using a commercial kit (Asan Pharmaceutical Co., Seoul, Korea).

Quantitative real-time PCR analysis

Total RNA was extracted by TRIzol reagent and the concentration was measured spectrophotometrically (Shimadzu Corporation, Kyoto, Japan); the concentration of RNA was determined by absorbance at 260 nm and the quality of the RNA was determined by the ratio of signals at 260 nm to 280 nm. For real-time PCR, 1 μg of extracted RNA was reverse transcribed into first-stand cDNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Then, amplification was performed in a cDNA mixture on a 7500 Real Time PCR system (Applied Biosystems) and SYBER Green PCR Master Mix (Applied Biosystems, Woolston, Warrington, UK) according to the manufac-

Table 1. PCR primer sequences used for real-time PCR analysis

Gene		Primer sequence
PPAR-α	Forward	5'-CCT GAA CAT CGA GTG TCG AAT AT-3'
	Reverse	5'-GGT CTT CTT CTG AAT CTT GCA GCT-3'
CPT-1	Forward	5'-AAA GAT CAA TCG GAC CCT AGA CA-3'
	Reverse	5'-CAG GGA GTA GCG CAT AGT CA-3'
ACO	Forward	5'-CCA ACA TGA GGA CTA TAA CTT CCT-3'
	Reverse	5'-TAC ATA CGT GCC GTC AGG CTT-3'
SCD-1	Forward	5'-CAT CAT TCT CAT GGT CCT GCT-3'
	Reverse	5'-CCA GTC GTA CAC GTC ATT TT-3'
β-actin	Forward	5'-AGC CTT CCT TCT TGG GTA TGG-3'
	Reverse	5'-CAC TTG CGG TGC ACG TA TGG-3'

turer's protocol. Relative quantification of gene expression with real-time PCR data was calculated relative to β-actin. The sequences of primers used in this study are given in Table 1.

Analyses of insulin and leptin

Serum insulin was measured using the insulin ELISA kit (SHIBAYAGI Co., Ltd., Shibukawa, Japan) and leptin concentrations were measured using the Quantikine® Immunoassay kit (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Data analysis was performed using SPSS version 16.0 (SPSS Institute, Chicago, IL, USA). Values are expressed as mean±standard deviation. The statistical significance of differences between two groups was determined by Student's t-test. When significant differences were observed, mean values were compared with a one-way ANOVA among the groups. The differences among groups were assessed using Duncan's multiple range tests. Statistical significance was considered at $P < 0.05$.

RESULTS

Body weight and food intake

Table 2 shows that initial body weight did not differ significantly among the groups. After the 8 week run-in period to achieve diet-induced obesity, the body weight in all high fat feeding groups (i.e., HD, BT, CO, and MO) was significantly higher than the ND group. Similarly, the final body weight of mice in the HD group was significantly higher than that of the ND group. Although significant differences were not observed among the high fat groups, body weight gain was significantly dif-

ferent in the MO group compared to the HD group. During the pre-obesity induction period (0 wk to 8 wk), food intake (g/d) was significantly higher in the normal group, whereas energy intake (kcal/d) was significantly higher in the groups fed a high fat diet compared to the group fed a normal diet. In the post-obesity induction period (9 wk to 16 wk), food intake was significantly higher in the normal diet group compared to the high fat diet fed group; however, energy intake was not different among the groups (Table 2).

Epididymal fat pads

Epididymal fat pad weights (Fig. 1) were significantly higher in the groups consuming the high fat diet compared to the group consuming the normal diet. Among the groups consuming the high fat diet, the MO group showed significantly lower epididymal fat pad weights compared to the BT group. However, there was no sig-
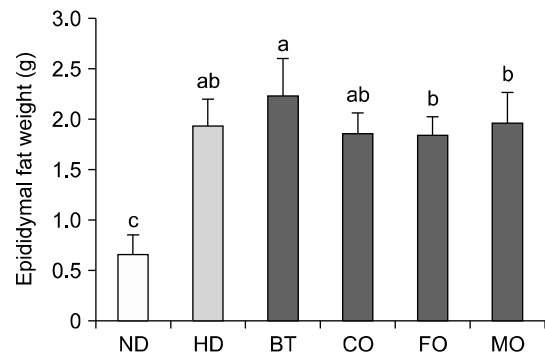

Fig. 1. Epididymal fat weight. All values are mean±SD. Values with different letters (a–c) are significantly different (ANOVA with Duncan's multiple range test at $P < 0.05$). ND, AIN-93 modified diet with 4% fat by weight (10% energy from fat); HD, AIN-93 modified high fat diet with 35% fat by weight (60% energy from fat); BT, high fat diet plus beef tallow; CO, high fat diet plus corn oil; FO, high fat diet plus fish oil; MO, high fat diet plus microalgal oil from *Aurantiochytrium* sp. KRS101.

