# **Brief Report**

# Evaluation of the effects of freeze-dried soybean curd intake on cholesterol levels using a novel biomarker

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ABSTRACT: To evaluate the effects of freeze-dried soybean curd intake on serum cholesterol levels, we examined the subclasses of cholesterol for healthy adult volunteers who continued to eat a piece of freeze-dried soybean curd each day along with their ordinary diet for four weeks. Of 12 subjects, the soybean curd diet proved effective in 2 cases; small dense LDL (sd-LDL) cholesterol levels were significantly reduced in association with the decrease in LDL cholesterol levels. These results suggested that daily intake of freeze-dried soybean curd may lead to an improvement in cholesterol metabolism in subjects with originally higher cholesterol levels and that sd-LDL cholesterol can be a novel biomarker for evaluation of the cholesterol lowering-action of functional food.

*Keywords:* Freeze-dried soybean curd, small dense LDL cholesterol

## 1. Introduction

The treatment strategy for metabolic syndrome has received increased attention because of the need to reduce cardiovascular events. Smoking cessation, lowering the levels of LDL-C, and blood pressure management are primary targets for risk reduction. Lifestyle interventions are the initial therapies recommended for treatment of metabolic syndrome. If lifestyle changes are not sufficient, then drug therapies for abnormalities in terms of individual risk factors may be indicated (1). Soybean protein is known to have components to help reduce total cholesterol (2) and the risk of myocardial infarction (3). Freeze-dried soybean curd (tofu) in particular has an even greater effect on reducing cholesterol and appears to be a beneficial food in terms of preventing lifestyle-related

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chronic diseases (4).

Recently, small dense low-density lipoprotein (sd-LDL) has been highlighted as a new risk factor for coronary heart disease (5,6). Sd-LDL is closely associated with insulin resistance and hypertriglyceridemia, suggesting a high prevalence of these atherogenic particles in metabolic syndrome. Therefore, sd-LDL cholesterol can be a useful marker for metabolic syndrome in patients with coronary artery disease. The effect of soybean protein intake on sd-LDL cholesterol has not been previously investigated. A preliminary study was conducted to examine the subclasses of cholesterol, including sd-LDL cholesterol, for healthy adult volunteers who continued to eat a piece of freeze-dried soybean curd each day over the course of 4 weeks.

# 2. Materials and Methods

# 2.1. Study subjects

A total of 12 volunteer subjects ages 24 to 58 was recruited after providing written informed consent, and this study was approved by the Ethics Committee of Sekino Clinical Pharmacology Clinic. All of the subjects had a wide range of serum lipid levels and were essentially healthy with no evidence of diseases related to atherosclerosis. None had been treated with lipid-lowering drugs.

### 2.2. Study diet

Seasoned, bite-size freeze-dried soybean curd was provided by Asahimatsu Foods Co., LTD (Nagano, Japan) for repeated daily intake over 28 consecutive days.

## 2.3. Study design

The subjects continued to eat a piece of freezedried soybean curd (18.5 g) each day for four weeks. Lifestyle and dietary habits were unchanged except for the equivalent caloric restriction corresponding to intake of the study diet. The subjects were also requested to

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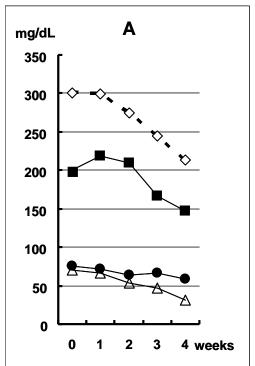
maintain a stable lifestyle throughout the duration of the trial with no intensive physical activity. Blood samples were collected after fasting (before breakfast) once every week for up to 4 weeks, including the start date. Serum was separated to measure serum levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides by standard laboratory procedures. Serum was also provided to asses liver functional tests such as ALT, AST,  $\gamma$ -GT, and Total bilirubin in order to evaluate the safety of food intake. Sd-LDL cholesterol assay was performed using a kit purchased from Denka-Seiken (Tokyo, Japan) (7).

#### 3. Results and Discussion

The present study was conducted to make a preliminary assessment of the effect of freeze-dried soybean curd intake on the levels of subclasses of cholesterol. Although statistical analysis of inter-individual variations was difficult due to the small number of subjects, two subjects showed significant changes over time (Figure 1). For both, the levels of total cholesterol decreased to an extent. Of note is the fact that sd-LDL cholesterol levels decreased significantly in association with the decrease in LDL cholesterol levels. That said, HDL cholesterol levels were fairly constant over time. Results for the subject depicted in Figure 1A also revealed a pronounced decrease in triglyceride levels. Laboratory data with regard to liver function showed no particular changes for all of the subjects, indicating the safety and tolerability of

the study diet. A meta-analysis of a randomized controlled study revealed that soy isoflavones significantly reduced serum total cholesterol and LDL cholesterol but did not change HDL cholesterol. Reductions in LDL cholesterol were greater in hypercholesterolemic subjects than in normocholesterolemic subjects (8). The present study, using sd-LDL cholesterol as a new marker, suggested that daily freeze-dried soybean curd intake may be beneficial for lipid metabolism in hypercholesterolemic subjects.

Sd-LDL cholesterol was identical to cholesterol in the denser LDL fraction. An increased concentration of small LDL particles is reported to be predictive of an increased risk of coronary heart disease (5,9). The therapeutical modulation of sd-LDL cholesterol has been shown to significantly reduce cardiovascular risk (10). Therefore, measurement of sd-LDL-C is useful at evaluating overall atherogenic risks associated with metabolic syndrome and may be applicable to routine clinical examination (6). To date, there is insufficient evidence for primary use of drugs that target the underlying causes of metabolic syndrome (1). If functional food such as soy products can be effective in reducing the sd-LDL-cholesterol level, it may prove beneficial as a form of first-line therapy. Although more detailed studies are required to verify the effectiveness of freeze-dried soybean curd intake on cholesterol homeostasis, sd-LDL cholesterol can be a novel biomarker for evaluation of the effect of functional food on improving lipid metabolism.



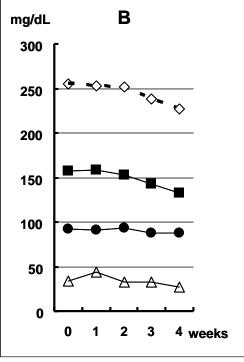


Figure 1. Changes in cholesterol levels during intake of the study diet.  $(\lozenge - - \lozenge)$ , total cholesterol; ( $\blacksquare - \blacksquare$ ), LDL cholesterol; ( $\bullet - \bullet$ ), HDL cholesterol; ( $\triangle - \triangle$ ), sd-LDL cholesterol; ( $\triangle - \triangle$ ), Subject with marked good response; (B), Subject with gradual good response.

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