Distribution and Effects of Selenoneine by Ingestion of Extract from Mackerel Processing Residue in Mice

Research

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Abstract

Selenoneine is an organic selenium compound contained in blood and dark muscle of fish. It has a strong antioxidative capacity and is considered useful as a new functional food material. However, the distribution and effects of selenoneine in the mammalian body have not been thoroughly examined. In this study, a selenoneine-rich mackerel extract was developed and fed to mice at 0.07% in standard rodent chow (ME diet) for 32 days to examine its distribution in the body. Selenoneine was distributed in the liver, kidney, and spleen in mice fed the mackerel extract, but it was not distributed in the plasma or erythrocytes. Moreover, concentrations of the major selenium-containing protein were not affected by the mackerel extract. The results of this study suggest that selenoneine is absorbed in the body following ingestion of low doses in crude material and preferentially accumulates in organs and later distributes in erythrocytes. Biochemical analyses of plasma in male mice showed that the glucose level was significantly increased and LDL-cholesterol level was significantly decreased by ME diet feeding. The results indicate that male mice are sensitive to ME diet.

Keywords

selenium, ergothioneine, OCTN1, spleen, kidney, erythrocyte

52 Introduction

53 Selenoneine (Fig. 1) is an organic selenium compound contained in seafood such as
54 tuna and mackerel (Yamashita and Yamashita 2010; Yamashita et al. 2011).
55 Selenoneine has stronger antioxidative capacity than its sulfur analogue,
56 ergothioneine, in the 1-diphenyl-2-picrylhydrazyl radical-scavenging assay (Yamashita
57 and Yamashita 2010). Recent studies reported that selenoneine has beneficial health
58 effects such as inhibition of melanin synthesis and amelioration of hepatocellular
59 injury and hepatic steatosis (Seko et al. 2020; Miyata et al. 2020). Moreover, in a study
60 in which fish were fed selenoneine containing diet, the oxidative–redox potential in
61 muscle was decreased and negatively correlated with the selenium concentration in
62 blood and muscle of them (Tofuku et al. 2021). These studies indicate that selenoneine
63 is considered to have potential as a useful new functional food or supplement.

64 Accumulation of selenoneine in tissues *in vivo* has been also studied. A study
65 conducted on a remote island in Kagoshima Prefecture, Japan, found high
66 concentrations of selenoneine in the erythrocytes of individuals who ate fish frequently
67 (Yamashita et al. 2013). A Canadian study also reported accumulation of selenoneine in
68 erythrocytes of individuals who ate seafood high in selenoneine (Achouba et al. 2019;
69 Little et al. 2019). In a study in which mice were fed purified selenoneine revealed
70 accumulation in erythrocytes and the liver (Miyata et al. 2020). These reports suggest
71 that selenoneine accumulates in the body following consumption of selenoneine-rich
72 foods. Selenoneine is taken up into cells via an ergothioneine transporter known as
73 OCTN1 (Yamashita et al. 2013). Thus, selenoneine is predicted to be taken up in
74 tissues expressing OCTN1 and accumulate with a distribution similar to that of
75 ergothioneine (Tang et al. 2018). However, the accumulation of selenoneine *in vivo* is
76 not fully understood.

77 Some Japanese companies have recently developed selenoneine-containing

78 ingredients for use in functional foods from fish residues such as tuna dark muscle or
79 mackerel heads and organs, as these sources contain high concentrations of
80 selenoneine (Yamashita and Yamashita 2010; Yamashita et al. 2011). However,
81 information regarding the accumulation, safety and health effects of selenoneine *in*
82 *vivo* following consumption of these crude materials is lacking.
83 In this study, mice were fed a powdered extract prepared from the residues of
84 canned mackerel as a source of crude selenoneine. Mice were fed the extract for 32
85 days, after which the accumulation of selenoneine in the blood and tissues and the
86 safety based on the body and tissue weights were examined. Biochemical analyses of
87 plasma were conducted to identify new functions of selenoneine-rich extracts from
88 mackerel residues.
89
90 Materials and methods
91 Preparation of powdered extract from mackerel
92 Powdered extract from mackerel was prepared in reference to a patent (Yamashita
93 et al. 2021) by BioChem. Corporation (Saitama, Japan). Fifty kilograms of frozen
94 residue of mackerel, including head, mid-bone, and internal organs, was chopped and
95 heated with 200 L of water at 80°C for 10 min. The preparation was then filtered using
96 a vibration sieve, 0.154-mm mesh, to remove solids. The resulting filtrate was mixed
97 with 5% charcoal, 2% Japanese acid clay (Fujifilm Wako, Tokyo, Japan), and 2% silica
98 gel at 55°C and filtered with 6 kg of diatomite and a filter cloth. The filtrate was
99 sterilized at 85°C for 10 min. A total of 185 L of the filtrate was mixed with 200 mg of
100 urease from Jack bean (Fujifilm Wako), and an NF270 reverse osmosis membrane
101 (Dow Chemical Co., MI, USA) was used to separate the filtrate into transparent and
102 non-transparent fractions. The two fractions were re-mixed, and 100 mg of pronase
103 (Roche, Basel, Switzerland) was added to the solution. The solution was separated

using a reverse osmosis membrane, and the non-transparent fraction was concentrated. The concentrated fraction was filtered using 1.0 kg of silica gel and No. 5A filter paper (Advantech, Tokyo, Japan) to remove the precipitate. The filtrate was concentrated using an evaporator and powdered with dextrin using a spray dryer.

Chemical analysis of the powdered extract

The water, crude protein, crude fat, carbohydrate, and crude ash contents of the powdered extract were measured by Shokukanken Inc. (Gunma, Japan). Water, crude protein, crude fat, and crude ash were measured by air drying method, combustion method (modified Duma's method), acid hydrolysis method, and direct ashing method at 550°C, respectively. Carbohydrates were calculated by the difference method, subtracting the total weight of water, crude protein, crude lipids, and crude ash. The methods were referenced from the Standard Tables of Food Composition in Japan (Ministry of Education, Culture, Sports, Science and Technology, Japan, 2015).

