Regulatory Effect of Dosiinpartner on High-Fat Diet-Induced Obesity in C57BL/6J Mice

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Dosiinpartner (DSP) is a newly developed dietary functional food to help control weight. The aim of this study was to evaluate whether DSP combined with a high-fat (HF) diet could influence body weight, fat accumulation, and plasma glucose levels. Mice were fed for 8 weeks with normal diet, HF diet, and HF+10% or 20% DSP diet. Body weight was recorded at 1 week, and plasma levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and glucose were analyzed at the end of the study. Weight increases in the 10% or 20% DSP group were significantly less than in the HF diet group (p<0.05). Plasma total cholesterol and LDL cholesterol levels decreased by 48.3% and 26.8% in the 10% DSP group and by 42.9% and 34.9% in the 20% DSP group, respectively. However, the HDL cholesterol level was unchanged. Glucose levels also decreased by 80.6% in the 10% DSP group but was almost the same in the HF and 20% DSP groups. Our findings indicate that DSP may be beneficial in the regulation of high-fat diet-induced overweight and other complications such as circulatory disorders and diabetes mellitus.

Key words dosiinpartner; high-fat diet; obesity; cholesterol; glucose

Obesity is a public health dilemma, especially in developed countries, and has steadily increased at an alarming rate in recent years. Morbid obesity increases the risk of hypertension, coronary artery disease, diabetes mellitus, cancer, sleep apnea, and osteoarthritis.1 Among various countermeasures, modifying the fat balance is a key therapy for obesity.2 Although obesity is usually thought to be simply the result of hyperphagia or energy intake/energy expenditure imbalance, there is evidence that obesity may occur without significant excess energy intake.3 Several studies in animal models of diet-induced obesity4–5 and in humans6,7 have demonstrated that increased levels of refined carbohydrates (e.g., sucrose) and/or saturated fat may lead to obesity in the absence of excessive energy intake. Furthermore, mechanisms underlying the development of obesity may include changes in skeletal muscle and adipose tissue enzymatic and/or receptor regulation (lipoprotein lipase, hormone-sensitive lipase, very low-density lipoprotein receptor) and/or hormonal regulation (i.e., insulin, growth hormone, catecholamine), resulting from physical inactivity and/or inappropriate macronutrient intake (i.e., high saturated fat and/or refined carbohydrates). Because obesity and its complications have become serious problems, numerous medicines and functional foods have been developed in many countries.

Dosiinpartner (DSP) is one functional food for weight loss. In the present study, we investigated the changes in fat accumulation, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and glucose levels in mice fed a 10% or 20% DSP diet and a high-fat (HF) diet. We also measured body weight to determine the effects of DSP on weight gain.

MATERIALS AND METHODS

Preparation of DSP DSP consists of 70% Hovenia dulcis stem, 25% Hovenia dulcis fructus, 2% Polyporus umbellatus sclerotium, 1.5% Zea mays stigma, and 1.5% Malva verticillata seed. H. dulcis stem was collected from 1–2-year-old H. dulcis (Gongju, Republic of Korea). H. dulcis fructus was collected from more than 10-year-old H. dulcis, which is from North Korea. P. umbellatus sclerotium, Z. mays stigma, and M. verticillata seed were purchased from the Oriental Drugstore, Daehak Oriental Pharmacy (Iksan, Republic of Korea), and authenticated by an Oriental pharmacist (Seung-Heon Hong). A voucher specimen was deposited at the Herbarium of the College of Pharmacy, Wonkwang University. An extract of DSP was prepared by decocting the dried combination of each herb with boiling distilled water. The duration of decoction was about 3 h. The decoction was filtered, lyophilized, and stored at 4 °C. Dilutions were made with saline, and then filtered through a 0.4-μm syringe filter.

Animal Experiments Male C57BL/6J mice weighing 19.4–19.8 g at the age of 4 weeks were purchased from the Dae-Han Experimental Animal Center (Eumsung, Republic of Korea). The animals were maintained under a 12-h light/dark cycle at a constant temperature of 23 ± 2 °C. Four groups of mice were fed for 8 weeks with 1) a standard laboratory diet (Samyang formula feed, Samyang Oil & Feed Co., Ltd.); 2) an HF diet (15% fat, 1.3% cholesterol, 0.5% Naccholate, CRF-1, Oriental Yeast Co., Ltd.); 3) the HF diet plus 10% DSP; and 4) the HF diet plus 20% DSP. Forty male C57BL/6J mice were divided into four groups of 10 mice each. The animals were given free access to food and tap water for 8 weeks. Body weight and food intake...
amount were recorded every week. At the end of this period, the animals were fasted overnight. The next day, they were anesthetized with ketamine and rompun 5:3 and then blood samples were collected by cardiac puncture.

