

## Original Article



# Blood selenium levels and contribution of food groups to selenium intake in adolescent girls in Iceland

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## Abstract

**Background/objectives:** Significant changes have been reported in dietary habits and food availability in Iceland that would be expected to compromise selenium intake and status, especially among young people. These include substantial decreases in the consumption of fish and milk, as well as the selenium content of imported wheat. The aim of this study was to assess selenium in the diet and whole blood of adolescent girls, as well as define the most important foods contributing to intake and blood concentrations of selenium.

**Design:** The subjects were 96 randomly selected girls, aged 16–20, who answered a validated food frequency questionnaire (FFQ) for dietary assessment. Selenium intake from each food group was calculated in  $\mu\text{g}/\text{day}$ . Blood samples were collected for measurement of whole blood selenium.

**Results:** Mean dietary selenium was  $51 \pm 25 \mu\text{g}/\text{day}$ . Milk/dairy products, including cheese, contributed  $36 \pm 14\%$  of total dietary selenium; fish  $18 \pm 12\%$ ; and bread/cereal products  $13 \pm 6\%$ . Mean whole blood selenium was  $117 \pm 12 \mu\text{g}/\text{l}$  (range 90–208); nearly 90% of subjects were above the optimal level of  $100 \mu\text{g}/\text{l}$ . Fish and bread/cereal products were the only foods significantly correlated with selenium in blood ( $r = 0.32$ ;  $P = 0.002$  and  $r = 0.22$ ;  $P = 0.04$ , respectively) while no correlation was found with milk and dairy products in spite of their greater contribution to total selenium intake.

**Conclusion:** In this population of Icelandic adolescent girls, selenium intake and status seem acceptable. Judging from associations between intake and blood levels, fish and cereals may be the most important contributors to blood selenium.

Keywords: *selenium; diet; micronutrient status; adolescent girls; fish consumption; milk consumption*

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Concern has been raised over decreased selenium intake and lowered blood concentrations in many European populations (1). Selenium is an essential trace mineral important to human health. Poor selenium status has been associated with increased risk of several chronic diseases, while the exact mechanism or even desirable intake is still under investigation (2, 3). In Iceland, significant changes have been reported in dietary habits and food availability in recent years that would be expected to compromise selenium intake and status, especially among young people and adolescents. These include substantial decreases in the consumption of fish

and milk (4) as well as changes in wheat imports, where selenium-rich wheat from North America has been replaced by selenium-poorer European wheat on the Icelandic market.

Environmental conditions and agricultural practices greatly affect the selenium content of many foods (5–7) which contributes to the extensive variability that has been observed in selenium intake and selenium status of different populations (1, 8). Iceland has been described as a selenium-poor area. Low concentrations of selenium have been found in hay and other vegetation, and selenium deficiency has been commonly reported in

live-stock (9). Iceland is self-sufficient with dairy, meat, and fish, while all grain and fruit as well as about 40% of all vegetables are imported into the country (10).

Selenium is present in foods in different forms with varying bioavailability and metabolic fate (5, 11, 12). Therefore, it is of interest, not only to determine total selenium intake and blood concentrations in the population but also which foods contribute most to intake and blood levels. In light of the great variability in selenium content of similar foods, an updated nutrient database is crucial for such a study, where foods are collected during similar periods as the biological samples for selenium determinations.

The main goal of the study was to assess selenium intake and blood concentrations of adolescent girls, the age and sex group showing the greatest changes in food habits in recent years in Iceland (4, 13), and determine the most important foods contributing to selenium intake and blood levels.

