

Identification and Determination of Selenoneine, 2-Selenyl- N_{α} , N_{α} , N_{α} -Trimethyl-L-Histidine, as the Major Organic Selenium in Blood Cells in a Fish-Eating Population on Remote Japanese Islands

Michiaki Yamashita · Yumiko Yamashita · Tetsuo Ando · Junji Wakamiya · Suminori Akiba

Received: 3 September 2013 / Accepted: 9 October 2013 / Published online: 8 November 2013
© The Author(s) 2013. This article is published with open access at Springerlink.com

Abstract Selenoneine is the major selenium compound in fish muscles, and fish appears to be an important source of selenium in the fish-eating population. Selenoneine has strong antioxidant activity and a detoxifying function against methylmercury (MeHg) toxicity. Dietary intake, bioaccumulation, and metabolism of selenoneine have not been characterized in humans. A nutritional survey was conducted in remote islands of the Kagoshima Prefecture in Japan. To evaluate the potential risks and benefits of fish consumption for health, we measured concentrations of selenoneine, total selenium, MeHg, inorganic mercury, and polyunsaturated fatty acid (LC-PUFA) in the blood of a fish-eating human population. The erythrocyte, leukocyte, and platelet residues following removal of serum (cellular fraction) contained 0.510 $\mu\text{g Se/g}$, 0.212 $\mu\text{g selenoneine Se/g}$, and 0.262 $\mu\text{g Se-containing proteins Se/g}$, whereas the serum contained 0.174 $\mu\text{g total Se/g}$. Selenoneine was highly concentrated in the cellular fraction in a manner that was dependent on subjects' frequency of fish consumption. Concentrations of selenoneine were closely correlated with concentrations of MeHg in the cellular fraction. Selenoneine is the major chemical form of selenium in the blood cells of this fish-

eating human population and may be an important biomarker for selenium redox status.

Keywords Selenium · Antioxidant · Selenoneine · Red blood cells · Methylmercury · Seafood · Fish

Introduction

Ocean fish, such as tuna, mackerel, and swordfish, possess a novel selenium-containing imidazole compound, selenoneine, which is the predominant form of organic selenium in tuna [1–3]. This compound has a unique selenoketone structure and is a powerful antioxidant [1–3]. The concentration of selenium in the red blood cells (RBCs) was correlated with the intake of selenium from animal foods and seafood, whereas the concentration of selenium in the serum or plasma was correlated with the intake of selenium from plant foods [4, 5]. Thus, a significant amount of selenoneine is thought to be incorporated through the consumption of seafood. Several studies have examined the bioavailability of selenium from fish and other food sources [6–13].

Fish consumption is also associated with the intake of methylmercury (MeHg) [14–21], which may cause adverse health effects. Concentrations of nutrients, including long chain polyunsaturated fatty acids (LC-PUFA) and specific chemical contaminants, such as MeHg and dioxins, have been characterized in a range of fish species [22, 23]. In addition, the health and nutritional benefits of fish consumption have been compared to the health risks associated with contaminants present in fish [22, 23]. Because selenium reacts with and detoxifies MeHg, its distribution in seafood and the detoxifying functions of dietary selenium from seafood should be characterized. The contents of selenoneine and total

M. Yamashita (✉) · Y. Yamashita
National Research Institute of Fisheries Science,
2-12-4 Fukuura, Yokohama, Kanagawa 236-8648, Japan
e-mail: mic@affrc.go.jp

T. Ando · S. Akiba
Department of Epidemiology and Preventive Medicine, Kagoshima
University Graduate School of Medical and Dental Sciences,
8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan

J. Wakamiya
Meisuien Minamata Municipal Welfare Institute for Minamata
Disease Patients, Minamata, Kumamoto 867-0008, Japan

selenium in fish are correlated with the content of total mercury [3]. The Se/Hg molar ratio in fish was estimated to be 3:1 and it varied among species, ranging from 1 to 217 [3]. Such ratios may be important for estimating the MeHg risks associated with fish consumption. Animal trials of feeding with both MeHg [0.5 or 50 $\mu\text{mol/kg}$ in diet] and sodium selenite showed that the toxicity of MeHg decreased with selenium intake for Se/Hg molar ratios of >0.2 [17, 18]. Selenoneine accelerated the excretion and demethylation of MeHg through the formation of secretory extracellular lysosomal vesicles via the specific organic cation/carnitine transporter-1 (OCTN1) [21]. Thus, the molar Se/Hg range of 1 to 217 found in the muscle of fish is considered to represent the normal physiological state in terms of MeHg bioaccumulation and metabolism [3]. Therefore, animal studies and human trials should examine various Se/Hg molar ratios and use them to compare the physiological and nutritional effects of fish consumption.

Here, we report the determination of selenoneine concentrations in human blood. Significant amounts of selenoneine accumulated in the cellular fraction, but not in the serum, of the fish-eating Japanese population were studied here. Concentrations of selenoneine and total selenium in the cellular fraction might be used as an important biomarker for fish consumption and selenium antioxidant functions.

Materials and Methods

Subjects A nutritional interview survey with medical checkup was conducted among rural residents living on remote islands in the Southwestern Islands in Kagoshima Prefecture, Japan, in 2009. A total of 217 persons from four islands participated in this study. Of the 217 persons, 50 were excluded because of missing forms or insufficient blood samples for chemical analysis. A total of 167 persons were covered in this study, comprising 75 males and 92 females, all over 20 years of age. Their mean ages were 63.2 years in both sexes. Information was collected regarding the frequency of consumption of seafood per week and the amounts of fish meat per meal. The study was approved by the Ethics Committee of the Kagoshima University Graduate School of Medical and Dental Sciences, and informed written consent was obtained from participants.

