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In Memoriam: Professor Andrzej Wojtczak, MD (1933 – 2020)

Prof. dr hab. med. Andrzej Wojtczak
(1933-2020)

With sincere sadness and regret we bid farewell to Professor Andrzej Wojtczak, a man who like no one else was able to reconcile and unit in common work, even those holding extremely different viewpoints. Being an outstanding physician and clinician he became one of the most influential propagators of public health, both in Poland and worldwide.

By co-chairing the 1989 Round Table negotiations on the Health Sector, he was able to achieve an effective compromise between deeply divergent parties.

Drawing on the attitude of a sincere, positivist-oriented patriotism formed in Poznań and holding high positions of responsibility at the Polish Ministry of Health, he was able to move freely amongst international institutions - at many levels within the World Health Organisation, Public Health Committee of the European Union, Health Committee of the Council of Europe in Strasbourg, as well as in numerous scientific societies.

Everywhere he worked, he left behind many loyal students who are now joined together in a sense of irreparable loss.

Public Health Committee of the Polish Academy of Sciences
National Institute of Public Health – National Institute of Hygiene

In Memoriam: Profesor Andrzej Wojtczak, MD (1933 – 2020)

Prof. dr hab. med. Andrzej Wojtczak
(1933-2020)

Ze szczerym smutkiem i żalem żegnamy Profesora Andrzeja Wojtczaka, Człowieka, który jak mało kto potrafił godzić i łączyć we wspólnej pracy przedstawicieli nawet skrajnie różniących się poglądów. Będąc wybitnym lekarzem – klinicystą stał się jednym z najbardziej wpływowych propagatorów zdrowia publicznego w Polsce i na świecie.

Współprzewodnicząc obradom Podzespołu ds. Zdrowia w negocjacjach Okrągłego Stołu w 1989 r. udało mu się doprowadzić do kompromisu głęboko zwaśnione strony.

Czerpiąc z postawy ukształtowanego w Poznaniu, szczerego, pozytywistycznie zorientowanego patriotyzmu i pełniąc odpowiedzialne funkcje w polskim Ministerstwie Zdrowia potrafił swobodnie poruszać się w międzynarodowych instytucjach – na różnych szczeblach Światowej Organizacji Zdrowia, w Komisji Zdrowia Publicznego Unii Europejskiej, w Komitecie ds. Zdrowia Rady Europy w Strassburgu, a także w licznych towarzystwach naukowych.

Wszędzie gdzie pracował, pozostawił po sobie wielu wiernych uczniów, którzy teraz łączą się w poczuciu niepowetowanej straty.

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REVIEW ARTICLE

THE LEGITIMACY AND SAFETY OF USING ALTERNATIVE DIETS IN CANCER

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ABSTRACT
Alternative diets are used by cancer patients, especially among those who are not treated with conventional methods. Due to worrying data published by the World Health Organisation and its Agenda, the International Agency for Research on Cancer and the International Cancer Union, as well as epidemiological data from all over the world, it has been concluded that cancer will be the main cause of death in the world and that, therefore, the popularity of alternative diets among cancer patients may increase. The paper reviews the scientific literature and assesses the legitimacy and safety of selected alternative diets, as well as the description of research in terms of assumed anticancer efficacy in the following diets: ketogenic, Dr. *Budwig* and macrobiotic. The article also contains a summary of the analyzed scientific research and conclusions concerning the legitimacy of their use by cancer patients.

Key words: *cancer, alternative diets, ketogenic diet, dr Budwig diet, macrobiotic diet, glucose metabolism*

STRESZCZENIE
Diety alternatywne są stosowane przez pacjentów onkologicznych, szczególnie wśród osób, którym konwencjonalne metody leczenia nie przynoszą oczekiwanych efektów. Dane Światowej Organizacji Zdrowia oraz jej Agendy, Międzynarodowej Agencji Badań nad Rakiem oraz Międzynarodowej Unii Przeciwrakowej, a także dane epidemiologiczne z całego świata wskazują, że wkrótce nowotwory będą główną przyczyną zgonów na świecie. W związku z tym popularność diet alternatywnych wśród pacjentów onkologicznych może ulec zwiększeniu.
Dokonano przeglądu piśmiennictwa naukowego wraz z oceną zasadności i bezpieczeństwa stosowania następujących diet alternatywnych: ketogennej, dr *Budwig* i makrobiotycznej, w aspekcie ich zakładanej skuteczności przeciwnowotworowej.

Słowa kluczowe: *choroby nowotworowe, diety alternatywne, dieta ketogenna, dieta dr Budwig, dieta makrobiotyczna, metabolizm glukozy*

INTRODUCTION

Cancer and cardiovascular diseases are the commonest causes of death, accounting for 70% of deaths in Poland [6]. In 2013, cancer was diagnosed in over 155 thousand Poles [27]. According to the forecasts of WHO and its Agendas (IARC and UICC) and epidemiological data, in 10-20 years cancer will be the main cause of death worldwide. It is expected that during this period an increase in the incidence of the disease to about 26.5 million and an increase in the number of deaths to 17.1 million will be recorded. [37].

Currently, the following methods are used in cancer therapy: surgery, chemotherapy, radiotherapy, immunotherapy and hormone therapy. Due to their ineffectiveness in many clinical cases, patients in advanced stages of the disease are interested in alternative methods, including special diets [59]. Still the authors of the study have not confirmed that the use of specialized diets or specific dietary models increase the chance of survival or cure. The relationship between diet and prognosis is still being studied and is still not entirely clear [50]. However, standard treatment and alternative therapies chosen by some patients may affect the effectiveness of conventional therapy and reduce its side effects on the patient [59].

There is no common knowledge about the validity and safety of their use. The aim of this study is to

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assess the validity of three alternative diets, i.e. ketogenic, Dr *Budwig* and macrobiotic in supporting cancer treatment, based on a review of the world literature.

CHARACTERISTICS OF ALTERNATIVE DIETS

Ketogenic diet

The ketogenic diet is successfully introduced in the treatment of drug-resistant epilepsy in children. Over time, it has been extended to patients with nodular sclerosis, brain tumors and neurodegenerative diseases such as *Alzheimer's* and *Parkinson's* disease. Many studies have also shown the neuroprotective properties of this diet [1, 5, 33, 52], binding it to molecular mechanisms and metabolism of ketone compounds and the ketosis they induce [33].

The ketogenic diet consists of high fat, moderate protein and very low carbohydrate content. This structure of the diet forces the patient's body to obtain energy from fat instead of glucose [1]. The ratio of fat, which accounts for 80% of energy requirements in a diet, to the sum of carbohydrates (5%) and protein (15%) is approximately 4:1. Ketosis can also be induced in the organism when the ratio of macro nutrients is 3:1 or 2:1, which is closely related to the patient's dietary tolerance and the expected results of the therapy. The main fat sources are long chain triglycerides (LTC) and medium chain triglycerides (MCT). A high MCT diet leads to higher ketosis, but at the same time causes more serious side effects for the patient (e.g. stomach pain) [10]. People who follow a ketogenic diet, fatty acid oxidation occurs in the liver, where is the absence of glucose, ketone compounds are formed, including: acetate, β-hydroxybutyrate and acetone. Low carbohydrate intake in the ketogenic diet may cause a slight decrease in blood glucose levels and support glycemic control resulting in lower level of A1c glycated haemoglobin (HbA1c) [59]. The effectiveness of the ketogenic diet can be controlled by testing the level of ketone compounds in blood serum and the β-hydroxybutyrate concentration in patient's urine [12].

Currently, research is also being conducted in the group of patients on the side effects of ketogenic diet and its importance in limiting the progression of disease [47]. However, the German Association of Urological Oncology (AUO), on the basis of a systematic review of the work on the use of a ketogenic diet, takes the view that a low-carbohydrate diet cannot be recommended to oncological patients because there are no prospective and randomized studies directly evidence of effectiveness [34].

Dr Budwig's diet

It was proposed in the 1950s by *Johanna Budwig* as a standard anti-hypertensive diet. However, the presented nutrition model is deficient in terms of iron and carbohydrate content. There is currently a lack of scientific researches on the safety and efficacy of this diet. The diet proposed by Dr *Johanna Budwig* does not support the development of correct eating habits according to current scientific guidelines. In addition, the use of this nutritional model over a longer period of time is unrealistic for the patient [26].

Dr. *Budwig's* diet is rich in omega-3 fatty acids as well as vegetables, fruit and dietary fiber [26]. The basic and one of the most important component of the diet is linseed oil, which contains large amount of α-linolenic acid (ALA) [38]. The basic product is cottage cheese paste with linseed oil. Banned products in this food model include meat (especially fried), butter, margarines, mayonnaise, sugar and other oils [61]. Dr. *Budwig* believed that the cancer process in the human body is associated with a large amount of trans-fatty acids and a coexisting deficit of *omega*-3 and *omega*-6 fatty acids [18].

Macrobiotic diet

The term „macrobiotics" comes from the Greek word *macros* (large, long) and *bios* (life). This diet is considered to be conducive to maintaining general health, preventing cancer and other diseases [7]. A lifestyle based on observing the macrobiotic diet was spread by *George Ohsawa* (1893-1966), Japanese philosopher. *Oshawa* believed that a healthy diet would ensure peace and harmony on Earth. The macrobiotic diet achieved its greatest popularity in the United States in 1960 thanks to *Michio Kushi*, *Oshawa* student promoting health as a source of peace [22]. The classic macrobiotic diet provides ingredients that are adapted to age, gender, level of physical activity and the needs of the patient and environment [25]. Due to the individual approach to the patient, this model of nutrition is not characterized by strictly defined rulet [22]. The basic rule of the macrobiotic diet is its use in balance with the seasons, the needs of your body and nature. According to traditional Chinese medicine, the fundamental philosophy of macrobiotics is to identify and equalise the energy properties of all products consumed, which can be yin (cooled, moistened, expanded) or yang (heated, dried and shrunk) [2].

A standard macrobiotic diet is mainly characterised by a high content of complex carbohydrates and low fat content. The menus of 50 people on a macrobiotic diet were analysed. Fats constituted 23% of energy, carbohydrates 65% of energy [31], saturated fatty acids 4.5% of energy, while polyunsaturated fatty acids provided 7.1% of energy. Average cholesterol intake in the examined persons was 76 mg/d [29].

The macrobiotic nutrition model is not strictly vegetarian, it allows small amount of animal meat. The basis of the diet is natural and unprocessed food. The macrobiotic diet consists of 40-60% cereals, mainly brown rice, barley, millet, oats, wheat, maize, rye and buckwheat. Cereal grains can germinate and, from a macrobiotic point of view, are rich in „young energy" for clear thinking [2]. 20-30% of the daily energy requirement is provided by vegetables, mainly grown locally and prepared in different ways [29], but cooked is preferred because of their easier digestion [2]. Various types of beans (azuki, peas or lentils) and dishes made from them, e.g. tofu, tempech and natto, provide 5-10% of energy during the day. In addition, the macrobiotic diet consists of sea plants and fruit, white fish meat, seeds and nuts [25, 28].

In the classical macrobiotic diet a minimum proportion of meat (including poultry), animal fat (butter, lard), eggs, dairy products, refined sugar is allowed. It is recommended that genetically modified foods, sweeteners, food additive products [35] and purified grains that increase the risk of cancer are excluded from the diet [29]. People living in a moderate climate should resign from exotic fruits [42].

Michio Kushi has created a macrobiotic nutrition pyramid that takes into account the structure of the sea plant, which distinguishes the macrobiotic diet from vegetarian [16] and Mediterranean diets [58]. Due to the difficult access to natural food, time-consuming meal preparation and the high cost of unprocessed food, this diet is difficult to maintain for oncological patients [35].

THE IMPORTANCE OF ALTERNATIVE DIETS IN CANCER

Supporting conventional therapies

Relatively recently, researches have begun on ketogenic diet as a nutritional model to support cancer therapy. These studies were carried out both on animal models and by analyzing the medical records of patients who had chosen unconventional therapy [1].

Another, more detailed case report concerned a 65-year-old woman with multiform glioma treated with standard therapy and a ketogenic diet with limited energy value. The patient was temporarily starving before starting conventional therapy and then introduced a restrictive 4:1 ketogenic diet. The energy value of the dietary model used was about 600 kcal/day, additionally the patient was taking vitamin and mineral supplements. The ketogenic diet was observed simultaneously with the traditional treatment. During the therapy, steroid drugs (dexamethasone) were discontinued by doctors. After two months of treatment, the patient's body weight decreased by 20%, whereas magnetic resonance imaging (MRI) and emission tomography with fluoro-deoxy-glucose (FDG-PET) did not detect cancer cells in the brain. In the patient's body, reduced blood glucose level and increased level of ketone compounds in urine were observed. MRI showed a recurrence of the tumor ten weeks after the suspension of strict dietary therapy [61].

The importance of ketogenic diet in cancer was also studied among pediatric oncological patients. At Cleveland University Hospital in the United States, research has been conducted to determine if the patient's ketosis condition reduces the availability of glucose to certain types of cancer, which could potentially disrupts tumor metabolism without adversely affecting the patient's overall nutritional status. Two girls with malignant stars at an advanced stage took part in the study. Patients have been on ambulatory observation for eight weeks. The state of ketosis was maintained by a diet consisting of 60% medium-chain fatty acids derived from oil. Metabolism of glucose in the tumor was evaluated with FDG-PET. Within seven days from the beginning of the ketogenic diet the blood glucose level of patients decreased to the lowest level of normal value, while the blood ketone concentration increased 20-30 fold. The results of the FDG-PET study showed a decrease in glucose uptake at the tumor site by an average of 28.7%. During the use of ketogenic diet by patients there was a 28.8% decrease in cancer standardized uptake values (SUV). In one of the patients participating in the study, a significant clinical improvement in the mood and development of new skills was observed. She continued her ketogenic diet for the next twelve months. During this period no progression of the disease was evident [39].

The survival rate of patients with multiform glioma (GBM) treated with conventional methods is 8-15 months. Promising results, increasing survival, were obtained on animal models with GBM combining chemotherapy and radiotherapy with dietotherapy. Ketones have been reported to reduce oxidative stress, probably by improving mitochondrial function, which may also contribute to anticancer activity. Anticancer effects of ketones and ketogenic diet were observed on several models of rodents. The prescribed ketogenic diet was with a limited energy supply. In animals a decrease in growth of transplanted brain tumor cells was noticed. Mice on a ketogenic diet with limited energy supply had higher blood ketone concentration and reduced brain tumor growth associated with increased apoptosis. The ketogenic diet has also increased the effectiveness of metabolic inhibitors during the treatment of starlings in rodent models. A decrease in the growth of multiform glioma cells was shown on cell lines that were treated with β-hydroxybutyrate, the main ketone produced in the ketosis state. A reduced growth of brain cancer and

higher animal survival were observed both in the group on a classical ketogenic diet and those enriched with MCT AIDS [47].

The effectiveness of different types of ketogenic diets was also analysed and compared. Standards of conduct during their application are being developed. In patients' blood, glucose and ketone levels must be monitored twice a day, and the patients' cooperation with a nutritionist is essential. Supporting GMB treatment with a ketogenic diet is possible during simultaneous radiotherapy and chemotherapy. The ketogenic model of nutrition was indicated as a complementary anticancer therapy for patients with malignant gliomas [47].

A research was also conducted to assess the toxicity and therapeutic efficacy of intranasally administered perillyn alcohol (POH) in combination with a ketogenic diet in patients with recurrent glioma. POH is a non-toxic, naturally occurring and hydroxylated monoterpene, showing cytotoxicity to glioma cells resistant to temosolomide. The study included 32 patients with recurrent GMB, 17 of the examined group were given a ketogenic diet and 15 were the control group. Each group received 55 mg POH four times a day for three months. Before the start of the clinical trial all patients were treated conventionally. After three months in the study group a partial response to treatment was observed in 77.8% (7 out of 9) of patients. They reported a reduction of the tumor in MRI, peritoneal oedema, as well as neurological stability, reduced demand for cicosteroids and general health improvement. Among the control group, a partial response to treatment was observed in 25.0% (2 out of 8) of patients, while the development of the disease occurred in 50.0% (4 out of 8) of patients. It appears that ketogenic diet with POH may be a form of complementary treatment in patients with recurrent multiform glioza [45].

In the therapy of breast cancer, caused by the overexpression of the human epidermal growth factor HER 2, transtuzumab (TRAS) is the first-line drug. This type of breast cancer belongs to the group of aggressive cancers, characterized by a high rate of metastases and difficulties in treatment. TRAS inhibits cancer growth in patients, but only in 12-26% of cases it causes cancer regression. Additionally, the response of patients' organisms to the therapy develops for one year, and in 5% of patients the drug is cardiotoxic. The aim of the study was to check whether combining a diet enriched with linseed oil with a low-dose TRAS could increase its therapeutic effectiveness. The study was carried out with mice that were given TRAS at a dose of 2.5 and 5 mg/kg body weight. The control diet contained 20% corn oil, and in turn the diet of study groups, cold-pressed linseed oil with 58% ALA. The control group tumors increased significantly in size by 187% compared to zero week. Tumors in mice in the TRAS 2.5 group did not differ significantly in size. compared to the size of the tumor at the beginning of the study. In the case of TRAS 5 and TRAS 2.5 groups and TRAS 5 group additionally taking linseed oil, a significant regression of the tumor was noted by 75%, 89% and 84% respectively. The tumor area in the fourth week of the study was significantly lower in the TRAS 2.5 group on a linseed oil diet, compared to the group with the same dose of the drug, but on a control diet. This result was no different from that achieved by mice treated with double dose of the drug while taking linseed oil or on a control diet. The authors of the study noted that the combination of low-dose (2.5 mg/kg body weight) TRAS treatment with linseed oil supplementation was as effective as double-dose (5 mg/kg body weight) TRAS treatment [36].

Growth and tumor metastasis inhibition

Clinical trials are conducted using a ketogenic diet in support of treatment of oncological patients. It has been shown that diets with very low carbohydrate content contribute to the reduction of tumors located in the head and neck [28]. *Otto Warbung* [56] observed that cancer tumors absorb large amount of glucose as an energy source. On this basis, he put forward a thesis that the growth and metabolism of cancer cells are closely related to the process of glycolysis. Restricted access to the energy substrate causes impaired tumor growth. Additionally, thanks to the reduction of cachexia and increased tolerance to chemo- and radiotherapy, the patient's general condition improves [32].

The reduction of carbohydrate intake in the diet of an oncologic patient limits the formation of oxygen free radicals and inflammation of tissues, which contributes to the protection against the spread of cancer and has a preventive effect on healthy body tissues. The ketogenic diet, apart from a short period of starvation for the body's metabolic adaptation, has an appropriate energy value, which enables it to function and at the same time protects tissues and muscles from catabolism [32].

Brain tumors are one of the most common causes of death among children with cancer. Surgical resection followed by radiotherapy and/or chemotherapy was the standard therapy used for over fifty years. *Stafford* et al. [51] have proven that a ketogenic diet inhibits the development of gliomas, slows tumor growth and reduces the number of reactive oxygen species that usually promote tumor growth. The ketogenic diet does not work only by reducing the amount of easily accessible glucose, but its therapeutic effects are additionally associated with modulation of both the intracellular signal cascade and heomeostatic mechanisms. Healthy brain cells easily adjust their

metabolism to use ketones as an alternative source of energy, while cancer cells have a lower capacity to change their energy metabolizm [51]. Glycological cells, as well as most other types of cancer, cannot omit glycolysis and use ketone compounds in the *Krebs* cycle (TCA), their metabolism depends on the glycolytic pathway [4]. The data presented show that a ketogenic diet may contribute to the regression of brain tumours and may have neuroprotective effects on healthy brain cells during cancer treatment instead of chemotherapy. Additionally, in the cancer tissue there is an intensive cells growth accompanied by the formation of new blood vessels. However, the growth of cancer cells precedes angiogenesis, so that part of the hypertrophied tissue does not have enough oxygen. To continue growth, invasive tissue cells take energy from anaerobic glycolysis. Inhibition of glycolysis by a ketogenic diet may be a significant factor inhibiting the development of cancer cells [48].

The authors of 62 studies recognized the use of a low carbohydrate diet as a supportive therapy in the treatment of various diseases, 11 were concerned with anticancer therapy. In Germany, at the University of Würzburg, patients whose conventional cancer treatments had been ineffective were included in the research on the effectiveness of a ketogenic diet. Preliminary reports indicate that patients who were able to continue their ketogenic diet therapy for more than three months have improved with stable physical condition and tumor contraction or slowing down its growth. However, the authors of the study cannot statistically determine the impact of this diet on cancer cells due to the small number of people who took part in the study and their heterogeneity. Pilot data from the study suggest that ketogenic diet may be effective even in patients with advanced metastatic cancer [46].

Gabor and *Abraham* [9] in their study showed the strengthening effect of linoleic acid (C18=2) and the growth of mammary gland tumor cells transplanted in rodents [9]. However, a study conducted by *Kamalia* et al. [23] showed that a diet rich in eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA) gave the opposite effect [23]. In another study in mice with breast cancer with metastases it was found that a diet with linseed oil rich in α-linolenic acid was more effective in inhibiting tumor growth and its metastases to the lungs than a diet rich in fish oil. In a different study on an animal model it was observed that linseed oil did not show any suppressing effect on the growth of cancer cells and their spread to other organs [8].

It was examined whether n-3 fatty acids derived from linseed oil will affect the cytolytic capacity of macrophages and eicosanoids production. Mice were fed a diet of 10% linseed oil or fish oil from herring or saffron oil for three weeks. In *vitro* and in *vivo* activated macrophages selected functions were evaluated. As predicted by the authors of the study, macrophages from mice fed on linseed oil and herring fish oil produced significantly less both prostaglandin and leukotrienes compared to macrophages from mice fed on saffron oil. Furthermore, macrophages from these mice were able to synthesise additional EPA leukotrien. However, the effect caused by linseed oil on mouse models was not as spectacular as with fish oil. For specific functions, macrophages from mice consuming linseed oil did not have an altered cytolytic capacity compared to macrophages from mice eating fish oil. The specific binding of macrophages to tumours, the production of nitric oxide and the production of tumour necrosis factor did not change after intake of linseed oil, which was observed after consumption of fish oil [19].

The aim of another study, carried out in the 1990s, was to determine if dietary supplementation with flax seeds, their ligan or oil fractions, starting from the thirteenth week after the cancer invasion, would reduce the size of nipple tumors (in the early stages of the disease) and the appearance of new tumors in rats. Ligands are diphenolic compounds present in products with high fibre content. They are assigned anticancer, antioxidant, low estrogenic (or anti-estrogenic) and anti-angiogenic properties. That suggests a diet rich in ligan precursors in mammals could protect them from cancer. Linseed is a good source of leagunes, both *in vivo* and *in vitro* researches. The authors of the study divided the animals into five groups. The control group received a diet with 20% corn oil, the next groups a diet with the addition of 2200 nmol/d of the ligano precursor sekoisodiol-diglycoside (S.D.) or 1.82% linseed oil, or 2.5% linseed or 5% linseed. After seven weeks it was found that the tumor volume was more than 50% lower in all therapeutic groups compared to control. Tumour volumes were smaller in groups on a diet with S.D. and 2.5% and 5% of linseed compared to groups whose diet was enriched with linseed oil. The authors of the study consider that taking S.D. present in linseed is beneficial in the phase of carcinogenesis promotion, while the effectiveness of linseed oil is observed after the growth of a tumor in the body. The beneficial effect of linseed oil on the cancer is attributed to its high ALA kontent [54].

In another study, it was evaluated which component of linseed reduces growth and metastases of human breast cancer in mice. The authors of the study wanted to determine whether the cancer inhibitory effect was caused by linseed oil, liganeskolaroglycol diglycoside or both at once. Eight weeks after the injection of cancer cells, an appropriate dietary model was introduced to the laboratory animals. Five different diets were used, a control diet with 20% corn oil and tested with 10% freshly milled linseed or with linseed oil or liganoescolaroglycol diglycoside or two at the

same time. Each of the prepared diets was isocaloric and high-fat. Noticeably lower tumor growth rate was observed in groups taking a diet with freshly ground linseed, linseed oil and two components simultaneously. However, there were no significant differences in the rate of cancer growth between the other two groups. Compared to the control group, the frequency of lung metastases decreased by 50.1%, 30.1%, 16.3% and 70.1%, respectively, in groups taking supplementation with freshly milled linseed, ligano-seskolaroglycol diglycoside, linseed oil and two components simultaneously. Among the study groups, only the group taking supplementation of both linseed components simultaneously had the lowest incidence of lung metastases. On the other hand, considering the occurrence of metastases to lymph nodes, only in the group supplemented with linseed oil a significant reduction of 52.5% was noted compared to the control group. The incidence of metastases in other organs, such as liver, bones, kidneys and abdominal cavity, decreased in all therapeutic groups, but in the group taking linseed oil and linseed oil with liganeskolaroglycol diglycoside metastases did not occur at all. The authors of the study believe that linseed oil, or linseed oil with liganeskolaroglycol diglycoside, contributes to the inhibitory tumour growth caused by the consumption of freshly ground linseed. Additionally, in the groups of mice taking linseed oil and two supplements at the same time, significantly lower proliferation of cancer cells was observed compared to the group of mice eating only freshly ground linseed, which suggests that both of these components increased the effect of freshly ground linseed causing reduced tumor growth rate [55].

The authors of the next study report that 80% of breast cancer patients use complementary or alternative therapy, including dietary supplements [3]. Linseed is the third most commonly used supplement. Linseed oil, which is rich in fatty acids from the n-3 family, is popularly used by patients with breast cancer because of its potential anticancer effects [36].

It was also found that these properties and principles of the macrobiotic diet, to some extent, contribute to the carcinogenic and therapeutic effects of diet on the patient's body [22].

Effects on patients' well-being and quality of life

The authors of clinical trials conducted among people at the terminal stage of the disease showed good tolerance of ketogenic diet by patients and its beneficial effect on their well-being, with no serious side effects [37, 48] (mainly constipation), even improved the emotional state of patients and reduced insomnia [48]. The ketogenic model of nutrition may additionally improve the quality of patients' life and their blood parameters [46]. However, other researchers report that side effects of radio and chemotherapy (nausea, vomiting or other stomach problems) can be further exacerbated by a ketogenic diet [47]. In one of the patients participating in the *Nebeling* et al. [39] study was observed to have a significant clinical improvement in mood and development of new skills [39].

Macrobiotic diet for people with cancer can result in weight loss, making it unsuitable for devastated oncological patients. Dietary enthusiasts believe that it improves health, but can cause nutrient deficiencies when the patient's food selection is too radical. The food chosen by patients should provide many nutrients, but there is a risk that the energy value of the diet, protein and fluid content may be insufficient [22].

Cancer protection

The American Institute for Cancer Research and the World Cancer Research Fund have prepared dietary recommendations on cancer prevention. The authors of the report suggest that a diet based on plant products, which simultaneously minimizes the consumption of red and processed meat and is rich in whole-grain cereal products, reduces the risk of cancer [20, 21]. Many components of the macrobiotic diet have been found to have an anti-cancer effect. Whole grain products belong to the basic group of food products in the macrobiotic diet. The importance of unprocessed grains in the prevention of cancer is not only attributed to the presence of increased amount of dietary fiber, but also includes their impact on the metabolism of estrogen, glucose, insulin and oxidative processes in the body [49]. The influence of regular consumption of various vegetables on reducing the risk of cancer was also appreciated [11]. An increase in the consumption of fruit and vegetables from 250 g to 400 g per day can reduce the risk of cancer by 23%. It has also been pointed out that sea vegetables included in the macrobiotic diet may reduce the risk of breast cancer [53, 60] and endometrial cancer [13]. Brown seaweed contains fucoiudan, a polysaccharide sulphate characteristic of this variety [19], and fucoxanthin, a carotenoid causing brown colouring of plants which are known to have anticancer properties [40, 41].

Legumes, whose daily supply in a macrobiotic diet should be about 5-10% of the daily energy intake. They can reduce the risk of cancer through the presence of inhibitors and saponins. Additionally, the phytoestrogens - ganistein and daidzein found in the seeds show antioxidant antiangiogenetic effects. Isoflavonoids can also affect signal transduction and inhibit the action of DNA topoisomerases [29].

The anticancer effect of the macrobiotic diet is also associated with minimizing the consumption of foods

with a pro-cancerogenic effect on the human body [30]. With the exception of fish, the macrobiotic feeding model minimises the consumption of red meat, which increases the risk of colon and rectal cancer [44], as well as prostate [49] and pancreatic cancer [16]. Moreover, the macrobiotic diet is based on natural and organic food, which is associated with a reduction in the body's exposure to pro-cancerogenic pesticides or artificial plant fertilizers [22].

At the beginning of the 21st century it has been shown that the increased consumption of whole-grain cereal products, soybean products and legumes causes a decrease in testosterone levels in patients. This is associated with a lower incidence of breast cancer in postmenopausal women. In addition, these products are conducive to weight normalization in overweight or obese patients [43].

Researchers from the Nutritional Data System for Research (NDSR) compared the nutrient and anti-inflammatory content of the macrobiotic and customary American diet (2009-2010 NHANES data) and the USDA recommendations and standards. The authors of the study used the previously developed indicator of dietary inflammation to compare nutritional models. The energy value, macronutrient content, 28 microelements and the score of the inflammatory index of the diet were analysed. In the study conducted in the next edition of the NHANES study, the macrobiotic diet was characterized by a lower percentage of energy from fat, a higher daily supply of dietary fiber and a higher content of most of the studied microelements. With the exception of vitamins D and B_{12} and calcium, the nutrients filled or exceeded the RDA recommendations. Macrobiotic diet has also been shown to have an anti-inflammatory effect due to its nutrient profile. It prevents diseases and supports their treatment. Additionally, the authors of the study observed a strong antibacterial effect of the diet, compared to the typical diet of Americans. The results of studies on cancer prevention showed a significant reduction of inflammation in the body of patients while eating foods with strong anti-inflammatory properties, which were included in the macrobiotic diet [15].

SUMMARY

Most of the scientific research in which cancer patients participated concerned the use of ketogenic diet. The authors of the study have observed the regression of neoplastic lesions after applying a strict low-carbohydrate diet, in patients who do not respond to conventional methods of treatment. It has been noticed that chronic hyperglycaemia is a risk factor for the development of cancer, as well as worsens the prognosis in already suffering patients and shortens the survival period. Cancer cells do not use ketone compounds as a source of energy as opposed to healthy cells. It suggests that the ketogenic diet could be used in the future as a therapy to support traditional methods of treatment. Due to insufficient number of studies with a large number of homogeneous cancer patients, ketogenic diet can still not be considered as anticancer dietary therapy.

A review of the literature has revealed many contradictory information concerning the actual effect of Dr. *Budwig's* diet on carcinogenesis. The cited scientific research evaluating the influence of linseed oil on cancer cells was conducted mainly on animal models. Some of the results collected are contradictory, but several quoted studies have shown that taking linseed oil together with conventional cancer therapy results in a faster reaction of the rodent organism to the treatment received, compared to laboratory animals taking only traditional treatment. Additionally, the authors of the study noticed that it is possible to reduce the dose of anticancer drugs (even by half) with simultaneous supplementation with linseed oil (or linseed) obtaining identical treatment results as with a higher dose of therapists. The authors of the study attributed beneficial changes caused by linseed oil supplementation in the examined mammals to ALA. Mice and rats are characterized by a different metabolism than humans, which does not allow for an unambiguous assessment of the effectiveness of the dietary model proposed by Dr. *Budwig.*

The analysis of the quoted literature on diet of *George Ohsawa* indicates that the macrobiotic dietary model is recommended primarily as prevention of cancer. For people with a diagnosis of cancer, a macrobiotic diet can have an adverse effect on their nutrition and health. Macrobiotic nutrition can contribute to weight loss and nutrient deficiencies, which excludes its use in oncological patients.

Alternative diets are very popular among oncologic patients, however, in the light of current and quoted studies, their use cannot be clearly considered as justified. However, numerous indications of their high effectiveness in supporting conventional therapy are very promising.

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REVIEW ARTICLE

RADIOFREQUENCY ELECTROMAGNETIC RADIATION FROM WI-FI AND ITS EFFECTS ON HUMAN HEALTH, IN PARTICULAR CHILDREN AND ADOLESCENTS

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ABSTRACT

Radiofrequency electromagnetic radiation emitted from Wi-Fi devices is nonionizing radiation. The frequencies used in wireless technology are similar to those applied in mobile telephony. Due to the much lower output power of devices using Wi-Fi compared to mobile phones, the degree of exposure to radiation is also lower. Most of the research on Wi-Fi has been carried out in less favorable or adverse conditions, involving higher power values of devices (peak values instead of average values) and smaller distances of working devices from measuring points. None of the studies conducted so far have indicated that there were the exceedances of the permissible values of radiofrequency electromagnetic radiation contained in the Polish and global legal regulations. Similar to the research related to the impact of mobile telephony on human health, the studies conducted until now focusing on exposure to Wi-Fi are considered ambiguous as they do not give a definitive answer on the possible negative (including carcinogenic) effects on human health. Because of the continuous development of wireless networks, there is a need for further research on this topic. Moreover, due to the high popularity of devices using Wi-Fi among children and adolescents, whose period of exposure to electromagnetic radiation is longer compared to adults, it is necessary to continuously observe these populations and subject them to careful analysis.

Key words: *electromagnetic wave, radiofrequency radiation (RFR), Wi-Fi, health aspects*

STRESZCZENIE

Promieniowanie elektromagnetyczne częstotliwości radiowych pochodzące od urządzeń korzystających z Wi-Fi jest promieniowaniem niejonizującym. Częstotliwości pracy wykorzystywane w technologii bezprzewodowej Wi-Fi są zbliżone do tych wykorzystywanych w telefonii komórkowej. Z powodu dużo niższej mocy urządzeń używających Wi-Fi w porównaniu do telefonii komórkowej, narażenie na promieniowanie jest również niższe. Większość badań dotyczących Wi-Fi przeprowadzona została w warunkach mniej korzystnych, uwzględniających wysoką moc pracujących urządzeń (wartości szczytowe zamiast wartości średnich) oraz mniejsze odległości urządzeń od punktów pomiarowych. W żadnym z przeprowadzonych dotychczas badań nie stwierdzono przekroczenia dopuszczalnych poziomów promieniowania elektromagnetycznego zawartych w polskich i światowych regulacjach prawnych. Podobnie do badań związanych z wpływem telefonii komórkowej na zdrowie ludzi, dotychczasowe badania dotyczące narażenia pochodzącego od Wi-Fi są uważane za niejednoznaczne, ponieważ nie dają ostatecznej odpowiedzi na temat możliwych negatywnych (w tym rakotwórczych) skutków dla zdrowia ludzi. Ze względu na ciągły rozwój sieci bezprzewodowych istnieje potrzeba dalszych badań. Ponadto, ze względu na dużą popularność urządzeń wykorzystujących Wi-Fi wśród dzieci i młodzieży, których okres ekspozycji na promieniowanie elektromagnetyczne jest dłuższy w porównaniu z osobami dorosłymi, konieczne jest ciągłe obserwowanie tych grup ludzi i poddawanie ich dokładnej analizie.

Słowa kluczowe: *fale elektromagnetyczne, promieniowanie elektromagnetyczne częstotliwości radiowych, Wi-Fi, aspekty zdrowotne*

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RADIOFREQUENCY ELECTROMAGNETIC RADIATION AND ITS SOURCES IN THE ENVIRONMENT

Radiation is the transfer of energy between an emission source and a receiver. It takes place through the transmission of particles or electromagnetic waves, without the involvement of a material medium. Electromagnetic waves can propagate not only through materials but also through a vacuum. These waves are created due to the movement of electric charge that generates an electric current, while a magnetic field appears around the resulting electric current. An electromagnetic wave is characterized by several physical quantities, which include the following: electric field strength – E (the SI unit is V/m); magnetic field strength – H (the SI unit is A/m); and frequency, which refers to the number of complete electric or magnetic field changes occurring per second (the SI unit is Hz) [13].

Electromagnetic radiation is inherent in an environment. The electric and magnetic fields are created naturally; for example, the constant electric field of the Earth arises from the potential difference between the negative charge of its surface and the positive charge of the ionosphere. The strength of the Earth's electric field varies between 100 and 150 V/m [17]. Artificial electromagnetic radiation is produced due to science and technological advances and human activities [16]. Each electrical device generates an electric and a magnetic field, increasing the level of electromagnetic radiation in the environment. A few examples of the sources of electromagnetic radiation are mobile phones and the Wi-Fi devices (e.g. routers or access points). With increased technological development, the issue of radiation exposure from Wi-Fi, which currently affects the majority of the population, has become a subject of research and analysis [14]. Radiation exposure covers not only the people using emitting devices but also those in the surrounding area. This problem is even more intensified in densely populated regions, especially large cities, where the number of Wi-Fi access points is comparatively higher.

LEGAL REGULATIONS IN POLAND AND WORLDWIDE

The high prevalence of the sources generating electromagnetic radiation (including Wi-Fi devices) in the natural environment has led to the implementation of legal regulations in Poland and worldwide for ensuring environmental protection. The basic legal act passed in Poland specifying environmental safety against radiation is the Act of 27 April 2001 – Environmental Protection Law (Journal of Law of 2019, item 1396, as amended) [24]. Pursuant to Article 122 of the Act, the levels of electromagnetic fields that can be permitted in the environment have been determined for areas intended for housing and those accessible to the public - Regulation of the Minister of Health of 17 December 2019 on permissible levels of electromagnetic fields in the environment (Journal of Law of 2019, item 2448) [18]. In the European Union, the basic legal act that was adopted for the protection of the population against electromagnetic radiation is Council Recommendation (1999/519/EC) of 12 July 1999 on the limitation of exposure of the general public to electromagnetic fields (0 Hz to 300 GHz) [3]. The reference levels for electromagnetic fields (electric field strength, magnetic field strength, and equivalent plane wave power density) in the whole frequency range that are set by the Polish legal regulations and the recommendation of the European Union are the same. In the frequency range from 2 to 300 GHz (radiofrequencies also used in Wi-Fi), an electric field strength of 61 V/m and an equivalent plane wave density of 10 W/m^2 are permissible. By the end of 2019, the levels of radiofrequency electromagnetic fields permitted by the Polish legal regulations became one among the most restrictive levels in Europe and in the world.

The legal acts define the basic restrictions (related to the phenomena that affect a person directly) and the reference levels (introduced to measure and evaluate the exceedances of basic restrictions). In the recommendation of the European Union, the basic restrictions are expressed by specific absorption rate (SAR) which is a measure of radiofrequency electromagnetic field absorbed by the human body (its unit is W/kg). As recommended in the legal acts passed by the European Union and the International Commission on Non-ionizing Radiation Protection (ICNIRP), the average SAR (for frequencies ranging from 10 MHz to 10 GHz) that is permissible for the entire human body is 0.08 W/kg, with the value for exposure of the head and torso being 2 W/kg and that of limbs being 4 W/kg [3, 7]. SAR is used to determine the exposure from the devices emitting electromagnetic radiation that are at short distances from the body [6]. The determination of SAR for humans is complicated by many factors including the thermoregulation potential of the human body (tissues have the ability to put off the thermal energy supplied to the body). The reference levels are expressed by the electric field strength (E), magnetic field strength (H), and equivalent plane wave power density (S). The basic restrictions will not be exceeded at a place where the exposure to electromagnetic radiation is within the reference levels, regardless of the length of stay.

WI-FI

Wi-Fi is a wireless transmission technique used for local area networking. It operates in the 2.4- and 5-GHz radio band, which is similar to the frequencies (microwave range) used in mobile telephony. In recent years, Wi-Fi has become ubiquitous in modern society. Wi-Fi devices support wireless local area networks (WLANs). The most common and known function of these devices is to provide internet access to portable computers, but they are also applied in the case of other communication devices, including electricity meters. Wi-Fi was initially developed as a wireless replacement for Ethernet cables which were previously used to connect computers to local networks. Currently, Wi-Fi is the basis for almost all the WLANs present in houses, offices, etc. Every modern laptop and mobile phone is equipped with Wi-Fi. In addition, domestic appliances (e.g. bathroom scales, gaming devices, audio equipment) are nowadays equipped with Wi-Fi to enable programming [6, 14]. Moreover, using Wi-Fi is very convenient because it allows moving and operating various mobile devices. Figure 1 presents a simple diagram of a WLAN.