Free amino acids analysis

The free amino acid composition was analyzed according to previous studies (Murata et al. 1994, 2020), with slight modifications. Powdered extract (0.2 g) was dissolved in 20 mL of distilled water, and the solution was diluted twice with 0.04 mol/L HCl and filtered with a 0.2-μm filter membrane (Millex-LG, Merck, Darmstadt, Germany) prior to analysis. Free amino acids were analyzed using an automatic amino acid analyzer (L-8900, High-Technologies Corp., Tokyo, Japan).

Total amino acids analysis

Total amino acids in the powdered extract were analyzed according to a previous study (Suzuki and Yasui 1995), with slight modification. The powdered extract

(80 mg) was dissolved in 1.0 mL of 6.0 mol/L HCl and heated at 145°C for 4 hours. The solution was filled with water to 50 mL and dried up with evaporator at 50°C for removing HCl. It was dissolved by 0.02 mol/L HCl and filtered with 0.2-μm filter membrane (Millex-LG, Merck, Darmstadt, Germany) prior to analysis. Amino acids in peptides were analyzed using an automatic amino acid analyzer (L-8900, High-Technologies Corp., Tokyo, Japan). Each analytical cycle was performed twice, and the mean of the two resulting values was used.

Main and trace elements analysis

Main and trace elements analysis was conducted in reference to a previous study (Iguchi et al. 2014) with some modifications. The powdered extract (30 mg) was dissolved in 5.0 mL of 61% HNO_3 and 3.0 mL of 30 % H_2O_2 and heated using a microwave digestion system, NovaWAVE (SCP SCIENCE, Quebec, Canada). The digestion program was rising to 100 °C for 10 min from a room temperature, kept for 10 min, followed by a rate of 8 °C/min, kept at 180 °C for 10 min, and followed by a rate of 6 °C/min, kept at 240 °C for 10 min. The solutions were diluted with MilliQ water and placed into 50-ml tubes with Be, Sc, Ge, In, and Bi as internal standards to give a concentration of 10 μg/L. Elements in the solution were analyzed by an inductively coupled plasma–atomic emission spectrometry (ICP-AES, ICPE-9000, Shimadzu, Kyoto, Japan) and inductively coupled plasma–mass spectrometry (ICP-MS, ELAN DRC II, PerkinElmer, Inc., Waltham, MA, USA) as per a previous study (Iguchi et al. 2014). Na, Mg, Ca and Fe were quantified by ICP-AES using an absolute calibration method. Trace elements (Li, V, Mn, Ni, Cu, Zn, As, Se, Rb, Mo, Cd, Ba, Pb) were quantified by ICP-MS using an internal standard method.

Online liquid chromatography–ICP-MS (LC-ICP-MS) conditions for selenoneine

156 analysis

157 Selenoneine in samples was quantified using online LC (GL Science, Tokyo, Japan)
158 with ICP-MS (ELAN DRC II, PerkinElmer, Inc., Waltham, MA, USA) using a system
159 equipped with a concentric quartz nebulizer (WE02-4371, PerkinElmer, Inc.) and a
160 sample injector (2-mm inner diameter, quartz) under previously reported LC conditions
161 (Yamashita and Yamashita 2010). Separation was achieved on an Ultrahydrogel-120
162 column (7.8 × 300 mm, Nihon Waters, Tokyo, Japan) equilibrated with 0.1 M
163 ammonium acetate aqueous solution containing 0.1% IGEPAL (Sigma-Aldrich, MO,
164 USA) at a flow rate of 1.0 mL/min; column temperature was maintained at 40°C using
165 a column oven. The ICP-MS conditions were as previously described, and *m/z* 82 was
166 monitored as selenium (Se-82). The plasma and auxiliary argon gas flow rates were 15
167 and 0.8 L/min, respectively. The nebulization argon gas flow rate was 1.13 L/min. The
168 radio-frequency power was 1500 W. Under these LC-ICP-MS conditions, selenoneine
169 eluted at a retention time of 9.6 min.

170

171 Feed preparation

172 A standard rodent chow diet, MF (Oriental Yeast Co., Tokyo, Japan), was used as
173 the control diet (Control). Powdered extract was mixed with the MF standard rodent
174 chow diet at 0.7% of total weight and designated the mackerel extract (ME) diet.

175

176 Animal experiments

177 Animal experiments were approved by the Fisheries Technology Institute,
178 National Research and Development Agency, Kanagawa, Japan (Permission number:
179 H29-1) and performed according to the Guideline for the Ethical Treatment of
180 Laboratory Animals of the institute. Male and female 5-week-old mice (Slc: ICR) were
181 purchased from Japan SLC, Inc. (Shizuoka, Japan). The male and female mice were

182 weighed and grouped according to equivalent body weight and fed the control diet for 1
183 week before beginning the dietary modification experiment. Male and female mice were
184 fed the control or ME diet for 32 days (control and ME mice, respectively). Both diets in
185 the feeder were changed twice each week. Body weight and food consumption were
186 monitored every 3 or 4 days. Mice were kept in an environmentally controlled room at
187 a temperature of 22°C and humidity of 55% with a 12-h light and dark cycle
188 throughout the experiment.
189
190 Sample preparation
191 After feeding for 32 days, the mice were fasted for 12 h and dissected under
192 isoflurane anesthesia (Pfizer, NY, USA). Blood was collected from the inferior vena
193 cava using a 1-mL syringe rinsed with heparin lithium (Wako, Tokyo, Japan). The
194 blood was centrifuged (1000 × g, 10 min, 20°C), and supernatant and precipitate (blood
195 cells) were used as plasma and erythrocytes (major cellular composition of blood cells),
196 respectively. The collected liver, spleen, and kidney were weighed. Samples were stored
197 at −80°C until analysis.
198
199 Sample preparation for LC-ICP-MS
200 Erythrocytes and plasma were diluted using a 10-fold volume of MilliQ water and
201 then centrifuged (1021 × *g*, 10 min, 4°C). Tissues were diluted with a 10-fold volume of
202 MilliQ water, homogenized by pestle, and centrifuged (1021 × *g*, 10 min, 4°C). The
203 supernatant was injected into the LC-ICP-MS instrument, and the concentration of
204 selenoneine was calculated from a standard curve of purified selenoneine synthesized
205 by genetically modified fission yeast (Seko et al. 2021).
206
207 Biochemical examination of plasma

