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Effect of *Undaria pinnatifida* Extract on Insulin Secretion from the Pancreas of Diabetic Rats

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We found previously that *Undaria pinnatifida* extract has an effect of lowering blood glucose levels in diabetic rats. Therefore, an effect of *Undaria pinnatifida* extract on the insulin secretion directly from the pancreas was examined in this study. Neonatal diabetes were induced by intraperitoneal injection of Streptozotocin (100 mg/kg body weight) at age of day 1. Rats were fed a rodent pellet diet until they were grown to adults (age of 7 weeks). Rats having a fasting serum glucose level over 250 mg/dL were used in this feeding study and they were divided into two diet groups as follows; a diet with *Undaria pinnatifida* extract (5%) and a diet without this extract (control group). Fasting (12 hr) blood glucose and serum insulin levels were measured before and after feeding a diet with *Undaria pinnatifida* extract for 4 weeks. At the last day of feeding, *in vitro* pancreas perfusion was performed. Pancreas was stimulated with a perfusate without glucose during a period of 0~10 minutes and with a perfusate containing 200 mg/dL glucose during a period of 11~40 minutes. Insulin amount was measured using a radioimmuno assay. In results, amount of the insulin secreted from the pancreas in the diabetic rats fed *Undaria pinnatifida* extract was significantly greater than that in the diabetic control group during the periods of the equilibration period (0~10 min) and the first phase (11~20 min) of the insulin secretion ($P<0.05$). It is concluded that *Undaria pinnatifida* extract increases insulin secretion from the pancreas in the neonatal diabetic rats. Therefore, the blood glucose lowering effect of the *Undaria pinnatifida* extract may be elucidated by mechanisms with promoted insulin secretion from the pancreas in diabetic rats.

Key Words: *Undaria pinnatifida*, Alginate, Diabetes mellitus, Insulin, Pancreas perfusion

INTRODUCTION

Diabetes mellitus is chronic burdensome disease involved serious defects in the glucose metabolism. There is considerable evidence on the beneficial effects of seaweeds; lowering blood glucose in humans with diabetes (Torsdottir et al., 1991) and in rats (Kimura et al., 1996). One of the popular seaweeds consumed favorably by Korean is *Undaria pinnatifida*. This seaweed contains a great amount of dietary fiber, and the major fiber is alginate (about 70~80%) that is abundant in the cell walls (Lee et al., 1998). Alginate is linear unbranched polymers containing β-(1→4)-linked D-mannuronic acid and α-(1→4)-linked L-guluronic acid residues. In the food industry, alginate is used extensively as a texture modifier duo to its viscous property. As alginate is a soluble dietary fiber, it has a similar physiological effect of dietary fibers such as lowering rate of small intestinal absorption of metabolizable nutrients and inhibiting activities of proteases and amylase, thereby reducing the glycemic load (Brownlee et al., 2005). Indeed, alginate supplement (5 gram, 75% soluble fiber) caused a reduction in postprandial rises in blood glucose (31%) in humans with diabetes, and this attenuated glycemic response was explained by a slow intestinal absorption as well as gastric emptying (Torsdottir et al., 1991). Dietary fiber's effect like this on the digestive system has been well known. However its role on the endocrine system, such as insulin

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secretion from the pancreas, has not yet studied. The purpose of the study is to investigate effects of *Undaria pinnatifida* extract, of which major component is alginate, on insulin secretion from the pancreas in order to explain an effect of lowering blood glucose levels.

MATERIALS AND METHODS

Animals and diets

Female pregnant Sprague-Dawley rats were obtained from Animals Co. (Hyo-Chang Science Co. Ltd, Korea) and resided them in the individual plastic cage in order to environmental acclimation for 7 days. Animals were maintained under the condition controlled with a 12 hour light-12 hour dark cycle (light in at 06:00 and light out at 18:00), temperature of 23±2℃, and humidity of 50±5%. The commercial rodent pellet diet and water were fed *ad libitum* until they delivered and weaned pups. At age of day 1 of the pups, they were injected Streptozotocin (i.p. inj., 100 mg/kg body weight, Sigma Chemical Co., St. Louis, MO, U.S.A.) dissolved freshly in 0.01 M citrate buffer (pH 4.5). Experimental procedure was approved by Inje University Animal Care and Use Committee (IUACUC), and was met all the guidelines in the Care and Use of Animals.

