ABSTRACT

Purpose of the study is to investigate food consumption in young people and the contribution of food to heavy metal pollution.

Methods: The object of the study are 60 healthy young people aged 19-29 years (average age – 23.9 ± 2.6) from Pleven and the Pleven district. Assessment of food intake was made according to a two-day, 24-hour diet recall method and Food Frequency Questionnaire (FFQ) method. A documentary method was used to assess the content of heavy metals- data provided by the Food Chain Risk Assessment Centre. 2,471 food samples were analyzed for lead and cadmium over a 7-year period.

Result: The food group importing complex carbohydrates could have a probable contribution to the dietary exposure to Pb and Cd, as they are consumed in the recommended amounts, although no content of lead and cadmium above the maximum permissible amounts was found in the studied foods. Intake of vegetables and fruits is significantly below recommended - vegetables and fruits would likely contribute to dietary exposure to Pb and Cd provided they were consumed as recommended. The average daily intake of dairy products is compared to the references. Game meat and offal could contribute to dietary exposure to both heavy metals, but these were also not consumed by the respondents. Fish and seafood likely contribute to dietary exposure to heavy metals, but their consumption is low and significantly below recommendations.

Conclusion: The obtained results confirm the importance of a healthy food model for the protection of human health, including protection from chemical hazards in food.

Keywords: daily consumption, Bulgarian young population, heavy metals,
metals below the limit of the maximum permissible amounts. However, a number of scientists claim that some pollutants, even within the limits of the maximum permissible amounts, lead to certain functional disorders and represent a burden on the body, especially with unhealthy eating patterns and an unhealthy lifestyle [6, 7].

OBJECTIVES

The objective of this ongoing study is to investigate food consumption in young people and their potential for possible contamination with the heavy metals lead and cadmium in the studied group.

MATERIALS AND METHODS

The object of the study are 60 healthy young people aged 19-29 years (average age – 23.9 ± 2.6) from Pleven and the Pleven region, randomly selected from the patient list of a general physician. The survey was conducted in the months of August and September 2022. The subjects did not report the intake of nutritional supplements and adherence to special dietary regimes.

To assess nutrition, the 24-Hour Dietary Recall Method was used for two non-consecutive days - one working day and one rest day. Food consumption was studied using the Food Frequency Questionnaire Method. The assessment of food consumption was made in accordance with the recommended weekly and daily frequencies of consumption of foods/food groups and average amounts of foods, indicated in the Food-Based Dietary Guidelines for adults in Bulgaria [2].

A documentary method was used to analyze the concentrations of the heavy metals lead and cadmium in the studied foods - data provided by the Food Chain Risk Assessment Center of the Ministry of Agriculture, Food and Forestry of the Republic of Bulgaria. The study covers 2,471 food samples for a period of 7 years up to and including 2019. All samples were analyzed for selected heavy metals (Pb and Cd) by graphite furnace atomic absorption spectrometry (GF-AAS) method in an accredited laboratory according to a standardized procedure.

The data were processed with the statistical software IBM SPSS Statistics v.24. for Windows.

Written informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee of Medical University-Pleven.

RESULTS

Cereals and potatoes

The average daily consumption of cereals corresponds to the recommendations for a healthy diet – 265.9 ± 277.8 g/day; Me – 164.4 g/day, but a decreasing trend is established. Bread (148.7±133.9 g/day; Me – 104.8 g/day) and pasta products – 74.7±86.1 g/day have the greatest contribution to the consumption of cereals; Me - 41.4 g/ day. More than half the amount of bread consumed (51.7%) for the day is whole grain – 76.7±90.6 g/day; Me-43.4 g/ day. The average daily consumption of rice met the recommendation. The dietary intake of potatoes is 89% of the recommended amounts(Fig. 1, Table 1). The trend for high consumption of cereals and potatoes is maintained, the proportion of whole grain bread is increasing, the consumption of pasta and fried potatoes is high.

In the tested samples of cereals and potatoes offered on the Bulgarian market, no Pb and Cd content above the maximum permissible amounts was found (Fig. 2). The group of foods, importers of complex carbohydrates, could have a probable contribution to the dietary exposure of Pb and Cd, since they are consumed in the recommended amounts (Table 1) and in 43.1% of the total number of samples examined, cereals and potatoes are found values above the limit of quantification (LOQ) (Fig. 3).