208 Total protein (TP), albumin (ALB), albumin-globulin ratio (A/G ratio), total
209 bilirubin (T-Bil), aspartate aminotransferase (AST), alanine aminotransferase (ALT),
210 alkaline phosphatase (ALP), total cholesterol (T-cho), triglycerides (TGs), creatinine
211 (Crea), calcium (Ca), glucose, sodium (Na), potassium (K), chloride (Cl), urea nitrogen
212 (UN), low-density-lipoprotein (LDL)-cholesterol and high-density-lipoprotein (HDL)-
213 cholesterol in plasma were analyzed by the Safety Research Institute for Chemical
214 Compounds Co., Ltd. (Hokkaido, Japan). TP was measured using the Biuret method.
215 ALB was measured using the BCG method. A/G ratio was calculated from TP and ALB
216 (A/G ratio = ALB/[TP-ALB]). T-Bil, T-cho, and Crea were measured using enzymatic
217 methods. AST, ALT, and ALP were measured according to the Japan Society of Clinical
218 Chemistry method. TGs were measured using the free glycerol elimination method. Ca
219 was measured using the *ortho*-cresolphthalein complexone method. Na, K, and Cl were
220 measured using the Ion Selective Electrode method. UN was measured using the
221 urease/glutamate dehydrogenase method. LDL was measured using the selective
222 solubilization method, and HDL was measured using the selective suppression method.
223 These analyses were carried out on a Hitachi 7080 Chemistry Analyzer (Hitachi, Ltd.,
224 Tokyo, Japan).
225
226 Statistical analyses
227 Values are expressed as the mean ± SD. Differences in means were analyzed using
228 the Student's *t*-test, $P < 0.05$ indicating significance. Data were analyzed using Excel in
229 Microsoft 365 (Microsoft, WA, USA) or GraphPad Prism, version 9 (GraphPad
230 Software, CA, USA).
231
232 Results
233 Components of powdered extract and feed for mice

The general components of the feed, including water, crude protein, crude fat, carbohydrates, and crude ash, are shown in Table 1; the main component was crude protein. The free amino acid and total amino acid compositions are shown in Table 2, and the total amount of free amino acids was 44.5 g/100 g and that of total amino acids was 63.4 g/100g. Non-protein nitrogen compounds were quantified in free amino acids analysis simultaneously (Table S2). Main and trace element compositions are shown in Table 3 and selenoneine concentration is 0.270 mg Se/100g also exhibited (Table 3). In addition to arsenic and cadmium (Table 3), harmful chemicals regarded as problematic in seafood including histamine and mercury were quantified and shown in supplement data (Table S2). Components of the control and ME diets are shown in Table 4. The components of control diet are in reference to the components of MF, as disclosed by Oriental Yeast Co. (Tokyo, Japan). The components of ME diet were calculated from those of the control diet and 0.7% powdered extract. There were no markedly differences between the diets except for selenoneine.

Food consumption and body weight gain

Daily food consumption did not differ between the control and ME diets in male and female mice (Fig. 2a). Daily body weight gain (%) from day 0 was not different between male and female mice on the ME diet (Fig. 2b).

Organ weight

Organ weights were expressed as the organ weight/body weight ratio (%) (Fig. 3). The ME diet had no effect on the organ weight ratios of the liver, spleen, and female kidney (Fig. 3a-c). The organ weight ratio of the kidney in male mice fed the ME diet was significantly lower than that of control mice (Fig. 3b).

Measurement of selenium compounds

Selenium included in protein and selenoneine in plasma, erythrocytes, liver, kidney (n = 5), and spleen (n = 4) were measured by LC-ICP-MS (Fig. 4). Selenium compounds in the spleen of one control mouse and one ME-fed mouse could not be quantified because good chromatograms could not be obtained due to poor storage condition of the spleens. Selenium in protein and selenoneine was quantified based on a standard curve of purified selenoneine. The values of limit of detection (LOD) and limit of quantification (LOQ) for selenium in protein and selenoneine were represented those for selenoneine. They were 0.259 nmol Se/g and 1.30 nmol Se/g calculated from the ratios of signal and noise (LOD: S/N = 2, LOQ: S/N = 10). The levels of selenium included in protein in plasma, erythrocytes, and organs were not affected by the ME diet (Table 5a). Selenoneine was not detected in plasma or erythrocytes from any of the mice, but it was detected in the liver, kidney, and spleen in both male and female ME-fed mice (Table 5b). Comparing the male and female mice fed the ME diet, the selenoneine level in the kidneys of female mice was 2.26-fold higher than that in male mice ($P < 0.05$). The distribution of selenoneine in the kidney and spleen relative to the liver was calculated from the selenoneine concentration (Table 5b), and the results are shown in Table 6. The relative distributions (liver: kidney: spleen) were 1.00: 0.275 ± 0.0794: 0.198 ± 0.0863 in male and 1.00: 0.434 ± 0.0974: 0.229 ± 0.0825 in female mice. The values for the kidney and spleen were not markedly different from those reported for ergothioneine calculated from a previous study (Tang et al. 2018, Table 6). The ratios of selenoneine accumulation in the organs relative to total intake calculated from food consumption data (Fig. 2a) are shown in Table 6. The values in the liver were 1.60 ± 0.431% in male and 1.85 ± 0.607% in female mice. Those in kidney were 0.432 ± 0.145% in male and 0.768 ± 0.128% in female mice, significantly higher than that in male mice. The values in the spleen were 0.270 ± 0.0798% in male and 0.377 ± 0.154%

286 in female mice.

