# Effect of Sodium Selenite Supplementation on Glucose Intolerance and Pancreatic Oxidative Stress in Type 2 Diabetic Mice under Different Selenium Status

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The objective of this study was to investigate the effect of various selenium (Se) status on glucose intolerance and pancreatic oxidative stress or the defense systems in Nagoya-Shibata-Yasuda (NSY) mice as the animal model for type 2 diabetes mellitus. To let the mice become Se-insufficient to Se-sufficient conditions, the NSY mice were given normal or Se-deficient diet with 0–7.0 mg/l $Na_2SeO_3$-containing drinking water for 6, 8 or 12 weeks. In NSY mice ingested normal diet, levels of blood glucose and plasma insulin after intraperitoneal glucose tolerance test (IPGTT) were not significantly affected by $Na_2SeO_3$-supplementation. In Se-deficient diet-treatment groups, however, the supplementation resulted in the decrease of blood glucose and the increase of plasma insulin after IPGTT. Although glutathione peroxidase (GPX) 1 activity in pancreas of the NSY mice ingested Se-deficient diet was augmented by $Na_2SeO_3$-supplementation, pancreatic glutathione was depressed by the supplementation, accompanying by the increase of 2-thiobarbituric acid-reactive substances. These results indicated that although the supplemented $Na_2SeO_3$ may not protect against oxidative stress in the pancreas of NSY mice under Se-insufficient condition, the Se compound improved glucose intolerance of the mice.

**Key words** —— sodium selenite, glucose tolerance, Nagoya-Shibata-Yasuda mice, type 2 diabetes mellitus, pancreas, glutathione peroxidase 1

## INTRODUCTION

Human type 2 diabetes mellitus is defined as a group of metabolic diseases developed by defects of insulin secretion from pancreatic β-cells and insulin action in the target tissues such as liver, muscle and adipose.[1–3] The development of this disorder is related to congenital factors such as the genetic trait of a host.[4] Host environmental factors such as obesity, overeating and the lack of exercise also contribute to the development of this disease.[5] Such deterioration of life-style also has been linked with oxidative stress in pancreas.[6] The resulting enhancement of reactive oxygen species (ROS) production, not only induces dysfunction of pancreatic β-cells, but also inhibits insulin signal transduction and then declines insulin action including the increase in glucose uptake and glycogen synthesis in liver.[7,8] However, the protection against oxidative stress occurring in the β-cells and insulin target tissues may be effective for prevention and treatment of type 2 diabetes mellitus.

Selenium (Se) is an essential trace element for mammals and many other forms of life and has been shown to regulate many intracellular functions by existing as a chemical component of selenoproteins.[9] The well-known selenoproteins are Se-dependent enzymes such as glutathione peroxidase (GPX) family (GPX1, GPX2, GPX3, GPX4, GPX5 and GPX6) and thioredoxin reductase (TR) family (TR1, TR2 and TR3), which have selenocysteine residues in the catalytic centers and function as critical enzymes in response to oxidative stress.[10,11] As well as catalase (CAT), GPX1 eliminates hydrogen peroxide ($H_2O_2$) which is intracellularly generated by oxidative stress or converted from superoxide anion by superoxide dismutase.[12] GPX1 is known as a principal antioxidant enzyme for oxidative stress, as CAT has low affinity for

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$H_2O_2$.[13] Therefore, Se-deficiency may deteriorate the pathological conditions of diabetes by depleting expression of GPX1. Many epidemiological studies have proved that serum Se levels in diabetic patients are lower than the levels in healthy individuals.[14–18] The atrophy and degeneration of pancreatic islets are observed in patients with a Se deficiency syndrome, Keshan disease.[19] Se-insufficient rats have low serum insulin levels, which are normalized by Se-supplementation.[20] Recently, however, it has been reported that the long-term dietary supplementation of Se increases incidence of type 2 diabetes.[21–23] Therefore, whether Se-supplementation has a beneficial role for prevention of type 2 diabetes mellitus is controversial.

The objective of this study was to investigate the effect of various Se status on glucose intolerance in Nagoya-Shibata-Yasuda (NSY) mice which were developed as the animal model for type 2 diabetes mellitus by impairing insulin secretion and enhancing insulin resistance.[24–27] NSY mice were given Se-deficient diet to avoid the influence of internal Se sources. To let the mice become Se-insufficient to Se-sufficient conditions, the NSY mice were given normal or Se-deficient diet with various concentrations of $Na_2SeO_3$-containing drinking water. To clarify the relationship between glucose intolerance and pancreatic oxidative stress or the defense system, GPX1 activity, reduced glutathione (GSH) content and 2-thiobarbituric acid-reactive substances (TBA-RS) value in the pancreas of NSY mice were determined.

