An Assessment of Niboshi (a Processed Japanese Anchovy) as an Effective Food Source of Selenium

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Niboshi is processed from the small Japanese anchovy with a 3–6 cm length by a several-minute boiling and subsequent drying. It is usually used in a variety of Japanese dishes. We assessed the Niboshi and its extract as food source of the micronutrient selenium (Se). The Se content in the Niboshi was 1.14 ± 0.01 μg/g and up to 60% of its total Se was found in the abdominal part. After the feeding of Niboshi-supplemented diets for 7 weeks to mice, the organ Se contents and hepatic cellular and plasma glutathione peroxidase (GPx) activities were comparable to those of selenious acid (SA)-fed mice. As the Niboshi is a processed foodstuff for extracting a base seasoning used in many Japanese dishes, the absorptivity of Se from its extract was further examined in dietary Se-depleted mice. The daily oral administration of the Niboshi extract to the mice for 7 days improved the Se contents in the blood and liver and the GPx activities. Thus, the Niboshi can serve as an efficient dietary source of Se in mice. Taking the daily intake habit of the Niboshi into consideration, it would contribute to the dietary intake of Se in Japan.

Key words —— fish, Japanese anchovy, Niboshi, selenium

INTRODUCTION

In 1957, Schwartz and Foltz[1] reported that inorganic selenium (Se) salts were effective in protecting against necrotic liver degeneration in vitamin E-deficient mice. Meanwhile, Mills[2] discovered the Se-dependent glutathione peroxidase (GPx) that protects hemoglobin from oxidative damage in the red cells. The early interest in Se was primarily related to its toxicity, but since then, Se was recognized as a dietary essential.[3] The importance of Se to human health is now recognized, and a reference nutrient intake (RNI) and a recommended dietary allowance (RDA) of Se are set as well as other micronutrients.[4]

Fish contains a relatively high amount of Se compared to other food materials and is potentially a good dietary source of Se for humans.[5,6] Se in trout is a highly bioavailable source of dietary Se in comparison to Se from yeast in humans.[7] Se from flounder was efficient at restoring its concentrations in the liver and skeletal muscles, while tuna was not sufficient after 9 weeks of recovery following a 6-week period of Se depletion.[8] Meltzer *et al.*[9] found no changes in the serum and platelet Se concentrations when the diet was supplemented with trout and mackerel. Other studies also reported a lower absorption from fish muscle compared with meat and offal in humans and rats.[10,11] Overall, the nutritional efficiency of Se from fish materials may be dependent on the kind and/or part of the fish used.

In Japan, a variety of seafood materials are ordinarily ingested in the diets. Se from fish made the largest contribution to the dietary Se intake (up to 60% of daily total) rather than that from the staple food rice and vegetables in Japan.[12,13] Thus, we addressed the nutritional evaluation of Se from the Niboshi [Fig. 1(a)] that is one of dried fish materials commonly used in the Japanese cuisine. The small Japanese anchovy (*Engraulis japonicus*) of 3–6 cm in length [Fig. 1(b)] is processed as the Niboshi by a several-minute boiling and subsequent drying. In the domestic cuisine, the commercial Niboshi is left in plain water for one night and then brought to a boil for not over 10 min. The obtained extract [Fig. 1(c)] is used as a base seasoning for a variety of Japanese dishes, like *the fond de veau* in French. Miso-soup is one of the most typical dishes using the Niboshi extract, which is ordinarily taken once or twice a day in the Japanese dietary custom. As the Niboshi can be directly eaten, it is also cooked

