Effect of Selenium Supplementation on the Selenium Content in Muscle and Liver of Finnish Pigs and Cattle

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Low selenium intake was considered to be a potential nutritional risk for the whole Finnish population. The Expert Working Group on Selenium recommended in 1983 that the selenium content of Finnish feed and food should be increased by adding selenium to fertilizers. The primary purpose of this action was to increase the selenium content of cereal grains from 0.01 to 0.1 mg/kg of dry matter. The limit was clearly often exceeded. Therefore, the Working Group decided in spring 1990 to decrease the selenium supplementation level so that only one level of 6 mg/kg is used in multinutrient general fertilizers. The highest selenium contents were reached in 1989, when selenium in pig muscle was 0.30 ± 0.06 mg/kg of wet wt, in pig liver 0.73 ± 0.13 mg/kg of wet wt, in cattle muscle 0.21 ± 0.05 mg/kg of wet wt, and in cattle liver 0.51 ± 0.18 mg/kg of wet wt. After 1989 the selenium contents gradually decreased. In 1995 the selenium content in pig muscle was 0.15 ± 0.03 mg/kg of wet wt, in pig liver 0.52 ± 0.06 mg/kg of wet wt, in cattle muscle 0.10 ± 0.02 mg/kg of wet wt, and in cattle liver 0.28 ± 0.05 mg/kg of wet wt.

Keywords: *Selenium; selenium in pigs and cattle; selenium supplementation*

INTRODUCTION

Selenium is a naturally occurring trace element that takes part in essential functions in living organisms. It is a constituent of glutathione peroxidase, a key enzyme in the antioxidant defence system of cells. Selenium deficiency has been shown to cause cardiomyopathy, known as Keshan disease, in the Chinese population (Keshan Disease Research Group, 1979). The relationship between selenium status and other cardiovascular diseases is less clear, but low serum selenium concentration may be associated with increased risk of death from cardiovascular causes (Virtamo and Huttunen, 1988, 1991; Suadicani et al., 1992).

Numerous experimental studies in animals have indicated that selenium supplementation in suitable doses decreases the incidence of certain types of cancer (Salonen et al., 1982; Virtamo et al., 1987; Knekt et al., 1990; Willet et al., 1983, 1991). In excessive doses, however, selenium is toxic. An intake of selenium of up to about 4 μg/kg of body weight could be considered as safe and tolerable. Organic-bound selenium in food is considered to be less toxic than inorganic selenium compounds, but little is known about the toxic potency of different selenium compounds in humans (Alexander, 1993).

Considerable regional variation occurs in selenium contents in soil. In Finland, the concentration of selenium in soil is low, as in the other Scandinavian countries. The low selenium concentrations can be explained by both geochemical and climatic factors. Because of the relatively low pH of soils, the low prevailing temperatures, and the high humidity characteristics of the climate in Finland, selenium is stored in reduced forms, which are poorly available to plants. Consequently, the natural selenium content of Finnish agricultural plants is very low, and therefore the selenium contents of all domestic agricultural products have been low (Koivistoinen and Huttunen, 1986). An investigation of 30 mineral elements including selenium in about 450 food items in Finland was performed in the 1970s. The results indicated that the selenium concentration of most domestic foods was very low: the daily intake varied from <20 μg/day to somewhat more than 30 μg/day, which was well below the safe, adequate intake level for adults (50−200 μg/day) later defined by the U.S. National Academy of Sciences (Koivistoinen, 1980).

The Expert Working Group on Selenium was appointed in 1983 by the Ministry of Agriculture and Forestry to evaluate the selenium problem in Finland. The low selenium intake was considered to be a potential nutritional health risk for the whole Finnish population. In its report of 1983 (Ministry of Agriculture and Forestry, 1983) the Working Group recommended that the selenium content of Finnish feed and food should be increased by adding sodium selenate to fertilizers. The most effective ways to increase the selenium content in cultivated crops are addition of selenites or selenates to fertilizers, spraying these salts onto the crops or treatment of the seeds with aqueous solutions of selenium compounds (Bisbjerg and Gissel-Nielsen, 1969; Gissel-Nilesen and Bisbjerg, 1970; Gissel-Nielsen, 1977; Yläranta, 1985). Selenium from selenate finds its way into plants more efficiently than that from selenite, irrespective of whether the selenium is applied to the soil or directly onto the plants. The reason for this difference is the high solubility of selenate (Yläranta, 1985). The addition of selenium in the form of selenate was introduced to all multinutrient fertilizers used in agriculture after July 1, 1984 (Ministry of Agriculture and Forestry, 1984). The selenium supplementation levels were 16 mg/kg to fertilizers used for cereal crops and 6 mg/kg to fertilizers used for grassland crops. The primary purpose of this action was to increase the selenium content of cereal grains from 0.01 to 0.1 mg/kg of dry matter. In practice, this limit was clearly often exceeded. Therefore, the Working Group