Fish Intake A food-frequency questionnaire was used to assess the average intake of fish meals including shrimp, crab, squid, octopus, and clam during the previous week. Participants were asked to indicate their usual rate of consumption choosing from six frequency categories. The categories range from “never or less than once/week” to “more than nine times per week”. The servings are specified in terms of units or common portions (e.g., one slice of fish

fillet). An average portion size was assigned to each questionnaire item. The subjects were divided into six categories consuming fish meal: less than once per week = f-time 0, once per week = f-time 1, two or three times per week (less than once per 2 days) = f-time 2, three or four times per week (once per 2 days) = f-time 3, everyday (five to eight times per week) = f-time 4, every time (more than nine times per week) = f-time 5, and categories of the amount of fish meat: 25 g per meal = f-mass 1, 50 g per meal = f-mass 2, 75–100 g per meal = f-mass 3, 150 g per meal = f-mass 4, 200 g or more per meal = f-mass 5.

Chemical Analysis of the Blood Samples Blood samples were obtained from the subjects, and the cellular fraction and serum of a collected blood sample were separated with blood serum separation tubes by centrifugation at $2,000\times g$ for 10 min. The cellular fraction and serum specimens were stored at $-80\text{ }^{\circ}\text{C}$ until use. Concentrations of MeHg and LC-PUFA in the blood were previously shown to be correlated with fish consumption [22–25]. Hence, we also analyzed whether the concentrations of selenium and selenoneine in the cellular fraction were correlated with the levels of MeHg and LC-PUFA.

Each sample (0.1 g) was placed in glass test tube and digested at $200\text{--}220\text{ }^{\circ}\text{C}$ in 1 mL of a 1:2 mixture of nitric acid and perchloric acid and subjected to a fluorometric assay using 2,3-diaminonaphthalene, as described previously [3, 26]. Detection limit was $0.006\text{ }\mu\text{g/g}$. For selenium speciation analysis, selenium compounds were detected through online liquid chromatography–inductively coupled plasma mass spectrometry (LC-ICP-MS) using an ELAN DRC II mass spectrometer (PerkinElmer, Waltham, MA) [1, 3]. The cellular fraction (20 mg) was placed in a 1.5-mL microcentrifuge tube and homogenized in 10 volumes of ultrapure water using a Pellet Mixer Pestle (Treff, Degersheim, Switzerland). After centrifugation at $10,000\times g$ for 5 min, the supernatant of cellular fraction was used for the analysis. Each sample (20 μL) of the cellular fraction supernatant and serum was analyzed using an Ultrahydrogel 120 gel permeation chromatography column ($7.8\times 250\text{ mm}$; Nihon Waters, Tokyo, Japan) equilibrated with 0.1 M ammonium acetate buffer containing 0.1 % (w/v) Igepal CA-630 (Sigma-Aldrich Japan, Tokyo, Japan), as described previously [1, 3]. During separation, selenoneine were eluted with retention times of 10.1 min, and Se-containing proteins including glutathione peroxidase (GPx), selenoprotein P, and other Se-containing proteins were eluted in the void volume of the GPC column at 5.4 min. The concentration of selenium was determined using bovine GPx1 (Sigma-Aldrich) as a standard of Se-containing proteins and bluefin tuna blood selenoneine. Detection limit was $0.002\text{ }\mu\text{g/g}$.

Concentrations of total mercury in the cellular fraction and serum specimens were determined using flameless atomic absorption spectrometry at 253.7 nm using an HG-310

mercury analyzer (Hiranuma, Tokyo, Japan) according to the manufacturer's instructions, following digestion of the sample material (0.1–0.5 g) with 2 mL of a 1:2:1 mixture of nitric acid/perchloric acid/sulfuric acid and dilution in water to 25–125 mL [3]. MeHg and inorganic mercury in the cellular fraction was determined using LC-ICP-MS with a C18 reverse phase column (Atlantis dC18, 3.6×100 mm; Waters Japan, Tokyo) following hydrochloric acid extraction, as described previously [27]. Detection limit was $0.0005 \mu\text{g/g}$.

Methyl-esterified fatty acids in the serum were analyzed using gas liquid chromatography on Shinchrom E71 (Shimadzu Co. Ltd., Kyoto) of glass column (3 mm diameter \times 3 m long).

[22]. Concentrations of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and other fatty acids in the serum were determined using heptadecanoate (C17:0) as an internal standard. The concentration of EPA plus DHA in the serum was used in the analysis as an index of LC-PUFA.

Statistical Analysis Analysis of variance (ANOVA) and correlation analysis were conducted using GraphPad Prism™ 5.03 (GraphPad Software Inc., La Jolla, CA). Multiple regression analysis was done using StatView (ver. 5, SAS Institute, Cary, NC). Statistical significance level was defined as $p < 0.05$.

Results

To characterize the selenium compounds in the RBCs, we determined organic selenium in the cellular fraction by a speciation analysis method based on monitoring ^{82}Se using LC-ICP-MS with a GPC column (Fig. 1). The contents of selenoneine, Se-containing proteins (including GPx, selenoprotein P, and others), and other unidentified selenium compounds in the cellular fraction were also determined; selenite, selenocysteine, and selenomethionine were not detected in the tissues examined (Table 1). Figure 1 shows how the selenoneine in the cellular fraction was eluted at 10.1 min after the bed volume of the column. The highest concentration of selenoneine was $2.38 \mu\text{g Se selenoneine/g}$ in the cellular fraction (Fig. 1a). In this subject, total selenium in the cellular fraction was $2.40 \mu\text{g Se/g}$ (Fig. 1a). In the serum of this subject, selenoneine was below the limit of detection, and selenium was at $0.255 \mu\text{g total Se/g}$. In contrast, in another subject, the lowest detected selenium concentrations in the cellular fraction were $0.006 \mu\text{g selenoneine Se/g}$ and $0.207 \mu\text{g total Se/g}$, respectively, and the selenium concentration in the serum was $0.160 \mu\text{g total Se/g}$ (Fig. 1b). Overall, the cellular fraction contained $0.510 \pm 0.389 \mu\text{g total Se/g}$, $0.212 \pm 0.356 \mu\text{g selenoneine Se/g}$, and $0.262 \pm 0.093 \mu\text{g Se}$

containing proteins Se/g, whereas the serum contained $0.174 \pm 0.038 \mu\text{g total Se/g}$.