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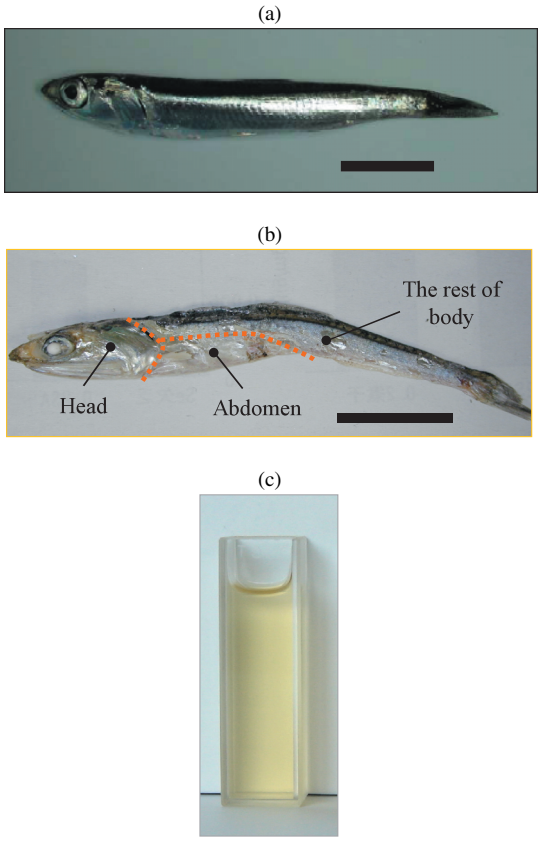

Fig. 1. Photographs of (a) Small Japanese Anchovy in Raw, (b) Niboshi and (c) Niboshi Extract
Scale bars in the photographs indicate 1 cm length.

and served as a snack. In this study, the Se contents in the organs and the GPx activities were investigated when Se-supplemented diets from the Niboshi were fed to mice in comparison to selenious acid (SA)-supplemented ones. In addition, the absorptivity of Se from the Niboshi extract was accessed in dietary Se-depleted mice.

MATERIALS AND METHODS

Materials —— Artificial additive-free Niboshi of 3–6 cm in length were purchased at a grocery shop and used as received. Nutrient content of Niboshi in 100 g: protein, 64.5; lipid, 6.2; carbohydrate, 0.3; fatty acids, 2.64; Na, 1.7; K, 1.2; Ca, 2.2; Mg, 0.23; P, 1.5 (g); vitamins D, 18; E, 0.9; B_1, 0.1; B_2, 0.1; niacin, 16.5; B_6, 0.28 (mg); B_{12}, 41.3 (μg).[14] A basal diet [torula yeast, 30; sucrose, 55.7; lard, 5; liver oil, 3; minerals, 5; vitamins, 0.9; methionine, 0.3; choline chloride, 0.2 (%); Se, 4 ng/g] was from Oriental Yeast Co. Ltd. (Tokyo, Japan).[15] H_2SeO_3 (SA) was obtained from Kanto Chemical Co. Inc. (Tokyo, Japan). H_2O_2 and *tert*-butyl hydroperoxide (*t*-BuOOH) were from Wako Pure Chemical Ind. Ltd. (Osaka, Japan) and Sigma Co. (St. Louis, MO, U.S.A.), respectively. Water used for all experiments was generated by a Milli-Q Biocel system (Millipore Corp., Billerica, MA, U.S.A.). All other chemicals were of commercial reagent grade and used as received. Three-week old male Institute of Cancer Research (ICR) mice (specific pathogen free) were purchased from Clea Japan Inc. (Tokyo, Japan) and cared for in accordance with the guidelines of Nagasaki University on Animal Care. Mice were housed 5 per cage on a 12 hr light/12 hr dark schedule at 23 ± 2°C and 60% relative humidity, and were given deionized water during all the experiments.

