

# APARATURA

## BADAWCZA I DYDAKTYCZNA

### The influence of constitutional factors on the daily intake of selenium on the example of Pomeranian population

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**Keywords:** selenium, dietary methodology, food intake, BMI, duplicate portion method

#### ABSTRACT

In the current work, dietary intake of selenium was studied among adults residents of Pomeranian District. Concentration of selenium in diet samples was determined using Hydride Generation Atomic Absorption Spectrometry (HGAAS) technique after microwave digestion.

The analyses of the 1 - day food ratios showed that the participants consumed insufficient levels of selenium with reference to Recommended Dietary Allowance what may constitute a risk for the deficiency of this element in human body. Moreover, the statistical analysis demonstrated a significant effect of the gender, age, and body mass index upon the dietary intake of selenium.

# Ocena wpływu czynników konstytucjonalnych na dzienne spożycie selenu na przykładzie mieszkańców województwa pomorskiego

**Słowa kluczowe:** selen, metodologia badań spożycia żywności, spożycie żywności, BMI, metoda podwójnej porcji

## STRESZCZENIE

W pracy dokonano oceny realizacji zapotrzebowania na selen w całodziennych racjach pokarmowych pobranych metodą podwójnej porcji wśród mieszkańców województwa pomorskiego. Oznaczenie Se przeprowadzono techniką generowania wodorków w połączeniu z absorpcyjną spektrometrią atomową (HGAAS) po uprzedniej mineralizacji próbek żywności.

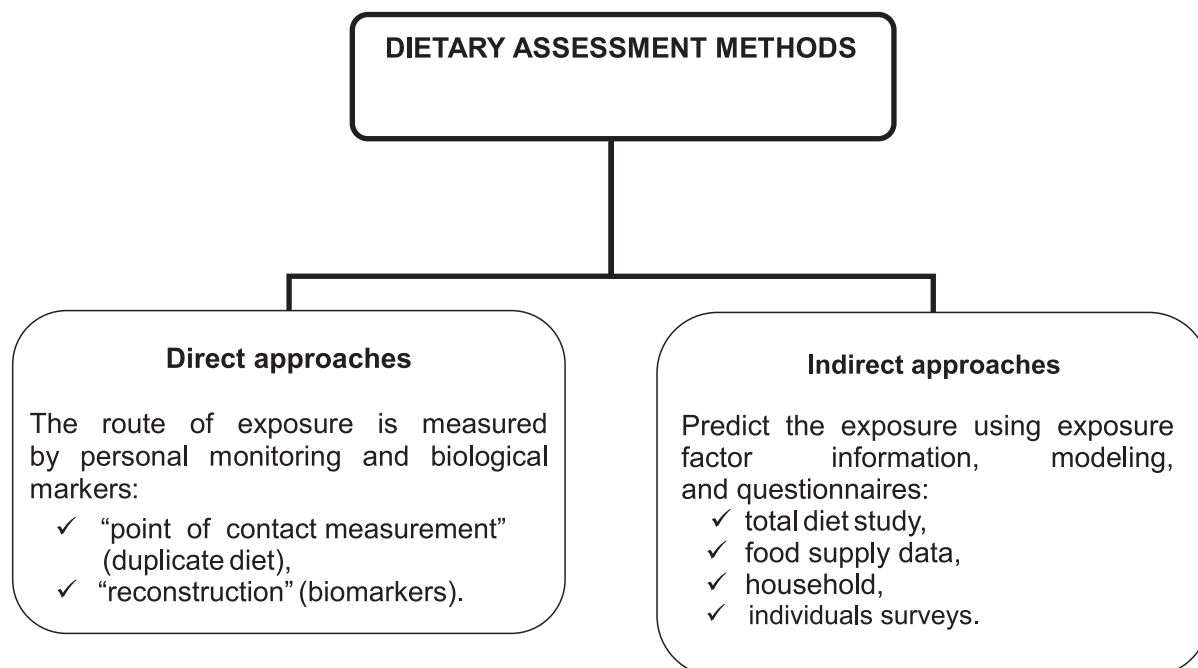
Analiza całodziennych racji pokarmowych pozwoliła na stwierdzenie, że ilość selenu przyjmowanego wraz z dietą jest niewystarczająca i może stanowić duże ryzyko niedoboru tego pierwiastka w organizmie. Ponadto, przeprowadzona analiza statystyczna wykazała wpływ płci, wieku oraz wskaźnika masy ciała na zawartość selenu w badanych próbkach żywnościowych.