## Subjects and methods

### Subjects

A total of 350 adolescent girls, born in the years 1987–1992 and living in the capital area in Iceland, were randomly selected from Statistics Iceland. Inclusion criteria were the ability to understand Icelandic and absence of disease. Six per cent of the original sample did not fulfil these criteria, and 13% could not be reached by phone, leaving 284 subjects. Of these, 145 girls (51% of the eligible sample) agreed to participate in the study. The main reasons for refusing to participate were lack of time or lack of interest. A total of 112 girls completed the study, but 16 blood samples were insufficient for selenium measurements, leaving 96 girls or 34% of the eligible sample. The study was approved by The National Bioethics Committee (VSNb2007040006) and The Icelandic Data Protection Commission (2007040320) and took place between June 2007 and May 2008.

### Anthropometric measures and lifestyle questionnaire

The subjects' weight and height were measured using a digital scale and a stadiometer (SECA, model 708, Hamburg, Germany) to the nearest 100 g and 1 mm, and body mass index (BMI, kg/m<sup>2</sup>) was calculated. The subjects answered a questionnaire on smoking habits and on oral contraceptive use. See Table 1 for overview of subjects' characteristics.

### Determination of blood selenium concentration

Blood samples were collected using K<sub>2</sub>EDTA anticoagulant for the measurement of whole blood selenium. Samples were stored frozen (−28°C) in plastic vials and thawed immediately before decomposition. Samples were decomposed by microwave heating under pressure in

**Table 1.** Characteristics of subjects (*n* = 96)

Age in years (SD)	18.3 (1.4)
Body weight in kg, mean (SD)	62.8 (11.7)
Body height in m, mean (SD)	1.67 (0.06)
BMI <sup>a</sup> , mean (SD)	22.4 (3.8)
Normal weight <i>n</i> (%)	71 (74.0)
Under weight <i>n</i> (%)	8 (8.3)
Overweight <i>n</i> (%)	12 (12.5)
Obesity <i>n</i> (%)	5 (5.2)
Current smoker <sup>b</sup> <i>n</i> (%)	14 (14.7)
Oral contraceptives <sup>b</sup> <i>n</i> (%)	34 (35.8)

<sup>a</sup>BMI: body mass index (kg/m<sup>2</sup>).

<sup>b</sup>Information from one subject was missing (*n* = 95).

45 ml Teflon cylinders (Parr, Moline, IL) in a microwave oven programmable in three heating steps (Electrolux Heatwave Autocook EMS 2371). An aliquot of approximately 0.5 ml whole blood was weighed into a cylinder, adding 1.0 ml 30% hydrogen peroxide and 1.5 ml 65% (w/w) nitric acid. Six cylinders were placed in the oven at a time, heating for 2 min at 450 W, 1 min at 150 W, and 7 min at 300 W. A clear colorless solution was thus produced, transferred quantitatively to a polystyrene tube (Sarstedt, Nümbrecht, Germany), and diluted with deionized water to 5 ml.

Selenium was determined by graphite furnace atomic absorption spectrometry using a Perkin Elmer Analyst 650 spectrometer equipped with a Zeeman background corrector. Selenium was quantified using a one-point standard addition technique, adding to each sample, alternatively a blank or a standard containing 30 µg/l of selenium. Both addition solutions contained 250 mg/l of platinum (as nitrate) and 0.25% (w/v) of citric acid for matrix modification. All determinations were carried out in duplicates, twice without and twice with added selenium, injecting each time 5 µl of sample plus 5 µl of the addition solution. Signals were evaluated as peak heights. Every tenth sample was a reagent blank. A linear regression of signals from all the blanks was used to estimate the zero level for all other measurements. Accuracy was tested using certified reference samples, Seronorm Blood Level-1 and Seronorm Blood Level-2 (Sero AS, Billingstad, Norway) with a stated selenium content of 80 µg/l and 123 µg/l, respectively. Eight determinations of each reference sample, spread throughout the measurements, yielded average values of 96 ± 19% and 101 ± 11%, respectively of the reference value.

