

Bioavailability of selenium from raw or cured selenomethionine-enriched fillets of Atlantic salmon (*Salmo salar*) assessed in selenium-deficient rats

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The bioavailability of Se from raw and cured selenomethionine-enriched (Se-enriched) salmon fillets was assessed in Se-deficient male albino rats (Mol: Wist). A low-Se Torula yeast feed was supplemented with 0, 50, 100, 150 or 200 µg Se/kg as sodium selenite or as Se from raw or cured Se-enriched salmon. The diets were fed to weanling rats for 10 and 30 d. Bioavailability of Se was assessed by metabolic balance, Se accumulation in femur, muscle, liver and plasma, and induction of Se-dependent glutathione peroxidase (EC 1.11.1.9; GSHpx) in plasma as response parameters. Except for the metabolic balance results, the slope-ratio method was used when calculating Se bioavailability from raw or cured Se-enriched fish fillets (test food) relative to sodium selenite (standard). The data for fractional apparent absorption and fractional retention showed differences ($P < 0.05$) among all three Se sources in the order raw salmon > cured salmon > selenite. At 10 d, Se from raw and cured Se-enriched fish fillets tended to be more bioavailable than selenite. This was supported by the observations for Se accumulation in femur and muscle and induction of GSHpx activity. At 30 d, all response parameters showed a higher bioavailability of Se from raw and cured Se-enriched fish fillets compared with selenite. Differences ($P < 0.05$) in Se accumulation in muscle at 10 and 30 d, and differences ($P < 0.05$) in fractional apparent absorption and fractional retention suggested that curing salmon altered the utilisation of Se. The experimental results showed that enrichment of fish fillets with selenomethionine yields fillets with high Se bioavailability.

Bioavailability of selenium: Selenomethionine-enriched salmon: Processed salmon: Selenium

Supplementing selenomethionine (SeMet) to the salmon feed has shown to increase the Se concentration in muscle tissue of farmed Atlantic salmon considerably (Lorentzen *et al.* 1994). The present study was undertaken to examine the bioavailability of Se from SeMet-enriched (Se-enriched) fish fillets. The term 'bioavailability' describes how efficiently a nutrient in the diet is utilised in a physiological process that is necessary to the organism, i.e. the amount of nutrient that is absorbed and utilised for metabolic function or storage (Fairweather-Tait, 1992). The reported bioavailability of Se from foods varies considerably. In general, the bioavailability of Se from foods of plant origin has been considered more available than Se of animal origin (Combs & Combs, 1986). However, more recent reports have shown that Se of animal origin may have a higher bioavailability than previously assumed (Shi & Spallholz, 1994a, b; Wen *et al.* 1997). Fish is known as a rich source of Se with a concentration ranging from 200 to 600 µg/kg wet weight

(Knudsen *et al.* 1992; Lie *et al.* 1994). Previous studies with rats have reported a lower bioavailability of Se from fish products than from other Se-containing foods (for example, see Douglass *et al.* 1981; Alexander *et al.* 1983). Other more recent reports have shown that Se from fish has a good bioavailability (Lorentzen, 1990; Knudsen *et al.* 1992; Wen *et al.* 1997). Processing methods have been found to enhance the bioavailability of Se (Shi & Spallholz, 1994a; Shen *et al.* 1997). Some processing methods may cause protein degradation and thus improve protein digestibility. This may facilitate release of bound Se and therefore increase Se bioavailability (Shi & Spallholz, 1994a; Shen *et al.* 1997). However, Se may be lost from the food item through heat processing (Higgs *et al.* 1972; Eskeland, 1988). Curing of fillets causes breakdown of proteins without the risk of losing Se. 'Gravlaks' (cured salmon) is a traditional Norwegian course where refrigerated salmon fillets are cured over a 2-d period. Cured salmon was chosen as a

Abbreviations: GSHpx, glutathione peroxidase; SeMet, selenomethionine.

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representative of processed fish because the process does not involve heating, it is relatively easy to produce and because the curing process causes breakdown of proteins. The purpose of the present study was thus to establish the bioavailability of Se from Se-enriched salmon, and to examine the effect of curing on the bioavailability of Se from the Se-enriched fish fillets.