Table 2. Body weight and food intake for the period during which obesity was induced and the experimental period

Groups	ND	HD	BT	CO	FO	MO
Initial body weight (g)	21.98±0.86	21.93±1.43	22.09±1.02	22.42±1.35	22.97±1.25	22.61±1.21
Body weight at 8 weeks (g)	29.19±1.01[b]	39.09±3.11[a]	38.45±2.90[a]	39.75±3.38[a]	39.01±2.53[a]	38.99±2.76[a]
Final body weight (g)	28.16±0.96[c]	41.58±2.53[ab]	43.88±1.02[a]	42.62±1.31[a]	41.50±1.78[ab]	39.94±2.71[b]
Body weight gain (g)[1]	1.41±1.17[d]	3.37±0.88[ab]	3.84±0.86[a]	3.84±0.86[bc]	2.60±1.68[abc]	1.96±0.72[cd]
Food intake (g/d)[2]	2.64±0.23[a]	2.37±0.02[b]	2.32±0.05[b]	2.29±0.07[b]	2.30±0.02[b]	2.24±0.10[b]
Energy intake (kcal/d)[2]	10.29±0.89[b]	12.33±0.08[a]	12.05±0.25[a]	11.89±0.34[a]	11.98±0.10[a]	11.65±0.50[a]
Food intake (g/d)[3]	2.77±0.04[a]	2.07±0.03[b]	2.03±0.13[b]	1.89±0.08[b]	1.91±0.10[b]	1.89±0.78[b]
Energy intake (kcal/d)[3]	10.81±0.15	10.78±0.15	10.55±0.66	9.84±0.40	9.91±0.50	9.85±0.40

All values are mean±SD.
Values with different letters (a–d) within the same row are significantly different (ANOVA with Duncan's multiple range test at $P < 0.05$).
ND, AIN-93 modified diet with 4% fat by weight (10% energy from fat); HD, AIN-93 modified high fat diet with 35% fat by weight (60% energy from fat); BT, high fat diet plus beef tallow; CO, high fat diet plus corn oil; FO, high fat diet plus fish oil; MO, high fat diet plus microalgal oil from *Aurantiochytrium* sp. KRS101.
[1]Body weight gain during experimental period.
[2]For the obesity induction period.
[3]For the experimental period.

nificant difference observed between the MO and HD groups.

Lipid concentrations in serum and liver

The effects of omega-3 PUFAs on hepatic and serum lipid profiles are described as following. The concentrations of hepatic TG and TC were significantly lower in the MO group compared to the HD, BT, and CO groups (Fig. 2A and B). As for the serum lipid profiles, serum TG was significantly lower in both the FO and MO groups compared to the HD group (Fig. 3A). Serum TC was significantly lower in the MO group compared to the BT and CO groups (Fig. 3B). The ratio of HDL-c to

TC was significantly higher in the FO and MO groups compared to BT and CO groups (Fig. 3C).

Hepatic mRNA levels

The expression level of genes involved in hepatic lipid metabolism is shown in Fig. 4. Peroxisome proliferator-activated receptor (PPAR)-α is a gene involved in lipid catabolism. It regulates the expression of carnitine palmitoyltransferase (CPT)-1 and acyl-CoA oxidase (ACO), which are involved in β-oxidation of lipids in the mitochondria and the peroxisomes, respectively. Although the expression pattern of all three genes remained similar, there was a small but significantly higher expres-
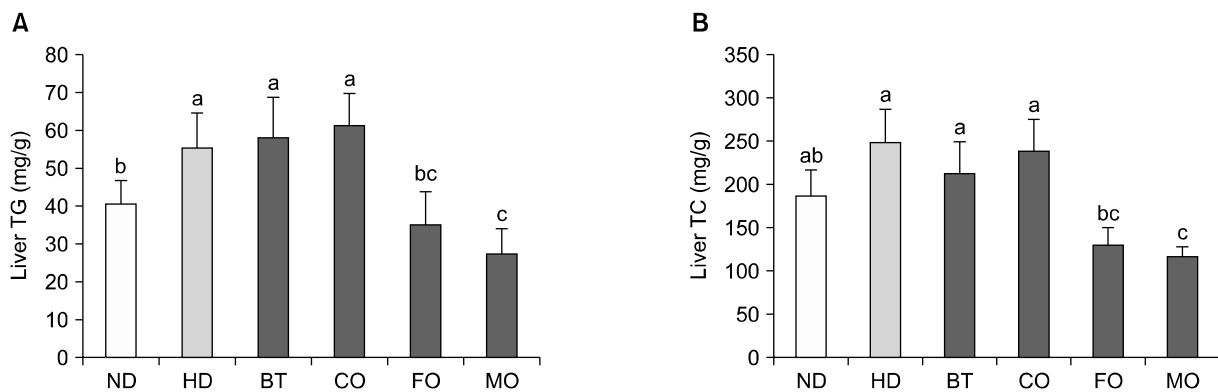