### Definitions of selenium status and recommended intake

Recommendations for selenium intake, including the Nordic Nutrient Recommendations (NNR), are generally based on the criterion of maximizing plasma glutathione-peroxidase activity (GPx3) (14, 15). Studies have shown

that GPx3 is saturated at plasma levels of 70–80 µg/l (16–18), which corresponds to 85–100 µg/l in whole blood according to Combs review (1). Selenium status is ideally determined by more than one indicator. Still, blood or serum levels alone are widely used in the literature, and whole blood selenium is considered to reflect long-term intake better than plasma or serum selenium (19–21). The current NNR for selenium are 40 µg/day for women, with 30 µg/day as the average requirement, 20 µg/day as the lower limit and 300 µg/day as the upper limit of intake (15).

#### Dietary assessment

Dietary intake was assessed using a validated semi-quantitative food frequency questionnaire (FFQ) (22, 23). It includes 130 food items from 17 food groups and is designed to reflect food intake over the previous 3 months. Portion sizes were estimated from pictures of three portion sizes of common food items and from general household measures. The questionnaire was answered in an interview.

The National Food Composition Database (ISGEM) (24) and ICEFOOD nutrient and food calculating program from the Icelandic Nutrition Council were used to calculate the intake of foods in g/day, selenium in µg/day, and contribution of each food group to total selenium intake.

ISGEM was updated in 2009 with respect to energy nutrients, minerals and trace elements, including selenium. The foods examined most extensively were milk, dairy products, cheese, meat (10), fish, cereals, and eggs. All food samples were collected in 2007 and 2008, with milk and dairy products sampled in the summer and winter 2008. The quality of the new data was evaluated, and the data obtained the highest confidence code

established in the EuroFIR project (25). Values for selenium in foods were also obtained from earlier Icelandic investigations and foreign sources (24).

#### Statistical analysis

Descriptive and statistical analysis was conducted using the statistical software package SPSS 11.0 (SPSS, Inc., Chicago, IL). Normality was tested with the one-sample Kolmogorov–Smirnov test. Variables for selenium in whole blood, total selenium intake, and selenium from bread/cereals and meat/poultry, and the percentage intake from milk/dairy products, fish/seafood and meat/poultry were normally distributed, whereas other variables were skewed. Data are presented as mean and standard deviation (SD) and percentiles, as appropriate (Table 2). Total selenium intake and selenium intake from the food groups contributing most to dietary selenium were categorized into highest and lowest quartiles (Q4) and (Q1) (Table 3). The association between blood selenium, total selenium intake and selenium intake from different food groups was assessed using Spearman's rho correlation. The differences in median blood selenium between Q4 and Q1 for total selenium intake, and selenium from different food groups were tested using the Mann–Whitney *U* test. Both tests were two-sided, and the level of significance was taken as  $P \leq 0.05$ .

#### Results

The main sources of dietary selenium were milk and dairy products, fish, bread, and cereals, as shown in Table 2. The combined contribution of milk, dairy, and cheese was  $36 \pm 14\%$  of total selenium intake while fish and cereals contributed less. Almost  $11 \pm 10\%$  came from mixed dishes, mostly pizzas (results not shown). Eighteen girls took vitamin and mineral supplements

**Table 2.** Food consumption from different food groups and contribution to selenium intake, and energy and selenium intake as well as blood selenium

	Percentiles				Contribution to selenium intake (%)	
	Mean	25th	50th	75th	Mean	SD
Food consumption (g/day)						
Milk/dairy products	550	227	418	798	27	14
Fish/seafood	14	6	11	19	18	12
Bread/cereal	166	102	137	205	13	7
Cheese	21	6	13	24	8	7
Meat/poultry	38	17	30	49	8	6
Eggs	12	3	7	12	6	6
Energy intake (kJ/day)	7648	5215	6890	9276		
Selenium intake (µg/day)	51	33	43	59		
Blood selenium (µg/l)	117	108	115	123		

The sum of mean selenium intake from other food groups including vegetables, fruits, sugar, drinks, and mixed dishes was 20% of the total selenium intake. Most from mixed dishes or approximately 11%.

