"CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY BUCHAREST
DOCTORAL SCHOOL

PHARMACY

Studies investigating the effects of a well-balanced nutrition and the importance of food quality on the maintenance of health

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2024

Table of contents

Introduction.....1

I. GENERAL PART.....5

1. Contamination of food products.....6

1.1 General information on food poisoning.....6

1.2 Contamination of food.....8

1.3 The main pathogens and the diseases they cause.....10

1.3.1. *Staphylococcus aureus*.....10

1.3.2. *Salmonella sp*.....11

1.3.3. *Bacillus cereus*.....12

1.3.4. *Escherichia coli*.....15

1.3.5. *Campylobacter sp*.....16

1.3.6. *Clostridium sp*.....23

1.4 The main chemical agents involved in food contamination…….....26

1.5 Legislative regulations.....28

1.6 Preventive measures.....30

II. PERSONAL RESEARCH.....33

2. Evaluation of the eating habits and lifestyle of the Romanian population in the context of the COVID-19 pandemic.....34

2.1. Working hypothesis and research objectives.....34

2.2. Materials and methods.....36

2.2.1. Data collection and analyzed groups.....36

2.2.2. Questionnaire validation.....37

2.2.3. Statistical analysis.....38

2.3. Results..... 39

2.3.1. Socio-demographic and anthropometric data.....39

2.3.2. Current eating habits.....42

2.3.3. Lifestyle and psycho-affective changes during the pandemic…….....46

2.4. Discussions.....52

2.5. Conclusions..... 56

3. Evaluation of the concentration of heavy metals and pesticides in dairy products and the safety of consumption.....58
3.1. Working hypothesis and research objectives.....58
3.2. Materials and methods.....60
3.2.1. Collection of evidence.....60
3.2.2. Analysis of heavy metals.....61
3.2.3. Analysis of pesticides.....62
3.2.4. Calibration of contaminant analysis devices.....62
3.2.5. Exposure assessment.....64
3.2.6. Characterization of consumption risk.....65
3.2.7. Statistical analysis.....66
3.3. Results.....66
3.3.1. Exposure assessment.....73
3.3.2. Risk characterization.....78
3.4. Discussions.....84
3.4.1. Monitoring the results.....84
3.4.2. Exposure assessment.....85
3.4.3. Risk characterization.....86
3.5. Conclusions..... 88
4. Conclusions and personal contributions.....89
Bibliography.....92
Appendices.....109

Lifestyle is currently not only a growing concern of the population worldwide but also a topic addressed and frequently mentioned in scientific studies in the medical field. Lifestyle is particularly important for physical and mental health, faster recovery from illness and normal physiological ageing. Adequate physical activity and nutrition are part of a healthy and balanced lifestyle. A balanced diet ensures the maintenance of an optimal nutritional state, is essential for the normal functionality of the body and for preventing or reducing imbalances caused by internal factors related to individual characteristics or external factors coming from multiple sources such as daily stress, pollution or various infections. Nutritional imbalances, even in conditions of optimal health, are well known for initiating inflammation, tissue stress, metabolic disturbances with long-term consequences, and triggering numerous ailments. A healthy daily diet means, on the one hand, a quantitatively adequate consumption ensuring the energy requirement, but also qualitatively by ensuring a nutritional balance, a varied chemical composition and that does not include components that can harm health.

Maintaining a healthy lifestyle has been a particular challenge during the coronavirus pandemic. In the case of elderly people, patients with multiple comorbidities and high levels of malnutrition experienced a much more serious infection state and higher mortality, which supports the importance of nutrition in maintaining a balanced immune system and effective body defence.

An unhealthy diet is considered one of the main factors responsible for approximately 26% of preventable mortality cases, according to the Global Nutrition Report of 2021. Also, the report shows that certain eating habits, correlated with geographic location, directly show a negative or positive impact on the general state of health of the population. The World Health Organization and other food regulatory bodies now recognize a number of nutritious foods in the vegetables and fruit category for which multiple daily servings are recommended, while salt, saturated fat and excess sugar are part of the foods for which the recommendation to avoid from daily consumption is supported. At the international level, the Global Nutrition Report reveals that more than 50% of the world's population does not consume a sufficient amount of food from the fruit and vegetable category. The situation at the European level is variable depending on the country. Romania is the European country for which the highest percentage of the population (over 60%) that does not consume any portion of fruit or vegetables daily is reported. At the opposite pole at the European level, we find Portugal, Spain, Italy, Belgium and Great Britain.