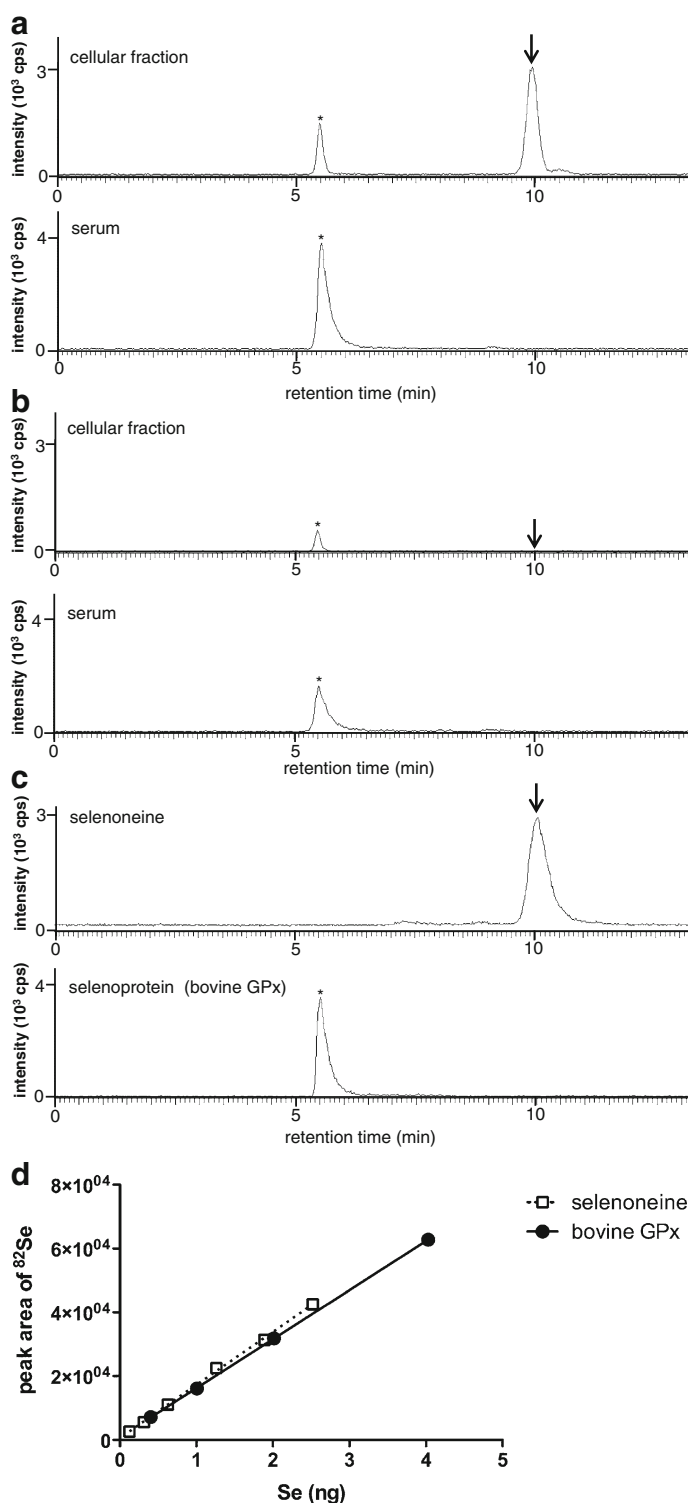
Because we considered the range of concentrations of selenoneine and total selenium observed in the cellular fraction to be due to fish consumption, we compared the concentrations of total selenium, Se-containing proteins, and selenoneine in the cellular fraction and the levels of selenium in the serum with the reported frequency (Fig. 2) and the amount of fish meat per meal (Fig. 3) of fish consumption. For all subjects, the mean concentrations of total selenium in the cellular fraction (p -trend 0.001), selenoneine in the cellular fraction (p -trend 0.005), Se-containing proteins in the cellular fraction (p -trend 0.004), MeHg in the cellular fraction (p -trend 0.008), and mercury in the serum (p -trend 0.009) increased with the frequency of fish consumption (Fig. 2). Subjects who reported consuming fish more than almost every day serving per week had significantly higher concentrations of selenoneine and total selenium in their cellular fraction than those who reported consuming fish under every other day. In addition, concentrations of MeHg in the cellular fraction of subjects who reported consuming more than one or more on the 2 days serving per week were higher than those of subjects who consumed fish under every other day serving of fish. Concentrations of inorganic Hg in the cellular fraction and total selenium and mercury in the serum were not significantly related to the frequency of fish consumption.

The amount of fish meat per meal is also closely related to the selenium and MeHg concentration in the blood. The mean concentrations of total selenium in the cellular fraction (p -trend 0.001), selenoneine in the cellular fraction (p -trend 0.005), Se-containing proteins in the cellular fraction (p -trend 0.004), MeHg in the cellular fraction (p -trend 0.008), and mercury in the serum (p -trend 0.009) increased with the categories of amount of fish meat per meal (Fig. 3). Concentrations of inorganic Hg in the cellular fraction and total selenium and mercury in the serum were not significantly related to the amount of fish meat per meal.