## MATERIALS AND METHODS

**Animals** —— All laboratory animals in this study were treated according to the Guidelines of the Committee for Ethical Use of Experimental Animals at Setsunan University, Osaka, Japan. Male NSY mice (about 12 g, 4-weeks-old) were purchased from Japan SLC Inc. (Shizuoka, Japan), and were randomly divided into groups of eight animals each. The room temperature was controlled at $25 \pm 2$°C and humidity at $55 \pm 10$%. Lighting was turned on and off at 12 hr intervals for day and night using a timer. Mice were given a Se-deficient diet based on torula yeast[28] (Oriental Yeast Co., Ltd., Tokyo, Japan) or a normal diet (Type MF, Oriental Yeast Co., Ltd.) throughout the experiment.

**Supplementation of $Na_2SeO_3$** —— $Na_2SeO_3$ (99.999%, Sigma-Aldrich Inc., St. Louis, MO, U.S.A.) was dissolved in sterile water and given to mice via drinking water at a dosage of 1.4, 2.8 and 7.0 mg/l for 6, 8 or 12 weeks. Mice were closely monitored during the experiment period, and the body weight of each mouse was monitored weekly. The consumptions of diet and water were measured three times weekly.

**Tissue Harvesting** —— After 6, 8 or 12 weeks of the $Na_2SeO_3$-supplementation regime, mice were fasted overnight and then blood was drawn from tail vein. Glucose was delivered to each mouse via intraperitoneal injection at a dosage of 2 g/kg body weight, and blood was collected from the tail vein after 2 hr of glucose loading. Blood was centrifuged at $700 \times g$ for 10 min at 4°C, and plasma was stored in −80°C until assay. After euthanasia, liver was perfused with ice-cold saline through the portal vein, then liver and pancreas were removed and immediately stored in −80°C for storage until assay.

**Determination of Blood Glucose Level and Plasma Insulin Level** —— Blood glucose level was measured directly by a glucose oxidase method using Glucose Vision (Adventure Healthcheck, LLC, West Carlsbad, CA, U.S.A.). Plasma insulin level was measured with the ELISA kit (Shibayagi Co., Ltd., Gunma, Japan).

**Determination of Se Content** —— Se content in liver, pancreas and plasma was determined by the fluorometric method using 2,3-diaminonaphthalene[29] after wet digestion with a mixed acid solution (nitric acid/perchloric acid, 2 : 1).

**Determination of GPX1 Activity** —— GPX1 activity was determined by a modification of the coupled assay procedure of Lawrence and Burk.[30] Mice pancreas was homogenized with 20 vol. ice-cold 0.2% Triton-X - 50 mmol/l phosphate buffer (pH 7.0) and homogenate was centrifuged at $13000 \times g$ for 15 min at 4°C. The supernatant was dialyzed with 8000 Molecular Weight (M. W.) cut off membrane (Pierce Biotechnology, Inc., Rockford, IL, U.S.A.) and Microdialyzer System 500 (Pierce Biotechnology, Inc.) against 50 mmol/l phosphate buffer (pH 7.0) to remove low molecular aldehydes such as methylglyoxal. The reaction mixture consisted of 50 mmol/l phosphate buffer (pH 7.0), 0.2 mmol/l reduced nicotinamide adenine dinucleotide phosphate (NADPH), 2 mmol/l GSH, 0.2 mmol/l sodium azide and 1 unit/ml glutathione reductase in a total volume of 850 µl. The dialyzed pancreas supernatant (100 µl) was added to the re-

action mixture and allowed to incubate 2 min at 25°C before initiation of the reaction by the addition of 50 µl 0.3 mmol/l $H_2O_2$ solutions. Absorbance at 340 nm was recorded for 1 min and the activity was calculated from the slope of these lines as nanomoles NADPH oxidized per min. Protein was determined by the Bradford method[31] using bovine serum albumin as the protein standard.

**Determination of GSH Content and TBA-RS Value** —— GSH content in pancreas was determined by HPLC using ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate as fluorogenic reagent according to the method of Toyo'oka and Imai.[32] TBA-RS value in pancreas was determined by the method of Ohkawa *et al.*[33] using 1,1,3,3-tetramethoxypropane as a TBA-RS standard.

**Statistical Analysis** —— Values in the figures are reported as means ± S.D. Group means were compared using one-way analysis of variance (ANOVA) with Bonferroni correction. The $p$ level was set at 0.05 or 0.01.