In recent years, there has been a growing interest in improving and accelerating local food production to reduce the negative impact of agricultural product discontinuities and their

price fluctuations. At the same time, in rural Romania, the cultivation of fruits and vegetables in households, the raising of animals and the utilization of animal products often represent the only source of food for families due to low incomes. At the same time, the World Health Organization draws attention to the fact that at the European level, the population's illness rate due to the consumption of contaminated food is approximately 23 million people annually, and the mortality rate is estimated at 4700 deaths.

Industrial and agricultural pollution, considered historic in certain areas, affects all producers and causes negative effects on consumers. There are several ways in which food can become contaminated. The main sources of chemical contamination in the food sector include contamination of water, soil and surfaces caused by human activities. Lack of proper hygiene and unsanitary conditions are the main factors contributing to pollution and the emergence of infectious diseases worldwide. The negative effects of water pollution are particularly pronounced due to its crucial role in food production. Within aquatic ecosystems, heavy metals are distributed between the water column and sediments, which can lead to their accumulation in fish and other edible aquatic organisms. Similarly, in terrestrial systems, pollutants can be absorbed and accumulate in plant tissues during the growing period. Numerous studies have consistently shown that leafy vegetables have higher bioaccumulation factors than other plants, indicating their robust ability to collect metals from soil.

Contamination of food by microbiological organisms can occur at any point in the food supply chain, from the agricultural field to the actual consumption. Therefore, it is imperative to follow proper hygiene and manufacturing procedures throughout the food supply chain to avoid microbiological contamination of food by microorganisms, which can lead to a significant increase in illness and death among consumers.

In this context, the main objectives of this doctoral thesis included:

1. Evaluation of changes in lifestyle and food patterns in the Romanian population one year after the onset of the global health crisis COVID-19.

2. Assessing the impact of the pandemic on psycho-affective states, including anxiety, depression and stress, among the community.

3. Investigating the relationship between dietary changes made during the pandemic, general health and body mass of respondents.

4. Determination of the concentrations of heavy metals (cadmium, copper, lead and zinc) and pesticide residues (DDE, DDD and DDT) in soil, milk and cow's cheese samples obtained from farms located in three geographical areas in Romania characterized by industrial and agricultural activities.

5. Analysis of the possible health risks related to consuming food products contaminated with heavy metals and pesticides, by determining some indicators that reflect the risk of nephrotoxicity, hepatotoxicity, hematotoxicity, cardiotoxicity and reproductive toxicity.

Thus, Chapter 1 analyzed the data on the main sources of contaminants with a negative effect on human health, discussing, at the same time, the current legislative regulations in the field of food safety. Food can be contaminated at every stage of the chain of production, processing, distribution, preparation and consumption. Another source of food contamination is environmental contamination (air, water, soil pollution), as well as improper food storage and processing.

In the context generated by the COVID-19 pandemic, the population has become increasingly aware of the need to adopt a healthy lifestyle and a balanced diet, primarily to support the immune system. Strong immunity is the best barrier against viral infections, including those with the new coronavirus. The body's immunity is affected by a large number of factors. Stress decreases the body's immune response to pathogens. Food quality is also an important factor affecting the body's reserves and resistance. The health of the body depends on the lifestyle a person adopts. Factors that influence a healthy lifestyle are diet, hydration, sleep time and physical activity. Sleep quality has an impact on both the state of health and the psyche. That is why sleep hours must be monitored; a balanced sleep schedule is needed, without excesses and without radical changes. Quality sleep is essential for the regeneration and optimal functioning of the immune system. During sleep, the body produces cytokines, which are proteins that help fight infection and inflammation. One way to strengthen the body's resistance is physical exercise, carried out constantly. Moreover, physical exercise also contributes to maintaining a good mental tone. Moderate exercise helps improve blood circulation, which allows immune cells and protective substances to circulate more efficiently through the body. Regular physical activity can reduce inflammation and improve the immune response. A healthy lifestyle includes following a schedule that includes three main meals each day, as well as eating healthy, vitamin-rich foods. Also, any excess should be avoided, especially when it comes to unhealthy foods or drinks.

