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Synthesis and quantification of long chain monounsaturated fatty acid positional isomers occurred in fish and their bioactivities  
(魚中で発生した長鎖モノ不飽和脂肪酸の合成と定量そしてそれらの生物活性)

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博士学位論文内容要旨  
Abstract

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論文題目 Title	Synthesis and quantification of long chain monounsaturated fatty acid positional isomers occurred in fish and their bioactivities (魚中で発生した長鎖モノ不飽和脂肪酸の合成と定量そしてそれらの生物活性)		

Fish oil rich in long chain monounsaturated fatty acids (LC-MUFA) such as *cis*-eicosenoic acid (*c*-20:1) and *cis*-docosenoic acid (*c*-22:1) have shown beneficial health effects in modulating the risk factors associated with cardiovascular diseases, diabetes and obesity related metabolic dysfunctions. Thus, LC-MUFA may partially contribute to the well-known functional health benefits of fish oil. Some pelagic fishes and marine mammals contain high quantity of LC-MUFA particularly *c*-20:1 and LC-MUFAs occur as a mixture of positional isomers (PIs) in fish oil. Limited data on health effects and occurrence of LC-MUFA PIs might be due to the unavailability of pure fatty acids (FAs) standards. The objectives of the current study were to chemically synthesize the common LC-MUFA PIs and quantitatively analyze the distribution of LC-MUFA PIs in fishes from the Indian, Pacific and Atlantic Ocean. Further, health effects among bioavailable LC-MUFA PIs in fish oil was compared using chemically synthesized *c*-20:1 and *c*-22:1 isomers to examine their isomer specific effect on cellular lipid metabolism.

Positional isomers of *c*-20:1 (*c*5 (double bond locates at 5th carbon atom from carbonyl side, namely  $\Delta$ 5 position), *c*7, *c*9, *c*11, *c*13, and *c*15) and *c*-22:1 (*c*7, *c*9, *c*11, *c*13, and *c*15) were synthesized by a series of chemical reactions. The reaction pathway consisted of seven main reactions namely; bromination, protection of hydroxyl group, coupling reaction, hydrolysis, bromination, and Grignard reaction. Most of reactions resulted a good yield (above 80% in average) with few exceptions. Synthesized PIs were analyzed by gas chromatography (GC)- flame ionization detector (FID) and GC-mass spectroscopy to determine the purity and confirm the synthesis. GC analysis of synthesized isomers using high polar capillary column showed an elution order of FA PIs with an increasing  $\Delta$  position. All the above-mentioned FA isomers except *c*5-20:1 were successfully synthesized with high purity (>97%) using the given protocol.

The occurrence and distribution of *c*-20:1 PIs in fishes from the Indian Ocean was investigated and compared to those from the Pacific and Atlantic Ocean. Lipids were extracted from the edible part of the fish and methyl esterified for GC analysis. The 20:1 methyl ester (ME) fraction was separated from total FAMES by reversed-phase HPLC and quantitatively analyzed using a GC-FID equipped with highly polar capillary column. The synthesized PIs in the previous study were used as standard to identify FA peaks in fish oil samples and *c*14-20:1 was used as an internal standard. The results indicated that high content of *c*-20:1 PIs were found in fishes from the Pacific Ocean (saury, 166.95±12.4 mg/g of oil), followed by the Atlantic Ocean (capelin, 162.7±3.5 mg/g of oil), and lastly from the Indian Ocean (goatfish, 34.39 mg/g of oil). With only a few exceptions, the most abundant 20:1 PI found in fishes of the Indian and Atlantic Ocean was the *c*11-20:1 isomer (>50%) followed by the *c*13-20:1 isomer (<25%). Unusually, the *c*7-20:1 isomer was predominantly found in some fishes such as the tooth ponyfish, longface emperor, and commerson's sole. The *c*9, *c*5, and *c*15-20:1 isomers were the least occurring in fishes from the Indian and Atlantic Ocean. In contrast, the *c*9-20:1 isomer was the principal isomer identified in fishes from the Pacific Ocean. The results revealed that the content and distribution of *c*-20:1 PIs varied among fishes in different oceans. The data presented in the current study are the first to report on the distribution of *c*-20:1 PIs in fishes from the Indian Ocean.