Determination of Se Contents —— The Se contents in the diets and isolated organs were fluorometrically determined using 2,3-diaminonaphtharene after digestion using a 1:4 mixture of $HClO_4$ and HNO_3.[16]

Niboshi-Supplemented Diets and Their Feeding to Mice —— The Niboshi were finely ground in a mortar with a pestle and sieved through a 150 μm-aperture screen. The ground Niboshi were mixed with the basal diet at 1:50 or 4 by weight. During the feeding experiments, these diets were freshly prepared for every 7-day. Ten milliliters of SA solution dissolved in saline (40 mM) was sprayed on 1 kg of the basal diet spread out on a plastic vat with vigorous agitation.[17]

Niboshi Extract and Its Administration to Dietary Se-Depleted Mice —— Three pieces of the Niboshi (up to 3 g) were placed in a non-woven fabric bag, and put into 30 ml of boiling water. After a 10 min extraction, the pale yellow extract was passed through a filter paper, and its volume was made up to 30 ml with Milli-Q water. The obtained extract was lyophilized once and then dissolved in an appropriate volume of Milli-Q water to adjust the dose of Se to the mice. The Niboshi extract or SA solution was orally administered to dietary Se-depleted mice once a day in 9–10 a.m. for 7 days at 0.3 μgSe/kg. The Se-depleted mice were prepared by the feeding of the Se-deficient diet for 6-week[17] and subsequently given the same diet during the administration of the sample solutions.

Determination of Enzyme Activity —— The hepatic cellular GPx (cGPx) activity was determined as follows: isolated livers were rinsed with saline and then homogenized by a probe-type sonicator 250D (Branson Ultrasonics Corp., Danbury, CT,