Materials and methods

Experimental diets

The Se-enriched fish fillets were produced in an experiment conducted at the Institute of Nutrition, Directorate of Fisheries, Norway. The experiment produced Atlantic salmon with graded Se levels in muscle tissue through SeMet supplementation. Data on Se speciation from the SeMet supplemented fish are available in an EU report (RIVO – The Netherlands Institute for Fisheries Research, 2000). The Se content of the fillets ranged from 240, for unfortified tissue, to 610 µg Se/kg wet weight (corresponding to 800–2050 µg Se/kg for the dry muscle meal) and were used as Se sources in the experimental diets. Cured salmon was made according to a traditional Norwegian 'gravlaks' recipe: approximately 30 g of a salt–sugar mixture (3:1, v/v) together with pepper, dill and 1 tablespoon of liquor ('Lord Calvert' whisky) was evenly distributed between two deboned fillets with skin. A pressure of approximately 10 g/cm² was provided on top of the fillets. The fillets were kept at 4°C for 2 d and were turned after 24 h. After removing the skin, the cured fillets were treated similar to the raw salmon fillets: each of the salmon fillets was homogenised in a kitchen blender (Dito Sama, Aubusson, France). The homogenised fillets were frozen at –20°C, freeze-dried (VirTis Genesis 25SE, New York, USA) and homogenised in a mill (Retsch ZM100, Haan, Germany). The salmon muscle meal was then stored for 1 week at –30°C in plastic bags filled with CO₂ to prevent rancidity. A Torula yeast-based feed low in Se (Low Selenium Diet; Ing. Heidenreich A/S, Oslo, Norway) was supplemented with 0, 50, 100, 150, and 200 µg Se/kg in the form of sodium selenite or Se from raw or cured salmon (the recommended dietary Se level for growing rats has been estimated to be 150 µg Se/kg; National Research Council (1995)). This provided a total of thirteen experimental diets. The composition of the Torula yeast feed is given in Table 1. A vitamin mixture, a mineral mixture, fish oil (*Clupea harengus*) and lard (*Adeps suillus*) were added in amounts sufficient to ensure that the diets contained equal amounts of nutrients and that all diets met the requirements for rats (National Research Council, 1995). The Torula yeast feed was low in vitamin E and methionine, 60 mg/kg DL- α -tocopheryl acetate and L-methionine equivalent to 0.4 g/kg (Sunde *et al.* 1981) of the Torula yeast feed was therefore added to all diets. The experimental diets were stored at 4°C in plastic bags filled with CO₂ until fed.

Experimental design

One hundred and thirty Se-depleted, weanling male albino rats (Mol: Wist; Møllegaard, Køge, Denmark) were

Table 1. Composition of the low-selenium basal diet

	g/kg
Torula yeast	300
Sucrose	590
Tocopherol-stripped lard	50
Salt mixture (Hubbel, Mendel & Wakeman)*	50
ICN vitamin diet fortification mixture†	10

* Provided (g/kg mix): CaCO₃ 543.00; MgCO₃ 25.00; MgSO₄ · 7 H₂O 16.00; NaCl 69.00; KCl 112.00; KH₂PO₄ 212.00; FePO₄ · 4H₂O 20.50; KI 0.08; MnSO₄ · H₂O 0.36; NaF 1.00; Al₂(SO₄)₃ 0.17; CuSO₄ · 5 H₂O 0.50.