When pups were grown up to age of 7 weeks, male rats (n=9) were fasted for 12 hours for measuring blood glucose levels. Only rats showing above 250 mg/dL of 12-hr fasting blood glucose level were used for the diabetic groups. The diabetic rats were divided into two diet groups as follows; a diet with 5% alginate extracted from the *Undaria pinnatifida* (ALG group, n=5), and a diet without alginate for the control group (CON group, n=4). Initial body weights were measured at week 0 and final body weight were measured at the end of feeding period of 4 weeks.

Fasting blood glucose and insulin levels

After rats were fasted for overnight 12 hrs, blood samples were collected at week 0 for the initial levels and week 4 for the final levels. Serums were separated and frozen at -20℃ for s-insulin assays. Blood glucose levels were measured by Accu-Check (Roche Diagnostics GmbH, Mannheim, Germany). Serum insulin levels were determined by the rat insulin radioimmunoassay kit (Linco Research, Inc. Saint Charles, U.S.A). Radioisotope detection was carried out using a Cobra auto gamma counting system (Hewlett Packard Instrument Co, U.S.A.). The cpms were counted from triplicated standard tubes and duplicated sample tubes, and the count of the nonspecific binding was subtracted. The bound/free triplicates for each point on the standard curve were averaged and plotted *vs* insulin amounts. For each sample duplicate, the bound/free was read off as insulin amounts (ng/mL) from the standard curve.

Pancreatic insulin amounts and insulin secretion rates

At the last day of feeding, *in vitro* pancreas perfusion was performed for insulin secretion dynamics. Briefly, the components of the perfusate were as follows: 0.18% albumin, 4.00% of dextran, 0.110 M of $CaCl_2$, 0.154 M of KCl, KH_2PO_4, $NaHCO_3$, NaCl, and $MgSO_4$. Glucose (200 mg/dl), a primary stimulator of insulin secretion, was added using an infusion pump during the 10~40 min of the total perfusion period. The perfusate was continually oxygenated with a 95% O_2~5% CO_2 mixture and maintained at 37℃ using an external heating source. Perfusate inflow rate was maintained at 5 ml/min. Pancreas was removed from the rats and perfused using a perfusion apparatus. The perfusate inflow began *via* the heparinized celiac arterial cannula. Total outflow was collected *via* a heparinized portal vein cannula, and its volume was measured. The total venous effluent was collected for 40 min with 2~5 min time intervals. The effluent samples were kept on ice until centrifuged (20 min at 4℃, 3000 rpm). The supernatant was decanted and frozen at -20℃ until insulin amounts were assayed. Insulin amounts in the perfusion effluent samples were determined by the rat insulin radioimmunoassay kit (Linco Research, Inc. Saint Charles, U.S.A.). Insulin secretion rates (ng/min) were calculated by using the measured perfusate effluent flow rate (ml/min) and the assayed insulin concentrations (ng/ml).

Statistical analysis

All data were expressed as means ± SE. Student's *t*-test and paired *t*-test were conducted to determine a statistical

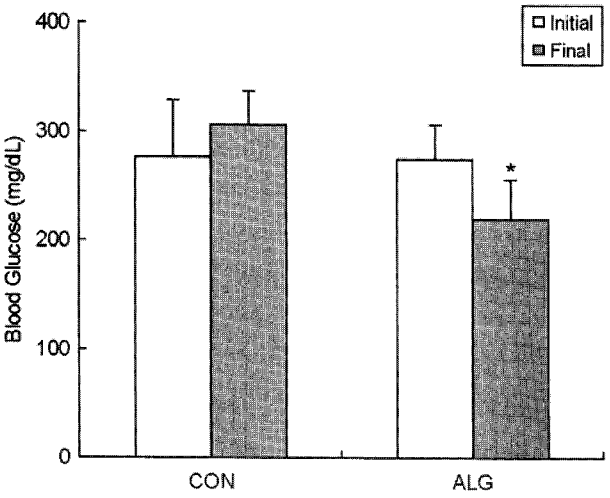

Fig. 1. Initial and final blood glucose levels of diabetic rats during the feeding period of 4 weeks
Data are expressed as the mean ± SE.
CON: rats fed a diet without alginate from *Undaria pinnatifida*
ALG: rats fed a diet with 5% alginate from *Undaria pinnatifida*
* Significant difference at $P<0.05$ using a paired *t*-test compared to the initial value in ALG group

