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Cadmium Measurements in Blood and Hair of Occupationally Non-Exposed Military Recruits and in the Foods of Plant Origin Produced in Slovenia

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Aim. To determine cadmium content in the most frequently consumed foods of plant origin grown in different regions of Slovenia, assess cadmium content in the biological materials (blood and hair) from Slovenian young men, and verify the hypothesis that the cadmium pollution of the environment varies across regions in Slovenia.

Methods. We analyzed cadmium concentration in 982 composite samples of foods of plant origin, and determined the cadmium burden in the population by analyzing cadmium content in whole blood and hair samples from 742 randomly selected healthy men aged 18-26 years.

Results. There were significant differences in cadmium content in the analyzed foods with respect to the regions in Slovenia where were the foods of plant origin were produced ($p<0.001$). The median cadmium content in 463 blood specimens was 0.5 μg/L, and 4 ng/g in 245 hair specimens. Cadmium content in the blood and hair samples from study participants varied significantly across regions in Slovenia ($p<0.001$ and $p<0.003$, respectively). Cadmium content in foods of plant origin and cadmium burden in the blood showed only a weak correlation (Spearman's $r=0.13$), whereas the correlation between cadmium content in the foods and hair was much stronger (Spearman's $r=0.55$).

Conclusion. Our research confirmed the hypothesis of regionally different environmental pollution with cadmium, and clearly showed the connection between cadmium burden in foods and in population in Slovenia.

Key words: *cadmium; blood; hair; food analysis; statistics; Slovenia*

The most important way of intake of harmful substances in the occupationally unexposed population groups is dietary intake (1). If the characteristics of absorption, distribution, and excretion of a substance are known, biological monitoring can be used for the exposure assessment of individuals.

The concentration of harmful substances in human biological material depends on the extent of person's exposure as well as on his or her genetic characteristics and individual habits, because these factors affect body intake, absorption, and concentration of such substances in the body (2,3). The long-term intake of harmful substances, even in relatively low concentrations, represents a substantial health risk (4). This particularly applies to substances that tend to accumulate in the body (5). One of these harmful substances that raise a special health concern is cadmium (5,6). Since biological accessibility of cadmium varies in different foods, exposure assessments can be unreliable because they concentrate on the exposure of population to substances in food (7). For the evaluation of recent exposure to cadmium, whole blood samples are considered the most appropriate material (8). Cadmium concentration in occupationally unexposed people mostly depends on the cadmium intake with food. Local environmental conditions have a significant impact on the amount of cadmium ingested, especially where food of plant origin is concerned. It is very easy for cadmium to transgress from the soil into plants (9). Thus, some plant types can absorb considerable quantities of cadmium even when its concentration in the soil is relatively low. An increased cadmium pollution of the soil increases plant cadmium uptake, which means increased cadmium ingestion with food of plant origin (7).

The cadmium uptake from the soil depends on the type of plant and the acidity of the soil – higher acidity of the soil increases cadmium intake (9). In acid rain areas, the pollution of plants with cadmium is greater than in alkaline or neutral rain areas (10). Thus, the presence of cadmium in plants can be an indicator of the degree of environmental pollution.

Since the food of plant origin represents the most important source of cadmium in human diet, we wanted to establish the correlation between the average cadmium content in plant origin foods from various Slovene regions, and the cadmium content in the blood and hair of the local population. Slovenia (21,000 km^2) is a central European country, with 2 million population, bordered by Italy, Austria, Hungary, and Croatia. We had no data available on cadmium concentrations in Slovene population until research conducted in 2001 among 64 blood donors, occupationally unexposed to cadmium intake (11). The study showed a low medium cadmium concentration of 0.3 μg/L in the blood of non-smokers and as high as 0.9 μg/L in the blood of smokers.

The aim of our study was to determine the cadmium content in the most frequently consumed foods of plant origin and assess the cadmium content in biological material, ie, blood and hair from the Slovenian young men. Furthermore, by showing different cadmium content in the biological material from men from nine different regions, we would confirm the hypothesis that the cadmium pollution of the environment varies regionally.