## 1. INTRODUCTION

A few elements that occur in the environment in trace or ultratrace amounts, often called essential microelements, have a number of biological functions in live organisms. One such element is selenium, which has been characterized as “two – faced” [1, 2]. Although selenium is necessary for proper functioning of live organisms, its excessive intake may result in homeostatic imbalance, and even death [3]. The ambivalent character of selenium attracted wide interest in the properties and influence on live organisms. Many epidemiological studies [4, 5] have reported an association between the improper nutrition (that may contribute to insufficient consumption of selenium) and etiology of disease processes or, in some cases, the intensification of an existing disease [6-8]. The critically low selenium intake, suggested as less than  $11 \mu\text{g day}^{-1}$  is definitely associated with a number of adverse health effects that may lead to increased morbidity and mortality rates [9]. The physiological daily requirements for selenium in adult diets have been estimated to be at least  $40 \mu\text{g}$  [10], whereas the supranutritional dose (as much as  $300 \mu\text{g day}^{-1}$ ) [10] has been found to be protective against some chronic diseases [10-12]. The possible beneficial effects of selenium, consumed in higher doses than those needed for the prevention of deficiency symptoms, include a chemoprevention of cardiovascular disease and certain types of cancer [13]. On the other hand, the excessive intakes of selenium, over  $900 \mu\text{g day}^{-1}$  taken over a prolonged period of time, may lead to toxic symptoms [14],

while a dose as small as  $5 \text{ mg day}^{-1}$  can be lethal for many humans. The consumption of selenium at level of about  $400 \mu\text{g day}^{-1}$  was suggested to be the maximum safe dietary selenium intake [15]. Taking into consideration food as the main source of selenium, its daily intake is dependent, among others factors, on food origin and thus on the level of selenium in soil, where the food was produced. Unfortunately, the soils of Poland generally feature with a low content of this microelement. According to reported data, the selenium content did not exceed  $123 \mu\text{g kg}^{-1}$  for the soils in the region of the western Pomerania [16]. The intake of selenium by the general population from drinking water appears to be insignificant and has been recommended to be  $10 \mu\text{g dm}^{-3}$  [17, 18]. The WHO recommendations assume that the drinking water is not regarded as a nutritional source of selenium, and intake from it should be therefore less than 10 % of the tolerable exposure [17]. According to the report on Environmental State Assessment the content of selenium in ground waters of Gdansk ranges from  $0.00020$  to  $0.0025 \text{ mg dm}^{-3}$  [19].

The assessment of nutritional status on Se can be realized using direct or indirect methods [Fig. 1]. Among them, the duplicate diet collection methodology is a particularly useful approach [20] since it provides the most accurate information on the ingested dose of nutrients. This technique pertains to the analysis of the exact duplicate portion of all food and beverages prepared for the consumption. An unusually significant issue is that duplicate diet methods account for specific food and water sources, types and quantities of



**Figure 1** Food intake measurement [21]

**Rysunek 1** Źródła informacji o spożyciu żywności [21]

food consumed, as well as cooking methods. In other words, it reflects the dietary habits of meal preparation of the surveyed population. It is extremely important, due to the fact, that selenium is sensitive to processing effects. Peeling, washing, cooking, frying, and other culinary activities can have a significant influence on the content of selenium in foodstuffs.

## 2. MATERIALS AND METHODS

### 2.1 Study populations

The study was conducted in two periods: between March and September 2006 and from February to May 2008 among randomly selected residents of Pomeranian District. 46 inhabitants were asked to collect the equivalent portions of all the food, beverages, and snacks they have consumed during the day of the study. Among participants were adults aged between 18 and 65. All subjects were requested to complete a questionnaire that asks, among other questions about the body weight and height. The self-report anthropometric data were then used to calculate BMI. The consumers were divided into two groups, according to gender. Details referring to the groups are summarized in Table 1.

It is important to mention, that none of the participants were on a special diet and no participant

was vegetarian. All of them consumed a mixed diet.