We also analyzed the levels of MeHg and LC-PUFA. In the 167 subjects tested, concentrations of MeHg ranged from 0.022 to $0.469 \mu\text{g total Hg/g}$ (mean \pm SD = $0.087 \pm 0.076 \mu\text{g/g}$). Concentrations of EPA plus DHA ranged from 24 to 585 mg/kg (mean \pm SD = $141 \pm 83 \text{ mg/kg}$). The content of inorganic Hg ranged from 0.0001 to $0.0549 \mu\text{g total Hg/g}$ (mean \pm SD = $0.013 \pm 0.008 \mu\text{g total Hg/g}$). Concentrations of total mercury in the serum ranged from 0.000015 to $0.0203 \mu\text{g/g}$ (mean \pm SD = $0.0042 \pm 0.005 \mu\text{g/g}$).

Concentrations of selenoneine in the cellular fraction were significantly correlated with concentrations of total selenium ($r = 0.91$, $p < 0.0001$; Fig. 4a) and MeHg ($r = 0.58$, $p < 0.0001$; Fig. 4b) in the cellular fraction and total selenium ($r = 0.46$, $p < 0.0001$; Fig. 4c) and LC-PUFA ($r = 0.45$, $p < 0.0001$; Fig. 4d) in the serum. In contrast, concentrations of

Fig. 1 Speciation analysis of organic selenium in the human blood by LC-ICP-MS. Water-soluble selenium compounds were analyzed in the cellular fraction and in the serum. **a** A sample that contains a high concentration of total selenium. **b** A sample that contains a low concentration of total selenium. **c** Standards. An *asterisk* indicates that Se-containing proteins including GPx were eluted close to the void volume of the column. An *arrow* indicates the elution of selenoneine. **d**, Standard curve for selenium detection by LC-ICP-MS. Total counts of ^{82}Se per min in a peak area were accounted for bovine GPx1 (*closed circle*) and purified selenoneine (*open square*)



selenoneine in the cellular fraction did not correlate significantly with concentrations of inorganic mercury or Se-containing proteins in the cellular fraction or levels of total mercury in the serum. The selenium–MeHg molar ratio in the cellular fraction ranged from 3.5:1 to 81.1:1 in all cases examined (mean=41.9:1) (Fig. 5).

Discussion

The present study demonstrates that selenoneine is the major selenium compound in the RBCs of a fish-eating population in Japan. When we compared the concentrations of selenoneine and total selenium in the cellular fraction and the

Table 1 Characteristics of subjects ($n=167$)

Age (years)	63.2 (29–92)
Se in the cellular fraction (μg total Se/g)	0.510 (0.099–2.99)
selenoneine in the cellular fraction (μg selenoneine Se/g)	0.212 (0.006–2.38)
Se-containing proteins in the cellular fraction (μg Se-containing proteins Se/g)	0.262 (0.087–0.777)
Se in the serum (μg total Se/g)	0.174 (0.115–0.316)
MeHg in the cellular fraction (μg total Hg/g)	0.087 (0.022–0.469)
inorganic Hg in the cellular fraction (μg total Hg/g)	0.013 (0.00015–0.0549)
Hg in the serum (μg total Hg/g)	0.0020 (0.000015–0.0203)
EPA + DHA in the serum (mg/kg)	141 (24–585)

Values indicate the geometric mean (range)

concentration of selenium in the serum with the frequency of fish consumption among the 167 people living in these remote islands, we found that levels of the selenoneine in the cellular fraction were closely related to the frequency of fish consumption. In the serum, we detected Se-containing proteins and a selenosugar-like low molecular weight compound, but not selenoneine, and concentrations of selenium in the serum were not correlated with the frequency of fish consumption. Therefore, selenoneine in the cellular fraction is identified as an important biomarker of the intake of selenium from fish consumption in this Japanese fish-eating population.

There have been several reports on concentrations of selenium in the blood of Japanese subjects. Imai et al. reported that concentrations of selenium in the RBCs and serum of

middle-aged Japanese subjects were 0.244 and 0.111 μg Se/g for males and 0.235 and 0.107 μg Se/g for females, respectively [5]. Fish consumption, age, and alcohol intake are related to levels of selenium in the blood. Suzuki et al. reported that concentrations of selenium in the RBCs and serum of Japanese female college students were 0.31 and 0.13 μg Se/g [4]. Alfthan et al. reported that supplementation of selenium-rich yeast increased the concentration of selenium in the plasma from 0.112 to 0.170 $\mu\text{g}/\text{g}$ and the concentration of selenium in the RBCs from 0.189 to 0.356 $\mu\text{g}/\text{g}$ after 11 weeks [12]. Excessive concentrations of selenium in the RBCs, from 0.36 to 1.32 $\mu\text{g}/\text{g}$, have been reported in residents of a high-selenium region of Venezuela with chronic selenosis [6]. The current findings for these remote islands in Kagoshima show total selenium levels of 0.510 $\mu\text{g}/\text{g}$ in the