† Provided (g/kg mix): vitamin A acetate (500.000 IU/g⁻¹) 1.80; vitamin D₂ (850.000 IU/g) 0.125; ascorbic acid 45.00; inositol 5.00; choline chloride 75.00; menadione 2.25; *p*-aminobenzoic acid 5.00; niacin 4.25; riboflavin 1.00; pyridoxine hydrochloride 1.00; thiamine hydrochloride 1.00; calcium pantothenate 3.00; biotin 0.02; folic acid 0.009; vitamin B₁₂ 0.0135.

randomly distributed among thirteen diet groups with ten rats in each group. The rats were housed in individual cages in a temperature- and light-controlled room (21 ± 2°C, 12 h light–dark cycle). At 10 d, five rats in each dietary group were killed. The groups given diets 4, 8 and 12 for 30 d were housed in metabolic cages (Techniplast Gazzada, Buguggiate, VA, Italy). The construction of the metabolic cages allowed for collection of urine and faeces from each rat and made accurate calculation of Se absorption and retention possible. Urine and faeces were collected daily, the samples were accumulated in separate urine and faeces containers for each rat, and stored at –20°C. The data on absorption and retention were obtained using the values from the five rats in each diet group. The apparent absorption was calculated as the difference between Se intake and Se in faeces:

$$\text{Apparent Se absorption } (\mu\text{g}) = \text{Se}_{\text{intake}} - \text{Se}_{\text{faeces}}$$

The fractional apparent absorption is Se absorption relative to Se intake and was calculated using the formula:

$$\begin{aligned} \text{Fractional apparent Se absorption } (\%) \\ = \frac{(\text{Se}_{\text{intake}} - \text{Se}_{\text{faeces}})}{\text{Se}_{\text{intake}}} \times 100 \end{aligned}$$

Total Se retention is the amount of Se retained in the body after Se excretion. The amount of Se retained was calculated using the formula:

$$\text{Se retention } (\mu\text{g}) = \text{Se}_{\text{intake}} - (\text{Se}_{\text{faeces}} + \text{Se}_{\text{urine}})$$

Fractional retention is total Se retention relative to Se intake and was calculated using the formula:

$$\begin{aligned} \text{Fractional Se retention } (\%) \\ = \frac{(\text{Se}_{\text{intake}} - (\text{Se}_{\text{faeces}} + \text{Se}_{\text{urine}}))}{\text{Se}_{\text{intake}}} \times 100 \end{aligned}$$

All rats had free access to ultrafiltered water and were fed *ad libitum*. Feed spill and leftovers were collected daily and taken into consideration when the feed intake for each rat was calculated. At sampling, the animals were killed by intraperitoneal injection of 0.1 ml Mebumal (50 mg/ml)/100 g body weight. Blood was collected from

the heart using syringes with heparinised needles and chilled on ice until centrifuged at 4°C in a cooling centrifuge (Jouan CR312, St. Mazaire, France), at 1300g for 10 min. The plasma was transferred to two Eppendorf tubes. One tube was frozen at -20°C for Se analysis and the other was frozen at -80°C for glutathione peroxidase (GSHpx) analysis. The femoral muscle, femur and liver were dissected, frozen at -20°C, freeze-dried, homogenised and kept dry until analysed.