## RESULTS

### Daily Se Intake and Se Contents in Tissues

In the present study, to obtain different Se status of NSY mice that were led into the insufficient to sufficient treatment conditions, the normal daily intake was first estimated. As the mice (4 weeks of age, 12 g of mean body weight) were daily fed an average of 4.7 g of normal diet (400 ± 15.6 ng Se/g), the normal daily Se intake was estimated to be 158 µg/kg (2 µmol/kg). This meant the same intake as that when the mice daily took an average of 3.0 ml (daily water consumption) of 1.4 mg/l $Na_2SeO_3$-containing drinking water with Se-deficient diet. Thus the mice fed the Se-deficient diet were supplemented with 0 [the deficient diet-feeding control (DC) group], 1.4 [the deficient diet-feeding low Se (DL) group] or 7.0 mg/l $Na_2SeO_3$-containing drinking water [the deficient diet-feeding high Se (DH) group]. To compare Se status of these groups with that of normal diet-treatment groups, the mice fed normal diet were supplemented with 0 [normal diet-feeding control (NC) group], 1.4 [normal diet-feeding low Se (NL) group], 2.8 [normal diet-feeding middle Se (NM) group] or 7.0 mg/l $Na_2SeO_3$-containing drinking water [normal diet-feeding high Se (NH) group].

Table 1 shows the total daily Se intakes of NSY mice that were calculated from the diet and water consumption of those ingesting normal diet or Se-deficient diet with $Na_2SeO_3$-containing drinking water for 6, 8 or 12 weeks. Se intake of the DC group was significantly lower than that of NC group. However, there was no significant difference in Se intake between Se-deficient diet- and normal diet-treatment groups that were supplemented with the same concentrations of $Na_2SeO_3$ containing water: DL *vs.* NL group or DH *vs.* NH group; and Se intake in these $Na_2SeO_3$-supplementation groups was augmented depending on the concentration of $Na_2SeO_3$ in the drinking water. Intake of the DH group was 3.8-fold higher than DL group, and that of NM and NH groups was 1.5 and 3.0-fold higher than NL group, respectively.

**Table 1.** Mean Daily Total Se Intake Calculated from Diet and Water Consumptions of NSY Mice

| Treatment group | Total Se intake (µg/kg) 6 weeks | 8 weeks | 12 weeks |
|---|---|---|---|
| DC | 2.4 ± 0.4 | 2.2 ± 0.5 | 1.9 ± 0.4 |
| DL | 98 ± 29** | 84 ± 29** | 96 ± 33** |
| DH | 350 ± 85** | 327 ± 69** | 361 ± 122** |
| NC | 42 ± 10** | 48 ± 7** | 41 ± 5** |
| NL | 126 ± 18†† | 111 ± 12†† | 106 ± 19†† |
| NM | 205 ± 41†† | 177 ± 24†† | 160 ± 30†† |
| NH | 379 ± 63†† | 327 ± 48†† | 322 ± 54†† |

Significantly different from DC group at $^{**}p < 0.01$. Significantly different from NC group at $^{\dagger\dagger}p < 0.01$.

There was no significant difference in body weight among the control groups and $Na_2SeO_3$-supplemented groups, regardless of ingestion of Se-deficient or normal diet (data not shown). No was any disorder such as hair loss or deformation of toenail by $Na_2SeO_3$-supplementation observed.

To investigate Se status in tissues when the mice ingested normal diet or Se-deficient diet with $Na_2SeO_3$-containing drinking water for 6, 8 or 12 weeks, Se contents in plasma, liver and pancreas were determined. In normal diet-treatment groups, as shown in Fig. 1A, Se contents in plasma of all the groups at every termination were significantly high compared with the mice before ingestion of the diet and the water. However, there was no significant difference in Se contents of liver and pancreas between before and after ingestion of such a diet (Fig. 1C and 1E). Se contents in these tissues were also not significantly increased by $Na_2SeO_3$-supplementation.

In Se-deficient diet-treatment groups, although significant decrease of Se content in plasma of the DC group was observed in comparison with the Se