Vegetables & Fruits

The intake of vegetables and fruits is within the minimum sufficient (400 g/day) and significantly below the recommendations (Fig 1). The average daily consumption of vegetables is 214.9 ± 248.5 g/day; Me-94 g/day. The average daily amount of fresh green leafy vegetables is 66.9±98.2 g/day; Me -56.4 g/day. The average daily consumption of other fresh vegetables was 124.5±186.9 g/day; Me – 82.4 g/day, i.e. 2.2 times greater, compared to the values for green leafy vegetables. The average daily values of the medians of carrots consumed are 2.4 g/day, eggplant - 3.5 g/day, mushrooms - 7.4 g/day and red peppers - 9.6 g/ day. The average daily consumption of canned vegetables and pickles is low with median values of 6 to 8 g/day. Average daily fruit consumption is 169.5±170.6 g/day; Me-136.5 g/day. The intake of canned fruit is 49% of the recommended average daily amounts.

From the group of vegetables and fruits offered on the Bulgarian market, content above the maximum permissible amounts of lead and cadmium was found in individual samples of spinach, (fresh, deep-frozen, canned), carrots, mushrooms, eggplants, peppers and apples, which represents 1.55% of all foods studied (Fig. 2). With values above the LOQ, 37.3% of the analyzed samples of vegetables and fruits offered on the Bulgarian market would have a probable contribution to food exposure to Pb and Cd, provided they were consumed, according to the recommendations. Samples with values above the LOQ were detected in spinach (fresh, deep-frozen, canned) and lettuce(Fig. 3).

A greater capacity for dietary exposure to lead and cadmium can be found in apples, where samples with values above the maximum permissible amounts are found and the average daily consumption of apples in the studied group is 93.1 ±143.4 g/day; Me- 69 g/day.

Milk and milk products

The average daily consumption of dairy products was 209.1±154.4 g/day; Me-165.3 g/day. The average daily intake of milk (fresh, sour) is 78% of the recommendations - 155.6±143.6 g/day; Me - 141.4 g/day, and for dairy products - cheese, yellow cheese, cottage cheese is 93% compared to the recommendation of 50 g/day, respectively - 46.4±53.3 g/day; Me -32 g/day (Fig. 1).

Regarding the content of Pb and Cd in the examined dairy foods, 4.8% of all samples did not correspond to the maximum permissible amounts, and values above the LOQ were found in 89.1% (Fig. 3).
Foods rich in protein - meat, fish, eggs, legumes, nuts

The average amount of meat consumed was 203.6±154 g/day; Me - 158.5 g/day. Average daily meat consumption is high. The consumption of meat products shows a tendency to restrict. The results show a low frequency of consumption of offal and game meat - once a month or less. The average daily consumption of fish and seafood is 28.4±15.4 g/day; Me - 13.5 g/day. The average daily intake of fish is low and the usual frequency of consumption in the studied group is once a week or 1-3 times a month. Seafood consumption (mussels, shrimps, crabs) was 2.97±21 g/day; Me - 0 (Fig. 1, Table 1).

The relative share of samples of meat and meat products, fish and seafood and pulses with established values of Pb and Cd above the maximum permissible amounts is 1.9% (Fig. 2), and with values above the LOQ are 71.5% of the total number of samples (Fig. 3). Game meat, horse meat and offal could contribute to dietary exposure to both heavy metals, but these were rarely or not consumed by the respondents during the study period. Pb and Cd in values above the maximum allowable amounts were found in investigated samples of fish and seafood, and in values above the LOQ, they were found in salmon, trout and bivalve molluscs offered on the Bulgarian market. Fish and seafood likely contribute to dietary exposure to heavy metals, but their consumption is low and significantly below recommendations.

The intake of eggs corresponds to the recommendations, and that of pulses and nuts is significantly below the recommendations. In the food samples tested for lead and cadmium content, there are no data on eggs and nuts.

Added fat

Average fat consumption was 36.7±24.3 g/day; Me - 30.1 g/day. In the food samples taken for the determination of Pb and Cd content, no fat was examined.