Chapter 2 looks at changes in the diet and lifestyle of survey respondents within a year of the onset of the epidemic. The objective of the paper was to investigate changes in lifestyle, eating habits and psycho-emotional state among the Romanian population one year after the onset of the COVID-19 pandemic. Adopting a healthy lifestyle and balanced diet offers significant benefits, both in the short and long term. They not only improve physical and mental health but also contribute to the prevention of chronic diseases, thus increasing the quality of

life and longevity. Implementing these principles in your daily life is an essential step towards a healthier and more balanced life. Between May 3 and June 6, 2021, a cross-sectional study was conducted among the Romanian population based on a questionnaire disseminated online using the Google Forms web platform to identify changes in lifestyle and diet in the first year after the start of the COVID-19 pandemic -19. The questionnaire contains 58 items that address the following aspects: socio-demographic data (age, sex, occupation, area of residence, occupation), anthropometric data (height, weight), eating habits, lifestyle and psycho-affective behaviour before and during the pandemic. The final database was downloaded into a Microsoft Excel spreadsheet.

Following the online distribution of the questionnaire, a total of 2054 responses were obtained, of which 2040 were valid responses (responses with incorrect anthropometric data were removed). 71.61% of respondents were female (1464) and 28.39% were male (576). According to the recorded answers, 1598 (78.14%) of the people who completed the questionnaire live in urban areas and 442 (21.86%) in rural areas. Anthropometric data (weight and height) were used to calculate BMI using the Quetelet equation [body mass (kg)/height (m2)] and interpreted according to World Health Organization criteria. Most of those who participated in the survey were of normal weight (57.78%), while 7.25% were underweight, 24.97% overweight, and 9.98% obese.

One year after the start of the pandemic, 39.8% of respondents said they had maintained a constant body weight, 38.4% said they had gained weight, and 32.6% said they had an increased appetite. 62.4% of the participants in the questionnaire stated that they did sports during the pandemic, and 26.4% stated that they were in quarantine due to infection with the new coronavirus. We noted a negative change in the quality of life of the population caused by the COVID-19 pandemic; the majority of respondents stated that the standard of living deteriorated during the pandemic (63.9%) and only 12% said that life improved. Of the people surveyed, 38.1% said they exercised at home during the pandemic, 22.5% outdoors and only 6% in the gym. Regarding the frequency of sports activity during the pandemic, the percentage of those who did not exercise at all or very rarely increased slightly, and the percentage of those who exercised daily before the pandemic decreased. Thus, 13.4% of respondents stated that they exercised daily during the pandemic, 15.9% stated that they exercised daily before the pandemic, and 26.8% stated that they exercised very rarely during the pandemic (compared to 25.6% before the pandemic). 37.6% of respondents declared that they did not exercise at all during the pandemic (compared to 34.1% before the pandemic). There was a sedentary lifestyle trend before the pandemic that intensified during the pandemic.

In Chapter 2, several aspects of daily habits among the Romanian population were analysed during a pandemic year compared to the previous period. The study concluded that the pandemic also had a positive impact on respondents' habits, namely that cooking at home and consumption of healthy foods, especially vegetables and fruits, increased. At the same time, it was found that the respondents began to pay more attention to the health and well-being of those close to them. However, the increase in the number of overweight or obese people and the limitation of human interactions with psychological consequences represented the most common negative consequences of the pandemic. Some respondents reported sleep problems, depression, anxiety, fatigue, increased alcohol consumption, excessive smoking or compulsive eating. Overburdening the health system has also limited the population's access to basic medical services.