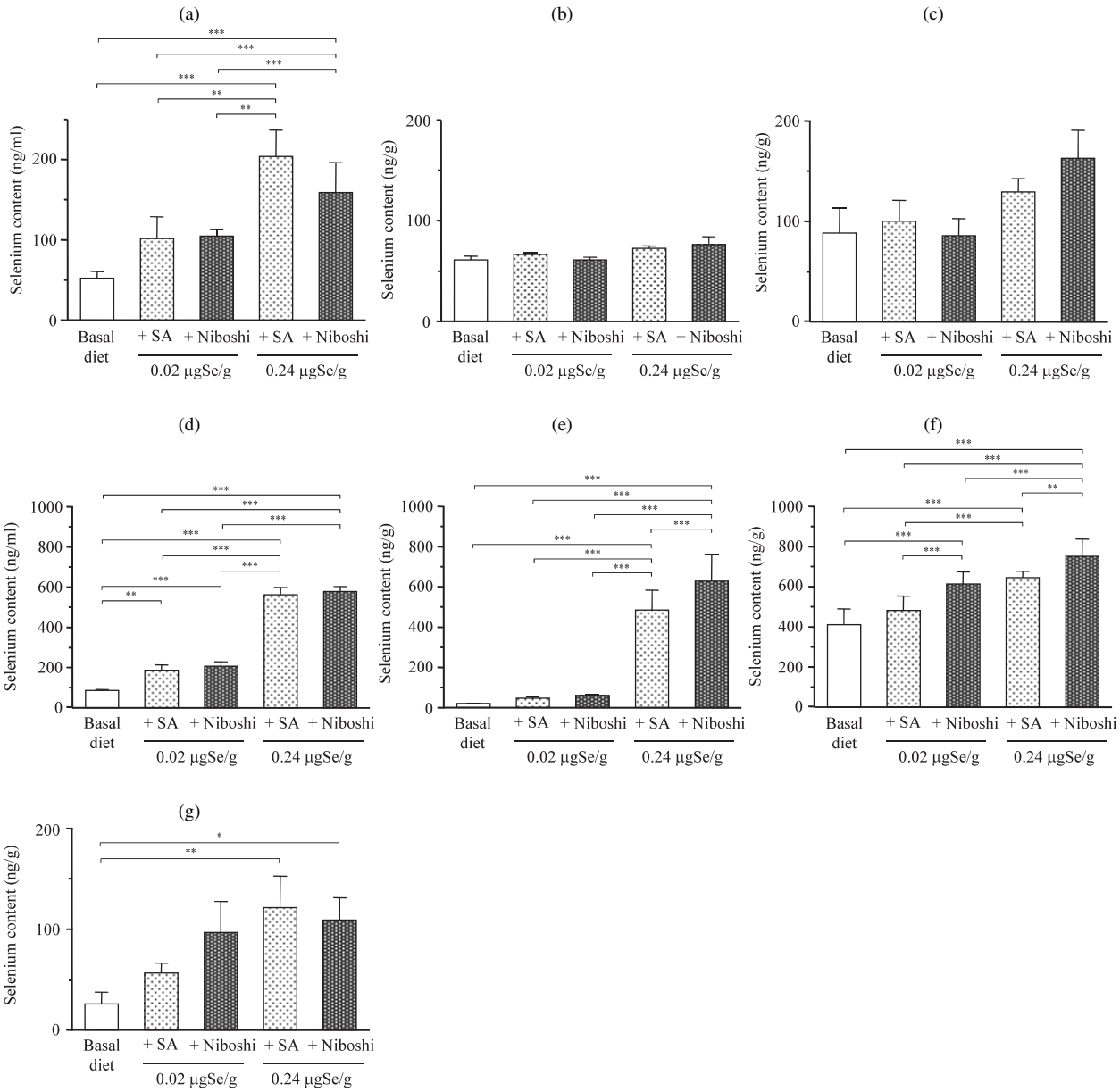

Fig. 2. Se Contents in Selected Organs after the Feeding of Niboshi- and SA-Supplemented Diets to 3-week Old Normal Mice for 7 Weeks

(a) Blood, (b) brain, (c) heart, (d) kidney, (e) liver, (f) testis, (g) thyroid. $^{*}p < 0.05$; $^{**}p < 0.01$, $^{***}p < 0.001$. Three-week old male mice were randomly divided into 5 groups of 5 mice each and given the diets *ad libitum* for 7 weeks.

U.S.A.) in saline (5 ml). The supernatant fractions were prepared by centrifugation at $105000 \times g$ and 4°C for 1 hr on a L-80 (Beckman Coulter, Inc., Fullerton, CA, U.S.A.). H_2O_2 and *t*-BuOOH were used as substrates. Catalase activity was blocked by the addition of 1 mM NaN_3. Absorbance at 340 nm due to the NADPH was recorded at every 10 sec just after mixing by inversion. The GPx activity was calculated using the following equation as mmoles NADPH oxidized per minute.[18] Activity (mmol/min·ml) = $-(DA_{Sample} - DA_{Reagent\ Blank}) \times 5/\varepsilon_{mM}$ (DA: difference in absorbance at 340 nm between 15 and 75 sec after addition of the substrates, 5: dilution factor, ε_{mM}: extinction coefficient for NADPH, 6.22). The hepatic glutathione *S*-transferase (GST) activity was determined with 2,4-dinitro-chlorobenzene as a substrate according to the procedure by meister.[19]