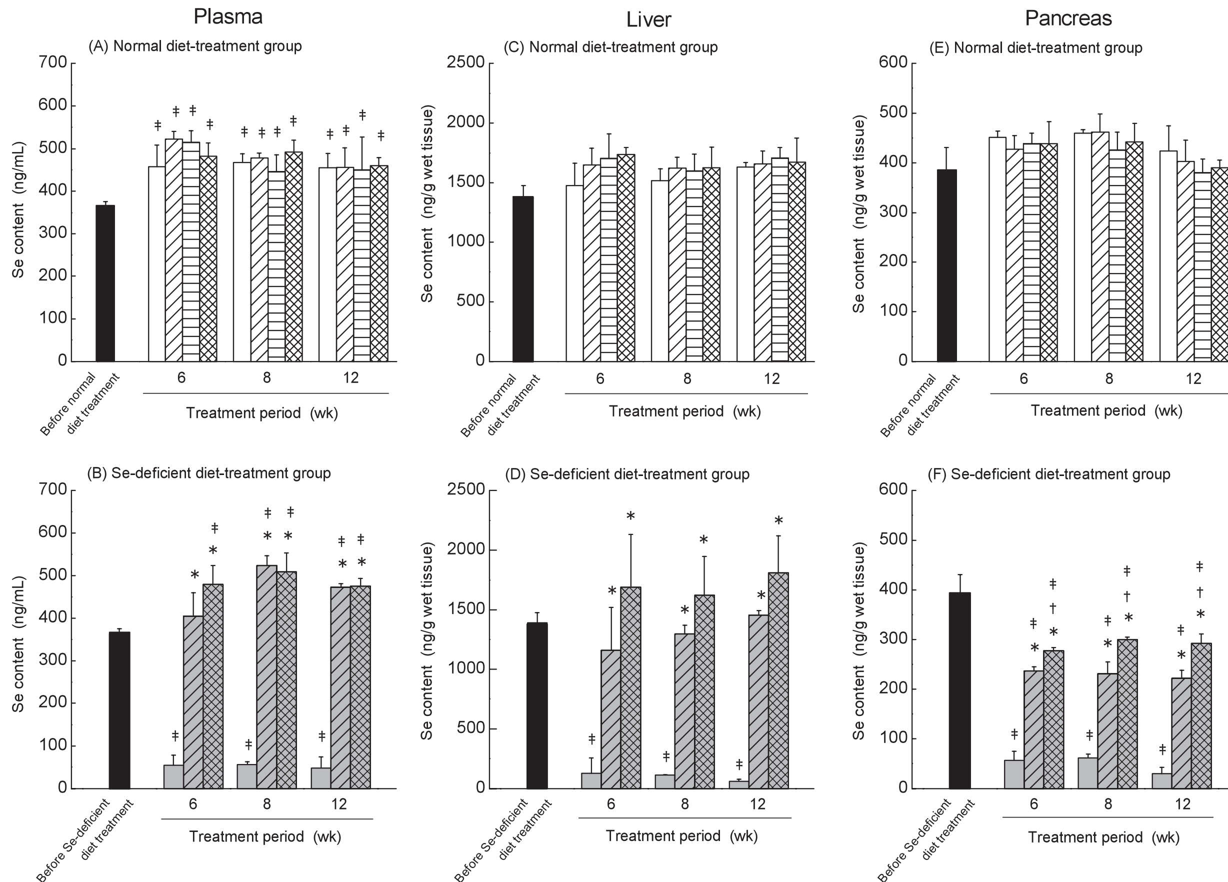


**Fig. 1.** Effect of $Na_2SeO_3$-supplementation on Se Content in Liver, Plasma and Pancreas of NSY Mice

The mice were given normal or Se-deficient diet with $Na_2SeO_3$-containing drinking water for 6, 8 or 12 weeks. NC (□), NL (▨), NM (▤), NH (▩), DC (▭), DL (▨), and DH (▩). The values are mean ± S.D. ($n$ = 4). Significantly different from the control in each diet-treatment group at $^{*}p < 0.05$. Significantly different from the tissual Se content of NL or DL group at $^{\dagger}p < 0.05$. Significantly different from the tissual Se content of the mice before each diet-treatment at $^{\ddagger}p < 0.05$.

content of the mice before treatment of the deficient diet, Se content of DL and DH groups was significantly increased at the termination of 8 and 12 weeks (Fig. 1B). Se content in liver of DC group at every feeding period largely declined compared with the mice before ingestion of the deficient diet (Fig. 1D). However, there was no significant difference in hepatic Se content of DL and DH groups between before and after ingestion of Se-deficient diet with $Na_2SeO_3$-containing drinking water. Se contents in pancreas of DC, DL and DH groups at every supplementation period were significantly lower than the level before feeding the deficient diet with $Na_2SeO_3$-containing drinking water (Fig. 1F). Se contents in these tissues at every supplementation period tended to augment depending on the dose of $Na_2SeO_3$.

## Levels of Blood Glucose and Plasma Insulin

To determine the effect of $Na_2SeO_3$-supplementation on glucose intolerance in NSY mice, levels of blood glucose and plasma insulin 2 hr after intraperitoneal injection of 2 g/kg glucose was measured. As shown in Fig. 2A, the blood glucose level in the normal diet-treatment groups was not affected by $Na_2SeO_3$-supplementation. However, in Se-deficient diet-treatment groups, the blood glucose level decreased depending on the dose of $Na_2SeO_3$ (Fig. 2B). As shown in Fig. 3A, there was no significant difference in plasma insulin level as well as the blood glucose level among normal diet-treatment groups, regardless of $Na_2SeO_3$-supplementation. However, augmentation of plasma insulin level by $Na_2SeO_3$-supplementation in Se-deficient diet-treatment groups was observed at the termination of 8 and 12 weeks depending on the dose (Fig. 3B).