Fig. 2. Lipid concentrations of triglyceride (A) and total cholesterol (B) in liver. All values are mean±SD. Values with different letters (a-c) are significantly different (ANOVA with Duncan's multiple range test at $P<0.05$). ND, AIN-93 modified diet with 4% fat by weight (10% energy from fat); HD, AIN-93 modified high fat diet with 35% fat by weight (60% energy from fat); BT, high fat diet plus beef tallow; CO, high fat diet plus corn oil; FO, high fat diet plus fish oil; MO, high fat diet plus microalgal oil from *Aurantiochytrium* sp. KRS101.

Fig. 3. Lipid concentrations of triglyceride (A) and total cholesterol (B), and HDL-c (high density lipoprotein)/TC (total cholesterol) (C) in serum. All values are mean±SD. Values with different letters (a-d) are significantly different (ANOVA with Duncan's multiple range test at $P<0.05$). ND, AIN-93 modified diet with 4% fat by weight (10% energy from fat); HD, AIN-93 modified high fat diet with 35% fat by weight (60% energy from fat); BT, high fat diet plus beef tallow; CO, high fat diet plus corn oil; FO, high fat diet plus fish oil; MO, high fat diet plus microalgal oil from *Aurantiochytrium* sp. KRS101.
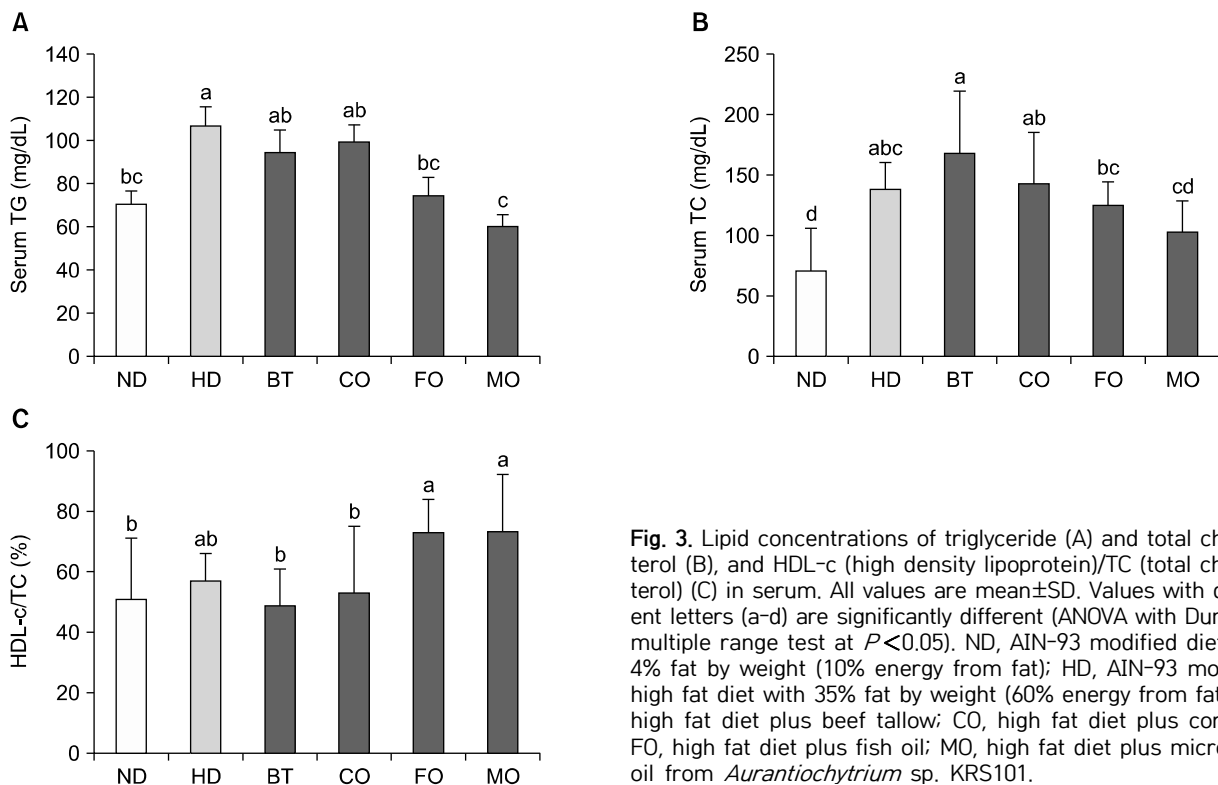
Fig. 4. mRNA levels of PPAR-α (A), CPT-1 (B), ACO (C), and SCD-1 (D) in liver. All values are mean±SD. Values with different letters (a–d) are significantly different (ANOVA with Duncan's multiple range test at $P<0.05$). ND, AIN-93 modified diet with 4% fat by weight (10% energy from fat); HD, AIN-93 modified high fat diet with 35% fat by weight (60% energy from fat); BT, high fat diet plus beef tallow; CO, high fat diet plus corn oil; FO, high fat diet plus fish oil; MO, high fat diet plus microalgal oil from *Aurantiochytrium* sp. KRS101.
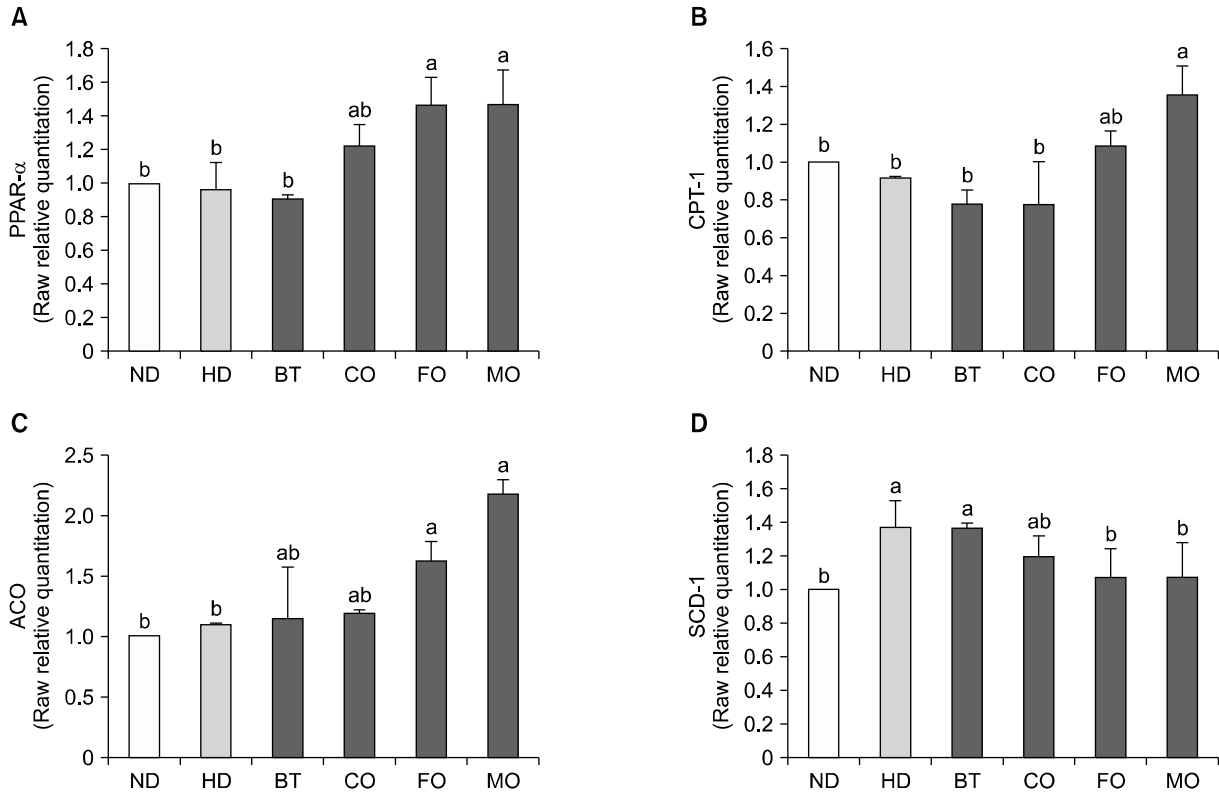