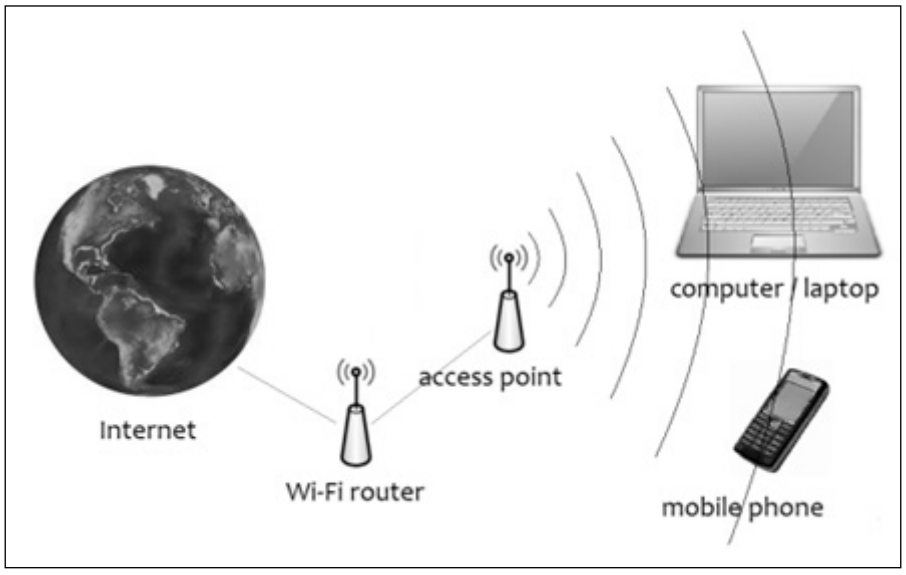

Figure 1. Wireless local area network (WLAN)

A Wi-Fi router can be integrated with an access point or can function independently. It is a device that allows connecting to a wireless network. Most Wi-Fi devices operate in the frequency range of 2.400–2.4835 GHz (2.4 GHz). For example, cell phones use a similar frequency band (the third-generation 3G network) of 2.1 GHz. The frequency range of Wi-Fi devices falls within the ISM (industrial–scientific–medical) band. Furthermore, the frequency band of 2.4–2.5 GHz is reserved for other devices, including those used for communication such as cordless telephones or domestic appliances such as microwave ovens [6]. A Wi-Fi network might also use a higher frequency range of 4.915–5.825 GHz (5 GHz) in some cases [23].

A simple model representing the propagation of electromagnetic radiation in free space can allow estimating the exposure to radiation emitted from a Wi-Fi device. In the case of an antenna emitting power (P) (excluding reflections from other surfaces), the power density (S) at a distance (R) is given as:

$$S = \frac{PG}{4\pi r^2} = \frac{EIRP}{4\pi r^2},$$

where:

- G is the gain of the antenna (expressed in dBi) which indicates the value (expressed in dB) at which the antenna gain is greater in relation to the hypothetical isotropic antenna (an infinitely small point in a vacuum, emitting isotropic electromagnetic radiation in each direction without reflections or losses);
- EIRP is the effective isotropic radiated power, which is the product of power (P) and gain of the antenna (G) and represents the power that the isotropic antenna would have to radiate in order to get the same level of the signal in the direction of the maximum radiation of the given antenna;
- R is the distance from the source of electromagnetic radiation [6].

For a simple lossless dipole antenna, which is typical of the antennas present in many Wi-Fi transmitters, the antenna gain (G) equals 1.65. Typical Wi-Fi devices that operate at a power of approximately 0.1 W produce a peak power density of almost 330 and 13 mW/m^2 at a distance of 20 cm and 1 m, respectively. The above equation does not take into account the reflections from surfaces, which is a significant phenomenon occurring during the propagation of radiofrequency electromagnetic waves inside buildings. However, it provides a fairly good

estimate of the wave propagation taking place inside buildings for a distance of several meters from the antenna. It should be noted that real Wi-Fi transmitters work at much lower levels of power than that indicated in the above equation [6].

The actual conditions of propagation of electromagnetic waves from devices, for example, Wi-Fi routers, in indoors are different from that in free space. The electromagnetic waves encounter various obstacles, such as walls, equipment, and even humans. Moreover, physical phenomena, including wave attenuation and wave reflection or diffraction at the edges of walls, should also be taken into consideration. Table 1 shows the attenuation values of some individual building elements for the 2.4-GHz frequency.

Table 1. Attenuation values of building materials for the 2.4-GHz frequency [10]

Name of the element	Material	Thickness [cm]	Attenuation [dB]
Internal wall	Brick	10	7
External wall	Brick	30	9
Partition wall	Plasterboards and glass wool	7	2
Ceiling	Concrete	30	11
Window	Glass	2 × glass + 1 cm gap	4.5
Door	Wood	4	2.5

For example, a 3-dB attenuation indicates a twofold decrease in signal strength, while a 9-dB attenuation means an eightfold decrease, and an 11-dB attenuation, over a 12-fold loss of a radio signal in a given band.

Antennas that are mounted inside laptops or other devices using Wi-Fi are usually very small in size in the order of a few centimeters, which is considered to be insignificant compared to the distance between the device and the user. This suggests that exposure to electromagnetic radiation emitted by the devices rapidly decreases as the user's distance from them increases (as exposure is inversely proportional to the square of the distance). However, it is worth noting that the users are at much greater distances from devices transmitting Wi-Fi signals than when using mobile phones (distance between the head and the device) [15].

RECENT STUDIES AND EXPERIMENTS ON THE IMPACT OF WI-FI ON HUMANS

In studies carried out so far analyzing the possible negative impact of electromagnetic radiation (radio- and microwave frequencies), exposure values were measured either from physical quantities such as electric field strength and equivalent plane wave power density, which are compared to the reference levels specified in the relevant legal regulations, or from SAR, which is calculated to determine the effects of radiation on human tissues [9]. The recent experiments that investigated the impact of electromagnetic radiation emitted from Wi-Fi devices had either of the following two objectives: assessing the exposure under normal conditions or assessing the exposure under adverse conditions (e.g. with antennas that emit electromagnetic radiation continuously or at a distance of only a few centimeters from the body). Both experimental studies and numerical simulations have been carried out, with some of them involving animals. The results of selected studies are described below.

A study conducted by *Karipidis* et al. in 2017 measured exposure to electromagnetic radiation from Wi-Fi and other sources at 23 schools in Australia. The mean and peak values of radiofrequency electromagnetic radiation were calculated by the authors. All the measurements revealed much lower levels compared to the reference ones specified in the legal regulations - Council Recommendation (1999/519/EC) of 12 July 1999 on the limitation of exposure of the general public to electromagnetic fields (0 Hz to 300 GHz). The values measured in empty classrooms and classrooms with students constituted from about 0.0001% (average) to about 0.01% (peak) of the limit values recommended by the European Council. The levels of exposure to electromagnetic radiation from Wi-Fi determined in the study were found to be much lower than those estimated for other working devices, such as radio, television, or mobile phones [11].

In another study conducted by Peyman et al. in 2011, the level of electromagnetic radiation (electric field strength) released from a total of 15 working laptops and 12 access points at UK schools was measured. The maximum values of this physical quantity at a distance of 0.5 m from the devices - laptops and Wi-Fi routers - were estimated to be 2.893 and 5.716 mV/m, respectively. These values represented 0.005% and 0.01%, respectively, of the reference levels recommended in the European Union. At a distance of 0.5 m, the equivalent plane wave power density was calculated as 22 mW/m^2 for laptops and 87 mW/m^2 for Wi-Fi access points. Moreover, these did not exceed the recommended values and constituted 0.22% and 0.87% of the reference levels specified in the Polish

and European legal regulations. Measurements were also made with respect to other distances (greater than 0.5 m), which showed that for a twofold increase in the distance (1 m), the values of equivalent plane wave power density decreased by about four times [15]. This finding is consistent with the fact that the decrease in the level of radiation depends on the distance from its source.

In 2007, *Foster* [5] conducted a study on the effects of electromagnetic radiation emitted from Wi-Fi in different environments and in various countries. A total of 365 measurements were taken in 55 cities of four countries (United States of America, France, Germany, and Sweden), in places occupied by people, such as offices, shops, and healthcare facilities. Measurements made at short distances from devices (1 m from laptops that use Wi-Fi) showed exposure levels that were much lower than those recommended in the international legal regulations. In addition, the measurements were taken while large files were downloaded (during which time the devices work at the highest power). However, it was found that the level of electromagnetic radiation (expressed by equivalent plane wave power density) was much lower than that contained in the Polish and international legal regulations. The measurements ranged from 0.001 to 0.01 W/m^2, which constitutes from 0.01% to 0.1% of the limit values, respectively.

Another study was carried out by *Schmid* et al. [20] in 2007 under the normal operating conditions of devices using WLANs in closed rooms such as cafes, airports, and open space (including public places with Internet access - downtown and residential area) in Germany. All the measured values of equivalent plane wave power density were several orders of magnitude lower than the limit values. It was found that even for a distance of 20 cm (for cafes), the measured values did not exceed the recommended limits. Similarly, in the case of open space (where the distances from the devices are much greater), the values measured were within the reference levels and were much lower than the measurements taken in rooms. Table 2 shows the results of these measurements.

In 2010, *Findlay* and *Dimbylow* [4] conducted an experiment on a voxel phantom (computer-simulated human body) of a ten-year-old child. Exposure to electromagnetic radiation from Wi-Fi devices was simulated - for the frequencies of 2.4 and 5 GHz. In all the measurements made in the experiment, the SAR values were found to be significantly lower than those specified in the relevant legal regulations. The highest measured SAR value for the whole body was 19.1 μW/kg (normalized value for 1 V/m). It was determined that in order to exceed the SAR limit (i.e. 0.08 W/kg for the whole body), a person should be exposed to electromagnetic radiation with an electric field strength of 64.7 V/m. Typical values of electric field strength measured at a distance of 1 m from Wi-Fi access points were below 2 V/m, which were so much lower than those at which SAR would be exceeded. SAR values were also measured depending on the distance from the antenna for the head and torso. Even at the smallest distances (in the study, the head–antenna distance was 3 cm), the maximum SAR value measured was 81.7 mW/kg. The SAR value was calculated taking into account a factor of 1/10 for laptop Wi-Fi antennas (assuming that 10 people use an access point simultaneously) and typical Wi-Fi antenna power, which does not exceed 100 mW, which showed an average power of 10 mW for laptop Wi-Fi antennas. The SAR values recommended by ICNIRP for the head are 2 W/kg; therefore, the values measured in the study were below the one mentioned in the guidelines (they represent a maximum of 4% of the limit values). All the SAR measurements carried out at different distances during the use of a computer with Wi-Fi access were not found to be exceeded and were significantly lower than those allowed in legal regulations.

In addition to the experiments carried out under the normal (standard) operating conditions of devices using Wi-Fi, some were carried out in more adverse conditions. These evaluated the exposure to electromagnetic radiation from devices that were very close to the measuring points (as if they were close to the user's body) or worked at maximum (or much higher than normally used) power. In a study conducted by

Table 2. Maximum measured values of equivalent plane wave power density at various measuring points [20]

Measurement location	Minimum distance from the antenna [m]	Maximum measured values of equivalent plane wave power density [mW/m^2]	The share of the measured value in relation to the limit value—10,000 mW/m^2
Cafe	0.2	183	1.83%
Airport	3.0	1.86	0.0186%
Outdoor 1—downtown	5.0	0.10	0.001%
Outdoor 2—downtown	5.0	0.34	0.0034%
Outdoor 1—residential area	50.0	0.002	0.00002%
Outdoor 2—residential area	50.0	0.004	0.00004%

Kuhn et al. in 2007, the levels of electric field strength and SAR values for 100 cm from the device using Wi-Fi were estimated under the worst possible operating conditions (devices worked at maximum power). In the case of Wi-Fi routers operating in the frequency range of 2.400–2.484 GHz, the electric field strength was 1.1 V/m, while for a frequency of 5.200–5.800 GHz, the strength equaled 0.9 V/m. The SAR values were 0.36 and 0.81 W/kg, respectively, which did not exceed the reference levels and basic restrictions. Close proximity to this type of equipment can be avoided by mounting them on the wall above users' heads [12].

In a study conducted by *Schmid* et al. in 2007, video surveillance systems for babies, Digital Enhanced Cordless Telecommunications (Digital European Cordless Telecommunications) - DECT, devices using the Bluetooth function, and WLANs were analyzed. The values of equivalent plane wave power density measured did not exceed the limit values in any of the checked points, and constituted a maximum (for average values) of 0.004% of the limit values [19]. In the same experiment, the SAR values for use under very unfavorable conditions were also measured for selected devices, including those using a WLAN (the device emitting electromagnetic radiation from the antenna was in contact with the phantom surface where the SAR level was measured). For a laptop using Wi-Fi, the maximum SAR value was found to be 0.05 W/kg which was lower than the limit value for limbs (4 W/kg) and that for the head and torso (2 W/kg) [3, 7].

In addition to numerous studies carried out over the years, in which the physical quantities related to exposure to high-frequency electromagnetic radiation were measured in various places and under different operating conditions of devices using Wi-Fi (antennas on laptops, Wi-Fi routers, access points), experiments involving living organisms such as animals and humans have also been conducted, investigating the effects of electromagnetic radiation on their behaviors and biological functions.

Studies focusing on the effects of long-term exposure to electromagnetic radiation from Wi-Fi signals (a frequency of 2.4 GHz) were carried out by *Banaceur* et al. [2] in 2013 using mice. The animals were exposed to radiation to reach an SAR of 1.6 W/kg for 2 hours a day for 1 month. The results of the study showed that exposure to electromagnetic radiation can improve memory in animals with *Alzheimer's* disease and also improve cognitive abilities.

Shokri et al. [21] conducted an experiment in 2015 on rats. The study animals were divided into three groups: the first group (control) which consisted of nine healthy individuals was not exposed to electromagnetic radiation from antennas (Wi-Fi devices), the second group which consisted of nine individuals was irradiated with electromagnetic radiation at a frequency of 2.45 GHz for 1 hour a day for 2 months, and the third group which consisted of nine individuals was irradiated with 2.45-GHz electromagnetic radiation for 7 hours a day for 2 months. The results of the study showed that depending on the length of exposure (the longer the exposure, the greater the negative impact), the quality of sperms in male rats deteriorated (with the worst quality observed in the third group). The study also showed the relationship between the other health effects and the time of exposure to the high-frequency electromagnetic radiation. The authors concluded that the effects of exposure of our body to electromagnetic radiation emitted from Wi-Fi antennas are time-dependent [21]. However, as a very small number of animals were tested in the study, the obtained results are considered unreliable.

A study conducted by *Hassanshahi* et al. in 2017 aimed to assess the impact of chronic exposure to electromagnetic radiation from Wi-Fi devices on learning ability in rats. The results showed that Wi-Fi signals impair the ability of animals to distinguish between a new and a known object. As this finding indicated a possible adverse effect of the electromagnetic radiation from Wi-Fi, it might be particularly interesting to analyze the potential effects of Wi-Fi on cognitive behaviors in both animals and humans [8].

Most studies on animals conducted thus far have shown the negative effects of electromagnetic radiation. However, they report contrasting results. Undoubtedly, electromagnetic radiation is related to the absorption of energy by living organisms. Thus, it can be emphasized that changes can be observed in the hormone levels or in the activity of the nerve ending of the brain in the organisms that are exposed to high-frequency electromagnetic radiation (with SAR values not causing thermal effects). With higher SAR values (about 6.8 W/kg), a significant shortening of the life expectancy can be expected in the examined animals.

Table 3 presents some examples of biological effects caused by high-frequency electromagnetic radiation for various organisms depending on the SAR value.

After absorbing low energies of electromagnetic radiation (before body temperature rises), thermoregulatory mechanisms can reduce the heat energy. In primates, behavioral changes may occur with reduction of locomotor activity or delay of reaction time. The limit values for the behavioral changes mentioned above are only indicative [22]. Research results reported to date have shown the high-frequency electromagnetic radiation as a weak biological factor [16, 17].

Due to ethical reasons, studies on the effects of radiofrequency electromagnetic radiation on human health are difficult to conduct. Moreover, ensuring the right conditions (full power range of working devices,

Table 3. Examples of biological effects caused by high-frequency electromagnetic radiation [17, 22]

Type of biological effect	Research subject	SAR [W/kg]	Temperature increase [°C]
Cataract	Rabbits	100–150	3–6
Congenital abnormalities	Rats	6–10	2–3
Hormonal responses	Rats, primates	3–4	1–2
Impaired performance of learned activities	Rats, primates	2–5	1
Reduction of locomotor activity	Primates	1–3	<1
Activation of thermoregulatory mechanisms	Primates	0.7–1	<1
Decreased metabolism	Primates	0.7–1	<1
Auditory hallucinations	Humans, rats	0.01–0.1	-
Electrocardiography changes	Rabbits	0.01–0.5	-
Increased permeability of the blood–brain barrier	Rats, rabbits	0.05–0.1	-

testing the same population for many years) is also challenging [16]. Therefore, it is only possible to carry out experiments on animals or on cell cultures. However, each of them may have some disadvantages. In addition, it is not known if conclusions can be drawn from animal studies and generalized directly to humans. Therefore, many of the studies conducted are considered ambiguous.

In a recent study carried out on humans by *Hosseini* in 2019, the possible impact of short-term Wi-Fi signals on the cognitive functions of the brain was analyzed. Consent for the study was obtained from the local Ethics Committee of Shiraz University of Medical Sciences in Iran, and the study was approved by the Iranian Registry of Clinical Trials. A total of 45 students from Shiraz University participated in the experiment which had two sessions: one involving exposure to electromagnetic radiation from Wi-Fi and the other without exposure (sham). Each session lasted two hours. After the session, the participants were subjected to reaction time test, short-term memory test, and reasoning skills test. No statistically significant differences were found between the average results of reaction time, short-term memory, and reasoning ability in both sessions (sham and exposure). During the experiment, the reference levels – electric field strength and equivalent plane wave density – were also measured. At a distance of 1.5 m and a height of 1.2 m (typical when using Wi-Fi devices), the values were calculated to be, respectively, 4.1 V/m and 0.0446 W/m^2 [9], which constituted 7% of the limit value for the electric field strength recommended in most of the countries of the European Union and 0.4% of the limit value for equivalent plane wave density.

Since the 1950s, many studies have been conducted on the biological effects of radiofrequency electromagnetic radiation on living organisms. These have assessed both the impact of mobile phones and Wi-Fi. However, so far, none of them have shown that there is proven risk associated with the operation of these devices, as long as the limits (basic restrictions and reference levels) specified in the relevant legal regulations are not exceeded [1, 6].

SUMMARY

Radiofrequency electromagnetic radiation is nonionizing radiation, which cannot affect the structures or molecules present in the biological systems as their energies are too low. However, high-frequency electromagnetic radiation can possibly cause, among others, an increase in tissue temperature (thermal effect).

When examining the impact of electromagnetic radiation from Wi-Fi devices, it should be noted that the output power resulting from a Wi-Fi router is much lower than that from mobile phones. In addition, both cell phones and Wi-Fi devices work in similar frequency ranges. Therefore, the degree of exposure to electromagnetic radiation from Wi-Fi is lower compared to mobile phones. Moreover, Wi-Fi routers are located at much greater distances from the human body than mobile phones, which are usually present at a few centimeters from the body or right next to the head. With the continuous progress of science and technology, higher frequencies of electromagnetic radiation are applied. However, the higher the frequency, a greater part of the radiation is absorbed by the human skin and a lesser part reaches the internal organs.

Most of the research related to the exposure to electromagnetic radiation from Wi-Fi has been carried out in adverse conditions (higher power values – peak values instead of average values, distances shorter than real). However, the results showed that the measured levels did not exceed the limit values contained in various legal regulations around the world. The biggest difficulty in interpreting and drawing conclusions from animal studies is to estimate the possible effects on human health using their results. For this reason,

many of the studies conducted so far are considered ambiguous as they do not give a definitive answer about the possible negative impact on human health. Due to the continuous development of wireless networks, there is a need to conduct further research on this topic.

Wi-Fi devices (including routers) came into existence only about 15–20 years ago. However, they have become ubiquitous in the modern world. Due to their high popularity among children and young people who are known to have a longer period of exposure (than adults) to radiofrequency electromagnetic radiation, these populations should be subjected to careful analysis. Despite the lack of unambiguous evidence for the carcinogenic effect of radiofrequency electromagnetic radiation, in recent years, several harmful environmental factors are identified to be detrimental to human health. Therefore, it is necessary to analyze whether the overlap of some of these harmful factors can cause deterioration of health and increase the risk of morbidity (e.g. cancer).

Conflict of interest

The authors declare no conflict of interests.

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ORIGINAL ARTICLE

FREQUENCY OF CONSUMING SELECTED PRODUCT GROUPS AMONG POLISH AND SPANISH PHYSICAL EDUCATION STUDENTS

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ABSTRACT

Background. Nutritional behaviours are determined by numerous individual and environmental factors.
Objective. The aim of the study was to analyse the frequency of consuming selected food product groups (with potentially beneficial and potentially detrimental effects on health) among physical education students depending on gender and home country.
Materials and methods. The study was conducted among 219 Polish and 280 Spanish physical education students, using a standardised questionnaire for obtaining information on views and eating habits for people aged 16-65 (Kom-PAN). In the statistical analysis, the *Mann-Whitney* U test and t-test with independent estimation of variance were incorporated, the significance level at $\alpha = 0.05$.
Results. Among Polish and Spanish students, significant differences in the frequency of consuming certain product groups depending on home country were noted, with Polish students significantly more frequently consuming recommended products (fruit, vegetables, whole-grains, milk, fromage frais, and poultry), but also those non-recommended (purified cereals, cheeses with high fat content, butter, fried foods, sweets and alcoholic beverages). Spanish students significantly more often consumed recommended meals including legume seeds and sea fish, but also non-recommended products (red meat, fast food, sweets and energy drinks). Moreover, significant differences in the frequency of consuming selected product groups depending on sex were observed, with an indication of the tendency for less rational food choices among male students than female students, especially regarding the consumption of: high-milled cereal products, processed and red meat, fatty cheeses, fried foods, lard, sweets and energy drinks. On the other hand, women consumed sweets significantly more often than men, and less often legume seeds and fish.
Conclusions. A limited prevalence of rational dietary choices among Polish and Spanish physical education students and their diversity depending on gender and home country have been demonstrated.

Key words: *frequency of food consumption, conditioning of food choices, physical education students*

STRESZCZENIE

Wprowadzenie. Zachowania żywieniowe są determinowane licznymi czynnikami osobniczymi i środowiskowymi.
Cel. Celem badań była analiza częstości konsumpcji wybranych grup produktów (o potencjalnie korzystnym i potencjalnie niekorzystnym wpływie na zdrowie) wśród studentów wychowania fizycznego w zależności od płci oraz kraju pochodzenia.
Materiał i metody. Badania przeprowadzono wśród 219 polskich i 280 hiszpańskich studentów wychowania fizycznego, z zastosowaniem standaryzowanego kwestionariusza do poglądów i zwyczajów żywieniowych dla osób w wieku od 16 do 65 lat (Kom-PAN). W analizie statystycznej zastosowano test U *Manna-Whitneya* i test t z niezależną estymacją wariancji, na poziomie istotności α=0,05.
Wyniki. Wśród polskich i hiszpańskich studentów wykazano istotne zróżnicowanie częstości konsumpcji niektórych grup produktów w zależności od kraju, przy czym polscy studenci istotnie częściej spożywali produkty rekomendowane (owoce, warzywa, zbożowe pełnoziarniste, mleko, sery twarogowe, mięso drobiowe), ale także niezalecane (zbożowe oczyszczone, tłuste sery, masło, potrawy smażone, słodycze, napoje alkoholowe). Hiszpańscy studenci istotnie częściej spożywali zalecane potrawy z nasion strączkowych i ryb morskich, ale także niezalecane (mięso czerwone, fast food, napoje słodkie i energetyzujące). Wykazano ponadto istotne zróżnicowanie częstości konsumpcji niektórych grup produktów w zależności od płci, ze wskazaniem na tendencję do mniej racjonalnych wyborów żywieniowych studentów niż studentek, szczególnie w zakresie konsumpcji: produktów zbożowych z wysokiego przemiału, przetworów mięsnych,

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mięsa czerwonego, tłustych serów, potraw smażonych, smalcu oraz napojów słodkich i energetyzujących. Kobiety natomiast istotnie częściej niż mężczyźni spożywały słodycze, a rzadziej nasiona strączkowe i ryby.
Wnioski. Wykazano ograniczone rozpowszechnienie racjonalnych wyborów żywieniowych wśród polskich i hiszpańskich studentów wychowania fizycznego oraz ich zróżnicowanie w zależności od płci i kraju pochodzenia.

Słowa kluczowe: *częstość spożycia żywności, uwarunkowania wyborów żywieniowych, studenci wychowania fizycznego*

INTRODUCTION

The correct nutritional model, taking age, gender, level of physical activity and health into account, is one of the important factors contributing to maintaining health potential and prevention of chronic diseases, including obesity, type 2 diabetes, cardiovascular diseases and some cancers. A rational diet should be diverse and balanced, including products with high nutritional value (fruit and vegetables, whole-grains, low-fat dairy products, fish and vegetable oils as well as nuts), with restrictions on animal fats, red and processed meat products, purified cereal products as well as confectionery and fast food products [20]. The rational nutrition model, as an important health determinant, in many countries, including Poland and Spain, has been included in the canon of basic aspects of a healthy lifestyle (Polish Pyramid of Physical Activity and Healthy Nutrition from 2016 and the Spanish Strategy for Nutrition, Physical Activity and Prevention of Obesity from 2008) [16].

A group with special nutritional needs includes people characterised by increased physical activity – i.e. athletes and physical education students who demonstrate a significantly higher level of physical activity than students of other university faculties [19, 21, 40]. Interest in this population group is also associated with the role of physical education students (future teachers) in the implementation of school health education [22]. In various studies, it has been confirmed that the intensification of teachers' pro-health attitudes increases the effectiveness of promoting a healthy lifestyle, including the implementation of a rational diet [3]. Nutritional behaviours are determined by numerous individual and environmental factors, including cultural ones [33]. Within the context of increased physical activity among P.E. students and their preparation for the future role of health educators of children and adolescents, assuming the geographical and cultural diversity of eating behaviours, research has been undertaken regarding selected determinants of the nutritional choices among physical education students with a profile focused on physical culture and health.

The aim of the study was to analyse the frequency of consuming selected product groups (with potentially beneficial and detrimental effects on health) among physical education students depending on gender and home country.

MATERIALS AND METHODS

Research was conducted between the years 2017-2019 among 499 2nd- and 3rd-year physical education students (B. A. studies), aged 18 to 35 (21.65±2.42). The study included 219 Polish students (University of Physical Education in Kraków, n=135; and University of Physical Education in Wrocław, n=84) and 280 Spanish students (University of Murcia, Faculty of Sports Sciences, n=127; and University in Granada, Faculty of Sports Sciences, n=153).

In the study, a standardised questionnaire was used to evaluate views and habits related to nutrition. This survey is dedicated to subjects aged 16 to 65 (KomPAN), and was developed by the Human Nutrition Science Committee of the Polish Academy of Sciences, in the part concerning the frequency of consuming selected groups of food products. The frequency of consuming products was assessed on an ordinal scale: never (1), 1-3 times a month (2), once a week (3), several times a week (4), once a day (5), several times a day (6) [13]. The general index of rational and incorrect nutrition choices was calculated as the total value of the average frequency of consuming individual products from a particular category. The group of products with potentially beneficial effects on health (n=10) includes: wholemeal bread; buckwheat, oatmeal, whole-grain pasta and other coarse grains; milk; beverages from fermented dairy products; fromage frais; dishes from the so-called white meat; fish; legume seed dishes; fruits and vegetables. The group of products with potentially adverse effects on health (n=14) are: white bread; white rice, plain pasta or fine-grained groats; fast food; fried foods; butter; lard; cheese; cold-cuts, sausages or hotdogs; red meat dishes; sweets; canned meat; sweetened carbonated or non-carbonated beverages; energy drinks and alcoholic beverages [13].

The IBM SPSS 21 program and *Newsom's* macro J.T. were used for statistical calculations. Basic statistics of the studied variables were calculated (mean, standard deviation, median, upper and lower quartiles). Differentiation in the frequency of product consumption (with potentially beneficial and potentially adverse effects on health) depending on gender and country of origin of students was assessed using the *Mann-Whitney* U test while the *t*-test with independent estimation of variance was applied to

compare the general index of rational and incorrect dietary choices. The adopted level of significance was $\alpha = 0.05$.

RESULTS

Among the products with potentially beneficial effects on health, the examined group of physical education students most often, usually several times a week (Me=4), consumed: fruit and vegetables, milk, fermented dairy products and poultry, while among products with potentially detrimental influence on health, the following were noted: white bread, other high-milled cereal products, cold-cuts, sausages and hotdogs (Table 1).

Statistical analysis of the results depending on home country showed that among products with a potentially beneficial effect on health, Polish students, significantly more often, consumed: fruit and vegetables, wholemeal bread ($p<0.001$), other low-milled cereal products ($p = 0.001$), milk ($p<0.05$), fromage frais ($p<0.001$) and poultry meat ($p<0.001$), while the frequency of consuming legume seeds and fish was significantly higher among the Spanish students ($p<0.001$) (Table 2).

Among products with potentially adverse effects on health, Polish physical education students consumed the following significantly more often: white bread ($p<0.01$), other high-milled cereal products ($p<0.05$), hard, blue and processed soft cheeses ($p<0.001$), fried foods ($p<0.001$), butter ($p<0.001$), sweets and confectionery ($p<0.001$) as well as alcoholic beverages ($p=0.001$), while Spanish students included the following products in the diets more frequently: red meat, fast food ($p<0.001$), sweetened carbonated beverages and energy drinks ($p<0.05$) (Table 3).

Statistical analysis of the results depending on sex showed that among the products with potentially beneficial effects on health, the surveyed men, significantly more often than women, consumed: legume seeds and fish ($p<0.05$), and among the products with potentially adverse effects on health: white bread ($p<0.05$), other high-milled cereal products ($p<0.01$), cold-cuts, sausages and hotdogs ($p<0.01$), red meat

Table 1. Frequency of consuming potentially beneficial and detrimental products among Polish and Spanish physical education students (n=499) (descriptive statistics)

Categories of food products M		Poland and Spain				
		M	Me	Q25	Q75	SD
Potentially beneficial influence on health	Fruit	4.02	4.00	3.00	5.00	1.49
	Vegetables	4.02	4.00	3.00	5.00	1.44
	Wholemeal bread	3.20	3.00	2.00	4.00	1.41
	Coarse-grained barley, oatmeal, whole-grain pasta	3.25	3.00	2.00	4.00	1.19
	Legume seeds	3.06	3.00	2.00	4.00	1.16
	Milk	3.55	4.00	2.00	5.00	1.66
	Fermented dairy products	3.39	4.00	2.00	4.00	1.34
	Fromage frais	2.89	3.00	2.00	4.00	1.15
	Poultry	3.68	4.00	3.00	4.00	1.09
	Fish	3.00	3.00	2.00	4.00	1.02
Potentially detrimental influence on health	White bread	3.32	4.00	2.00	4.00	1.54
	White rice, finely-ground barley	3.60	4.00	3.00	4.00	1.08
	Hard, blue, soft cheeses, processed cheese spreads	3.00	3.00	2.00	4.00	1.16
	Cold-cuts, sausages, hotdogs	3.54	4.00	3.00	4.00	1.21
	Red meat	3.12	3.00	2.00	4.00	1.13
	Canned meat	2.26	2.00	2.00	3.00	1.04
	Fried meals (farinaceous and meat)	3.27	3.00	2.00	4.00	1.11
	Butter	2.90	2.00	2.00	4.00	1.38
	Lard	1.94	2.00	1.00	2.00	1.05
	Fast food	2.49	2.00	2.00	3.00	0.93
	Sweets, confectionary	3.09	3.00	2.00	4.00	1.22
	Sweetened carbonated and non-carbonated beverages	2.57	2.00	2.00	3.00	1.15
	Energy drinks	2.33	2.00	2.00	3.00	1.14
	Alcoholic beverages	2.61	2.00	2.00	3.00	1.03

M- arithmetic mean; Me- median; Q25- lower quartile; Q75- upper quartile; SD- standard deviation

Table 2. Frequency of consuming products with potentially beneficial influence on health among Polish (n=219) and Spanish (n=280) physical education students depending on home country (descriptive statistics and *Mann-Whitney* U test)

Food products	Spain					Poland					*p*
	M	Me	Q25	Q75	SD	M	Me	Q25	Q75	SD	
Fruit	3.71	4.00	2.00	5.00	1.69	4.41	4.00	4.00	5.00	1.05	<0.001
Vegetables	3.58	4.00	2.00	5.00	1.53	4.58	4.00	4.00	5.00	1.08	<0.001
Wholemeal bread	2.95	2.00	2.00	4.00	1.43	3.52	4.00	2.00	4.00	1.33	<0.001
Coarse-grained barley, oatmeal, whole-grain pasta	3.10	3.00	2.00	4.00	1.24	3.44	4.00	3.00	4.00	1.10	0.001
Legume seeds	3.33	3.00	3.00	4.00	1.20	2.70	3.00	2.00	3.00	1.00	<0.001
Milk	3.34	4.00	1.00	5.00	1.89	3.81	4.00	3.00	5.00	1.25	0.021
Fermented dairy products	3.34	4.00	2.00	4.00	1.47	3.45	4.00	3.00	4.00	1.15	0.438
Fromage frais	2.68	2.00	2.00	3.50	1.11	3.16	3.00	2.00	4.00	1.15	<0.001
Poultry	3.50	4.00	3.00	4.00	1.23	3.91	4.00	4.00	4.00	0.82	<0.001
Fish	3.17	3.00	2.50	4.00	1.08	2.79	3.00	2.00	3.00	0.88	<0.001

M - arithmetic mean; Me - median; Q25 - lower quartile; Q75 - upper quartile; SD - standard deviation; *p* - significance

Table 3. Frequency of consuming products with potentially detrimental influence on health among Polish (n=219) and Spanish (n=280) physical education students depending on home country (descriptive statistics and *Mann-Whitney* U test)

Food products	Spain					Poland					*p*
	M	Me	Q25	Q75	SD	M	Me	Q25	Q75	SD	
White bread	3.14	3.00	2.00	4.00	1.61	3.54	4.00	2.00	4.00	1.41	0.004
White rice, finely-ground barley	3.51	4.00	3.00	4.00	1.11	3.72	4.00	3.00	4.00	1.04	0.026
Hard and blue, soft cheeses	2.74	2.00	2.00	4.00	1.14	3.33	4.00	2.00	4.00	1.11	<0.001
Cold-cuts, sausages, hotdogs	3.45	4.00	3.00	4.00	1.27	3.65	4.00	3.00	4.00	1.12	0.130
Red meat	3.28	3.00	2.00	4.00	1.17	2.90	3.00	2.00	4.00	1.05	<0.001
Canned meat	2.60	2.0	2.0	3.0	1.01	1.83	2.0	1.0	2.0	0.91	<0.001
Fried meals (farinaceous and meat)	2.95	3.00	2.00	4.00	1.02	3.68	4.00	3.00	4.00	1.10	<0.001
Butter	2.54	2.00	2.00	3.00	1.05	3.36	4.00	2.00	5.00	1.60	<0.001
Lard	2.25	2.00	2.00	2.00	1.03	1.53	1.00	1.00	2.00	0.93	<0.001
Fast food	2.64	2.00	2.00	3.00	0.98	2.31	2.00	2.00	3.00	0.82	<0.001
Sweets, confectionary	2.75	3.00	2.00	4.00	1.11	3.53	4.00	3.00	4.00	1.20	<0.001
Sweetened carbonated and non-carbonated beverages	2.65	2.00	2.00	3.00	1.07	2.47	2.00	1.00	3.00	1.25	0.036
Energy drinks	2.41	2.00	2.00	3.00	1.08	2.22	2.00	1.00	3.00	1.20	0.012
Alcoholic beverages	2.50	2.00	2.00	3.00	1.02	2.75	3.00	2.00	3.00	1.03	0.001

M - arithmetic mean; Me - median; Q25 - lower quartile; Q75 - upper quartile; SD - standard deviation; *p* - significance

($p<0.001$), canned meat ($p<0.01$), hard, blue and processed soft cheeses ($p<0.001$), fried foods ($p<0.05$), lard ($p<0.01$), and sweetened carbonated beverages as well as energy drinks ($p<0.001$), while women more often reached for sweets and confectionery ($p=0.001$) (Tables 4 and 5).

Statistical analysis showed that Polish students were characterised by a significantly higher level of rational food choices ($p<0.001$), but at the same time, they consumed products with potentially adverse effects on health more often than students from Spain ($p<0.05$) (Table 6).

DISCUSSION

The discussed research showed a limited prevalence of rational food choices among Polish and Spanish physical education students and their differentiation depending on gender and home country.

The indicated nutritional mistakes of all the surveyed students were particularly associated with the low frequency of consuming selected the products recommended for one's daily diet (fruit and vegetables, whole-grain cereals, fermented dairy products), as well as the relatively frequent consumption of meat

Table 4. Frequency of consuming products with potentially beneficial influence on health among female (n=189) and male (n=309) physical education students from Poland and Spain (descriptive statistics and *Mann-Whitney* U test)

Food products	Men					Women					*p*
	M	Me	Q25	Q75	SD	M	Me	Q25	Q75	SD	
Fruit	3.99	4.00	3.00	5.00	1.47	4.07	4.00	3.00	5.00	1.51	0.398
Vegetables	3.99	4.00	3.00	5.00	1.37	4.07	4.00	4.00	5.00	1.53	0.243
Wholemeal bread	3.11	3.00	2.00	4.00	1.37	3.35	4.00	2.00	4.00	1.47	0.090
Coarse-grained barley, oatmeal, whole-grain pasta	3.22	3.00	2.00	4.00	1.20	3.31	3.00	2.00	4.00	1.17	0.432
Legume seeds	3.14	3.00	2.00	4.00	1.15	2.93	3.00	2.00	4.00	1.16	0.048
Milk	3.55	4.00	2.00	5.00	1.65	3.54	4.00	2.00	5.00	1.67	0.973
Fermented dairy products	3.34	4.00	2.00	4.00	1.33	3.47	4.00	3.00	4.00	1.35	0.260
Fromage frais	2.86	3.00	2.00	4.00	1.14	2.94	3.00	2.00	4.00	1.16	0.441
Poultry	3.73	4.00	3.00	4.00	1.12	3.59	4.00	3.00	4.00	1.04	0.139
Fish	3.08	3.00	2.00	4.00	1.01	2.88	3.00	2.00	4.00	1.02	0.020

M- arithmetic mean; Me- median; Q25- lower quartile; Q75- upper quartile; SD- standard deviation; *p* - significance

Table 5. Frequency of consuming products with potentially detrimental influence on health among female (n=189) and male (n=309) physical education students from Poland and Spain (descriptive statistics and *Mann-Whitney* U test)

Food products	Men					Women					*p*
	M	Me	Q25	Q75	SD	M	Me	Q25	Q75	SD	
White bread	3.42	4.00	2.00	5.00	1.56	3.15	3.00	2.00	4.00	1.50	0.037
White rice, finely-ground barley	3.72	4.00	3.00	4.00	1.07	3.41	4.00	3.00	4.00	1.09	0.003
Hard and blue, soft cheeses	3.00	3.00	2.00	4.00	1.17	2.99	3.00	2.00	4.00	1.14	0.967
Cold-cuts, sausages, hotdogs	3.66	4.00	3.00	4.00	1.22	3.34	4.00	3.00	4.00	1.16	0.002
Red meat	3.35	3.00	3.00	4.00	1.09	2.73	3.00	2.00	4.00	1.10	<0.001
Canned meat	2.34	2.0	2.0	3.0	1.03	2.14	2.0	1.0	3.0	1.04	0.017
Fried meals (farinaceous and meat)	3.36	4.00	2.00	4.00	1.11	3.13	3.00	2.00	4.00	1.11	0.041
Butter	2.85	2.00	2.00	4.00	1.29	2.98	3.00	2.00	4.00	1.51	0.548
Lard	2.00	2.00	1.00	2.00	1.02	1.81	2.00	1.00	2.00	1.05	0.005
Fast food	2.54	2.00	2.00	3.00	0.92	2.41	2.00	2.00	3.00	0.89	0.069
Sweets, confectionary	2.95	3.00	2.00	4.00	1.16	3.33	3.00	2.00	4.00	1.27	0.001
Sweetened carbonated and non-carbonated beverages	2.77	3.00	2.00	4.00	1.16	2.24	2.00	1.00	3.00	1.06	<0.001
Energy drinks	2.46	2.00	2.00	3.00	1.11	2.11	2.00	1.00	2.00	1.15	<0.001
Alcoholic beverages	2.64	2.00	2.00	3.00	1.08	2.55	2.00	2.00	3.00	0.94	0.652

M - arithmetic mean; Me - median; Q25- lower quartile; Q75 - upper quartile; SD - standard deviation; *p* - significance

Table 6. Comparison of the level of rational and incorrect nutritional choices among physical education students from Poland (n=219) and Spain (n=280) (*t*-test)

Nutritional choices	M Poland	M Spain	t	df	*p*	SD Poland	SD Spain	Quotient F of variance	*p* of variance
Rational	35.76	32.71	4.956*	489.7*	<0.001*	5.60	8.12	2.10	<0.001
Incorrect	40.84	39.41	2.19	497	0.029	7.47	7.11	1.10	0.441

* - *t*-test with independent group estimation; df - degree of freedom; M - arithmetic mean; SD - standard deviation; *p* - significance

products not recommended for a healthy diet (cold-cuts, sausages and hotdogs). Insufficient consumption of fruit and vegetables may reduce the nutritional content and health values of diet, through a lower supply of dietary fibres (including lipid-soluble fractions) and mineral salts (including potassium and magnesium), and reducing the diet's antioxidant potential (an important factor in preventing, among others, cardiovascular and cancer diseases). Low consumption of whole-grain cereal products may reduce the supply of fibres (especially insoluble fractions) and B vitamins, catalysing, among others, metabolic processes [20]. In various studies, it has been confirmed that a diet rich in fresh fruit and vegetables, whole-grain cereals, legumes as well as sprouts and seeds, is an effective way to cover the body's antioxidant needs, including those for people with increased physical activity [11]. Low consumption of fermented dairy products may reduce the dietary intake of calcium (an early prevention factor for osteoporosis) and probiotic bacteria that enrich the intestinal microflora (and eubiosis state), with numerous pro-health values [4]. Relatively frequent (according to the median, several times a week) consumption of processed meat products (cold-cuts, sausages and hotdogs) may, in turn, increase the supply of saturated fatty acids and cholesterol, which in excess, have hyperlipidemic and hypertensive properties. The share of excess processed meat products in the etiology of cancer should also be emphasised [20].