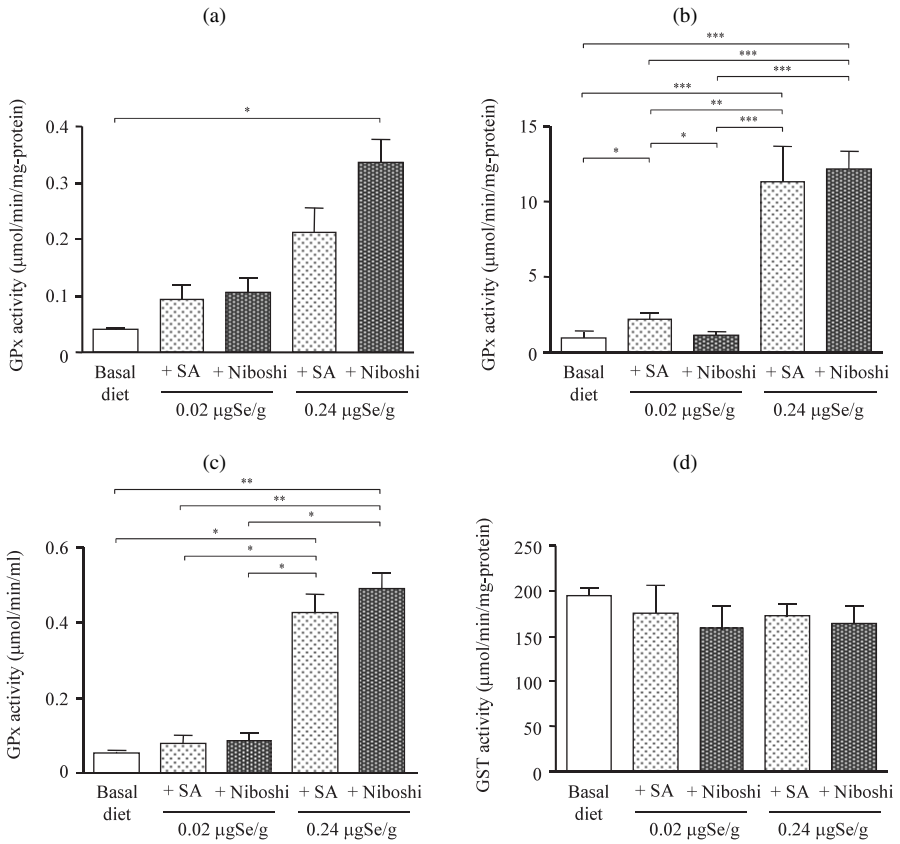

Fig. 3. cGPx and pGPx Activities after the Feeding of Niboshi- and SA-Supplemented Diets to 3-week Old Normal Mice for 7 Weeks

(a) cGPx activity for H_2O_2, (b) cGPx activity for *t*-BuOOH, (c) pGPx activity for *t*-BuOOH. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Three-week old male mice were randomly divided into 5 groups of 5 mice each and given the basal, SA- or Niboshi-supplemented diet *ad libitum* for 7 weeks.

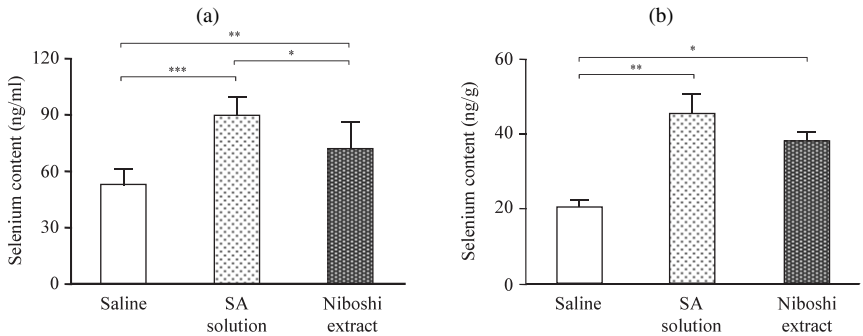

Fig. 4. Se contents in the Blood and Liver after Daily Oral Administration of the Niboshi Extract and the SA Solution to 9-week Old Dietary Se-Depleted Mice at a Dose of 0.3 μg/kg for 7 days

(a) Blood, (b) liver. $^{*}p < 0.05$; $^{**}p < 0.01$, $^{***}p < 0.001$. Three-week old male ICR mice were randomly divided into 3 groups of 5 mice each and fed with the Se-deficient basal diet *ad libitum* for 6 weeks. The dietary Se-depleted mice were subsequently given the same diet during the administration of the sample solutions.

Statistical Analysis —— All data were presented as the mean ± standard error ($n = 5$ or more). Statistical analyses were performed using a program PRISM 4 (GraphPad Software Inc., San Diego, CA, U.S.A.). Multiple mean values were compared by a two-way ANOVA with a Bonferroni post-hoc test with treatment and Se concentration in the diets as factors. Comparisons were considered statistically significant at $p < 0.05$.

