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Dietary Supplementation of Sea Tangle (*Laminaria japonica*) Improves Blood Glucose and Lipid Metabolism in the Streptozotocin-induced Diabetic Rats

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Abstract The purpose of this study was to investigate the effect of dietary supplementation of sea tangle (*Laminaria japonica*) on the blood glucose and lipid metabolism in streptozotocin (STZ)-induced diabetic rats. Male Sprague-Dawley rats were divided into 3 groups fed control, sea tangle powder (15%, w/w), or sea tangle water extract (4%, w/w) diet. Diabetes was induced by a single injection of STZ (60 mg/kg, i.p.) in citrate buffer. The animals were fed each of the experimental diet for 13 weeks. Serum insulin was increased by dietary supplementation of sea tangle in diabetic rats. Dietary sea tangle reduced blood glucose level of diabetic rats compared to the diabetic rats fed control diet. Dietary sea tangle also reduced the serum total cholesterol, low density lipoprotein (LDL)-cholesterol, and triglyceride in the diabetic rats. While hepatic lipids were reduced, fecal excretion of lipids was increased by supplementation with dietary sea tangle in the diabetic rats. These results indicate that dietary sea tangle decreased blood glucose and improved lipid metabolism in STZ-induced diabetic rats and this effect might be exerted by increases in serum insulin and fecal excretion of lipids.

Keywords: diabetes, sea tangle, blood glucose, serum insulin, lipid metabolism

Introduction

Diabetes mellitus is a major cause of morbidity in developed countries. It is a metabolic disorder caused by an absolute or relative lack of insulin. The metabolism of all fuels including carbohydrate, fat, and protein is altered in diabetes and patients have lipoprotein disorders and the increased risk of diabetic vascular complications (1-3). Hyperglycemia, hyperglycouria, hyperlipidemia, and glycation/glycoxidation are among the characteristics of Type I and Type II diabetes (4,5). Other than insulin injection, increased intake of dietary fibers or plants, which have phytochemicals, has been demonstrated to lower hyperglycemia and glycouria (5). Controlling blood glucose level is the most important approach in the management of complications of diabetes and poor glycemic control is the most important risk factor for the development and progression of diabetic complication (6). The influence of diabetes mellitus on lipid metabolism is well established. The association of hyperglycemia and alteration of lipid parameters presents a major risk of cardiovascular disease in diabetic patients. Abnormalities in lipid metabolism are also known to contribute to the pathogenesis of coronary heart disease and progressive renal disease (6). The incidence of cardiovascular mortality in diabetic subjects without a clinical history of previous cardiac events is as high as the incidence in nondiabetic subjects with a history of myocardial infarction (7).

Seaweeds have been consumed in Asia since ancient times (8,9). Sea tangle (*Laminaria japonica*), a kind of seaweeds mainly consumed in Korea and Japan, is a rich source of dietary fibers, minerals, carotenoids, xanthophylls, and proteins (10,11). Dietary fibers of seaweeds, which are little or non-digested by human (9,11), are particularly rich in the soluble fractions such as alginates, fucans, and laminarans. Especially fucans and alginic acid derivatives are known to exhibit different biological properties: anticoagulant, anti-inflammatory, antiviral, or anti-tumoral activities (11-15).

The present study was done to extend the current information on the hypoglycemic and hypolipidemic effects of sea tangle in diabetic rats. We found the alteration of blood glucose level and lipid metabolism, and elucidated the mechanisms of the hypolipidemic effect in the diabetic rats supplemented with dietary sea tangle.