In other research, nutritional mistakes among academic youth, including physical education students, both in Poland [28, 32, 36] and in other countries, e.g. in Spain and Romania [6] and Chile [39], have also been confirmed. Errors in nutrition have also been described among students of other university faculties, including those Polish [14, 18, 24, 26, 38], Spanish [5, 17, 29, 42], Chinese [15], Brazilian [2] and American [41] , Filipino [1], furthermore, from Zamora [31] and Bahrain [27]. Low vegetable consumption among students was also confirmed in a systematic review of works from 2009-2018 [34]. For example, the results of the author's research correspond to the tendencies described among 1st- and 2nd-year physical education students from Biała Podlaska, which showed a low level of both positive and unfavourable food choices, meaning that students did not choose products with a potentially beneficial effect for their health often enough, but at the same time, they restricted the consumption of unhealthy products, however, more beneficial choices were found among graduating students than those beginning their studies (more frequent consumption of fruit and vegetables vs. white bread, butter, cold-cuts and sweets) [32]. In another study conducted among students from the Warsaw University of Physical Education, the most common eating errors, in addition to too few meals and irregular consumption, were insufficient consumption of vegetables, fruit, wholemeal bread, milk and dairy products, and too frequent consumption of sweets, carbonated and alcoholic beverages as well as instant products and fast food [28], which also partly corresponds to the results of the author's research. Similarly, Chilean physical education students reported low consumption of vegetables and dairy products (men) and excessive consumption of sweet snacks (women) [39].

The discussed research conducted among Polish and Spanish physical education students also showed statistically significant differences in the frequency of consuming some product groups depending on the subjects' home country. Among Polish students, there was a tendency towards more frequent consumption of some products recommended in a healthy diet (fruit and vegetables, whole-grain cereal products, milk and fromage frais as well as poultry), but also more frequent consumption of some non-recommended products (purified cereal products, fatty, hard, blue and processes cheeses, butter, fried foods, sweets and confectionery as well as alcoholic beverages). At the same time, Spanish students significantly more often consumed the recommended legume seeds and sea fish, but also non-recommended products (red meat, fast food products as well as sweetened carbonated and non-carbonated beverages and energy drinks). Thus, nutrition errors were confirmed both among Polish and Spanish physical education students. It is impossible to clearly determine the direction and degree regarding the regularity of students' food choices depending on their country of origin, as Polish students were characterised by a significantly higher level of rational food choices, but at the same time, they consumed products with potentially adverse effects to health more often than students from Spain. Less rational nutritional behaviours of the Polish students may have reduced the supply of fibre while increasing the supply of saturated fatty acids. Spanish students could have also increased the risk of oversupply of saturated fatty acids, trans isomers and simple sugars that lower their health potential.

In another study, the aim of which was to compare Polish and Spanish (Gdańsk vs. Murcia) health behaviours of physical education students, the diversity of eating behaviours depending on home country was also confirmed. Students from the University of Murcia consumed seafood and dairy products more often, while sweetened carbonated and alcoholic beverages less often. In turn, students from Gdańsk consumed more vegetables. Regardless of the geographical factor, however, the authors of the study suggested the need to rationalise the diets (and other aspects of lifestyle) of students in a way that promotes

early prevention of chronic diseases, including obesity and diabetes. At the same time, they suggested that the found differences may result from differences in culture, traditions and climatic factors (Northern vs. Southern Europe), but also from the implementation of an effective health policy since 2000 [25].

The results of research among Spanish medical students, which showed an excessive supply of saturated fatty acids (SFA) and a low supply of monounsaturated fatty acids (MUFA), also correspond to the relatively more frequent consumption of red meat, fast food products and vitamin D as well as E intake, suggesting deviations from the Mediterranean nutrition model [5]. Similarly, assessment of the diet of students from the University of Granada showed that eating habits, which deteriorate as the academic year progresses, are characterised by high consumption of protein products and fats, rich in saturated acids, with low consumption of products rich in dietary fibres [29]. There is also a corresponding tendency towards more frequent consumption of fruit and vegetables by Polish physical education students, which confirmed that the intake of flavonoid was more than twice as high among Polish than Spanish students (801 vs. 297 mg/day). The most common sources of flavonoids were: vegetables, fruits (oranges), drinks (tea, orange juice) and spices (dried parsley, oregano). The demonstrated supply of flavonoids, in comparison to other population studies, was assessed as sufficient in both groups of students [23].

In other comparative studies on the geographical context concerning the implementation of the Mediterranean diet assumptions among physical education students from universities in Spain and Romania, it was shown that Spanish students followed the principles of the Mediterranean diet more than their Romanian peers [6]. Research in Catalonia also confirmed that students' eating habits deviate from the recommendations of the Mediterranean diet, because a high percentage of students followed a nutritional pattern characterised by low consumption of fruit (73.9%), vegetables (39.8%), wholemeal cereal products (92.6%) and excessive consumption of red meat (84%). These types of nutritional trends have been increasing in recent years in Spain and other industrialised countries [35]. A very small scale of implementing the recommendations of the Mediterranean diet in terms of limiting the consumption of fresh and processed meat (only 3.8%) was also described in another group of students from eleven Spanish universities (n=9,862) [30]. Nutritional blunders, particularly related to insufficient consumption of dairy products as well as fruit and vegetables, were also confirmed in other studies undertaken among students of other university centres in Poland and abroad [38]. Also, other research on the determinants of the nutritional behaviour of students from four European countries (Germany, Denmark, Poland and Bulgaria) has shown that food consumption patterns vary from country to country. Bulgarian students were distinguished by frequent consumption of sweets, cakes and fast food products, and Polish students by low consumption of vegetables and fruit [9].

The discussed research among Polish and Spanish physical education students also indicated statistically significant differences in the frequency of consuming some product groups depending on gender, with an indication of the tendency for less rational food choices of male students than female students, especially with regard to the consumption of high-milled cereal products, cold-cuts, sausages and hotdogs, red and canned meat, hard, blue and processed cheeses, fried foods, lard, sweetened carbonated beverages and energy drinks. Different trends concerned the more frequent consumption of sweets and confectionery products, and the less frequent consumption of legume seeds and fish by women. Less rational dietary behaviours among males, physical education students, increased the risk of a lower supply of fibres and a higher supply of saturated fatty acids. Less rational nutritional behaviours among women, students of physical education, may have increased the supply of simple sugars, while reducing the supply of polyunsaturated acids (PUFA) *omega*-3.

The demonstrated tendency towards more rational dietary choices among women than men is confirmed by the results of research among students of seven universities in Chile [8]. Also, research conducted among students of the Medical University of Wrocław demonstrated numerous nutritional mistakes according to gender, with a higher percentage of men eating less than the recommended 3 meals a day, less frequently consuming 5 portions of fruit and vegetables daily or choosing low-fat dairy products not as often as they should, while including products with high GI and meat dishes more frequently than women. In addition, they used large amounts of salt or sugar to sweeten drinks more often [18]. In another study, variation in eating behaviours has also been shown depending on gender, including the greater incidence of fruit and vegetable consumption among women [10]. More rational dietary choices among women than men were also described in other population groups, including athletes (more frequent consumption of raw vegetables and dairy products with reduced fat content) [12]. Also, other research on the conditioning nutritional behaviours of students from four European countries (Germany, Denmark, Poland and Bulgaria) showed diversity of eating habits according to sex, while in all countries except for Bulgaria, men declared significantly more frequent consumption of snacks than women [9].

In the research, it is generally confirmed that students constitute a population group exposed to making nutritional mistakes, and their diet largely depends on living conditions/factors, in particular, income, knowledge about nutrition, education, housing conditions and professional work [37]. In the author's research, the predictive significance of the geographical context (country of origin) was also highlighted and the gender-specific diversity was confirmed. The nutritional profile of students indicates the need for nutritional education and the promotion of a healthy nutritional model in order to prevent the development of chronic diseases at later stages of ontogenesis, including obesity, cardiovascular as well as other diseases, which are also emphasized by other authors [7]. Nutritional education of physical education students is also important within the context of their future professional work, because as physical education teachers, they will be included in the implementation of school health education, the effectiveness of which may depend, among others, on the intensification of their individual pro-health attitudes, also towards rational nutrition [3].

CONCLUSIONS

Nutritional mistakes have been demonstrated among Polish and Spanish physical education students, particularly due to insufficient consumption of products with potentially beneficial effects on health (fruit, vegetables, whole-grain cereals, fermented milk products), as well as relatively frequent consumption of non-recommended meat products.

Among the Polish and Spanish students, there was significant differentiation in the frequency of consuming selected product groups according to country of origin, with Polish students consuming the recommended products more often (fruit and vegetables, whole-grains, milk, fromage frais and poultry), but at the same time, non-recommended products as well (purified cereals, fatty cheeses, butter, fried products, sweets and confectionery products as well as alcoholic beverages).

Among the Polish and Spanish physical education students, significant differentiation in the frequency of consuming selected product groups depending on gender was noted, with an indication of less rational dietary choices among men than women, associated with more frequent consumption of: purified cereal products, red meat, processed meat, fatty cheeses, fried foods, lard, and sweetened as well as energy drinks.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

DIETARY PATTERNS OF HEALTH SCIENCES STUDENTS IN REGARDING TO PHYSICAL ACTIVITY LEVELS AND SOMATIC INDICATORS OF NUTRITIONAL STATUS

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ABSTRACT

Background. In extensive world literature, there is no relationship has been noted between dietary patterns defined a posteriori ("data driven") and metabolic risk indicators, especially among medical and health science students.

Objective. The aim of the study was recognition of dietary patterns defined a posteriori ("data driven") among students in discipline of Health Sciences in regarding to their levels of physical activity, as well as selected nutritional status indicators.

Material and methods. The studied population group consisted of 609 respondents aged 19 - 30. Data was collected on: the frequency of consumption of 16 groups of food products, levels of physical activity, body weight and hight. Based on the data, the somatic indicators were calculated: BMI (Body Mass Index), WC (Waist Circumference) and WHtR (Waist to Hight Ratio). Four dietary patterns were identified using factor analysis, i.e. including two health-promoting factors, "vegetables and fruits" and "milk, fermented milk drinks and cottage cheese", and two non-health-promoting factors, "carbonated drinks, energy drinks, alcohol and canned food" and "fast food and confectionery products". The relationship between levels of physical activity, somatic indicators and dietary patterns was tested using the Chi-square test.

Results. Respondents with a high level of physical activity were statistically significantly more often characterized by high intensity of all health-promoting dietary patterns and low intensity of one unhealth-promoting dietary patterns such as "fast food and confectionery products". There was no statistically significant differentiation between underweight and normal body weight according to the BMI criteria or differentiation according to the severity of separate dietary patterns, but such a difference was found between overweight according to the BMI criteria and obesity according to the BMI and WC criteria. There was often a statistically significant relationship between overweight and obesity according to the BMI and visceral obesity with a high risk of metabolic complications according to the WC index, and a high intensity of unhealthy dietary patterns was more often observed than a high intensity of health-promoting dietary patterns.

Conclusion. Effective programs and methods of nutritional education and motivation to change health behaviours should be implemented among students of Health Sciences, especially those who are overweight or obese, or have lower activity level values.

Key words: *dietary patterns, physical activity, somatic indicators, students*

STRESZCZENIE

Wprowadzenie. W literaturze światowej nie dostrzega się badań oceniających związek wzorów żywieniowych zdefiniowanych a 'posteriori („data-driven") ze wskaźnikami ryzyka chorób metabolicznych szczególnie wśród studentów medycyny i nauk o zdrowiu.

Cel badań. Celem badania było rozpoznanie wzorów żywieniowych zdefiniowanych a posteriori ("data driven") wśród studentów Nauk o Zdrowiu w odniesieniu do ich poziomów aktywności fizycznej i wybranych wskaźników stanu odżywienia.

Materiał i metody. Badaną grupę populacyjną stanowiło 609 respondentów w wieku 19-30 lat. Zebrane dane dotyczyły: spożycia 16 grup produktów żywnościowych, poziomów aktywności fizycznej, masy ciała i wzrostu. Na podstawie zebranych danych obliczono wskaźniki somatyczne: BMI (Body Mass Index), WC (Waist Circumference) and WHtR (Waist to Hight Ratio). Za pomocą analizy czynnikowej zidentyfikowano 4 wzory żywieniowe, obejmujące dwa czynniki sprzyjające zdrowiu, tj.: "owoce i warzywa" oraz "mleko, mleczne napoje fermentowane i twarogi" i dwa czynniki nie sprzyjające zdrowiu, tj.: "napoje gazowane, energetyczne, alkohole i konserwy" oraz "fast food i wyroby cukiernicze". Związek między poziomem aktywności fizycznej, wskaźnikami somatycznymi i wzorami żywieniowymi badano z użyciem testu Chi-kwadrat.

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Wyniki. Respondenci o wysokim poziomie aktywności fizycznej statystycznie istotnie częściej charakteryzowali się wysoką intensywnością wszystkich wzorów żywieniowych sprzyjających zdrowiu oraz niską intensywnością wzoru żywieniowego nie sprzyjającego zdrowiu tj.: "fast food i wyroby cukiernicze". Nie stwierdzono statystycznie istotnego zróżnicowania między niedowagą i prawidłową masą ciała według kryteriów wskaźnika BMI a nasileniem wzorów żywieniowych, ale stwierdzono takie zróżnicowanie w przypadku nadwagi według kryteriów wskaźnika BMI oraz otyłością według kryteriów wskaźników BMI i WC. Częściej obserwowano statystycznie istotną zależność między nadwagą i otyłością według kryteriów wskaźnika BMI oraz otyłością brzuszną z wysokim ryzykiem powikłań metabolicznych według wskaźnika WC i wysoką intensywnością wzorów żywieniowych nie sprzyjających zdrowiu, niż wysoką intensywnością wzorów żywieniowych sprzyjających zdrowiu.
Wnioski. Skuteczne programy i metody edukacji żywieniowej oraz metod zmiany zachowań żywieniowych powinny być wdrożone wśród studentów Nauk o Zdrowiu, szczególnie wśród tych z nadwagą i otyłością lub niskim poziomem aktywności fizycznej.

Słowa kluczowe: *wzory żywieniowe, aktywność fizyczna, wskaźniki somatyczne, studenci*

INTRODUCTION

Dietary patterns represent a set of many characteristics, common features describing the diet of different people or population groups. These features can determine the type and amount of nutrients, food or food groups, as well as the frequency of having meals or preferring or avoiding specific foods [11]. Analysis of dietary patterns is considered a holistic approach to assessing the relationship between diet and the risk of chronic disease. Instead of looking for the effects of individual nutrients or food, dietary patterns evaluate the impact of an entire diet and its complexity [11, 15, 29].

Studies on dietary patterns are carried out mainly to assess the relationship between them and the occurrence of chronic diseases [11]. In the world literature, there is a relationship between health-promoting dietary patterns defined *a priori* („hypothesis driven"), e.g. HEI (*Healthy Eating Index*), AHEI-2010 (*Alternative Healthy Eating Index 2010*) HDI (*Healthy Diet Index*), DQI (*Diet Quality Index*), AMED (*the Alternative Mediterranean Diet* score), or DASH (*the Dietary Approach to Stop Hypertension*), and risk indicators of metabolic diseases, e.g. somatic [23], biochemical [11] or physical activity [16, 18]. Studies assessing the relationship of dietary patterns defined *a posteriori* („data driven") with metabolic disease risk indicators, especially among medical and health sciences students, are not seen.

From the perspective of the above data, the purpose of the work was to identify dietary patterns selected on the basis of the dataset characteristics ("data driven") for students of Health Sciences in regarding to their levels of physical activity and selected somatic indicators of the nutritional status of these students.

MATERIAL AND METHODS

This cross-sectional study was carried out between October and December 2018 among full-time students. The studied population group consisted of 609 respondents aged 19−30. Students represented two universities. The surveyed students were educated in six fields in the discipline of Health Sciences, i.e. cosmetology – 233 people, dietetics – 165 people, physiotherapy – 96 people, emergency medical services – 57 people, nursing – 49 people and electroradiology – 9 people. The detailed characteristics of the studied population group in terms of the level of physical activity and selected somatic indicators, taking into account the sex and age of the subjects, are presented in Table 1.

The study was conducted using a questionnaire. The study sample was selected arbitrarily, i.e. a survey was conducted, and the waist circumference (WC) of all students present at the university who agreed to participate was measured on the day of the study. As a result of the study, 609 surveys were collected, including 609 waist circumference measurements.

The survey used in the study consisted of two research tools. The first of these was the QEB questionnaire for researching nutritional behaviours and opinions on food and nutrition developed by the Group of Behavioural Nutrition Conditions of the Committee on Science of Human Nutrition PAN. Based on the QEB questionnaire, intake was assessed for 16 groups of food products by using a 6-grade intake frequency scale, where grade 1 meant "never" and grade 6 "several times a day". The second research tool was the shortened version of the IPAQ (*International Physical Activity Questionnaire*), enabling the collection of information on various forms of physical activity of respondents over the last 7 days. The collected data on weekly physical activity were calculated according to a strictly defined scheme into MET units (*Metabolic Equivalent of Task*) [12]. On the basis of physical activity expressed in MET units (MET-minutes/week), people with moderate (600−3000 MET-minutes/week) and high levels of physical activity (>3000 MET-minutes/week) were distinguished. None of the subjects had low levels of physical activity (<600 MET-minutes/week). In addition, as part of the survey, respondents were asked

Table 1. Characteristics of the studied population group [%]

Features of the population group	Generally (n = 609)	Gender		Age (in years)			
		men n = 106)	women n = 503	19–21 n = 430	22–24 n = 128	25–27 n = 34	28–30 n = 17
Generally	100.0	17.4	82.6	70.6	21.0	5.7	2.7
Level of physical activity							
moderate	24.7	16.0	26.4	23.5	28.3	20.0	42.9
high[P, W]	75.3	84.0	73.6	76.5	71.7	80.0	57.1
Selected somatic indicators							
BMI							
underweight	10.0	1.9	11.7	11.4	6.3	8.6	5.9
norm	73.8	59.4	76.9	73.7	76.4	77.1	52.9
overweight[P, W]	13.8	33.0	9.7	13.0	12.6	14.3	41.2
obesity[P, W]	2.4	5.7	1.6	1.9	4.7	0.0	0.0
WHtR							
norm	79.5	65.1	82.9	82.3	72.4	77.1	64.7
visceral obesity[P]	20.5	34.9	17.1	17.7	27.6	22.9	35.3
WC							
norm	78.8	75.5	79.5	81.6	70.1	80.0	70.6
*visceral obesity with IR of MC	14.1	17.9	13.3	12.6	18.1	17.1	17.6
**visceral obesity with HR of MC[W]	7.1	6.6	7.2	5.8	11.8	2.9	11.7

* Visceral obesity with an increased risk of metabolic complications; ** Visceral obesity with a high risk of metabolic complications

[P, W] Statistically significant differences between the feature of the population group according to gender (P) and age (W); level of significance $p \leq 0.05$ *(Chi*-squared test)

to provide body weight in kilograms and body height in centimetres. WC was measured in a standing position using anthropometric tape at the greatest narrowing of the torso at the waist, with the abdominals relaxed.

In the statistical analysis, the number and percentage of students with a given feature were used to describe the population structure. During statistical analysis the „data-driven" method was used to extract dietary patterns. The method does not require preliminary assumptions and dietary patterns to be discovered on the basis of the features of the data set [15, 29]. Factor analysis was used as the method of dietary pattern construction, wherein 6-step response scales reflecting the frequency of consumption were treated as quantitative scales, assuming the existences of a fixed. As a result of the application of the main components method among 16 features (food product groups), four factors (main components) identified on the basis of the correlation coefficient $r \geq 0.5$ were separated. Varimax rotation with normalization was used and achieved convergence in nine iterations (Table 2).

The factors selected as a result of the analysis were described as dietary patterns wherein factor 1 described unhealthy patterns of the type "carbonated drinks, energy drinks, alcohols and canned products"; factor 2, health-promoting patterns of the type "fruits and vegetables"; factor 3, unhealthy patterns of the type "fast food and confectionery products" and factor 4, health-promoting patterns of the type "milk, fermented milk drinks and cottage cheese". In addition, after triacylic division of the factor values, three qualitative variables of each factor were distinguished and described as low factor intensity (first percentile), moderate factor intensity (second percentile) and high factor intensity (third percentile).

When assessing the somatic parameters (body weight, height, WC), basic nutritional assessment indicators were calculated, i.e. BMI (body mass index), WC (*waist circumference*) and WHtR (*waist-to-height ratio*). WHO recommendations were adopted as BMI diagnostic criteria for assessing nutritional status [30]. According to these criteria, BMI <18.5 kg/m² indicates underweight, 18.5–24.9 kg/m² normal weight, 25.0–29.9 kg/m² overweight and ≥30.0 kg/m² obesity. The WHO Report 2008 regarding WC recommendations were adopted as diagnostic criteria for the assessment of abdominal obesity and the risk of metabolic complications associated with it [28]. According to this report, a WC above 94 cm for men and 80 cm for women is associated with abdominal obesity with a significant risk of metabolic complications, and values above 102 cm for men and 88 cm for women indicate abdominal obesity with a very high risk of metabolic complications. As the WHtR diagnostic criterion for abdominal obesity and an increased risk of heart disease and diabetes, we adopted the cut-off point of 0.5. A WHtR value ≥0.5 indicates abdominal obesity and an increased risk of these complications [3, 5].

Table 2. Factor load table after varimax rotation with normalization

Food groups	Main components			
	Factor 1	Factor 2	Factor 3	Factor 4
Wholemeal bread	-0.081669*	0.365991	-0.031295	0.276615
Milk (including flavoured milk)	-0.121347	-0.001969	0.231113	0.605702
Fermented milk drinks	-0.023804	0.144385	0.048491	0.779949
Cottage cheese (incl. homogenized and grainy)	0.114069	0.166751	-0.002955	0.713511
Fish preparations and dishes	0.324804	0.271738	-0.397893	0.370605
Legumes dishes	0.231068	0.558948	-0.275638	0.072277
Fruits	-0.193276	0.834602	0.043821	0.125781
Vegetables	-0.112542	0.868646	-0.023847	0.034972
Fast food	0.384493	-0.188068	0.549160	-0.171010
Fried dishes	0.154927	-0.078421	0.603051	0.106602
Cheese	0.157405	0.049426	0.505363	0.246706
Sweets and confectionery products	0.023817	-0.015154	0.711206	0.059633
Canned food of all kinds	0.592384	-0.068808	-0.059689	0.296348
Carbonated drinks	0.596532	-0.082963	0.462091	-0.130269
Energy drinks	0.590367	-0.095931	0.204770	-0.063556
Alcoholic drinks	0.604830	0.012824	0.112486	-0.056424

*Value of the correlation coefficient between the feature – a group of food products and the main component

The comparison of qualitative features i.e. the BMI, WC and WHtR criteria, and factors (dietary patterns), including the evaluation of statistically significant differences, was performed by using the *Chi*-squared test. The significance level was assumed to be 0.05.

Statistical analysis was performed by using the Statistica Basic 13 PL statistical program.

RESULTS

Slightly fewer than 25% of the respondents were characterized by moderate and over 75% by high levels of physical activity. A high level of physical activity in respondents statistically significantly corresponded to the intensity of three dietary patterns. A statistically significantly higher percentage of surveyed students with a high level of physical activity was characterized by a high intensity of dietary patterns favouring the health-promoting type "fruit and vegetables" as well as "milk, fermented milk drinks and cottage cheese". An inverse dependence was demonstrated for the unhealthy dietary pattern type "fast food and confectionery". In this case, a statistically significantly higher percentage of students with a high level of physical activity was characterized by a low intensity of this dietary pattern (Table 3).

A comparative analysis of the BMI, WC and WHtR criteria showed that approximately 75% of respondents had somatic values characteristic of body weight due. Overweight and obesity were present 16% of the surveyed population according to the BMI criteria. Of those surveyed, 10% were underweight according to the criteria of the same indicator. Over 20% of respondents were characterized by abdominal obesity according to the criteria of the WC and WHtR indices. The underweight according to the criteria of the BMI index did not statistically significantly differentiate the severity of the identified dietary patterns. Due body weight according to all indicators did not statistically significantly differentiate the intensification of identified dietary patterns. The same situation also applied to the WHtR criterion indicating visceral obesity. A statistically significantly higher percentage of respondents with a high intensity of the unhealthy dietary pattern type "carbonated drinks, energy drinks, alcoholic drinks and canned" was characterized by obesity according to the BMI. In the case of the unhealthy dietary pattern type "fast food and confectionery", a statistically significantly higher percentage of subjects with a high intensity of this pattern was overweight according to the BMI index and abdominal obesity with a high risk of metabolic complications according to the WC index and in the case of moderate severity of this pattern of obesity according to the BMI index. However, a statistically significantly higher percentage of subjects with a high intensity of the health-promoting dietary pattern type "fruit and vegetables" was characterized by overweight and obesity according to the BMI index and visceral obesity with a high risk of metabolic complications according to the WC index. In addition, the moderate severity of the health-promoting dietary pattern type "milk, fermented milk drinks and cottage cheese" was related to a statistically significantly higher proportion of obese subjects by the BMI index (Table 3).

Table 3. Characteristics of dietary patterns (factors) of the studied population group, taking into account its level of physical activity and selected somatic indicators of nutritional status [%]

Features of the population groups	Generally	Dietary patterns (factors)											
		Factor 1			Factor 2			Factor 3			Factor 4		
		unhealthy pattern *type „carbonated and energy drinks, alcohol and canned"*			health-promoting pattern type „fruit and vegetables"			unhealthy pattern *type „fast-food and confectionery"*			health-promoting pattern *type „milk, fermented milk drinks and cottage cheese"*		
		L*	M	H	L	M	H	L	M	H	L	M	H
n	609	73	516	20	212	299	98	173	307	129	174	309	126
Generally	100	12.0	84.7	3.3	34.8	49.1	16.1	28.4	50.4	21.2	28.6	50.7	20.7
Physical activity													
Physical activity													
moderate	24.7	27.4	24.5	20.0	22.6	27.1	21.4	20.2	26.7	25.6	24.7	25.2	23.8
high[F2, F3, F4]	75.3	72.6	75.5	80.0	77.4	72.9	78.6	79.8	73.3	74.4	75.3	74.8	76.2
Selected somatic indicators of nutritional status													
BMI													
underweight	10.0	8.2	10.3	5.0	11.3	7.7	14.3	7.5	11.4	10.1	10.9	7.1	15.9
norm	73.8	82.2	72.8	73.5	74.5	76.3	65.3	74.6	74.3	72.1	78.2	74.4	66.7
overweight[F2, F3]	13.8	8.2	14.4	17.5	11.3	14.7	16.3	16.2	11.4	16.3	9.2	15.7	15.9
obesity[F1, F2, F3, F4]	2.4	1.4	2.5	5.0	2.9	1.3	4.1	1.7	2.9	1.5	1.7	2.8	1.5
WHtR													
norm	79.5	83.6	78.6	85.0	81.6	77.9	79.6	79.2	81.1	75.9	85.1	77.3	77.0
visceral obesity	20.5	16.4	21.4	15.0	18.4	22.1	20.4	20.8	18.9	24.1	14.9	22.7	23.0
WC													
norm	78.8	83.6	78.1	80.0	81.6	76.3	80.1	79.8	79.5	76.0	88.5	75.3	73.8
**visceral obesity with IR	14.1	8.2	15.0	15.0	12.3	16.4	11.2	13.3	13.7	16.3	8.0	16.7	16.7
***visceral obesity with HR[F2, F3,	7.1	8.2	6.9	5.0	6.1	7.3	8.7	6.9	6.8	7.7	3.5	8.0	9.5

* Intensity of the factor (dietary pattern) – tertile distribution; L – low intensity of the factor (first percentile), M - moderate intensity of the factor (second percentile), H – high intensity of the factor (third percentile);

** Visceral obesity with an increased risk of metabolic complications;

*** Visceral obesity with the high risk of metabolic complications;

[F1, F2, F3, F4] Statistically significant differences between the feature of the population group and the factors 1-4

DISCUSSION

There is a general decrease in physical activity in the population. A particularly steep decline in this type of activity concerns young people taking up studies [17, 25]. *Brey* and *Born* [4] showed that 1/3 of active high school students became inactive after starting academic life. In the case of Polish students of the Medical University, research by *Dąbrowska-Galas* et al. [6] reported that, depending on the field of study, 0−26% of respondents were characterized by low levels of physical activity, 52−86% of respondents by moderate levels of physical activity and 8−46% of respondents by high levels of physical activity. *Angyán* et al. [2] indicated that medical students were characterized by low levels of physical activity. However, *Frank* et al. [9] reported that American medical students had higher levels of physical activity than their peers.

Low levels of physical activity and unhealthy dietary patterns are the determinant factors of overweight and obesity [8, 14]. In turn, the impact of physical activity on dietary patterns is poorly understood [16]. Some cross-sectional studies suggest that young adults who are physically active tend to consume foods with a higher nutritional density and lower energy density, e.g. higher consumption of fruit or dairy products and lower consumption of fast food [7, 13, 22]. In addition, the analysis of three observational cohort studies on the *a priori* dietary patterns AHEI, AMED and DASH showed that those subjects who adhered to these dietary patterns were more likely to have a higher level of physical activity than those who less adhered to these dietary patterns [8]. Similarly, in our study, it was observed that a statistically significantly higher percentage of people with a high level of physical activity was characterized by a high intensity of the health-promoting dietary pattern of the types "fruits and vegetables" and "milk, fermented milk drinks and cottage cheese" and at the same time with a low intensity of the unhealthy nutrition pattern type "fast food and confectionery". Nevertheless, studies by *Koehler* et al. [16] suggest that the beneficial effect of increased physical activity on dietary patterns exists only when switching from low to moderate and then from moderate to high levels of physical activity. There is no change in dietary patterns when changing from high to very high physical activity.

In our study, 16.2% of the student population was characterized as overweight or obese according to the BMI criteria. Similarly, according to another Polish study conducted by *Likus* et al. [18], overweight and obesity was present in 13.4% of students. In addition to obesity, another equally important problem for young people is underweight, which affected up to 10% of respondents in the present study and 9.6% of students in medicine and health sciences in *Likus* et al. [18]. It should be pointed out that, while the study showed a relationship between dietary patterns and overweight and obesity according to the BMI and visceral obesity, with a high risk of metabolic complications according to the WC index, no statistically significant difference between the respondents' nutritional patterns and underweight or body mass norm according to the BMI, body mass norm according to WC and WHtR, or visceral obesity according to WHtR.

A statistically significantly higher percentage of respondents with a high intensity of the unhealthy dietary pattern type "carbonated drinks, energy drinks, alcoholic drinks and canned goods" was characterized by obesity according to the BMI. In the case of unhealthy dietary pattern type "fast-food and confectionery", a statistically significantly higher percentage of subjects with a high intensity of this pattern were overweight according to the BMI and had visceral obesity with a high risk of metabolic complications according to the WC and in the case of moderate severity of this pattern, obesity according to the BMI. Several studies have found a significant relationship between health-promoting *a priori* dietary patterns and weight loss and visceral obesity [26]. Especially often, such a relationship is observed between the AMED-type dietary pattern and anthropometric indicators [23]. Most cross-sectional studies report a strong inverse relationship between adherence to the Mediterranean diet and the prevalence of obesity, and this has been confirmed by cohort studies [23]. The analysis of three observational cohort studies on *a priori* dietary patterns AHEI, AMED and DASH showed that those subjects who adhered more closely to these nutrition patterns had lower BMI than those who adhered less to these dietary patterns [10]. The research of *Sahrai* et al. published in 2019 demonstrated that AMED-type pro-healthy dietary patterns statistically significantly corresponded to the lower WC and WHR indicators but did not correspond to the BMI indicator [23]. Such observations are confirmed by the EPIK-PANACEA study, which showed a significant relationship between adherence to the Mediterranean diet and lower waist circumference in men and women, while the relationship between the Mediterranean diet and BMI was not demonstrated [21]. In turn, the results of intervention dietary studies showed that compliance with the Mediterranean diet was associated with lower BMI and significantly lower visceral obesity [1, 10, 19]. In the *Sahrai* et al. study [23], there was no significant relationship between the HEI or DQI type dietary patterns and the anthropometric indicators WC, WHR and BMI. Regarding HEI-type dietary patterns, a study of Mexican Americans also found no relationship between HEI and waist circumference in

women, but the results were significant in men [23]. A recent study showed that compliance with DQI was associated with a decrease in WC and BMI in men, while there was no relationship in women [20]. These gender-based differences can be explained by differences in fat storage between men and women. Women tend to store fat around the hips and lower extremities, and men in the abdominal area [31].

Our own study also showed a statistically significantly higher percentage of subjects with obesity according to the BMI index and visceral obesity, with a high risk of metabolic complications according to the WC index characterized by a high intensity of the health-promoting of dietary pattern type "fruit and vegetables" and a moderate severity of the health-promoting dietary pattern type "milk, fermented milk drinks and cottage cheese", but only for those with obesity according to BMI. This relationship can be explained by the fact that obese study participants can implement a specific diet, e.g. low energy, by consuming more vegetables and fruits [22]. In addition, obese subjects can provide socially desirable answers by underestimating unhealthy foods and overstating healthy foods such as vegetables and fruits [24], which can also be applied to the consumption of milk, fermented milk drinks and cottage cheese.

The main limitations of the study were its design (cross-sectional) and the method used (mainly survey). In addition, the survey showed an exceptionally high proportion of respondents with high levels of physical activity and no respondents with low levels of physical activity in relation to data on the level of student activity in Poland [6]. Most students of the surveyed universities declared that they performed work outside of academic activity, which had an impact on the results of the assessment of the levels of physical activity examined and which could significantly underestimate the relationship between dietary patterns and anthropometric indicators of the respondents. However, the study used the international, standardized IPAQ questionnaire, the most commonly recommended tool for measuring the level of physical activity [12, 27] and the research sample was selected arbitrarily and concerned all students in two universities present on the day of the study. Furthermore, only the WC indicator was measured. The body weight and height of respondents were recorded in the study based on self-reported values, which could further reduce the reliability of BMI and WHtR indicators.

CONCLUSIONS

In conclusion, effective programs and methods of nutritional education and motivation to change health behaviours should be implemented among students of Health Sciences, especially those who are overweight or obese, or have lower activity level values.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

URIC ACID ALTERATIONS BY CONSUMPTION OF GLUTEN-FREE BAKERY PRODUCTS IN RELATION TO CARDIOVASCULAR AND METABOLIC SYNDROME RISK FACTORS

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ABSTRACT

Background. Serum uric acid (UA) levels are one of the determinants of the cardiovascular disease and metabolic syndrome but none of criteria for that syndrome include serum UA. Consumption of bakery products (with or without gluten) is associated with an increasing prevalence of overweight/obesity and hyperuricemia frequently occurs in subjects with overweight and obesity.

Objective. The aim of the study was to find out how 6-weeks consumption of gluten-free bakery products can affect risk factors for cardiovascular disease and metabolic syndrome, and especially uric acid levels.

Material and Methods. The group was composed of 27 female volunteers consuming gluten-free bakery products during 6-week period. The biochemical parameters levels were measured by Biolis 24i Premium, the anthropometric parameters by InBody 720 and blood pressure by OMRON Microlife.

Results. We found a non-significant increase in total cholesterol and decrease in triglycerides, in the case of LDL cholesterol a significant reduction in values and increase of HDL cholesterol. Glucose level increased significantly, but uric acid has not changed significantly. We found the highest total cholesterol, triglyceride, and LDL concentrations in the third UA quartile. The highest glucose concentrations were found in the lower UA quartiles, while the lowest in the highest quartiles. Linear increases in UA concentrations were not observed in any of the parameters. Evaluation of the anthropometric parameters showed that while values of BMI, VFA, fat mass and waist circumference were the highest at the beginning of the study in the second quartile, after intervention the highest values were shifted to the third quartile.

Conclusions. Due to the consumption of gluten-free bakery products the risk values of the monitored parameters shifted to higher UA quartiles.

Key words: *uric acid, gluten-free, lipid profile, blood pressure, weight gain rate*

INTRODUCTION

Uric acid (UA) is the end-product of purine metabolism in humans [53]; product of an exogenous pool of purines and endogenous purine metabolism [14]. Its levels vary significantly within humans as the results of the factors that increase generation (e.g. high purine or protein diet) or decrease excretion (e.g. reduction in glomerular filtration rate) [55]. The production and catabolism of purines are relatively constant between 300 and 400 mg per day [14]. Exogenous sources that can increase serum UA include fatty meat, organ meat, and seafood [33] and fructose is another source of exogenous UA. Higher serum UA levels are found in men (with older age, higher blood pressure, increasing cholesterol level and creatinine, and higher body mass index) [24, 36].

Hyperuricemia is usually defined as 6.5 or 7.0 $mg.dL^{-1}$ in men and 6.0 $mg.dL^{-1}$ in women. This condition frequently occurs in subjects with overweight and obesity, insulin resistance, glucose intolerance, dyslipidemia, hypertension, fatty liver, etc. [23, 60].

Some observational studies have suggested that serum UA levels are one of the determinants of the metabolic syndrome and cardiovascular diseases [4]. Metabolic syndrome has become health problem worldwide due to its relationships with cardiovascular disease and type 2 diabetes. It rises with aging and women are more affected than men [50]. The diagnostic criteria for metabolic syndrome vary [3, 28], but three or more of these manifestations are needed to diagnose:

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for European individuals waist circumference ≥94 and ≥80 cm in men and women respectively; serum triglycerides 1.7 $mmol.L^{-1}$; high-density lipoprotein cholesterol <1 $mmol.L^{-1}$ and 1.3 $mmol.L^{-1}$ in men and women respectively; blood pressure ≥140/90 mmHg; and fasting blood glucose >6.1 $mmol.L^{-1}$ [2, 3, 8]. None of these criteria include serum UA although elevated levels are in subjects with metabolic syndrome [62] and it has therefore been suggested that serum UA is one of the determinants of syndrome [46]. Whilst hyperuricemia is not accepted as factor for the diagnosis of metabolic syndrome, many studies have suggested that serum UA levels are uniformly elevated [11, 47]. Body composition including muscle mass and fat mass are associated with serum UA levels. In adults with normal body mass index, the metabolic syndrome is 10 times higher in those having serum UA ≥10 $mg.dL^{-1}$ compared to those with <6 $mg.dL^{-1}$ [17]. The hazard ratio of incident metabolic syndrome shows a steady increase when normal adults were allocated into four quartiles according to serum UA [63]. *Shani* et al. [52] found that high normal serum UA was also associated with future development of type 2 diabetes among lean healthy and normoglycemic women. Elevated serum UA predicted diabetes mellitus and insulin resistance [35]. Uric acid is also commonly associated with hypertension and the risk to develop the hypertension rises greater in hyperuricemic male and female subjects; this chance augments in older age [58, 61]. *Leiba* et al. [39] found that those with serum UA higher than 3 $mg.dL^{-1}$ had a greater chance to develop hypertension and the higher the serum UA within the normal range, the greater was the risk to develop hypertension. The risk is stronger in younger ages and in females and high serum UA is one of the major predictors of worse blood pressure control [18, 38, 56]. Increased serum UA was also appointed as independent risk factor for overall and cardiovascular mortality. The relationship is higher in the lowest and highest quintiles in both men and women [59].