287

288 Biochemical analyses of plasma

289 Results of biochemical analyses of plasma are presented in Table 7. The glucose
290 level of male mice was significantly increased by ME diet feeding (P = 0.0394), but that
291 of female mice tended to decrease with ME diet feeding (P = 0.0548). The LDL-
292 cholesterol level in male mice was significantly decreased by the ME diet (P = 0.0370)
293 but not affected in female mice. The T-cho level in male mice tended to decrease with
294 ME diet feeding (P = 0.0707).

295

296 Discussion

297 In the present study, mice were fed a selenoneine-containing ME for 32 days, after
298 which the accumulation of selenium-containing protein and selenoneine in blood and
299 tissues was evaluated. The effects of the ME diet on the body were examined by
300 biochemical analyses of plasma.

301 Crude protein was the main component of the powdered ME, at 77.8 g/100 g crude
302 protein (Table 1), including 63.4 g/100 g amino acids (Table 2). Free amino acids except
303 tryptophan were contained in the total amino acids and the rest of total amino acids
304 was considered to be derived from peptides because the extract did not contain proteins
305 in the production procedures. The values of phenylalanine and tyrosine in total amino
306 acids were lower than ones in free amino acids (Table 2). That was predicted to be
307 caused by the hydrolysis using concentrated HCl for total amino acids analysis
308 (Kenmochi and Tamura 1967). The rest subtracted the total amino acids and the free
309 tryptophan from the crude protein would be the non-protein nitrogen compounds such
310 as taurine, urea, ammonia (Table S1) and nucleic acids. The ME contained 0.270
311 mg/100 g (= 34.2 nmol/g) of selenoneine. Previous studies have reported that the blood

312 and white muscle of Pacific mackerel (*Scomber japonicus*) contain 437 ± 159 nmol/g
313 and 0.6 ± 0.2 nmol/g of selenoneine, respectively (Yamashita and Yamashita 2010;
314 Yamashita et al. 2011). The extract concentrated selenoneine from mackerel residues,
315 although the concentration of selenoneine was not as high as that in the blood. No
316 histamine or mercury was detected in the extract (Table S2), but arsenic and cadmium
317 were detected at 0.582 mg/100 g and 0.0277 mg/100 g, respectively (Table 3). These
318 results suggest that arsenic and cadmium are also contained in mackerel residues and
319 concentrate along with selenoneine. The concentrations of arsenic and cadmium in the
320 ME diet were calculated at 0.0409 mg/kg and 0.00194 mg/kg, respectively. The
321 concentration of arsenic was <0.20 mg/kg, the maximum allowable level of inorganic
322 arsenic in rice in the European Union (European Commission, 2006). Moreover, most
323 arsenic found in marine fish is the safer organic form of arsenic, arsenobetaine, which
324 has a lethal dose 50 value by oral administration of 10,000 mg/kg body weight in male
325 mice (Francesconi, 2010; Joint FAO/WHO Expert Committee on Food Additives, 2011).
326 The concentration of cadmium was <0.003 mg/L, the maximum allowable level in
327 natural mineral water (Codex Alimentarius, 2007). The total amount of main and trace
328 elements was lower than that of crude ashes because phosphorus and sulfur, which
329 would have been present in higher amounts, were not quantified (Table1, Table 3).
330 Thus, the ME diet was judged to be safe for mice. The components and energy of the
331 ME differed in comparison to the control diet only in terms of selenoneine (Table 4).
332 Indeed, food consumption and weight gain did not differ significantly between mice fed
333 the control and ME diet for each sex (Fig. 2).
334 The effect of the ME diet on major organs was evaluated based on the ratio of
335 organ to body weight (Fig. 3). The proportional weight of the liver, spleen, and kidney
336 in female mice was not affected by the ME diet, but that of the kidney in male mice
337 decreased significantly (Fig. 3b). A decrease in kidney weight was also identified in a

338 previous study in which KK-Ay mice were administered selenoneine at 62 μg/100 g as a
339 mackerel peptide for 7 weeks (Hase and Matsumoto, 2020). Another previous study
340 showed that oral administration of 16 mg/kg sodium selenite from water led to a
341 decrease in kidney weight of male mice and suppressed weight gain (National
342 Toxicology Program, 1994). Those results suggested that selenoneine is more toxic than
343 sodium selenite, as it exerted toxicity at a lower level, 1.89 μg Se/100 g (= 0.0189 mg
344 Se/kg), compared with sodium selenite. Compared with male mice, more selenoneine
345 accumulated in the kidneys of female mice, but a decline in kidney weight was not
346 observed in female mice (Table 5b). Selenoneine is reportedly less cytotoxic to B16
347 melanoma cells than sodium selenite or selenocystine (Siwek et al. 1994; Seko et al.
348 2020). Thus, the present result suggests that selenoneine is specifically toxic to the
349 kidney in male mice, but we could not conclude that the effect was due only to
350 selenoneine due to inconsistent results (Table 5b, Siwek et al. 1994; Seko et al. 2020)
351 and the observation that the crude extracts contained compounds not only selenoneine.
352 To elucidate the mechanism of the selenoneine-induced decrease in kidney weight,
353 more detailed experiments using purified selenoneine are needed.
354 The effects of selenium compounds on the blood and organs were evaluated by
355 selenium quantitation and speciation using LC-ICP-MS with size exclusion
356 chromatography (Yamashita and Yamashita 2010). This method was used in a previous
357 study to separate selenium-containing compounds and identified two main peaks as
358 selenium in protein and selenoneine in blood and organs (Yamashita et al. 2011). In the
359 present study, the amount of selenium in protein was not significantly affected by the
360 ME diet in either male or female mice (Table 5a). In mammals, organic selenium
361 compounds such as selenomethionine and selenocysteine from foods are metabolized
362 and incorporated into proteins (Yang et al. 2017). Selenomethionine substitutes non-
363 specifically for methionine in proteins to form selenium-containing proteins, and