## Parameters for Oxidative Stress in Pancreas

Activity of GPX1 as a selenoenzyme in re-

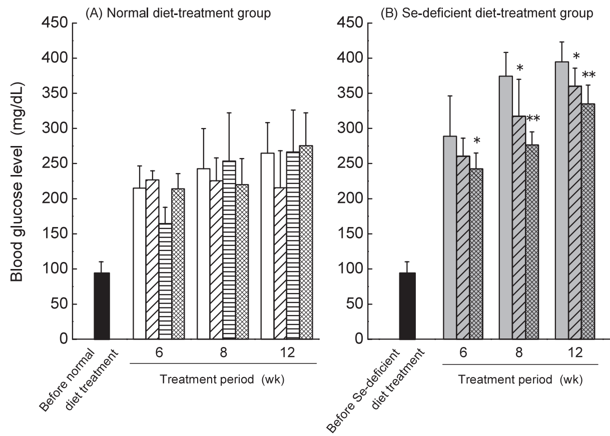


**Fig. 2.** Effect of $Na_2SeO_3$-supplementation on Blood Glucose Level after Intraperitoneal Glucose Loading in NSY Mice

The mice were given normal or Se-deficient diet with $Na_2SeO_3$-containing drinking water for 6, 8 or 12 weeks. These blood glucose levels were measured 2 hr after intraperitoneal glucose loading of 2 g/kg. NC (□), NL (▨), NM (▤), NH (▩), DC (■), DL (▨), and DH (▩). The values are mean ± S.D. ($n = 8$). Significantly different from the control in each diet-treatment group at $^{*}p < 0.05$; $^{**}p < 0.01$.

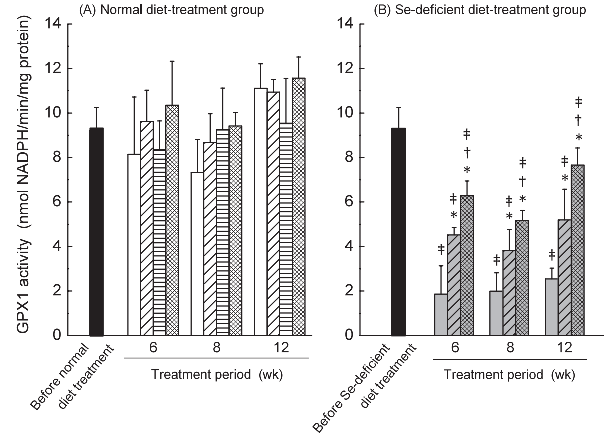


**Fig. 4.** Effect of $Na_2SeO_3$-supplementation on GPX1 Activity in Pancreas of NSY Mice

The mice were given normal or Se-deficient diet with $Na_2SeO_3$-containing drinking water for 6, 8 or 12 weeks. NC (□), NL (▨), NM (▤), NH (▩), DC (■), DL (▨), and DH (▩). The values are mean ± S.D. ($n = 4$). Significantly different from DC group at $^{*}p < 0.05$. Significantly difference from DL group at $^{\dagger}p < 0.05$. Significantly different from the tissual GPX1 activity of the mice before each diet-treatment at $^{\ddagger}p < 0.05$.

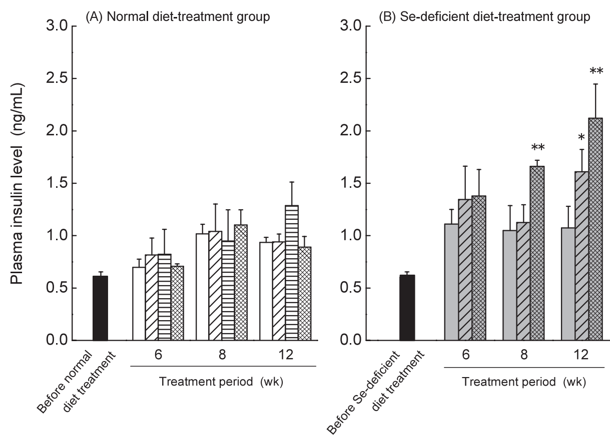


**Fig. 3.** Effect of $Na_2SeO_3$-supplementation on Plasma Insulin Level after Intraperitoneal Glucose Loading in NSY Mice

The mice were given normal or Se-deficient diet with $Na_2SeO_3$-containing drinking water for 6, 8 or 12 weeks. These plasmas were collected 2 hr after intraperitoneal glucose loading of 2 g/kg. NC (□), NL (▨), NM (▤), NH (▩), DC (■), DL (▨), and DH (▩). The values are mean ± S.D. ($n = 4$). Significantly different from the control in each diet-treatment group at $^{*}p < 0.05$; $^{**}p < 0.01$.