**Table 3.** Correlation (Spearman's rho) between whole blood selenium status ( $\mu\text{g/l}$ ) and selenium intake from each food group ( $\mu\text{g/day}$ ), total selenium intake ( $\mu\text{g/day}$ ) and total selenium intake excluding dietary selenium from fish ( $\mu\text{g/day}$ ) ( $n=96$ )

	Correlation intake vs. status		Median selenium concentration in whole blood ( $\mu\text{g/l}$ ) per Q1 group intake ( $\mu\text{g/day}$ )	Difference in median selenium concentration ( $\mu\text{g/l}$ ) between Q4 and Q1 ( $\mu\text{g/day}$ )	$P^a$
	$r$	$P$			
Total selenium intake	0.20	0.05	109	7	0.04
Milk/dairy products	-0.003	0.98	111	3	0.56
Fish/sea food	0.32	0.002	111	10	0.01
Bread/cereal	0.22	0.04	110	6	0.07
Meat/poultry	0.10	0.32	113	2	0.56
Cheese	0.08	0.43	116	0	0.40
Eggs	-0.02	0.88	114	-4	0.69
Total selenium excluding from fish	0.10	0.34	113	2	0.55

Median selenium status ( $\mu\text{g/l}$ ) in Q1 per intake group. Difference in median selenium status ( $\mu\text{g/l}$ ) between Q4 and Q1 in selenium intake from each food group, total selenium and total selenium intake excluding from fish  $P$  value for the difference in median selenium status between Q4 and Q1 (Mann-Whitney  $U$  test). Significant at the  $P < 0.05$  level (two-sided).

<sup>a</sup> $P$  value for the difference in median selenium status between Q4 and Q1 in selenium intake from Mann-Whitney  $U$  test.

at least once a week while 10 reported daily usage (data not shown).

Total selenium intake and blood selenium are shown in Table 2. Median intake was  $43 \mu\text{g/day}$  (range 20–135), and about 85% of the subjects were above the average requirement of  $30 \mu\text{g/day}$ . Mean whole blood selenium concentration was  $117 \pm 16 \mu\text{g/l}$  (range 90–208). Table 3 shows correlations between blood selenium and selenium intake from each food group (Spearman's rho). There was a modest, but significant, correlation between selenium levels and total selenium intake as well as selenium from cereal products and from fish.

No association was found between dairy consumption ( $\text{g/day}$ ) and blood selenium ( $r=0.005$ ;  $P=0.002$ ) or between selenium from milk/dairy and selenium levels in blood ( $r = -0.003$ ;  $P=0.98$ ). Excluding selenium from milk, dairy products and cheese resulted in a slightly stronger and more significant correlation between selenium intake and blood concentrations ( $r=0.30$ ;  $P=0.003$ ) (data not shown).

The median selenium values in whole blood ( $\mu\text{g/l}$ ) for Q1 and the differences between the median values in Q4 and Q1 selenium intake groups are shown in Table 3. There were significant differences in median selenium concentration between Q4 and Q1 for total selenium intake and dietary selenium from fish/seafood,  $P=0.04$  and  $P=0.01$ , respectively. The difference was not significant ( $P=0.55$ ) when dietary selenium from fish/seafood was excluded from total selenium intake (Mann-Whitney  $U$  test).

Blood selenium was not associated with BMI or energy intake (Spearman's rho), smoking habit or use of contraceptive pills (Mann-Whitney  $U$  test).

## Discussion

In this population of Icelandic adolescent girls, mean selenium intake was above NNRs, and approximately 85% of the subjects had an intake above the average requirement (15). In none of the subjects was the selenium concentration in blood below  $85 \mu\text{g/l}$ , which is the lower level associated with GPx3 saturation (1, 16) and nearly 90% were above  $100 \mu\text{g/l}$ , the upper level reported for the enzyme saturation (17, 18).