364 selenocysteine is inserted into the amino acid sequence to form the active center of
365 selenoproteins (Thiry et al. 2012). Sodium selenite and selenomethionine are
366 reportedly to have elevate selenoproteins in mice, but selenoneine did not elevate them
367 in the present study. This result suggests that selenoneine undergoes a different route
368 of metabolism than other selenium compounds. Selenoneine was detected in the liver,
369 kidney, and spleen of the ME-fed mice, but it was not detected in the plasma or
370 erythrocytes. This is the first report of the detection of selenoneine in the kidney and
371 spleen of mice (Fig. 4). Other studies have found selenoneine in porcine and seabird
372 kidney and bluefin tuna spleen (Yamashita and Yamashita 2010, El Hanafi et al. 2022),
373 but it has not been reported in spleen of mammals, which is an important finding for
374 evaluating the functionality of selenoneine in the body.
375 Ergothioneine is also distributed to the kidney and spleen (Tang et al. 2018).
376 Selenoneine is predicted to accumulate in other organs and tissues in which
377 ergothioneine accumulates, as selenoneine is incorporated into cells via OCTN1,
378 similar to ergothioneine (Yamashita et al. 2013). Moreover, in the present study,
379 selenoneine was detected in the male and female liver at 0.411 and 0.562 nmol/g,
380 respectively, by feeding a diet containing 0.0189 mg Se/kg selenoneine for 32 days (Fig.
381 4, Table 5b). Those values were under the LOQ value because the baseline noise level
382 of LC-ICP-MS were high but the chromatograms showed the existence of selenoneine
383 obviously (Fig. 4, Table 5). A previous study showed that selenoneine accumulated in
384 the liver at a concentration of 8.11 nmol/g by feeding a diet containing 0.3 mg Se/kg
385 selenoneine for 4 months (Miyata et al. 2020). Therefore, the present results indicate
386 that a lower amount of selenoneine in crude material can accumulate in the liver with
387 shorter-term feeding of a lower concentration of selenoneine compared with the
388 previous study (Miyata et al. 2020).
389 The distribution of selenoneine in the kidney and spleen relative to the liver was

390 calculated from the selenoneine concentration (Table 5b), and the results are shown in
391 Table 6. The values for the kidney and spleen were not markedly different from those
392 reported for ergothioneine calculated from a previous study (Tang et al. 2018, Table 6).
393 The total intake of selenoneine as calculated from food consumption data (Fig. 2a) was
394 48.1 and 40.8 nmol/mouse in male and female mice, respectively. The ratios of
395 selenoneine accumulation in the organs relative to total intake are shown in Table 6.
396 More than 95% of the selenoneine contained in the ME diet was not accounted for.
397 Most of the non-accounted for selenoneine was thought to have been excreted into the
398 urine or not absorbed in the body, as the relative distribution of ergothioneine was the
399 highest in the liver (Tang et al. 2018) and selenoneine was also thought not to
400 accumulate in other organs at a concentration higher than that in the liver. Moreover,
401 no selenoneine was detected in erythrocytes in the present study (Fig. 4), contrary to
402 previous studies (Yamashita and Yamashita 2010; Yamashita et al. 2013; Miyata et al.
403 2020). This result suggests that selenoneine preferentially accumulates in organs and
404 is only distributed to erythrocytes later when animals are fed a low concentration of
405 selenoneine.
406 Biochemical analyses of plasma revealed the effect of the ME diet on general
407 health parameters of mice (Table 7). In male mice, blood glucose was significantly
408 elevated by the ME diet ($P = 0.0394$). However, the blood glucose level of 202 ± 20.7
409 mg/dL did not meet the criterion for diabetes for general diabetes model mice
410 (Yagihashi et al. 2010). Elevated blood glucose causes renal dysfunction and kidney
411 hypertrophy (Gross et al. 2005), but the kidneys of male ME-fed mice in the present
412 study became smaller (Fig. 2b). Thus, the plasma biochemical data could not explain
413 this decrease in kidney size. In contrast to male mice, the blood glucose level in ME-fed
414 female mice tended to decrease ($P = 0.0548$). The LDL-cholesterol level in male mice
415 decreased with ME diet feeding ($P = 0.0370$). This represents a beneficial effect of the

ME diet, as blood LDL is oxidized by reactive oxygen species, leading to arteriosclerosis (Steinbrecher et al. 1990). However, this effect was not observed in female mice. These results indicate that male mice are more sensitive to the ME diet than female mice.

In conclusion, selenoneine is distributed to the liver, kidney, and spleen in mice fed an ME diet containing a low concentration of selenoneine for a shorter term compared with previous studies. This is the first study to report the distribution of selenoneine to the kidney and spleen in mice. Male mice were more sensitive to the effects of the ME diet on plasma parameters compared with female mice.

Acknowledgments

This work was supported by the “Advanced technology deployment project for revitalization of food production areas” funded by the Ministry of Agriculture, Forestry and Fisheries of Japan.

Funding

This work was funded by the Ministry of Agriculture, Forestry and Fisheries of Japan.

Author Contribution

Takuya Seko designed, managed and conducted the experiments, and wrote the manuscript. Yoko Sato, Michiko Kuniyoshi, Yuko Murata, Kenji Ishihara conducted the experiments. Yumiko Yamashita conceived and supervised the study, and managed and conducted the experiments. Sanjuro Fujiwara and Tomohiro Ueda provided and analyzed samples. Michiaki Yamashita conceived and supervised the study, and proofed the manuscript.