Sugar products, herbal infusions and spices

The consumption of added sugar and sugar products is high - 36.1±70.7 g/day; Me -19.4 g/day. The average daily consumption of bee honey by the study participants ranges from 0.5 g to 30 g/day. In the tested sugar products for Pb and Cd content, 1.9% of the samples exceeded the maximum permissible amounts (Fig. 1). Values above the LOQ were found in 79.2% of the studied honey samples (Fig. 2; Fig 3). Of the beverages, a suspected contribution to dietary exposure to heavy metals could be herbal infusions. Tea consumption in the studied group was low - 60 ±235.7 ml/day; Me - 0, of herbal teas is 35±157.5 ml/day; Me - 0, with only 6% of the subjects drinking herbal tea (250 ml) once a day. Exceeding the permissible limit from the group of beverages was found in 8.1% of all samples, which is due to herbal tea infusion (Fig. 2). Values above the LOQ were in 47.5% of the samples (Fig. 3).

The average daily consumption of the spices most often used by the respondents (parsley, dill, savory and celery) is 1.5±8.35 g/day; Me - 0 g/day with minimum-maximum intake amounts for parsley and dill from 0.04 g to 7 g/day, for savory from 0.04 g to 8 g/day and for celery from 0 to 17 g/day. In the tested samples of spices offered on the Bulgarian market, values above the maximum permissible amounts of Pb and Cd were found.

Fig. 1. Distribution of average daily consumption of foods (in % of recommendations) among respondents in the study.
**Table 1.** Average daily consumption of foods (grams/day) with lead and cadmium values above the limit of quantification (LOQ)

<table>
<thead>
<tr>
<th>Food items</th>
<th>Mean±SD</th>
<th>Me</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cereals and potatoes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>24,1 ±31,2</td>
<td>9,34</td>
</tr>
<tr>
<td>Potatoes</td>
<td>97,6 ±101,6</td>
<td>84,5</td>
</tr>
<tr>
<td><strong>Vegetables&amp;Fruits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrots</td>
<td>9,7±14</td>
<td>2,4</td>
</tr>
<tr>
<td>Spinach in salads</td>
<td>14,2±45</td>
<td>3,9</td>
</tr>
<tr>
<td>Spinach in dishes</td>
<td>20,7±14</td>
<td>4,1</td>
</tr>
<tr>
<td>Lettuce</td>
<td>61,6±52,5</td>
<td>53</td>
</tr>
<tr>
<td><strong>Milk and milk products</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>155,6±143,6</td>
<td>141,4</td>
</tr>
<tr>
<td><strong>Foods rich in protein</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td>161,2±139,6</td>
<td>134,8</td>
</tr>
<tr>
<td>Meat products</td>
<td>32,6±45,1</td>
<td>15</td>
</tr>
<tr>
<td>Inwards</td>
<td>4,19 ±18,6</td>
<td>0</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In this study, we assessed the diet of healthy young people and, based on the detected amounts of lead and cadmium in 2471 food samples offered on the Bulgarian market, over a seven-year period, we tried to assess how much the dietary pattern could contribute to heavy metal exposure in the studied group.

Our results confirm trends established by national surveys after 1998, such as high consumption of cereals and potatoes, an increase in meat consumption significantly above recommendations, insufficient consumption of milk (fresh and sour) and fish in the population over
19 years of age [8]. In the current study, consumption of fruit and vegetables was within the minimum sufficient amounts, and of added fat, sugar and confectionery was 102-103% of the recommendations. It found, consistent with the recommendations, consumption of whole grain bread and eggs, lower consumption of meat products at the expense of increasing the proportion of meat, more frequent consumption of chicken meat, but also low consumption of pulses and nuts.

The analysis of the results shows that the foods offered in the Bulgarian market have relatively low levels of contamination with heavy metals. The relative share of samples with Pb and Cd values above the maximum permissible amounts is below 5% of the total number of food samples, with the exception of herbal infusions, where their share is 8,1%. Values above the LOQ were found in 89% of the milk samples and 71,5% of meat, fish and pulses.

The relative share of milk samples (cow, sheep) with Pb and Cd values above the maximum permissible amounts is 4,8%. The consumption of milk and milk products in the studied group occupies 78-93% of the recommendations. Low yogurt consumption by study respondents did not favor the interaction between Pb, Cd and the gut microbiome, which is particularly sensitive to environmental pollutants. Exposure to heavy metals slows growth and alters the structure of intestinal microvilli. Lactobacilli in dairy foods can inhibit intestinal absorption and increase the activity of enzymes that metabolize heavy metals [9,10]. Unlike a number of regions around the world, heavy metals in milk in the European market and North American countries are below the maximum permissible amounts, due to established regulatory monitoring and control mechanisms. The concentration of Pb and Cd in milk is higher in regions with a developed mining and metallurgical industry, as well as in raw full-fat cow’s milk from a conventional production system, compared to that from the organic production system. Meta-analyses have shown that increasing concentrations of Pb, Cd, Ni and Cu in raw cow’s milk are positively associated with adverse health effects [11, 12]. The health risk of dietary exposure to Pb and Cu is higher in children compared to adults, due to the more frequent consumption of milk and the peculiarities of children’s metabolism. A study in Peru found a dietary hazard ratio for Pb and Cd <1 for milk consumption for individuals over 19 years of age [13].