**Cholesterol Analysis** Plasma was separated immediately after blood sampling by centrifugation at 10000×g for 10 min. Levels of total cholesterol, HDL cholesterol and LDL cholesterol were determined using the colorimetric enzymatic method of Allain et al., with the modifications of Badham and Trinder, using an autoanalyzer (Hitachi 747, Hitachi, Japan). Plasma concentrations of total cholesterol and HDL cholesterol were determined using automated enzymatic methods, and LDL cholesterol was calculated using the Friedewald et al. formula. The decrement ratio of cholesterol was calculated as follows:

\[
\text{decrement ratio of cholesterol} = \left( \frac{\text{A} - \text{C} \text{ or D}}{\text{A} - \text{B}} \right) \times 100
\]

where A is control, B is HF diet, C is 10% DSP diet, and D is 20% DSP diet.

**Glucose Assays** Glucose levels were determined using enzymatic colorimetric glucose oxidase assay kits commercially available from Roche (formerly Boehringer Mannheim). The basic principle is:

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glucose + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{glucose oxidase} \rightarrow \text{gluconate} + \text{H}_2\text{O}_2
\]

Subsequently, there is a reaction between \(\text{H}_2\text{O}_2\) and aminophenazone and phenol to produce a detectable color change. A 10-μl plasma sample was mixed with 2 ml of reagent and incubated for 30 min at room temperature (20—25 °C). The absorbance of the sample was read at a wavelength of 500 nm on a PC800 Colorimeter (Brinkmann, Westbury, NY, U.S.A.). If dilution was required, the sample was diluted 1:2 in 0.9% saline as per assay instructions.

The decrement ratio of glucose was calculated as follows:

\[
\text{decrement ratio of glucose} = \left( \frac{\text{A} - \text{C} \text{ or D}}{\text{A} - \text{B}} \right) \times 100
\]

where A is control, B is HF diet, C is 10% DSP diet, and D is 20% DSP diet.

**Liver Function Tests** Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assessed to evaluate hepatic dysfunction. Enzyme activities were measured by means of standard spectrophotometric methods, and the results are expressed in International Units per liter.

**Statistical Analysis** Results are expressed as the mean±S.E.M. of independent experiments, and statistical analysis was performed using one-way ANOVA to determine differences among groups.

**RESULTS**

**Effects of DSP on HF Diet-Induced Weight Increase** As shown in Fig. 1, mice fed the HF diet gained significantly more weight than those fed the standard diet \((p<0.05)\). On the other hand, weight increases in the 10% or 20% DSP group were significantly less than in the HF diet group \((p<0.05)\). The 20% DSP group showed less weight increase than the 10% DSP group (Fig. 1A). As shown in Fig. 1B, differences in food intake and weight were investigated among groups over time. The feed gain ratio was calculated by dividing the weight increase by food intake. Taking the standard diet group values as 100%, the HF group, 10% DSP group, and 20% DSP group had feed gain ratios of 154.5%, 108.2%, and 105%, respectively (Fig. 1C).

**Effects of DSP on Total Cholesterol Levels** The total cholesterol levels in the HF diet mice were compared with those in the standard diet group, while the levels in the DSP diet groups were compared with those in the HF diet group. In the mice fed the HF diet, total plasma cholesterol levels increased by 63.4% compared with the standard diet mice. In the HF diet plus 10% and 20% DSP groups, the HF-induced increase in total cholesterol level was decreased by 48.3% and 42.9%, respectively (Fig. 2), compared with the HF group.

**Effects of DSP on Plasma Lipid Levels** The lipid levels in the HF diet mice were compared with those in the standard diet group, while levels in the DSP groups were compared with those in the HF group. The HF diet increased the plasma LDL cholesterol level by 10-fold compared with
standard diet. In the HF plus 10% and 20% DSP groups, the HF-induced increase in LDL cholesterol level was decreased by 26.8% and 34.9%, respectively (Fig. 3). However, the HDL cholesterol levels were similar among groups (data not shown).

Effects of DSP on Glucose Levels As shown in Fig. 4, the plasma glucose levels in mice fed the HF diet were higher than in mice fed the standard diet. In the HF diet plus 10% DSP group, the HF-induced increase in glucose level was decreased by 80.6% compared with the HF group (Fig. 4). However, the glucose level in the 20% DSP group was almost the same as that in the HF diet group.