442 Declarations

443 Conflict of Interest　The authors declare no competing interests.

444

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536

537 Table 1 General composition of powdered mackerel extract

components	g/100 g
water	5.80
crude protein	77.8
crude fat	0.500
carbohydrates	8.30
crude ash	7.60

538

539 Table 2 Free and total amino acid composition of powdered mackerel extract

amino acid	free amino acid (g/100 g)	total amino acid (g/100 g)
isoleucine	2.69	3.39
leucine	4.68	5.27
lysine	3.36	4.69
methionine	1.41	1.86
cystine	0.320	0.418
phenylalanine	2.65	2.52
tyrosine	1.16	0.983
threonine	2.30	3.35
tryptophan	0.810	-
valine	3.01	3.89
arginine	4.48	5.04
histidine	1.50	2.19
alanine	3.43	4.55
aspartic acid	3.65	6.14
glutamic acid	3.54	8.56
glycine	1.49	3.92
proline	1.62	3.06
serine	2.43	3.59
total	44.5	63.4

540 "-" means not analyzed.

541

542 Table 3 Main and trace elements composition in powdered mackerel extract

main elements	g/100 g	trace elements	mg/100 g
Na	0.633	Li	0.0799
Mg	0.224	V	0.523
Ca	0.167	Mn	0.196
		Fe	13.5
		Ni	1.28
		Cu	0.190
		Zn	7.17
		As	0.582
		Se	0.684
		(selenoneine 0.270 mg Se/100g)	
		Rb	0.309
		Mo	0.190
		Cd	0.0277
		Ba	0.133
		Pb	0.0213
total	1.03	total	24.9

543

544

545 Table 4 Components of the control and ME diets

components of feed (g/100g)	control	ME	% of control
water	7.90	7.89	99.8
crude protein	23.1	23.5	102
crude fat	5.10	5.01	99.4
carbohydrates	58.1	57.7	101
crude ash	5.80	5.82	100
isoleucine	0.890	0.908	102
leucine	1.74	1.77	101
lysine	1.24	1.26	102
methionine	0.440	0.450	102.3
cystine	0.360	0.360	100.1
phenylalanine	1.04	1.05	101.0
tyrosine	0.680	0.682	100.3
threonine	0.890	0.907	101.9
tryptophan	0.280	0.284	101.3
valine	1.08	1.10	101.8
arginine	1.42	1.45	101.8
histidine	0.600	0.611	101.9
alanine	1.20	1.22	102.0
aspartic acid	2.14	2.17	101.3
glutamic acid	3.99	4.02	100.8
glycine	1.18	1.20	101.6
proline	1.31	1.32	100.9

serine	1.11	1.13	101.6
selenoneine (µg Se/100 g)	0.00	1.89	
energy (kJ)	1502	1502	100.0

546

547 Table 5a Concentrations of selenium in protein in blood and tissues (plasma,

548 erythrocytes, liver, and kidney: n = 5, spleen: n = 4)

	selenium in protein (nmol Se/g)			
	male		female	
	control	ME	control	ME
plasma	16.0 ± 2.31	16.6 ± 1.78	15.0 ± 0.579	14.3 ± 0.973
erythrocyte	11.6 ± 1.29	10.3 ± 1.80	13.5 ± 1.26	14.6 ± 1.45
liver	17.3 ± 2.78	17.6 ± 1.11	19.8 ± 1.44	20.8 ± 1.05
kidney	6.77 ± 0.909	6.39 ± 0.550	7.79 ± 0.639	7.57 ± 0.543
spleen	9.24 ± 0.833	8.99 ± 1.07	9.27 ± 0.577	9.55 ± 0.557

549

Table 5b Concentrations of selenoneine in blood and tissues (plasma, erythrocytes, liver, and kidney: n = 5, spleen: n = 4)

	selenoneine (nmol Se/g)			
	male		female	
	control	ME	control	ME
plasma	-	-	-	-
erythrocyte	-	-	-	-
liver	-	0.411 ± 0.121	-	0.562 ± 0.203
kidney	-	0.323 ± 0.0910	-	*0.731 ± 0.151
spleen	-	0.939 ± 0.0920	-	1.10 ± 0.347

"-" means the peak of selenoneine was not detected. "*" means significant difference between male and female ($P < 0.05$). The LOD value was 0.259 nmol Se/g (S/N = 2).

555 Table 6 Relative distribution and ratio of selenoneine accumulation (liver and kidney: n

556 = 5, spleen: n = 4)

		selenoneine of ME fed mice		ergothioneine (calculated from Tang et al., 2018)	
		male	female	male (basal)	male (maximum)
relative distribution	liver	1.00	1.00	1.00	1.00
	kidney	0.275 ± 0.0794	0.434 ± 0.0974	0.284	0.539
	spleen	0.198 ± 0.0863	0.229 ± 0.0825	0.388	0.231
accumulation ratio (%)	liver	1.60 ± 0.431	1.85 ± 0.607		
	kidney	0.432 ± 0.145	*0.768 ± 0.128		
	spleen	0.270 ± 0.0798	0.377 ± 0.154		

557 "*" means significant difference between male and female ($P < 0.05$). Male (basal)

558 means basal ergothioneine concentration in mice administered with single dose of

559 saline and male (maximum) means maximum ergothioneine concentration in mice fed

560 with 70 mg/kg/day ergothioneine for 28 days (Tang et al., 2018).