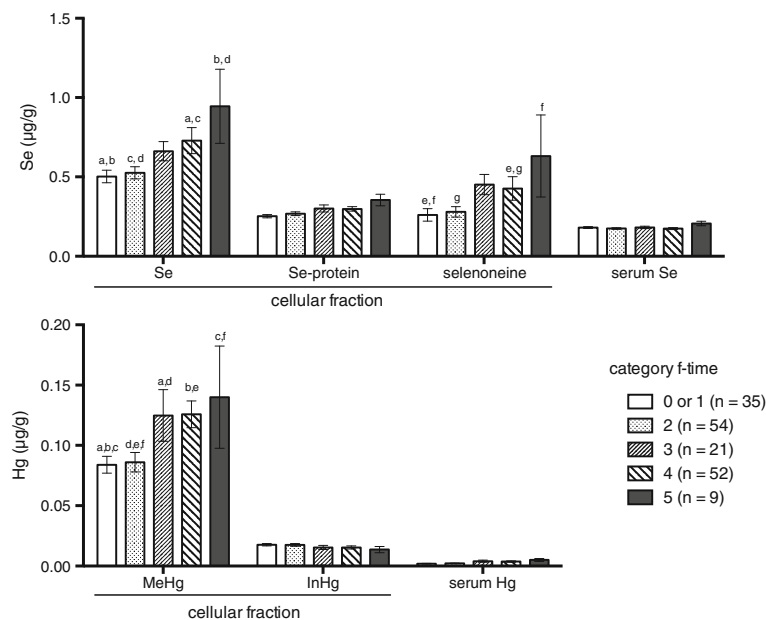
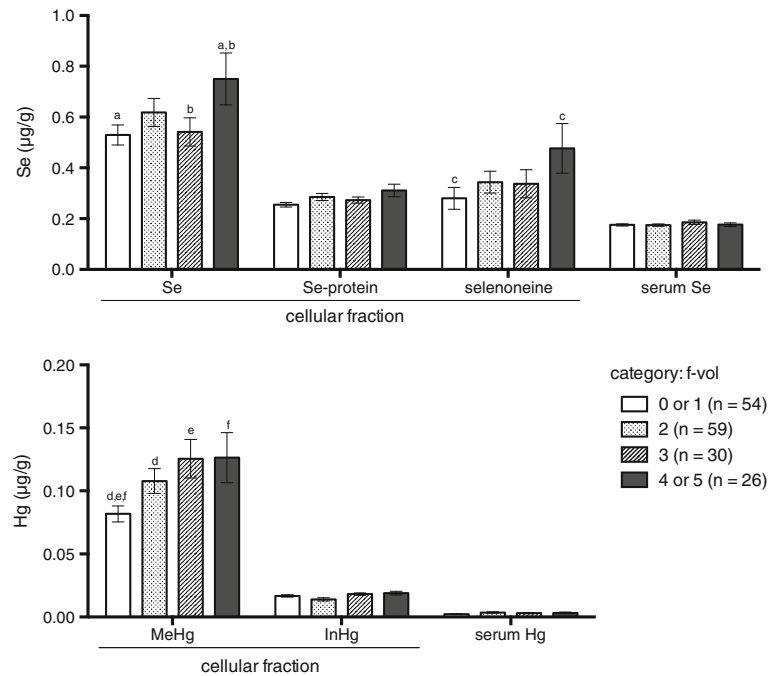


Fig. 2 Mean selenium and mercury concentrations of groups categorized by frequency of fish consumption. Concentrations of total selenium, selenoneine, and Se-containing proteins (Se protein) in the cellular fraction, total selenium in the serum, MeHg and inorganic mercury in the cellular fraction, and total mercury in the serum were compared to the frequency of fish consumption. The subjects were divided into six

categories consuming fish meal: less than once per week = f-time 0, once per week = f-time 1; two or three times per week (less than once per 2 days) = f-time 2, three or four times per week (once per 2 days) = f-time 3, everyday (five to eight times per week) = f-time 4, every time (more than nine times per week) = f-time 5. a–g, significant differences between the mean values (ANOVA)

Fig. 3 Mean selenium and mercury concentrations of groups categorized by volume of fish consumption. Concentrations of total selenium, selenoneine, and Se-containing proteins (Se protein) in the cellular fraction, total selenium in the serum, MeHg and inorganic mercury in the cellular fraction, and total mercury in the serum were compared to the volume of fish consumption. The subjects were divided into five categories of the amount of fish meat per meal: 25 g per meal = f-mass 1, 50 g per meal = f-mass 2, 75–100 g per meal = f-mass 3, 150 g per meal = f-mass 4, 200 g or more per meal = f-mass 5. a–f, significant differences between the mean values (ANOVA)

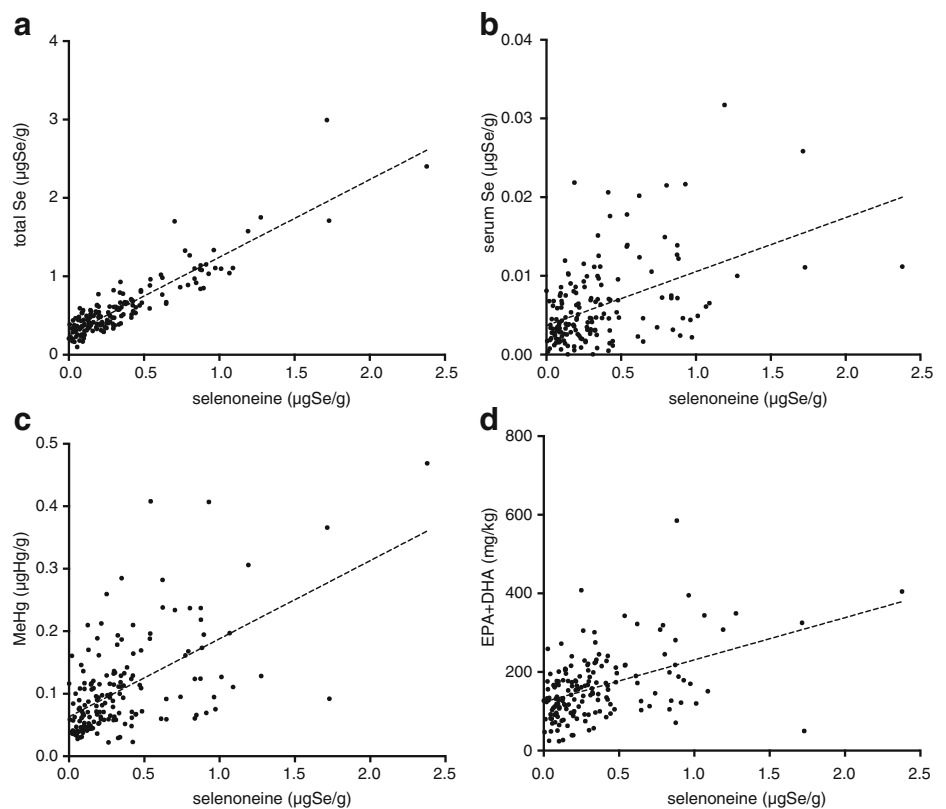


cellular fraction and $0.174 \mu\text{g/g}$ in the serum, representing the highest exposure to selenium among previous reports on chronic selenosis in the USA, China, Colombia, and Venezuela. In the case of selenosis, selenite or selenomethionine were incorporated [6]. However, the current results indicate that the chemical form of selenium was

selenoneine derived from fish consumption, which is distinct from the other reported cases of selenosis [6].