Analytical methods

The Se content of the diets and tissues was determined by flow injection analysis with hydride generation atomic absorption spectroscopy. Briefly, samples for element analyses (0.25 g freeze-dried material or 0.5 ml plasma or urine) were digested in 2 ml HNO₃ (Merck, Oslo, Norway) and 0.5 ml H₂O₂ (Merck, Oslo, Norway) using a microwave oven (MLS-1200 MEGA, Sorisol, Italy). The digested samples were diluted to a known volume and the Se content determined by hydride generation atomic absorption spectroscopy: Se ions in solution were reduced with NaBH₄ (Merck, Oslo, Norway) under acidic conditions, 10% HCl (1.2 M) (Merck, Oslo, Norway), and formed volatile Se hydrides (Nakahara, 1995). The hydrides were carried with Ar as the carrier gas through a 1.0 µm Ø membrane filter (TE37; Schleicher & Schuell, Dassel, Germany) to a heated quartz cell where the hydrides dissociated to form free Se atoms. The Se atoms were then quantified by atomic absorption spectroscopy using an external standard curve made from different dilutions of a 1000 mg Se/l in 2.5% HNO₃ (0.3 M) stock solution (Teknolab AS, Drøbak, Norway). The analyses were performed using a Perkin Elmer 3300 AAS (Perkin Elmer, Norwalk, CT, USA) coupled with a Perkin Elmer AS 90 autosampler (Perkin Elmer, Ueberlingen, Germany). The 'flow injection system' used was a Perkin Elmer FIAS-200 (Perkin Elmer, Ueberlingen, Germany). Accuracy and precision of the methods were controlled by concomitant analyses of the standard reference materials non-fat milk powder (SRM 1549; National Bureau of Standards, Washington, DC, USA) and bovine muscle (SRM 184; Community Bureau of Reference, Brussels, Belgium). The activity of plasma GSHpx was determined spectrophotometrically in an NADPH-coupled reaction using a slightly modified version of the method described by Flohé & Günzler (1984). In this assay, GSHpx reduces peroxides using glutathione as an H donor. Oxidised glutathione is regenerated in an NADPH-coupled reaction catalysed by glutathione reductase. The consumption of NADPH is directly proportional to the consumption of the peroxide, hence it is also proportional to the activity of GSHpx. Briefly, 100 µl of 1 M Tris-HCl buffer with 5 mM EDTA (pH 8), 20 µl of 0.1 M reduced glutathione (Sigma, Oslo, Norway), 100 µl of 2 mM in 0.1% NaHCO₃ (12 mM) (Merck, Oslo, Norway), 100 µl of 10 U/ml glutathione reductase (Sigma, Oslo, Norway) and 10 µl buffer-diluted plasma sample was mixed with 660 µl distilled water and incubated at 37°C before addition of 10 µl of 10 mM NADPH pre-warmed *t*-butyl hydroperoxide (Aldrich Chemie, Steinheim, Germany). The decrease in NADPH

concentration was measured at 37°C (Haake E 15 waterbath; Karlsruhe, Germany) and 340 nm (Shimadzu Graphicord UV-240; Kyoto, Japan). The enzyme assays were performed under conditions where product formation was linear with respect to the amount of protein. Before the GSHpx analyses, the protein concentration of the plasma samples was determined as described by Bradford (1976) using a commercial protein assay (Bio-Rad Laboratory, Richmond, CA, USA). Total lipid content of the diets was measured gravimetrically after extraction with ethyl acetate. Total N content of the diets was determined in homogenised, freeze-dried samples as described by Crooke & Simpson (1971) and protein was calculated as N × 6.25. Ash and DM content of the diets was determined gravimetrically as described by Mortensen & Wallin (1989). The carbohydrate content of each diet was calculated as the residue when the amount of water, fat, protein and ash had been subtracted from 1000 g diet. The energy content of each diet was calculated using Atwater's constants, which gives 17 kJ/g protein, 16 kJ/g carbohydrate and 36 kJ/g fat. The results are summarised in Table 2.

Statistical analyses

One way ANOVA was conducted for the data on Se intake, apparent absorption, fractional apparent absorption, total retention and fractional retention. A Kolmogorov-Smirnov test was used to assess normality of distribution and homogeneity of variance was tested using Levene's test. All tests were conducted using Statistica™ Software, Release 4.5 (Statsoft Inc., USA, 1993). A slope-ratio assay (Finney, 1978) was used to compare the bioavailability of Se from raw or cured salmon compared with sodium selenite. In this assay, linear regression lines are generated for both test and standard food and test regression line: standard regression line expresses the relative bioavailability. For a satisfactory application of this method, it is important that the regression lines are linear and that they have a common origin (Finney, 1978). Regression lines are generated when a selected measure of response, e.g. plasma GSHpx activity, is plotted against the Se intake for each rat. A linear dose-response relationship is expected if the following assumptions are fulfilled: (1), the diets contain graded levels of Se; and (2), the Se intake does not exceed the amount required to replete the chosen response parameter. The model also requires that the regression lines intercept at a common origin. A good approximation to this requirement is found when the regression lines converge at 'zero' dose. A basal diet with no Se was therefore used in the generation of all regression lines. The linear regression equations were thus obtained by plotting Se intake against Se concentration in femur, muscle, liver, plasma or GSHpx activity in plasma, and compared using Tukey's test as described by Zar (1996). The ratio of the slopes was calculated manually as described by Finney (1978). The significance level was set at 95%.