Participants and Methods

Participants

The study focused on the population of military recruits called up for military service between August and November 2001. All recruits have to undergo an obligatory vaccination against tick-borne meningoencephalitis, which is performed at the Public Health Institute in the region of their permanent residence. The recruits, who came for vaccination successively upon receiving call-ups, were invited to participate in our research of the cadmium content in blood and hair. They were informed on the purpose and course of our research, and asked to fill out a questionnaire about their age, profession, hobbies, eating habits, and the quantity of alcohol and cigarettes consumed per week.

Taking into account the expected number of recruits and data on the prevalence of smoking among the young population, we determined that study's enrollment period should last at least 4 months before each regional group of occupationally unexposed nonsmokers would consist of at least 40 participants. Due to a very high response rate of 93.7% (742 out of 792 recruits agreed to co-operate), the number of non-smoking participants in each regional group was higher than the minimum of 40.

To assess the intake of cadmium with food as accurately as possible, we excluded anyone who declared themselves as smokers, drank more than 20 g of alcohol per day, had special eating habits (vegetarians or vegans), or could be occupationally exposed to increased cadmium intake (12). Thus, miners, painters, and metal (especially color-metal) industry workers were excluded due to their possible professional exposure to cadmium. Eventually, the analysis of blood cadmium concentrations included samples from 464 participants aged 18-27 years from nine different regions in Slovenia (Table 1). There was no statistically significant difference in the median age of the recruits from different regions.

The call-up of recruits takes place randomly, depending on the space capacities of the military barracks and the age of recruits, so we considered the participants in our study a randomly selected sample. Therefore, the information gathered by our research could be used to describe the characteristics of the Slovene male population corresponding by sex, age, eating habits, and smoking status to the population segment included into our research.

Food Samples

The samples of foods of plant origin were collected from 1999 until 2000. To obtain a nationally representative data, random samples of foods were taken from both regional farmers and shops in all major cities, taking into account the seasonal variation of food consumption. The sampled food originating from nine regions of Slovenia (ordered geographically from the west to the east): Nova Gorica, Koper, Kranj, Ljubljana, Ravne, Novo mesto, Celje, Maribor, and Murska Sobota. From each region, at least six random composite samples of individual foods were obtained. A composite sample was obtained by taking three to six (depending on the size of a region) separate foods samples and compounding them into a single one. A considerable quantity of the composite samples was reduced in the laboratory as prescribed, and then treated in the manner customary for food preparation before cooking. The analysis included foods of plant origin consumed by adult Slovenes in quantities of at least 2 kg a year.

Table 1. Number of participants and their age by regions in Slovenia

Region	No. of participants	Median age (range, years)
Ravne	59	20 (18-25)
Murska Sobota	59	20 (18-26)
Nova Gorica	51	20 (19-27)
Celje	44	20 (19-27)
Kranj	57	20 (18-27)
Novo mesto	57	20 (18-27)
Koper	40	20.5 (18-26)
Ljubljana	48	21 (18-26)
Maribor	49	21 (18-26)
Slovenia	464	20 (18-27)

Blood Samples

Blood samples of up to 2 mL were drawn into a 5-mL syringe (with added ethylene diamine tetracetat acid) from the cubital vein. Prior to the sample taking, the skin was thoroughly cleansed with a disinfectant (70% alcohol or Cetavlon). Every blood sample carried an identification number, identical to the number of the questionnaire completed by the participant before the blood sample taking. The samples were stored in a refrigerator until analysis. If the analysis did not taking place within 48 h, they were frozen and stored at -20°C.

Hair Samples

A bundle of 50-100 hairs was cut from the nape of the neck as close to the scalp as possible. Every hair sample was allocated an identification number, identical to the number of the questionnaire completed by the participant. After being washed in n-hexane-ethanol, the hair was fixed between two pieces of adhesive tape, and a 2-3 mm segment on the proximal end of the bundle was cut off for cadmium content determination.