Recent findings from human trials have raised concern that high long-term selenium supplementation may be associated with type 2 diabetes or insulin resistance [28, 29]. Type 2 diabetes is associated with oxidative stress attributable to the

Fig. 4 Relationships between concentrations of selenoneine and total selenium in the cellular fraction (a), total selenium in the serum (b), MeHg in the cellular fraction (c), or LC-PUFA (EPA + DHA) (d)



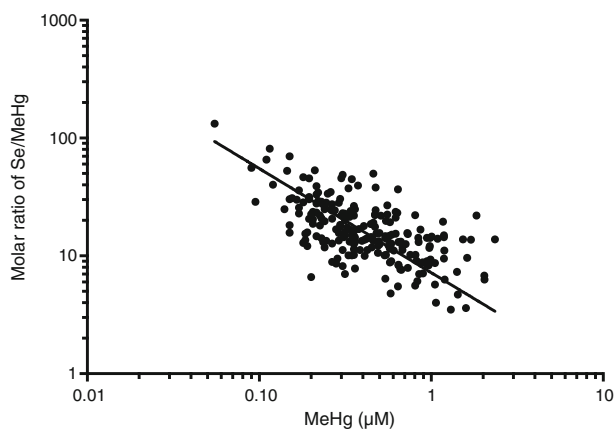


Fig. 5 Relationship between MeHg concentration and Se/MeHg molar ratio in cellular fraction

production of excess levels of reactive oxygen species in hyperglycemia [30–32]. Selenium supplementation induces selenoproteins, such as GPx1 and selenoprotein P [31, 32]. High activity of GPx1 can interfere with insulin signaling by removing hydrogen peroxide that acts as a second messenger [30–32]. Selenoprotein P is also implicated in insulin resistance [33]. Selenoprotein P impaired insulin signaling in the liver and skeletal muscle in mouse and induced glucose intolerance [33]. In contrast, mice deficient in selenoprotein P showed improved glucose tolerance and enhanced insulin signaling [33]. Selenium yeast is predominantly selenomethionine, a form that is not abundant in ocean fish [34]. Since long-term selenium supplementation with selenium yeast is linked to the development of type 2 diabetes, further research is required to characterize whether higher selenium intake by fish and seafood consumption may be associated with type 2 diabetes risk. According to a recent large-scale, population-based cohort study in Japan, fish consumption was associated with a lower risk of type 2 diabetes in men but not in women [35]. Fish intake was not associated with an increased risk of type 2 diabetes in either men or women [35]. Therefore, selenoneine intake by fish consumption might not associate with the increased risk of type 2 diabetes that found in long-term selenium supplementation with selenium yeast.

Selenoneine is incorporated into animal cells through the OCTN1 transporter [21]. RBCs express the OCTN1 transporter and accumulate selenoneine. The RBCs of marine animals, such as bluefin tuna [15] and dolphins (Yamashita et al., unpublished), contain high levels of selenoneine ($>1 \mu\text{g Se/g}$). Anan also reported selenoneine in the liver of turtle [36]. Klein et al. reported a selenoneine-derivative S-methylselenoneine in human urine [37]. Mammalian OCTN1 is thought to be involved in the secretion of cations in renal proximal tubules and the small intestine [38]. Mutations and variations of the OCTN1 gene and selenium status are linked to Crohn's disease [39–41]. Further investigation will require surveying a relationship between the susceptibility to Crohn's

disease in patients with OCTN1 variations and the dietary selenium intake from seafood.

Previously, contents of selenium in the serum and levels of total selenium in hair and toenail samples were measured as a biomarker of selenium in a human population [42–46]. Plasma selenoprotein P and GPx levels were used as biomarkers for selenium supplementation [42]. However, the current study clearly demonstrates that the level of selenoneine in the RBCs should be determined as a biomarker for selenium intake and redox status by fish consumption.

Furthermore, levels of selenoneine in the cellular fraction are closely related to levels of MeHg in the cellular fraction and levels of LC-PUFA in the serum. Previously, mercury levels in the cellular fraction showed a significant positive correlation with selenium levels, suggesting that Se potentially can work to moderate the toxicity of MeHg [47]. Blood MeHg and LC-PUFA are also confirmed to be correlated with fish consumption [22–25]. The selenium–MeHg molar ratio in the cellular fraction ranged from 3.5:1 to 81.1:1 (mean=41.9:1), suggesting that excess amounts of selenium against MeHg contents might be present in the cellular fraction and reduce MeHg toxicity (Fig. 5). High mercury exposure risks by fish consumption may depend on dietary selenium intakes and selenium status, as pointed out by Raymond et al. [18]. To estimate the MeHg risks associated with fish consumption, animal trials of feeding with both MeHg and selenoneine for selenium–MeHg molar ratios of $>3.5:1$ in the cellular fractions will be conducted. Selenoneine mediated MeHg demethylation and excretion in human cultured cells and zebrafish embryos [21]. Further study is required to obtain evidences that inorganic mercury might be produced by exosomal secretion mechanisms mediated by selenoneine and OCTN1 in humans.

In conclusion, selenoneine was the major chemical form of selenium in the cellular fraction of this fish-eating human population. Concentrations of selenoneine and total selenium in the cellular fraction might be used as an important biomarker for fish consumption and selenium antioxidant and detoxification functions.