sponse to oxidative stress in pancreas and GSH content as the relating indicator were determined. In normal diet-treatment groups, as shown in Fig. 4A, there was no significant difference in pancreatic GPX1 activity at any feeding period between before and after ingestion of the diet with $Na_2SeO_3$-containing drinking water, and these GPX1 activities also did not show a dose-dependency by $Na_2SeO_3$-supplementation. In all of the Se-deficient diet-treatment groups, pancreatic GPX1 activity was significantly low at every termination compared with the mice before ingestion of the deficient diet. However, these GPX1 activities showed a dose-dependency by $Na_2SeO_3$-supplementation (Fig. 4B).

In normal diet-treatment groups, as shown in Fig. 5A, there was neither significant difference in pancreatic GSH content at every feeding period between before and after ingestion of the diet with $Na_2SeO_3$-supplementation nor any effect by the supplementation, because the content showed a comparative fluctuation. In all of the Se-deficient diet-treatment groups, however, the GSH content was significantly low at every termination compared with the mice before ingestion of the deficient diet (Fig. 5B).

TBA-RS value as the indicator of oxidative stress in pancreas was examined. In normal diet-treatment groups, there was no significant difference in pancreatic TBA-RS value at any feeding period before and after ingestion of the diet with $Na_2SeO_3$-containing drinking water, and the value was not altered by $Na_2SeO_3$-supplementation (Fig. 6A). Of Se-deficient diet-treatment groups, as shown in Fig. 6B, pancreatic TBA-RS value in DC group elevated depending on the treatment period. However, the TBA-RS values in DL and DH groups tended to be lowered by $Na_2SeO_3$-supplementation.

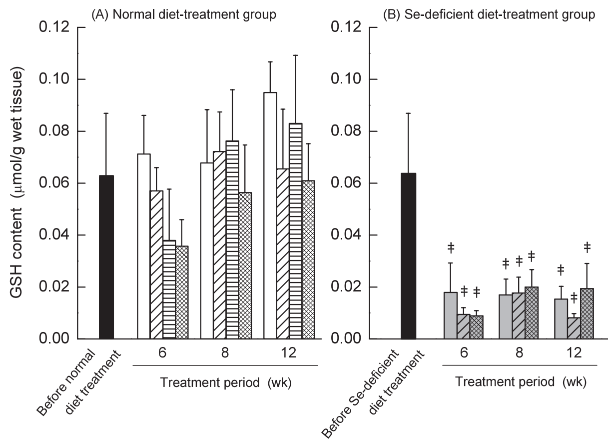


**Fig. 5.** Effect of $Na_2SeO_3$-supplementation on GSH Content in Pancreas of NSY Mice

The mice were given normal or Se-deficient diet with $Na_2SeO_3$-containing drinking water for 6, 8 or 12 weeks. NC (□), NL (▨), NM (▤), NH (▩), DC (■), DL (▨), and DH (▩). The values are mean ± S.D. ($n = 4$). Significantly different from the tissual GSH content of the mice before each diet-treatment at $^{‡}p < 0.05$.

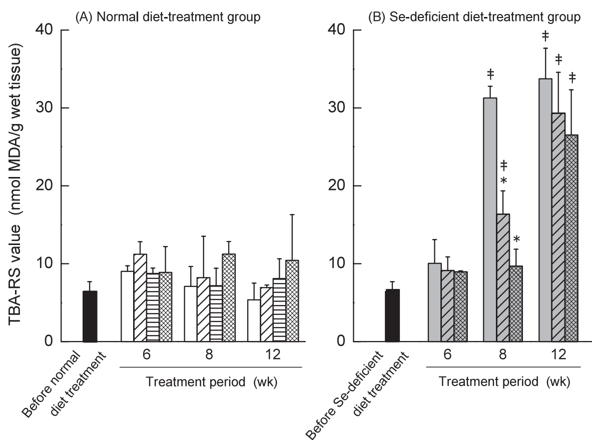


**Fig. 6.** Effect of $Na_2SeO_3$-supplementation on TBA-RS Value in Pancreas of NSY Mice

The mice were given normal or Se-deficient diet with $Na_2SeO_3$-containing drinking water for 6, 8 or 12 weeks. NC (□), NL (▨), NM (▤), NH (▩), DC (■), DL (▨), and DH (▩). The values are mean ± S.D. ($n = 4$). Significantly different from DC group at $^{*}p < 0.05$. Significantly different from the tissual TBA-RS value of the mice before each diet-treatment at $^{‡}p < 0.05$.