Analysis of Food Samples of Plant Origin

The cadmium concentration was determined with the use of electro-thermal atomic absorption spectroscopy (SpectrAA-20, Varian, Mulgrave, Australia). The digestion was carried out in a laboratory microwave oven under pressure, and concentrated HNO_3 and 30% H_2O_2 were added. The results were reported in μg/kg of fresh sample. The quantification limit for cadmium in food samples of plant origin was 5 mg/L.

Analysis of Blood Samples

Blood samples were diluted before analysis with the solution of 0.25% ascorbic acid and 1% Triton X-100, applied ratio 1:5. The prepared samples were analyzed directly with electrothermal atomic absorption spectroscopy (SpectrAA-20, Varian, Mulgrave, Australia). Argon gas was used for blowing of graphite tube atomizers.

During the ashing stage, air was introduced into the graphite furnace instead of argon to burn the organic matrix. Cadmium absorbance spectrum was detected at 228.8 nm, and the background correction was performed by using the deuterium bulb. The quantification limit for blood cadmium concentration was 0.5 mg/L (13,14).

Analysis of Hair Samples

The concentration of cadmium in hair sample (ng/g) was measured with solid-sample electrothermal atomic absorption spectroscopy on the laboratory-assembled electrothermal atomic absorption spectrometer (Biotechnical Faculty, Ljubljana, Slovenia). The quantification limit for hair cadmium concentration depended on the amount of hair in the bundle, and was between 2 and 7 ng/g (15).

Analytical Quality Assurance and Control

The calibration with the method of standard addition took place before sample measurement and afterwards every ten samples. In this way, it was possible to ensure that all the samples and standards were prepared in the same way and with the same chemicals. The blank probe was prepared in the same manner.

Each sample was prepared and analyzed in pairs, so the final value represented the mean value of both measurements. Standard deviation was calculated from ten measurements of different paired samples. The reliability of measurements was considered satisfactory if the difference between parallel determinations did not exceed two standard deviations; the measuring was repeated if the difference was greater.

The accuracy of the method was verified by an analysis of the certified reference material produced by CEC (Brussels, Belgium): CL-1 (cabbage leaves), BCR 189 (wholemeal), and BCR 184 (bovine muscle). The resulting coefficient of variation for the analysis of cadmium was 6.5%.

BCR-CRM 194 (CEC) certified reference material was used in monitoring the correctness of the blood analysis method. The analysis results for the reference material were satisfactory (observed values 94-103% of certified value, coefficient of variation 4.7%).

To check the accuracy of the hair analysis, the GBW 09101 certified reference material (Peking, China) was used. The analysis results for the reference material were satisfactory: observed values were 96-108% of certified value, coefficient of variation 7.1%.

Statistical Analysis

The cadmium blood, hair, and food concentrations are either quantitative or semi-quantitative, depending on how the values beyond the quantification limits are treated. In our study, we used the following procedure for the imputation of data beyond the quantification limits: to the values lower than that of the quantification limit, the value of half of the quantification limit was assigned.

Box-and-whisker plots – summary plots based on the median, quartiles, and extreme values – were used for statistical description of cadmium values in foods, blood, and hair.

We used Spearman's method of correlation to assess the relation between cadmium in foods and blood and cadmium in foods and hair. For assessing the differences in cadmium values among nine Slovene geographical regions, we used Kruskal-Wallis H test, a nonparametric equivalent to one-way ANOVA (16).

P-value of less than 0.05 was considered significant. SPSS for Windows (Version 11, SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

Cadmium in Foods of Plant Origin

The contents of cadmium in foods of plant origin were determined in 982 samples collected in the 1999-2000 period (Table 2). The highest median cadmium concentrations were found in the spinach/mangel samples, followed by flour, potatoes, and lettuce (Table 2).

In other kinds of foods, the median cadmium concentration varied between 3 and 12 mg/kg; in the fruit samples it was even lower. The cadmium content in individual samples varied widely, especially in the food groups prone to contain a greater concentration of cadmium.