Gluten-free eating patterns are frequently perceived to be healthier than gluten-containing ones and good health is the primary reason given for a gluten-free diet adoption in healthy population. In recent years there has been an increasing interest on gluten-free foodstuffs [34]. The proportion of people on a gluten-free diet exceeds in more than five times the number of those who require gluten exclusion as a treatment [13]. However, the nutritional value and quality of gluten-free products is questionable. *Lucisano* et al. [41] reported that most gluten-free products had poor cooking quality compared with their wheat counterparts and there is a need to improve the nutritional quality of these products. Researchers found an excess intake of total energy, animal protein and fat and a lower intake of dietary fibre, magnesium and folic acid by following a gluten-free diet [44, 66]. Gluten-free breads had a higher glycaemic index than the conventional breads and had great divergences in fat [19]. Almost all the gluten-free products were high in available carbohydrates [42]. Gluten-free bread provides twice as much fat, mainly saturated fat in comparison to its equivalents with gluten [44]. Consumption of bread and bakery products (with or without gluten) is associated with an increasing prevalence of overweight and obesity. It was demonstrated that the obesity risk is increased in celiac on gluten-free diet because of the high glycemic index of the gluten-free diet [37].

Therefore, the aim of the study was to find out how six weeks of consumption of gluten-free bakery products can affect selected risk factors for cardiovascular disease and metabolic syndrome, and especially uric acid, whose elevated levels are a significant predictor of these diseases.

MATERIALS AND METHODS

Characteristics of the participants

Twenty-seven female volunteers were included in the study. The requirement for participation was the consent of individuals with whole study and measurement conditions which they will have to complete during the research. The group of participants was composed of volunteers from the general population, consuming gluten-free bread and gluten-free bakery products during 6-week period; however the participants of the study were not allowed to be on total and strict gluten-free diet. Participants with the present severe disease or with recommended special dietary regimen were excluded prior to the start of the study. The amount of bread and bakery products was determined according to the recommended dietary allowance for the Slovak population 150-200 grams per day. All participants were asked not to change their eating habits and also not to change their habits related to the physical activity. Volunteers completed a total of 2 measurements (1^{st} measurement before consumption as a control, 2^{nd} measurement after the 6-week consumption). The trial was approved by the Ethic Committee at the Specialized St. Svorad Hospital Nitra Zobor (Slovakia); (protocol no. 012911/2016).

Dietary Assessment

For study purposes, we monitored the nutritional intake (Table 1) of study participants in order to evaluate the recommended nutritional doses and to better assess the potential impact of consumption of gluten-free bakery products on the blood concentrations of the monitored parameters. Dietary intake was assessed using 3-day 24-h food recalls, two on weekdays and one at the weekend. We used the

Table 1. Nutrient intake of participants

	Energy (kJ)	Carbohydrates (g)	Lipids (g)	Proteins (g)
mean ± SD	7840 ± 3241	229.54 ± 113	79.34 ± 37	68.75 ± 28
max	17290.35	643.82	208.42	161.33
min	2378.35	66.75	25.11	21.2
med	7218.25	195.54	69.7	66.09
mod	ND	ND	93.48	ND
	Dietary fibre (g)	Polyunsaturated fatty acids (g)	Monounsaturated fatty acids (g)	Saturated fatty acids (g)
mean ± SD	19.04 ± 11	11.57 ± 6	22.06 ± 11	24.92 ± 14
max	58.45	36.38	53.34	63.66
min	4.8	1.13	5.84	7.8
med	17.72	10.3	19.1	21.39
mod	58.45	13.3	36.07	47.48
	Phosphorus (mg)	Magnesium (mg)	Sodium (mg)	Iron (mg)
mean ± SD	1026.35 ± 501	335.89 ± 541	3974.77 ± 2772	12.65 ± 7
max	2923.66	5004.57	15147.43	36.44
min	162.22	78.94	967.53	3.33
med	909.75	258.12	3248.25	11.36
mod	ND	ND	ND	36.44
	Calcium (mg)	Potassium (mg)	Pyridoxine (mg)	Thiamine (mg)
mean ± SD	775.62 ± 414	2332.23 ± 1148	1.20 ± 1	1.02 ± 1
max	2111.8	6024.09	4.23	3.8
min	185.87	675.95	0.34	0.14
med	747.32	2055.11	1.12	0.94
mod	ND	ND	1.32	0.93

Table 2. Compliance with the standard for nutrient intake (according to *Kajaba* et al. [32])

	Energy (%)	Carbohydrates (%)	Lipids (%)	Proteins (%)
mean	89	63	124	133
max	503	183	321	316
min	25	18	39	41
	Dietary fibre (%)	Polyunsaturated fatty acids (%)	Monounsaturated fatty acids (%)	Saturated fatty acids (%)
mean	81	55	104	116
max	244	182	267	318
min	5	7	26	36
	Phosphorus (%)	Magnesium (%)	Sodium (%)	Iron (%)
mean	87	83	256	78
max	244	203	939	243
min	21	23	65	21
	Calcium (%)	Potassium (%)	Pyridoxine (%)	Thiamine (%)
mean	85	52	67	100
max	746	134	240	280
min	19	15	19	14

nutritional software program Mountberry – Nutrition & Fitness Software (2011, Version 1.1, Slovakia). Mountberry provides a complete analysis of food, meals, recipes based on an updated food database and nutritional recommendations for nutrient intake, health insights, dietary guidelines, and individual user needs. The average nutrient intake for participants was assessed according to the Recommended Dietary Allowance (OVD in Slovakia) updated in 2015 [32] (Table 2). During the trial we focused on basic parameters such as energy, proteins, carbohydrates, fats and also minerals (phosphorus, magnesium, sodium, iron, calcium and potassium) and vitamins (pyridoxine and thiamine).

Blood samples

Blood samples were obtained before and after intervention. Venous blood was collected in the morning after 8 h of fasting using 2.5 mL EDTA solution and in a 2x7.5 mL serum gel tube. After the separation of blood serum, the parameters levels were measured by automatic biochemical analyser Biolis 24i Premium (Tokyo Boeki Machinery Ltd., Japan) using direct ion selective electrodes methods in the laboratory of the Department of Human Nutrition (Slovak University of Agriculture in Nitra). We focused on changes in serum lipid profile (total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol), glucose and uric acid. The serum UA concentration was divided into four quartiles as follows: 1st quartile $\leq$3.5 mg.dL^{-1}, 2nd quartile 3.6-4 mg.dL^{-1}, 3rd quartile 4.1-4.6 mg.dL^{-1} and 4th quartile $\geq$4.7 mg.dL^{-1} [63].

Anthropometric measurements

Body height was measured in a standing position without shoes on the electronical medical scales Tanita WB-300 while shoulders were in normal alignment and the data were recorded to the nearest 0.1 cm. Weight was measured in light clothing without shoes using a standard scale and recorded to the nearest 0.1 kg. Waist circumference was measured at the umbilical level and that of the hip at the maximum level over light clothing, using a stretched tape meter, without any pressure to body surface and measurements were recorded to the nearest 0.1 cm. BMI (kg.m^{-2}) was calculated as weight (kg) divided by square of the height (m^2).

The anthropometric measurements were made by using InBody 720 (Biospace Co. Ltd., Seoul, Republic of Korea). Body composition was diagnosed by multi-frequency bioelectrical impedance analysis, which measures the total impedance at frequencies of 1, 5, 50, 100, 500, 1000 kHz. Each of the participants was informed with the measurement procedure, explained the possible risks of measuring in the case of pregnancy or having an artificial pacemaker at the heart. Before the measurement, participants were asked to excrete and refrain from drinking excessive amounts of water. At the same time each participant signed informed consent for the measurement procedure and also agreed to the processing of personal data. The Lookin'Body 3.0 software was used to process the results. We focused especially on visceral fat area (VFA, cm^2) and fat mass (FM, kg / %).

Measurement of blood pressure

Blood pressure (systolic and diastolic) was measured by using OMRON Microlife AG, 9443 (Widnau/Switzerland) with fully automatic operation and the possibility of using both the classic and elongated inflatable cuff on the arm. Blood pressure was measured after the body fluid had settled, resting, sitting. The reference limits were for systolic pressure 120-129 mmHg, diastolic pressure 80-84 mmHg and pulse 60-90 beats per minute.

Statistical analysis

We evaluated the collected data from the measurements statistically and graphically in Microsoft Office Excel 2010 (Los Angeles, CA, USA). The changes between biochemical and anthropometric measurements were performed using paired *Student's t*-test and the data were presented as mean ± standard deviation (SD). Statistical analyses were performed using the program STATISTICA Cz version 10. Differences among data were also tested with a one-way analysis of variance (ANOVA) and were compared using *Tukey's Post Hoc* Test. The levels of statistical significance were set at P <0.05 (*), P <0.01 (**), P <0.001 (***). We used also *Pearson's* correlation analysis between parameters.

RESULTS AND DISCUSSION

Changes of the biochemical and anthropometric parameters after intervention are shown in Table 3. Within the lipid spectrum, we found a non-significant increase in total cholesterol from 5.82 ± 0.94 mmol.L^{-1} to 5.93 ± 0.91 mmol.L^{-1} and a decrease in triglycerides from 0.98 ± 0.41 mmol.L^{-1} to 0.91 ± 0.46 mmol.L^{-1}. However, in the case of LDL cholesterol, we found a significant reduction in values from 3.29 ± 0.75 mmol.L^{-1} to 2.97 ± 0.71 mmol.L^{-1} (P <0.001) and increase of HDL cholesterol from 1.93 ± 0.40 mmol.L^{-1} to 2.02 ± 0.41 mmol.L^{-1} (P <0.05). In this regard, we can conclude that an increase in total cholesterol may have been due to an increase in HDL, but it is important for the assessment of cardiovascular risk in which HDL and LDL subfractions are predominant, since not every increase in HDL or decrease in LDL must clearly predict increased or decreased cardiovascular risk. Therefore, further analyses are needed.

Table 3. Changes of the biochemical and anthropometric parameters after intervention

Parameters	Baseline			After 6 weeks			
	mean ± SD	max	min	mean ± SD	max	min	*P* value
Age (years)	29.11 ± 7	45	23				
Height (cm)	167.1 ± 5.6	176	156				
Weight (kg)	63.51 ± 11.93	101.4	48.5	63.29 ± 11.88	100.5	48.1	*0.1550*
Uric acid (mg.dL^{-1})	3.7 ± 0.72	5.28	2.55	3.71 ± 0.81	5.48	2.38	*0.9293*
Total cholesterol (mmol.L^{-1})	5.82 ± 0.94	8.50	4.41	5.93 ± 0.91	7.94	4.71	*0.3066*
Triglycerides (mmol.L^{-1})	0.98 ± 0.41	2.05	0.48	0.91 ± 0.46	2.63	0.48	*0.2595*
LDL cholesterol (mmol.L^{-1})	3.29 ± 0.75	5.10	1.78	2.97 ± 0.71	4.55	1.75	*<0.001*
HDL cholesterol (mmol.L^{-1})	1.93 ± 0.40	2.95	1.18	2.02 ± 0.41	2.97	1.27	*0.0166*
Glucose (mmol.L^{-1})	4.55 ± 0.39	5.23	3.89	4.85 ± 0.51	5.84	3.92	*<0.001*
Body mass index (kg.m^{-2})	22.75 ± 4.12	35.50	17.75	22.67 ± 4.13	35.19	17.68	*0.1797*
Visceral fat area (cm^2)	74.10 ± 30.16	153.94	37.80	74.77 ± 31.37	167.22	41.07	*0.3732*
Fat mass (kg)	18.70 ± 9.44	51.30	8.70	18.66 ± 9.27	50.50	9.80	*0.8080*
Percentage of body fat (%)	28.16 ± 8.02	50.59	16.51	28.22 ± 7.70	50.21	19.10	*0.8155*
Waist circumference (cm)	82.08 ± 10.99	110.30	68.40	82.31 ± 11.30	114.90	69.90	*0.4023*
Systolic blood pressure (mmHg)	121 ± 9.82	141	103	119 ± 9.82	142	101	*0.2138*
Diastolic blood pressure (mmHg)	79 ± 9.74	97	64	77 ± 8.61	94	63	*0.3363*

Table 4. Changes of the biochemical and anthropometric parameters according to quartiles of serum uric acid concentration

UA quartiles	UA (mg.dL^{-1})		T-C (mmol.L^{-1})		TAG (mmol.L^{-1})	
	baseline	after 6 weeks	baseline	after 6 weeks	baseline	after 6 weeks
<3.5 mg.dL^{-1}	3.0	3.0	5.64	5.72	0.92	0.81
3.6-4 mg.dL^{-1}	3.7	3.9	5.62	5.68	1.02	0.86
4.1-4.6 mg.dL^{-1}	4.3	4.4	6.39	6.72	1.09	1.26
>4.7 mg.dL^{-1}	5.0	5.0	5.76	6.20	0.93	0.94
UA quartiles	LDL (mmol.L^{-1})		HDL (mmol.L^{-1})		G (mmol.L^{-1})	
	baseline	after 6 weeks	baseline	after 6 weeks	baseline	after 6 weeks
<3.5 mg.dL^{-1}	3.24	2.80	1.83	2.00	4.53	5.01
3.6-4 mg.dL^{-1}	3.39	2.60	1.73	2.08	4.66	4.53
4.1-4.6 mg.dL^{-1}	3.48	3.77	2.23	1.97	4.52	5.13
>4.7 mg.dL^{-1}	2.88	3.29	2.11	2.05	4.43	4.55
UA quartiles	BMI (kg.m^{-2})		VFA (cm^2)		FM (kg)	
	baseline	after 6 weeks	baseline	after 6 weeks	baseline	after 6 weeks
<3.5 mg.dL^{-1}	22.37	22.36	70.91	71.49	17.25	17.66
3.6-4 mg.dL^{-1}	24.21	21.13	86.82	65.52	23.16	15.50
4.1-4.6 mg.dL^{-1}	22.46	26.34	66.98	101.30	17.10	27.70
>4.7 mg.dL^{-1}	21.33	22.35	70.34	72.80	16.80	17.60
UA quartiles	WC (cm)		SBP (mmHg)		DBP (mmHg)	
	baseline	after 6 weeks	baseline	after 6 weeks	baseline	after 6 weeks
<3.5 mg.dL^{-1}	81.4	81.2	119	118	77	75
3.6-4 mg.dL^{-1}	83.30	78.40	120	117	73	78
4.1-4.6 mg.dL^{-1}	79.25	92.9	124	126	83	80
>4.7 mg.dL^{-1}	80.23	81.2	126	121	88	82

Footnotes: *UA* - uric acid; *T-C* - total cholesterol; *TAG* - triglycerides; *LDL* - low-density lipoproteins; *HDL* - high-density lipoproteins; *G* - glucose; *BMI* - body mass index; *VFA* - visceral fat area; *FM* - fat mass; *WC* - waist circumference; *SBP* - systolic blood pressure; *DBP* - diastolic blood pressure

Glucose level increased significantly during the intervention from 4.55 ± 0.39 mmol.L^{-1} to 4.85 ± 0.51 mmol.L^{-1} (P <0.001). Uric acid has not changed significantly (3.70 ± 0.72 mg.dL^{-1} vs 3.71 ± 0.81 mg.dL^{-1}; P >0.05).

Although the assessment of anthropometric parameters showed a non-significant decrease in BMI (22.75 ± 4.12 vs 22.67 ± 4.13 kg.m^{-2}) and fat mass (18.7 ± 9.44 vs 18.66 ± 9.27 kg), but also an increase in visceral fat area (74.10 ± 30.16 vs 74.77 ± 31.37 cm^2) and waist circumference (82.08 ± 10.99 vs 82.31 ± 11.30 cm).

In the case of blood pressure, we observed a non-significant decrease in systolic pressure from 121 ± 9.82 to 119 ± 9.82 mmHg and diastolic pressure from 79 ± 9.74 to 77 ± 8.61 mmHg (P >0.05).

The monitored parameters were also evaluated by individual UA quartiles (Table 4). We found that in the case of the lipid profile, women had the highest total cholesterol, triglyceride, and LDL concentrations in the third quartile (UA between 4.1-4.6 mg.dL^{-1}). The values of all the above mentioned parameters increased in the third quartile after six weeks of consumption of gluten-free bread and bakery products. In the case of HDL, the initial value was highest in the third quartile, but after the intervention in the second quartile. Linear increases in UA concentrations were not observed in any of the parameters. Similarly to other studies, in our case, the highest glucose concentrations were found in the lower UA quartiles, while the lowest in the highest quartiles.

Evaluation of the anthropometric parameters showed that while values of BMI, VFA, fat mass and waist circumference were the highest at the beginning of the study in the second quartile (UA between 3.6-4 mg.dL^{-1}), after intervention the highest values were shifted to the third quartile (UA between 4.1-4.6 mg.dL^{-1}). From this point of view we can conclude that due to the consumption of gluten-free bakery products the risk values of the monitored parameters shifted to higher UA quartiles.

Mean systolic blood pressure values did not exceed the limit of 140 mmHg in any quartile of UA, with the highest values after intervention in the third quartile. The mean diastolic blood pressure values did not exceed the limit of 90 mmHg during the study, with the highest values in the fourth quartile of UA in both cases (before and after intervention).

According to correlations we found significant relationships between total cholesterol and triglycerides, LDL, HDL, systolic and diastolic blood pressure (P<0.01; Table 5). Significant correlation was also found between triglycerides and LDL (P<0.05), both of which had a significant relationship to systolic and diastolic blood pressure (P<0.01). Correlation analysis showed a strong relationship between LDL

Table 5. Correlation between biochemical and anthropometric parameters after intervention (n = 27)

	Correlation coefficients												
	UA	T-C	TAG	LDL	HDL	G	BMI	VFA	FM	WC	SBP	DBP
Uric acid/UA (mg.dL^{-1})		0.272	0.219	0.362	-0.017	-0.259	0.070	0.094	0.085	0.093	0.107	0.224
Total cholesterol/T-C (mmol.L^{-1})	0.272		**0.482****	**0.856****	**0.613****	-0.068	0.161	0.169	0.186	0.227	**0.675****	**0.629****
Triglycerides/TAG (mmol.L^{-1})	0.219	**0.482****		**0.466***	0.143	0.201	0.250	0.224	0.199	0.261	**0.613****	**0.638****
Low-density lipoprotein/LDL (mmol.L^{-1})	0.362	**0.856****	**0.466***		0.136	0.031	**0.414***	**0.448***	**0.448***	**0.497****	**0.639****	**0.492****
High-density lipoprotein/HDL (mmol.L^{-1})	-0.017	**0.613****	0.143	0.136		-0.179	-0.286	-0.333	-0.289	-0.299	0.238	**0.398***
Glucose/G (mmol.L^{-1})	-0.259	-0.068	0.201	0.031	-0.179		0.349	**0.393***	**0.385***	0.358	0.140	-0.025
Body mass index/BMI (kg.m^{-2})	0.070	0.161	0.250	**0.414***	-0.286	0.349		**0.967****	**0.980****	**0.966****	0.191	0.098
Visceral fat area/VFA (cm^2)	0.094	0.169	0.224	**0.448***	-0.333	**0.393***	**0.967****		**0.986****	**0.987****	0.186	0.099
Fat mass/FM (kg)	0.085	0.186	0.199	**0.448***	-0.289	**0.385***	**0.980****	**0.986****		**0.977****	0.176	0.106
Waist circumference/WC (cm)	0.093	0.227	0.261	**0.497****	-0.299	0.358	**0.966****	**0.987****	**0.977****		0.209	0.108
Systolic blood pressure/SBP (mmHg)	0.107	**0.675****	**0.613****	**0.639****	0.238	0.140	0.191	0.186	0.176	0.209		0.657**
Diastolic blood pressure/DBP (mmHg)	0.224	**0.629****	**0.638****	**0.492****	**0.398***	-0.025	0.098	0.099	0.106	0.108	**0.657****	

P values by *Pearson´s* correlation analysis between parameters; * P< 0.05 and ** P< 0.01

and anthropometric parameters (BMI, visceral fat area, fat mass; $P<0.05$) and waist circumference ($P<0.01$). Glucose had a correlation with visceral fat area and fat mass ($P<0.05$). BMI had a significant relationship with VFA, fat mass, waist circumference ($P<0.01$) and visceral fat area with fat mass and waist circumference ($P<0.01$). Blood pressure systolic and diastolic was associated with lipid parameters (total cholesterol, triglycerides, LDL; $P<0.01$).

In terms of metabolic syndrome prevalence, at least one risk factor was found in 52% and 48% of respondents at the beginning and end of the study. Metabolic syndrome would be diagnosed in 11% of participants before intervention, but only in one case after intervention. The most female participants with one risk factor occurred in the first and fourth quartiles of the UA. Volunteers with two or more risk factors for metabolic syndrome (after intervention) were found in the second and third quartiles. Prior to intervention, the highest incidence of risk factors was in the second quartile. The most frequently occurring risk factors were increased waist circumference and diastolic blood pressure.

In terms of cardiovascular risk, total cholesterol, waist circumference, and LDL (elevated concentrations in 81%, 48% and 67%, respectively) were the most critical parameters before and after the intervention. Most risk factors occurred in the first quartile (between 1 and 4 risk factors per participant). The highest incidence of risk factors per participant occurred in the third quartile.

The gluten-free diet is for people suffering from celiac disease, for which the only form of treatment is the strict exclusion of gluten from the diet [9, 57]. The early development of gluten-free products has been associated with many technological and rheological problems, with the nutritional value of these products being secondary. Therefore, gluten-free products were nutritionally unbalanced [12]. Many studies have confirmed higher levels of saturated fatty acids, salt, low dietary fibre [1, 26]. These facts support the suspicion that in such nutritional composition of gluten-free products, consumers are at risk of higher intake of risk food components involved in the development of cardiovascular, metabolic and other diseases [64].

However, considering gluten-free products suitable for weight loss could lead to overconsumption of these energy-rich products and could result in promoting weight gain [27]. Obesity is a serious health condition significantly associated with higher mortality and morbidity [30] and highly prevalent metabolic disorder that is characterized by excessive body fat mass. Abdominal obesity and excess visceral fat are independent risk factors for cardiovascular diseases [21], diabetes mellitus and total mortality [29, 51]. Visceral fat area is important factor used in the assessment of cardio-metabolic risk and is correlated with the metabolic syndrome even at the normal body mass index indicating the absence of obesity [6].

Elevated serum UA levels are commonly seen in association with individual cardiovascular and metabolic syndrome risk factors such as hypercholesterolemia, hypertriglyceridemia, hypertension, hyperglycemia, and obesity [23].

Many studies reported that the association between serum UA levels and metabolic syndrome was stronger in females than in males. Women with a higher serum UA concentration had a higher incidence of hypertension, hypertriglyceridemia and low HDL as well as increased cardiovascular morbidity and mortality compared to that in men [7, 15, 24, 60].

Yang et al. [60] observed that higher levels of serum UA were significantly associated with increasing BMI, waist circumference, systolic blood pressure, serum total cholesterol, LDL, and triglycerides but fasting plasma glucose and reduced HDL were significantly negatively related to serum UA concentration.

In the study of *Zhang* et al. [65] participants with metabolic syndrome were more likely to have higher levels of BMI, waist circumference, systolic and diastolic blood pressure, total cholesterol, triglycerides, fasting glucose and lower HDL levels in both men and women. The prevalence of metabolic syndrome increased according to the quartiles of serum UA concentration. In women, the prevalence raised from 24.98% among the participants with serum UA concentrations <3.5 mg.dL^{-1} to 55.71% among those with concentrations ≥ 4.7 mg.dL^{-1}. In the fourth quartile, 9.65% in women exhibited five metabolic components. There was a positive association between serum UA concentration and central obesity, hypertriglyceridemia, low HDL and high blood pressure in men and women.

The prospective study of *Liu* et al. [2014] conducted in US reported that subjects with a high vs low serum UA concentrations were 2.29 times more likely to have metabolic syndrome in women. In addition, the association between serum UA and metabolic syndrome was stronger among women than men, consistently with other studies [16, 54].

Hyperuricemia and hypertension may both result from the common pathway hyperinsulinemia due to insulin resistance, which increases urine sodium retention and decreases renal uric acid clearance [25].

In prospective study of a Chinese population *Yang* et al. [60] found that there was a graded increase in the incidence of metabolic syndrome among individuals with increasing levels of serum UA. Findings suggest that there was a sex-related association, and therefore hyperuricemia is a significantly independent risk factor for the development of metabolic syndrome in women and tended to interact additively with elevated

blood pressure and elevated waist circumference. Postmenopausal women have higher uric acid levels than younger ones due to their lack of estrogens, which are naturally uricosuric and favour uric acid excretion. Serum UA concentrations are always lower in women than in men at any age albeit less markedly so with aging [20]. These differences are caused by other determinants as estrogens.

Zurlo et al. [67] found in their study that women with higher baseline serum UA concentrations had a higher incidence of both hypertriglyceridemia and high blood pressure than men. They also found out that high baseline serum UA concentrations were able to predict the onset of metabolic syndrome only in older women, but not in men. At the baseline, higher serum UA levels were significantly associated with more abdominal obesity in both genders and high triglyceride levels, but only in men.

Obesity is a well-recognized marker and risk factor for type 2 diabetes, but many individuals with diabetes are not obese [31]. Results of *Krishnan* et al. [35] suggest that elevated serum urate concentration may be one of the risk factor. Data from the Rotterdam Study showed that the age and gender adjusted hazard ratio for diabetes was greatest among persons in the highest quartile of serum urate level [22].

Hyperuricemia have been proposed as novel risk factors for diabetes, but the results from epidemiologic studies have been not clear [10, 49]. The role of serum UA as a risk factor for diabetes is gender related and due to different dietary patterns, genetic factors and the influence of sex hormones [43]. Although higher than normal serum UA levels are positively associated with diabetes, some studies suggest that patients with recently-diagnosed diabetes tend to have lower serum UA than non-diabetics [5]. *Nan* et al. [45] reported a bell-shape association between serum UA and fasting glucose, which showed an increasing trend in UA up to fasting glucose of 7.0 mmol.L^{-1}. Thereafter, the serum UA started to decrease along with further increases in fasting glucose.

CONCLUSIONS

The results of our study showed that consumption of gluten-free bread and bakery products during six weeks had no significant effect on the observed cardiovascular and metabolic syndrome risk parameters. But we found that in the case of the lipid profile, women had the highest total cholesterol, triglyceride, and LDL cholesterol concentrations in the third quartile of UA and the values of all the above mentioned parameters increased in the third quartile after six weeks of consumption of gluten-free bread and bakery products. The highest glucose concentrations were found in the lower UA quartiles, while the lowest in the highest quartiles. In terms of metabolic syndrome prevalence, prior to intervention, the highest incidence of risk factors was in the second quartile. The most frequently occurring risk factors were increased waist circumference and diastolic blood pressure. In terms of cardiovascular risk, the highest incidence of risk factors per participant occurred in the third quartile.

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Conflict of interest

The authors declare no conflict of interests.

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ORIGINAL ARTICLE

MEASURING AND COMPARING THE WATER ACTIVITY AND SALT CONTENT IN PARENICA CHEESES MADE BY TRADITIONAL AND INDUSTRIAL TECHNOLOGY

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ABSTRACT

Background. Slovenská Parenica is one of the most traditional and ever-popular sheep´s milk cheese specialities. This cheese has been registered as a geographical indication (PGI) in the EU. Parenica cheese is produced also from cow´s milk, but without the trade name "Slovenská/Slovak".

Objective. The aim of our research was was statistical reporting and results visualization of water activity analysis and salt content in cow´s milk Parenica cheeses from 8 small and medium-sized Slovak dairy producers.

Material and method. A total of 320 samples of smoked and non-smoked Parenica cheeses made from cow´s milk using traditional and industrial technology were examined during the 10-month period. Each cheese was analysed immediately after sampling (A) and subsequently after 7 days of storing at 4°C (B). The salt content was measured on the Chloride analyser M 926 and the water activity on the Fast-Lab meter. Due to the hierarchical design of the experiment, the linear mixed models via the R statistical environment to compare the differences in the water activity and salt content were used.

Results. Statistical reporting and visualization of water activity measurements showed significant differences between samples A and B ($p = 0.0129$) and between kinds of Parenica cheese ($p = 0.0196$). The value of water activity ranged from 0.908 to 0.975 (A) with the increasing trend after storing in both kinds of Parenica cheese. The impact of dairy producer type was not significant. The higher content of NaCl was found in fresh Parenica cheese from small farms (non-smoked: 2.51 ±1.12 g/100 g, smoked: 1.97 ±0.89 g/100 g). The average salt content in cheeses from industrial dairies was 1.65 ±0.34 g/100 g (non-smoked) and 1.96 ±0.43 g/100 g (smoked). Results showed lower variability of salt content in cheeses from industrial dairies.

Conclusions. It can be concluded that especially the small producers can have probably problem in noncompliance with the technological processes, non-implementation of standardized procedures and underestimation of hygiene regulations.

Key words: *Parenica cheese, salt, water activity, dairies, statistical models*

STRESZCZENIE

Wprowadzenie. „Słowacka Parenica" to jeden z tradycyjnych i bardzo popularnych serów wytwarzanych z owczego mleka. Produkt ten został zarejestrowany w systemie ochrony regionalnych produktów rolnych (PGI) przez Unię Europejską. Ser „Parenica" produkowany jest również w wersji z mleka krowiego, ale bez nazwy „słowacka".

Cel. Celem naszych badań było oznaczenie i porównanie aktywności wody i zawartości soli w serach „Parenica" pochodzących z 8 małych i średnich słowackich producentów mleka.

Material i metoda. W ciągu 10 miesięcy zbadano ogółem 320 próbek wędzonych i niewędzonych serów Parenica wytworzonych z mleka krowiego przy użyciu technologii tradycyjnej i przemysłowej. Analizowano sery zaraz po pobraniu

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próbki (A) oraz po 7 dniach przechowywania w temperaturze 4°C (B). Zawartość soli oznaczano na analizatorze chlorków M 926, a aktywność wody w aparacie Fast-Lab. Ze względu na hierarchiczną strukturę eksperymentu zastosowano liniowe modele mieszane w środowisku statystycznym R do porównania różnic w aktywności wody i zawartości soli.
Wyniki. Analizy statystyczne i wykresy pomiarów aktywności wody wykazały statystyczne różnice między próbkami A i B ($p = 0.0129$) oraz między rodzajami sera Parenica ($p = 0.0196$). Wartość aktywności wody wahała się w zakresie od 0.908 do 0.975 (A), wykazano trend wzrostowy tego parametru podczas przechowywania obu rodzajów sera Parenica. Nie stwierdzono statystycznych różnic pomiędzy poszczególnymi producentami serów. Wyższą zawartość NaCl stwierdzono w świeżym serze Parenica produkowanym w małych gospodarstwach (niewędzone 2.51 ±1.12 g/100 g, wędzone 1.97 ±0.89 g/100 g). Średnia zawartość soli w serach produkowanych przemysłowo wyniosła 1.65 ±0.34 g/100 g (niewędzone) a 1.96 ±0.43 g/100 g (wędzone). Badania wykazały mniejszą zmienność zawartości soli w serach z mleczarni przemysłowych.
Wnioski. Można stwierdzić, że szczególnie mali producenci mogą mieć trudności z utrzymywaniem standardów i ujednoliceniem procedury technologicznej oraz przestrzeganiem rezimu higienicznego podczas wytwarzania produktu.

Słowa kluczowe: *ser Parenica, sól, aktywność wody, mleczarnie, modele statystyczne*

INTRODUCTION

Traditional cheeses represent the heritage and are the result of accumulated empirical knowledge passed on from generation to generation. Every traditional cheese is connected to the territory of its origin and to the prevailing environmental conditions [5].

Among traditional cheeses are also steamed cheeses, which are characterized by a unique production. They usually have a flat-cylindrical shape, no holes, and straw-yellow to yellow colour. Traditional steamed cheeses are made from milk of cows, goats, sheep, or buffalo cows [36].

Steamed cheeses are currently produced in Slovakia and have received the status of 'Protected Geographical Indication' within the European Union. Products with this designation include e.g. Slovenská parenica [12], Oravský korbáčik [14], Zázrivský korbáčik [15], Slovenský oštiepok [13], Slovenská Parenica is a traditional Slovak cheese. The name comes from the Slovak word for steaming. Parenica is a soft, steamed cheese made from unpasteurized sheep's milk of the *Wallachian, Cigaya*, *East Friesian*, and improved *Wallachian* breeds. The cheese can also be made using a mixture of raw sheep's and cow's milk, where the content of sheep's milk must be at least 50%. The fat content in the dry mater is at least 50% and the salt content should not exceed 3% [9, 27, 28].

Slovenská Parenica is a steamed, lightly smoked or non-smoked cheese wound into two opposed rolls 0.06 – 0.08 m in diameter and 0.05 – 0.08 m high, forming „S" shape bulk. Moreover, the rolls are bound with cheese string. Prior to rolled up, the cheese strips are 0.002 – 0.003 m thick, 0.05 – 0.08 m wide, and 4 – 6 m long [10, 11, 24]. Cows´ cheese version is also produced but without trade name Slovenská.

Salt plays an important role in cheese production. The salt addition has three basic functions: (1) acts as a preservative, (2) has an effect on cheese taste, and (3) is a source of sodium. The addition of salt further regulates the water content of the cheese, reduces the water activity, and affects the ripening [3, 36]. *Everett and Auty* [19] reported that salt limits the action of bacteria in cheese, as well as has the secondary effect of flavor enhancement. Saltiness is one of the most important flavor attributes of cheese and is directly correlated to overall desirability by consumers.

The salt content of the product may be concentrated due to the gradual loss of water and increasing the dry matter content. This effect was observed by several authors [8, 18, 25, 40]. NaCl influences cheese ripening principally through its effects on water activity. Among the principal effects of salt are control of microbial growth and activity; control of the various enzyme activities in cheese; syneresis of the curd and thus in a reduction in cheese moisture, which also influences the above; and physical changes in cheese proteins which influence cheese texture, protein solubility and probably protein conformation [21].

Water activity is a critical parameter that determines the shelf life of products. It is a very important measurement to maintain the chemical and microbiological stability of the food, to improve the shelf life of products. Measuring water activity enables us to predict which microorganism is or is not a potential source of spoilage. Most bacteria do not multiply at water activities below 0.91 and most moulds do not multiply below 0.80 [1]. The a_w factor controlling the type and number of microorganisms in cheese plays a vital role in respect of safety and also affect the metabolic pathways leading to flavor development in steamed cheese [16]. Measuring the water activity is an important Critical Quality Control (CQC) step during cheese making as well as storing [45].

Each type of cheese may have a slightly different technological process and subsequent water activity level. It is important for manufacturers and companies to be aware of the differences and treat each cheese variety with the quality and care it deserves. Measuring a_w of cheese essentially gives the manufacturer control of the cheese process [4].

In context with the above mentioned, the goal of this study was statistical reporting and visualization of results of water activity analysis and salt content in cow´s milk Parenica cheeses from various Slovak dairy producers.

MATERIAL AND METHODS

Samples of fresh non-smoked and smoked Parenica cheeses made from cow´s milk were analysed monthly during the 10-month period. Samples no. 2, 4, 5 and 7 were obtained directly from dairy producers of small size – farm dairy, other samples (no. 1, 3, 6 and 8) were taken from dairy producers of medium size – industrial dairy. The characteristics of analysed samples are given in the Table 1.

mg/l (milligrams per liter Chloride) or mg % (milligrams percentage) salt as Sodium Chloride.

The water activity was analysed on the Fa-st lab apparatus (O.K. Service, BioPro). The water activity meter Fa-st lab uses the hygrometric technology that is also called dew point technology. During the measurement, the instrument shows the measurement time, the sample temperature, a_w mean, and a_w flash when a flash is activated. a_w mean corresponds to the mean of the last 40 instantaneous measurements done during the last 20 seconds.

All chemicals and standards were analytical grade and obtained from Sigma Aldrich.

To compare dairy producers in terms of the consistency of salt content and water activity of their products, the coefficient of variation (cv) was used.

Table 1. Specification of the analysed smoked and non-smoked Parenica cheeses from Slovak dairies

Cheese sample	Cow´s milk	Cheese characteristics	Declared parameters
1	pasteurized	semisoft, semi-skimmed	dry matter: min. 45% fat in dry matter: 35% salt: max. 2.5%
2	non-pasteurized	semisoft, semi-skimmed	undeclared
3	pasteurized	semisoft, semi-skimmed	dry matter: min. 46% fat in dry matter: 40%, salt: max. 2%
4	non-declared	soft, whole	undeclared
5	pasteurized	Bio Parenica	dry matter: min. 41% fat in dry matter: 45% salt: max. 2%
6	pasteurized	semisoft, semi-skimmed	fat in dry matter: 37% salt: max. 2%
7	non-pasteurized	semisoft, semi-skimmed	fat in dry matter: 45% salt: max. 4.5%
8	pasteurized	undeclared	dry matter: min. 35% fat in dry matter: 44% salt: max. 2%

Analysis of salt content and the water activity were realized on the fresh cheese samples (A) and subsequently after 7 days of storing in the original packing at 4 °C (B). The expiration dates of the cheeses, listed by the producers, varied from 7 to 28 days. Together 320 cheese samples were analysed in triplicates.

The salt content (based on the chloride concentration) was measured on the Chloride analyser M 926 (O.K. Service, BioPro). It is an instrumental analog of "Argentometry", the traditional titrimetric methods using Silver Nitrate reagent. The analyser automatically titrates chloride ions by passing a known constant current between two silver electrodes which provides a constant generation of silver ions. The sample volume is 0.5 ml and results are displayed on a digital readout in

The hierarchical configuration of the experiment required a multilevel model to analyse differences within the water activity and salt content. Statistical computing in R language [38] with using the nlme package [7] was used for these linear mixed models (LMM). Gaussian (normal) distribution was applied therefore in both dependent variables the continual measurements were characterized by symmetric errors that were located in the sufficient distance from the logical limits (from zero). Both models have used the following independent variables: time of storing (A/B), type of cheese (non-smoked/smoked), size of dairy producer (small/medium size), and their interactions. Random factor reflected data correlation in replicated measurements in each cheese within each locality (locality/cheese/measurement). Random factor

also allowed random variability of individual slopes versus population slopes for individual differences in time (A/B). In both cases it was necessary to reflect and include an account for heteroskedasticity between the measurements in a different time (A/B). Analysis of salt required also heteroskedasticity between size of dairy producers (small/medium). Both models were fitted with a restricted maximum likelihood (REML) approach. The level of significance was set to $p<5$ %.

RESULTS

To produce a stable and sensory attractive cheese there should be monitoring performed many physico-chemical, sensory, and microbiological analysis during the production according to the HACCP regulations. Among others, the a_{w-}value and salt content measurement.

In the context of the above, two parameters – salt content and water activity were measured in smoked and non-smoked Parenica cheese immediately after sampling from 8 dairies (A) and subsequently after 7 days of storage (B) in the packed state at 4 °C.

The results of salt content as well as water activity in fresh Parenica cheeses are presented in Table 2.

The higher content of NaCl was found in fresh Parenica cheese from small farms (non-smoked: 2.51 ±1.12 g/100g, smoked: 1.97 ±0.89 g/100g). The average salt content in cheeses from industrial dairies was 1.65 ±0.34 g/100g (non-smoked) and 1.96 ±0.43 g/100g (smoked). Results showed lower variability of salt content in cheeses from industrial dairies.

In our research differences in salt content between dairy producers were mainly in producer no. 5 with the highest overall values of salt content, and in producer no. 7 with the lowest (Figure 1). Except for producer no. 6, we can see general higher salt content in non-smoked cheeses.