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Table 1. Results of the Analyses of Certified Reference Materials

method	certified reference material (bovine)	no.[a]	Se concn (mg/kg, mean ± 1 SD[b]) our results	certified value
fluorometric	NIST 1577 b liver	219	0.71 ± 0.06	0.71 ± 0.07
AAS/hydride	NIST 1577 b liver	49	0.78 ± 0.06	0.71 ± 0.07
AAS/hydride	BCR 184 muscle	46	0.173 ± 0.025	0.183 ± 0.012
AAS/hydride	BCR 185 liver	75	0.474 ± 0.035	0.446 ± 0.0133

[a] Number of analyzed samples. [b] SD, standard deviation.

Table 2. Results of Two-Tail *t*-Test of Pig and Cattle Samples[a]

1985−1986	muscle	liver	1989/1991	muscle	liver	1994−1995	muscle	liver
				Pig				
t	−8.88	−2.42	*t*	2.10	3.26	*t*	−2.92	−1.08
t critical	2.03	2.01	*t* critical	2.01	2.00	*t* critical	2.00	2.01
$P(T \le t)$	1.05E−10	0.020	$P(T \le t)$	0.041	0.002	$P(T \le t)$	0.005	0.287
				Cattle				
t	−3.50	−2.60	*t*	4.13	2.81	*t*	−0.93	1.95
t critical	2.01	2.01	*t* critical	2.00	1.99	*t* critical	2.01	2.02
$P(T \le t)$	0.001	0.012	$P(T \le t)$	0.0001	0.007	$P(T \le t)$	0.357	0.058

[a] Significance level is 5%.

decided in the spring of 1990 to decrease the selenium supplementation level so that only one level of 6 mg/kg is used in multinutrient general fertilizers. This directive came into force June 16, 1990 (Ministry of Agriculture and Forestry, 1990).

A control system was established to monitor the consequences of using selenium fertilizers. The Expert Working Group on Selenium has evaluated and reported annually on the effects of the selenium fertilization. It was foreseen that the selenium levels of other agricultural products would also increase. The selenium contents of fertilizers, soils, animal feeds, basic foodstuffs, and human blood have since been analyzed regularly. The results have been published as Working Group reports.

The selenium contents of muscle and liver of pigs and cattle have been analyzed in the Department of Chemistry in the National Veterinary and Food Research Institute (EELA). This paper reports the results of these analyses.

MATERIALS AND METHODS

Samples from pigs and cattle were obtained from 8 and 17 Finnish slaughterhouses, respectively. The samples were collected regularly every month according to an annual plan, from animals selected randomly from the slaughter line. The samples were packed separately, immediately frozen, and sent to the laboratory within 24 h in temperature-controlled chambers containing coolant canisters frozen before dispatch. The samples were stored at −18 °C until analyzed monthly. Sampling was carried out by official inspectors (veterinarians).

The concentrations of selenium were measured until 1990 according to the fluorometric method with 2,3-diaminonaphthalene using a digestion mixture containing nitric and perchloric acids (Stabel-Taucher, 1977). In 1991 a new method was introduced. The sample, about 2 g, is weighed into a 100 mL quartz crucible, mixed with 10 mL of concentrated nitric acid and 10 mL of saturated $Mg(NO_3)_2 \cdot H_2O$ solution, and left on a sand bath overnight. Thereafter, the sample is heated until completely dry and brown fumes are no longer evolved. The crucible is then heated in a muffle furnace (Nabertherm + Program Controller) at 450°C overnight. After cooling, 30 mL of 7 M hydrochloric acid is added. This solution is then heated gently on a hot plate for 10 min to reduce the selenium(VI) to selenium(IV). After cooling, the solution is made up to 50 mL with 7 M hydrochloric acid (Neve et al., 1980). The selenium concentration is measured by hydride atomic absorption spectrometry at 196.0 nm [Perkin-Elmer AAS 2380 + Perkin-Elmer MHS-20 + electrodeless discharge lamp (EDL) + EDL power supply + Perkin-Elmer R 100A recorder]. Reductant (ca. 6 mL) is dispensed into the sample solution (10 mL), where it reacts to liberate hydrogen. The selenium ions are reduced to the volatile hydride. The hydrogen stream flushes the hydride into the heated (900 °C) quartz cell, where it is decomposed and the absorption of the selenium measured. The selenium determination is carried out by direct comparison with standard solutions in 5 M hydrochloric acid. The selenium standard solutions are made up from Titrisol ampules (Merck, Art. 9915).