Assessment of Environment Pollution with Cadmium in Individual Slovene Regions

The state of environment pollution with cadmium was assessed on the basis of average cadmium content in plant origin foods produced in individual regions. The differences in the cadmium burden between the regions where the foods were produced were statistically significant ($p<0.001$).

The highest cadmium concentrations in foods of plant origin were those in Kranj (median, 10 μg/kg; inter-quartile range, 5-16; 10th-90th percentile, 2-49) and Celje (median, 10 μg/kg; inter-quartile range, 6-17; 10th-90th percentile, 3-40). The lowest median cadmium content in foods of plant origin was found in the Nova Gorica (median, 8 μg/kg; inter-quartile range, 4-11; 10th-90th percentile, 2-16) and Koper region (median, 9 μg/kg; inter-quartile range, 4-10; 10th-90th percentile, 2-25), followed by in Ljubljana and Murska Sobota regions (Fig. 1).

Cadmium in Blood

Cadmium concentrations determined in 463 blood samples (one sample was lost due to laboratory manipulation) were relatively low, ranging from 0.25 to 24 μg/L. As many as 38.2% of the participants had a blood cadmium concentration lower than 0.5 μg/L, whereas in 28.2% it was up to 1 μg/L. The median concentration of blood cadmium was 0.5 μg/L (inter-quartile range, 0.25-1; 10th-90th percentile, 0.25-2).

Table 2. Median concentrations on cadmium in 982 food samples of plant origin in Slovenia, 1999-2000

		Cadmium concentrations (μg/kg of fresh food)			
Sample	No. of samples	median	Q_1-Q_3	10th-90th percentile	%LOQ*
Spinach/mangel	60	25	16-65	12-121	0
Flour	52	17	12-23	5-34	11.5
Potatoes	60	15	9-34	6-43	0
Lettuce	68	14	8-27	6-58	0
Red beet	59	12	6-21	5-26	0
Turnip	50	11	6-16	4-20	0
Rise	17	11	9-20	6-22	11.8
Paprika	60	10	6-14	4-17	1.7
Carrots	64	9	4-22	2-51	1.6
Onion	60	8	4-15	1-33	0
Tomato	55	6	4-10	3-12	0
Cabbage	54	4	2-7	1-9	0
Legumes	81	3	2-7	1-12	29.6
Pears	48	3	2-4	1-8	12.5
Cucumber	64	3	2-5	2-8	4.7
Pumpkin	67	3	2-5	1-8	20.9
Apples	63	2	1-2	1-8	55.6

*LOQ – percentage of samples where the cadmium concentration was below the limit of quantification.

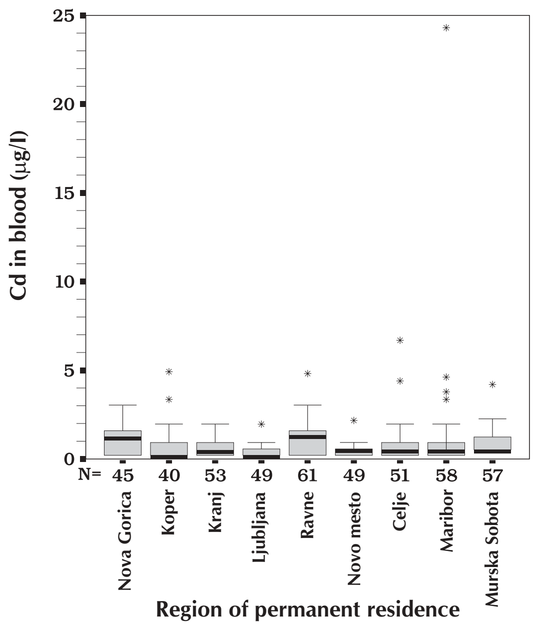

Figure 1. The median, interquartile range, and extreme values of cadmium concentrations in foods in different geographical regions of Slovenia (from west to east). The box represents the interquartile range, which contains the 50% of values. The box length represents the interquartile range. The whiskers represent the highest and lowest values, excluding outliers (cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box) and extreme values (cases with values more than 3 box lengths from the upper or lower edge of the box). A line across the box indicates the median.