Materials and Methods

Animals Male Sprague-Dawley rats (180-230 g) were purchased from Jung-Ang Lab. Animal Inc. (Korea- SLC, Inc., Seoul, Korea). The animals were housed in the cages under standard conditions (12:12-hr light/dark cycle, 50% relative humidity at 21°C) and given free access to a semi-purified diet (Table 1) and water. After 7 day acclimation, experimental diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) (Sigma-Aldrich, St. Louis, MO, USA) in citrate buffer at a dose of 60 mg/kg body weight. Fasting blood sample was obtained from the tail vein at 72 hr after STZ injection and blood glucose was determined. Rats with fasting blood glucose levels above 250 mg/dL were used as the diabetic animals. Three groups of diabetic rats were maintained on each experimental diet *ad libitum* for 13 weeks. The sea tangle-supplemented diet was made of AIN-76 based control diets with either sea tangle powder (15%, w/w) or water extract of sea tangle (4%, w/w) (Table 1) (16). Food and water consumptions

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Table 1. Composition of experimental diet

Component	Control diet	Sea tangle powder diet	Sea tangle water extract powder diet
Casein	20.0	18.5	20.0
DL-Methionine	0.3	0.3	0.3
Corn starch	54.7	47.2	50.7
Corn oil	15.0	14.8	15.0
α-Cellulose	5.0	0.0	5.0
Sea tangle powder[1)]	0.0	15.0	0.0
Sea tangle water extract	0.0	0.0	4.0
Mineral mix[2)]	4.0	3.7	4.0
Vitamin mix[3)]	1.0	1.0	1.0
Total (%)	100.0	100.5	100.0

[1)]Composition of sea tangle powder (g/15 g of sea tangle powder): Crude protein, 1.5; carbohydrate, 7.5; crude fat, 0.2; NaCl, 0.3.
[2)]AIN 76 Mineral mixture. Nutritional Biochemicals, ICN Life Science Group, Cleveland, OH, USA. Composition of mineral mixture, g/kg mixture: Calsium phospate didasic 500.00 g, sodium chloride 74.00 g, potassium citrate monohydrate 220.00 g, potassium sulfate 52.00 g, magnesium oxide 24.00 g, manganous carbonate (43-48% Mn) 3.50 g, ferric citrate (16-17% Fe) 6.00 g, zinc carbonate (70% ZnO) 1.06 g, cupric carbonate (53-55% Cu) 0.30 g, potassium iodate 0.01 g, sodium selenite 0.01 g, chromium sulfate 0.55 g, sucrose, finely powdered 118.0 g.
[3)]Nutritional Biochemicals, ICN Life Science Group. Vitamin mixture is composed of: vit. A acetate (500,000 IU/g) 1.8 g, vit. D conc. (850,000 IU/g) 0.125 g, α-tocopherol (250 IU/g) 22.0 g, ascorbic acid 45.0 g, inositol 5.9 g, choline chloride 75.0 g, menadione 2.25 g, P-aminobenzoic acid 5.0 g, niacin 4.25 g, riboflavin 1.0 g, pyridoxine hydrochloride 1.0 g, calcium pantothenic acid 3.0 g, biotin 0.02 g, folic acid 0.09 g, vit. B_{12} 0.00135 g, and extrose to 1 kg.

were measured every day and weight gain was measured every week. At the end of 13 weeks, the animals were decapitated after overnight fasting, and serum was prepared. Liver was quickly excised, weighed, and then stored at –70°C until analyzed. Feces were collected for the last 3 days of the experiment. Collected samples were stored at –70°C for analysis.

Serum insulin Serum insulin was measured by commercially available rat insulin kit (Rat insulin ELISA kit; Shibayagi, Co., Shibukawa, Japan), which is for quantitation of insulin by sandwich technique of enzyme immunoassay (EIA).

Blood glucose Blood glucose was measured from the blood of all the animals, taken from the tail vein using kit (Medisense Optimum, Abingdon, UK) every week.

Serum, hepatic and fecal lipids Serum, hepatic and fecal total cholesterol and triglyceride concentrations, and serum high density lipoprotein (HDL)-cholesterol were determined using a commercial kit (Asan Pharm. Co., Hwaseong, Korea) at the end of the experiment. Serum low density lipoprotein (LDL)-cholesterol was calculated from the equation (17). Liver was homogenized and lipid was extracted with chloroform/methanol (2/1, v/v) according to the method of Folch *et al.* (18). Dried lipid residue was dissolved in 1 mL of isopropyl alcohol for cholesterol and triglyceride assay. The lipid of feces was extracted using the procedure of Folch *et al.* (18).