Before the hydride method was adopted, samples were analyzed with both the fluorometric method and the hydride method to check that the two methods are comparable.

The hydride method has been accredited by the Centre of Metrology and Accreditation of Finland. The laboratory fulfills the requirements of the following standards: SFS-EN 45001 and ISO/IEC Guide 25.

Certified reference materials are analyzed together with each sample series. The analysis results of certified reference materials are shown in Table 1. Blank samples are also analyzed with each sample series to check for contamination. Calibration is continuously checked using standard samples with different concentrations. The recovery is studied by adding known amounts of standard solution to samples. The amounts added are selected so that they would be close to the amounts normally found in meat and liver. The recoveries in different sample materials are 88−99% in muscle and 80−102% in liver for the fluorometric method and 78−117% in muscle and 79−117% in liver for the AAS/hydride method.

RESULTS AND DISCUSSION

The *t*-test (Analysis Tools; Two-Sample Assuming Equal Variances and Two-Sample Assuming Unequal Variances) was used to determine whether there were statistically significant differences between selenium contents in two different years. The *F*-test (Two-Sample for Variances) was used to compare the variances of two populations (Table 2). The selenium contents of muscle and liver of pigs and cattle are shown in Tables 3 and 4.

The selenium contents of muscle and edible offals of Finnish livestock were low compared to those recorded in other countries (Frost, 1971; Ku et al., 1972; Norrmann, 1977) when the supplementation of fertilizers with selenium began. The selenium fertilization began to affect the selenium contents of cattle in 1985, but in pig samples no increase was observed, and in fact the selenium content of pig liver in 1981 was higher than in 1985. After 1985, the selenium content in pig

Table 3. Selenium Concentrations (Milligrams per Kilogram, Wet Weight) of Muscle and Liver in Pig

	muscle			liver		
year	n^a	x^b	SD[c]	n	x	SD
1974[d]	40	0.11	0.03	60	0.45	0.14
1981[e]	48	0.08		60	0.56	
1985	23	0.08	0.01	23	0.49	0.09
1986	24	0.12	0.02	24	0.56	0.10
1987	30	0.23	0.04	30	0.64	0.10
1988	30	0.26	0.06	30	0.7	0.12
1989	34	0.30	0.06	34	0.73	0.13
1990	50	0.29	0.05	50	0.68	0.11
1991	30	0.26	0.09	30	0.62	0.13
1992	30	0.19	0.04	30	0.57	0.08
1993	30	0.19	0.03	30	0.56	0.06
1994	30	0.13	0.03	30	0.50	0.09
1995	31	0.15	0.03	30	0.52	0.06

[a] n, number of samples. [b] x, mean. [c] SD, standard deviation. [d] Stabel-Taucher (1977). [e] Salmi and Hirn (1984).

Table 4. Selenium Concentrations (Milligrams per Kilogram, Wet Weight) of Muscle and Liver in Cattle

	muscle			liver		
year	n^a	x^b	SD[c]	n	x	SD
1974[d]				60	0.10	0.06
1981[e]	60	0.04		60	0.16	0.06
1985	26	0.07	0.03	26	0.28	0.10
1986	25	0.10	0.02	25	0.34	0.09
1987	40	0.16	0.04	40	0.42	0.18
1988	29	0.16	0.04	29	0.45	0.16
1989	35	0.21	0.05	35	0.51	0.18
1990	47	0.19	0.09	47	0.46	0.13
1991	30	0.16	0.04	30	0.40	0.15
1992	30	0.16	0.04	30	0.37	0.10
1993	30	0.14	0.02	30	0.35	0.12
1994	30	0.09	0.03	30	0.32	0.11
1995	30	0.10	0.02	30	0.28	0.05

[a] n, number of samples. [b] x, mean. [c] SD, standard deviation. [d] Stabel-Taucher (1977). [e] Salmi and Hirn (1984).