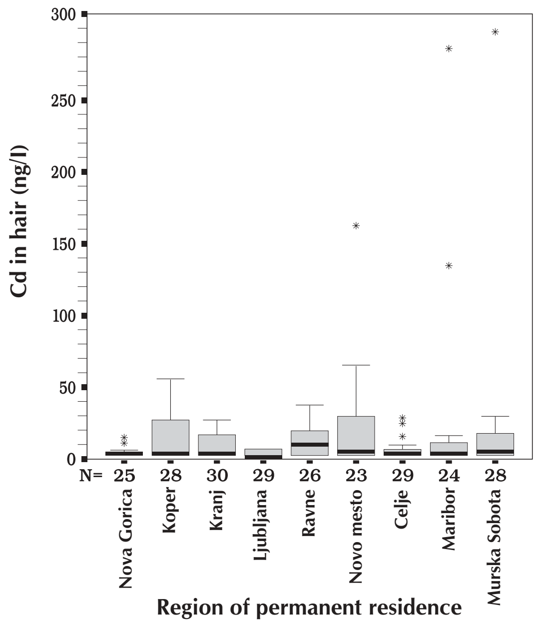

Figure 2. The median, interquartile range, and extreme values of cadmium concentrations in blood of Slovene military recruits by regions of their permanent residence (from west to east). The box represents the interquartile range, which contains the 50% of values. The box length represents the interquartile range. The whiskers represent the highest and lowest values, excluding outliers (cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box) and extreme values (cases with values more than 3 box lengths from the upper or lower edge of the box). A line across the box indicates the median.

The median concentrations of blood cadmium varied across the regions (Fig. 2). The highest median concentration of blood cadmium was observed in the residents of the Nova Gorica region, where the median concentration of blood cadmium was 1 μg/L (inter-quartile range, 0.24-1.86; 10th-90th percentile, 0.25-2.5), and only a bit lower in residents of the Ravne and Murska Sobota regions. The lowest median blood cadmium concentration of 0.25 μg/L was found in the population of the Ljubljana and Koper regions (inter-quartile range, 0.25-0.5; 10th-90th percentile, 0.25-0.5; and inter-quartile range: 0.25-0.5; 10th-90th percentile, 0.25-2.5, respectively). The differences in blood cadmium concentration among the regions were statistically significant ($p<0.001$).

Cadmium in Hair

Cadmium content was determined in 245 hair samples. Hair samples could not be taken from all participants because many had a very short haircut. The cadmium concentration was determined in the proximal part of the hair, which means that the established cadmium content was primarily a reflection of the organism's contamination with cadmium, and not of external contamination. The median concentration of cadmium in hair samples was very low, 4 ng/g (inter-quartile range: 3-19; 10th-90th percentile, 3-41).

The number of the participants who gave hair samples varied per region between 23 (Nova Gorica) and 30 (Kranj). In the large number of samples, the cadmium content was below the quantification level allowed by our method. In as many as 53.7% of hair samples, the cadmium content in the proximal part of the hair was below 5 ng/g, whereas in 11% it was up to 10 ng/g.

The median cadmium content in the hair varied greatly across regions ($p=0.003$). The lowest median content was found in the participants from the Nova Gorica region. It was also relatively low in participants from Ljubljana, Celje, and Kranj. The highest median cadmium content was found in the hair of participants from the Murska Sobota and Ravne regions (Fig. 3).

Correlation Analysis

The non-parametric Spearman correlation test between the cadmium content in the blood and hair showed that the correlation was not statistically significant (correlation coefficient, 0.126). The correlation between the level of environmental pollution with cadmium, ie, the median cadmium content in foods of plant origin from individual regions, and cadmium concentration in the blood of the study participants, was weak (Spearman's rho=0.133, $p=0.732$). The correlation between the cadmium content in foods of plant origin and that in the hair was much stronger, but still not significant (Spearman's rho=0.546, $p=0.128$).