From the variability perspective (Figure 2), we can see a higher inconsistency of fresh, non-smoked cheeses in producer no. 7 and lowest in no. 4. After 7

Table 2. Salt content and water activity of fresh non-smoked and smoked Parenica cheeses from Slovak dairies

Parameter	Dairy	Cheese sample	Min.	Max	Mean ±SD	cv (%)
NaCl (g/100 g)	small (farm)	non-smoked	0.75	4.52	2.51 ±1.12	44.62
		smoked	0.50	4.04	1.97 ±0.89	45.17
	medium (industrial)	non-smoked	1.03	2.60	1.65 ±0.34	20.61
		smoked	1.21	3.62	1.96 ±0.43	21.94
aw	small (farm)	non-smoked	0.917	0.975	0.956 ±0.02	2.09
		smoked	0.911	0.975	0.943 ±0.02	2.12
	medium (industrial)	non-smoked	0.910	0.971	0.960 ±0.02	2.08
		smoked	0.908	0.969	0.952 ±0.01	1.05

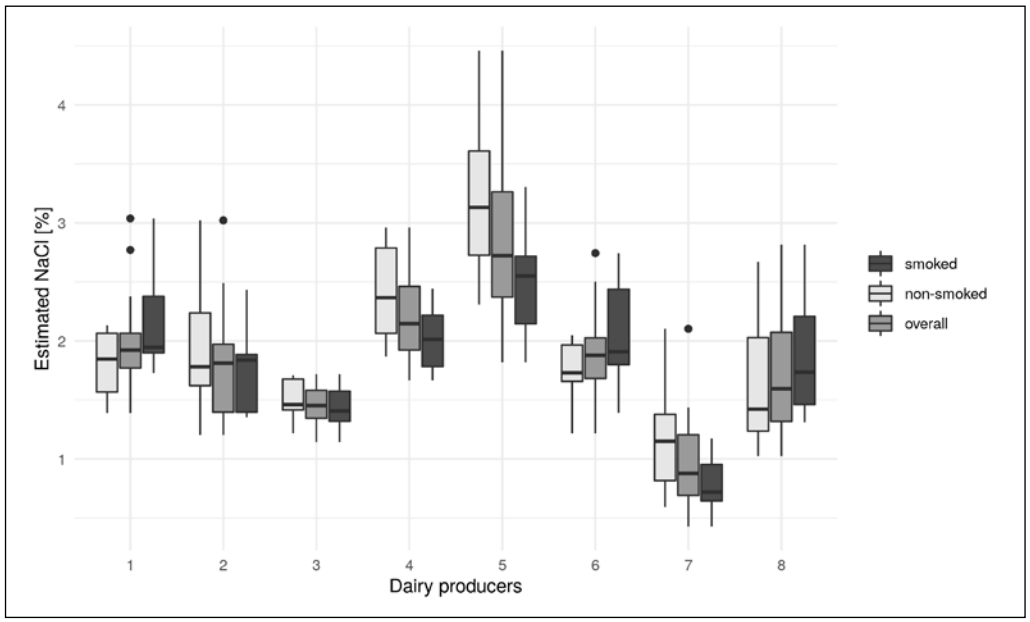

Figure 1. Distribution (median with the 25th and 75th percentiles) of salt content values in measured Parenica cheeses for smoked, non-smoked and overall (smoked+non-smoked) per every dairy producers

days of measurements, was the highest variation in no. 8. For smoked kinds was variation relative consistent, except for extreme small variation in fresh samples from producer no. 2. Heteroscedasticity between A and B was found in the small cheesemakers in comparison with the medium ones.

Generalized results from LMM showed significant differences between the main effect of the storing (fresh cheese A vs. stored B) ($F_1 = 11.89$; $p = 0.0010$) as well as interaction between measurements in different storage length and type of cheesemaker (small/medium producer) ($F_1 = 6.51$; $p = 0.013$) and interaction between measurements in different storage length and type of cheesemaker and type of cheese (smoked/non-smoked) ($F_2 = 5.95$; $p = 0.0041$). What suggests that the salt content is changing depending on all three factors.

The overall mean content of NaCl after storing (B) is lower than in fresh samples (A) by 0.704 unit per 1% NaCl. This global decline can be seen in all categories except non-smoked cheeses from medium-size producers (Figure 3).

The water activity of smoked Parenica cheese ranged from 0.908 to 0.975 and of non-smoked from

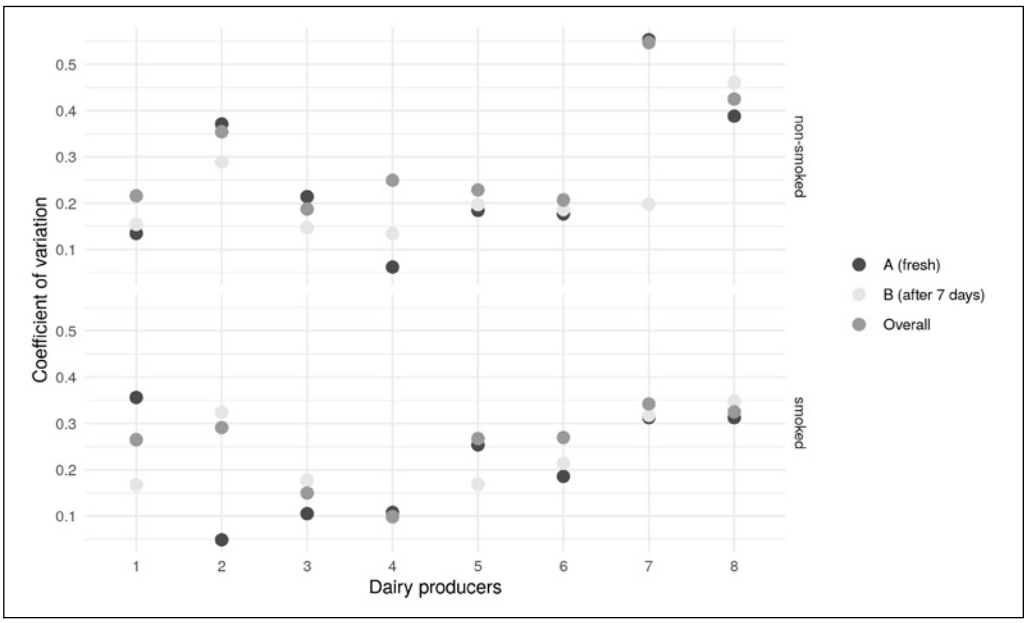

Figure 2. Variability of salt content values in measured Parenica cheeses for fresh (A), after 7 days storing (B) and overall (A+B) per dairy producers

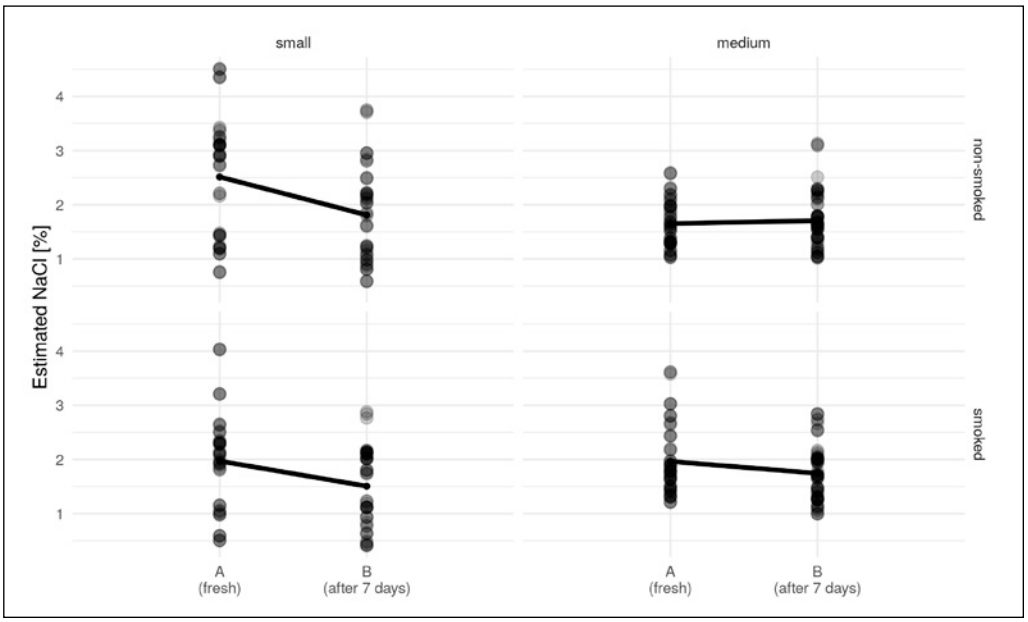

Figure 3. Salt content values with marginal mean differences between fresh (A) and after 7 days storing (B) for smoked and non-smoked Parenica cheeses depending on the producer size (small/medium)

0.910 to 0.975. The mean value of water activity in non-smoked Parenica cheeses taken directly (A) from small dairies was 0.956 ±0.02. A slightly higher value was found in cheeses from the medium dairies (0.960 ±0.02). The values of the coefficients of variation were also comparable (2.09 and 2.08%). In smoked versions of cheese were found slightly lower values of water activity (0.943 ±0.02 – small dairies and 0.969 ±0.01 – medium dairies). Differences in water activity (a_w) between small and medium-size dairy producers were not significant (p>0.05), however, small variability was observed. The lowest water activity values were found in the cheeses from the dairy producer no. 2 and the highest a_w had cheeses from the producers no. 3 and 7 (Figure 4). There is also an overall higher value of a_w in non-smoked cheeses.

From Figure 5 we can see that variability depends not only on the dairy producer, but also on the type of Parenica cheese (smoked; non-smoked) and its freshness (A; B).

In the case of smoked, fresh cheese was the highest variability observed in producer no. 7 and the lowest in no. 5 and 3. In, after 7 days measurements (B), were most inconsistency in producer no. 6 and least in no. 3.

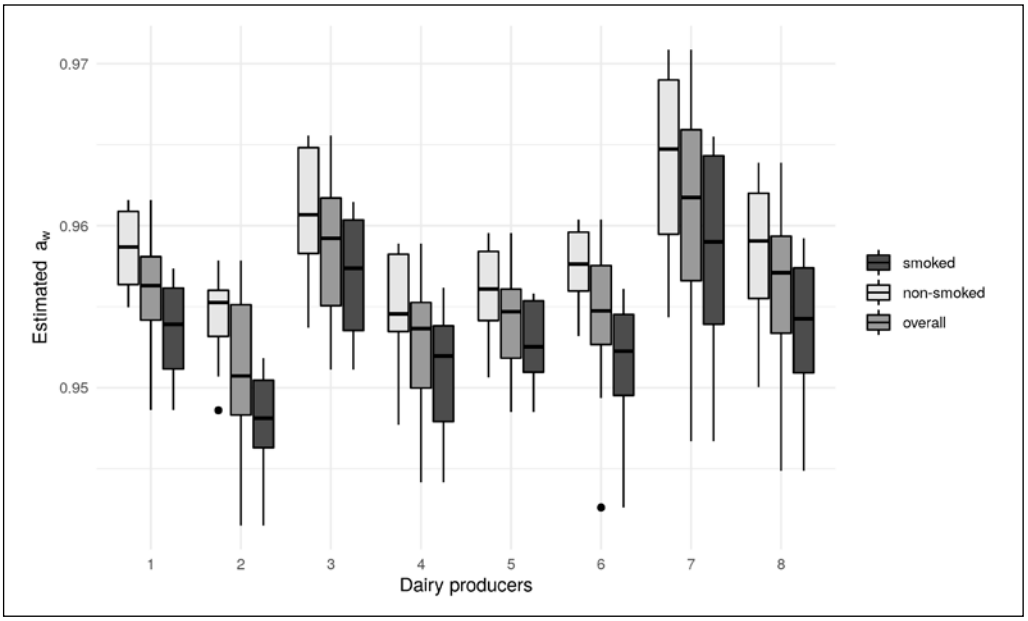

Figure 4. Distribution (median with the 25th and 75th percentiles) of water activity values in measured Parenica cheeses for smoked, non-smoked and overall (smoked+non-smoked) per every dairy producers

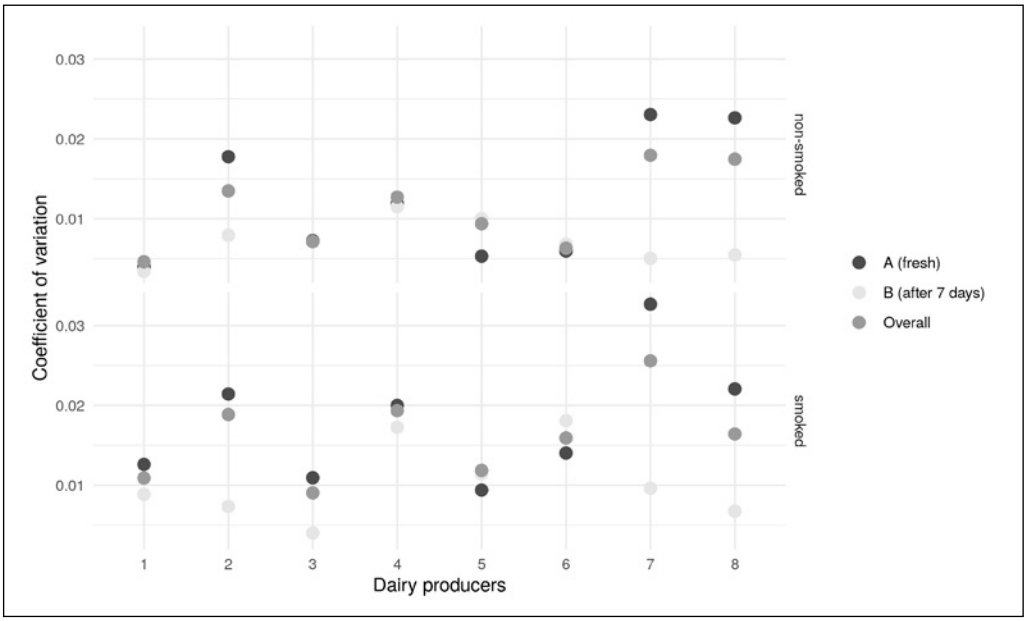

Figure 5. Variability of water activity values in measured Parenica cheeses for fresh (A), after 7 days storing (B) and overall (A+B) per dairy producers

For non-smoked, fresh cheese, we can see the highest variability in no. 7 and 8 and lowest in no. 1. After 7 day´s measurements (B), was the highest variability in no. 4 and lowest again in producer no 1.

Overall consistency (showing the smallest differences between fresh and 7 days old Parenica cheese) was the highest in producer no. 1, for non-smoked and no. 3 for smoked cheese. Contrariwise highest overall variability was in no. 7 for smoked and non-smoked cheese.

From Figure 5 we can also see an obvious general decline in variation between fresh and 7 days old cheese, except producers no. 5 and 6, were on the contrary variability in increases.

After accounting for variations between dairy producers and heteroskedasticity between freshness, result from LMM showed a significant general increase between samples A/B ($F_1 = 6.5$; $p = 0.0129$) and between kinds of Parenica cheese ($F_1 = 5.7$, $p = 0.0196$) (Figure 6).

The overall mean of water activity in B samples is by 0.0059 higher than in A samples and by 0.0046 higher for non-smoked samples than for smoked. Although an interaction between these variables was not significant, we can suggest slightly higher values of water activity after 7 days, rather in smoked Parenica cheese than in non-smoked.

Dependence between the water activity and the salt content showed to be antagonistic. With increasing, salt content decreased water activity values approximately by decreased 0.0038 unit per 1% of NaCl.

DISCUSSION

The quality of the traditional product may be different from farm to farm and dairies [44]. This claim is in line with the results published in similar studies in which there were significant differences in traditional product quality detected *Čuboň* et al. [7].

Measurement of the salt content of cheese is an important quality control step in cheese production. In cheese making, salt is highly relevant due to several factors, including microbiological control through the reduction of water activity, participation in cheese syneresis and mineral balance, regulation of biochemical processes, and contribution to taste [34].

Salt content is limited by the legislation of the Slovak Republic to 2.5 g/100g [35]. Each manufacturer in our research declared on the packaging label the dry matter, fat, and salt content. The most manufacturers reported a salt content of max. 2% (Table 1). The Parenica cheeses from two small dairy producers exceeded the declared values of salt content as a whole during the entire experimental period. 33% of the non-smoked cheeses and 25% of smoked cheeses were in accordance with data declared on the package.

The variation of the salt content of the non-smoked Parenica cheeses from small producers can be explained as follows. In industrial dairies, milk is standardized in fat content, the process of acidification of the curd is controlled by pH measurement. In this way, it is possible to ensure a more or less constant value of dry matter content or very low variation in dry matter content. The production of the cheese is continuous and thoroughly controlled. After the steaming, the curd is pulled mechanically, by

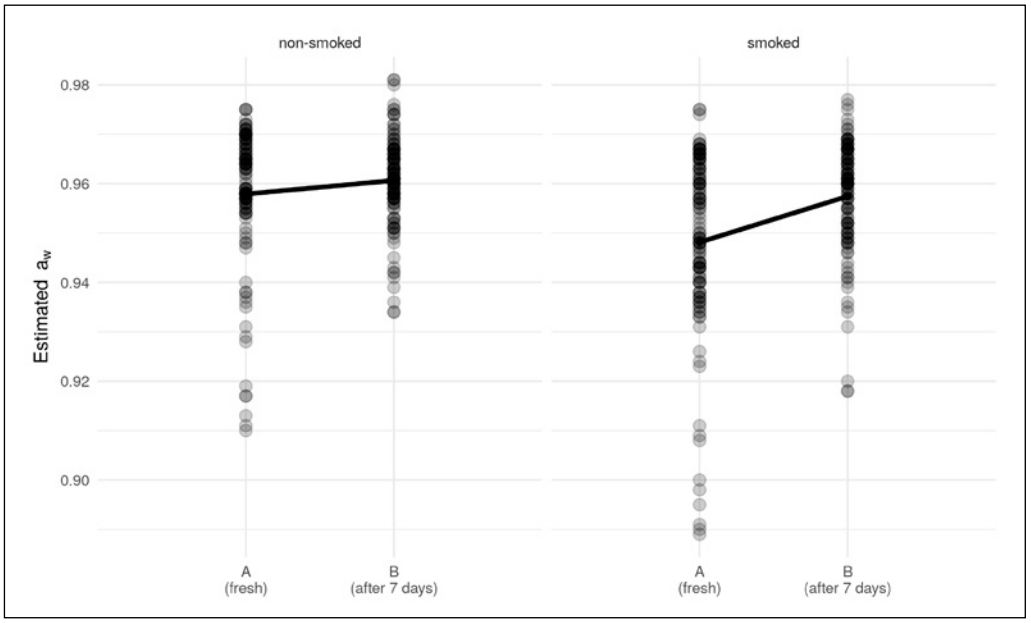

Figure 6. Water activity values with marginal mean differences between fresh (A) and after 7 days storing (B) for smoked and non-smoked Parenica cheeses

machine, and pass through the brine in which the salt content is always adjusted before the processing. As the production is machine-made, the cheese is in brine for the same time.

In the case of farmer's Parenica cheeses, the milk is not standardized before the production, which means that during the year the cheese has a fat-varying content, which is also reflected in the dry matter variation. Obviously, the fatter parenica cheeses may have lower water content and hence the salt content will vary in such cheeses. The curd is steamed and pulled by hands and put in the brine bath where the cheese is also cooled. Farmers usually do not have special hydrometers to adjust the salt content of the brine, so they prepare the brine intuitively. This results in the fact that the cheese does not usually have a constant salt content and that is also the reason for the variation of the salt content. In the case of smoked cheese, other factors that cause variations in the quality of the cheeses are taken into account.

In industrial dairies, they have a smoke chamber equipped with a generator of the smoke that is subsequently blowing and circulating into the chamber where the cheeses are placed on the grates. During the controlled process, the temperature and smoking time can be adjusted and maintained. This is also reflected in the colour of the smoked cheese, which is uniform. While this method of smoking also increases dry matter, our measurements also show that the change in dry matter varies minimally. In addition to one of these dairies, *Ducková* et al. [8] found that the increase in dry matter is not statistically significant.

In farm production, cheeses are smoked in chambers without a smoke generator and usually do not have temperature and smoking time regulators. This explains why some Parenica cheeses were smoked more and some less. Another factor is that they do not control the temperature. As a result of higher temperatures, more fat is released from the cheeses, which of course must also be reflected in the dry matter. The salt that is water-soluble, is not removed with fat, and regarding this fact, the cheese of these producers is saltier.

The steamed cheeses may be salted during the steaming process (a hot salt solution is used as the steaming water), the salt may be added to the steamed dough at the exit of the steaming machine or cheeses may be salted in a cold salt bath, to keep their shape [43, 47].

When large amounts of salt are added to curd during cheese manufacture, the ability of starter bacteria to metabolize lactose to lactic acid is impaired, which would also contribute to stabilizing the cheese pH [31].

In the research of *Estrada* et al. [18] was found that the whole cheese reached a homogeneous salt distribution at 180 days of ripening. Brining conditions did not have an influence on the rate of salt penetration, but on the final sodium chloride (NaCl) content. Cheese with higher salt content (3%) showed increased proteolysis and lipolysis as compared to cheese with lower salt content (2.2%).

Ducková et al. [8] analysed Parenica cheeses from farm dairy and found that no samples of non-smoked parenica cheese and only 33.3% of smoked Parenica cheeses were in accordance with a declared salt content of 2%. The mean value of salt content was in non-smoked cheeses 3.12 ±0.73% and in smoked cheeses 2.62 ±0.73%. However, the coefficient of variation for both sample types was relatively high (23.44% and 27.85%, respectively).

On the contrary, *Maľová* et al. [28] stated that the Slovak samples of smoked and non-smoked steamed cheeses were in accordance with the content of salt declared by the producer on the package and their contents ranged from 1.5% to 2.5%.

The maximum limit of NaCl content of 3%, which is stated in the national specification of traditional Slovenská Parenica, is published by the Industrial Property Office of the Slovak Republic and Official Journal of European Commission [10, 26]. It is important to note that our cheeses were made from cow milk – without trade name Slovenská.

Čuboň [6] found that the NaCl content in traditional Slovak steamed-formed Parenica cheese made from lump cheese was 1.82 ±0.42% (after 1st week of maturing) and 1.58 ±0.28% (after 3rd week of maturing).

Compared to other traditional cheeses produced in Slovakia, the salt content of traditional Parenica cheese is lower. The NaCl content of traditional Slovenský oštiepok cheese was 2.85 ±0.96% and in traditional Slovenský korbáčik cheese after the first week of cheese ripening it was 3.78 ±0.33% and after the second week of cheese ripening it was 2.93 ±0.76% [44].

Sodium chloride is traditionally added to cheeses as a preservative and to improve flavor. However, there is considerable evidence that high salt (sodium chloride) intake has been linked to health complications [2, 17, 20]. For these reasons, it is necessary to regularly monitoring whether food producers comply with the legal limits set for the application of salt in food production.

There are two ways to influence water activity. The water content, more specifically the amount of "free" water, and the amount of solutes can be controlled to a great extent by the cheesemaker. The further factors to set the water content are such as syneresis, the course of acidification, and the structure of curd grain [41].

As was studied from various sources [21, 23, 34] cheese varieties have typical values of a_w. During the cheese production, the a_w-value and salt content

must be carefully considered for optimal cheese quality. For most cheese varieties, the salt to moisture ratio is the most important and easy to control parameter to influence the water activity [29].

It is well documented that pathogens grow more easily in cheese with higher moisture, high pH, and low salt content [42]. Milk enzymes and starter cultures gradually hydrolyse milk compounds and lower the water activity. Such transformations are relatively small in fresh and soft cheese but very distinctive in semi-hard and hard cheese. The treatment with salt and the loss of water during the storage have an additive effect to lower the water activity [46].

Influence of NaCl on the water activity (a_w) of cheese was studied by many other authors as *Roos* [39], *McSweeney* [32], *McSweeney* [33], *McCarthy* et al. [30], *Hanauer* et al. [22], *McSweeney* [34], *Estrada* et al. [18], etc.

CONCLUSIONS

Production of traditional cheese specialties in Slovakia is not negligible. The methods of milk processing have developed and changed considerably. Today, cheeses are produced in large quantities on automatic production lines in dairies and these products have balanced quality and product safety is regularly monitored. However, small-scale production continues in small farms, private dairies, or households where they have their own milk obtained from livestock. It is retail companies – small dairies – that produce most of the national cheese specialities, such as Slovenská bryndza, Slovenský oštiepok, or Slovenská parenica.

Our results of the salt content lead to the assumption that more frequent problems with maintaining of salt content were found in Parenica cheeses produced in small dairies. Higher salt content can have a negative effect on the health of consumers. Dependence between the water activity and the salt content showed to be antagonistic. With increasing, salt content decreased water activity values approximately by decreased 0.0038 unit per 1% of NaCl.

The results showed that measuring the physical-chemical parameters during the cheese-making process is important for the product of standard quality. It can be concluded that especially the small producers can have probably problem in noncompliance with the technological processes, non-implementation of standardized procedures and underestimation of hygiene regulations.

All establishments, irrespective of capacity and produced range, must meet the basic requirements of the so-called "Hygiene package" of the European Union. They must comply with the legal regulations in the field of technological milk processing and must not underestimate the regular control of quality and hygiene-health parameters. The Association of Sheep and Goat Breeders in Slovakia has developed a hygiene manual based on HACCP principles for small producers which contain technological procedures for the production of these traditional products. A lot of training activities should be performed by government or professional associations focused on ensuring traditional cheese production.

Conflict of interest

The authors declare no conflict of interests.

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ORIGINAL ARTICLE

LEAD AND CADMIUM IN INFANT MILK AND CEREAL BASED FORMULAE MARKETED IN NIGERIA: A PROBABILISTIC NON-CARCINOGENIC HUMAN HEALTH RISK ASSESSMENT

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ABSTRACT

Background. There has been concern on the likelihood of contamination of infant formulae and consequent health risk in children.

Objective. This study has assessed and compared the lead and cadmium levels in infant formulae commonly consumed in Nigeria with international regulatory limits. It has also compared the estimated daily intake with Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Proposition 65 Provisional Tolerable Daily Intake.

Material and Methods. Lead and cadmium levels in 26 different brands of infant formulae purchased from stores in Port-Harcourt, Rivers state, Nigeria in January 2018 were assayed by Atomic Absorption Spectrophotometry.

Results. The lead and cadmium levels in milk based infant formulae ranged from 0.61-3.50 mg/kg and 0.01-0.55 mg/kg respectively whereas the range of the lead and cadmium levels in the cereal and cereal mix based were 0.29-1.95 mg/kg and 0.02-0.37 mg/kg, and 0.47-2.34 mg/kg and 0.001-0.46 mg/kg respectively. The mean lead level in the milk-based formulae (1.49 0.89 mg/kg) was slightly higher than other groups of formulae but the difference was not significant ($p<0.05$). The mean level of cadmium (0.17 mg/kg) in milk-based infant formulae was higher than levels in cereal and cereal mix but there was no significant statistical difference ($p<0.05$) between the samples. The lead and cadmium level in milk, cereal and cereal mixed based infant formulae were above the food safe limits.

Conclusions. The consumption of infant formulae may add to the body burden of cadmium and lead of children with attendant public health implication. Regular monitoring and safety assessment of metals contamination of these infant formulae is advised.

Key words: *infant formulae, cadmium, lead, risk assessment, regulatory toxicology, child health, Nigeria*

INTRODUCTION

Lead is a known potent neurotoxin and reproductive toxin with permanent irreversible effects and the brain of infants and children are particularly vulnerable to its deleterious effect [7, 47]. Cadmium is a cumulative toxin and given its very long half life in the body, even very low exposure in children is associated with neurodevelopmental defects [11]. Cadmium is listed by the International Agency of Research on Cancer (IARC) as a category 1 carcinogen and has been shown to cross the placenta [4, 24]. Cadmium is primarily toxic to the kidney and can also cause bone mineralization [45]. Food contamination is a regular source of exposure to heavy metals like cadmium and lead [43] and health concern associated with this duo known for their multiorgan (neurotoxic, nephrotoxic, reprotoxic, etc.) in infant and children food was recently emphasized in French total diet study [24, 46]. The elevated blood lead levels among children in Nigeria are known to be multifactorial [32, 38, 54]. Infant formulae, candies and pediatric syrups sold in Nigeria have been reported to be contaminated with lead and cadmium [22, 35].

The California Office of Environmental Health Hazard Assessment [8] has set a new and stricter

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maximum allowable oral dose level of 0.5 μg/day for lead and 4.1 μg/day for cadmium (also called Proposition 65) with respect to the reproductive toxicity of lead and cadmium. So far, the maximum allowable limits of lead in foods are premised on its ubiquity and unavoidability due to its natural occurrence in soil. There is still a challenge to fine tune a realistic achievable limit given the emerging evidence of its deleterious cognitive effects even at much lower and commonly-observed exposure levels [7, 47]. The actual dietary intakes of these metals should be estimated and compared with corresponding toxicological reference intake such as the provisional tolerable daily intake (PTDI) and provisional tolerable weekly intake (PTWI) in order to assess the risk to children's health arising from the presence of these metals in food. The extent of exposure of children to contaminants in food is still patchy due to the scarcity of child-specific data on food consumption.

In the absence of Nigerian regulatory standards for lead and cadmium in baby foods and infant formulae, this study has examined the levels of lead and cadmium contamination in milk, cereal and cereal mixed based infant formulae sold in Nigeria and their comparisons with some international regulatory limits namely US FDA (CFR Title 21 – FDA) [50], International Diary Federation (IDF) [21], Joint FAO/WHO Food Standards Programme Codex Committee 2016 [17], US DA & China National Food Standard GB 2762-2012 [9]. This work has also compared the estimated daily intake (EDI) of lead and cadmium from these infant formulae with the California Office of Environmental Health Hazard (Proposition 65) and Joint FAO/WHO Expert Committee on Food Additives (JECFA).

MATERIALS AND METHOD

Sampling

Twenty-six samples of different brands of commonly consumed infant formulae (age range from birth up to first year of life and above) in Nigeria, were purchased from stores in Port-Harcourt, Rivers state, Nigeria in January 2018. These 26 different brands which represent about 65% of infant formulae in Nigerian market were divided into three types with group codes as milk based infant formula coded as: M1 to M9, Cereal based coded as: C1 to C7 and Cereal mix based coded as: CM1 to CM10. These included infant formulae and follow-on formulae samples; soy-based infant formulae; milk and rice-based products for infants; rice gruel, wheat gruel and mixed cereal for infant (all products sold as powder) vegetable meals and fruit-based desserts.

Infant formulae preparation and determination of metals

The infant formulae samples (1–2 g) were weighed with plastic materials to prevent contamination with metals and digested using the hot-block digestion as in our previous publication described [36]. Briefly, approximately 9 mL of 65% concentrated nitric acid (HNO_3) and 3 mL of perchloric acid was added in a ratio of 3:1 prior to heating and the solution was transferred to a hot plate and heated to a temperature of 120°C for about 5 h. The sample was introduced into an oven under a temperature that was gradually increased by 10°C every 60 min until the final temperature of 450°C was attained with white ashes obtained after 18 h. Afterwards the samples were left to cool, and the white ash was dissolved in 5 mL of 1.5% nitric acid (HNO_3) and a final volume of 25 mL was made by addition of deionized water. Metal concentrations were assayed with atomic absorption spectroscopy (Model 205, Buck Scientific, East Norwalk, CT, USA). Samples were analyzed in triplicates [36].

Quality control

The instrument was recalibrated after every ten runs. The analytical procedure was checked using the spike recovery method (SRM). A known standard of the metals was introduced into already-analyzed samples and re-analyzed. The results of the recovery studies for lead and cadmium were more than 97% [36]. The relative standard deviation between replicate analyses was less than 4%. The limit of detection (LOD) for lead and cadmium was 0.005 mg/kg, with blank values reading as 0.00 mg/kg in deionized water with an electrical conductivity value of less than 5 μS/cm. The limit of quantification (LOQ) for lead and cadmium was 0.04 mg/kg.

Estimation of risk of exposure to lead and cadmium from infant formulae

The non-carcinogenic health risks of a single metal via consumption of infant formulae were assessed based on the estimated daily intake (EDI) which can be calculated as the following from equation *(Bargellini* et al.) [5] :

$$EDI = C \times DIR / BW$$

where: EDI - is the estimated daily intake of lead and cadmium (mg/kg/day); *C* - is the mean concentration of lead or cadmium in infant formulae samples (mg/kg); DIR - is the daily intake rate of infant formulae per kg body weight per day; BW- is the body weight.

The estimated daily intake (EDI) of lead and cadmium in different infant formulae was calculated using the actual lead and cadmium levels from this study to multiply the recommended consumption/ intake rate by manufacturers divided by body weight. The EDI was calculated for 0-12 months old of 3.5 - 10.5 kg body weight [45]. For the exposure assessment of lead and cadmium in infant formulae the percent of lead and cadmium of provisional tolerable daily intake (PTDI) and California Office of Environmental Health Hazard (Proposition 65) were calculated using the lowest and highest EDI of lead and cadmium as shown in Table 2. The estimated daily intake were compared with the California Office of Environmental Health Hazard (Proposition 65) [8] and WHO Provisional Tolerable Daily Intake (PTDI) of lead and cadmium. The highest and lowest value of EDI from all the three types of infant formula were used to calculate the percent lead and cadmium contribution to PTDI.

Percentage of lead and cadmium in infant formulae to California Office of Environmental Health Hazard (Proposition 65) [8] and WHO Provisional Tolerable Daily Intake (PTDI) were calculated by the following:

$$\% \text{ PTDI} = (\text{EDI} / \text{PTDI}) \times 100$$

$$\% \text{ Proposition 65} = (\text{EDI} / \text{Proposition 65}) \times 100$$

This study compared the percentage of estimated daily intake (EDI) with California Office of Environmental Health Hazard (Proposition 65) [8] and WHO Provisional Tolerable Daily Intake (PTDI) set by JECFA of lead and cadmium to characterize the extent of exposure.

Statistical analysis

Statistical analysis was carried out using the Graphpad prism version 6.5. All results were expressed as mean ± standard deviation (SD). The data were analyzed using one-way analysis of variance (ANOVA) and Turkey *post hoc* test at 95% confidence level. $P<0.05$ was considered as statistically significant.

RESULTS

Table 1 shows the brand names, types, manufacturers and packaging, of lead and infant formulae. Three types of infant formulae were used namely: milk, cereal and mixed cereal based with aluminum foil as the commonest packaging. Most of the infant formulae were manufactured in Nigeria with less than ten percent imported.

The comparisons of the Pb and Cd levels in the infant formulae with international regulatory limits namely US FDA (Pb 0.4 mg/kg, Cd 0.1 mg/kg),

Table 1. Types, brand names, manufacturers, packaging of infant formulae involved in this study

Sample code	Brand name of infant formulae	Type	Manufacturer	Packaging
M_1	Pre NAN	Milk based	Nestle	Aluminum
M_2	Sma	Milk based (starter powdered milk)	Wyeth nutritionals	Aluminum
M_3	Peak Baby	Milk based (starter powdered milk)	Friesland campina	Aluminum
M_4	Lactogen 1	Milk based (starter powdered milk)	Nestle	Aluminum
M_5	Nutristart	Milk based (follow-on milk)		Hard cardboard external with formulae in Al foil
M_6	Sma Pro	Milk based (follow-on milk)	Nestle	Aluminum
M_7	Sma Pro	Milk based (starter milk)	Wyeth nutritionals	Aluminum
M_8	Cowbell Tina	Milk based (follow-up milk)	Cowbell	Aluminum
M_9	My Boy Eldorin	Milk based		Aluminum
C_1	Nestle Cerelac	Cereal based	Nestle	
C_2	Nestum Baby Cereal	Cereal based	Nestle	Hard cardboard external with formulae in Al foil
C_3	Golden Country Baby Cereal	Cereal based	Sun mark Ltd.	Aluminum
C_4	Friso Gold	Cereal based	Friso Gold	Aluminum

C_5	Cerelac Infant Cereal	Cereal based		Aluminum
C_6	Pediasure: Grow And Gain	Cereal based		Hard cardboard external with formulae in Al foil
C_7	Aptamil: Organic Rice	Cereal based		Hard cardboard external with formulae in Al foil
Cm_1	Nutriban	Cereal based (Mix)	Nutrimental	Hard cardboard external with formulae in Al foil
Cm_2	Ridielac (Vina Milk)	Cereal based (Mix)	Vietnam dairy products	Hard cardboard external with formulae in Al foil
Cm_3	Nutriben	Cereal based (Mix)	Alter farmacia	Hard cardboard external with formulae in Al foil
Cm_4	Ninolac	Cereal based (Mix)	Ninolac maroc SARL	Hard cardboard external with formulae in Al foil
Cm_5	Gerber	Cereal based (Mix)	Nestle	Plastic
Cm_6	Heinz Dinners	Cereal based (Mix)	Heinz	Hard cardboard external with formulae in Al foil
Cm_7	Gerber Oatmeal	Cereal based (Mix)	Nestle	Plastic
Cm_8	Heinz Summer Fruits	Cereal based (Mix)	Heinz	Plastic with aluminum lining
Cm_9	Nutrilac Infant Cereal	Cereal based (Mix)		Hard cardboard external with formulae in Al foil
Cm_{10}	Cerelac Infant Cereal	Cereal based (Mix)		Aluminum

International Diary Federation (IDF) (Cd 0.026 mg/kg), Joint FAO/WHO Food Standards Programme Codex Committee (Pb 0.01 mg/kg), US FDA & China National Food Standard (0.02 mg/kg) [9] is shown in Figure 1.

The highest level of lead was found in formulae milk based M_9 (3.50 mg/kg) and the highest-level cadmium was in cereal based mix brand Cm_8 with a concentration of 0.46 mg/kg. The lead and cadmium levels in milk based infant formulae ranged from 0.61-3.50 and 0.01-0.55 mg/kg respectively whereas the range of the lead and cadmium levels in the cereal and cereal mix based were 0.29-1.95 and 0.02-0.37, and 0.47-2.34 and 0.001-0.46 mg/kg respectively. At least

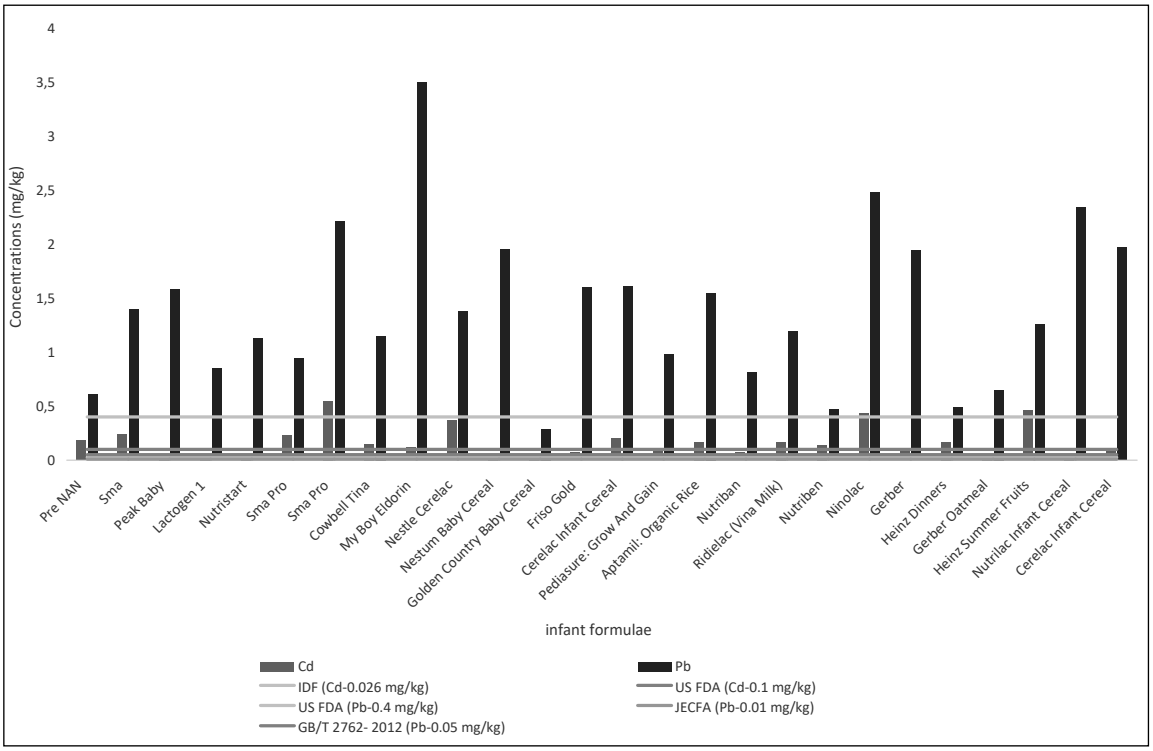

Figure 1. Comparison of mean concentrations (mg/kg) of lead and cadmium in infant formulae to international standard regulatory limits

96.15% of infant formulae violated the permissible limit of 0.4 mg/kg Pb set by US FDA, while 53.85% of infant formulae were above the US FDA maximum limit of 0.1 mg/kg Cd. International Dietary Federation (IDF) established a maximum limit of 0.026 mg/kg for Cd which was violated by 76.92% of infant formulae. One hundred percent of the infant formulae violated the limit set of 0.01 mg/kg and 0.05 mg/kg set by JECFA and GB/T2762-2012 for Pb respectively.