Results

All rats appeared healthy and grew well throughout the experiment. The rats given the basal and selenite diets showed a lower weight gain than the rats given raw or cured

Table 2. Selenium level, dry matter, fat, protein and ash content, and calculated carbohydrate and energy content of nutrients in the experimental diets*

(Mean values)								
Se source	Diet no.	Se level ($\mu\text{g}/\text{kg}$)	DM (g/kg)	Fat (g/kg)	Protein (g/kg)	Ash (g/kg)	Carbohydrates (g/kg)†	Energy (kJ/g)‡
Basal diet	1	17	975	79	143	59	694	16.4
Raw salmon	2	57	978	76	147	55	699	16.4
	3	72	979	81	147	56	694	16.5
	4	97	980	77	153	51	698	16.6
	5	145	978	79	149	51	699	16.6
	6	80	978	87	144	55	694	16.6
Cured salmon	7	95	977	83	145	55	692	16.7
	8	106	978	84	145	53	696	16.6
	9	139	978	83	136	57	702	16.5
Sodium selenite	10	56	960	75	142	57	686	16.1
	11	86	960	70	142	57	691	16.0
	12	119	963	76	145	56	686	16.2
	13	148	963	75	142	57	688	16.1

* For details of diets and procedures, see Table 1 and p. 14.

† The carbohydrate content of each diet was calculated as the residue when the amount of water, fat, protein and ash had been subtracted from 1000 g of diet.

‡ The energy content of each diet was calculated using Atwater's constants, which gives 17 kJ/g protein, 16 kJ/g carbohydrate and 36 kJ/g fat.

salmon at both 10 and 30 d. The weight gain showed a high correlation to feed intake (r 0.77 and r 0.71) whereas the correlation between weight gain and Se intake was r 0.34 and r 0.40 for the rats fed for 10 and 30 d respectively. Apparent absorption, fractional apparent absorption, total retention and fractional retention were calculated for the rats in metabolic cages given diets 4, 8 and 12 which contained 97, 106 or 119 μg Se/kg as raw salmon, cured salmon or selenite, respectively. The apparent absorption ranged from 29 to 31 μg Se, and there were no significant differences among the three diet groups. The fractional apparent absorption ranged from 74 to 81 %, and all three diet groups were significantly different from each other ($P < 0.05$). The total retention ranged from 14 to 26 μg , and the raw and cured salmon diet groups were significantly higher than the selenite diet group ($P < 0.01$). The fractional retention ranged from 36 to 73 % and all three diet groups were significantly different from each other ($P < 0.05$). The group given salmon retained most and the group given selenite retained least Se. The results are given in Table 3. The

relative bioavailability of Se was assessed by the slope-ratio method using sodium selenite as a reference. Slopes with a coefficient of determination (R^2) > 0.70 were arbitrarily considered linear. All slopes had P values < 0.001 for the significance of the regression coefficient (slope $\neq 0$). Fig. 1 shows an example of slope-ratio comparison for Se accumulation in muscle at 30 d. When the regression lines showed a saturation tendency, the data points for the group given the highest dietary level of Se was removed to ensure linearity (femur at 30 d given selenite and plasma at 30 d given raw salmon or selenite). At 10 d, the data points for the selenite regression line for plasma GSHpx activity showed an exponential tendency, while at 30 d a saturation tendency was observed. The data points for the groups given the highest dietary level of selenite at 10 and 30 d were therefore removed to ensure linearity. Fig. 2 shows the exponential tendency for GSHpx at 10 d and the saturation tendency for GSHpx at 30 d. There were significant differences ($P < 0.05$) in bioavailability among all three Se sources at both 10 and 30 d as measured by Se accumulation

Table 3. Selenium intake, apparent selenium absorption, fractional apparent selenium absorption (relative to intake), total selenium retention, and fractional selenium retention (relative to intake) for rats in metabolic cages given diets 4, 8 and 12*

(Mean values and standard errors of the mean for five rats per group)