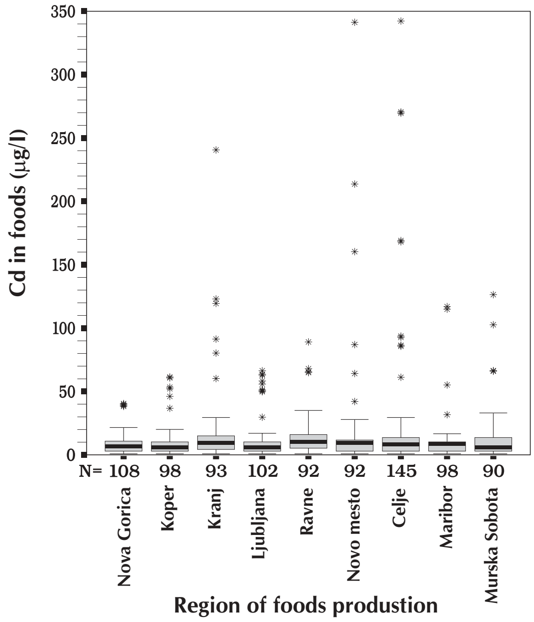

Figure 3. The median, interquartile range, and extreme values of cadmium concentrations in the proximal part of hair of Slovene military recruits by regions of their permanent residence (from west to east). The box represents the interquartile range, which contains the 50% of values. The box length represents the interquartile range. The whiskers represent the highest and lowest values, excluding outliers (cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box) and extreme values (cases with values more than 3 box lengths from the upper or lower edge of the box). A line across the box indicates the median.

Table 3. Reference concentrations of cadmium in blood and hair in different studies

Population	Country	Ref. No.	Cadmium concentration* M	GM	AM
Values in blood (μg/L):					
women	Japan, 1980	18			3.6
women	Japan, 1990	18			1.98
men	China	19			1.31
men and women	Korea	20			1.27
men and women	Sweden	21			0.9
men and women	Israel	22			0.85
children	Germany	23	0.3	0.36	0.69
men and women	Spain	24			0.26
men and women	Finland	25			0.1
men	Singapore	26		0.21	
women	Singapore	26		0.26	
men and women	London, UK	27	0.7		
young men (our study)	Slovenia		0.5		
Values in hair (ng/g):					
women	France	28	380		
men	USA	29			240
men and women	Japan	30			280
women	Russia	31			290
women	Poland	33			710
children	USA	32	270		410
children 2-6 years of age	Albania	34	87		95
children	Germany	23	50	48	96
young men (our study)	Slovenia		4		

*Abbreviations: M – median; GM – geometric mean; AM – arithmetic mean.

Discussion

Cadmium Concentration in Blood and Hair

The median blood cadmium concentration in our study was slightly higher than that observed by other researchers investigating unexposed population. Moderately exposed persons usually have the blood cadmium concentration of up to 4 μg/L, mostly around 1 μg/L or less, depending on whether they are smokers, non-smokers, or passives smokers, respectively (Table 3).The mean concentration of cadmium in the blood of smokers is usually twice as high as that in non-smokers (22,27). In our research, we did not have any information regarding passive smoking, which could increase the average cadmium content in the blood. In a study of the influence of active and passive smoking on the blood cadmium concentration conducted in Germany, the mean cadmium concentration observed in non-smokers was 0.85 μg/L, and in passive smokers it was 0.93 μg/L (22).

