

Positional characteristics of triacylglycerol in soft-shelled turtle oil and its effects on lipid metabolism in hamsters fed a high fat diet

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Abstract

Soft-shelled turtle is a highly economic species and plays a mystical role in traditional Chinese medicine. The present study analyzed the positional distribution in triacylglycerol (TG) of soft-shelled turtle oil (STO) and its effects on lipid metabolism in hamsters fed a high fat diet. Analysis showed that TG made up the maximum fragments among lipid classes of STO, with eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) integrated in the *sn*-1,3 position of TG. For the animal study, forty male hamsters were randomly divided into five groups and fed a control diet (5% fat), high fat diet (HF, 10% fat) and HF diets containing 2% (STO2), 4% (STO4) or 6% (STO6) soft-shelled turtle oil for 28 days. The body weight gain and food intakes were comparable between hamsters fed HF diet and HF diet containing STO from 2% to 6% during the experiment. Hamsters fed HF diets containing 2–6% STO showed decreased liver TG, and HF diets containing 4% or 6% STO showed decreased serum TG but increased serum total cholesterol. In addition, enzyme activities of lipogenesis in hepatic malic enzyme and glucose-6-phosphate dehydrogenase were lower in hamsters fed 4% or 6% STO than in those fed the high fat diet. These results suggest that dietary 4% or 6% STO, with different intramolecular distributions of n-3 PUFA, might decrease serum and liver TG concentrations by diminishing enzyme activities involved in liver fatty acid synthesis.

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Keywords: Soft-shelled turtle oil; TG structure; Lipid metabolism; Hamsters

1. Introduction

The soft-shelled turtle (*Pelodiscus sinensis*) is a highly economic product in Asian countries and is a high-valued aquaculture species in Taiwan. They are highly prized as a luxury food item and as a nutritional supplement, playing a mystical role in traditional Chinese medicine. Feng et al. (1991) showed that orally repeated administration of the soft-shelled turtle powder had a therapeutic effect on carbon tetrachloride-induced liver injury, by improving liver function and protein synthesis. In addition, they also pointed out that soft-shelled turtle powder attenuated fatigue and accelerated recovery from stress in mice (Feng, Matsuki, & Saito, 1996a), and decreased the growth of solid tumors – possibly by activating the host immune sys-

tem (Feng, Yamazaki, Matsuki, & Saito, 1996b). However, there is still limited information about the biological function of soft-shelled turtle as a dietary component.

It is well known that hypercholesterolemia appears critical to the atherogenic process and tends to lead to serious cardiovascular disease (CVD). Moreover, plasma triacylglycerols (TG) were also an independent risk factor for CVD in many epidemiological studies (Pan & Chiang, 1995; Hopkins, Wu, Hunt, & Brinton, 2005). Over the past three decades, CVD has become the major leading cause of death in developing and developed countries. Epidemiological investigations and animal studies show that n-3 polyunsaturated fatty acids (PUFA), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish oils, have a protective effect with respect to coronary heart disease (Davis, Bridenstine, Vesselinovitch, & Wissler, 1987; Kromhout, Bosschieter, & Coulander, 1985). On the other hand, dietary seal oil, which contains EPA and

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DHA mainly at the *sn*-1 and -3 positions of the triacylglycerols (TG), more effectively reduced plasma and liver TG concentrations than did fish oil in rats (Yoshida et al., 1999). Soft-shelled turtle is an amphibious reptile and its oil contains *n*-3 PUFA. There are few published studies about the oil characteristics of soft-shelled turtle oil (STO) or its effects on lipid metabolism of humans or animals. Therefore, the present study was aimed, first, to analyze the positional characteristics of fatty acids in the STO TG fraction, and second, to investigate the effect of STO on serum and liver lipids, and hepatic enzyme activities of fatty acid synthesis.

2. Materials and methods

2.1. Positional distribution of fatty acids in TG of STO

The content and positional distribution of fatty acids in triacylglycerol (TG) fractions of STO were analyzed according to Myher and Kuksis (1979), and as modified by Paterson, Weselake, Mir, and Mir (2002). Briefly, lipid classes were isolated by thin-layer chromatography (TLC) on silica gel 60 (Merck, Darmstadt, Germany), using petroleum ether–diethyl ether–acetic (82/18/1, v/v/v), containing 0.02% propyl gallate, as the solvent system. The TG fraction was scraped off and then digested by pancreatic lipase (Sigma) in a Tris–HCl buffer (pH 8) to produce *sn*-2 monoacylglycerol (2-MG). The lipids were extracted and 2-MG was isolated using boric acid TLC plates with a developing solvent of CHCl₃–acetone (88/12, v/v). The *sn*-1/3 fatty acids in TG were calculated after extraction of 2-MG and analysis of the fatty acid composition.