Milk/dairy products and cheese contributed approximately one third of dietary selenium, a higher proportion than reported from other European countries as far as we have discovered (2, 26). While milk consumption has decreased substantially in Iceland over the last decades, it still averaged  $550 \text{g/day}$  in these adolescent girls. The selenium content of Icelandic milk is also relatively high compared to neighboring countries (27, 28) ( $2.4 \mu\text{g}/100 \text{g}$ ) (24), which is noteworthy as Iceland is considered a low selenium area (7). Low concentrations of selenium are generally found in hay in Iceland, and selenium deficiency has been reported in Icelandic livestock (9). High selenium content of supplemental feed for dairy cows is a plausible explanation for the high content found in milk, but the selenium content was 18% lower in summer than in winter milk (10).

Interestingly, no association was found between the intake of milk and dairy products and selenium in whole blood in spite of their substantial contribution to selenium intake. It is well known that the absorption and metabolism of selenium from foods varies, depending on the chemical forms as well as on selenium status (8, 29). Consequently, no simple relationship exists between total selenium intake and selenium concentration in blood. Particularly when the level in plasma exceeds  $70 \mu\text{g/l}$ ,



the concentration depends greatly on the form consumed (14, 17, 30). The chemical speciation of selenium in cow's milk is largely determined by the forms present in the feeds (31) and selenite, the form used in feed for dairy cows in Iceland, has been reported not to affect selenium status in selenium-replete individuals (12, 32, 33). However, the chemical speciation of selenium in Icelandic cow's milk has not yet been analysed. The lack of correlation between blood selenium and milk and dairy products is in sharp contrast to our study of iodine status of these same adolescent girls (13). In the iodine status study dairy was not only the food group contributing most to iodine intake, but also the only one that correlated significantly with iodine status.

In contrast, selenium from bread and cereals and from fish, which contributed much less to total selenium intake, did correlate significantly with blood levels. This is in partial agreement with other studies, including a recent Danish study, showing that seafood intake was associated with serum selenium values, while milk consumption showed no such association (34). In that study dairy selenium contributed significantly less to total selenium intake than in our study while fish accounted for a comparable proportion of intake.

The significant difference in selenium concentrations between the highest and lowest quartiles of fish intake further supports the contribution of selenium from fish, while no difference was seen between quartiles for other food groups. Comparable associations with selenium status and fish and seafood have been seen in several studies (29, 35–38) but not in others (39, 40), possibly resulting from differences in selenium forms and content in different fish species.

Selenium is abundant in fish, especially ocean fish (41), and according to the ISGEM database the selenium content in haddock (*Melanogrammus aeglefinus*), the species accounting for 80% of total fish intake in this study (13), is 38.8 µg/100 g (wet weight) (24). In cod, a close relative of haddock, 70% of the selenium is selenomethionine (11, 42), a form that is readily absorbed and taken indiscriminately into body tissues. Selenomethionine is also the form abundant in wheat (11), and, interestingly, selenium from grain was associated with selenium in blood. Wheat selenomethionine has been shown to increase plasma levels even in selenium-replete individuals (12, 43) while no similar increases in plasma are observed with other biochemical forms of selenium. Considering the adequate selenium status of our subjects, the observed association with fish and cereal intake but not with milk and dairy products, may result from the form of selenium in these foods.

Cereals contributed only 13% of total selenium, reflecting low cereal consumption in Iceland. Practically no grain is grown in the country for human consumption, and cereals have traditionally been sparse in the Icelandic

diet. Nowadays most of the wheat imported to Iceland comes from low-selenium areas in Europe with a mean concentration of 3.3 µg/100 g. American wheat, which was until recently the most commonly imported wheat to the country, contains approximately 10 times more selenium (24, 28). As a result, when older selenium values from the Icelandic nutrient database were used to calculate these same intake data, cereals accounted for 25 ± 10% of total selenium intake (results not shown) rather than the 13 ± 7% reported presently.