There are considerable differences in the mean blood cadmium concentrations among regions in Slovenia, which follow two patterns. The difference between west and east versus central part of Slovenia without Ravne region shows one pattern, and the difference between the Ravne, Murska Sobota, and Nova Gorica regions versus all others shows another. The burden of cadmium contamination in regions with the highest blood concentration of cadmium, as found in recruits, could be explained by the fact that lead smelter plant is located in the Ravne region, whereas the Murska Sobota and Nova Gorica regions are viniferous and rural regions. Staessen et al (27) studied the cadmium burden of the population in London and found a similar mean concentration of blood cadmium as we did: the median concentration in investigated London population was 0.7 μg/L, ranging from 0.4 μg/L to 8.5 μg/L. Interestingly, they found higher mean cadmium concentration in women than in men. Considering that we eliminated interference factors in our study, such as smoking and alcohol consumption, the reasons for a higher mean blood cadmium concentration in our population could lie in a larger share of vegetables in the nutrition. Watanabe et al (23) concluded that the decrease in the mean cadmium concentration in the blood of Japan women over the 1980-1990 period was a consequence of considerably lower cadmium content in rice produced in 1990 than that produced in 1980, due to the implementation of environmental measures.

The cadmium concentration in the hair samples in our study was very low. A comparison with results reported by other authors was difficult because the studies varied in the hair-taking methodology, preparation of samples for analysis, and the procedure and methodology of the analysis. Also, the higher cadmium content in hair found by other researchers was probably influenced by the fact that electrothermal atomic absorption spectrometry analyses usually employ hair samples of 1-3 cm in length. Consequently, in addition to the internal cadmium burden, they also measure the one resulting from external contamination. Hair analysis by the method we employed, however, allows the use of very short hair samples (2 mm in length), which makes it the only method so far that also allows measuring of the concentration gradient of metal in hair. It also provides more reliable data on the actual metabolic trace element incorporation than the conventionally used methods, which determine average trace element content in the entire hair length (1-3 cm). A great advantage of this method lies in the fact that the risk of sample contamination during the preparation and performance of the analysis is essentially reduced. Furthermore, it also has a lower limit of quantification of the substance analyzed (15).

Assessment of Environment Pollution with Cadmium in Individual Regions

The differences in the cadmium burden in foods of plant origin across regions in Slovenia were statistically significant. The cadmium content in these foods largely reflects the level of cadmium burden in the soil, and depends on other soil characteristics, especially acidity. This makes cadmium a sensitive indicator of the degree of environmental pollution (9).

Cadmium content in foods of plant origin, however, cannot be the only criterion for the assessment of the degree of exposure to harmful substances through food. Inhabitants of a certain area or region also eat foods grown elsewhere, which can differ in content of harmful substances. There are also variations in the quantity of non-local foods consumed in a particular environment; they depend on the quantity of foods of plant origin produced in a certain region, the size of the region, social and economic conditions, and migration rate. In areas where the share of farm production is high and accompanied by bad social and economic status, the share of at-home grown foods in one's diet is larger. In Slovenia, the only region that meets these criteria is that of Murska Sobota.

The correlation between the level of environmental pollution with cadmium and cadmium content in blood and hair is weakened by the fact that many young men who participated in our research were students. This means that many lived in one region and studied in another, e.g., Ljubljana or Maribor, which belong to less polluted Slovene regions at least as far as cadmium is concerned.

Influence of Cadmium Content in Environment on Its Concentration in Biological Samples

We also performed the cross-sectional ecological epidemiological analysis (a correlation epidemiological study). The bias characterizing this epidemiological method of work has been partly reduced by use of the questionnaire, which supplied us with several important pieces of information. For example, we were able to exclude smokers, who are more exposed to cadmium intake. However, the important drawbacks of the study still remain: we studied the cadmium content in the foods that had not been consumed by our participants, and we did not have any information on the kinds and quantities of foods they consumed.

The analysis of the correlation between the environmental pollution with cadmium (determined on the basis of the average cadmium content in foods of plant origin) and the cadmium content in the blood and hair showed that it was not statistically significant. However; this is primarily due to the fact that the number of the geographical units studied (9) was extremely small. Had it been possible to assess the degree of environmental pollution, e.g., in Slovene administration units (with the analysis of biological samples from their population), the chances of establishing a statistical significance would have been much higher as the number of the units studied would be 65. The results obtained, however, support the hypothesis that there is an actual correlation between the degree of environmental pollution and the cadmium content in the blood and especially in hair of the population.

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