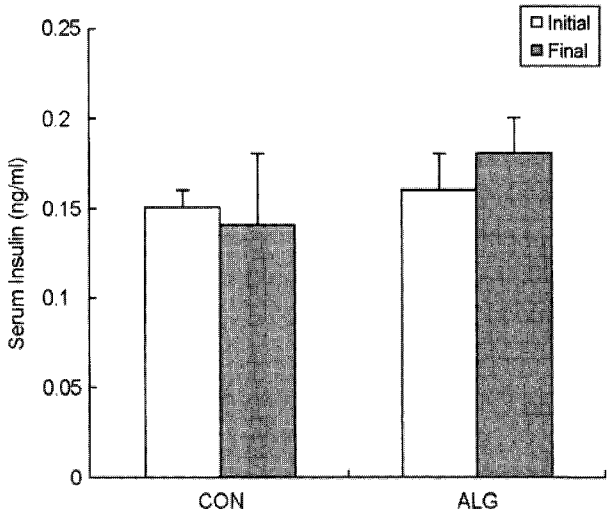

Fig. 2. Initial and final serum insulin levels of diabetic rats during the feeding period of 4 weeks
Data are expressed as the mean ± SE.
CON: rats fed a diet without alginate from *Undaria pinnatifida*
ALG: rats fed a diet with 5% alginate from *Undaria pinnatifida*
No significant differences between the CON and ALG groups

significance in difference at $P<0.05$ among mean values of groups.

RESULTS

Body weights, fasting blood glucose and serum insulin levels

Initial body weight was 298.0±7.7 g in the CON and 340.0±19.0 g in ALG group. And final body weight was 323.5±9.2 g in the CON and 354.0±17.7 g in ALG group. Initial and final body weights in ALG group seemed to be greater than those in CON group, but there were no statistical significances between two groups. The initial fasting blood glucose levels were similar between CON (276.0±52.0 mg/dL) and ALG (306.0±31.8 mg/dL) groups. However, the final blood glucose levels were significantly different as 274.0±34.0 mg/dL in CON group and 219.8± 35.4 mg/dL in ALG group ($P<0.05$). Therefore, blood glucose levels were increased in CON group after 4 weeks of feeding period, while those levels were decreased in ALG group. The initial fasting serum insulin levels did not differ significantly between the CON and ALG groups. The final fasting serum insulin levels seemed to be greater in ALG group than in CON group, but there was no significant difference between these two groups.

Amount of pancreatic insulin secretion and its dynamics

Amount of insulin secretion from the pancreas during the first 10 minutes of the equilibration period in the ALG group was greater compared to the CON group (31.3±7.4 ng, 9.8±2.7 ng respectively, $P<0.05$). During the next 10 minutes of the glucose stimulation period, amount of insulin secretion in the ALG group was also greater compared to the CON group (69.4±3.7 ng, 32.8±6.5 ng, respectively, $P<0.05$). However, amounts of pancreatic insulin did not differ during the period of 21~40 minutes between the CON and ALG groups.

Dynamic of insulin secretion was shown in Fig. 5. The insulin secretion rate in the diabetic ALG group was greater at 5, 14, 16, 18 and 20 min compared those corresponding levels in the control group ($P<0.05$). Secretion curve of ALG group clearly showed a typical biphasic secretory pattern. However, the first peak and first phase were lessened in CON group. These results indicate that the dynamics of insulin secretion is recovered by a feeding *Undaria pinnatifida*, which may explain an effect of lowering blood glucose level in diabetic rats.