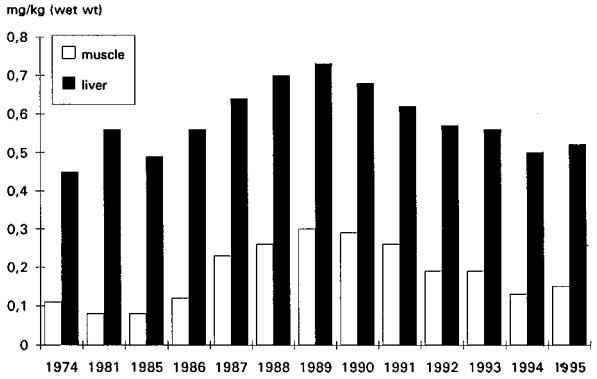

Figure 1. Levels of selenium in muscle and liver of pigs.

samples also began to increase. The reason for this difference was probably that the cattle were fed partly in outside pasture, whereas pigs were fed only inside using feed predating the selenium fertilization. The selenium contents of samples analyzed in 1986 differed significantly from the samples analyzed in 1985 (Table 2). The increase both in pigs and in cattle was highest in samples collected in 1987 compared to the selenium content the year before. A clear increase was also observed in 1989. The highest selenium contents were reached in 1989, when the selenium content in pig muscle was 0.30 ± 0.06 mg/kg, in pig liver 0.73 ± 0.13 mg/kg, in cattle muscle 0.21 ± 0.05 mg/kg, and in cattle liver 0.51 ± 0.18 mg/kg (Figures 1 and 2). The recommended selenium content in pig muscle is 0.060−0.320 mg/kg, in pig liver 0.40−1.20 mg/kg, in cattle muscle

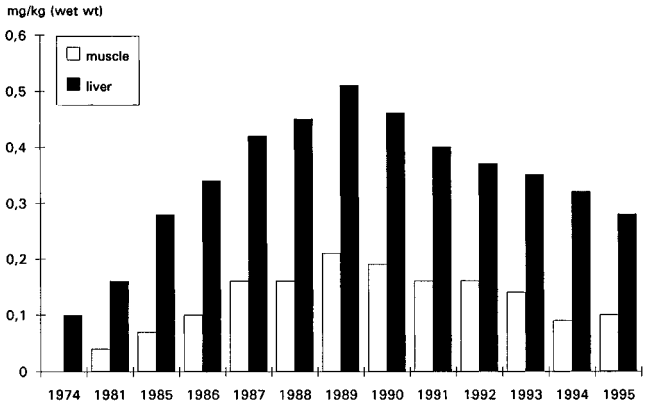

Figure 2. Levels of seleniuim in muscle and liver of cattle.

0.070−0.150mg/kg, and in cattle liver 0.25−0.50 mg/kg (Puls, 1989). The selenium content of liver had increased to the upper limit of the recommended range, and in muscle the content exceeded the recommendation. The selenium content in pigs was rather high even before selenium fertilization began, probably because the pig feed had been supplemented with selenium since the early 1960s to prevent muscular dystrophy.

After 1989, the selenium contents gradually decreased. The reduction of the amount of selenate in fertilizers affected the selenium content in muscle more slowly than that in liver. In samples collected in 1991 the selenium contents differed significantly from the contents in 1989. A very clear decrease was also observed in 1992 in pig samples and in both pigs and cattle in 1994 compared to the selenium contents the year before. In 1995 the selenium concentrations in pig samples and in cattle muscle again increased slightly. In pig samples there was statistical difference (significance level 5%) in selenium contents between 1994 and 1995. The selenium content in pigs and in cattle is currently within the recommended range.

The daily human intake of selenium in 1994 was 0.08 mg/day as calculated from Finnish food consumption statistics at an energy level of 10 MJ (Agricultural Economics Research Institute, 1993). This is almost exactly the level that was originally the target of selenium supplementation. The recommendations of the United States are 0.055 mg/day for women and 0.070 mg/day for men (National Academy of Science, 1989), and the Scandinavian recommendation is 0.03−0.06 mg/day (Nordic Council of Ministers, 1989). The average selenium intake is still higher in Finland than in most other European countries and is at almost the same level as in some parts of the United States and Canada (Ekholm, 1995). According to the Expert Working Group on Selenium, it is recommended to continue the selenium fertilization at the current level.

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