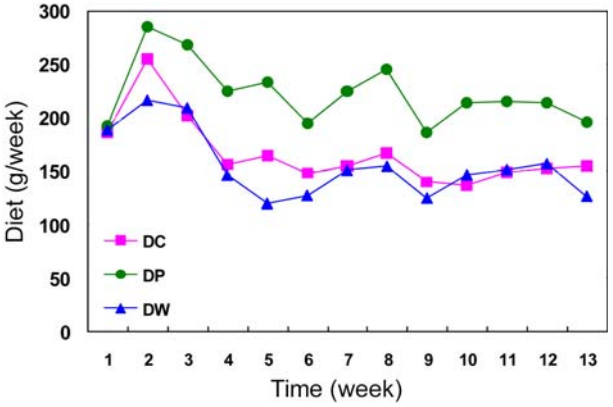

Fig. 1. Effect of dietary sea tangle on the food intake in diabetic rats. DC, diabetic rat+control diet; DP, diabetic rat+sea tangle powder diet; DW, diabetic rat+sea tangle water extract diet.

Statistical analyses All data are expressed as the mean± standard error (SE). The statistical analyses were performed on an SPSS program (Version SPSS/PC 11.5, Chicago, IL, USA). The group comparisons were done using a variance analysis followed by Duncan's multiple-range test. Statistical significance was considered at $p<0.05$.

Results and Discussion

Body weight, food intake, and water intake Diabetic rats fed the control diet and supplemented with water extract of sea tangle showed no significant difference in food intake. Food intake of diabetic rats supplemented with sea tangle powder (DP) tended to be higher than that of other groups (Fig. 1). Dietary sea tangle did not show any appreciable influence on water intake and body weight by the diabetic animals (data are not shown).

Serum insulin and blood glucose Diabetes is a chronic metabolic syndrome (19). So, we have performed this study for 13 weeks and it is relatively long period compared to other studies concerned with the effect of dietary supplementation on the metabolism of diabetic rats. The result of present study showed that sea tangle, both of powder and water extract, improved diabetic status in terms of fasting serum insulin and blood glucose. Fasting blood glucose in diabetic rats became lower when the rats had been fed sea tangle for 3 weeks and was significantly lower than that of the diabetic rats fed the control diet at the end of experiment (Fig. 2). Streptozotocin causes massive reduction in insulin release, through the selective destruction of β-cells of the islets of Langerhans, probably by a free radical-mediated mechanism which is responsible for high blood glucose seen in STZ-induced diabetic animals (20,21). In the present study, we have observed significant increases in the fasting insulin level, when STZ-induced diabetic rats were supplemented with sea tangle (Fig. 3). This could be due to potentiation of the insulin effect by increasing the pancreatic secretion of insulin from existing β-cells or its release from bound insulin. We can also consider the possibility that sea tangle protects the functional β-cells from further deterioration so that more β-

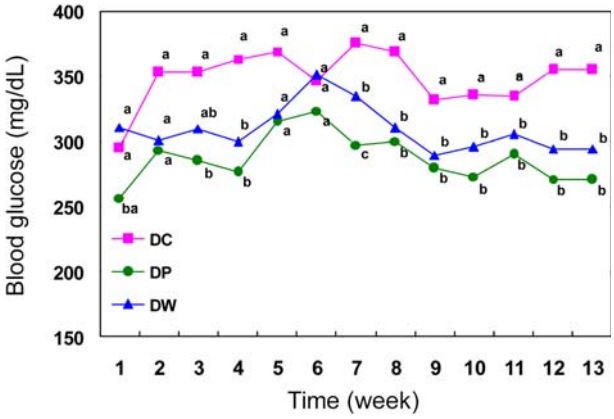

Fig. 2. Effect of dietary sea tangle on the fasting blood glucose in diabetic rats. DC, diabetic rat+control diet; DP, diabetic rat+sea tangle powder diet; DW, diabetic rat+sea tangle water extract diet. Means with different letters are significantly different by Duncan's multiple-range test ($p<0.05$).