Effects of DSP on ALT and AST Levels The ALT and AST levels in the HF diet group were 42.5±0.5 and 72.7±3.8 IU/l, respectively. In the HF diet plus 10% and 20% DSP groups, ALT and AST levels were 41.0±6.4 and 74.3±4.5 and 42.0±2.0 and 75.8±3.8 IU/l, respectively. ALT and AST levels in the DSP groups were similar to those in the HF diet group. These results indicate that DSP did not cause liver injury.

DISCUSSION

We demonstrated that DSP inhibits weight increase in mice fed an HF diet. In addition, we found that DSP decreased total cholesterol, LDL cholesterol, and glucose levels. These results indicate that DSP is a good candidate for the treatment of HF diet-induced obesity and other complications such as circulatory disorders and diabetes mellitus.

DSP is composed of the five herbs H. dulcis stem, H. dulcis fructus, P. umbellatus sclerotium, Z. mays stigma, and M. verticillata seed. H. dulcis lowers plasma glucose and may be an effective antidiabetic herb. P. umbellatus sclerotium has been used for the treatment of edema and other water metabolism disorders in Oriental medicine. Z. mays stigma has an antidiabetic effect. M. verticillata seed has been reported to have hypoglycemic activity. This combination in DSP was based on the theory of traditional Korean medicine to maximize its efficacy. However, experiments using individual herbs or DSP with one or two herbs excluded should be performed in future studies.

Compelling evidence links overweight and obesity with serious disorders, in particular cardiovascular disease, diabetes mellitus, musculoskeletal problems, and cancer. In recent studies, overweight and obesity have been associated with the risk of death from all cancers and with death from many specific cancers. It is estimated that 90000 deaths due to cancer could be prevented each year in the U.S.A. if men and women could maintain normal weight. Overweight and obesity have become a major public health concern. Figures for the U.S.A., using the World Health Organization classification of obesity, indicate that more than one in five of the general adult population can be classified as obese. Obesity has become one of the most important avoidable risk factors for morbidity, with considerable costs directly attributable to this condition.

In this study, the ratio of weight increase in the DSP diet groups was lower than that in the HF diet group. As a result, we speculate that DSP can prevent the obesity induced by an HF diet. When we analyzed the results, the body weight in the 10% and 20% DSP diet groups in week 6 suddenly decreased. The reason is not clear, but the sudden decrease in body weight in week 6 may have been influenced by less food intake, because food intake in that week decreased sharply compared with other weeks. There are reports that a decline in food intake and decrease in body weight may be caused by metabolic changes that occur in animal experiments. Further study is needed to clarify the precise mechanism of this phenomenon.

In the relationship between weight loss and plasma lipid levels, weight loss was shown to reduce total cholesterol and LDL cholesterol and to increase HDL cholesterol at stabilized weight. However, when individuals were actively losing weight, HDL cholesterol levels decreased. The present study showed that DSP in an HF diet tends to reduce total
cholesterol and LDL cholesterol levels. HDL cholesterol levels in the DSP groups were similar to those in the HF diet group. We assume that when we evaluated the lipid levels, DSP-treated mice were actively losing weight, and thus the HDL cholesterol levels were unchanged. The level of LDL cholesterol in plasma is the major determinant of the risk of vascular disease, and lowering the level of LDL cholesterol diminishes that risk, both in those with and those without symptomatic vascular disease.24—26 Fully understanding the factors that govern the concentration of plasma LDL cholesterol is therefore one of our most important challenges. Reduced plasma LDL cholesterol levels have been recognized as a strong independent risk factor for atherosclerotic cardiovascular disease.27—29 Obesity and diabetes mellitus have a close relationship. There is convincing evidence that obesity leads to the development of type 2 diabetes mellitus.30 The metabolic syndrome (syndrome X or insulin-resistance syndrome) has been defined as a cluster of conditions including obesity and insulin resistance that are frequently seen together and which impart increased risk of the development of type 2 diabetes mellitus.31

Thus the vast majority of persons with type 2 diabetes mellitus are obese, and weight loss may be one of the best methods both to prevent and treat type 2 diabetes mellitus.32 DSP has glucose-reducing effects as well as weight loss effects. It will probably thus prevent diabetes as well as obesity.

In our study, total cholesterol and glucose levels in the DSP diet groups showed a decreasing tendency compared with the HF diet group, although the effects of DSP were not dose dependent or significant. Therefore further study is needed to clarify how DSP acts on lipids, cholesterol, and glucose.

In conclusion, the observed decrease in cholesterol, glucose, and weight due to DSP indicate that the product has potential medicinal value for obese patients.

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REFERENCES