561

562 Table 7 Biochemical analyses of plasma from mice fed the control or ME diet (n = 5)

	male			female		
	control	ME	*P* value	control	ME	*P* value
TP (g/dl)	4.94 ± 0.313	5.06 ± 0.297	0.551	4.64 ± 0.207	4.66 ± 0.434	0.929
ALB (g/dl)	1.60 ± 0.0707	1.64 ± 0.114	0.527	1.56 ± 0.0548	1.56 ±0.152	1.00
A/G ratio	0.480 ± 0.0210	0.479 ± 0.0174	0.960	0.507 ± 0.0196	0.503 ± 0.0218	0.786
T-Bil (mg/dl)	0.148 ± 0.0568	0.108 ± 0.0455	0.255	0.0980 ± 0.0179	0.118 ± 0.0303	0.248
AST (units/l)	59.4 ± 10.4	49.0 ± 10.7	0.158	51.8 ± 4.32	52.2 ± 7.73	0.923
ALT (units/l)	26.0 ± 4.06	26.8 ± 2.95	0.732	24.2 ± 3.42	23.2 ± 3.83	0.675
ALP (units/l)	217 ± 60.2	243 ± 74.0	0.560	220 ± 60.7	253 ± 51.6	0.385
T-cho (mg/dl)	134 ± 13.1	116 ± 13.3	0.0707	72.8 ± 15.9	70.0 ± 23.4	0.831
TGs (mg/dl)	95.2 ± 60.0	103 ± 10.2	0.777	74.6 ± 16.7	57.4 ± 8.41	0.0865
Crea (mg/dl)	0.0960 ± 0.0181	0.118 ± 0.0466	0.369	0.0640 ± 0.0270	0.0660 ± 0.0207	0.899
Ca (mg/dl)	9.10 ± 0.718	9.16 ± 0.358	0.873	8.64 ± 0.288	8.66 ± 0.195	0.901
Glucose (mg/dl)	169 ± 21.4	202 ± 20.7*	0.0394	167 ± 19.8	140 ± 18.0	0.0548
Na (mmol/l)	143 ± 2.39	144 ± 4.39	0.500	145 ± 3.58	145 ± 2.70	1.00
K (mmol/l)	8.43 ± 2.10	8.01 ± 1.25	0.715	5.64 ± 0.496	5.75 ± 1.12	0.848
Cl (mmol/l)	106 ± 2.78	107 ± 3.91	0.593	107 ± 3.29	108 ± 2.28	0.668
UN (mg/dl)	23.1 ± 3.46	24.0 ± 3.51	0.713	21.8 ± 4.22	25.1 ± 6.79	0.396
LDL (mg/dL)	17.0 ± 2.92	10.2 ± 5.02*	0.0370	11.6 ± 2.97	11.6 ± 5.98	1.00
HDL (mg/dL)	117 ± 10.2	103 ± 13.0	0.101	59.4 ± 14.9	57.8 ± 20.4	0.891

563 "*" means significant difference between control and ME fed mice in each gender ($P <$

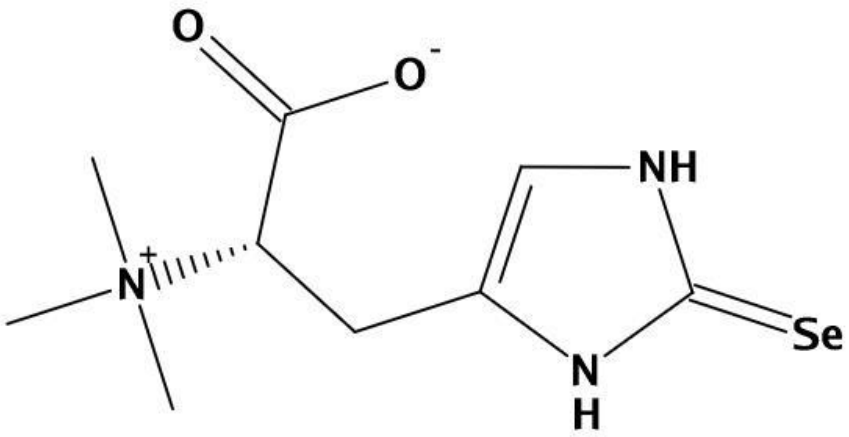
564 0.05)

565

566 Figures

567

568 Figure1



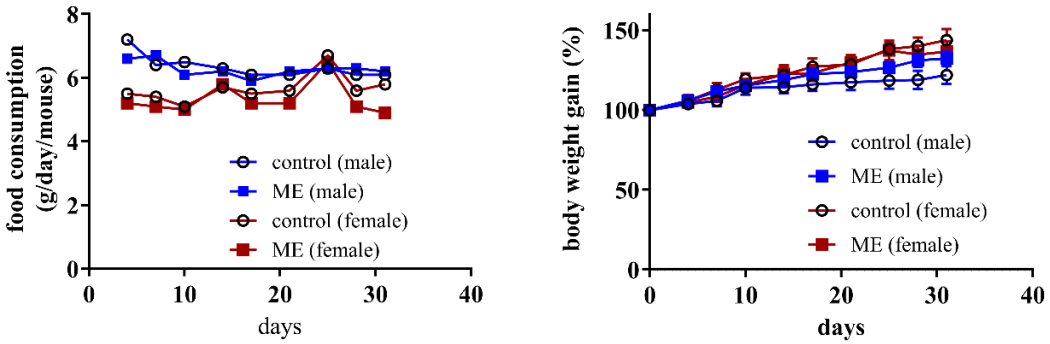
569

570 Fig. 1 Structure of reduced selenoneine

571

572 Figure 2

573 a b



574

575 Fig. 2 Food consumption and body weight gain in mice

576 **a** Food consumption (g/day/mouse) calculated from the weight loss of feed relative to

577 cage weight. **b** Body weight gain presented as percentile from day 0. Values are the

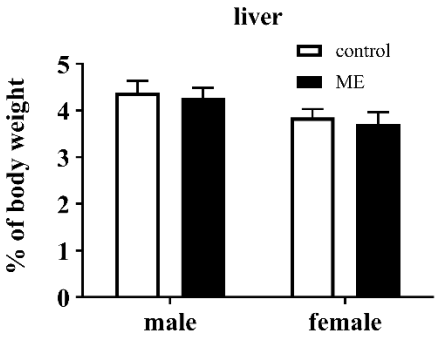
578 mean ± SD (n = 5). Circles indicate control mice, and squares indicate ME-fed mice.