## DISCUSSION

Type 2 diabetes mellitus in NSY mice is developed by impairments of the Adenosine triphosphate (ATP)-sensitive $K^+$-channel and voltage-dependent $Ca^{2+}$-channel in the cascade of insulin secretion in pancreatic $\beta$-cells.[24] Although the detailed mechanism is not clear, enhancement of insulin resistance in the target tissues is also related to development of diabetes mellitus in the mice.[25–27] We therefore used NSY mice as the animal model for type 2 diabetes mellitus to investigate the effect of Se status on the glucose intolerance since the pathognomonic mechanisms of these mice are similar to those of human. In this study, to avoid effects of internal Se sources, the mice were given Se-deficient diet. We utilized $Na_2SeO_3$ as the Se-supplementation source because the Se compound had been used in many nutritional studies. To produce various Se status from Se-insufficient to Se-sufficient conditions, the mice were given the deficient diet with several concentrations of $Na_2SeO_3$-containing drinking water.

The total daily Se intake of NSY mice in normal diet- and Se-deficient diet-treatment groups that was calculated from the diet and water consumption increased depending on the concentrations of $Na_2SeO_3$ in drinking water. The theoretical total Se intake of DH group was estimated to be 5.0-fold higher than that of DL group, and the intake of NM and NH groups was 2.0 and 5.0-fold higher than that of NL group, respectively. As shown in Table 1, however, the actual intakes of these groups were lower than the theoretical intakes, as the consumption of the $Na_2SeO_3$-containing drinking water decreased in the higher dose groups.

The Se content in plasma of NC group was significantly high, compared with the mice before ingestion of the diet and $Na_2SeO_3$-containing water (Fig. 1C). This fact may be due to increase of selenoprotein P which is a major Se source in plasma.[34,35] However, there was neither significant difference among Se contents in liver nor pancreas of NC, NL, NM and NH groups (Fig. 1A and 1E). It is reported that $Na_2SeO_3$-supplementation does not influence Se content in various tissues for animals under adequate Se condition.[36] These evidences mean that normal diet-feeding mice have already become Se-sufficient condition, regardless of extra supplementation of the Se compound.

Se contents in plasma, liver and pancreas of DC group were markedly depressed at all termination periods, compared with mice before ingestion of Se-deficient diet (Fig. 1B, 1D and 1F). This result suggests that the mice are led into Se-insufficient condition by the ingestion of Se-deficient diet for at least 6 weeks. This is also supported by reports of Se contents in various tissues of mice largely declining by continuous ingestion of Se-deficient diet for more than 4 weeks.[37,38] The Se contents in all the tissues of DL and DH groups largely increased compared with that of DC group (Fig. 1B, 1D and 1F). However, the Se contents in pancreas

of these groups were low compared with that before feeding the deficient diet (Fig. 1F). This result indicates that pancreas of DH group becomes Se-sub-sufficient condition, suggesting that the inorganic Se source may not be incorporated into the tissue as much as liver and plasma. It is also reported that $Na_2SeO_3$ has low bioavailability to pancreas compared with liver and plasma.[39,40] These results therefore suggest that mice of DC and DH groups are Se-insufficient and sub-sufficient, respectively, and that of DL group is in a medium Se condition between these two groups.

We then investigated the effect of Se status on blood glucose and plasma insulin levels of NSY mice after intraperitoneal glucose tolerance test (IPGTT), as they were led into various Se conditions. In normal diet-treatment groups, the blood glucose levels after IPGTT were not significantly affected by $Na_2SeO_3$-supplementation (Fig. 2A); however, this level of DC group at every termination was higher than that of NC group (Fig. 2A and 2B). This result indicates that ingestion of Se-deficient diet in NSY mice promotes the glucose intolerance. It is also reported that the onset of diabetes mellitus in NSY mice is accelerated by ingestion of sucrose or high fat diet.[27] As the Se-deficient diet contains 55.7% sucrose, the high intake of sugar may deteriorate the diabetic symptoms of the mice. In Se-deficient diet-treatment groups, the blood glucose levels at every termination after IPGTT decreased depending on the dose of $Na_2SeO_3$ (Fig. 2B). $Na_2SeO_3$-supplementation mitigates the symptoms of the experimental diabetic animals.[19,41]