**Table 1** Baseline characteristics of the population participated in the diet study

**Tabela 1** Charakterystyka badanej populacji

Characteristics	Male	Female
No. of subjects (n)	25	21
Mean age (range) (years)	39 (18-58)	39 (18-65)
Mean BMI (range) (kg m <sup>-2</sup> )	22 (18-30)	27 (18-38)

### 2.2 Sample preparation and analysis

The samples of diet were collected and stored in plastic containers. Individual duplicate food portions were weighted, homogenized, aliquoted and kept frozen until lyophilized. The dried sample was then powdered and stored in refrigerator until the consecutive stage of pre-analysis. About 1 g of lyophilized material was digested (under the optimized conditions described in Table 2) with HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> in the microwave digestion system. The blank digestions were carried out in the same way. The samples of each portion of daily food were analyzed in duplicate or triplicate. After cooling, the digested solutions were diluted with deionized water to 25 mL.

**Table 2** Digestion conditions of the food samples in the microwaves digestion system

**Tabela 2** Warunki mineralizacji mikrofalowej próbek żywności

Power [W]	Time [min]	Pressure [Pa]	Temperature [°C]
300	15	5.2·10 <sup>6</sup>	180
480	15	5.2·10 <sup>6</sup>	200

After the digestion, the solution was treated with 1 mL of 8 % of (NH<sub>2</sub>)<sub>2</sub>CO, and placed into a 20 mL beaker. Afterward, 0.8 mL of HClO<sub>4</sub> was added and heated in a thermostated mineralizer at 120-130°C for 90 min until the HNO<sub>3</sub> was completely evaporated. Subsequently, the resulting samples (0.6-0.8 mL) were diluted with redistilled water to 5 mL; and after adding of 5 mL of concentrated HCl, the mixtures were heated on a water bath at 80°C for 30 min. After reduction of Se<sup>4+</sup> to Se<sup>2+</sup> with NaBH<sub>4</sub>, the final determination by HGAAS was performed. An Avanta Σ atomic absorption spectrometer (GBC Scientific Equipment, USA) equipped with an automatic hydride generator with a continuous-flow generation system HG 3000 was used for selenium quantification.

### 2.3 Quality assurance of the results

The accuracy was assessed by the determination of selenium in the certified reference material (NIST SRM 1548a Typical Diet), which was digested analogous to the food samples. The mean selenium concentration (n=10) obtained for the reference material (0.233±0.062 mg kg<sup>-1</sup>) agreed with the certified value (0.245±0.028 mg kg<sup>-1</sup>) at the 95 % probability level.

### 2.4 Statistical analysis

Statistical analyses were performed with the Statistica software, version 8.0. Before starting the assessment of the potential influences on the daily dietary selenium intake, the distribution characters were investigated for data of all reflected factors. Since the data on selenium level were not normally distributed; the non-parametric tests (Manna Whitney U test for two unpaired variables, Kruskal Wallis test for three-group unpaired variables, and Spearman rank correlation coefficient) were applied to assess any relationships between concentrations of selenium found in the duplicate diets and several factors.

## 3. RESULTS AND DISCUSSIONS

### 3.1 Daily dietary intake of selenium in Pomeranian District

The mean daily intake of selenium for the healthy subjects living in various areas of the Pomeranian District was 45 µg day<sup>-1</sup>. According to the Recommended Dietary Allowance (RDA = 55 µg day<sup>-1</sup>), the participants' diets were deficient in selenium. In women, mean daily intake of this element amounted 96 % of the RDA level, whereas in men 70 %. A low selenium intake was found in the case of 57 % females and 76 % males.

A comparison of daily intake of selenium by the investigated population with the reported values in other countries indicated that the obtained mean value is comparable to those found in Belgium [22], Sweden [23], Greece [24], and France [25].

### 3.2 Influence of constitutional factors on the daily intake of selenium

In Table 3 was shown the variables to be significantly related ( $\alpha < 0.050$ ) to selenium levels in diet samples.

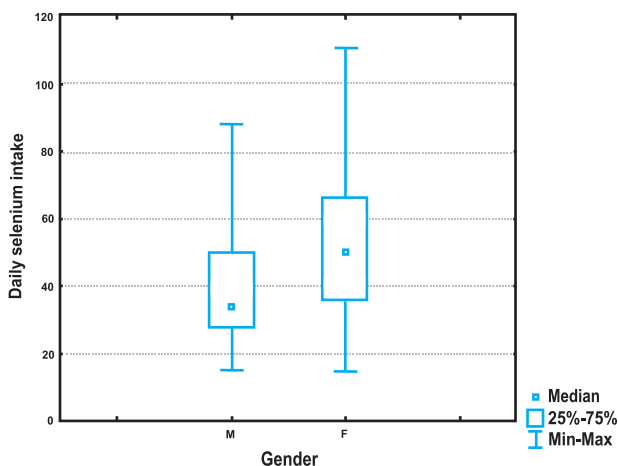
The statistical analysis demonstrated a significant effect of the gender upon the dietary intake of selenium ( $\alpha = 0.044$ ). It turned out that intake of selenium in diet of females was statistically higher than men (Fig. 2). Probably the most important factor leading to this finding was taking the supplements by participants. The exclusion