Acknowledgments We thank T. Suzuki and T. Hara for selenium and mercury analysis. This work was supported in part by grants from the Japan Society for the Promotion of Science, the Fisheries Research Agency, and the Ministry of Agriculture, Forestry and Fisheries of Japan.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. Yamashita Y, Yamashita M (2010) Identification of a novel selenium-containing compound, selenoneine, as the predominant chemical form of organic selenium in the blood of bluefin tuna. *J Biol Chem* 285(24):18134–18138

2. Yamashita Y, Yabu T, Yamashita M (2010) Discovery of the strong antioxidant selenoneine in tuna and selenium redox metabolism. *World J Biol Chem* 1(5):144–150
3. Yamashita Y, Amlund H, Suzuki T, Hara T, Hossain MA, Yabu T, Touhata K, Yamashita M (2011) Selenoneine, total selenium, and total mercury content in the muscle of fishes. *Fish Sci* 77(4):679–686
4. Suzuki T, Hongo T, Ohba T, Kobayashi K, Imai H, Ishida H, Suzuki H (1989) The relation of dietary selenium to erythrocyte and plasma selenium concentrations in Japanese college women. *Nutr Res* 9: 839–848
5. Imai H, Suzuki T, Kashiwazaki H, Takemoto T, Izumi T, Moji K (1990) Dietary habit and selenium concentrations in erythrocyte and serum in a group of middle-aged and elderly Japanese. *Nutr Res* 10: 1205–1214
6. Gerald F Jr, Combs SB (1986) *The role of selenium in nutrition*. Academic, New York
7. Fairweather-Tait SJ, Collings R, Hurst R (2010) Selenium bioavailability: current knowledge and future research requirements. *Am J Clin Nutr* 91(5):S1484–S1491
8. Hagmar L, Persson-Moschos M, Akesson B, Schütz A (1998) Plasma levels of selenium, selenoprotein P and glutathione peroxidase and their correlations to fish intake and serum levels of thyrotropin and thyroid hormones: a study on Latvian fish consumers. *Eur J Clin Nutr* 52(11):796–800
9. Fox TE, Van den Heuvel EG, Atherton CA, Dainty JR, Lewis DJ, Langford NJ, Crews HM, Lutén JB, Lorentzen M, Sieling FW, van Aken-Schneyder P, Hoek M, Kotterman MJ, van Dael P, Fairweather-Tait SJ (2004) Bioavailability of selenium from fish, yeast and selenate: a comparative study in humans using stable isotopes. *Eur J Clin Nutr* 58(2):343–349
10. Fox TE, Atherton C, Dainty JR, Lewis DJ, Langford NJ, Baxter MJ, Crews HM, Fairweather-Tait SJ (2005) Absorption of selenium from wheat, garlic, and cod intrinsically labeled with Se-77 and Se-82 stable isotopes. *Int J Vitam Nutr Res* 75(3):179–186
11. McAdam PA, Orville LA (1987) Chronic toxicity and retention of dietary selenium fed to rats as D- or L-selenomethionine, selenite, or selenate. *Nutr Res* 7:601–610
12. Alfthan G, Aro A, Arvilommi H, Huttunen JK (1991) Selenium metabolism and platelet glutathione peroxidase activity in healthy Finnish men: effects of selenium yeast, selenite, and selenate. *Am J Clin Nutr* 53(1):120–125
13. Rayman MP, Infante HG, Sargent M (2008) Food-chain selenium and human health: spotlight on speciation. *Br J Nutr* 100(2):238–253
14. Ganther HE, Goudie C, Sunde ML, Kopecky MJ, Wagner P (1972) Selenium: relation to decreased toxicity of methylmercury added to diets containing tuna. *Science* 175(4026):1122–1124
15. Cuvin-Aralar ML, Furness RW (1991) Mercury and selenium interaction: a review. *Ecotoxicol Environ Saf* 21(3):348–364
16. Suzuki T, Imura N, Clarkson TW (1991) *Advances in mercury toxicology*. Plenum, New York
17. Ralston NV, Ralston CR, Blackwell JL 3rd, Raymond LJ (2008) Dietary and tissue selenium in relation to methylmercury toxicity. *Neurotoxicology* 29(5):802–811
18. Raymond LJ, Ralston NVC, Seale LA (2011) Seafood selenium in relation to assessments of methylmercury exposure risks. In: Hatfield DL, Berry MJ, Gladyshev VN (eds) *Selenium: its molecular biology and role in human health*. Springer, New York, pp 399–408
19. FAO (2011) *Fisheries and Aquaculture Report No. 978 Report of the Joint FAO/WHO Expert Consultation on the Risks and Benefits of Fish Consumption*
20. Mozaffarian D (2009) Fish, mercury, selenium and cardiovascular risk: current evidence and unanswered questions. *Int J Environ Res Public Health* 6(6):1894–1916
21. Yamashita M, Yamashita Y, Suzuki T, Kani K, Mizusawa N, Imamura S, Takemoto T, Hara T, Hossain MA, Yabu T, Touhata K (2013) Selenoneine, a novel selenium-containing compound, mediates detoxification mechanisms against methylmercury accumulation and toxicity in zebrafish embryo. *Mar Biotechnol* 15(5):559–570
22. Andoh T, Uda H, Yoshimitsu N, Hatano H, Ueda T, Iwamatsu Y, Akiba S (1997) The sex differences in cord-blood cholesterol and fatty-acid levels among Japanese fetuses. *J Epidem* 7(4):226–231
23. Wakisaka I, Ando T, Yanagihashi T (1986) Interrelationships among hair mercury levels, fish-eating habits and fatty acid composition of serum lipid. *Jpn J Hyg* 41:828–835
24. Wakisaka I, Ando T, Yanagihashi T, Tominaga K (1986) Fatty acid composition of serum lipids of remote islanders in Kagoshima Prefecture. *Jpn J Public Health* 33:11
25. Tsuji M, Ando T, Kitano T, Wakamiya J, Koriyama C, Akiba S (2012) Relationship between RBC mercury levels and serum n3 polyunsaturated fatty acid concentrations among Japanese men and women. *J Environ Pub Health* 2012; Article ID 849305
26. Hasunuma R, Ogawa T, Kawanishi Y (1982) Fluorometric determination of selenium in nanogram amounts in biological materials using 2,3-diaminonaphthalene. *Anal Biochem* 126:242–245
27. Friberg L, Mottet NK (1989) Accumulation of methylmercury and inorganic mercury in the brain. *Biol Trace Elem Res* 21: 201–206
28. Stranges S, Marshall JR, Natarajan R, Donahue RP, Trevisan M, Combs GF, Cappuccio FP, Ceriello A, Reid ME (2007) Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. *Ann Intern Med* 147(4):217–223
29. Rayman MP, Stranges S (2013) Epidemiology of selenium and type2 diabetes: can we make sense of it? *Free Radic Biol Med*. doi:10.1016/j.freeradbiomed.2013.04.003
30. Ezaki O (1990) The insulin-like effects of selenite in rat adipocytes. *J Biol Chem* 265(2):1124–1128
31. Steinhilber H, Speckmann B, Pinto A, Sies H (2011) High selenium intake and increased diabetes risk: experimental evidence for interplay between selenium and carbohydrate metabolism. *J Clin Biochem Nutr* 48(1):40–45
32. Chung SS, Kim M, Youn BS, Lee NS, Park JW, Lee IK, Lee YS, Kim JB, Cho YM, Lee HK, Park KS (2009) Glutathione peroxidase 3 mediates the antioxidant effect of peroxisome proliferator-activated receptor gamma in human skeletal muscle cells. *Mol Cell Biol* 29(1): 20–30
33. Wang XD, Vatamaniuk MZ, Wang SK, Roneker CA, Simmons RA, Lei XG (2008) Molecular mechanisms for hyperinsulinaemia induced by overproduction of selenium-dependent glutathione peroxidase-1 in mice. *Diabetologia* 51(8):1515–1524
34. Schrauzer G (2006) Selenium yeast: composition, quality, analysis, and safety. *Pure Appl Chem* 78(1):105–109
35. Nanri A, Mizoue T, Noda M, Takahashi Y, Matsushita Y, Poudel-Tandukar K, Kato M, Oba S, Inoue M, Tsugane S (2011) Japan public health center-based prospective study group. Fish intake and type 2 diabetes in Japanese men and women: the Japan public health center-based prospective study. *Am J Clin Nutr* 94(3):884–891
36. Anan Y, Ishiwata K, Suzuki N, Tanabe S, Ogra Y (2011) Speciation and identification of low molecular weight selenium compounds in the liver of sea turtles. *J Anal At Spectrom* 26:80–85
37. Klein M, Ouerdane L, Bueno M, Pannier F (2011) Identification in human urine and blood of a novel selenium metabolite, S-methylselenoneine, a potential biomarker of metabolism in mammals of the naturally occurring selenoneine, by HPLC coupled to electrospray hybrid linear ion trap-orbital ion trap MS. *Metallomics* 3(5):513–520
38. Kato Y, Kubo Y, Iwata D, Kato S, Sudo T, Sugiura T, Kagaya T, Wakayama T, Hirayama A, Sugimoto M, Sugihara K, Kaneko S, Soga T, Asano M, Tomita M, Matsui T, Wada M, Tsuji A (2010) Gene knockout and metabolome analysis of carnitine/organic cation transporter OCTN1. *Pharm Res* 27(5):832–840