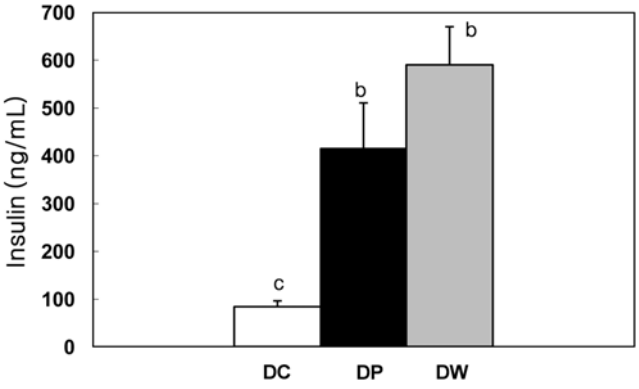

Fig. 3. Effect of dietary sea tangle on the insulin in diabetic rats. DC, diabetic rat+control diet; DP, diabetic rat+sea tangle powder diet; DW, diabetic rat+sea tangle water extract diet. Means with different letters are significantly different by Duncan's multiple- range test ($p<0.05$).

cells remain active and produce insulin. Medical plant such as *Azardirachta indica* (22), *Syzigium cumini* (23), *Trigonella feonum graecum* (24), *Tribulus terrestris*, and *Curcuma longa* (25) were also reported to have stimulatory effects on insulin release. Diabetes mellitus has distinct pathogenesis but hyperglycemia and various life-threatening complications resulting from long-term hyperglycemia are the most common features (21). In the current study, supplementation of dietary sea tangle reduced blood glucose in diabetic rats (Fig. 2). The hypoglycemic effect of sea tangle may be caused by the increased serum insulin and the enhancement of peripheral metabolism of glucose (26).

Serum, hepatic and fecal lipids Serum total cholesterol, LDL-cholesterol, and triglyceride were lowered by dietary sea tangle in diabetic rats. Especially water extracts of sea tangle significantly decreased serum total and LDL-cholesterol. Serum triglyceride tended to be decreased by water extracts of sea tangle. Dietary sea tangle did not

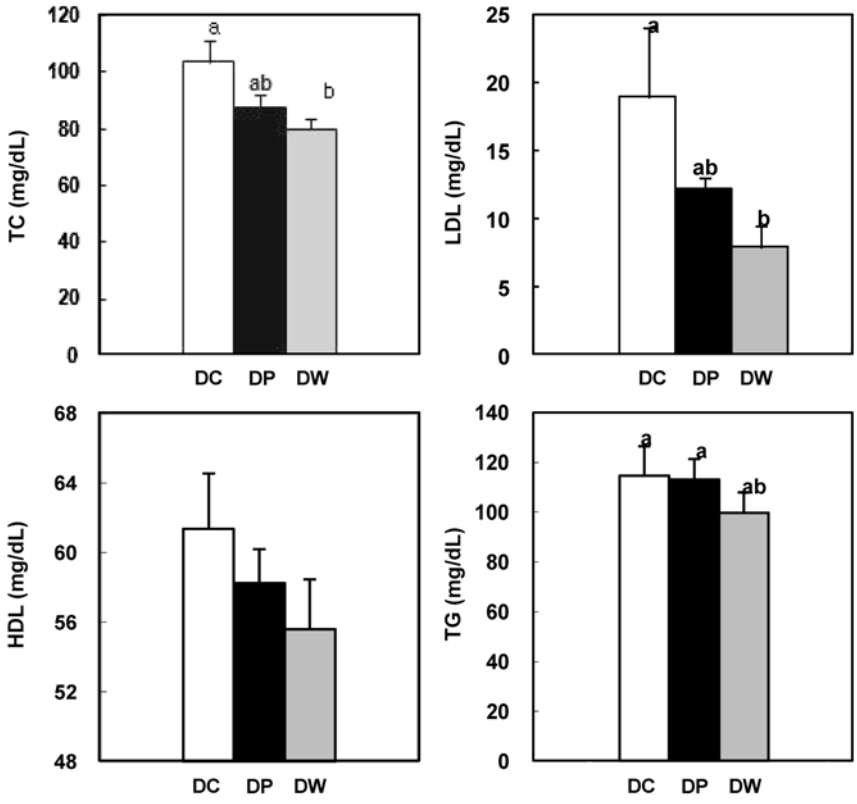

Fig. 4. Effect of dietary sea tangle on the serum lipids in diabetic rats. DC, diabetic rat+control diet; DP, diabetic rat+sea tangle powder diet; DW, diabetic rat+sea tangle water extract diet. Means with different letters are significantly different by Duncan's multiple-range test ($p<0.05$).