The mean levels of lead and cadmium in the three different groups of infant formulae is shown in Figure 2. The mean lead level in the milk-based formulae (1.49 0.89 mg/kg) was slightly higher than other kinds of formulae but the difference was not significant ($p<0.05$). The mean level of cadmium (0.17 mg/kg) in milk-based infant formulae was higher than levels in cereal and cereal mix but there was no significant statistical difference ($p<0.05$) between the samples.

The estimated daily intake EDI (mg/kg bw/day) of lead and cadmium in different groups of infant formulae in different age groups (0-12 months) of body weights 3.5 – 10 kg and daily intake rates (DIR) of 0.075 - 0.135 kg is shown on Table 2. The EDI of lead and cadmium in the milk-based infant formulae ranged from 0.02 to 0.035 mg/kg bw/day and 0.002 to 0.004 mg/kg bw/day respectively. The EDI of lead and cadmium in the cereal-based infant formulae ranged from 0.018 to 0.032 and 0.002 to 0.003 mg/kg bw/day

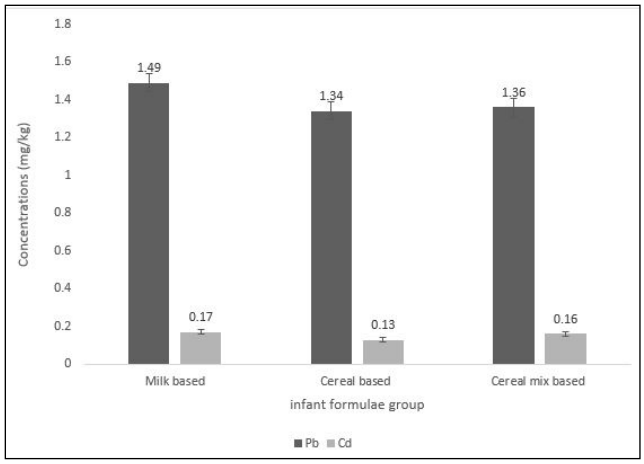

Figure 2. Mean concentrations of lead and cadmium (mg/kg) in different infant formulae groups

Table 2. Estimated daily intake (mg/kg bw/day) of lead and cadmium in different types of infant formulae

Age	DIR (kg)	Bw (kg)	Pb	Cd
Milk based				
0-2 weeks	0.075	3.5	0.032	0.004
2-4 weeks	0.1	4.2	0.035	0.004
2 months	0.11	4.7	0.035	0.004
4 months	0.145	6.5	0.033	0.004
6 months	0.135	7.5	0.027	0.003
6-12 months	0.135	10	0.020	0.002
Cereal based				
0-2 weeks	0.075	3.5	0.029	0.003
2-4 weeks	0.1	4.2	0.032	0.003
2 months	0.11	4.7	0.031	0.003
4 months	0.145	6.5	0.030	0.003
6 months	0.135	7.5	0.024	0.002
6-12 months	0.135	10	0.018	0.002
Cereal mix based				
0-2 weeks	0.075	3.5	0.029	0.003
2-4 weeks	0.1	4.2	0.032	0.004
2 months	0.11	4.7	0.032	0.004
4 months	0.145	6.5	0.030	0.004
6 months	0.135	7.5	0.024	0.003
6-12 months	0.135	10	0.018	0.002

DIR- daily intake rate, Bw - Body weight (*Sipahi* et al 2014)

Table 3. Percentage of lead and cadmium in infant formulae to California Office of Environmental Health Hazard (Proposition 65) and WHO Provisional Tolerable Daily Intake (PTDI)

	Pb	Cd		Pb	Cd
California Office of Environmental Health Hazard (Proposition 65) (µg/kg bw/day)	0.5	4.1	WHO Provisional Tolerable Daily Intake (PTDI) (µg/kg bw/day)	3.6	0.83
Age	% California Office of Environmental Health Hazard (Proposition 65) (µg/kg bw/day)		Age	% PTDI	
0-2 weeks	6385.7	88.85	0-2 weeks	886.9	438.9
2-4 weeks	7095.2	98.722	2-4 weeks	985.5	487.7
2 months	6974.5	97.042	2 months	968.7	479.4
4 months	6647.7	92.495	4 months	923.3	456.9
6 months	5364	74.634	6 months	745.0	368.7
6-12 months	4023	55.976	6-12 months	558.8	276.5

whereas in the cereal mix based, the EDI of lead and cadmium 0.018 to 0.032 and 0.002 and 0.004 mg/kg bw/day respectively.

Table 3 shows the percentage of lead and cadmium in infant formulae to California Office of Environmental Health Hazard (Proposition 65) [8] and WHO Provisional Tolerable Daily Intake (PTDI) in the different age group. The highest EDI of lead and cadmium across all age groups were used to calculate the percentage. For all the age groups, the percentage of lead and cadmium in infant formulae to California Office of Environmental Health Hazard (Proposition 65) was highest in age group 2-4 weeks. The EDI of lead and cadmium exceeded the PTWI set by JECFA across all age groups. The highest percentage contribution was in age group 2-4 weeks with a percentage value of 985.5% for lead while cadmium percentage contribution was highest in 2-4weeks with a value of 487.7%.

The mean levels of lead and cadmium in milk-based infant formulae were found to be highest of the three types of infant formulae considered in this study. Some workers have reported lead levels in various groups of infant formulae in the range of 1 ng/g to 10 ng/g [13]; 143 ng/g [18] and even 450 ng/g [52]. Lead and cadmium levels in the infant formulae in this present study were higher than the threshold values of Asian standards (0.05 mg/kg, 0.026 mg/kg) [9, 21]. The level of lead in all the infant formulae samples were higher than the limits in both China (0.05 mg/kg) and the EU (0.02 mg/kg). Similarly, the lead levels in this study were higher than those from Turkey, Ethiopia, Egypt, Pakistan and Canada [1, 12, 23, 28, 45]. Cadmium was observed in all our infant formulae samples to exceed the values reported in Pakistan (0.0042-0.0123 mg/kg), Iran (0.0403-0.058 mg/kg), and EU market [25, 29].

Table 4. Percentage of highest and lowest estimated daily intake (EDI) to California Office of Environmental Health Hazard (Proposition 65) and WHO Provisional Tolerable Daily Intake (PTDI) of lead and cadmium

	Pb	Cd		Pb	Cd
California Office of Environmental Health Hazard (Proposition 65) (µg/kg bw/day)	0.5	4.1	WHO Provisional Tolerable Daily Intake (PTDI) (µg/kg bw/day)	3.6	0.83
EDI Range (mg/kg bw/day)	% California Office of Environmental Health Hazard (Proposition 65) (µg/kg bw/day)		EDI Range (mg/kg bw/day)	% PTDI	
Highest EDI	0.035 (7000%)	0.004 (97.5%)	Highest EDI	0.035 (972%)	0.004 (481.9%)
Lowest EDI	0.018 (3600%)	0.002 (48.7%)	Lowest EDI	0.018 (500%)	0.002 (241%)

The percentage of highest and lowest estimated daily intake (EDI) to California Office of Environmental Health Hazard (Proposition 65) and WHO Provisional Tolerable Daily Intake (PTDI) of lead and cadmium are shown on Table 4. For the percent PTDI, highest and lowest EDI of lead and cadmium were 500 - 972% and 241 - 481% respectively.

DISCUSSION

Food is an important source of metal exposure in man. The poorly developed food surveillance system in sub-Saharan Africa does not have the capacity to monitor food sources to effectively safeguard public health. This study has revealed unacceptable levels of lead and cadmium in commonly consumed infant formulae sold in Nigeria with values exceeding the maximum permissible limits of various regulatory bodies.

Although exceeding the PTDI occasionally does not indicate a health risk per se; but in the present study the estimated daily intakes were more than the California Office of Environmental Health Hazard (Proposition 65) and JECFA PTDI. Infant formulae considered in this study may be adding to the body burden of lead and cadmium in children. The low body weights of infants and of course higher nutrient requirements predispose them to higher sensitivity to dietary contaminants because of the very efficient luminal absorption prior to full development of organs like liver and kidney tend to exaggerate the toxicity over a life time [37]. Higher blood lead levels have been seen in formula-fed children compared with the breast-fed counterparts [37].

Developing nations are particularly at high risk of lead poisoning and carry the highest burden of this hazard [6]. Childhood lead poisoning is a commonplace in Nigeria with long-term neurological impairment including blindness and deafness [14, 32, 40]. Infants

and children are considered as a separate group in risk assessments due to their different consumption patterns and lower body weights.

Consequently, higher gastrointestinal absorption in combination with the consumption of infant formulae composed of ingredients with higher lead and cadmium levels than breast milk, may result in increased internal lead and cadmium exposure of these infants. One study in Nigeria, reported elevated blood lead levels in children with mean 8.974.8 μg/dL, the median 7.8 μg/dL, and the range 1–52 μg/dL [32]. Other studies based in Nigeria recorded mean BLL 11 μg/dL for children in Kaduna, a medium-size city in Northern Nigeria [33, 34] and 15±1.4 μg/dL for 218 children in Jos [38]. Blood lead level BLL may impair erythropoiesis by inhibiting protoporhyrin synthesis and impairing iron absorption thus increasing the risk of anaemia [19]. It is also a well-known neurotoxin and irreversibly affects the brain of infants [7]. Bioavailability plays a significant role in the assessment and management of risk posed by food contaminants. Although intestinal absorption of heavy metals is proportional to the concentration in the diet, there are other factors that may influence the rate of intestinal absorption and organ retention of heavy metals like lead and cadmium [42]. Since humans who have low iron status are likely to absorb more cadmium and lead than those with adequate iron status the consumption of infant formulae with high levels of lead and cadmium may have exaggerated health implications in anemic children.

The estimated daily intake of lead (for 6-12 months old infant weighing 10 kg) was calculated as 0.02 mg/kg bw/day which is 558% of PTDI in violation of the PTDI of lead recommended by JECFA. The percent PTDI of lead and cadmium from this study for infants aged 0-2 weeks to 6-12 months ranged from 558.8 to 985.5 and 276.5 to 487.7 respectively. Cadmium intake should not exceed 7 μg/kg of body weight over the course of one-week i.e. the PTWI [15]. The estimated daily intake and % PTDI of cadmium for different infant formulae at all age groups from 0-12 months were observed to be above the JECFA PTDI. Infant exposure to cadmium is very deleterious because cadmium elimination half-life from the blood is approximately 100 days and this accumulates in the proximal tubules of kidneys leading to kidney dysfunction [24]. The European Food Safety Authority (EFSA) in 2009 set for cadmium a Tolerable Weekly Intake (TWI) at 2.5 μg/kg body weight [24].

In addition to cadmium accumulation in the proximal tubules of kidneys, cadmium can also cause considerable changes in the renal cortical levels of many metals to constitute nephrotoxicity with early biomarkers as urinary beta-2-microglobulin which may be associated with enzymuria, aminoaciduria, glycosuria, hypercalciuria, hyperphosphaturia [39]. Hypercalciuria, hyperphosphaturia can ultimately lead to urolithiasis. Decline in bone mineral density is the main feature of cadmium toxicity in the skeleton [2, 31]. According to the International Agency of Research on Cancer cadmium is classified as a Category 1 carcinogen, and placental transfer has been demonstrated [3, 4].

In sub-Sahara Africa and many other places, infant formulae constitute vital source of food for infants and small children. It is of immense importance that the nutritional quality and status of infants and small children foods are not compromised. Raw materials especially agricultural produce, storage, packaging to tainted tap water constitute the likely sources of contamination of the infant formulae [26, 41].The risk assessment in this study has not considered the percentage of lead and cadmium that is bio-accessible in human body from infant formula and furthermore levels of lead and cadmium represent only the batch we studied.

CONCLUSION

Infant formulae sold in Nigeria may add to the body burden of lead and cadmium in children. Given the health implications of lead and cadmium, every effort should be made by manufacturers to reduce their levels to an achievable practical minimum and should be compelled to indicate the levels of these contaminants in the infant formulae labels stating the possible dangers particularly in nursing infants with renal insufficiency or other disorders that might favor accumulation of these metals.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

ASSESSMENT OF CADMIUM AND LEAD CONTENT IN TOMATOES AND TOMATO PRODUCTS

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ABSTRACT

Background. Cadmium and lead are completely redundant in the human body and any amount of these elements ingested poses a risk of adverse health effects. In non-occupational exposure the highest amount of xenobiotics enters the body with food. Valued for their taste, universal culinary application and health benefits tomatoes and tomato products are consumed almost every day by a large proportion of society. In order to protect consumers' health it is very important to monitor cadmium and lead content in food products.

Objective. The aim of the study was the health assessment of cadmium and lead content in tomatoes and tomato products in relation to their acceptable maximum levels in the relevant legislation.

Material and methods. Fresh fruits of the tomato plant and tomato products (juices, purées, concentrates, sauces) were analysed. Heavy metal content (Cd, Pb) was determined by flameless atomic absorption spectrometry (AAS). Before the AAS determination the samples were subjected to pressure mineralisation using microwave energy.

Results. Cadmium and lead contents in the studied food products were within the allowed range (the maximum level of cadmium and lead contamination of tomatoes is 0.05 mg/kg and 0.1 mg/kg of fresh mass). The limit for cadmium was exceeded only in a canned tomato concentrate (0.064 mg/kg of fresh mass). The average cadmium content in raw tomatoes and tomato products was: 0.017 mg/kg fresh weight, and lead 0.021 mg/kg fresh weight.

Conclusions. Despite the low cadmium and lead contamination of the study samples of tomatoes and tomato products, it seems desirable to constantly monitor the content of these elements in food due to their ability to accumulate in the body and the risk of adverse health effects developing after many years of exposure, even to small doses.

Key words: *tomatoes, vegetable products, heavy metals, contamination*

STRESZCZENIE

Wprowadzenie. Kadm i ołów są całkowicie zbędne dla organizmu ludzkiego, każda ilość tych pierwiastków jaka zostaje przyjęta przez człowieka stwarza ryzyko wystąpienia niekorzystnych skutków zdrowotnych. W narażeniu pozazawodowym największa ilość ksenobiotyków trafia do organizmu drogą pokarmową. Pomidory oraz ich przetwory cenione za smak, uniwersalne zastosowanie w kulinariach, a także właściwości prozdrowotne są spożywane niemal każdego dnia przez dużą część społeczeństwa. Ze względu na zapewnienie bezpieczeństwa zdrowotnego konsumentów, niezwykle ważne jest monitorowanie zawartości kadmu i ołowiu w produktach spożywczych.

Cel badań. Celem pracy była ocena zawartości kadmu i ołowiu w pomidorach i przetworach pomidorowych w aspekcie zdrowotnym, w odniesieniu do maksymalnych dopuszczalnych limitów tych zanieczyszczeń w badanych produktach.

Materiał i metody. Analizie poddano świeże owoce pomidora zwyczajnego, a także ich przetwory (soki, przeciery, koncentraty, sosy pomidorowe). Zawartość metali ciężkich (Cd, Pb) oznaczono za pomocą bezpłomieniowej spektrometrii absorpcji atomowej (AAS). Przed analizą AAS próbki poddawano mineralizacji ciśnieniowej przy użyciu energii mikrofalowej.

Wyniki. Zawartość kadmu i ołowiu w badanych produktach mieściła się w zakresie wartości dopuszczalnych (maksymalny poziom zanieczyszczenia kadmem i ołowiem pomidorów wynosi 0,05 mg/kg i 0,1 mg/kg świeżej masy). Limit kadmu został przekroczony tylko w koncentracie pomidorowym w puszkach metalowych (0,064 mg/kg świeżej masy). Średnia zawartość kadmu w surowych pomidorach i produktach pomidorowych wynosiła: 0,017 mg/kg świeżej masy, a ołowiu 0,021 mg/kg świeżej masy.

Wnioski. Pomimo niewielkiego zanieczyszczenia badanych próbek kadmem i ołowiem, celowym wydaje się dążenie do stałego monitorowania zawartości tych pierwiastków w żywności, ze względu na ich zdolność do kumulacji w organizmie i możliwość wystąpienia niekorzystnych skutków zdrowotnych po wielu latach narażenia, nawet na małe dawki.

Słowa kluczowe: *pomidory, przetwory warzywne, metale ciężkie, zanieczyszczenie*

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INTRODUCTION

Tomatoes are one of the most popular vegetables among consumers. Their annual production dominates among that of vegetables produced in EU countries and totalled 21 million tonnes in 2015. According to Statistics Poland, a statistical agency of the Polish government, the annual tomato consumption per capita is approximately 10 kg, which has been a stable figure over the last 15 years [23, 26].

The large popularity of tomatoes is associated with their wide application in many dishes: raw tomatoes are added to salads and processed tomatoes are the base for juices, sauces, purées, concentrates or jams. An increase in consumer awareness of the role of proper nutrition in a healthy lifestyle has led to the introduction of new products with functional additives on the market in which tomato processing by-products are used [21].

Tomato processing makes it possible to preserve healthy nutrients during periods of limited access to fresh vegetables (the winter season). After processing tomatoes are characterised by better health properties than their raw counterparts due to higher bioavailability of lycopene present in these vegetables [29].

Lycopene is a red and orange pigment belonging to the carotenoid group and is one of the strongest antioxidants. Anti-cancer properties are attributed to tomatoes such as protection against cell damage as well as reduction of prostate, lung, breast and gastrointestinal cancer and leukaemia risk. Multiple studies also demonstrate that the consumption of lycopene-rich products reduces cardiovascular risk [4, 17].

Apart from lycopene, tomatoes are also a rich source of vitamins C, E, D, K, E, B1, B2, B3 and B6, folic acid, fibre, macro- and micronutrients (potassium, sodium, phosphorus, magnesium, calcium, iron, copper, zinc and manganese), phenolic acids, flavonoids and phytosterols [3, 24].

Apart from high nutritional value, food should primarily be safe, both in the microbiological and chemical sense. Pollution emitted from industry, transport and waste contribute to the deterioration of the environment, including soil, and, consequently, food. Heavy metals are particularly dangerous for human health. They are characterised by a long life and are not subject to degradation or decomposition, which makes them linger in the environment for many years [27].

Cadmium and lead are classified as heavy metals and are completely redundant in the human body; any amount of them which is ingested by a human poses a risk of adverse health effects. These elements have the ability to accumulate in the body, particularly in bone tissue, but also in solid organs such as the liver and kidneys, which take part in their detoxification [14, 18].

The high toxicity of heavy metals manifests itself primarily in damage to internal organs (liver, kidneys) or abnormalities in the nervous system, hematopoietic system (anaemia), bones and cardiovascular system (arterial hypertension) [22].

Heavy metal exposure can cause abnormalities in protein synthesis and ATP production, which can lead to disease, including cancer. The toxic effects of elements discussed in the present study on the body depend on their chemical form, solubility in body fluids and lipids as well as the duration of exposure and resistance of a given body [18].

Cadmium is classified by the International Agency for Research on Cancer (IARC) as a Group 1 human carcinogen and teratogen. It can also cause anaemia, calcium and vitamin D metabolism disturbances, iron, copper and zinc deficiencies as well as abnormal function of reproductive glands and the immune system [28].

The harmful effects of lead on the human body involve mainly the nervous system: neurological and psychological abnormalities, IQ decrease, memory deterioration, aggression, easy fatigue and muscle paralysis occur. Kidneys and the liver as well as the cardiovascular and respiratory systems become damaged. Lead also causes reproductive disorders, has toxic effects on the embryo and is probably carcinogenic to humans according to IARC (Group 2A) [15].

The consumption of food contaminated with heavy metals is the primary route of exposure for humans. It is estimated that lead and cadmium enter the human body with plant products in approximately 60% and more than 80%, respectively [19].

Tomatoes and tomato products are valued for their taste, fragrance and health benefits. As such, they constitute part of everyday diet of a large proportion of society. The determination of heavy metal content in products which are consumed frequently can help in the assessment of consumer exposure to these xenobiotics.

The aim of the study was the health assessment of cadmium and lead content in tomatoes and tomato products in relation to their acceptable maximum levels in the relevant legislation.

MATERIALS AND METHODS

The study material were tomato fruits (*Solanum lycopersicum* L.) and tomato products (juices, purées, concentrates, sauces). The products were purchased in a retail chain shop and at a local vegetable market. The studied tomatoes differed in terms of variety, method of cultivation (conventional or organic farming) and

county of origin. Tomato products were selected for the study based on different levels of processing and types of packaging (glass jars, metal cans, carton). For unprocessed tomatoes, cadmium and lead content was assayed both in fresh and dry mass of the product. Table 1 presents detailed characteristics of the study material.

Table 1. Characteristics of the studied products

No	Product	Kind, variety, type	Country of origin	Form
1.	tomato concentrate	concentrate in a glass jar	Poland	fresh mass
2.	tomato concentrate	canned concentrate	Poland	fresh mass
3.	cherry tomato	cherry tomato, "Sasari" variety size 25–35 mm	Poland	dry mass
4.	cherry tomato	cherry tomato, "Sasari" variety size 25–35 mm	Poland	fresh mass
5.	small-fruited strawberry tomato	cherry tomato, "Sunstream" variety	Poland	dry mass
6.	small-fruited strawberry tomato	cherry tomato, "Sunstream" variety	Poland	fresh mass
7.	"Koralik" cherry tomato	organic cherry tomato	Poland	dry mass
8.	cherry tomato on a twig	cherry tomato on a twig size 15–40 mm	Poland	dry mass
9.	cherry tomato on a twig	cherry tomato on a twig size 15–40 mm	Poland	fresh mass
10.	"Ożarowski" pink tomato	pink tomato, "Ożarowski" variety	Poland	dry mass
11.	"Lima" tomato	"Lima" variety	Poland	dry mass
12.	orange tomato	"Blush Tiger", organic tomato	Poland	dry mass
14.	mini plum tomato	"Vespolino" variety, size 20–30 mm	Poland	dry mass
15.	mini plum tomato	"Vespolino" variety, size 20–30 mm	Poland	fresh mass
13.	oblong plum tomato	"Romanella" variety	Poland	fresh mass
16.	oblong plum tomato	"Romanella" variety	Poland	dry mass
17.	large-fruited "Gargamel" tomato	"Gargamel" variety	Poland	dry mass
18.	"Jawor" tomato	organic tomato	Poland	fresh mass
19.	dried tomatoes with spices	dried tomatoes with spices (salt, garlic, oregano)	Turkey	dry mass
20.	dried tomatoes	organic dried tomato	Turkey	dry mass
21.	canned tomatoes	canned tomatoes (whole)	Greece	fresh mass
22.	tomato purée	tomato purée in a carton	Greece	fresh mass
23.	tomato juice	tomato juice, glass container	Poland	fresh mass
24.	tomato sauce	canned sauce	Poland	fresh mass
25.	tomato sauce (ketchup)	mild tomato sauce (ketchup), plastic container	Poland	fresh mass

The analysed products were subjected to pressure mineralisation using microwave energy (Magnum II microwave mineraliser, ERTEC, Poland). Heavy metal content (Cd, Pb) was determined by flameless atomic absorption spectrometry (AAS) using the SavantAA Sigma spectrometer (GBC, Poland) and the GF 3000 graphite furnace (GBC, Poland). Assays were performed at the following wavelengths: 228.80 nm for cadmium and 217.00 nm for lead. For every sample measurements were taken three times and the final result is the arithmetic mean of the three readings.

Due to the differences in water content between different tomato varieties, some of the study material was subjected to drying. Samples of unprocessed (raw) tomatoes were dried in a laboratory forced air circulation oven (UFP 500, Memmert, Germany) at 60ºC (preliminary drying for 2 hours) and then at 105ºC until solid mass was obtained. The dried material was ground using a laboratory vibration mill (LWM-s, Testchem, Poland). Samples prepared in this way were subjected to mineralisation according to the methodology described above; subsequently, cadmium and lead content was assayed. Blind samples were also analysed.

Statistical analysis was performed on the results obtained using Microsoft Excel 2010 spreadsheet software; means and standard deviations were determined.

RESULTS

According to the Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs (OJ L. 364, 20.12.2006, as amended) [7], the maximum level of cadmium and

lead contamination of tomatoes is 0.05 mg/kg and 0.1 mg/kg of fresh mass, respectively.

Cadmium and lead content in raw tomatoes and tomato products was within the allowed range. The limit for cadmium (0.05 mg/kg of fresh mass) was exceeded only in a canned tomato concentrate (0.064 mg/kg of fresh mass), (Table 2).

The highest heavy metals content in products subjected to drying was found in the "Koralik" cherry tomato (cadmium: 0.071 mg/kg of dry mass; lead: 0.139 mg/kg of dry mass), "Lima" tomato (cadmium: 0.216 mg/kg of dry mass), an organic orange tomato (cadmium: 0.206 mg/kg of dry mass), "Gargamel" tomato (cadmium: 0.076 mg/kg of dry mass), dried tomatoes with spices from Turkey (0.055 mg/kg of dry mass) and organic dried tomatoes from Turkey (0.107 mg/kg of dry mass), (Table 3).

In order to compare the results regarding cadmium and lead content in products subjected to drying with the maximum contamination levels set in the Commission Regulation (EC) the results were converted to fresh mass (based on mean water content in a given product). After these calculations no product was found to have cadmium and lead levels exceeding the allowed values.

DISCUSSION

Vegetables with edible fruits, such as tomatoes, contain relatively the lowest amounts of heavy metals.

Table 2. Cadmium and lead content in raw tomatoes and tomato products, in mg/kg of fresh mass

No	Product	Cd	Pb
1.	tomato concentrate (glass jar)	0.034 ± 0.004	< LOQ
2.	tomato concentrate (can)	0.064 ± 0.003	0.005 ± 0.001
3.	cherry tomato	0.028 ± 0.035	0.020 ± 0.002
4.	small-fruited strawberry tomato	0.006 ± 0.001	0.018 ± 0.002
5.	cherry tomato on a twig	0.007 ± 0.001	0.014 ± 0.004
6.	mini plum tomato	< LOQ	0.022 ± 0.003
7.	oblong plum tomato	< LOQ	0.007 ± 0.001
8.	"Jawor" tomato (organic farming)	< LOQ	< LOQ
9.	canned tomatoes	0.006 ± 0.001	0.006 ± 0.001
10.	tomato purée	0.024 ± 0.004	< LOQ
11.	tomato juice	0.018 ± 0.003	< LOQ
12.	tomato sauce	0.008 ± 0.001	0.006 ± 0.001
13.	tomato sauce (ketchup)	0.013 ± 0.002	< LOQ

Explanatory notes:
The table shows mean values ± standard deviations; LOQ for Pb = 0.004 mg/kg; LOQ for Cd = 0.004 mg/kg

Table 3. Cadmium and lead content in tomatoes subjected to drying, in mg/kg of dry mass

No	Product	Cd	Pb
1.	cherry tomato	< LOQ	< LOQ
2.	small-fruited strawberry tomato	< LOQ	< LOQ
3.	"Koralik" cherry tomato (organic farming)	0.071 ± 0.004	0.139 ± 0.004
4.	cherry tomato on a twig	0.012 ± 0.004	< LOQ
5.	"Ożarowski" pink tomato	0.015 ± 0.002	< LOQ
6.	"Lima" tomato (organic farming)	0.216 ± 0.004	0.026 ± 0.002
7.	orange tomato (organic farming)	0.206 ± 0.012	0.031 ± 0.040
8.	mini plum tomato	< LOQ	< LOQ
9.	oblong plum tomato	< LOQ	< LOQ
10.	large-fruited "Gargamel" tomato	0.076 ± 0.004	0.087 ± 0.002
11.	dried tomatoes with spices	0.055 ± 0.002	< LOQ
12.	dried tomatoes (organic farming)	0.107 ± 0.005	< LOQ

Explanatory notes:
The table shows mean values ± standard deviations; LOQ for Pb = 0.004 mg/kg; LOQ for Cd = 0.004 mg/kg

However, considering frequent consumption of tomatoes by consumers, these vegetables can pose a health risk since heavy metals can accumulate in the body and adverse health effects can be delayed by many years [5].

The use of mineral fertilisers and crop protection products in organic farming is limited to a significant extent; therefore, foodstuffs coming from organic farming should contain lower levels of heavy metals compared to conventional farming. In their study, *Ilić* et al. [11] compared cadmium and lead contents in Greek tomatoes cultivated using traditional and organic farming. Organic vegetables contained less cadmium than the conventional ones, whereas no such relationship was observed for lead. It is worth emphasising the fact that the analysed tomatoes were characterised by very low levels of both metals: lead content did not exceed 0.014 mg/kg of fresh mass, while cadmium did not exceed 0.00027 mg/kg of fresh mass.

Compared to the study cited above [11], organic tomatoes from the present study contained relatively high levels of cadmium: from 0.071 to 0.206 mg/kg of dry mass, while the content of lead was between < 0.088 mg/kg of dry mass and 0.139 mg/kg of dry mass.

Cadmium and lead content in selected organic and conventional produce was also investigated in a study by *Staniek* et al. [25]. The analysed products included cherry tomatoes in which cadmium level was found to be 0.0833 mg/kg of dry mass (organic farming) and 0.0086 mg/kg of dry mass (conventional farming). Regardless of the type of production system lead content was very low (below the method's sensitivity threshold: < 0.0005 mg/kg of dry mass). The comparison of cadmium content in tomatoes from both farming systems revealed that organic vegetables were nearly 10 times more contaminated with this element than conventional ones [25].

In their study, *Bressy* et al. [6] evaluated the content of selected elements in tomatoes of different varieties and levels of plant maturity. Cadmium content was the highest in the "Italy" tomato variety, which were at the last stage of maturation (0.21 μg/g of dry mass); in the early stage of maturity cadmium level in this type of tomato was nearly 6 times lower (0.0362 μg/g of dry mass). The comparison of the studied varieties revealed that the Khaki tomato variety contained the least amount of cadmium (< 0.0092 μg/g of dry mass). Tomatoes at the early stage of maturity in the study by *Bressy* et al. [6] contained a lower amount of cadmium than at the final stage of maturity, except for "Cherry" tomatoes for which the level of cadmium at the early stage was 0.027 μg/g of dry mass, whereas at the final stage it was < 0.008 μg/g of dry mass; this figure was the same as that in the present study.

In addition, the "Italy" variety of tomatoes was cultivated in conventional as well as organic farming systems. In both types of farming the level of cadmium in tomatoes was lower at the early stage of maturity than at the final stage. Conventional vegetables were characterised by higher levels of the investigated elements than organic ones [6].

The content of trace elements, including cadmium and lead in tomato fruits was also analysed by *Kleiber* et al. [12]. Their study aimed to determine the relationship between manganese concentration in a nutrient solution used for tomato cultivation and the levels of heavy metals, among other substances. Cadmium was found to be present in the range of 0.38–0.41 mg/kg of dry mass regardless of the manganese concentration used in the nutrient solution, while lead levels were 0.833–0.908 mg/kg of dry mass. In comparison to the results of the present study regarding cadmium and lead content in tomatoes, the mean cadmium content was nearly 2 to 25 times lower, while lead content was 6 to 32 times lower.

Vegetables become contaminated with toxic elements not only as a result of using fertilisers which may contain heavy metals, but also due to the location of the farm. High plant contamination is found in industrialised areas, along busy transport routes and near human dwellings (low-altitude emissions, municipal pollution) [13].

Osma et al. [20] compared the content of selected elements in tomatoes cultivated in various regions of Turkey, in suburban and industrial areas, among others. Cadmium content fell in the range of 0.17 to 0.40 μg/g of dry mass, while lead content was between 4.31 and 5.51 μg/g of dry mass. The researchers note that depending on the region of cultivation, washing vegetables before consumption can reduce cadmium and lead contamination: more than 5 to nearly 42 times for cadmium and nearly 2 to almost 20 times for lead [20].

Adefemi and *Awokunmi* [1] found very high levels of cadmium and lead in tomatoes in their study. Cadmium content fell in the range of 3.8–4.4 mg/kg of dry mass, while lead content was between 9.0 and 9.6 mg/kg of dry mass.

Lead contamination has also been investigated for plant products originating from the Legnica and Głogów region, Poland, in which a copper foundry operates. Cereal grains (barley, wheat, triticale, rye), vegetables (tomatoes, carrots, beetroots, potatoes, cabbage, parsley root and leaves) and fruit (apples, pears) were analysed. The limits were not exceeded in tomato, carrot, beetroot, potato, cabbage, apple and pear samples. Excessive amounts of lead were found in cereal grains and parsley leaves (135% and 436% of the maximum level, respectively) [19].

Apart from environmental sources (air, water, soil), food can also become contaminated with toxic elements during technological processes or storage (packaging contamination). Canned foods are a group of products to which heavy metals can migrate from packaging. Canning is a very popular form of food storage due to a long shelf life without refrigeration. Canned foods are ideal as a basis for meal preparation away from home and on trips; canned foods are also resistant to damage during transport [16]. Research investigating heavy metal content in canned foods has been conducted in a city in northern Jordan, among other places, where the following products were analysed: tomato sauce (ketchup), string beans, carrot and pineapple juice. The mean cadmium content in tomato sauce was 0.49 mg/kg of dry mass and that of lead was 2.95 mg/kg of dry mass [16].

A study in Nigeria demonstrated the following heavy metal contamination levels in canned tomatoes: lead from 0.1301 to 0.1701 mg/kg of dry mass and cadmium from 0.0091 to 0.0115 mg/kg of dry mass [8]. In another study conducted in the same country cadmium concentration in a canned tomato concentrate was below the level of detection, while lead concentration was found to be between below LOQ and 0.68 mg/kg of dry mass [9].

In their study, *Al-Maylay* et al. [2] found that canned tomato concentrates contained between 0.0 and 0.23 mg/kg of dry mass of cadmium and between 86 mg/kg and 138 mg/kg of dry mass of lead [2].

The comparison of the results of different studies on heavy metal concentrations in canned foods indicates a high level of variability of cadmium and lead content in these products; this may be determined by the source of food itself, the type of material from which a can is produced, the type of material used to join different parts of a can (soldering) and the presence of rusting inside the can body [29].

In order to protect consumers' health safety it is of particular importance to monitor toxic element content in food products. The results of research assessing cadmium and lead contents in tomatoes and tomato products indicate a low health risk associated with consuming this group of foodstuffs.

Due to the ability of heavy metals to accumulate in the human body and cause adverse health effects many years after exposure, it is advisable to conduct further studies aiming to determine the magnitude of exposure to the elements discussed. Since any amount of heavy metals in food is a potential health hazard, it seems desirable to strive for the minimisation of their presence in food.

CONCLUSIONS

The analysed tomatoes and tomato products were characterised by cadmium and lead levels below the maximum values set in the relevant Commission Regulation (EC). The limit for cadmium was slightly exceeded only in a canned tomato concentrate.

In order to protect consumers' health it is desirable to monitor the levels of cadmium and lead in food products, particularly those which constitute part of everyday diet.

Conflict of interest

The authors declare no conflict of interests.

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ORIGINAL ARTICLE

DIFFICULTIES AND FACTORS INFLUENCING PURCHASE DECISION. THE PERSPECTIVE OF FAMILIES WITH CHILDREN WITH AUTISM SPECTRUM DISORDERS ON A GLUTEN-FREE AND CASEIN-FREE DIET. PRELIMINARY STUDY

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ABSTRACT

Background. Gluten-free and casein-free diet is frequently used in the support of therapy of children with autism spectrum disorders. In addition, many parents restrict the consumption of simple sugars for their children.

Objective. The aim of this paper was to understand factors influencing purchase decision in case of family with children with autism spectrum disorders on gluten-free and/or casein-free and/or sugar-free diet and the difficulties associated with this type of nutrition.

Material and methods. The study covered a group of 40 families with children with autism spectrum disorders (32 boys and 8 girls) aged 3 to 10 years. Data were collected with questionnaire included questions concerning overall characteristics of caregivers, availability of foods used in the special diet, factors affecting decision on the purchase of products, difficulties in maintaining the child nutrition method.

Results. The factors having strongest impact on parents' decisions on the purchase of products were product composition, presence of a certificate confirming the absence of gluten and/or milk and taste values. Exclusion diet constituted a considerable obstruction for traveling, social gatherings and resulted in conflicts with family and the environment. The limited range of healthy gluten-free, casein-free and sugar-free foods, low taste quality and unsatisfactory quality impeded purchase and preparation of varied meals.

Conclusions. The surveyed parents were aware consumers, paying attention primarily to product composition and safety. The respondents were looking for healthy, organic and nutritionally valuable products with low sugar content. Further development of the gluten/casein/ sugar free products market may considerably improve certain aspects of family's life with children with autism spectrum disorders.

Key words: *children with autism, gluten-free diet, casein-free diet, purchasing decision*

STRESZCZENIE

Wprowadzenie. We wspomaganiu terapii dzieci z zaburzeniami ze spektrum autyzmu często stosowana jest dieta bezglutenowa i bezmleczna (bezkazeinowa). Wielu rodziców ogranicza także swoim dzieciom spożycie cukrów prostych.

Cel. Celem badania była ocena wymagań i preferencji konsumenckich rodziców dzieci z zaburzeniami ze spektrum autyzmu będących na diecie bezglutenowej i/lub bezkazeinowej i/lub bezcukrowej oraz trudności związanymi z takim sposobem żywienia dziecka.

Materiał i metody. W badaniu wzięło udział 40 rodziców dzieci z zaburzeniami ze spektrum autyzmu (32 chłopców i 8 dziewczynek) w wieku od 3 do 10 lat. Dane zebrano za pomocą ankiety zawierające pytania dotyczące m.in. ogólnej charakterystyki opiekunów, rodzaju stosowanej diety eliminacyjnej, dostępności środków spożywczych używanych w diecie specjalnej, trudności w utrzymaniu danego sposobu żywienia dziecka, czynników wpływających na decyzje o zakupie produktów stosowanych w diecie.

Wyniki. Czynnikami mającymi największe znaczenie dla respondentów przy zakupie produktów stosowanych w diecie dziecka były: skład produktu opisany na etykiecie, oznakowanie warunkujące wykluczenie glutenu i/lub mleka z pro-

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duktu oraz walory smakowe Dieta eliminacyjna wpłynęła negatywnie na różne aspekty życia codziennego respondentów, takie jak podróżowanie, spotkania towarzyskie, powodowała nieporozumienia rodzinne oraz konflikty z otoczeniem. Ograniczony asortyment stosowanej żywności specjalnej, niskie walory smakowe i niezadawalająca jakość zamienników żywności tradycyjnej utrudniały skomponowanie urozmaiconych posiłków.
Wnioski. Badani rodzice byli świadomymi konsumentami zwracającymi uwagę przede wszystkim na skład produktu i bezpieczeństwo. Respondenci szukali zdrowych, ekologicznych i wartościowych odżywczo produktów o niskiej zawartości cukru. Dalszy rozwój rynku produktów bezglutenowych, bezmlecznych i bezcukrowych może znacznie poprawić niektóre aspekty życia rodzin z dziećmi z zaburzeniami ze spektrum autyzmu.

Słowa kluczowe: *autyzm, dzieci, dieta bezglutenowa, dieta bezmleczna, decyzje o zakupie*

INTRODUCTION

Autism is classified among neurodevelopmental disorders, referred to as autism spectrum disorders (ASD), characterized by autism triad, including inhibition of social development, disorders and dysfunctions in verbal and non-verbal communication as well as behavioral disorders [1]. The incidence rate of ASD have been continuously increasing globally for the last several decades. According WHO globally this disorder affects 1 in 160 people worldwide [2], whereby it should be noted that estimates of the US Center for Disease Control and Prevention the number of children in the USA is diagnosed with ASD increased from approximately one in 150 children in 2000 to one in 59 in 2014 [14]. These disorders do not have a single etiologic agent, but are caused by a set of different conditions of genetic and environmental background [17]. Digestive tract changes can be one of the factors favoring occurrence and intensification of neurological abnormalities. Some authors indicate that autistic children may exhibit abnormal digestion of gluten proteins and casein leading to formation of peptides with opioid properties (i.a. β-casomorphin from milk and gliadomorphin from gluten proteins) affecting the central nervous system function [16]. Gastrointestinal symptoms in ASD children may be caused by the commonly occurring in this group hypochlorhydria and reduced secretion of the gastric juice, lower activity of amylolytic enzymes and intestinal disaccharidases as well as mucosal inflammation of the stomach and intestines. Moreover, ASD patients have increased incidence of elevated permeability of intestinal mucosa and disorders of intestinal microflora [9, 15]. A suitable nutrition of ASD patients may have a considerable role in alleviating both digestion, metabolic and mental symptoms. Thus, apart from the primary psychological, educational and pharmacological treatment, attempts at dietary interventions were made. Gluten-free (GF) and casein-free diet (CF) are most frequently used in autistic disorders therapy, excluding consumption of gluten proteins and casein. It equals abandonment of consuming wheat, rye and barley based products as well as milk and its products, and all products that may contain even traces of these ingredients. Given mentioned prevalence of autism spectrum disorder, families with children with ASD, next to a group of people with celiac disease are group potentially represents a significant customer group on the gluten-free market. A large proportion of parents also restrict consumption of simple sugars (SF-sugar-free diet) for their children, which aims at reducing overgrowth of yeasts *Candida albicans* in the intestinal flora, whose proliferation frequently disturbs ASD children functioning. There are about 50 thousand people with autism in Poland, with children representing 1/5 of that number [9]. No detailed data exist on the number of individuals with ASD in Poland and in the world using GF and/or CF diet. This is certainly an important group of consumers, but so far it has not been investigated.