Fig. 5. Insulin (A) and leptin (B) concentrations in serum. All values are mean±SD. Values with different letters (a–d) are significantly different (ANOVA with Duncan's multiple range test at $P<0.05$). ND, AIN-93 modified diet with 4% fat by weight (10% energy from fat); HD, AIN-93 modified high fat diet with 35% fat by weight (60% energy from fat); BT, high fat diet plus beef tallow; CO, high fat diet plus corn oil; FO, high fat diet plus fish oil; MO, high fat diet plus microalgal oil from *Aurantiochytrium* sp. KRS101.
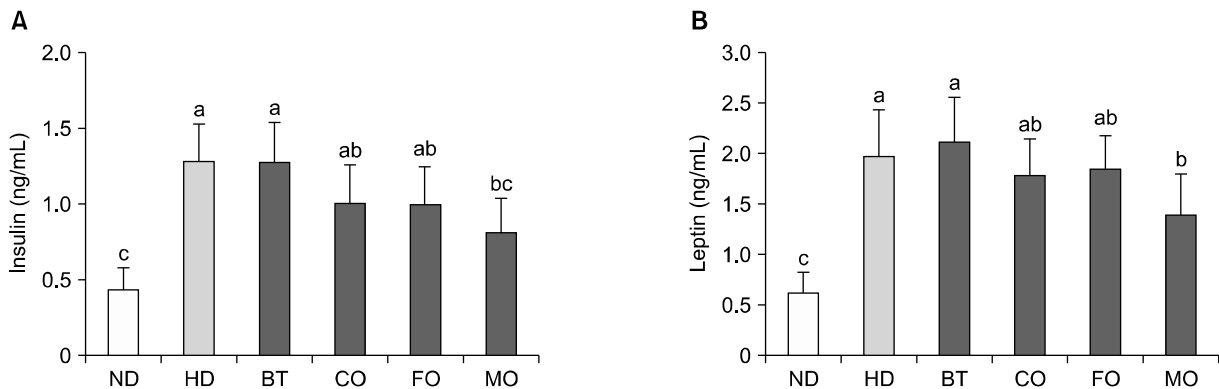
sion of PPAR-α and ACO in the MO and FO groups compared to the HD group (Fig. 4A-C). At the same time, the BT, CO, HD, and ND groups showed a similar expression level of all three genes. The lipid anabolic gene, stearoyl-CoA desaurase enzyme (SCD)-1, is involved in lipogenesis. It showed significantly lower expression in the MO and FO groups compared to the HD and BT groups (Fig. 4D).

Insulin and leptin levels in serum

As shown in Fig. 5A and B, the levels of serum insulin and leptin were significantly higher in the HD group compared with the ND group. Supplementation with MO significantly reduced the rise in serum insulin and leptin compared to other groups consuming the high fat diet.

DISCUSSION

Many studies on humans and animals have demonstrated the beneficial effects of omega-3 PUFAs from fish oil (4,5). Hence, it is well known that omega-3 PUFAs improve serum lipid levels and are beneficial against obesity. Specifically, omega-3 PUFAs differ from other fatty acids in relation to their storage as triglycerides in mature adipocytes. An excess of triglyceride storage promotes hyperplasia and hypertrophy of adipocytes leading to obesity, (21) and omega-3 PUFAs, especially EPA and DHA, can inhibit adipocyte hypertrophy and decrease the lipid content of adipose tissue (22).

Earlier reports on microalgal oil suggested it could be an alternative to omega-3 fish oils; however, most of these studies failed to observe any significant differences in obesity-related markers other than changes in the serum lipid profiles. Therefore, in order to gain insight into the benefits of microalgal oil, we induced obesity in mice by feeding them a high fat diet for 8 weeks, followed by supplementation with different oils (along with the same high fat diet) for 8 more weeks. Since the energy demand changes due to the estrous cycle in female mice, leading to variations in food intake, we used male mice in this current study (23-26). The anti-obesity effects of the microalgal oil supplementation were quantified in high-fat diet-induced obese C57BL/6J mice by assessing body weight changes, body fat pad weights, serum and hepatic lipid profiles, serum insulin and leptin concentrations, and changes in hepatic mRNA levels related to lipid metabolism. Since fish oil is a common omega-3 fatty acid supplement, it was used as a positive control; beef tallow, rich in saturated fatty acids, and corn oil, a good source of omega-6 fatty acids, served as negative controls.