Of the protein-rich foods, the European Rapid Alert System for Food and Feed for the period 1980-2016 rati- fied the most notifications related to fish and food contact materials, in addition to fruits and vegetables, seafood gifts, and diet food. Fish and seafood in our study had a low frequency of consumption, but given the high est number of notifications for Hg and Cd content, the possible reproductive health risk in young women should not be underestimated [14]. Although the exposure assessment showed a relatively low health risk from meat and meat product consumption, Han et al. reported increasing dangers regarding heavy metals in foods of animal origin, given the intensive industrial activity [15]. The high consumption of meat and meat products by the survey respondents and the detected Pb and Cd concentrations above the LOQ in approximately 2/3 of the studied samples may demonstrate the potential of the unhealthy dietary pattern to dietary exposure to heavy metals.

Fruits and vegetables are one of the most frequently reported food groups - accumulators of lead and cadmium. Growing them on soils contaminated with heavy metals adversely affects the nutritional value of vegetables and fruits and that combined with insufficient dietary intake, may increase the risk of serious nutritional deficiencies of vitamins, minerals and antioxidants among the population [16,17]. A study by Chinese scientists on the contamination of plant foods with 6 types of heavy metals (Cu, Pb, Zn, Cr, Cd, As and Hg) shows the following degree of accumulation in descending order - leafy vegetables > tuberous crops > non-leafy vegetables > legumes > fruits > cereals [18]. Our results show Cd and Pb content above the maximum permissible amounts and above the LOQ in green leafy vegetables (spinach and lettuce), carrots, eggplant, mushroom, red peppers and apples. The intake of vegetables and fruits below the recommendations, the failure to meet the requirement for a daily intake of a variety of fruits and vegetables by season, combined with the low consumption of pulses in the study group, may contribute to the likely dietary exposure to heavy metals and to the likely risk of nutritional deficiencies in young people.

Cereals accumulate the least amount of heavy metals of all plant foods. Thabit et al. found no content of As, Cd, Hg and Pb in wheat and barley grains of European countries for the 2018 season. Single samples of Cd in wheat and traces of Cd in barley were detected [19]. According to literature data on cereals, higher values of Pb are found in rice, followed by Cd, especially in rice imported from China, India and Pakistan [20]. Cereals were consumed in the recommended amounts by the study participants and the results obtained for Pb and Cd content, except for rice, would not be likely contributors to heavy metal exposure.

CONCLUSIONS
At the established average daily consumption of the food groups, a probable contribution to the dietary exposure of Pb and Cd and possible nutritional deficiencies related to the content of heavy metals in them may be:

- Rice and potatoes;
- Vegetables&fruits - green leafy vegetables (spin-
ach, lettuce), red peppers, carrots, eggplant, mushrooms, apples;

- Milk and milk products - cow’s and sheep’s milk, as well as the low consumption of yogurt as a source of probiotic microorganisms;
- Foods rich in protein - the high consumption of meat and meat products - significantly above the recommendations, bioaccumulate heavy metals, especially game (boar), offal, fish and seafood, but only 43% of the recommendations are consumed.
- Honey, herbs, spices and herbal teas.

The obtained results confirm the importance of a healthy food model for the protection of human health, including protection from chemical hazards in food. Further studies are needed to make an accurate assessment of the risk to human health, and to evaluate the potential risks associated with the consumption of foods and the content of heavy metals in them. In this sense, the evaluation of dietary exposure to heavy metals in different age groups is an essential step in the assessment of the risk of metals in food, since the concentration of heavy metals in food, the amount of food consumed, eating habits and individual characteristics of the individual can critically affect the bioaccumulation of heavy metals from foods.

In conclusion, with the established eating pattern of the studied group, a probable risk of bioaccumulation of the heavy metals - lead and cadmium is created by the high consumption of meat and meat products, but also the inadequate dietary intake of vegetables & fruits and yogurt, which are related to the biotransformation of heavy metals in the body and the occurrence of possible nutritional deficiencies.

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