The purpose of this paper is to understand factors influencing food purchase decision in case of family with children with ASD using GF, CF and/or SF diet and the difficulties associated with this type of nutrition.

MATERIAL AND METHODS

The study was carried out between July 2017 and September 2018 at the Synergis Therapy Center in Warsaw (the institution dealing with the diagnosis and therapy of children with: autism, Asperger syndrome, pervasive developmental disorder and educational problems) and during 3 therapeutic camps for ASD children organized in Wisła by the Synergis Therapy Center and the Tourist Office „Tairon" (the office in Lublin dealing with tourism and rehabilitation of disabled people). There were two criteria for inclusion in the study: 1) parents of children diagnosed with ASD (by a psychiatrist according to the ICD-10 criteria [19]), 2). children on special restriction diet – GF and/or CF and/or SF for at least 6 month.

To obtain the data, the direct PAPI (Paper & Pen Personal Interview) survey method was used. The specifically developed, anonymous questionnaire was used consisted of 16 questions. It included a combination of one option, multiple-choice and open questions concerning, i.e. general characteristics of

the caregivers (age, education, place of residence) and on the use of exclusion diets in child, availability of food products, difficulties in maintaining the method of child nutrition, factors affecting decision on the purchase of products. To assess respondents' opinions on product availability and labeling, a 5-point hedonic Likert scale was used with the associated verbal terms, where 1 meant the lowest grade (difficult access/poorly labeled) and 5 - the highest (easy access/well labeled). The survey has been disseminated by the following channels: 1. Face-to-face: face-to-face interviews were done when it was possible, 2. E-mail: the survey was sent to the respondents by e-mail (after a previous personal or telephone call). Because of ability to reach the target group the study had a pilot character and was based on 40 completed questionnaires, out of 65 target respondents (61,5% return on correctly completed surveys). All participants gave their informed consent prior to inclusion in the survey and were informed of its purpose. Elements of descriptive statistics were used to analyze and present the results.

RESULTS

The study covered a group of 40 families with children diagnosed with ASD (32 boys and 8 girls) aged 3 to 10 years (7,24± 1,47 years). The average age of boys was 7,55±1,16 years, while girls was 6,0 ±1,87years. The questionnaire was filled by mothers (aged 32 – 46), because they are mostly engaged in the purchase of products used and preparation of meals. A considerable majority of the surveyed received higher education (88.9%) and the reaming had professional (11.1%). Over 77% resided in large cities (>100 000 residents), 11.1% in medium sized cities and the equal number in small cities (≤40 000 residents). Among the respondents 90% did not belong to any associations and support groups for parents of ASD children. Parents participating in the study used various exclusion diets for their children (Table 1). The largest group (33 individuals) comprised of children using GF diet, including 10 children additionally using CF diet, and 22 on GF, CF and SF diet. The period of special diets ranged between 7 months and 7 years (49,98±21,49 months). At times, parents added an additional exclusion diet to the nutrition model of their child, e.g. after a more detailed diagnosis of an allergy or gastrointestinal intolerance. Decision on the introduction of diet occurred at different times from the moment of ASD diagnosis (Table 2). Over half of the surveyed used exclusion diet along with ASD diagnosis or within the period of 1 year from the diagnosis. Some respondents pointed out to gastrointestinal problems of the child prior to the ASD diagnosis and used the diet due to allergy to dairy products. Thirty percent of parents started a special diet 4 to 5 years after ASD diagnosis.

Table 1. Type of exclusion diet (GF-gluten-free, CF-casein-free, SF-sugar-free)

Type of diet	Number of children
GFCFSF	21 (52,5%)
GFCF	10 (25%)
CFSF	4 (10%)
SF	1 (2.5%)
GFSF	1 (2.5%)
CFSF, no wheat	1 (2.5%)
vegetarian with gluten, milk and sugar restrictions	1 (2.5%)
GFCFSF, no eggs	1 (2.5%)

Table 2. Time of introducing of the exclusion diet in relation to ASD diagnosis

Time of introducing the diet	Number of answers
Before diagnosis	4 (10%)
along with diagnosis up to 1 year after diagnosis	21 (52.5%)
1-2 years after diagnosis	4 (10%)
2-3 years after diagnosis	4 (10%)
3-4 years after diagnosis	0 (0%)
4-5 years after diagnosis	12 (30%)

The number of responses does not sum up to 40 and 100%, because sometimes subsequent diet modifications were made by parents at different times to get ASD diagnosis

A considerable majority of the respondents (72%) stated that the exclusion diet is solely used for ASD child, whereas in 17% families all members of the family switched to the special diet. In the remaining cases only mother with the ASD child or the child's neurotypical younger sibling were using the diet.

The Internet, scientific literature and health care personnel were primary sources of information on the used diet and food products (Table 3). In addition, 40% of respondents used the advice of a dietician.

Respondent's opinions were divided on the issue of labeling and access to food in the used special diet (Table 4). 25% respondents believed that access to such food products is easy, rather easy (15%) or sufficient (5%). 55% of parents were of the opposite opinion. Over 50% of the caregivers believed that GFCF and SF products are well labeled (17,5% - well, 27,5% -rather well, 7,5%- well enough), whereas the rest of the surveyed criticized this issue.

Due to the size of the sample, no in-depth analyzes were conducted regarding the customer behavior, nevertheless an important aspect of the conducted study was determination of what factors influence the

Table 3. Source of information on the used diet

Initial source of information	Number of answers
Internet	24 (60 %)
Scientific literature	22 (55%)
Health care personnel	20 (50%)
Dietician	16 (40%)
Television	11 (28%)
Celiac society	4 (10%)
Other: therapist, diagnostic center	4 (10%)

The number of responses does not sum up to 40 and 100%, because one parent could provide several answers

child or during rehabilitation camps, gathering people from all over the country, who have access to different products.

Many respondents (17.5%) looked for products without gluten-free wheat starch, due the origin of the ingredient and the concerns about the insufficient purifying starch of gluten.

An additional element affecting product purchasing decision, important for 22% of the surveyed parents, was product's price.

Only 3 persons among the surveyed stated that they look for products labeled as bio/organic, the question whether they prefer foods from organic farming was met 80% positive answers. The most frequent reason

Table 4. Opinions on availability and labeling of food products in the used diet

Evaluated aspect	Value of the positions applicable for the assigned verbal terms (nr of indications and % share of indications of a given value)					Statistical measures		
						Mean	SD	Median
Access	1- difficult	2- rather difficult	3- sufficient	4- rather easy	5- easy	3	1.43	2
	4 (10%)	18 (45%)	2 (5%)	6 (15%)	10 (25%)			
Labeling	1- poorly	2- rather poorly	3- well enough	4- rather well	5- well	3	1.38	3
	6 (15%)	13 (32.5%)	3 (7.5%)	11 (27.5%)	7 (17.5%)			

decision of the parents on selection and purchasing food products used in the exclusion diet (Table 5). For 95% of the surveyed, food product composition described on the label was important. The majority of respondents (62.5%) further noted whether the given product possesses a certificate confirming the absence of gluten or milk in its composition. Half of the parents considered taste preferences of their child. A significant factor convincing the surveyed consumers about good quality of a product was its recommendation by their acquaintances. Such 'marketing' among parents of children with ASD often takes place during therapeutic classes for the

Table 5. Factors determining the choice and purchase of food products in the exclusion diet

Factors determining the choice and purchase of food products	Number of answers
Product composition - label	38 (95%)
Gluten-free/dairy- free certificate	25 (62.5%)
Taste preferences	20 (50%)
At the recommendation of friends	11 (27.5%)
Price	9 (22.5%)
Without gluten-free wheat starch	7 (17.5%)
Other: bio/ecolabeling	3 (7.5%)

The number of responses does not sum up to 40 and 100% because one parent
could provide several answers

for selection of organic foods was the belief that this type of products is of higher quality, caused by lower contamination with chemicals and increased supervision by certification bodies. Parents, who provided negative answer to this question explained their opinion with high price of organic products (3 persons) and lack of access to such products (2 persons). Three respondents did not have opinion on organic foods.

The surveyed indicated different places for purchasing food products used in the diet. A considerable majority made purchases in 'healthy foods' shops (82.5%), at discount stores (77.5%) and on Internet (60%). Half of the parents purchased products necessary for the diet in supermarkets, and over 27% in delicatessen.

Introducing diet eliminating such popular cereals as wheat, rye, barley, often oats and dairy products requires obtaining a specialized knowledge and considerable organizational and financial effort. Such actions change life of the entire family and bring numerous difficulties (Table 6). The surveyed parents mostly complained about restricted possibilities of eating during travel. Only few restaurants, bars and hotels in Poland and abroad offer GF meals, and the additional dietary exclusions make eating out practically impossible. Even on rehabilitation camps for children with autism, where GFCFSF diet is considered, issues related to eating occur for various reasons. One of the reasons for difficulties

Table 6. Parents' experiences associated with the use of exclusion diet in their child

Difficulties in maintaining the diet	Number of answers
Eating out, during vacation, travel - lack of restaurants, bars with foods ensuring the diet, difficulties with purchasing food outside of home	23 (57.5%)
Lack of understanding from the society, diet disregarded by employees of pre-school facilities, other members of the family, e.g. grandparents or parent - primarily father (in the event of parents' divorce)	15 (37.5%)
Many products have a composition like the „periodic table", they contain many E- ingredients	15 (37.5%)
Limited range, absence of suitable quality replacement products suitable quality, it is difficult to find products labeled as GFCFSF at the same time	12 (30%)
Taste values (typically problems with bread)	11 (27.5%)
Social isolation - own food has to be brought to gatherings, celebrations	7 (17.5%)
Siblings have sweets which are not allowed in the diet - great temptation and willingness to try, peers can eat food containing gluten/milk - feeling of one's difference	6 (15%)
Cooking in line with the diet for one child and "normally" for remaining family members	4 (10%)
Purchasing with child- the child chooses forbidden products	4 (10%)
Buying in various places – no full assortment in one shop	4 (10%)
Adaptation of diet products proportions in non-diet recipes	4 (10%)
Need for self-catering	4 (10%)
Limited access to products with healthy foods in smaller towns	4 (10%)
Difficulty in preparing varied foods	3 (7.5%)
Lack of diet meals at school canteen - difficult to adapt foods to the child's daily schedule	2 (5%)
Refusing the child food that he/she knows and used to eat before introducing the diet	2 (5%)

The number of responses does not sum up to 40 and 100% because one parent could provide several answers

of the diet is the poor products range resulting from higher cost of such foods and lack of knowledge of persons dealing with gastronomy. The second aspect of this situation concerns the ASD children. They often have an extremely limited number of acceptable foods, anxiety against novelties, sensory disruption, concentrate on details, rituals resulting from the anxiety and insecurity, further intensified by new place and unknown people.

Another serious issue pointed out by parents (37.5%) was the small selection of nutritionally valuable GFCF foods with low sugar content and simple composition (with the so-called clean label). The low diversity of these products was a major nuisance to caregivers (30% of respondents) who wanted to find quality diet replacements for traditional foods. In addition, the available products, especially bread, were assessed negatively by over 27% of parents and their children in terms of taste values.

Some surveyed parents (37.5%) experienced lack of understanding from their environment, including lack of agreement on the diet, e.g. with the kindergarten personnel or family members.

Due to difference of the diet, some of the respondents (17.5%) experienced sense of social isolation, particularly during social gatherings, various celebrations, caused by the need of bringing and consuming own food. The negative experiences were also associated with purchasing products used in diet and the need of preparing meals by themselves, often cooking separately for ASD child and the rest of the family.

DISCUSSION

Following the shock and breakdown caused by ASD diagnosis for the child, parents commence the combat for improvement of her/his functioning and enabling. Having a child with ASD, a disorder with unknown etiology, and often not accepting any therapy offered by the conventional medicine, forces parents to look for other solutions. ASD children caregivers often lean toward unconventional and alternative medicine [3, 18]. GFCF diet is one of the available solutions. Scientific research has not provided evidence for the need of routinely introduction of this diet to the therapeutic protocol of ASD patients, but some parents have observed its positive impact on autistic symptoms in their children [5, 18]. An adverse effect of introducing an elimination diet in children with ASD is the risk of deficiency of some vitamins (e.g. from group B) and minerals e.g. calcium, magnesium, zinc. This makes it difficult to balance the diet and is often associated with the need to use dietary supplements [5, 6, 9, 11]. In the present study, the information on the GFCF diet was primarily obtained by the surveyed parents from such media as the

Internet, television, scientific literature, and largely from health care personnel. This issue was not further detailed in the survey, but according to discussions with the respondents, the majority of physicians recommending GFCF diet belonged to DAN! movement (Defeat Autism Now!), pioneered by Dr. *Bernard Rimland* from the Autism Research Institute. Nowadays, this group of physicians raises much controversy in the medical community. Similarly, in the study of Cornish the majority of caregivers of ASD children learned about the possibility of trying the GFCF diet in the therapy of their children in television, the Internet, parent support groups, from friends and acquaintances. However, only 8% of the surveyed indicated the medical environment as the source of information on the diet. Regarding assistance during the use of GFCF diet, respondents also placed the primary care physician and pediatrician last, and chose to use the care of a dietitian (40%) and, to a considerable extent, support group devoted to autism [4]. 17 years later, in the study of Trudeau et al. health care professionals were the most frequent information source (65%) regarding the use of elimination diets and supplements in ASD treatment, however, it is not known to what extent these data relate to the GFCF diet itself [18]. ASD children caregivers often concealed the use of unconventional medicine therapy, including GFCF from the treating physician, primarily due to the reluctance of the medical community and lack of knowledge about therapies other than traditional [3, 18]. Furthermore, *Hozyasz* et al. raises the issue of physicians rejecting the possibility of considering gluten-free diet in juvenile patients with autism, which raised frustration and feeling of loss in their parents [8].

In the present study, over half of the respondents introduced exclusion diet for their child within 1 year from the ASD diagnosis. This is typically the most intensive time in terms of the search for suitable research, specialists and therapeutic methods. 30% the surveyed parents made the decision about the diet at a later date, approx. 5 years after diagnosis. This could have been caused by too slow and/or lack of progress of the child in the conventional therapy used thus far or emergence of new problems. According to Hall and Riccio the child's functioning level is associated with selection of medical regimen, i.e. the more severe the form of autism, the more desperate caregivers are in seeking other, alternative solutions [7]. An increased length of time since the diagnosis may be associated with greater frustration among the families, giving risen to alternative medicine use.

The use of exclusion diet has a considerable impact on the daily functioning of the child with ASD and the entire family. A positive, expected effect means improved well-being of the patient, autistic symptoms alleviation, giving caregivers better motivation in overcoming the difficulties associated with maintaining the special diet [4, 5]. In the present study, areas in which the parents experienced the greatest discomfort included: eating out, travels and lack of understanding from the society. A considerable obstacle in maintaining the diet consisted in the low range of products, their low nutritional and taste values and purchasing. Limitations to traveling and social gatherings were heavily underlined by caregivers of ASD children on GFCF diet in the study conducted 18 years ago [4]. This problem is still current and has been widely described in the literature on patients with coeliac disease on GF diet. However, in the case of patients with ASD the difficulties are greater, because diet is more stringent. Additionally, these patients constitute a particularly difficult consumer group due to strongly outlined culinary preferences, extremely limited number of acceptable foods, sensory and gastrointestinal disorders, schematism and focusing on details [21].

In our study efforts to maintain special diet were impeded by relatives or teachers for close to 38% of mothers. The respondents pointed out difficult relations with grandparents or ex-spouse, who did not agree on the diet and did not observe it when the child was visiting them. The surveyed individuals with coeliac disease faced the lack of support from the family to a lesser degree, and the understanding increased with the duration of the diet [20]. This likely stems from the fact, that gluten-free diet has been approved as the sole treatment of coeliac disease, whereas the use of this diet in autism therapy is a contentious issue in the medical community.

In the present study low selection of GFCFSF products, low taste values, particularly of bread and insufficient quality of replacements to the traditional diet were the sources of significant problems. According to Cornish, a wide range of food products and improved taste values are indispensable for the approval and maintaining GFCF diet in children with ASD [4]. An important factor affecting compliance with the diet is the physical availability of food, but also economic. This aspect is one of the particularly significant problems met by people using GF diet, indicated in numerous studies [4, 8, 10, 12, 13]. In Poland the cost of gluten-free food ration, assuming only the replacement of traditional cereal products with gluten-free components, increases by 30% [13]. In our study, price constituted an element determining decision on the purchase of a product for over 1/5 of the respondents, however, the high cost of special food was not seen as a factor causing difficulties in maintaining the diet. It is possible, that in terms of the material status, the group of parents participating in the study was not representative due to low number of respondents, who mostly received higher education and resided in large cities. Further research should

cover a more diverse group of respondents in terms of education and take into account the material conditions this type of families.

There are a couple areas to consider when examining the limitations of this study. While the study provides novel information about the selected aspects of daily life families with ASD children on GFCF diet, the study is limited as the all participants are from Poland, therefore may not be generalizable to other geographic regions. The number of participants was small and the survey was anonymous, but unlike other anonymous surveys, the authors had personal contact with each respondent and were able to discuss certain issues and doubts in more detail.

CONCLUSION

The study results demonstrate that the surveyed parents were aware consumers, paying attention primarily to product composition and safety. They were looking for healthy, organic and nutritionally valuable gluten and casein free products with low sugar content. Further studies should examine whether this group of consumers correctly understands the information on the product label, or whether they can consciously choose healthy GFCFSF foods, e.g. find "hidden" sources of sugar. The exclusion diet had a considerable impact on various aspects of the daily life of respondents and their families, was a major obstacle in traveling, socializing and caused conflicts with family and the environment. The limited range of GFCFSF food products, their low taste values and unsatisfactory quality impeded composing varied meals and indicate the need for further development of the market of GFCF products including the special needs of this kind customers. This would give also greater possibilities to dietitians supporting families with children with ASD in choosing the right menu taking into account the specific nutritional, sensory and behavioral difficulties of each patient.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

EMERGENCY MANAGEMENT OF DENTAL INJURY; PREPAREDNESS AMONG SCHOOL TEACHERS IN BHUBANESWAR, INDIA

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ABSTRACT

Background. School is the place, identified with a noticeable risk of Traumatic Dental Injuries (TDI) in children which have functional, esthetic and psychological effects.

Objectives. To assess the preparedness of concerning traumatic dental injuries and their management among school teachers and also to empower the clinician to frame a set of instructions for school teachers to handle the emergencies effectively at the site of the incident.

Material and Methods. A cross-sectional study was conducted among 330 school teachers enrolled in government and private schools of 24 randomly selected schools in Bhubaneswar city. A self-administered questionnaire was distributed to collect information on participants demographic characteristics, knowledge, attitude and practice about emergency management of Traumatic Dental Injury. Chi-square test with level of significance set at 5% was used for statistical analysis.

Results. Statistically significant ($p<0.05$) correct responses were provided by 66.7% males and 35.6% females and 100% younger age teachers. Larger population perceived that their level of knowledge was not satisfactory as they responded for inadequate and don't know options which was significant in relation to gender and age ($p<0.05$) but not with respect to the type of school ($p>0.05$). All the respondents expressed the need for a training program.

Conclusions. This study highlights the instantaneous need for tailor made dental health educational and preventive programs for school teachers in order to effectively manage the Traumatic Dental Injuries.

Key words: *knowledge, management, school teachers, Traumatic Dental Injury*

INTRODUCTION

Childhood is a social construction; whose boundaries shift with time and place and this has implications for vulnerability to injury. Epidemiological studies have shown that prevalence of Traumatic Dental Injury (TDI) ranges between 11% to 30% and 4% to 58% in primary and permanent dentition respectively [13, 28]. Luxation injuries are the most common TDIs in primary dentition, whereas crown fractures are more commonly reported for permanent teeth. This variability may have various causes, including the type of study, trauma classification, strategy, study size and population, geographical area, and contrasts in cultural behavior [13].

Most dental injuries result from simple falls, accidents, sports activities, or childish pranks, which were not intended to cause harm [3, 13, 28]. Children with TDIs can experience emotional stress, teasing, and distress, influencing their self-image both at the time of mishap, as well as later during treatment [1, 21]. Moreover, there is a relationship between dental issues and scholastic accomplishment and learning in children [22].

Dental trauma is one of the most important oral health problems in children and is considered a dental public health problem because: (a) incidence of TDI is high world-wide; (b) trauma to oro-facial structures can cause severe damage to teeth, supporting structures, and craniofacial structures; (c) oro facial injuries can occur at a young age, and treatment may continue for rest of the patient's life.

Children spend large span of time in school where accident from falls is very common and is the main etiological factor of TDI at school [3, 28]. School teachers are the first ones contacted by the child after accident. Hence, they are expected to have proper knowledge and awareness, which will help them

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to carry out expedite procedures in time. Further, proper diagnosis, treatment planning and follow-up assures a favourable outcome. Therefore, the present investigation was set out with the following objectives: (1) to assess the knowledge, attitude and practice towards the emergency management of dental trauma among school teachers of Bhubaneswar (India) and (2) to enable the clinician to formulate a set of instructions which can be given to school teachers, such that the emergencies can be handled effectively.

MATERIAL AND METHODS

Study design and ethical approval

This cross-sectional descriptive survey was steered from July to August 2018 among 330 primary and secondary school teachers of Bhubaneswar, India. School teachers who were permanent residents of Bhubaneswar city, aged over 18 years and were logically and physically proficient of responding to questionnaire in English language were included in the study. Teachers who did not give their consent to participate and those do not present on the day of survey were excluded.

The survey procedure was approved by Institutional Ethical Committee (Ref No/DMR/IMS.SH/SOA/180074). Prior to initiation of primary investigation, certified list of all schools (Government and Private) of Bhubaneswar city was attained from District Education Office (DEO), Bhubaneswar. An official authorization was attained from corresponding Heads of designated schools. Filling out the questionnaire itself was considered consent by participating teachers.

Compilation of study subjects

School teachers were enrolled by a two-stage stratified random sampling technique. In the primary stage, Bhubaneswar city was alienated into four geographical areas, and six schools (two Government and four private) from each area were randomly chosen. Of the total number of Government (163) and private schools (319), eight Government and 16 private schools were randomly picked. In the subsequent stage, qualified school teachers were stratified by age and gender and randomly selected in proportion to the aggregate number of teachers accessible in each school which accomplished for a sample of 330.

Pre-testing survey

A self-administered structured questionnaire was developed in English and pretested on a group of 15 school teachers. Face validity specifies whether the instrument seems to be evaluating the anticipated qualities. When face validity was assessed, it was detected that 96% of participants found the questionnaire to be easy. Assessment of content validity reproduces a decision whether the instrument samples all the relevant or significant domains. Mean content validity ratio was intended as 0.89 based on the thoughts stated by a panel of four academicians. Test of reliability encompassed two components: Question-question reliability, which was calculated by percentage of agreement (88%) and internal reliability for the responses to questions, which was evaluated using Cronbach's alpha coefficient (0.86).

Questionnaire

Ultimate questionnaire comprised of 36 questions under subsequent sections:

Section I: incorporated five questions to gather information related to teacher's demographic characteristics.

Section II: integrated 13 multiple choice questions to assess the knowledge in emergency management of TDI.

Section III: comprised of 12 questions, which aimed to assess the attitude of teachers toward the emergency management of TDI. Responses were recorded on a five-point Likert scale as "strongly agree", "agree", neither agree nor disagree", "disagree" and "strongly disagree".

Section IV: contained six multiple choice questions aimed to investigate the practice of teachers regarding emergency management of TDI.

Data collection

The nature and purpose of study were enlightened to the teachers. Its voluntary nature was accentuated, and confidentiality was guaranteed**.** Questionnaire was distributed to school teachers by a single investigator, offered adequate time to fill it and collected on the spot after completion. During this process the investigator was available to teachers for any clarifications with the questionnaire, simultaneously assured that the respondents answered sincerely without any access to other sources and also established that every question was answered.

Statistical analysis

The data collected was subjected for statistical analysis using SPSS version 21.0 (SPSS Inc., Chicago, Illinois, USA). For the purpose of analysis, proportion of KAP scores was used to assess the level of knowledge, attitude and practice among the participants. Correct responses of <40%, 40-60%,

60-80% and >80% were categorized as having poor, average, good and very good KAP scores respectively. Descriptive statistics were used to summarize the data. *Chi*-square test (χ^2) was used to assess the relationship between categorical variables. For all tests, confidence interval and 'p-value' were fixed at 95% and <0.05 correspondingly.

RESULTS

A total of 330 school teachers, 60 (18.2%) males and 270 (81.8%) females consented and participated in the study. More than half, 175 (53%) the study population were in the age group of 30 to 39 years and the mean age was 34±2.3 years. A proportion of 81.8% and 18.2% represented the private and government schools respectively (Table 1).

Table 1. Demographic characteristics of study participants (n=330)

Characteristics	n (%)
Gender:	
Male	60 (18.2)
Female	270 (81.8)
Age Group (years):	
20 -29	39 (11.8)
30-39	175 (53.0)
40-49	78 (23.6)
>50	38 (11.5)
Type of school:	
Private	270 (81.8)
Government	60 (18.2)

Regarding knowledge on avulsion of tooth (Table 2), 66.7% males and 35.6% females and 100% younger age teachers provided the correct response which was statistically significant (p<0.05). Contradictory responses were obtained for necessity of saving permanent tooth (66.7% v/s 35.6%), right method of carrying the affected tooth to dentist (66.7% v/s 14.8%). On the basis of school type, government teachers (60%) gave higher correct responses but the difference was not statistically significant (p>0.05). For all other knowledge-based questions, majority of males have given correct response which was statistically significant (p<0.05). Responses on the basis of age has shown that a greater number of right answers were provided by younger age population. Government school teachers demonstrated better knowledge than their counterparts which was statistically significant (p<0.05). Larger population perceived that their level of knowledge was not satisfactory as they responded for inadequate and don't know options which was significant in relation to gender and age (p<0.05) but not with respect to type of school (p>0.05).

Male school teachers showed a significantly improved attitude than females (p<0.05). More of negative attitude was observed in elderly age group subjects compared with other groups. Responses categorized with reference to school type was almost alike in both private and government teachers which was statistically not significant (p>0.05) (Table 3). A statistically significant (p<0.05) observation was found across all groups wherein they felt the emergency management and their timely intervention towards dental injuries was essential. Every participant expressed the need for receiving more information on proper management of traumatic dental injuries.

Practices followed by study population differed significantly between groups for all characteristics (Table 4). Additional healthier practices were reported in males than females for appropriate management of avulsed tooth. Across all age groups again a greater number of younger teachers were found to practice better methods which was statistically significant (p<0.05). Similar findings were observed which did not differ much in both private and government school teachers.

Table 2. Responses to knowledge-based questions according to gender, age and type of school

Questions	Gender			Age (years)					Type of school		
	Male n=60 (%)	Female n=270 (%)	χ^2 P	20-29 n=39 (%)	30-39 n=175 (%)	40-49 n=78 (%)	>50 n=38 (%)	χ^2 P	Private n=270 (%)	Government n=60 (%)	χ^2 P
What is avulsion of a tooth?											
Correct response	40 (66.7)	96 (35.6)	19.61	39 (100)	39 (22.3)	58 (74.4)	0 (0)	143.52	100 (37)	36 (60)	63.245
Incorrect response	20 (33.3)	174 (64.4)	0.001	0 (0)	136 (77.7)	20 (25.6)	38 (100)	0.002	170 (63)	24 (40)	0.201
Do you have any prior knowledge about the management of avulsed tooth?											
Yes	20 (33.3)	19 (7)	32.57	20 (51.3)	19 (10.9)	0 (0)	0 (0)	73.98	35 (13)	4 (6.7)	1.867
No	40 (66.7)	251 (93)	0.001	19 (48.7)	156 (89.1)	78 (100)	38 (100)	0.001	235 (87)	56 (93.3)	0.17
*What is your source of information on avulsion***?**											
Internet source	40 (66.7)	82 (30.4)		34 (87.1)	69 (39.4)	15 (19.2)	4 (10.5)		102 (37.8)	20 (33.3)	
Health talks on television or radio	5 (8.3)	33 (12.2)		2 (5.1)	20 (11.4)	8 (10.3)	8 (21)		28 (10.4)	10 (16.7)	
Magazines/Newspapers	13 (21.7)	140 (51.9)	74.40	3 (7.7)	86 (49.1)	49 (62.8)	15 (39.5)	308.69	126 (46.7)	27 (45)	90.743
Others (specify)	2 (3.3)	15 (5.5)	0.001	0	0	6 (7.7)	11 (28.9)	0.001	14 (5.2)	3 (5)	0.001
Do you think it is necessary to save avulsed permanent tooth?											
Yes	40 (66.7)	96 (35.6)	19.61	39 (100)	59 (33.7)	38 (48.7)	0 (0)	88.15	119 (44)	17 (28.3)	5.020
No	20 (33.3)	174 (64.4)	0.001	0 (0)	116 (66.3)	40 (51.3)	38 (100)	0.001	151 (56)	43 (71.7)	0.025
If you were at a site where the child knocked out his permanent front tooth, would you advise the parents to look for the avulsed tooth?											
Yes	60 (100)	270 (100)	-	39 (100)	175 (100)	78 (100)	38 (100)	-	270 (100)	60 (100)	-
No	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	
*What would you do if the tooth was in child's mouth, however, out of place***?**											
Correct response	0 (0)	39 (14.4)	9.83	0 (0)	19 (10.9)	20 (25.6)	0 (0)	24.78	33 (12.2)	6 (10)	0.233
Incorrect response	60 (100)	231 (85.6)	0.002	39 (100)	156 (89.1)	58 (74.4)	38 (100)	0.001	237 (87.8)	54 (90)	0.630
If you did not put the knocked-out tooth back into its socket, would you advise the parents to take the tooth to the dentist?											
Yes	60 (100)	250 (92.6)	4.73	39 (100)	155 (88.6)	78 (100)	38 (100)	18.86	256 (94.8)	54 (90)	1.999
No	0 (0)	20 (7.4)	0.03	0 (0)	20 (11.4)	0 (0)	0 (0)	0.001	14 (5.2)	6 (10)	0.157

If you decide to put the knocked-out tooth back into its socket, but it has fallen onto the ground and is covered with dirt, what would you do?											
Correct response	0 (0)	98 (36.3)	111.85	19 (48.7)	39 (22.3)	40 (51.3)	0 (0)	60.85	81 (30)	17 (28.3)	0.097
Incorrect response	60 (100)	172 (63.7)	0.001	20 (51.3)	136 (77.7)	38 (48.7)	38 (100)	0.001	189 (70)	43 (71.7)	0.953
If you decide to visit the dentist how would you carry the tooth to him?											
Correct response	40 (66.7)	40 (14.8)	78.57	20 (51.3)	60 (34.3)	0 (0)	0 (0)	189.326	66 (24.4)	14 (23.3)	3.512
Incorrect response	20 (33.3)	230 (85.2)	0.001	19 (48.7)	115 (65.7)	78 (100)	38 (100)	0.001	204 (75.6)	46 (76.7)	0.476
How to hold an avulsed tooth?											
Correct response	0 (0)	115 (42.6)	39.23	19 (48.7)	19 (10.9)	58 (74.4)	19 (50)	105.14	88 (32.6)	27 (45)	3.329
Incorrect response	60 (100)	155 (57.4)	0.001	20 (51.3)	156 (89.1)	20 (25.6)	19 (50)	0.002	182 (67.4)	33 (55)	0.068
Is extra oral time for putting the knocked-out tooth back into its socket important?											
Yes	20 (33.3)	77 (28.5)		0 (0)	97 (55.4)	0 (0)	0 (0)		77 (28.5)	20 (33.3)	
No	0 (0)	0 (0)	0.548	0 (0)	0 (0)	0 (0)	0 (0)	121.68	0 (0)	0 (0)	0.548
Don’t know	40 (66.7)	193 (71.5)	0.46	39 (100)	78 (44.6)	78 (100)	38 (100)	0.001	193 (71.5)	40 (66.7)	0.459
When should the tooth be put back, if it had been knocked out of the mouth?											
Correct response	0 (0)	19 (7)	50.14	0 (0)	19 (10.9)	0 (0)	0 (0)	238.04	15 (5.6)	4 (6.7)	14.835
Incorrect response	60 (100)	251 (93)	0.002	39 (100)	156 (89.1)	78 (100)	38 (100)	0.003	255 (94.4)	56 (93.3)	0.005
What do you think is your level of information about traumatic dental injuries?											
Adequate	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	
Inadequate	40 (66.7)	231 (85.6)	11.93	39 (100)	116 (67.4)	78 (100)	38 (100)	63.63	221 (81.9)	50 (83.3)	0.073
Don’t know	20 (33.3)	39 (14.4)	0.001	0 (0)	59 (22.7)	0 (0)	0 (0)	0.001	49 (18.1)	10 (16.7)	0.786

Table 3. Responses to attitude-based questions according to gender, age and type of school

Questions	Gender			Age (years)					Type of school		
	Male n=60 (%)	Female n=270 (%)	χ^2 P	20-29 n=39 (%)	30-39 n=175 (%)	40-49 n=78 (%)	>50 n=38 (%)	χ^2 P	Private n=270 (%)	Government n=60 (%)	χ^2 P
A teacher is not responsible for posttraumatic dental injuries											
Correct	0 (0)	40 (14.8)	15.96	0 (0)	20 (11.4)	20 (25.6)	0 (0)	236.08	32 (11.9)	8 (13.3)	94.85
Incorrect	60 (100)	230 (85.1)	0.001	39 (100)	155 (88.6)	58 (74.4)	38 (100)	0.002	238 (88.1)	52 (86.7)	0.001
Time consciousness for emergency management of dental trauma can play a vital role in improving tooth prognosis											
Correct	60 (100)	270 (100)		39 (100)	175 (100)	78 (100)	38 (100)		270 (100)	60 (100)	
Incorrect	0 (0)	0 (0)	-	0 (0)	0 (0)	0 (0)	0 (0)	-	0 (0)	0 (0)	-
A tooth after avulsion will be lost definitely, so there is no need for treatment											
Correct	40 (66.7)	250 (92.6)	40.05	39 (100)	135 (77.1)	78 (100)	38 (100)	152.85	237 (87.8)	53 (88.3)	10.83
Incorrect	20 (33.3)	20 (7.4)	0.001	0 (0)	40 (22.9)	0 (0)	0 (0)	0.001	33 (12.2)	7 (11.7)	0.004
Dental trauma emergency management must become one of the educational priorities for teachers											
Correct	60 (100)	213 (78.9)	16.30	39 (100)	156 (89.1)	78 (100)	0 (0)	267.201	236 (87.4)	37 (61.7)	24.18
Incorrect	0 (0)	57 (21.1)	0.001	0 (0)	19 (10.9)	0 (0)	38 (100)	0.001	34 (12.6)	23 (38.3)	0.001
Dental trauma emergency is not an emergency situation											
Correct	40 (66.7)	58 (21.5)	11.39	39 (100)	116 (66.3)	78 (100)	19 (50)	114.69	203 (75.2)	49 (81.7)	2.33
Incorrect	20 (33.3)	212 (78.5)	0.003	0 (0)	59 (33.7)	0 (0)	19 (50)	0.001	67 (24.8)	11 (18.3)	0.312
Teacher intervention in school dental injuries may play a key role in traumatized tooth											
Correct	60 (100)	251 (93)	15.31	39 (100)	175 (100)	59 (75.6)	38 (100)	98.71	251 (93)	60 (100)	4.50
Incorrect	0 (0)	19 (7)	0.001	0 (0)	0 (0)	19 (24.4)	0 (0)	0.001	19 (7)	0 (0)	0.105
Emergency management of dental trauma requires special education and therefore there is no need for teacher intervention											
Correct	60 (100)	156 (57.8)	38.70	39 (100)	80 (45.7)	78 (100)	19 (50)	102.62	172 (63.7)	44 (73.3)	2.768
Incorrect	0 (0)	114 (42.2)	0.001	0 (0)	95 (54.3)	0 (0)	19 (50)	0.001	98 (36.3)	16 (26.7)	0.251

Wearing a mouth guard should be compulsory in all contact sports											
Correct	60 (100)	270 (100)	15.31	39 (100)	175 (100)	78 (100)	38 (100)	21.23	270 (100)	60 (100)	1.61
Incorrect	0 (0)	0 (0)	0.001	0 (0)	0 (0)	0 (0)	0 (0)	0.001	0 (0)	0 (0)	0.204
Due to legal considerations, it is advisable that a teacher refrains from intervening in such scenarios											
Correct	20 (33.3)	192 (71.1)	35.16	19 (48.7)	97 (55.4)	58 (74.4)	38 (100)	137.75	169 (62.6)	43 (71.7)	10.68
Incorrect	40 (66.7)	78 (28.9)	0.001	20 (51.3)	78 (44.6)	20 (25.6)	0 (0)	0.002	101 (37.4)	17 (28.3)	0.005
Having some short pertinent educational experiences, educators can provide better assistance in traumatic dental injuries											
Correct	60 (100)	270 (100)	4.73	39 (100)	175 (100)	78 (100)	38 (100)	18.86	270 (100)	60 (100)	1.99
Incorrect	0 (0)	0 (0)	0.030	0 (0)	0 (0)	0 (0)	0 (0)	0.003	0 (0)	0 (0)	0.157
Learning about traumatic dental injuries is											
Essential	60 (100)	251 (93)	7.30	39 (100)	175 (100)	59 (75.6)	38 (100)	93.67	251 (93)	60 (100)	4.963
Not essential	0 (0)	19 (7)	0.026	0 (0)	0 (0)	19 (24.4)	0 (0)	0.001	19 (7)	0 (0)	0.084
Would you like to receive more information in order to properly manage traumatic dental injuries?											
Yes	60 (100)	270 (100)		39 (100)	175 (100)	78 (100)	38 (100)		270 (100)	60 (100)	
No	0 (0)	0 (0)	-	0 (0)	0 (0)	0 (0)	0 (0)	-	0 (0)	0 (0)	-

Table 4. Evaluation of practices according to gender, age and type of school

Questions	Gender			Age (years)					Type of school		
	Male n=60 (%)	Female n=270 (%)	χ^2 P	20-29 n=39 (%)	30-39 n=175 (%)	40-49 n=78 (%)	>50 n=38 (%)	χ^2 P	Private n=270 (%)	Government n=60 (%)	χ^2 P
Have you ever come across a patient with avulsed tooth (knocked out)?											
Yes	20 (33.3)	39 (14.4)	11.92	20 (51.3)	39 (22.3)	0 (0)	0 (0)	57.207	49 (18.1)	10 (16.7)	0.073
No	40 (66.7)	231 (85.6)	0.001	19 (48.7)	136 (77.7)	78 (100)	38 (100)	0.001	221 (81.9)	50 (83.3)	0.07
Would you refer the child or instruct the parents to visit the dentist after avulsion?											
Yes	60 (100)	270 (100)		39 (100)	175 (100)	78 (100)	38 (100)		270 (100)	60 (100)	
No	0 (0)	0 (0)	-	0 (0)	0 (0)	0 (0)	0 (0)	-	0 (0)	0 (0)	-
If yes, when will you advise the parents to go the dentist?											
Correct response	40 (66.7)	231 (85.6)	11.92	20 (51.3)	155 (88.6)	58 (74.4)	38 (100)	41.69	220 (81.5)	51 (85)	0.414
Incorrect response	20 (33.3)	39 (14.4)	0.001	19 (48.7)	20 (11.4)	20 (25.6)	0 (0)	0.001	50 (18.5)	9 (15)	0.52
A boy comes to you with a knocked out permanent anterior tooth in his hand after the accident. What will you do with the tooth?											
Correct response	40 (66.7)	115 (42.6)	15.68	39 (100)	58 (33.1)	58 (74.4)	38 (100)	147.23	140 (51.9)	15 (25)	15.946
Incorrect response	20 (33.3)	155 (57.4)	0.001	0 (0)	117 (66.9)	20 (25.6)	0 (0)	0.001	130 (48.1)	45 (75)	0.002
If you decided to save the tooth, what will you do with it?											
Correct response	20 (33.3)	38 (14.1)	16.11	20 (51.3)	19 (10.9)	0 (0)	19 (50)	111.21	35 (13)	23 (38.3)	21.881
Incorrect response	40 (66.7)	232 (85.9)	0.001	19 (48.7)	156 (89.1)	78 (100)	19 (50)	0.001	235 (87)	37 (61.7)	0.001
Would you care if the tooth that has been knocked out was a primary tooth?											
Yes	20 (33.3)	154 (57)	11.07	19 (48.7)	77 (44)	59 (75.6)	19 (50)	22.14	135 (50)	39 (65)	4.431
No	40 (66.7)	116 (43)	0.001	20 (51.3)	98 (56)	19 (24.4)	19 (50)	0.001	135 (50)	21 (35)	0.035

DISCUSSION

Dental trauma is injury to mouth, including teeth, lips, gums, tongue, and jawbones. Traumatic dental injuries (TDIs) may include knocked-out teeth (dental avulsion), cracked or craze (fractured), displaced teeth (dental luxation, lateral displacement, or extrusion), pushed up into the jawbone (dental intrusion), or loosened teeth (subluxation or dental concussion). TDIs leading to avulsion can be defined as complete displacement of tooth out of socket [8]. Among all types of TDIs, avulsion is the most complicated. Avulsion of permanent teeth is seen in 0.53% of all dental injuries. Several studies have demonstrated that this injury is one of the most thoughtful dental injuries and the prognosis is very much dependent on the actions taken at the place of accident and promptly after the avulsion and hence, we decided to assess the knowledge of management of avulsion among school teachers.

