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The regulatory effects of fish oil and chitosan on hepatic lipogenic signals in high-fat diet-induced obese rats

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ABSTRACT

The present study investigated the regulatory effects of fish oil and chitosan on the signals of hepatic lipid metabolism and the postulated mechanism in high-fat diet-induced obese rats. Diet supplementation of chitosan and fish oil efficiently suppressed the increased weights in body and livers of high-fat diet-fed rats. Supplementation of chitosan and fish oil significantly decreased the activities of hepatic lipid biosynthesis-related enzymes and efficiently regulated plasma lipoprotein homeostasis. Both chitosan and fish oil significantly ameliorated the alterations in the protein expressions of hepatic lipogenic transcription factors (LXRα and PPARα), and could also significantly regulate the downstream hepatic lipogenic genes (*FAS*, *HMGCR*, *CYP7A1*, *FATP*, *FABP*, *AOX*, and *ABCA*) expressions in high-fat diet-fed rats. These results suggest that both fish oil and chitosan exerts downregulative effects on hepatic lipid metabolism in high-fat diet-induced obese rats via the LXRα inhibition and PPARα activation, which further affect the expressions of hepatic lipogenesis-associated genes.

1. Introduction

Obesity is a metabolic syndrome-related complications, caused by the presence of an energy imbalance involving excessive energy storage and inadequate energy expenditure. The worldwide prevalence of overweight [body mass index (BMI) $\geq$ 25 kg/m^2] and obesity (BMI $\geq$ 30 kg/m^2) in 2014 are 39% (38% of men and 40% of women) and 13% (11% of men and

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15% of women), respectively, in adults aged 18 and over [1]. Moreover, obesity is also known as a high risk of accompany comorbid disorders, such as cardiovascular disease, diabetes, and nonalcoholic fatty liver disease, leading to poor quality of human life and higher financial burden on the state [2,3]. Notably, dysregulation of hepatic lipid metabolism is an obvious and major etiology of obesity, including a reduced secretion of very-low-density lipoproteins (VLDL) [4], an increase in free fatty acid (FFA) flux into the liver and in *de novo* lipogenesis of more FFA [5]. Therefore, the strategy to improve the imbalance of hepatic lipid metabolism is concerned with antiobesity food and food ingredients against human obesity.

The concept of functional food is widely accepted as the ability to beneficially impact body functions by possessing advantageous physiological effects and reducing the threat of diseases [6]. Chitosan, a marine functional food, is partially or fully deacetylated from the chitin composed of N-acetyl-D-glucosamine [7]. Several studies have shown that the chitosan acted as a drug delivery carrier or a dietary fiber against hypertension, hypercholesterolemia, and obesity [8–11]. The other well-known marine functional food, fish oil, has been reported to possess the beneficial effects on cardiovascular diseases and obesity, which are attributed to omega-3 fatty acids, particularly to eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids [12,13]. Our previous studies have also shown that supplement of chitosan alleviates lipid accumulation in the livers and adipose tissues in the type-2 diabetic rat model and high-fat (HF) diet-induced obese rat model [14,15]. In addition, foods and functional foods/dietary supplements has been found to exert synergistic or additive effects on health promotion and disease prevention [16]. Recently, the nuclear receptors liver X receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs) have been identified as regulators of the cholesterol and phospholipid in the liver, especially LXRα and PPARα. The activated LXRs have been reported to increase fatty acid synthesis through induction of the sterol regulatory element-binding protein-1c (SREBP1c) triggering the downstream signaling expression of fatty acid synthase (FAS) [17]. In order to energy homeostasis, hepatic fatty acid oxidation, as well called β-oxidation, occurs under the condition of elevated fatty acid (FA) levels through induction of PPARα/acyl-CoA oxidase 1 (AOX1) signaling pathway [18]. For cholesterol regulation, a high fat diet may trigger the cholesterol synthesis and metabolism through the activation of liver LXRα/3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and the induction of cytochrome P450 7A1 (CYP7A1) [17,19]. However, the mode of action and the possible molecular mechanism of synergistic or additive effects between chitosan and fish oil on hepatic lipid responses and lipid metabolism still remain unclear. Therefore, we hypothesized that chitosan combined with fish oil might comediate hepatic lipid metabolism profile in obesity conditions. In this study, we aim to investigate the synergistic or additive effects and mechanisms of chitosan combined with fish oil on hepatic lipid metabolism in HF diet-fed rats, which are commonly used as an *in vivo* obesity model.

2. Materials and methods

2.1. Materials

High molecular weight (MW) chitosan from crab shell was supplied from Koyo Chemical Co. (Tokyo, Japan). The concocted processing of chitosan was involved in the demineralization, deproteinization, and deacetylation of crab shell. The average MW and degree of deacetylation (DD) of chitosan were measured by high-performance liquid chromatography and Fourier transform infrared spectroscopy, respectively. The viscosity of chitosan was detected by a viscometer (CV20, Haake Mess-Technik GmbHu, Karlsruhe, Germany). The DD of chitosan was ~90.7%, and the average MW and viscosity of chitosan were ~642 kDa and 304.7 cP, respectively. Cellulose was supplied from Sigma-Aldrich (St. Louis, MO, USA). Fish oils were supplied from Sentosa Co. (Taipei, Taiwan).

2.2. Animals and diets

Six-week-old male Sprague-Dawley (SD) rats were supplied from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan) and were fed a chow diet (Rodent Laboratory Chow, Ralston Purina, St. Louis, MO, USA) for 1 week. Rats were randomly divided into five groups ($n = 8$ of each group): (1) standard rodent diet-fed rats with 5% cellulose (ND); (2) HF diet-fed rats with 5% cellulose; (3) HF diet-fed rats with 5% fish oils (HF + O); (4) HF diet-fed rats with 5% high-MW chitosan (HF + CS); and (5) HF diet-fed rats with 5% high-MW chitosan and 5% fish oils (HF + CS + O). The formulation of the experimental diets and the fatty acid composition of fish oils are shown in Tables 1 and 2, respectively. Rats were housed in individual stainless-

Table 1 – Composition of experimental diets (%).

Ingredient (%)	ND	HF	HF + O	HF + CS	HF + CS + O
Casein	20	20	20	20	20
Lard	3	18	13	18	13
Soybean oil	2	2	2	2	2
Fish oil			5		5
Vitamin mixture[a]	1	1	1	1	1
Salt mixture[b]	4	4	4	4	4
Cholesterol		0.5	0.5	0.5	0.5
Choline chloride	0.2	0.2	0.2	0.2	0.2
Cholic acid		0.2	0.2	0.2	0.2
Corn starch	64.8	49.1	49.1	49.1	49.1
Cellulose	5	5	5		
Chitosan[c]				5	5

DD = degree of deacetylation; HF = high fat diet (18% Lard + 2% soybean oil); HF + CS: high fat diet + 5% chitosan; HF + CS + O: high fat diet + 5% chitosan + 5% fish oil; HF + O: high fat diet (13% lard + 2% soybean oil) + 5% fish oil; MW = molecular weight; ND = normal control diet.

[a] AIN-93 vitamin mixture.

[b] AIN-93 mineral mixture.

[c] The average MW and viscosity of chitosan ~6.42×10^5 Dalton and 304.7 cps, respectively. DD was ~90.7%.