Plasma insulin levels after IPGTT in normal diet-treatment groups were also not significantly affected by $Na_2SeO_3$-supplementation (Fig. 3A). There was no significant difference between the plasma insulin levels of NC and DC groups after IPGTT (Fig. 3A and 3B). In Se-deficient diet-treatment groups, however, the plasma insulin levels after IPGTT dose-dependently increased following such supplementation (Fig. 3B). These results suggest that $Na_2SeO_3$ may enhance insulin secretion from pancreatic islet $\beta$-cells under Se-insufficient condition. This is also supported by the report that the serum insulin level of Se-insufficient rat increases by such supplementation.[20] The plasma insulin levels of DL and DH groups after IPGTT were higher than those of NL and NH groups, respectively (Fig. 3A and 3B). This result suggests that the insulin secretion from pancreatic $\beta$-cells in response to increased blood glucose may be delayed, since plasma insulin levels after glucose loading of patients with impaired glucose tolerance are observed to be higher than those of healthy controls.[42] Thus $Na_2SeO_3$-supplementation for mice maintained under Se-insufficient condition by feeding Se-deficient diet is indicated to improve the glucose intolerance. However, the improvement of glucose intolerance by supplemented $Na_2SeO_3$ might be observed under the glucose intolerance that was enhanced by intake of sugar contained at the high level in Se-deficient diet.

To clarify the relationship between glucose intolerance and oxidative stress or the defense systems in pancreas of NSY mice, we focused on selenoenzymes in response to oxidative stress, GPX1, GPX4 and TR. However, there was little activity of GPX4 and TR in the pancreas (data not shown). The mRNA expression levels of these enzymes in pancreas are also shown to be lower than those in the other tissues.[43] Thus GPX1 is supposed to be important as the defense system in pancreas of NSY mice supplemented with $Na_2SeO_3$. However, we have previously confirmed that methylglyoxal, which is highly detected as a glucose decomposition product in blood of diabetic patients and experimental animal models,[44,45] interferes with the determination of GPX1 activity (unpublished data). In this study, to avoid this influence, the dialyzed samples of tissue homogenates were used for the determination. There was no significant difference among GPX1 activity in pancreas of NC, NL, NM and NH groups (Fig. 4A). However, in Se-deficient diet-treatment groups, the GPX1 activities in pancreas of DC, DL and DH groups were lower than those before ingestion of Se-deficient diet (Fig. 4B). This result indicates that bioavailability of $Na_2SeO_3$ may be lower than that of the organic Se sources contained in normal diet. The GPX1 activities in pancreas of Se-deficient diet-treatment groups were augmented by $Na_2SeO_3$-supplementation (Fig. 4B). This result indicates that $Na_2SeO_3$-supplementation induces an activation of pancreatic GPX1.

GSH content and TBA-RS value in pancreas of normal diet-treatment groups were not significantly affected by $Na_2SeO_3$-supplementation (Figs. 5A and 6A). In Se-deficient diet-treatment groups, however, decrease of GSH content and increase of TBA-RS value by ingestion of the-deficient diet were observed, regardless of $Na_2SeO_3$-supplementation (Figs. 5B and 6B). Chronic hyperglycemia decreases expression of pancreatic $\gamma$-glutamylcysteine

ligase which is the rate-limiting enzyme for glutathione synthesis.[46] Both evidences may be associated with depression of GSH in pancreas of NSY mice fed Se-deficient diet. These findings also suggest that GSH provision-decreased GPX1 system does not contribute to the protection against oxidative stress in the pancreas under Se-insufficient condition. Chronic hyperglycemia enhances ROS production in pancreatic $\beta$-cells.[47] The pancreatic $\beta$-cells are especially vulnerable to ROS, as the expression levels of antioxidant enzymes such as GPX1, superoxide dismutase (SOD) and CAT in the cells are low.[48] It is also reported that chronic exposure of glucose and ROS to pancreatic $\beta$-cells causes insulin synthesis to diminish.[49] Hence as the GPX1 system induced by Se-supplementation does not seem to function as the antioxidant enzyme system because of a low level of GSH, $Na_2SeO_3$ may not enhance insulin synthesis in pancreatic $\beta$-cells. Therefore, supplemented $Na_2SeO_3$ may improve glucose intolerance of the NSY mice preferably by exhibiting the insulin-like action in insulin target tissues such as muscle and adipose tissue.

In conclusion, supplementation of $Na_2SeO_3$ resulted in the decrease of blood glucose and the increase of plasma insulin after IPGTT in NSY mice that had ingested Se-deficient diet. Although pancreatic GPX1 activity increased by $Na_2SeO_3$-supplementation, the GSH level was depressed. Thus supplemented $Na_2SeO_3$ improved glucose intolerance of NSY mice under Se-insufficient condition. However, the improvement of glucose intolerance by $Na_2SeO_3$-supplementation may not be caused by the enhancing insulin synthesis in pancreatic $\beta$-cells via protection against oxidative stress. Further study is needed to clarify the detailed mechanisms, for example, whether $Na_2SeO_3$ exerts the insulin-like action in insulin target tissues such as muscle and adipose cells.

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