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Study on the chemical and nutritional significance of microalgae lipids

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Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that have been extensively used as the promising ingredients in functional foods. Microalgae can produce high content of lipids, pigments, proteins, vitamins, and other biomolecules exploited for commercial use. The nutritional values of microalgae are often related to their lipid content and fatty acid composition. For this reason, the total lipid content, lipid class distribution and fatty acid composition of different microalgae species were evaluated in the present study. The results in Chapter 1 revealed that the total lipid and fucoxanthin contents as well as the lipid class and fatty acids varied remarkably among the eleven species of microalgae. *Pavlove lutheri* showed the highest lipid content (313.59 mg/g dry weight), with high level of eicosapentaenoic acid (EPA). *Chaetoceros gracilis* exhibited the highest fucoxanthin content (5.19 mg/g dry weight). *Nannochloropsis oculata* showed the highest level of EPA (26.21%), while *Isochrysis galbana* contained the highest level of docosahexaenoic acid (DHA, 8.76%) in the present study. On the other hand, all of the microalgae used in this study contained fucoxanthin except for *Spirulina*. In addition, no EPA or DHA was found in *Spirulina* lipids, whereas it was rich in gamma-linolenic acid (GLA, 18.54%) and carotenoids such as β-carotene and zeaxanthin.

Health beneficial effects of fucoxanthin, EPA and DHA have been well known; therefore, it will be interesting to analyze the physiological effect of microalgae lipids rich in these components. However, it was too difficult to prepare enough amounts of samples from these microalgae for animal experiment. Only *Spirulina* could be obtained in large quantity. Furthermore, great attention and extensive studies have been devoted to evaluate the therapeutic
benefits of *Spirulina* on various diseased conditions including reduction in blood cholesterol, protection against some cancers, suppression of oxidative stress, enhancement of the immune system and reduction of hyperlipidemia and obesity. However, little information has been known on the effect of the total lipids extracted from *Spirulina*. Thus, in the present study, the physiological effect of *Spirulina* lipids was evaluated by animal experiments, especially focusing on the improvement effect of the lipids on obesity induced dysfunction such as hyperlipidemia and oxidative stress. It has been reported that the extraction solvents markedly affected the content and effectiveness of the extracted bioactive compounds. For this reason, SOC (*Spirulina* oil extracted with chloroform/methanol (2:1, v/v)) and SOE (*Spirulina* oil extracted with ethanol) were prepared, and then, the physiological effect of both extracts was analyzed. The results showed that the fatty acid composition of SOC and SOE were almost similar, but the GLA level in SOE was little higher than that in SOC. In addition, the carotenoids content of SOE was also higher than SOC, but the difference was not significant.

Diet is one of the main environmental factors that contribute to the development of obesity. Therefore, the high-fat and high-sucrose diet (HFD) induced obese C57BL/6J mouse model was used as model animals to evaluate the effect of *Spirulina* oil. The findings in Chapter 2 indicated that long-term supplementation with 4% *Spirulina* oil (12 weeks) markedly reduced the body weight, mesenteric white adipose tissue (WAT) weight and serum triacylglycerol (TAG) level in mice fed the HFD. The data also manifested that *Spirulina* oil supplementation did not alter the blood glucose level in obese mice, suggesting that dietary *Spirulina* oil has no effect on hyperglycemia. Dietary *Spirulina* oil also significantly decreased the hepatic total lipids, TAG and total cholesterol (TC) levels in obese mice. The underlying mechanism of *Spirulina* oil on hepatic lipid metabolism was also investigated. Dietary *Spirulina* oil suppressed the mRNA expression of sterol regulatory element binding protein-1c (SREBP-1c), fatty acid synthase (FAS) as well as stearoyl-CoA desaturase 1 (SCD-1) and then resulted in a low rate of hepatic lipid synthesis. A decrease in fatty acid β-oxidation is also considered as being responsible for the pathogenesis of hepatic steatosis. Peroxisome proliferator-activated receptor alpha (PPAR α) regulates the transcription activation of several genes that are involved in peroxisomal and mitochondrial β-oxidation of fatty acids, predominantly in liver. As for the target genes of PPAR α, acyl-CoA oxidase 1 (ACOX-1) is a key factor to regulate the peroxisomal β-oxidation, while
carnitine palmitoyltransferase 1alpha (CPT-1α) and CPT-2 are rate-limiting enzymes of fatty acid \(\beta\)-oxidation. The results revealed that long-term supplementation with *Spirulina* oil significantly increased the hepatic mRNA levels of PPAR \(\alpha\) and CPT-1α in diet-induced obese mice. The findings also showed that *Spirulina* oil had an effect on cholesterol metabolism. Dietary *Spirulina* oil significantly suppressed the hepatic mRNA level of SREBP-2, a main nuclear transcription factor that is essential for cholesterol metabolism.