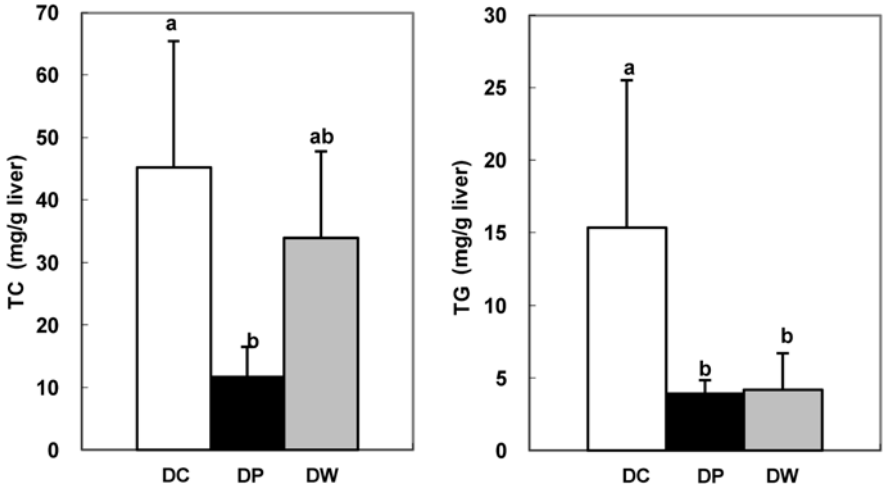

Fig. 5. Effect of dietary supplementation of sea tangle on the hepatic concentrations of cholesterol (TC) and triglyceride (TG) in the STZ-induced diabetic rats. DC, diabetic rat+control diet; DP, diabetic rat+sea tangle powder diet; DW, diabetic rat+sea tangle water extract diet. Means with different letters are significantly different by Duncan's multiple-range test ($p<0.05$).