Se source...	Raw salmon		Cured salmon		Sodium selenite	
	Mean	SEM	Mean	SEM	Mean	SEM
Se intake (μg)	35.6	1.1	39.8	0.7	39.0	2.5
Apparent absorption (μg)	29.0	1.0	31.2	0.6	29.0	2.0
Fractional apparent absorption (% of intake)	81.5 ^a	0.7	78.7 ^b	0.5	74.4 ^c	0.6
Retention (μg)	26.0 ^a †	1.4	26.2 ^a	0.6	14.1 ^b	1.6
Fractional retention (% of intake)	73.4 ^a †	1.1	65.8 ^b	0.7	35.7 ^c	2.3

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see Tables 1 and 2, and p. 14.

† Four rats per group due to loss of urine sample.

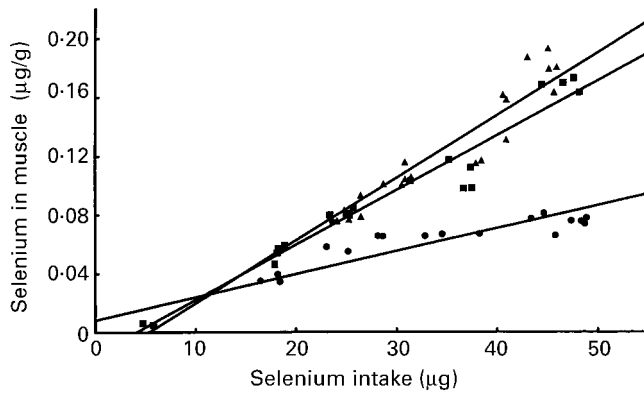


Fig. 1. Example of slope-ratio assay (here given for selenium accumulation in muscle at 30 d) for selenium bioavailability in rats given diets with graded levels of selenium from raw salmon (■), cured salmon (▲) or sodium selenite (●).

in muscle. At 30 d, there were significant differences ($P < 0.05$) between cured salmon and selenite, but not between raw salmon and selenite, as measured by Se accumulation in liver. At 10 d, Se from raw and cured salmon tended to be more bioavailable than selenite as measured by Se accumulation in femur and induction of GSHpx activity. Se from cured salmon tended to be more bioavailable than Se from raw salmon as measured by Se accumulation in femur and plasma. Se from raw and cured salmon tended to be less bioavailable than selenite as measured by Se accumulation in liver, and Se from raw salmon tended to be less bioavailable than selenite as measured by Se accumulation in plasma. At 30 d, Se from raw and cured salmon tended to be more bioavailable than selenite as measured by induction of GSHpx activity. Se from cured salmon tended to be more bioavailable than Se from raw salmon as measured by Se accumulation in femur and liver, and Se from raw salmon tended to be more bioavailable than from cured salmon as measured by Se accumulation in plasma. The bioavailabilities of Se from raw and cured salmon relative to selenite at 10 and 30 d are given in Table 4.

Discussion

The concept of bioavailability is important when considering the nutritive value of a nutrient in foods. The bioavailability of dietary Se is affected by several factors. The biochemical form of Se and the total protein and fat content of the diet are important in this context. The absorption of Se seems to be higher in high-protein diets than in low-protein diets (Greger & Marcus, 1981). Further, the fat content and type of fat seem to affect the utilisation of Se (Mutanen & Mykkänen, 1984). The diets in the present study were therefore designed to be as equal as possible with regard to the protein and lipid content (Table 2). The Se-enriched salmon fillets, which contained graded levels of Se, provided a unique possibility of producing isonitrogenous diets with Se levels increasing from approximately 15 to 150 µg Se/kg. The National Research Council (1995) recommends a minimum dietary concentration of 150 µg Se/kg for growing rats. However, Smith & Picciano (1987) showed that 250 µg Se/kg given as SeMet and 500 µg Se/kg