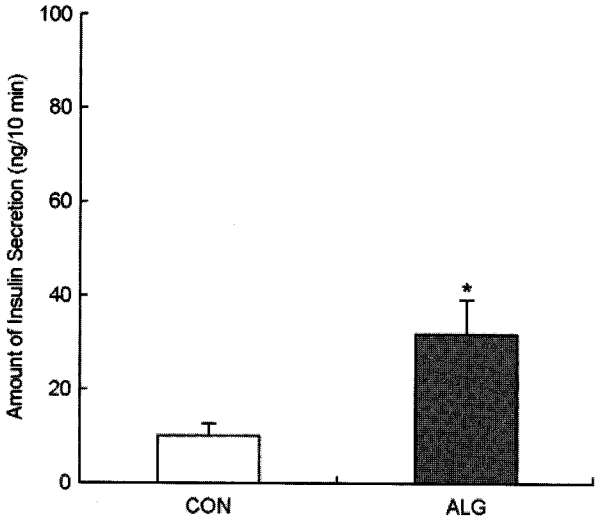

Fig. 3. Insulin amounts secreted from the pancreas during the perfusion period of 0~10 min in the diabetic rats
Data are expressed as the mean ± SE.
CON: rats fed a diet without alginate from *Undaria pinnatifida* for 4 weeks
ALG: rats fed a diet with 5% alginate from *Undaria pinnatifida* for 4 weeks
0~10 min: period of perfusion with no glucose in the perfusate
*: Significant difference at $P<0.05$ using a Student's *t*-test compared to the CON

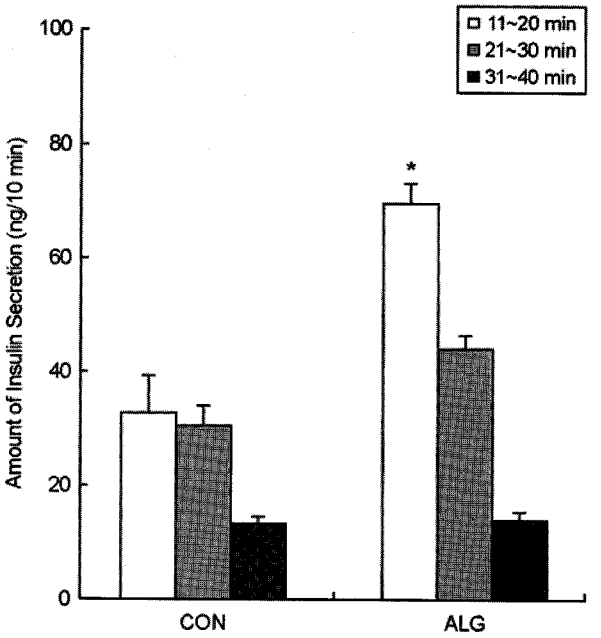

Fig. 4. Insulin amounts secreted from the pancreas during the 11~40 min of perfusion period in the diabetic rats
Data are expressed as the mean ± SE.
CON: rats fed a diet without alginate from *Undaria pinnatifida* for 4 weeks
ALG: rats fed a diet with 5% alginate from *Undaria pinnatifida* for 4 weeks
11~40 min: period of perfusion with 200 mg/dL glucose in the perfusate
*: Significant difference at $P<0.05$ using a Student's *t*-test

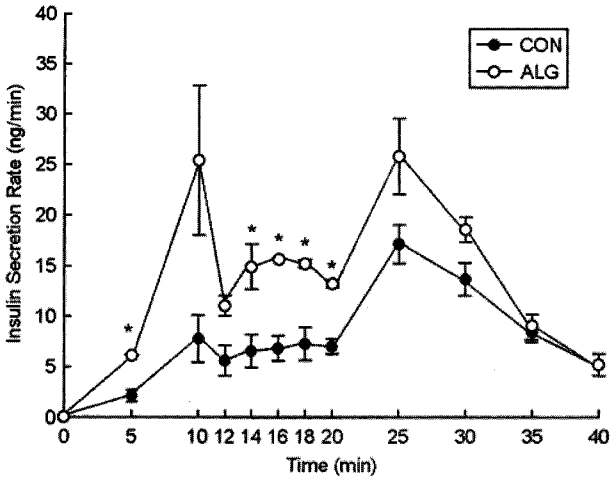

Fig. 5. Dynamics of insulin secreted from the pancreas during a total 40 min-perfusion period in diabetic rats
Data are expressed as the mean ± SE.
CON: rats fed a diet without alginate from *Undaria pinnatifida* for 4 weeks
ALG: rats fed a diet with 5% alginate from *Undaria pinnatifida* for 4 weeks
*: Significant different from the value of the CON group at $P<0.05$ using a Student's *t*-test
0~10 min: period of perfusion with no glucose in the perfusate
11~40 min: period of perfusion with 200 mg/dL glucose in the perfusate