Accumulating evidence indicates that obesity is characterized by increased accumulation of fat into adipose tissues leading to oxidative stress and chronic inflammatory status. In Chapter 3, the effect of *Spirulina* oil on oxidative stress status was also investigated. The result showed that the hepatic lipid hydroperoxide level was dramatically reduced by *Spirulina* oil feeding, suggesting the increase in hepatic antioxidant capacity of diet-induced mice fed *Spirulina* oil. Several studies indicate that the first line defense against reactive oxygen species (ROS) in the organism is formed by the antioxidative enzymes and molecules such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), which convert active oxygen molecules into non-toxic compounds. In the present study, SOD activity in liver of mice fed HFD was elevated on feeding *Spirulina* oil. In addition, dietary *Spirulina* oil also decreased the GSSG/GSH ratio in liver and epididymal WAT. The antioxidant effect of *Spirulina* oil is further supported by the mRNA expression of SOD1, SOD2, glutathione peroxidase 1 (GPx-1) and CAT in liver and epididymal WAT. The results revealed that the hepatic mRNA levels of these genes were elevated by addition of *Spirulina* oil. In epididymal WAT, the SOD2 mRNA level in *Spirulina* oil group increased significantly. These findings indicate that dietary *Spirulina* oil attenuates the oxidative stress status in diet-induced obese mice. It has been also suggested that the HFD-mediated chronic inflammation is marked by increased pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-\(\alpha\)), interleukin-1beta (IL-1\(\beta\)), and IL-6 in the circulation. The findings in Chapter 3 showed that the mRNA levels of TNF-\(\alpha\) and monocyte chemotactic protein-1 (MCP-1) in liver and epididymal WAT were markedly decreased by addition of *Spirulina* oil. The anti-inflammatory effect of *Spirulina* oil may be due to the synergistic combination of GLA and carotenoids.

The anti-obesity and antioxidant activities of *Spirulina* oil in diet-induced obese C57BL/6J mice were observed in this study. However, the effect of *Spirulina* oil on hyperglycemia is still
not well understood. Therefore, a spontaneous obese/diabetic KK-\(A^v\) mouse model was used to evaluate the effect of \textit{Spirulina} oil on hyperglycemia as well as obesity and oxidative stress status in Chapter 4. It was found that addition of 2\% \textit{Spirulina} oil had no anti-obesity effect in KK-\(A^v\) mice. However, a significant decrease in final body weight was observed in KK-\(A^v\) mice fed 4\% \textit{Spirulina} oil as compared to control group. This finding is consistent with the results in Chapter 2, which showed that dietary 4\% \textit{Spirulina} oil led to reduction of body weight in diet-induced obese C57BL/6J mice. It suggests that a diet contained more than 4\% \textit{Spirulina} oil is necessary to show the significant anti-obesity effect in KK-\(A^v\) mice. The effect of dietary \textit{Spirulina} oil on serum lipid parameters in KK-\(A^v\) mice were also investigated. Serum TC, HDL-C, LDL-C and phospholipids levels of mice fed \textit{Spirulina} oil increased slightly as compared as control group. Although serum TAG level was found to be decreased by dietary \textit{Spirulina} oil, no significant difference was observed.

After 4 weeks of feeding, \textit{Spirulina} oil did not alter the glucose level as compared to control mice. This is consistent with the previous findings in Chapter 2. This finding may indicate that \textit{Spirulina} oil supplementation has no effect on hyperglycemia. The results in this Chapter also revealed that supplementation with \textit{Spirulina} oil dramatically decreased the hepatic total lipids, TAG and TC levels in KK-\(A^v\) mice. These results suggest that \textit{Spirulina} oil supplementation ameliorates the hepatic steatosis in KK-\(A^v\) mice.

In Chapter 3, \textit{Spirulina} oil showed a potential antioxidant activity in diet-induced obese C57BL/6J mice. Similar results were also observed in Chapter 4. Dietary \textit{Spirulina} oil significantly increased the hepatic SOD activity, and lowered the ratio of GSSG/GSH in both of liver and epididymal WAT. These results provide the evidence that \textit{Spirulina} oil indeed exerts its effects on antioxidant defense mechanism both in diet-induced obese C57BL/6J mice and spontaneous obese/diabetic KK-\(A^v\) mice.

In conclusion, in the present study, it was found that \textit{Spirulina} oil showed potent beneficial effects on obesity and oxidative stress in two types of obese/diabetic mouse model, while the effects of SOC and SOE were basically comparable.