In the food sector, food safety and quality are of paramount importance as they have a direct impact on the health and well-being of consumers. Food safety encompasses the precautions put in place to protect food products from contamination, while food quality refers to the characteristics of food products that influence their appeal and acceptability to customers. Although the food sector has made notable progress in ensuring food safety and quality, there are still obstacles to overcome to maintain high standards.

Fresh milk is the indispensable food for newborns and has an important nutritional value for children in the first years of life. For many patients, convalescents, the elderly and active people working in the toxic environment, milk is a valuable and necessary food with health benefits due to its complex composition in the main macronutrients (proteins, lipids and carbohydrates), essential minerals and vitamins. Dairy products contain most nutrients in a balanced proportion that are easily assimilated by the body.

Chapter 3 represents a study that evaluated the contamination with heavy metals and pesticides in dairy products in different areas of Romania. It aimed to determine the levels of the heavy metals cadmium, copper, lead and zinc, as well as the pesticides DDE, DDD, DDT, in the soil of three peripheral urban areas in Romania. Two of these areas have a tradition in different sectors of industrial activity, while the third has an agricultural tradition. Concentrations of the same contaminants were determined in cow's milk and derived cheese samples collected from small producers and households. Furthermore, the study aims to correlate the level of contaminants in soil with food samples and ultimately determine the health risks to consumers after consuming these products.

Forty-two samples were collected from small farms: twenty-eight samples of dairy products (14 fresh cow's milk and 14 cheeses) and 14 soil samples from pastures belonging to

cow farms. All samples were collected in triplicate. The sampling date was between March and June 2019. The samples were collected from three different regions of Romania (14 from each area): a region with intense industrial activity, another with medium industrial activity and a third area with an agricultural and dairy tradition, without industrial activity.

The concentrations of heavy metals (copper, cadmium, lead and zinc) in the samples were determined using a Perkin-Elmer Analyst 700 atomic absorption spectrometer: cadmium ($\lambda = 228.8$ nm), copper ($\lambda = 324.7$ nm), zinc ($\lambda = 213.9$ nm) and lead ($\lambda = 283.3$ nm). The levels of lead and cadmium in the samples were determined using an HGA graphite furnace with argon as the inert gas. Other measurements were performed in an air/acetylene flame. A standard solution was prepared for each investigated element and used for calibration.

Organochlorine pesticides in samples such as 2,4-dichlorodiphenyltrichloroethane (DDT), 2,4-dichlorodiphenyldichloroethylene (DDE), 2,4-dichlorodiphenyldichloroethane (DDD) were detected using a gas chromatograph equipped with an electron detector (ECD).

Three urban areas were considered for the study: area 1 - urban area with medium industrial and agricultural activity, area 2 - urban area with intensive industrial activity and medium agriculture and area 3 - urban area with agricultural activity. In all cases, there is a tradition of producing dairy products for family consumption and local small trade. Area 1 and are 2 are characterized by traditional industrial activity and medium agricultural activity with farms and crops. Meanwhile, area 2, in particular, is recognized as one of the most polluted cities in Romania, with worrying medical reports in recent years regarding the increase in the number of patients with cancer, respiratory diseases, cardiovascular complications and diabetes. In the case of area 2 with intensive and traditional industrial and agricultural activities, very high levels of all heavy metals determined in soil were recorded, followed by area 1 and area 3. The concentration of heavy metals was higher in milk samples, the values being correlated in all cases with the soil concentration. In all cheese samples, the level of contamination was lower compared to soil or milk.

The level of pesticides (DDD, DDE, DDT) in the soil was higher in the case of area 2 followed by area 1. The lowest level of pesticides in the soil was determined in the case of area 3 compared to area 2 and area 1 ($p<0.001$). In the case of food samples, the level of pesticides was significantly higher in cheese samples compared to milk, and the level of contaminants was correlated with the level of pesticides in the soil in all areas tested.

As a result, the presence of heavy metals and pesticides above the maximum allowed limits in some milk and cheese samples analyzed could be mainly due to air pollution and less to the soil level.