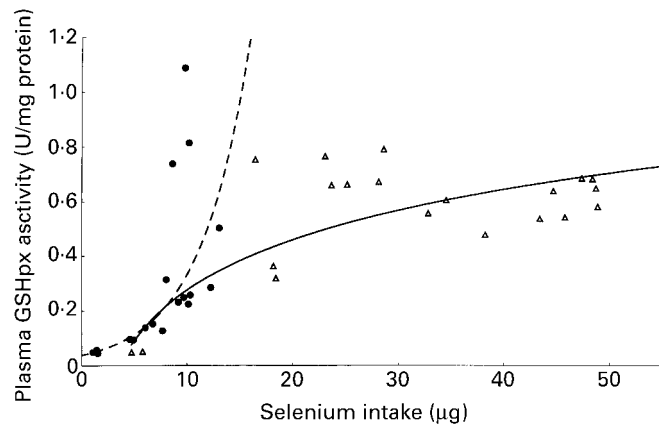


Fig. 2. Induction of plasma glutathione peroxidase (GSHpx; nmol NADPH/min per mg protein) in rats fed the selenite diets for 10 (●, - - -) and 30 (▲, —) d.

given as sodium selenite was necessary to optimise the activity of GSHpx in tissues in both dams and pups, something that indicates that 150 µg Se/kg might be too low. In the experimental design used in the present study it was important to keep the dietary Se levels below the requirement to achieve maximum response when the nutrient was fed.

Apparent absorption and total retention of Selenium

Both fish Se and selenite had a high degree of absorption, something that is in accordance with earlier observations (Whanger *et al.* 1976; Ringdal *et al.* 1985; Windisch *et al.* 1998). The fish used in the present study were enriched with SeMet and most probably contained a relatively high amount of SeMet. SeMet appears to have a higher absorption than selenite (Bopp *et al.* 1982; Swanson *et al.* 1991; Windisch *et al.* 1998) and this may explain why fish Se had the highest fractional apparent absorption. Another possibility is that the differences in fractional apparent absorption are connected with the differences in the concentration of Zn in the diets. House & Welch (1989) observed a decreasing absorption of Se with increasing Zn intake. In the present study, the Zn concentrations of the sodium selenite diets were slightly higher (45 µg Zn/g) than the diets with Se-enriched fish (32 µg Zn/g). However, the differences found in the present study were small and probably have little biological relevance. The difference in total retention between the rats given Se from Se-enriched fish and the rats given sodium selenite seemed to be in accordance with the observation that inorganic Se has a higher renal clearance than organically-bound Se (Swanson *et al.* 1991). Se from fish was more highly retained than sodium selenite and this is in accordance with the findings of Miller *et al.* (1972); Ringdal *et al.* (1985); Hassan *et al.* (1987, 1993).

Organ accumulation of Selenium and induction of glutathione peroxidase in plasma

The tissue Se accumulations in the rats fed Se-enriched fish were probably partly due to non-specific incorporation of

Table 4. Coefficients of regression lines (RC), coefficient of determination (R^2), and relative bioavailability (RBA) of raw salmon and cured salmon relative to selenite as measured by selenium accumulation in femur, muscle, liver and plasma, and induction of glutathione peroxidase (GSHpx) activity in plasma at 10 and 30 d*

Response parameter	Se source	RC at day 10	R^2 at day 10	RBA at day 10 (%)	RC at day 30	R^2 at day 30	RBA at day 30 (%)
Se accumulation in femur	Raw salmon	0.008	0.88	108	0.002	0.87	113
	Cured salmon	0.009	0.85	131	0.0024	0.81	135
Se accumulation in muscle	Selenite	0.007	0.76	100	0.0017	0.75	100
	Raw salmon	0.008	0.97	211 ^a	0.003	0.96	225 ^a
	Cured salmon	0.007	0.89	177 ^b	0.004	0.95	274 ^b
	Selenite	0.004	0.91	100 ^c	0.001	0.84	100 ^c
Se accumulation in liver	Raw salmon	0.050	0.89	87	0.020	0.97	115 ^{ab}
	Cured salmon	0.050	0.82	81	0.020	0.97	130 ^a
Se accumulation in plasma	Selenite	0.060	0.85	100	0.010	0.89	100 ^b
	Raw salmon	12.9	0.83	83	5.6	0.82	125
	Cured salmon	15.8	0.74	102	5.0	0.92	113
	Selenite	15.6	0.84	100	4.4	0.81	100
Induction of GSHpx activity	Raw salmon	0.060	0.75	142	0.018	0.80	139
	Cured salmon	0.070	0.71	147	0.018	0.70	142
	Selenite	0.050	0.63	100	0.012	0.90	100

a,b,c: Mean values within a column for a response parameter with unlike superscript letters were significantly differently ($P < 0.05$).