The major source of information reported was internet which is readily accessible to everyone through the development of technology. Similar findings of internet for young people and television for elderly people were observed by *Al-Sane* et al. [7]. Recently, *Al-Musawi* et al. [5] established the provision of information on emergency management of TDI through the use of smartphones.

The current study revealed insufficient proportion of knowledge about avulsion of tooth which can be comparable to previous similar studies. Around half the teachers think that it is necessary to save the avulsed tooth which is in accordance with the similar study conducted by *Mohandas and Chandan* [19] at Bangalore (49.6%). This shows the positive attitude of teachers in Bhubaneswar towards the necessity of saving avulsed tooth.

The transport media also play an important role. The most popular choice of storage medium during transportation was antiseptic solution 72.6%. Storing the tooth in an antiseptic solution is not recommended because it will compromise the periodontal ligament cells and thus adversely affect the prognosis of replanted tooth. Many teachers were not aware that dry storage during transportation would seriously prejudice survival of the replanted tooth; the prognosis being dependent on avoidance of injury to periodontal membrane during the time the tooth is out of its socket [29]. The perfect storage medium should be proficient of preserving cell vitality, adherence, and clonogenic capacity and must be readily accessible at the site of accident or easily reachable [9]. Milk has a favourable osmolarity and composition for maintaining the viability of periodontal ligament cells and has been recommended for temporary storage of avulsed teeth before replantation. In addition to being readily available, it preserves cell viability for up to 3 hours [27]. In the present study most of the teachers were unaware of the right method in transporting the affected tooth to dentist which is almost similar to the study conducted by *Shamarao* et al., [24]. Contrary to this, studies conducted by *Singh* et al., [26]; *Mohandas* and *Chandan* [19]; *Ahluwalia* et al., [2]; *Alluqmani* and *Omar* [4]; *Chandukutty* et al. [11] and *Taranath* et al. [27] found that a good number of school teachers, i.e. 87%, 49.6%, 47.4%, 45.3%, 40%, and 35.7%, respectively, were aware of correct storage medium.

It was surprising to notice that school teachers of Bhubaneswar were not aware of the importance of extra oral time in managing a dental injury. This was evident from their response regarding the urgency of seeking professional help, where a vast majority were not in favor of seeking immediate professional help. Opposing results were obtained from studies conducted *by Chandukutty* et al. [11], *Taranath* et al., [27], *Al-Obaida* [6] and *Kaul* et al. [15] as 45.9%, 33.6%. 50% and 78.3% respectively.

In our study, it was noteworthy to witness that majority of the participants (mostly male teachers) were having better knowledge which reflected in their positive attitude and practice as compared to the female counterparts regarding the need for treatment of loss of avulsed tooth. Contrary results were obtained in other studies conducted by *de Lima Ludgero* et al. [12] at Brazil (60.4%) and *Hashim* [14] at the UAE (44.1%). This can be owed to caring nature and closeness of females to children.

Responses based on age has shown that a greater number of right answers were provided by younger age population and were also found to practice better methods. This might be because the younger age group is more connected with various sources of information like technology and mass media. Similarly, though statistically not significant the government school teachers demonstrated better awareness which again may be related to improved knowledge in them.

Larger population perceived that their level of knowledge was not satisfactory as they responded for inadequate and don't know options which was similar to other studies [10, 18]. This indicates the need of organizing educational promotive and preventive programs on dental trauma. Statistically significant ($p<0.05$) observation was found across all groups wherein they felt the emergency management and their timely intervention towards dental injuries was essential.

When teachers were asked if they would like to receive more information to manage such cases, everyone (100%) answered 'yes' and almost similar responses were obtained in studies conducted by *Mohandas* and *Chandan* (96.5%) [19], *Olatossio* et al. (93.4%) [20], *Chan* (81.9%) [10] and *Uppal* et al., (76.4%) [30].

In our study, less than half the teachers have come across the cases of avulsed tooth. Such situations were responded to be managed by referring to nearby dentists and majority were aware of the immediate actions to be taken without wasting time which is analogous with the study conducted in Saudi [6]. In contrast majority of the teachers would call the parent and ask them to take the child to a dentist, while one-third were not able to decide on what to do under such circumstances [23]. It was appreciable to observe that at least half the subjects would care even if the avulsed tooth was a primary tooth. Early loss of a primary tooth may affect the physiological sequence of permanent teeth and may be an etiological factor for malocclusion.

Reports show that more than half of all children suffer traumatic dental injuries in school [16, 25] and this highlights the importance of experienced/ trained school staff, who are often required to respond initially to traumatic event. Instantaneous response to dental injury can reduce the level of children's and their parents' apprehension with respect to succeeding dental treatment [17]. Therefore, teachers who work with children must be aware of the importance of emergency treatment and of how to proceed in cases of tooth avulsion. Educational programs and dental camps can prove to be beneficial in this regard.

LIMITATIONS

As participants' responses were self-reported it may be considered as a limitation and caution must be exercised with generalization of these findings. It's also advisable to include teachers from all the schools in Bhubaneswar and consider many more variables.

CONCLUSION

This study has demonstrated the lack of knowledge among school teachers regarding correct handling, transportation, and storage of an avulsed tooth and stressed the immediate need for dental health education to be stepped up among school teachers. By increasing knowledge on how avulsed teeth are to be dealt with at the site of the accident, the risk of permanent tooth loss is minimized. The prognosis of an avulsed tooth is good if it is replanted under ideal circumstances, and the tooth can often be retained for life.

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ORIGINAL ARTICLE

RELATIONSHIP BETWEEN FARMERS' KNOWLEDGE AND ATTITUDES TOWARDS PESTICIDE USE AND THEIR SOCIODEMOGRAPHIC CHARACTERISTICS: A CROSS-SECTIONAL STUDY FROM NORTHWESTERN TURKEY

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ABSTRACT

Background. The determination of farmers' knowledge and attitudes towards pesticide use is very important in terms of preventing pesticide use-related short- and long-term damages.

Objective. This study was aimed at investigating the relationship between the sociodemographic characteristics of farmers in Karacabey District of Bursa, a province in northwest Turkey, and their knowledge and attitudes towards pesticide use.

Material and methods. This cross-sectional study was conducted in Karacabey district between December 2018 and April 2019. The sample size was calculated as 1924 using the Epi info program by assuming the prevalence as 50%, type I error as 5%, standard deviation as 3% and design effect as 2. The dependent variable of the study was the farmers' knowledge of and attitudes towards pesticide use whereas the independent variables were their sociodemographic characteristics. The relationship between the dependent and independent variables was analyzed using the chi-square test and binary logistic regression model.

Results. The mean score the participants obtained from the Knowledge and Attitude Index was 12.8±2.8 and the median value was 13. While 49.5% of the participating farmers obtained a score lower than the median, 51.5% of them obtained a score equal to or above the median. While advanced age increased the possibility of getting a low score from the Knowledge and Attitude Index 2.7 times, not being married increased it 35.7 times, not getting formal education increased it 30.1 times, living in a non-crowded household increased it 2.1 times, and low income (2000 Turkish liras equal to ≤$310 according to April 2019 exchange rates) increased it 3.1 times.

Conclusion. The study indicated that the participating farmers' knowledge and attitudes towards the proper use of pesticides were inadequate, and that there was a strong relationship between their Knowledge and Attitude Index scores and their sociodemographic characteristics.

Key words: *pesticide, knowledge, attitude, sociodemographic characteristics, public health*

INTRODUCTION

Excessive, illogical and unconscious use of pesticides which have been widely used in agricultural activities since the early 1970s, [6, 13] is an important public health problem threatening human and environmental health [13]. The fact that the number of people working in agriculture is high all over the world but in particular in developing countries places the pesticide use-related short- and long-term damage at the forefront of the current agenda as a serious problem [5, 17, 25, 31]. According to United Nations (UN), an average of about 200,000 people die from the toxic exposure of pesticides per year across the World [15]. Pesticides also create a serious burden of disease (The disability-adjusted life-year (DALY) in many countries, especially in developing countries [16]. In addition, the use of highly toxic pesticides, forbidden in developed countries, is widespread in developing countries, which increases the risk of pesticide-related deaths in those countries [22]. Due to factors such as unnecessary use of pesticides [1], inappropriate use of pesticides [3, 4, 7, 8, 22], not using or misusing protective equipment [3], due to lack of knowledge

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/ attitude, farmers face serious health problems [8, 13, 21]. Since farmers who do not have the accurate knowledge of pesticide use and display poor attitudes towards it are in an at-risk group [3], it is important to identify their knowledge / attitude levels and determinants of these low levels in order to clarify the situation and to guide public health interventions.

In the literature, according to studies conducted to investigate farmers' knowledge and attitudes towards pesticide use, the rate of farmers whose knowledge / attitude level is considered as sufficient ranges between 34% and 85% [3, 7, 17, 18, 19, 27, 28, 29]. As is reported in the literature, knowledge / attitude score is affected by factors such as age [28], income level [28], total length of time spent in farming [29], educational status [26, 27, 29] and cultural characteristics [33]. In Turkey, approximately one-fifth of the labor force, most of whom are men, is employed in agriculture [30]. In Turkey, the number of population-based studies designed to assess farmers' knowledge and attitudes towards pesticides is very limited and the samples of the available studies include a small number of farmers [7, 21].

In the present study conducted in Karacabey District of Bursa, a province in northwest Turkey, it was aimed to investigate the relationship between the sociodemographic characteristics of farmers who were registered in the Chamber of Agriculture and their knowledge and attitudes towards pesticide use. To our current knowledge, our study is the first study conducted on this issue in this region.

MATERIALS AND METHODS

This cross-sectional study was carried out within the scope of the Balikesir University scientific research project titled "Determination of farmers' knowledge and attitudes and behaviors towards pesticide use" (BAUN BAP No: 2018/169). This project has three stages: The first stage is the assessment of the knowledge and attitudes of the farmers registered in the Chamber of Agriculture in Karacabey District regarding the use of pesticides. The second stage is the determination of the behavioral characteristics of farmers who use pesticides during agricultural activities and actively work in the spraying of pesticides, and the frequency of pesticide poisoning. The third stage is the provision of training on the health effects of pesticides and ways of protection against the harmful effects of pesticides. In this study, the findings obtained from the first stage of the project are presented. The study was carried out in Karacabey district of Bursa between December 2018 and April 2019. Karacabey is a district with a population of 89,000 people. The district has three million decares (about 741316 acres) of fertile land where vegetables and fruits are intensively cultivated [10] (Figure 1).

The population of the study comprised 9750 people registered in Karacabey Chamber of Agriculture. The sample size was calculated as 1924 using the Epi-info Statcalc Program by assuming the prevalence as 50%, type I error as 5%, standard deviation as 3% and design effect as 2. During the study, 2100 people were reached using the multi-stage sampling method. In the

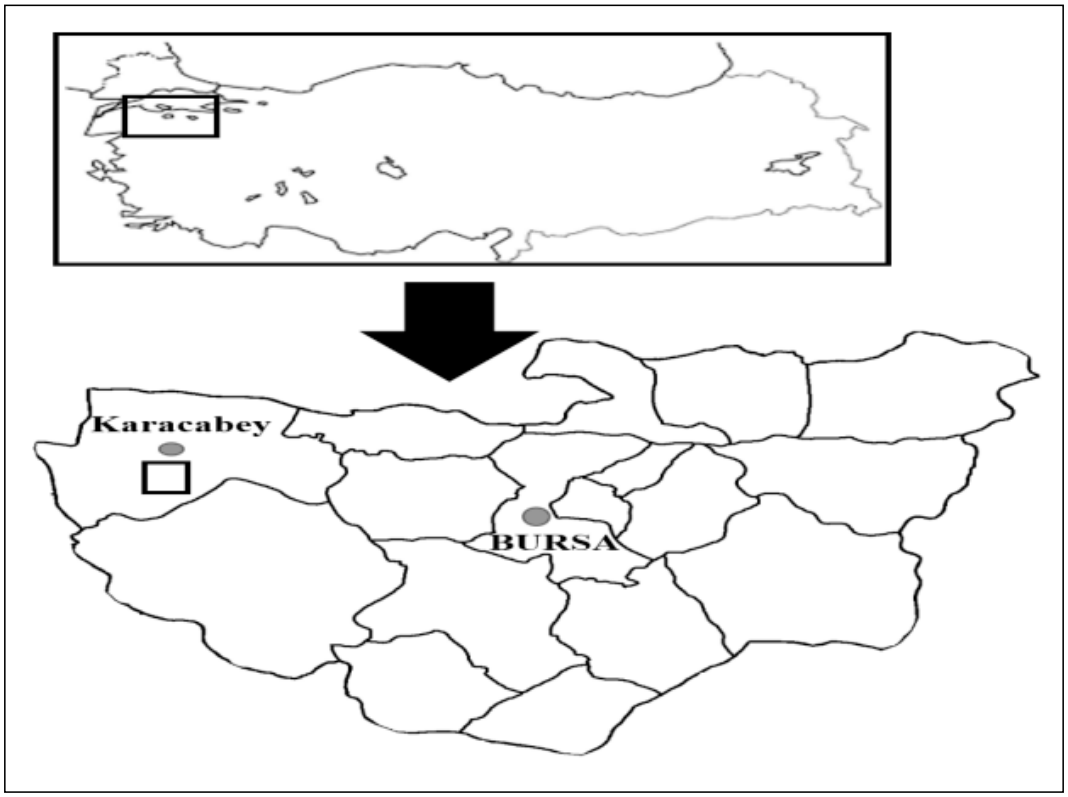

Figure 1. Study area representing Karacabey, in Turkey.

study, firstly, the neighborhoods where the farmers registered in the Karacabey Chamber of Agriculture lived were determined. Each of the 85 neighborhoods in Karacabey district was considered as a cluster. The number of farmers targeted to be reached in each cluster was determined in proportion to the farmer population in the neighborhood. Interviews were started from the first house in each cluster and continued by skipping the second house and visiting the third house in a row. If there was no farmer in the house, then the household in the next house was interviewed.

The dependent variable of the study was the participating farmers' knowledge of and attitudes towards pesticide use score. The score was determined using the Knowledge and Attitude Index developed by the researchers based on the pertinent literature [3, 8, 14, 17, 29]. The independent variables of the study were the place of residence, age, marital status, educational status, the number of households, monthly income and total length of time spent in farming. The study data were collected using the Personal Information Form and the Knowledge and Attitude Index.

Personal Information Form: The form developed by the researchers based on the literature consists of 7 items that question the participants' socio-demographic characteristics (age, marital status, education etc.) [19, 24, 32].

The Knowledge and Attitude Index: Included 22 items questioning the participants' knowledge and attitudes regarding the effects of pesticides on human and environmental health, pesticide selection, pesticide application time, use of protective equipment during spraying, smoking status, hand washing and taking a shower after spraying, how to dispose of empty pesticide boxes. Responses included three options: "yes", "no" and "I don't know". While responses indicating that the participant's knowledge and attitudes were correct were given "1 point", responses indicating that the participant's knowledge and attitudes were incorrect were given "0 points". If the participant selected the "I don't know" option, "0 points" were given too. Therefore, the highest and lowest possible scores that can be obtained from the index were 22 and 0 respectively. After the scores obtained by the farmers were calculated, then the median value which was used as the cutoff point was determined. While the scores below the median value indicated that the knowledge and attitude levels were "low", the scores equal to or higher than the median value indicated that the levels were "high". The Knowledge and Attitude Index used in the study was administered to 10 farmers who were not included in the study sample for pilot testing, and then the necessary revisions were made in the index.

The data obtained in the study were summarized as percentage distribution. The relationship between the dependent and independent variables was analyzed in the SPSS 25.0 program using the chi-square test and binary logistic regression model. P-values less than 0.05 were considered statistically significant.

Ethical approval was obtained from the Ethics Committee of Balikesir University (Dated April 04, 2018, Numbered 2018/73). The farmers to participate in the study gave their verbal consent indicating that they volunteered to participate in the study.

RESULTS

Within the scope of the study, 2100 farmers were reached. All the participating farmers were vegetable and fruit growers, and applied pesticides to their crops. Of the participating farmers, 78.5% lived in a village. 24.1% were ≥55 years old, 3.4% were single, 14.1% did not have any formal education. 15.9% earned more than 2000 Turkish liras ($357 according to April 2019). exchange rates) per month and 43.9% had been engaged in farming for more than 20 years (Table 1).

Table 1. Sociodemographic characteristics of the participating farmers

Variables	n	%
Place of residence		
District center	451	21.5
Village	1649	78.5
Age		
≤44 years	378	18.0
45-54 years	1215	57.9
≥55 years	507	24.1
Marital status		
Married	2028	96.6
Single	72	3.4
Education level		
No formal education	298	14.1
Primary school	1020	48.6
Junior High School	644	30.7
Senior High school	138	6.6
Family size (n)		
≤4 people	1277	60.8
≥5 people	823	39.2
Income per month (USD)*		
≤$357	1766	84.1
>$357	334	15.9
Total length of time spent in farming		
≤20 years	1179	56.1
>20 years	921	43.9
Total	2100	100.0

*$1 = 5.74 Turkish Liras according to April 2019 exchange rates

While a great majority of the farmers (86.8%) stated that pesticides would increase productivity in agriculture, 22.0% stated that pesticides were harmful to human health and 83.0% said that cheaper pesticides should be preferred more. A very small number of the farmers thought that protective clothing such as masks and special overalls should be worn during the application of pesticides. While 62.0% of the farmers stated that empty pesticide containers should be buried in the ground, 57.9% of them stated that the containers might be left in the environment, 35.1% of them said that the containers should be left in outdoor garbage cans (Table 2).

The minimum and maximum scores the participating farmers obtained from the Knowledge and Attitude Index were 4 and 19 respectively. While the mean scores the participants obtained from the Knowledge and Attitude Index was 12.8 ± 2.8 (min: 4 max: 19), the median value was 13. As is seen in table 3, of the participating farmers, 49.5% obtained a score lower than the median value, and 51.5% obtained a score equal to or above the median value.

Among the variables which increased the possibility of getting a low score from the Knowledge and Attitude Index were the advanced age (2.7 times), not being married (35.7 times), not getting formal education (30.1 times), living in a non-crowded household (2.1 times), and low income (≤2000 Turkish liras per month equal to ≤$357 according to April 2019 exchange rates) (3.1 times) (Table 3).

DISCUSSION

This community-based study with a large sample is the first study conducted in Turkey to determine farmers' knowledge of and attitudes towards pesticide use, and related factors, and to provide decision-makers with data on this issue. The mean scores the participants obtained from the Knowledge and Attitude Index was 12.8 ± 2.8 (min: 4, max: 19) and

Table 2. The participating farmers' knowledge and attitudes towards pesticide use

	Yes		No		I do not know	
	n	%	n	%	n	%
Pesticides increase productivity in agriculture	1822	86.8	278	13.2	0	-
Pesticides are harmful to human health	462	22.0	864	41.1	774	36.9
Pesticides are harmful to the environment	1427	68.0	19	0.9	654	31.1
Pesticides can leave residue in vegetables and fruit	1368	65.1	658	31.3	74	3.5
Pesticides should only be used for the product for which it is licensed.	903	43.0	562	26.8	635	30.2
Pesticide selection can be made (pesticides to be used can be selected) according to the recommendation by a friend /neighbor / relative	1375	65.5	408	19.4	317	15.1
Cheaper pesticides should be preferred.	1742	83.0	303	14.4	55	2.6
Pesticide selection should be based on the disease / pest	1252	59.6	491	23.4	357	17.0
The dosage of the pesticide should be adjusted as stated on the information label on the package of the pesticide	1456	69.3	300	14.3	344	16.4
If necessary, more than the recommended dose of pesticide can be sprayed	1390	66.2	187	8.9	523	24.9
Spraying should be performed just before harvesting	815	38.8	892	42.5	393	18.7
Spraying should be done out of noon hours	699	33.3	1319	62.8	82	3.9
Gloves should be worn during spraying	1423	67.8	548	26.1	129	6.1
A mask should be worn during spraying	677	32.2	886	42.2	537	25.6
Special overalls should be worn during spraying	809	38.5	503	24.0	788	37.5
Boots should be worn during spraying	1077	51.3	531	25.3	492	23.4
One can smoke during spraying	900	42.9	788	37.5	412	19.6
Hands should be washed after spraying	1442	68.7	129	6.1	529	25.2
One should take a shower after spraying	1377	65.6	545	26.0	178	8.5
Empty pesticide containers should be buried in the ground	1303	62.0	641	30.5	156	7.4
Empty pesticide containers might be left in the environment	1216	57.9	677	32.2	207	9.9
Empty pesticide containers should be put in a plastic bag and then in an outdoor garbage can	737	35.1	1121	53.4	242	11.5

Table 3. Correlation between the farmers' Knowledge and Attitude Index scores and their sociodemographic characteristics

Variables	Knowledge and Attitude Index score		Univariate analysis		Multivariate Logistic regression		
	low	high					
	n (%*)	n (%*)	X^2	p	OR	p	%95 GA
Place of residence							
District center	203 (45.0)	248 (55.0)					
Village	837 (50.8)	812 (49.2)	4.679	0.031	0.9	0.587	0.7-1.1
Age							
≤ 54 (ref)	665 (41.7)	928 (58.3)					
≥ 55	375 (74.0)	132 (26.0)	159.712	0.001	2.7	**0.001**	1.9-3.8
Marital status							
Married (ref)	970 (47.8)	1058 (52.2)					
Single	70 (97.2)	2 (2.8)	67.856	0.001	35.7	**0.001**	8.6-147.4
Educational status							
No formal education	293 (98.3)	5 (1.7)	330.819	0.001	30.1	**0.001**	11.9-76.2
Primary school and above (ref)	747 (41.5)	1055 (58.5)					
The number of households							
≤ 4	810 (63.4)	467 (36.6)	252.070	0.001	2.1	**0.001**	1.7-2.6
≥ 5 (ref)	230 (27.9)	593 (72.1)					
Income per month (USD)**							
≤$357	963 (54.5)	803 (45.5)	111.322	0.001	3.1	**0.001**	2.2-4.4
>$357 (ref)	77 (23.1)	257 (76.9)					
Total length of time spent in farming							
≤ 20 years (ref)	477 (40.5)	702 (59.5)					
> 20 years	563 (61.1)	358 (38.9)	88.386	0.001	0.8	0.122	0.67-1.1

* Row percentage; OR: Odds Ratio

**$1 = 5.74 Turkish Liras according to April 2019 exchange rates.

the median value was 13. Approximately half of the farmers' (49.5%) score was lower than the median value. The variables which increased the possibility of getting a low score from the Knowledge and Attitude Index were advanced age, not being married, not getting formal education, living in a non-crowded household, and low income.

In the present study, the rate of the farmers who thought that pesticides were harmful to human health was 22% which was lower than that in the literature [12]. On the other hand, 68% of them thought that pesticides were harmful to the environment which was consistent with the literature [12, 32] and 69.3% stated that labels should be read, which was also consistent with the literature [23]. In the present study, the rate of the farmers who took a shower immediately after spraying was 65.6%, which was slightly lower than that in the literature [12, 25]. Consistent with the literature, two-thirds of the farmers in our study stated that the empty pesticide containers might be left in the environment [21, 28]. In our study, the rates of those who thought that protective clothing such as masks and special overalls should be worn during spraying were 32.2% and 38.5% respectively. However, these rates ranged between 32.2% and 68.8% in the literature [4, 7, 17, 21, 23, 25, 28].

In our study, the Knowledge and Attitude Index scores of the majority of the participating farmers were low. The review of studies in the literature indicated that the rate of the participants whose knowledge / attitude was sufficient ranged between 34% and 85% [3, 7, 17, 18, 19, 27, 29]. The results of our study are consistent with those of studies in the literature. However, the fact that knowledge / attitude levels were low in half of the farmers in the present study conducted in an agriculture intensive region located in the West of Turkey is an important issue. While knowledge / attitude levels of 33% (n = 70) of the participating farmers in a study conducted by *Derafsi* et al. in a more developed region of Turkey, and 65.9% (n = 56) of the participating farmers in a study carried out by *Saeed* et al. in Pakistan were lower than were those of the participants in our study [7], in Thao et al.'s study carried out in the USA, 85% (n = 28) of the participating farmers had sufficient knowledge / attitude levels. These differences may be due to

differences between the participants' educational levels and cultural backgrounds, and regions they live in [29].

In the present study, advanced age increased the possibility of getting a low score from the Knowledge and Attitude Index 2.7 times. The results of *Sharafi* et al.'s [28] study conducted with 311 farmers in Iran. On the other hand, in *Derafshi* et al.'s study including 70 farmers, unlike the present study, as the age increased so did the level of knowledge/attitude, which is probably due to the fact that educational status of the farmers in *Derafshi* et al.'s study was higher and that no further analysis was performed [7].

In the present study, not getting formal education increased the possibility of getting a low score from the Knowledge and Attitude Index 30.1 times. Educational status is a factor which not only increases the level of knowledge/attitude regarding the reading of the labels on pesticides, understanding the instructions on how to use protective equipment, knowing the harmful effects of pesticide use [19, 20, 27], but also reduces pesticide poisoning [26, 28, 29]. In *Abollahzadeh* et al.'s [2], *Sharafi* et al.'s [9] and *Fuhriman* et al.'s [28] studies, similar to our study, the knowledge/attitude levels of those with a high level of education were high.

In the present study, low income (≤2000 Turkish liras per month equal to ≤$357 according to April 2019 exchange rates) increased the possibility of getting a low score from the Knowledge and Attitude Index 3.1 times. The lower the income level was the lower the participant's knowledge/attitude score was, which was consistent with that in the literature [18, 28], which caused those with low income to have inappropriate sanitation conditions [5] and to be exposed to pesticides more [7, 13].

In the present study, being single increased the possibility of getting a low score from the Knowledge and Attitude Index 2.1 times, which was consistent with the results of several studies in the literature. This might be due to the fact that farmers who were single or lived with a small number of people did not care about the potential effects of pesticides [11] and that their perception of risk was low [24]. On the other hand, contrary to the finding of our study, in Muleme et al.'s study with a sample size of 167 people, marital status and the number of people living in the household did not affect the knowledge and attitude score [18]. This difference between the studies probably stemmed from the differences between the characteristics and cultural backgrounds of the study groups [7, 33].

CONCLUSIONS

The most noteworthy result of our study is that although it was conducted in a region in the west of Turkey, where people's education is higher, about half of the participating farmers' knowledge and attitude levels were inadequate. The mean scores the participants obtained from the Knowledge and Attitude Index was 12.8 ± 2.8 (min: 4, max: 19) and the median was 13.

Among the factors which caused the participants' mean Knowledge and Attitude Index score to be lower than the median value was advanced age, not being married not getting formal education, living in a non-crowded household, and low-income level. Given the participants' mean knowledge/attitude score was lower, it is recommended that farmers should be trained on harmful effects of pesticides on human health and disposal of hazardous pesticide-related waste and empty containers, and that they should be informed where they can receive information on this issue. They should also be taught about the importance of the use of protective equipment because the participating farmers' tendency to use protective equipment was low. Moreover, public health interventions for farmers should be planned, and training programs for the Pesticide Management Process should be implemented.

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Conflict of interest
The authors have no potential conflict of interest.

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ORIGINAL ARTICLE

ASSESSING THE EFFECT OF ORAL DISEASES ON ORAL HEALTH RELATED QUALITY OF LIFE OF INSTITUTIONALIZED ELDERLY USING ORAL HEALTH IMPACT PROFILE (OHIP-14) QUESTIONNAIRE: A PILOT STUDY

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ABSTRACT

Background. India establishes a geriatric population of about 80 million which is 7.2% of the total population. The geriatric population is often faced by a negative impact on their quality of life due to poor oral health.

Objectives. To assess the impact of oral disease on daily activities and quality of life among the institutionalized elderly in Kanpur, India.

Materials and Method. A total of fifty-six patients were purposively selected from two old age homes through convenience sampling technique. To assess the oral health status WHO basic oral health survey form (1997) was used. Short version of Oral Health Impact Profile (OHIP - 14) questionnaire was used to assess the Oral Health Related Quality of Life (OHRQoL). Independent t-test and ANOVA test was done to determine the relationship between the groups.

Results. Presence or absence of grossly decayed teeth, chronic periodontitis, based on edentulism and remaining sound teeth status produced no significant differences in any of the domains. In comparison with males, females experienced greater impact of oral diseases with respect to mean OHIP-14 score; however, it was non – significant (p=0.45).

Conclusion. Oral health status of the institutionalized subjects in Kanpur city is poor, with edentulism and periodontitis. The effect of oral diseases on the lives of elderly is comparatively low and is non-significant.

Key words: *elderly, OHIP-14, OHRQoL, impact of oral diseases*

INTRODUCTION

The geriatric population is portrayed by extraordinary conditions because of physiological changes normal for maturing, just as diseases and psychosocial and dietary factors that impact their nourishing status [2].

Generally, the aged population is at greater risk of malnutrition due to inadequate food intake (amount) [11], poor selection of food (quality), illnesses that may lead to nutrient loss and also decrease nutrition absorption, also in elder people the nutrition deficiency can be a cause of physiological, psychological, pathological and social factors [5, 8]. This situation is aggravated when institutionalized, "the occurrence of nutritional disorders in institutionalized elderly ranging from 30% to 80%, with a consequent negative impact on their health" [4].

"Oral health is an essential element for general health and quality of life throughout an individual's life course", as written in a WHO report from 2006 about oral health in elderly. Good oral health is a state of being free from oral diseases, infections and pain that restricts normal function and quality of life. The world's population is ageing. Society will face a Challenge in treating oral and general diseases in older individuals as to provide an appropriate treatment, diagnosis of the disease at an early stage is required. According to *Razak et* al. [14] few factors such as illness and health related factors, socio-demographic factors, service-related factors and subjective factors are required for utilization of dental services.

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Due to remarkable increase in the geriatric population, the world is now facing a demographic revolution. According to 2011 census, there are nearly 104 million elderly persons (aged 60 years or above) in India. It is expected that by 2050, India's population of elderly may increase to 323 million [13].

With increasing age, the problems related to health also increases. Oral health is an integral part of general health. Poor oral health consisting dental problems such as, dental caries, periodontal diseases, mobile tooth, missing tooth and many more leads to compromised general health [7]. Because if one can't eat properly their body suffers. Similarly, the different systemic problems also may have adverse effects on oral health. Poor oral health negatively affects the quality of life.

Also, lately the number of elderlies receiving institutional care has increased tremendously, such residents often have to depend upon caregivers for their general and oral health care. Adequate access to dental care can affect their oral hygiene, oral health function that may lead to compromised overall health and also can affect the quality of life [16].

According to the National Oral Health Survey [12] it was observed that poor oral health led to 29.3% of high level of tooth loss, 84.7% of dental caries, 79.4% of periodontal disease, 10 % of mucosal lesions and 0.5% of oral cancer.

Surveys suggest limited utilization of dental services by geriatric population. This may be because of numerous considerable factors. Age related compromised mobility is the main factor for limited use of oral health care facility. Along with that financial dependence, physical dependence, physical health are other contributing barriers. The elderly living alone, who are financially deprived or abandoned from their houses look for alternative homes for living and some choose old age homes [3]. Such elderly does not have facilities for oral health care.

The fact is that we do not have much records about elderly in India concerning the oral disease burden and treatment needs. Moreover, the degree to which oral diseases influences the general health and quality of lives of the geriatrics have not been widely studied. Hence, the present study was conducted to assess the effect of oral diseases on daily activities and quality of life among the institutionalized elderly in Kanpur, India.

MATERIALS AND METHODS

A cross-sectional study was executed among the institutionalized elderly residing in old age homes at Kanpur, India. Reviewing of the study protocol was done and was approved by Institutional Review Board. Residents of two old age homes aged 60 years or more and who gave the written informed consent were included in the study. Inmates with cognitive impairment and with severely debilitated and hearing and/or speech impairment were excluded. A list was arrived at, after short listing through convenience sampling technique and a total of 56 patients were purposively selected.

A self-administered questionnaire was given to the inmates' who participated in the study. The information collected by the questionnaire included: Oral health related quality of life (OHRQoL) by using short version of questionnaire Oral Health Impact Profile (OHIP - 14) [18] and Oral health status of subjects by using Basic Oral Health Survey Form (1997) [19].

The OHIP-14, includes 14 questions which was introduced by *Slade* in 1997 [18]. The objective of OHIP is to present certain types of numerical data for different situations in terms of health and treatment consequences. It covers seven dimensions: functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability and handicap. Five-point Likert scale was used to grade two questions out of each seven subscales.

The data was collected through personal interview (among those who were illiterate or had other impediments in responding to the questionnaire), by filling in the specially designed questionnaire. The interview included assessment of the effect of oral diseases on the daily living and quality of life of the participants using the socio-dental indicator Oral Health Impact Profile. Using mouth mirror and CPI probe, the clinical examinations were conducted in a well illuminated area at the old age homes. Prior to the start of data collection, the investigator was trained in recording the Basic Oral Health survey proforma and intra examiner reliability was calculated as ($\kappa \geq 0.8$).

Along with the oral health the general health of the participants was also reviewed by the general physician. The clinical records of the inmates were likewise analyzed to get data in regards to the major systemic conditions that influenced them and the treatment given. The questionnaire required 25 to 30 minutes to be completed by each subject.

Statistical analysis was done using computer with the aid of Statistical Package for Social Science (SPSS), version 16, USA. Information procured were esteemed suitable for utilizing parametric tests, since the outcomes were normally distributed as seen by Shapiro Wilk test. Descriptive statistics was used to summarize the variables. Independent 't' and ANOVA tests were done to determine the relationship between the groups. For all tests, confidence interval and p value were set at 95% and <0.05 respectively.

RESULTS

A total of 56 old people aged between 71-80 years (mean 74.2±1.3 years) were examined. Among them, 37 were males and 19 were females. On recording the DMFT index (Table 1) it was found that maximum number of study subjects had sound teeth (9.12±4.12) followed by missing teeth (5.96±2.69), filled teeth (3.30±1.06) and decayed teeth (3.39±0.78).

Mean OHIP scores were higher among males across all domains but the difference was not statistically significant (p>0.05). The overall mean OHIP score was 22.65±6.22 and 21.37±5.46 among males and females respectively. This association was not statistically significant (p>0.05) (Table 3).

Similarly, the mean scores were higher among >80 years age group across all the domains and the difference was statistically not significant (p>0.05).

Table 1. Description of study subjects according to caries status

Components	n	Minimum	Maximum	Mean	Std. Deviation
Decayed	56	2.00	4.00	3.39	0.78
Missing	56	2.00	12.00	5.96	2.69
Filled	56	2.00	5.00	3.30	1.06

Table 2 shows the distribution of subjects according to the periodontal status as recorded according to the CPITN index. Only 5.3% subjects had healthy periodontium. Majority of the subjects had shallow pockets (42.8%) followed by calculus (19.6%), deep pockets (16%) and bleeding (12.5%).

The overall mean OHIP score was 20.70±6.15, 23.04±6.07 and 23.80±4.89 among <70, 70-80 and >80 years subjects respectively. It was not statistically significant (p>0.05) (Table 4).

Table 2. Description of study subjects according to periodontal status

Codes	n	%
0 = Healthy	3	5.3
1 = Bleeding	7	12.5
2 = Calculus	11	19.6
3 = Shallow Pockets	24	42.8
4 = Deep Pockets	9	16
X/9 = Not Recorded	2	3.5

Table 3. Gender-wise comparison of OHIP-14 scores and its domains

Domain	Gender	n	Mean	SD	t value	p value
Functional limitation	Male	37	3.14	1.00	0.658	0.514
	Female	19	2.95	1.03		
Physical pain	Male	37	3.19	1.00	0.852	0.398
	Female	19	2.95	1.03		
Physical discomfort	Male	37	3.19	1.00	0.852	0.398
	Female	19	2.95	1.03		
Physical disability	Male	37	3.24	0.89	0.787	0.435
	Female	19	3.05	0.78		
Psychological disability	Male	37	3.30	0.97	0.502	0.618
	Female	19	3.16	1.01		
Social disability	Male	37	3.30	0.97	0.502	0.618
	Female	19	3.16	1.01		
Handicap	Male	37	3.30	0.97	0.494	0.624
	Female	19	3.16	1.01		
OHIP-14	Male	37	22.65	6.22	0.759	0.451
	Female	19	21.37	5.46		

Table 4. Age-wise comparison of OHIP-14 scores and its domains

Domain	Age (in years)	n	Mean	SD	F value	p value
Functional limitation	< 70	23	2.78	1.00	1.770	0.180
	71 – 80	23	3.22	1.00		
	> 80	10	3.40	0.97		
Physical pain	< 70	23	2.78	1.00	2.161	0.125
	71 – 80	23	3.30	0.97		
	> 80	10	3.40	0.97		
Physical discomfort	< 70	23	2.78	1.00	2.161	0.125
	71 – 80	23	3.30	0.97		
	> 80	10	3.40	0.97		
Physical disability	< 70	23	2.96	0.88	1.378	0.261
	71 – 80	23	3.30	0.88		
	> 80	10	3.40	0.70		
Psychological disability	< 70	23	3.13	1.01	0.318	0.729
	71 – 80	23	3.30	0.97		
	> 80	10	3.40	0.97		
Social disability	< 70	23	3.13	1.01	0.314	0.709
	71 – 80	23	3.30	0.97		
	> 80	10	3.40	0.97		
Handicap	< 70	23	3.13	1.01	0.312	0.701
	71 – 80	23	3.30	0.97		
	> 80	10	3.40	0.97		
OHIP-14	< 70	23	20.70	6.15	1.341	0.270
	71 – 80	23	23.04	6.07		
	> 80	10	23.80	4.89		

DISCUSSION

Dental caries was found to be the major problems among the elderly. In our present study mean decayed teeth was reported as 3.39±0.78 which is in accordance with the study findings of *Shivakumar* et al. [17] (mean 3.5). A slightly lesser prevalence was reported by *Khanal* et al. [9] (2.6), *Agarwal* et al. [1] (1.51) and *Shaheen* et al. [16] (1.17) among elderly populations.

The prevalence of periodontitis in our study was (58.8%) which is comparatively less than that reported by *Sha* et al. [15] (71.8%) and in the National Oral Health Survey [12] (79.4%). Other studies conducted by *Khanal* et al. [9] (31%) and *Shaheen* et al. [16] (47.5%) reported a lower prevalence of periodontal disease amongst their study population.

In comparison to males, females experienced greater impact of oral diseases in all the domains of OHIP-14 and this is corroborating with the findings from a previous study conducted [6, 16]. Handicap, psychological disability and social disabilities are most common domains reported by our study participants. Whereas in the study conducted by *Krishnappa* et al. [10] functional limitations and physical pain were the domains reported by the participants which affect their life.

Our study is a cross-sectional study and included only two old age homes of Kanpur city in India, which represented a certain geographic area of the city. In future a longitudinal study on larger sample size is advocated so as to assess the impact of oral diseases on oral health related quality of life of institutionalized elderly people and its wider implication on masses.

CONCLUSION

Poor oral health status was observed in the institutionalized elderly in Kanpur, India, with edentulism and periodontitis which is associated with more OHIP scores. Elderly residing at old age home do not have healthy body and oral health because they lack care and funds. Almost everyone and especially elderly require some sort of dental treatment. Involvement of government, non-government organizations and also private organizations may improve their oral health and general health conditions.

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Authors contributions
AS, RN and DN contributed with the conception, design, acquisition, analysis and interpretation of data and took part in drafting of the manuscript, critical revision and final approval. MA and GR contributed with design, analysis and interpretation of data, drafting of the manuscript and revised it critically until final approval. All authors listed on the title page have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission.

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