学位論文内容の要旨

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学 位 論 文 題 目

Dietary Effect of Squalene and Farnesol on the Lipid Metabolism of Obese/diabetes KK-A^y Mice and Wild-type C57BL/6J Mice

（肥満/糖尿病 KK-Ayマウスと野生型 C57BL/6J マウスの脂質代謝に及ぼす

スクワレンとファルネソールの効果）

Cardiovascular diseases (CVD) are a group of disorders of the heart and blood vessels. A report from world health organization (WHO) indicated that in 2016, an estimated 17.9 million people died from CVD, representing 31% of all global deaths. CVD have become the number 1 cause of death globally. In many cases, CVD is caused by atherosclerosis, a chronic vascular disease that generally occurs in the aorta and muscular-type arteries such as coronary arteries, brain arteries, renal arteries and carotid arteries. Although the exact motive of atherosclerosis is still unknown, modification and deposition of lipids in the vascular wall can induce this event.

Olive oil intake is known to show health beneficial effects including cardioprotection and these effects have been recognized to be, in part, derived from olive oil minor compounds, mainly phenolic compounds. In addition, due to the relatively high content in olive oil as compared with other vegetable oils, squalene (SQ) has been also regarded as a contributing factor for the observations of reduced risk of diseases associated with olive oil intake; however, details has not been yet clear on the cardioprotective activity of SQ. Not just in the olive oil, SQ is also found to be very rich in shark liver oil. The shark *Centrophorus squamosus* has been reported to contain around 14% liver oil in which mainly is SQ (nearly 80% of the oil). Therefore, SQ, used as a unique nutraceutical obtained from marine products for the cardioprotection, will be interesting. The present study has been conducted to make clear the effect of SQ on lipid metabolism, especially plasma lipids, and fatty acid (FA) metabolism in animal models.

In **Chapter 1** and **Chapter 2**, the comparison has been done on the effect of SQ on the obese/diabetes KK-A^y mice and normal C57BL/6J mice.

Epidemiological studies have revealed an inverse correlation between high-density lipoprotein cholesterol (HDL-C) levels and the risk of CVD and of atherosclerosis. Every 1 mg/dL increase in HDL is associated with a 2% to 3% decrease in CVD risk, independent of low-density lipoprotein cholesterol (LDL-C) and triacyglyecerol (TAG) levels. In the present study, we have found a significant increase in plasma HDL-C of obese/diabetic KK-A^y mice fed SQ with no significant

difference in the total cholesterol (TC) and nonHDL-C levels. The increase in HDL-C was also found in the plasma of normal C57BL/6J mice, but the difference was not significant. This result suggests the anti-atherosclerosis effect of SQ, especially on subjects with metabolic disorders.

In the present study, different results on TC level were observed between the obese/diabetic KK-A^y mice and normal C57BL/6J mice. In KK-A^y mice, SQ administration decreased TC level in the liver and almost did not affect plasma TC. The decrease tendency in the TC level in the liver of KK-A^y mice might due to the down-regulation of HMG-CoA reductase activity and its gene expression. In C57BL/6J mice, both hepatic and plasma TC were increased. Few studies in animals and human had revealed discrepant effects of SQ on plasma cholesterol. Further research will be needed to clarify the discrepant influence of SQ on animals and humans.

On the other hand, significant increase in hepatic neutral lipid (NL), TAG and total lipid (TL) by SQ intake was observed in KK-A^y mice, and a same increase tendency was also observed in C57BL/6J mice. TAG can be accumulated in liver via the process of hepatic steatosis and hepatic steatosis has been proposed to be associated with liver disease, type 2 diabetes mellitus, arterial hypertension and metabolic syndrome. The results on hepatic NL, TAG and TL by SQ may illustrate that SQ administration can aggravate hepatic steatosis. Although SQ administration could reduce the risk of CVD by increase in HDL-C, it is noteworthy that SQ may also increase the risk of hepatic steatosis.

In KK-A^y mice, the contents of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and PUFA in hepatic NL were significantly higher in SQ supplementation groups, probably as a result of increment of liver TAG and NL. However, FA in hepatic phospholipid (PL) was affected slightly or was not affected by SQ supplementation. In C57BL/6J mice, 2% SQ supplementation significantly increased the contents of SFA, MUFA and PUFA in hepatic PL, while FA in hepatic NL was affected slightly or was not affected by SQ supplementation. Further research will be needed to clarify this completely opposite results found between two animal models.

In **Chapter 3**, farnesol and sphingomyelin (SM) were used as experiment sample to assess their effects on lipid metabolism of KK-A^y mice. Plasma TC and HDL-C levels were significantly increased by 0.1% farnesol administration. These results show that farnesol may also be a positive factor for cardioprotection. Administration of 0.5% SM down-regulated plasma and hepatic TC levels. However, plasma HDL-C level also decreased by SM supplementation. As several prospective studies on different racial and ethnic groups worldwide have confirmed that HDL-C is a strong, consistent and independent predictor of incident cardiovascular events, administration of SM may not be a good factor for cardioprotection.

Stearoyl-CoA desaturase (SCD) is a key and highly regulated ER enzyme that catalyzes the biosynthesis of MUFA from SFA. In this study, plasma and hepatic MUFA composition has been increased by farnesol treatment. In the present study, hepatic SCD-1 expression was significantly increased in farnesol treated mice. However, significant changes on SREBP-1c, NR1H3 and NR1H2 expression were not observed. This result may illustrate that farnesol serve as a SCD-1 activator in liver of KK-A^y mice.

In conclusion, the present study showed that SQ supplementation to obese/diabetes KK-A^y mice and

normal C57BL/6J mice increased plasma HDL-C level, important and independent anti-atherosclerotic factor. It is noteworthy that this effect of SQ was found more clearly in obese/diabetes model mice as compared with normal mice. Although more research is needed to make clear the effect of SQ on the lipid metabolism and dynamics related to atherosclerosis, the present study suggests the anti-atherosclerotic effect of SQ especially on subjects with metabolic disorders. In addition, the effect of farnesol has been also examined in the present study, since farnesol is known to be an important intermediary metabolite in SQ biosynthesis. As a result, it has been found that farnesol could also have ability to increase plasma HDL-C level as seen in SQ. Furthermore, farnesol supplementation to KK-A^y mice up-regulated the SFA conversion to corresponding MUFA. This effect would be due to the promotion of SCD-1 expression in the mice.