In the third study, six bioavailable *c*-20:1 PIs were examined for their effects on adipogenesis and

lipogenesis using 3T3-L1 preadipocytes. Cells were cultured in the presence of experimental i.e. *c*-20:1 PIs-added (50  $\mu$ M) or control (*c*9-18:1, 50  $\mu$ M or no FAs) growth medium. The treatment of *c*-20:1 PIs, decreased the cellular triglyceride (TG) content compared to that of *c*9-18:1-treated cells. Although, the levels of cellular phospholipids, total cholesterol, and non-esterified FAs were not altered significantly ( $p < 0.05$ ) among treatments. FA composition indicated that cells uptake *c*-20:1 PIs into cells at significantly ( $p < 0.05$ ) different percentages and has altered the FA metabolism in cells. Among the tested *c*-20:1 PIs, *c*15 isomer down-regulated the transcriptional factors for adipogenesis (peroxisome proliferative activated receptor gamma (PPAR $\gamma$ ) and CCCAT enhancer binding protein alpha (C/EBP $\alpha$ )) and lipogenesis (sterol regulatory element binding protein-1, SREBP-1) compared to that of *c*9-18:1 and other *c*-20:1 PIs.

The effect of selected LC-MUFA PIs with different chain length was compared through an *in vitro* study using 3T3-L1 and HepG2 cells. Common LC-MUFA PIs, i.e. *c*-18:1 (13 isomers), *c*-20:1 (6 isomers) and *c*-22:1 (5 isomers), were screened based on their individual effect on the cellular lipid accumulation. Three LC-MUFA PIs (*c*5-18:1, *c*9-18:1, *c*11-18:1, *c*9-20:1, *c*15-20:1, *c*11-20:1, *c*7-22:1, *c*9-22:1, and *c*11-22:1) from each chain length were selected for further studies of their effect on the cellular lipid metabolism. The selected LC-MUFA PIs influenced differently on TG accumulation in 3T3-L1 cells. The lowest and highest TG accumulation were showed in cells treated with *c*15-20:1 and *c*9-22:1, respectively. Further, cells treated with *c*9-20:1 followed by *c*15-20:1 indicated the lowest expression of transcription factors related to adipogenesis (PPAR $\gamma$  and C/EBP $\alpha$ ). The cells treated with *c*15-20:1 indicated the lowest expression of transcription factors related to lipogenesis (SREBP-1). The results were significantly different ( $p < 0.05$ ) among treatments. The cells treated with *c*15-20:1 had a low level of mRNA expression of genes related to lipid synthesis (stearoyl-Co-A desaturase-1 and FA synthase) compared to other PIs. In addition, experimental PIs showed different effects on the levels of mRNA expression of gene related to FAs  $\beta$  oxidation (carnitine palmitoyltransferase 1a). Comparison of the functionality among *c*9 and *c*11 isomers of 18:1, 20:1, and 22:1 showed the changes in effects of LC-MUFAs with different chain lengths and same double bond position (same  $\Delta$  position) influenced on their functionality and exhibited significantly ( $p < 0.05$ ) different effect among the selected LC-MUFA PIs. It was apparent that some experimental LC-MUFA PIs such as *c*15-20:1 and *c*9-20:1 improved the cellular lipid metabolism, by suppression of adipogenesis, lipogenesis, and enhancing the cellular FAs  $\beta$  oxidation.

In conclusion, LC-MUFA PIs, i.e. *c*-20:1 and *c*-22:1, were chemically synthesized with a high purity (>97%) and used as standards for GC analysis and *in vitro* studies. The occurrence and distribution of *c*-20:1 PIs varied among fishes in different oceans, where *c*11-20:1 contributed to high proportion in the Indian and Atlantic Ocean fishes and *c*9-20:1 was predominant in the Pacific Ocean fishes. Results revealed that common *c*-20:1 PIs affected differently on adipogenesis and lipogenesis in 3T3-L1 cells and the position of the double bond influenced on the functionality. The selected LC-MUFA PIs from different chain lengths had different effect on the lipid metabolism at cellular level and *c*15-20:1 showed comparatively good anti-adipogenic, anti-lipogenic effect, and improvement of cellular lipid metabolism. Results revealed that the double bond position and number of carbon atoms in LC-MUFA influenced on their functionality at the cellular level.