We observed that oil from the microalgae *Aurantiochytrium* sp. KRS101, which is rich in long chain omega-3 fatty acids, was effective against further weight gain in diet-induced obese mice compared to beef tallow or corn oil. The treatment of the obese animals with MO for a period of 8 weeks caused a decrease in the rate of weight gain compared to the other groups in the high fat diet regimen; this was true despite significant differences in food intake. We also found that animals treated with either omega-3 fatty acid source (i.e., FO or MO oil) had less epididymal adipose tissue compared to those in the HD, BT, and CO groups.

Dietary omega-3 fatty acids are regarded as down regulators of anabolic lipid genes and activators of catabolic lipid genes. Specifically, omega-3s have been shown to activate PPAR-α (27), which belongs to a superfamily of steroid nuclear receptors and plays a crucial role in regulating lipid homeostasis (4). In addition, PPAR-α activation leads to the induction of genes encoding for pro-

teins involved in lipid transport and oxidation including CPT-1 and ACO (28). CPT-1 and ACO are rate-limiting enzymes involved in mitochondrial and peroxisomal β-oxidation, respectively (29,30). In addition, SCD-1 is a lipogenic enzyme involved in the biosynthesis of cholesterol and triglyceride in the liver (31-33). Higher expression of hepatic SCD-1 promotes lipogenesis and formation of hepatic lipid droplets (31). In a study with SCD-1 knockout mice, a higher expression of PPAR-α, CPT-1, and ACO was observed. Consequently, there was an increase in lipid oxidation and an enhanced availability of free fatty acids for mitochondrial β-oxidation, thereby reducing TG synthesis and storage (34). Similarly, in our study the mRNA expression levels of PPAR-α, CPT-1, and ACO were significantly higher and the expression of SCD-1 was significantly lower in the FO and MO groups compared to the HD and BT groups. This observation suggests that the omega-3 fatty acid-rich microalgal and fish oils were effective at lowering triglyceride and cholesterol synthesis and accumulation in the liver. Also, a higher HDL-c/TC ratio and lower level of serum TG and TC were also observed in the group supplemented with MO compared to the other groups. These results suggest that microalgal oil has hypocholestrolemic and hypolipidemic properties.

Consuming a high fat diet causes hyperinsulinemia (35) as a result of the rise in adiposity (36). PPAR-α activation has been shown to improve insulin sensitivity by lowering the circulating lipids and lowering adiposity (37). In our study, one of the reasons that serum insulin was lower in the MO group could be due to the activation of PPAR-α and improvement of insulin sensitivity by omega 3-fatty acids. Supporting our study, other reports have also indicated that higher intakes of dietary omega-3 fatty acids improve insulin sensitivity (38,39). Leptin is an adipokine secreted by adipose tissue. It was reported that epididymal adipose tissue secretes the highest quantity of leptin compared to adipose tissues depots (40). Moreover, circulating leptin concentrations are proportional to the amount of adipose tissue mass (41). In this current study, a lower level of epididymal adipose tissue was observed in the MO and FO supplemented groups compared to the other groups fed a high fat diet. Therefore, it could be possible that a lower circulatory leptin level in the MO and FO groups could be a result of the lower amount of epididymal adipose mass in these groups. Moreover, serum insulin and leptin showed a similar tendency, suggesting that the beneficial effects of microalgal oil against hyperinsulinemia and hyperleptinemia could be attributable to the protective effects against obesity. In conclusion, our results demonstrated that oil from microalgae *Aurantiochytrium* sp. KRS101 had beneficial effects against high fat diet-induced obesity. Regular supplementation of microalgal

oil in obese rodents significantly up-regulated the mRNA expression of genes related to lipid catabolism (i.e., CPT-1, ACO, and PPAR-α) while lowering the expression of a lipid anabolic gene (SCD-1). The change in the expression pattern of lipid metabolic genes resulted in a significant decrease in serum hyperlipidemia along with a reduction in hepatic lipids. Moreover, epididymal adipose tissue mass was significantly decreased as a result of microalgal oil intake, and this result was reflected in lower levels of serum leptin and insulin. Microalgal oil supplementation, rich in omega-3 fatty acids, has anti-obesity actions; however, appropriate human studies must be conducted to explore its use as an alternative to fish oil for the purpose of controlling obesity.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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