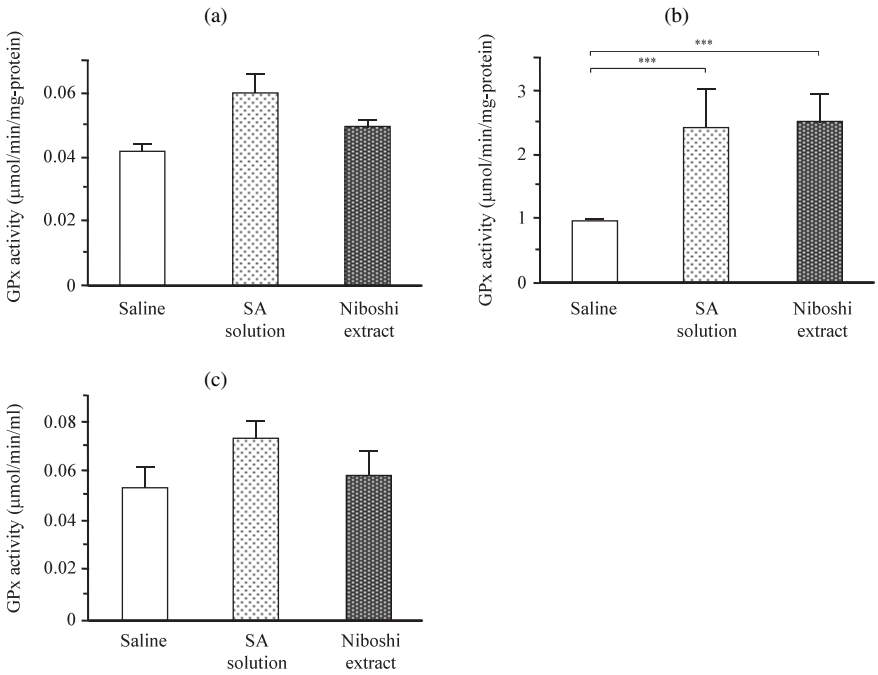

Fig. 5. cGPx and pGPx Activities after the Once-Daily Oral Administration of Niboshi Extract and SA to 9-week Old Dietary Se-Depleted Mice at a Dose of 0.3 μg/kg for 7 days

(a) cGPx activity for H_2O_2, (b) cGPx activity for *t*-BuOOH, (c) pGPx activity for *t*-BuOOH. *** $p < 0.001$. Three-week old male ICR mice were randomly divided into 3 groups of 5 mice each and fed with the Se-deficient basal diet *ad libitum* for 6 weeks. The dietary Se-depleted mice were subsequently given the same diet during the administration of the sample solutions.

RESULTS AND DISCUSSION

Total Content and Distribution of Se in Niboshi

The total content of Se in the Niboshi was 1.14 ± 0.01 μg/g (one gram approximately for one piece of the Niboshi). As the Niboshi is processed from small Japanese anchovy, all parts of the body are utilized without removing the internal organs, bone and head. Thus, the Niboshi was carefully divided into three parts with a long-nose tweezers along the curves shown in Fig. 1(b), and their Se distribution rates were compared. More than half of the total Se (56.4 ± 2.1%) was found in the abdominal part including gills. The Se distribution of the head part and the rest of the body were 14.4 ± 0.7% and 29.2 ± 1.6%, respectively. In most fish used for food, the abdominal part including the internal organs is usually discarded. However, the whole body of the Niboshi is utilized in the Japanese cuisine. Therefore, Se species not only from the fish muscle, but also from the abdominal part in the Niboshi were thought to be a potential source of Se. Se in migratory fishes (*e.g.*, tuna) forms a complex with Hg and/or proteins for detoxification,[20,21] and such a complex Se species seems to be poorly bioavailable as a nutrient. The total Hg content in the Niboshi was less than 2 mol% of Se as determined by cold vapor atomic absorption spectrophotometry.