579 Blue and red lines indicate male and female mice, respectively.

580

Figure 3

a



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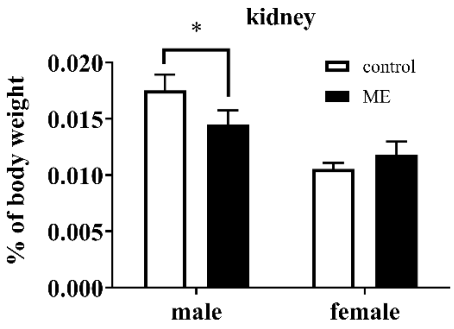

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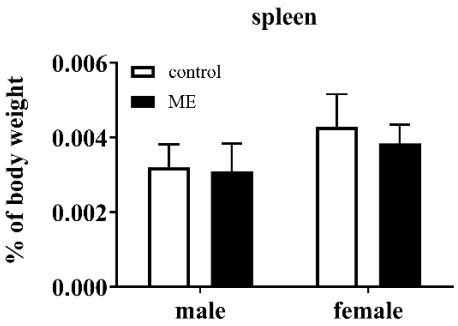
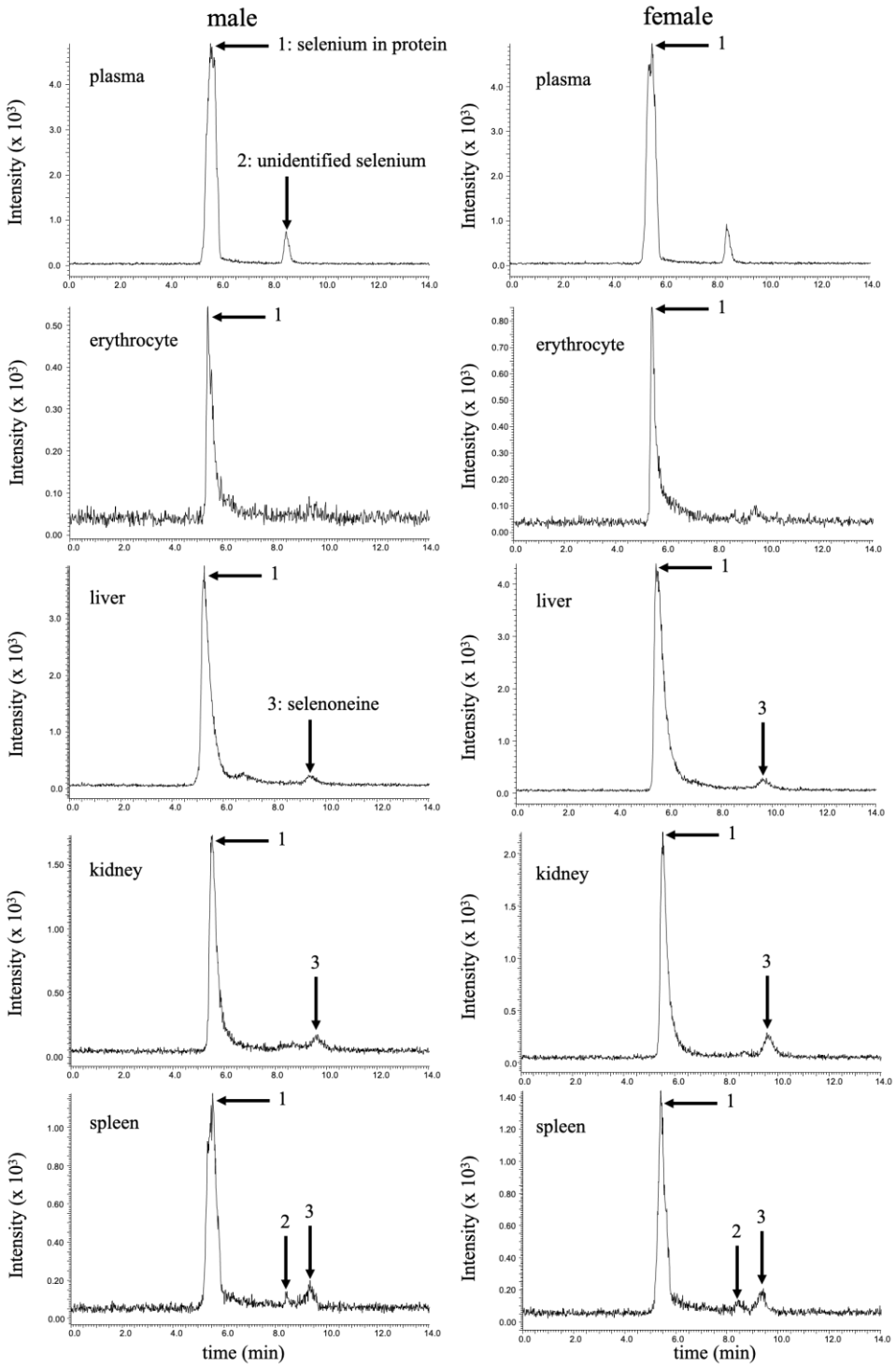


Fig. 3 Organ/body weight ratios (%) of mice fed the control or ME diet

a, c Ratios for the liver and spleen did not differ between control and ME-fed mice. **b** Ratio for the kidney of male mice was significantly decreased by ME diet ($P = 0.05$). Values are the mean SD (n = 5). Data were analyzed using the Student's *t*-test.

591 Figure 4



592

593 Fig. 4 LC-ICP-MS chromatograms of selenium (Se-82) in the plasma, erythrocytes,

594 liver, kidney, and spleen of ME-fed mice

595 Peak 1 was identified as selenium in protein and eluted with a retention time of 5.5
596 min. Peak 2 represents an unidentified selenium compound detected in the plasma and
597 spleen. Peak 3 was identified as selenoneine and eluted with a retention time of 9.5
598 min. Column: Ultrahydrogel-120, 7.8 × 300 mm; mobile phase: 0.1 M ammonium
599 acetate aqueous solution containing 0.1% IGEPAL; flow rate: 1.0 mL/min.