In the study, we determined the levels of heavy metals such as cadmium, copper, lead, and zinc, along with the detected levels of DDT and its derivatives DDE and DDD in soil, milk and cheese samples collected from 3 different regions. Human exposure to heavy metals results from various causes, from anthropogenic activities to air, water and food chain pollution. For Pb and Cd the food chain is an important source for humans and can lead to toxic effects, mainly due to the accumulation potential (Karri et al., 2016; Patra et al., 2008). Cu and Zn are essential metals for several processes in the human body, but in excess they can also be involved in toxic manifestation (Nishito and Kambe, 2018). Contaminated food and water are also the main routes of exposure for humans (Khaniki, 2007). The levels observed in soil samples from all 3 regions do not exceed the legal limits for heavy metals and DDT and DDE, but exceed the levels for DDD (Order 756/1997). The increase of heavy metals in the industrialized areas of Romania was previously reported and was correlated with the blood levels of heavy metals in people living in these regions. Soil is the main source of heavy metals, DDT and its derivatives that can transfer and accumulate in plants and from plants to farm animals. Accumulation of these contaminants in adipose tissue or excretion in milk makes the food chain the main contributor to human exposure to heavy metals and persistent organic pollutants.

For the exposure assessment, a new proposed methodology was used (Goumenou and Tsatsakis, 2019) on the basis of which we calculated the corrected total daily dietary exposure (cEDI), taking into account the global exposure from all relevant food products containing the specific toxicant and the exposure from milk and cheese as if they contain the MRL (maximum residue level). An innovative approach was used for risk characterization, grouping chemicals into hazard groups based on specific chemical hazards and using RfDs (reference doses) for these specific hazards. Six toxicity groups were identified, of which 5 have HIA (Specific Hazard Index for Adversity) above 10 or between 1 and 10 depending on the region, showing that the contamination of milk and cheese with heavy metals and the amount of DDT pose risks of nephrotoxicity, hepatotoxicity, hematotoxicity, cardiotoxicity and infertility.

Reducing pesticides and heavy metals in dairy products requires a series of preventive and control measures applied throughout the supply chain, from feed production to milk processing. An essential step is the strict monitoring of the quality of the fodder used to feed the animals. It is important that feed comes from safe sources and is grown using sustainable farming methods. Farmers should be encouraged to adopt organic farming practices, which involve minimal use of pesticides and chemical fertilizers. Crop rotation and the use of organic compost can reduce the accumulation of heavy metals in the soil.

In conclusion, dairy products, despite their nutritional value, may present contamination risks that must be managed to ensure consumer safety. Contaminants in dairy can include pathogenic microorganisms, antibiotic residues, pesticides, heavy metals and natural toxins. Pesticides and heavy metals can get into milk through animal feed. Pesticides used in agriculture to protect crops can contaminate feed, and heavy metals from soil can be ingested by animals and subsequently excreted in milk. Long-term consumption of milk contaminated with these substances can have adverse health effects, including neurological toxicity and increased cancer risks. Strict monitoring of feed quality and the environment in which the animals live is crucial to reduce this risk. The study showed that stricter monitoring of heavy metal and pesticide levels in milk samples from local farmers is needed to protect consumers.

The reduction of pesticides and heavy metals in dairy products can be achieved through a number of preventive and control measures. It is essential to strictly monitor the quality of feed used to feed animals, ensuring that it comes from safe sources and is grown with sustainable farming methods that minimize pesticide use and exposure to contaminated soils. Implementing organic farming practices and crop rotation can reduce the accumulation of heavy metals in the soil. Also, regular testing of animal feed and water for contaminants is crucial. Continuing education and training of farmers on the proper management of pesticides and avoiding sources of contamination contributes significantly to the prevention of food chain pollution. By adopting these measures, safer and higher quality milk production can be ensured.

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1. **Năstăsescu V**, Mititelu M, Goumenou M, Docea AO, Renieri E, Udeanu DI, Oprea E, Arsene AL, Dinu-Pîrvu CE, Ghica M. Heavy metal and pesticide levels in dairy products: Evaluation of human health risk. Food Chem Toxicol. 2020;146:111844.(IF-6,023/WoS)

https://doi.org/10.1016/j.fct.2020.111844

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https://doi.org/10.3390/nu14030504