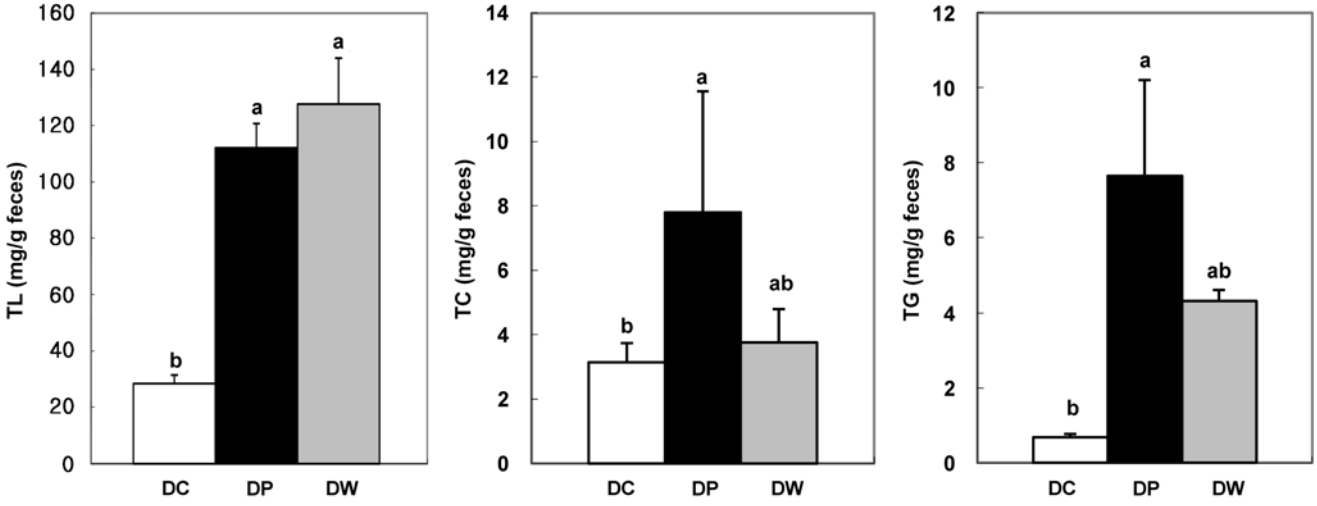

Fig. 6. Effect of dietary supplementation of sea tangle on fecal excretion of cholesterol (TC), triglyceride (TG) and total lipid (TL) in normal and STZ-induced diabetic rats. Experimental conditions are same as Fig. 1. Means with different letters are significantly different by Duncan's multiple-range test ($p<0.05$).

affect serum HDL-cholesterol in diabetic rats (Fig. 4).

Hepatic concentration of triglyceride was significantly decreased in the rats supplemented with sea tangle powder and water extract as compared with the rats fed control diet. Hepatic concentration of total cholesterol showed the similar tendency to the triglyceride level but was not significantly different among the groups (Fig. 5).

Sea tangle increased fecal excretion of cholesterol and triglycerides in diabetic rats. Fecal excretion of total lipid was also increased significantly in diabetic rats by supplementation with sea tangle (Fig 6). In the present study, dietary sea tangle reduced serum total cholesterol, LDL-cholesterol, and triglyceride in diabetic rats. The possible underlying mechanisms by which sea tangle can exert its lipid lowering activities have not been totally elucidated. At this stage of the study, we can propose several fundamental mechanisms to explain our results.

First, STZ rats show an important lipolytic activity, due to the insulinopenic state, which contributes to maintain the abnormally elevated plasma triglyceride and cholesterol levels (20). In this study, increased insulin by dietary sea tangle contributed to the decreases in the serum lipids in diabetic rats. It is also well known that level of glycemic control is the major determinant of plasma VLDL and triglycerides (20). The lowering effect of sea tangle on serum lipid in diabetic animals might be associated with a good glucose control result from increased insulin.

Second, conversion of cholesterol to bile acids is the major pathway of cholesterol elimination and it accounts for about 50% of daily cholesterol excretion (21). It is reported that soluble fiber increases bile acid synthesis and secretion (27). In this study, we observed increased fecal excretion of cholesterol and triglyceride in the sea tangle supplemented diabetic rats (Fig 6). With increased cholesterol entry into the bile acid synthetic pathway, less cholesterol enters the lipoprotein synthetic pathway and less VLDL is available for secretion into the circulation (28). This could be one of the mechanisms by which dietary

sea tangle decreased serum lipid levels, since VLDL-cholesterol is a major reservoir of serum triglyceride and a precursor of LDL-cholesterol (16). Increased lipid excretion by sea tangle could be also caused by a reduction of intestinal cholesterol absorption. A reduction in intestinal cholesterol absorption prevents the accumulation of cholesterol in the liver (29). In the present study, we also observed decreased hepatic cholesterol and triglyceride by dietary sea tangle (Fig. 5). Because the expression of LDL receptor is controlled by feedback inhibition of intracellular cholesterol (30), reductions in hepatic cholesterol accumulation in turn stimulate the production of more high affinity LDL receptors. This results in an increase in clearance of cholesterol from the circulation by LDL receptor and thus lowers blood cholesterol (6).

From our results, we can hypothesize that sea tangle supplementation increased serum insulin and improved blood glucose and lipid metabolism. Dietary sea tangle might increase bile acid excretion and reduced cholesterol concentrations because plasma or liver cholesterol would be used to maintain the bile acid pool. We can also consider the possibility the dietary sea tangle might prevent lipid absorption, thereby correct the elevated serum lipid in diabetic rats. Our results suggest that dietary supplementation of sea tangle may be beneficial for correcting hyperglycemia and hyperlipidemia, and preventing diabetic complications.

Acknowledgments

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