39. Taubert D, Grimberg G, Jung N, Rubbert A, Schömig E (2005) Functional role of the 503F variant of the organic cation transporter OCTN1 in Crohn's disease. *Gut* 54(10):1505–1506
40. Rannem T, Ladefoged K, Hylander E, Hegnhj J, Jarnum S (1992) Selenium status in patients with Crohn's disease. *Am J Clin Nutr* 56(5):933–937
41. Xuan C, Zhang BB, Yang T, Deng KF, Li M, Tian RJ (2012) Association between OCTN1/2 gene polymorphisms (1672C-T, 207G-C) and susceptibility of Crohn's disease: a meta-analysis. *Int J Colorectal Dis* 27(5):11–19
42. Burk RF, Norsworthy BK, Hill KE, Motley AK, Byrne DW (2006) Effects of chemical form of selenium on plasma biomarkers in a high-dose human supplementation trial. *Cancer Epidemiol Biomarkers Prev* 15(4):804–810
43. Mozaffarian D, Shi P, Morris JS, Spiegelman D, Grandjean P, Siscovick DS, Willett WC, Rimm EB (2011) Mercury exposure and risk of cardiovascular disease in two US cohorts. *N Engl J Med* 364(12):1116–1125
44. Longnecker MP, Stampfer MJ, Morris JS et al (1993) A 1-y trial of the effect of high-selenium bread on selenium concentrations in blood and toenails. *Am J Clin Nutr* 57(3):408–413
45. Longnecker MP, Stram DO, Taylor PR, Levander OA, Howe M, Veillon C, McAdam PA, Patterson KY, Holden JM, Morris JS, Swanson CA, Willett WC (1996) Use of selenium concentration in whole blood, serum, toenails, or urine as a surrogate measure of selenium intake. *Epidemiology* 7(4):384–390
46. Koriyama C, Campos FI, Yamamoto M, Serra M, Carrasquilla G, Carrascal E, Akiba S (2008) Toenail selenium level and gastric cancer risk in Cali, Colombia. *J Toxicol Sci* 33(2):227–235
47. Sakamoto M, Murata K, Kubota M, Nakai K, Satoh H (2010) Mercury and heavy metal profiles of maternal and umbilical cord RBCs in Japanese population. *Ecotoxicol Environ Saf* 73(1):1–6