2.2. Analysis of fatty acid composition

Total lipids of diets were extracted according to Folch, Lees, and Sloane-Stanley (1957). The fatty acids from diet lipids or TG and 2-MG fractions of STO were transmethylated (Fang, Chen, Huang, & Lu, 2004). Fatty acid methyl esters were separated by gas chromatography in a Shimadzu GC-14A gas chromatograph (Tokyo, Japan) fitted with a 30 m × 0.25 mm Rtx-2330 capillary column, 0.20 µm film thickness (Restek Co.). Nitrogen at 40 ml/min was used as the carrier gas and the split/splitless injector was used with a split/splitless ratio of 30:1. Injector and detector temperatures were 250 °C. The column oven temperature was maintained at 160 °C for 5 min after sample injection and was programmed to 220 °C at 3 °C/min, and then held there for 15 min. The separation was recorded with a Shimadzu C-R6A integrator. Fatty acid methyl esters were identified by comparison with previously run standards.

2.3. Animals and diets

Six-week-old male Syrian hamsters obtained from the National Laboratory Animal Center (Taipei, Taiwan) were

used. The animals were fed a laboratory rodent diet, #5001 (PMI Feeds, Inc., Brentwood, MO) for about 7 days until their body weight reached a range of 80–90 g. Forty animals were divided into five groups of eight animals and assigned to one of the diets shown in Table 1 for 28 days ad libitum. The experimental diets were made basically according to the AIN-76 (1977); the control and high fat (HF) diets were provided at 5% and 10%, respectively, at the expense of carbohydrates. STO2, STO4 and STO6 were HF diets containing 2%, 4% and 6% soft-shelled turtle oil (kindly provided by Chia Jei Technology Business Co. Ltd., Kaohsiung, Taiwan), respectively. Fatty acid compositions of the experimental diets are shown in Table 2.

Table 1
Composition of experimental diet

Composition (%)	Groups ^a				
	Control	HF	STO2	STO4	STO6
Casein	20	20	20	20	20
Lard	3	6	4.8	3.6	2.4
Corn oil	2	4	3.2	2.4	1.6
Sucrose	20	20	20	20	20
Corn starch	45	40	40	40	40
Alpha-cellulose	5	5	5	5	5
Vitamin mixture ^b	1	1	1	1	1
Mineral mixture ^b	3.5	3.5	3.5	3.5	3.5
DL-Methionine	0.3	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2	0.2
Soft-shelled turtle oil	0	0	2	4	6

^a Control: basal diet, HF: high fat diet, STO2, STO4 and STO6 were HF diets containing 2%, 4% and 6% soft-shelled turtle oil, respectively. All diets were supplemented with 5 mg DL- α -tocopherol/kg and stored in the dark at –20 °C.

^b AIN-76.

Table 2
Fatty acid compositions of experimental diet

Fatty acids	Groups ^a				
	Control (wt.%)	HF (wt.%)	STO2 (wt.%)	STO4 (wt.%)	STO6 (wt.%)
14:0	1.0	1.1	1.2	1.7	2.1
16:0	19.9	19.0	19.8	19.0	20.0
16:1	1.6	1.7	2.3	3.4	4.7
18:0	10.5	9.6	9.4	7.9	7.0
18:1(n-9)	38.6	37.0	36.4	35.3	33.8
18:2(n-6)	26.8	29.2	25.9	23.3	19.8
18:3(n-3)	0.4	1.0	1.2	1.5	1.7
20:5(n-3)	–	–	0.5	1.2	1.7
22:5(n-3)	–	–	–	0.9	1.1
22:6(n-3)	–	–	1.7	3.5	5.8
P/S	0.9	1.0	1.0	1.1	1.0
PUFA ^b	27.2	30.2	29.3	30.4	30.1
MUFA	40.2	38.7	38.7	39.7	38.5
SFA	31.4	29.7	30.4	28.6	29.1
n-3 PUFA	0.4	1.0	3.4	7.1	10.3

^a Control; normal fat diet, HF; high fat diet, STO2, STO4 and STO6 were HF diets containing 2%, 4% and 6% soft-shelled turtle oil, respectively.

^b PUFA: polyunsaturated fatty acid; MUFA: monounsaturated fatty acid; SFA: saturated fatty acid. Fatty acids less than 1% in all the groups were not shown.

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