DISCUSSION

Control of blood glucose level is crucial in the treatment of diabetes mellitus among other symptoms and complications. Recently, natural functional foods consumed by the diabetes in Korea have been searched in a study (Park and Wang, 2008) of 192 diabetic patients. They reported that 83.9% of the diabetes had been treated with oral hypoglycemic medications and 70.8% had consumed functional foods. The most frequently ingested food item was silkworm, and next ones are ginseng, black bean, pine tree leaves in order (Kim et al., 2000). Polysaccharide from the edible plants has been reported as anti-diabetic agents (Park et al., 2007; Kim et al., 2008). Abnormal glucose control is mainly due to deficiency in insulin secretion from the pancreas in the type 1 diabetes. In other experiment, we found that pancreas of rats fed a diet with alginate extracted from *Undaria pinnatifida* for 4 weeks had greater amounts of pancreatic insulin than the pancreas of rats fed a diet without this extract (data not shown). In present study,

insulin amounts secreted from the pancreas during the first 0~10 min were significantly greater in the diabetic ALG group compared with the CON group. This first 10 min was a period of pancreas stimulated without glucose in the perfusate, and insulin secretion in this period was from the stored pool in the secretory vesicles of the β cells (Curry and Maclachlan, 1987). The stimulation-secretion coupling in the pancreatic β-cells is affected by glucose (Miller, 1981; Curry, 1986; Rossetti et al., 1990; Yki-Jarvenen, 1992). In our results, amount of the insulin secreted from the pancreas during the first secretory phase was greater as 3.2 times in the ALG group compared with that in the control group ($P<0.05$). Moreover, the amount of insulin secreted from the pancreas during the 11~20 min of the second secretory phase was also greater as 2.1 times in the ALG group compared with that in the control group ($P<0.05$). Even though absolute amount of insulin in the diabetic ALG group is not sufficient to cure diabetes, these data suggest that intakes of ALG might improve pancreatic β cells response to the high levels of glucose challenge. Our study could not explain how ALG affects β cells' insulin secretion activity in the pancreas. Possible explanation is that ALG might protect some pancreatic β-cells from further deterioration or even enhance the remaining β cell function. Other explanation could be that insulin receptor substrate-2 mediates replication and survival of the β cells (Hennige et al., 2003; Park et al., 2006), therefore β cell failure shown in the diabetes could be restored by more expression of insulin receptor substrate-2. While this study has implications in terms of reducing blood glucose levels and increasing insulin secretion, studies on the long-term effects of ALG may be worthy to perform in the future.

It has been reported that disaccharides digestive enzyme activities in small intestine were altered in diabetic rats (Yoo et al., 2002; Jeong et al., 2005). Activities of maltase and sucrase in diabetic mice were as double as those in normal mice, and maltase and sucrase activities in the jejunum was correlated with plasma glucose ($r=0.643$, $P<0.01$; 0.622, $P<0.01$) (Jeong et al., 2005). Therefore, digestive system may try to adjust and compensate to the low glucose availability in the tissues. On the other hand, feeding a diet with 10% sodium alginate to normal rats for 5 weeks did not affect pancreatic amylase activity in the pancreas tissue (Song et al., 1996). In this present study, we evaluated dynamics of insulin secretion using *in vitro* pancreas perfusion, therefore any influence of the digestive alteration *in vivo* was definitely excluded. In conclusions, our results showed that an increase in the insulin secretion directly from the pancreas may partially explain the beneficial effect of alginate extracted from *Undaria pinnatifida* on lowering the blood glucose in the diabetes mellitus.

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