**Table 3** Selenium content (x) in diets [µg day<sup>-1</sup>] according to different characteristic

**Tabela 3** Średnie zawartości selenu (x) w dietach [µg day<sup>-1</sup>] w zależności od charakterystyki danej grupy

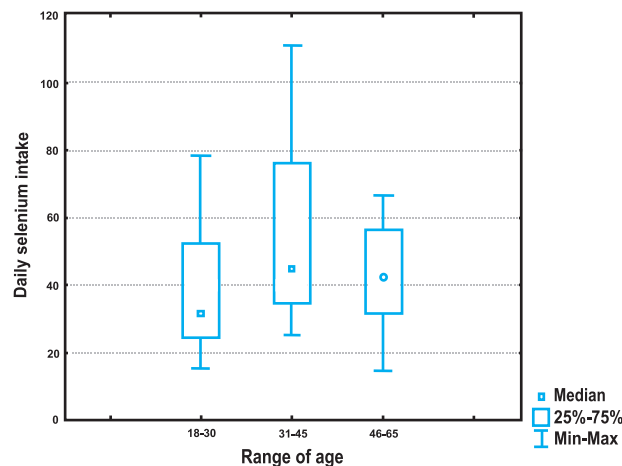
Characteristic	n	x ± SD	Median
Gender			
male	25	39±17	34
female	21	53±24	50
Age (years)			
18-30	13	39±19	31
31-45	18	54±26	44
46-65	15	41±15	42
BMI (kg m <sup>-2</sup> )			
<18.5	4	24±7	23
18.5-25.0	23	46±22	42
25.0-30.0	12	45±21	42
>30.0	7	56±21	58





**Figure 2** Dietary intake of selenium [ $\mu\text{g day}^{-1}$ ] of males (M) and females (F)

**Rysunek 2** Średnie spożycie selenu [ $\mu\text{g day}^{-1}$ ] w grupie mężczyzn (M) i kobiet (K)



**Figure 3** Dietary intake of selenium [ $\mu\text{g day}^{-1}$ ] of participants in different age groups

**Rysunek 3** Średnie spożycie selenu [ $\mu\text{g day}^{-1}$ ] w różnych grupach wiekowych

of the supplement users from the both gender groups have contributed to obtain contrary results. For participants who did not take selenium supplements there was no significant difference in selenium intake ( $\alpha=0.31$ ) between two genders.

The daily selenium intake depends on many internal and external factors such as its concentration in eaten food, the amount of food consumed, chemical form of the mineral, gender, age, etc. It is important to mention that men participated in this study were characterized by comparatively higher food consumption compared to females. The averages dry weight of food consumed by the males and females included in the present study were 1500 g and 1200 g, respectively. Despite this fact, after exclusion of supplement users there were no differences between the average selenium intakes between genders. So, it can be concluded that females ate more food items rich in selenium than males.

Dividing of the participants into three subgroups according to age (18-30, 31-45, 46-65 years)

showed a statistically significant difference between the first (18-30 years) and the second (31-45 years) age group ( $\alpha=0.046$ ) (Fig. 3). As it can be seen in Table 3, the average daily selenium intake is almost equal the RDA value in the case of second subgroup.

Nevertheless, the analyses conducted separately for females and males did not show an effect of age differences in selenium intake (Tab. 4). Furthermore, no significant differences were observed when such comparisons were made between genders for each age group, separately ( $\alpha=0.092$ ,  $\alpha=0.33$ ,  $\alpha=0.64$ , respectively, for the following age groups: 18-30, 31-45, 46-65 years). An application of the Spearman test to percentage of fulfillment of RDA value and participants' BMI showed a statistically significant correlation (the Spearman correlation coefficient  $r=0.35$ ,  $\alpha=0.018$ ) (Fig. 4). In general, higher BMI was associated with higher total selenium intake.