* For details of diets and procedures, see Tables 1 and 2, and p. 14.

SeMet into proteins. This incorporation may be explained mainly by the inability of the protein metabolism to distinguish between S amino acids and their seleno analogues (Ochoa-Solano & Gitler, 1968; Behne *et al.* 1991). Se accumulation should therefore occur in tissues with high methionine content, such as skeletal muscle (Windisch *et al.* 1998), as was also shown in the present study. Tissue accumulation of Se may be interpreted as a sign of superior bioavailability of SeMet over selenite. However, the level of tissue Se does not adequately measure the physiological Se status of the organism because SeMet is incorporated into general body proteins. The organism does not recognise SeMet as Se and is not able to mobilise Se from tissues containing SeMet (Windisch *et al.* 1998). However, this tissue SeMet probably serves as an important long-term source of Se as body proteins turn over (Sunde *et al.* 1981). This is supported by the observations for Se accumulation in liver and plasma: after 10 d, selenite seemed the most available species for synthesis of selenoproteins in the liver. After 30 d, protein turnover seemed to have made more Se from Se-enriched fish available for transformation in the liver, and hence, more retained than selenite.

The hypothesis that processing increases the bioavailability of Se from the Se-enriched fish fillets does not receive unambiguous support from the results for organ accumulation of Se. First, the differences observed for Se accumulation in muscle between raw and cured salmon at day 10 were reversed at day 30. Se accumulation in liver at day 30 showed that cured salmon was more available than selenite, while Se accumulation from salmon did not show significant differences from selenite. It seems evident that the curing process affected Se bioavailability, but the results seem unclear.

The induction of plasma GSHpx activity is probably the most significant parameter of Se bioavailability since it gives an indication of how much Se is available for biosynthesis of biologically-active selenoproteins. At 10 d, Se from Se-enriched fish tended to be more bioavailable than selenite, as measured by induction of plasma GSHpx. However, the regression curve for selenite at day 10 did not satisfy the self-imposed demand for linearity. To make the curve more linear, the group given the highest dietary level of selenite was removed. If this group had been included, the data points would have shown an exponential tendency (Fig. 2). This shape may be interpreted biologically as a 'delay' in the induction of GSHpx activity. This delay may be explained by the observation that plasma GSHpx has a low priority in Se deficiency (Behne *et al.* 1995). The regression lines for salmon and 'gravlaks' also seemed to exhibit some delay, but not as clearly as the regression line for selenite. The absence of this delay may be interpreted as a sign of a better bioavailability of Se from Se-enriched fish than selenite. No differences in bioavailability of Se could be traced between raw salmon and cured salmon at 10 and 30 d however.

At 30 d, Se from Se-enriched fish was more bioavailable than selenite as measured by all response parameters. This suggests that after 30 d, protein turnover has made more Se from SeMet available for synthesis of selenoproteins. Although the results for absorption, total accumulation of

Se, and Se accumulation in muscle and liver indicated that there were differences in the utilisation of Se from raw salmon and cured salmon, the results for induction of plasma GSHpx activity showed that these Se sources had equal bioavailability.

Thus, the results from the present study indicated that the bioavailability of Se from Se-enriched fish fillets was higher than the bioavailability of selenite. Further, processing of the Se-enriched fish fillets seemed to alter the bioavailability of Se, although no unambiguous conclusions could be reached at this stage. Further work is necessary to clarify the effect of processing on fish fillets with regard to the bioavailability of Se. The present study indicates that enrichment of fish fillets with SeMet yields fish fillets with high Se bioavailability.

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