Se Absorptivity from Niboshi-Supplemented Diets in Mice

The Niboshi was ground to less than 150 μm in diameter and mixed into the basal diet at two concentrations. The Se contents in the Niboshi-supplemented diets were determined as 0.02 ± 0.00 and 0.24 ± 0.01 μg/g (SA-supplemented diets used as controls: 0.02 ± 0.00 and 0.24 ± 0.02 μg/g). The Niboshi-fed mice favorably gained body weight over the period of feeding, similar to those of the other diet-fed mice. The body weights of the Niboshi-fed mice at 10-week old (39.5 ± 1.0 and 41.2 ± 0.8 g) were not significantly different from those of age-matched mice fed with a regular diet (40.0 ± 1.6 g).

Se contents of selected organs of the Niboshi-fed mice were compared to those of the SA-fed ones (Fig. 2). The feeding of the Niboshi-supplemented diets provided higher Se contents in all the organs, except for the brain and heart, than the basal diet. These results were similar to those obtained from the SA-fed mice. The cGPx and plasma GPx (pGPx) activities were compared as better measures

for the bioavailability of Se. The cGPx activity of the Niboshi-fed mice for both H_2O_2 and *t*-BuOOH significantly increased with an increase in the Se content in the diets [Fig. 3(a) and 3(b)]. The Se from the Niboshi also gave similar effects for the pGPx activity [Fig. 3(c)]. The cGPx activity of mice fed with the Niboshi-supplemented diets correlates with the Se contents in the liver. These results with respect to the GPx activity were comparable with those obtained in the SA-fed mice. GST is a Se-independent GPx and shows an increase in intrinsic GST activity plus GPx-like activity in the Se-depleted states.[22,23] As the hepatic GST activity of the Niboshi-fed mice was similar to that of the basal diet-fed mice [Fig. 3(d)], the GPx activity in the Niboshi-fed mice is attributed to the Se delivery from the Niboshi to the Se-dependent GPxs.

Se Absorptivity from Niboshi Extract in Dietary Se-Depleted Mice

The Se content in the Niboshi extract that was prepared according to the usual extraction procedure was 110 ng/g-Niboshi (30 ng/ml-extract). Analyses of the fish extracts showed that the distribution of the low- and high-molecular-weight compounds varies in the different species, *e.g.*, the plaice and mackerel muscle contains a high amount of low-molecular-weight species.[24] When the Niboshi extract was separated by dialysis through a molecular weight cutoff 6–8 kDa membrane, 90% of the Se in the extract traversed the membrane and emerged in the dialysate outside the bag. The administration of the Niboshi extract to the Se-depleted mice resulted in increases in the Se contents in the blood and liver (Fig. 4). The obtained values were comparable to those by the administration of a highly absorbable source compound SA (oral absorptivity, 87%).[25] The Niboshi extract administration improved the cGPx activities, as well as SA (Fig. 5). The pGPx activity also tended to increase due to the administration of the Niboshi extract. These facts demonstrate that the Niboshi extract can serve as an efficient food source of Se in mice.

In conclusion, we demonstrated that the Niboshi and its extract are available as Se source in mice in this study. Taking the daily intake habit of the Niboshi into consideration, it would contribute to the dietary intake of Se in Japan that there is so far no report yet on a Se-deficiency in humans. It is noted that the entire body of the Niboshi anchovy is utilized without removing the Se-rich abdominal part that contains up to 60% of the total content. As we have a dietary habit to ingest small dried fish, there would be other fish foodstuffs that could efficiently supply Se like the Niboshi anchovy. Although little is so far known about the chemical forms of Se species in the Niboshi anchovy, the Se is probably associated with proteins and/or organic materials and is unlikely to be present as inorganic species. The structural characterization of Se species in the Niboshi extract is now in progress.

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