学位論文内容の要旨

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学 位 論 文 題 目

Study on the chemical and nutritional significance of microalgae lipids

（微細藻類脂質の化学的、栄養的重要性に関する研究）

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 content as well as the lipid class and fatty acids varied significantly among the eleven species of microalgae. All of the microalgae used in this study contained fucoxanthin except for *Spirulina*. In addition, no eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) was found in *Spirulina* lipids, but it was rich in gamma-linolenic acid (GLA) 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. On the other hand, 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 result 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 finding in Chapter 2 showed that long-term supplementation with 4% SOC (12 weeks) effectively reduced the body weight, mesenteric white adipose tissue (WAT) weight and hepatic steatosis in mice fed the HFD. Although dietary SOE and fish oil (FO) also tended to suppress the increases of body and total WAT weight of mice fed HFD, no statistical differences were observed. Nonetheless, there were no significant differences among the FO, SOC and SOE groups. The present study also demonstrated that the suppression of hepatic steatosis by *Spirulina* oil may be through regulating the sterol regulatory element binding protein-1c (SREBP-1c) and SREBP-2-mediated TAG and cholesterol synthesis pathway as well as via the enhancement of peroxisome proliferator-activated receptor alpha (PPAR α)-mediated β-oxidation.

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. The present results revealed that long-term supplementation with SOC, SOE and FO effectively decreased the hepatic lipid hydroperoxide levels as well as increased the activities and mRNA levels of antioxidant enzymes in HFD-induced obese mice. In addition, dietary SOC, SOE and FO also markedly decreased the mRNA expression of pro-inflammatory cytokines in liver and epididymal WAT of mice fed HFD, suggesting the anti-inflammatory action of *Spirulina* oil. These finding suggest that the anti-inflammatory effect of *Spirulina* oil may be due to the synergistic combination of GLA and carotenoids. Moreover, the antioxidant and anti-inflammatory effects of *Spirulina* oil and fish oil were basically identical.

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^y mouse model was used to evaluate the effect of *Spirulina* oil on hyperglycemia as well as obesity and oxidative stress status in Chapter 4. A significant decrease in final body weight was observed in KK-A^y mice fed 4% *Spirulina* oil as compared to control group. After 4 weeks of feeding, *Spirulina* oil did not alter the glucose level as compared to control mice. The results in this Chapter also revealed that supplementation with *Spirulina* oil dramatically decreased the hepatic total lipids, TAG and TC levels in KK-A^y mice. These results suggest that *Spirulina* oil supplementation ameliorates the hepatic steatosis in KK-A^y mice. Dietary *Spirulina* oil also significantly increased the hepatic superoxide dismutase (SOD) activity, and lowered the ratio of GSSG/GSH in both of liver and epididymal WAT. These results provide the evidence that *Spirulina* oil indeed exerts its effects on antioxidant defense mechanism in spontaneous obese/diabetic KK-A^y mice. These findings are consistent with the previous results.

In conclusion, in the present study, it was found that *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. To the best of our knowledge, this is the first study to highlight the biological activity of *Spirulina* oil on obesity and oxidative stress in diet-induced obese mice and spontaneous obese/diabetic KK-A^y mice. These encouraging findings indicate that *Spirulina* oil has a potential for human consumption as a functional food ingredient to reduce the risk of obesity associated metabolic syndrome.