The only previous study on blood selenium in Iceland published in 1983 showed mean whole blood selenium of 127 ± 20 µg/l in healthy adults ( $n=43$ ) (44), compared with 117 ± 16 µg/l in our study. Direct comparisons are difficult because of the age and sex difference, as well as different methodology. In the earlier study, blood selenium was determined by fluorometry and the accuracy was not reported. However values would be expected to be lower now than earlier considering decreased fish intake in Iceland and decreased selenium in wheat. According to national nutrition surveys, mean adult fish intake decreased from 73 g/day in 1990 to 40 g/day in 2002, and that year the amount consumed by young girls was the lowest of any age group, or 15 g/day (4), which is comparable to the 14 g/day observed in this study.

An accepted optimal reference has not been established for blood or plasma selenium and appropriate biomarkers for selenium status are still under debate. In our study, the mean blood level of 117 µg/l is within the range associated with maximal GPx3 saturation, and no girl had levels associated with selenium insufficiency. However, only about 30% of the girls reached the blood selenium concentrations of 120 µg/l associated with maximal expression of selenoprotein P (2, 45), and 5% were above 150 µg/l, the level that has been associated with decreased mortality and potential cancer protection (1, 46).

No association was found between selenium in whole blood and BMI, energy intake, smoking habits, or use of contraceptive pills. The effect of lifestyle factors on selenium status has not been studied extensively. However, smoking has been reported to increase oxidative stress and may thus increase selenium requirements (47). The number of daily smokers in our study may not have been sufficient to demonstrate any putative relationship between smoking and selenium levels.

A limitation to our study is the low participation rate, i.e. 34% of the eligible sample. The girls participating in the study possibly had better diets and selenium status than girls of this age in general, thereby biasing the results. However, this group of girls was found to be representative of Icelandic adolescent girls with respect to overweight and obesity prevalence (4). Also, their food intake, including intake of fish and milk, resembled the average intakes in Iceland in this age group, according

to the most recent National Nutrition Survey (4). We therefore conclude that their food intake may adequately reflect that of the original sample.

The use of a FFQ to assess food and selenium intake may be considered a limitation. However, a validation study has demonstrated acceptable assessment of all the major food groups and nutrients (22, 23). The foods most likely to be underestimated are sweets and soda – foods that do not contribute significantly to selenium intake. Furthermore, FFQs including more than 100 food items have been shown to adequately assess selenium intake (48). Food supplements were included in intake calculations. However, most common supplements in Iceland do not contain any selenium. Another limitation is the use of selenium in whole blood for measuring selenium status. Other measures may be more appropriate, such as selenoprotein P (45). Also, a combination of two or more biomarkers may better reflect both nutritional status and intake from selected foods (2, 47). However, selenium concentrations in blood are widely used and available for comparisons between populations and time periods. Also blood levels may be a simple way to detect possible insufficiency in a population for public health purposes.

An important strength of the study includes the careful analysis of selenium in foods for the National database, with food sampling and analysis carried out during the same time period as the blood was collected from the subjects. As the selenium content of foods, including milk, meat, and cereals, varies greatly according to feeding practices of cattle as well as soil conditions, the levels registered in food tables may lead to invalid conclusions regarding intake when measurements are not quite up to date.

### Conclusion

In this population of Icelandic adolescent girls, selenium intake and blood levels appear acceptable even though they do not reach the levels associated with possible cancer risk protection. Milk and dairy products contribute most to selenium intake, while fish and cereals appear to be the most important contributors to blood selenium, judging from associations between intake and blood levels in this population of mostly selenium-replete subjects.

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L.S. and I.G. were responsible for the study design, collection of data and interpretation of the results, and they supervised the writing of the paper. I.G. was also responsible for statistical analysis. A.T. was responsible for the measurements and analysis of the blood samples, O.R. for the Icelandic Food Composition Database and H.G. for food analysis. I.T. was involved in the study design, interpretation of

the results and writing of the paper. E.Y.G. was involved in the data analysis and writing of the manuscript.

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