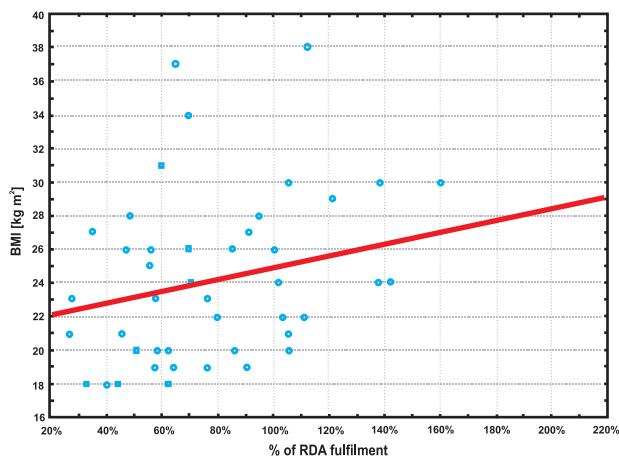
Dividing of the participants into four subgroups according to BMI (<18.5, 18.5-25, 25-30, >30  $\text{kg m}^{-2}$ ) showed a statistically significant difference

**Table 4** Daily intake of selenium ( $\bar{x}$ ,  $\mu\text{g day}^{-1}$ ) in age groups for both genders

**Tabela 4** Dzielne spożycie selenu ( $\bar{x}$ ,  $\mu\text{g day}^{-1}$ ) w różnych grupach wiekowych obu płci

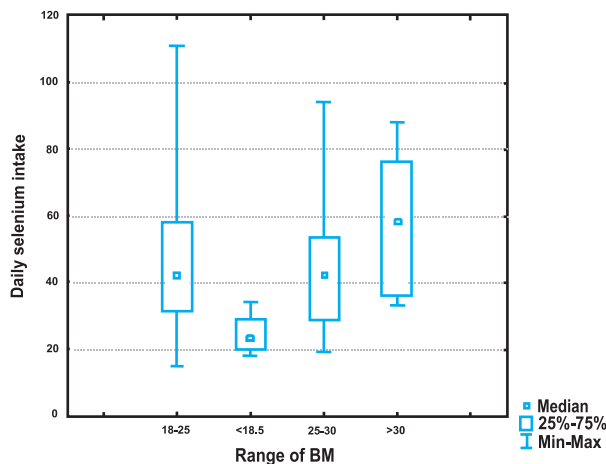
Group	Age groups [years]									$\alpha$
	18-30			31-45			46-65			
	<i>n</i>	$\bar{x}\pm\text{SD}$	Median	<i>n</i>	$\bar{x}\pm\text{SD}$	Median	<i>n</i>	$\bar{x}\pm\text{SD}$	Median	
Females	5	50 $\pm$ 20	52	10	59 $\pm$ 29	52	6	44 $\pm$ 18	46	0.66
Males	8	32 $\pm$ 14	31	8	47 $\pm$ 21	42	9	39 $\pm$ 13	35	0.23

$\alpha$  - The  $\alpha$  value for the differences in daily selenium intake between age groups for each gender



**Figure 4** Correlation of % of selenium RDA fulfillment and BMI of participants

**Rysunek 4** Korelacja pomiędzy pokryciem normy zalecanego dziennego spożycia (RDA) selenu, a wartościami BMI



**Figure 5** Dietary intake of selenium [ $\mu\text{g day}^{-1}$ ] of participants in different BMI groups

**Rysunek 5** Średnie spożycie selenu [ $\mu\text{g day}^{-1}$ ] w grupach o różnych wartościach BMI

between the first ( $>18.5 \text{ kg m}^{-2}$ ) and the fourth ( $>30 \text{ kg m}^{-2}$ ) BMI group ( $\alpha=0.032 \text{ kg m}^{-2}$ ) (Fig. 5). As it can be seen in Table 2, the average dietary intake of selenium in the case of fourth subgroup is within the recommended daily intake. The differences between the total selenium intakes observed among BMI categories were expected since; in general, people with larger body size tend to consume more food.

#### 4. CONCLUSIONS

The presented data do not suggest that the studied population is immediately endangered by an extremely low selenium status. However, the insufficient selenium daily nutritional intake levels may call for attention and consideration.

#### Acknowledgements

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#### The operating parameters for HG AAS analysis

Wavelength	196,0 nm
Measurement mode	Integration
Lamp current	10,0 mA
Read time	3,0 s
Slit width	1,0 nm
Time constant	1,0 s
Slit Height	Normal
Repetitions	2

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