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REVIEW ARTICLE

SPENT YEASTS AS NATURAL SOURCE OF FUNCTIONAL FOOD ADDITIVES

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ABSTRACT

Spent yeasts are by-products arising from beer and wine production which over many years have been chiefly used as feed additives for livestock. They contain many valuable and bioactive substances which has thereby generated much interest in their exploitation. Up till now, the main products obtained from beer-brewing yeasts are β-glucans and yeast extracts. Other like foodstuffs include dried brewer's yeast, where this is dried and the bitterness removed to be fit for human consumption as well as mannan-oligosaccharides hitherto used in the feed industry. β-glucans constitute the building blocks of yeast cell walls and can thus be used in human nutrition as dietary supplements or serving as food additives in functional foods. β-glucans products obtained via post-fermentation of beer also exhibit a high and multi-faceted biological activity where they improve the blood's lipid profile, enhance immunological status and have both prebiotic and anti-oxidant properties.
Yeast extracts are currently being used more and more to enhance flavour in foodstuffs, particularly for meat and its products. Depending on how autolysis is carried out, it is possible to design extracts of various meat flavours characteristic of specific meats. Many different flavour profiles can be created which may be additionally increased in combination with vegetable extracts. Within the food market, yeast extracts can appear in various guises such as liquids, pastes or powders. They all contain significant amounts of glutamic acid, 5'-GMP and 5'-IMP nucleotides together with various amino acids and peptides that act synergistically for enhancing the flavour of foodstuff products. Recent studies have demonstrated additional benefits of yeast extracts as valuable sources of amino acids and peptides which can be used in functional foods and dietary supplements. These products possess GRAS status (*Generally Recognised As Safe*) which thereby also adds further as to why they should be used as natural food additives that are functional.

Key words: *spent yeasts, β-glucans, yeast extracts, functional food additives, food additives, foodstuffs.*

STRESZCZENIE

Drożdże pofermentacyjne są produktem ubocznym przy produkcji piwa i wina stosowanym przez wiele lat głównie jako dodatek paszowy. Zawierają one w swym składzie wiele cennych składników bioaktywnych stąd też obserwuje się duże zainteresowanie ich wykorzystaniem. Głównymi produktami otrzymywanymi od niedawna z pofermentacyjnych drożdży piwowarskich są β-glukany i ekstrakty drożdżowe. Produkowane są również spożywcze preparowane suszone drożdże piwowarskie, które na drodze odgoryczenia i suszenia zostają przygotowane do bezpośredniego spożycia oraz mannooligosacharydy stosowane dotychczas w przemyśle paszowym. b-glukany będące elementem budulcowym ścian komórkowych drożdży, mogą być stosowane w żywieniu człowieka jako suplementy diety lub jako dodatki do rożnego rodzaju produktów spożywczych z grupy żywności funkcjonalnej. Preparaty β-glukanów otrzymane z pofermentacyjnych drożdży piwowarskich wykazują wysoką, wielokierunkową aktywność biologiczną, związaną przede wszystkim z poprawą profilu lipidowego krwi, statusu immunologicznego organizmu oraz z oddziaływaniem prebiotycznym i antyoksydacyjnym.
Ekstrakty drożdżowe znajdują coraz szersze zastosowanie jako substancje polepszające smak rożnego rodzaju produktów głównie o profilu mięsnym. W zależności od sposobu przeprowadzenia procesu autolizy możemy otrzymać ekstrakty o specyficznym smaku mięsa rożnego pochodzenia. Ilość profilów smakowych jest bardzo duża i może być dodatkowo zwiększona poprzez kombinacje z ekstraktami z warzyw. Ekstrakty drożdżowe mogą występować w rożnych postaciach handlowych takich jak płyn, pasta czy proszek. Zawierają one w swym składzie znaczne ilości kwasu glutaminowego, nukleotydów 5'GMP i 5'IMP, oraz różne aminokwasy i peptydy o synergistycznym oddziaływaniu polepszającym smak produktu. Ostatnio wykonane badania wskazują na możliwości dodatkowego wykorzystania ekstraktów jako cennego źródła aminokwasów i peptydów mogących znaleźć zastosowanie w żywności funkcjonalnej i suplementach diety. Preparaty drożdżowe posiadają status GRAS (*Generally Recognised As Safe*), co intensyfikuje możliwości ich zastosowania jako naturalnych dodatków funkcjonalnych.

Słowa kluczowe: *drożdże pofermentacyjne, β-glukany, ekstrakty drożdżowe, dodatki funkcjonalne, dodatki do żywności, żywność*

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INTRODUCTION

Along with the scientific and technological advances made in human nutrition, the food industry is increasingly turning towards natural food additives that complement foodstuff products with nutrients, improve flavour, stabilise texture and increase shelf life. Over many years the use of yeasts, obtained as by-products from the brewing industry, have been investigated as being natural sources of nutritionally bioactive components [27, 20, 44]. Up till now, most of the spent brewer's yeast has been considered an inconvenient waste by the brewing industry and had been used in livestock feed production; being a source of protein, vitamins and minerals [27, 48, 50, 51].

At present however, increasingly more food companies process waste yeast slurries into intermediates that are further used in foodstuff production. More and more published studies demonstrate the pro-health effects of spent yeasts and isolated β-glucans thereof, as well as their application to human nutrition [7, 11, 22, 41]. Another large and rapidly growing area of interest is focused on yeast extracts, particularly those derived from autolysis using the endogenous cellular enzymes present, or when further augmented by adding proteolytic preparations [12, 16, 17]. A drawback of other methods for yeast extraction, such plasmolysis or acid hydrolysis, is the high salt content, which in the latter cases also bears the risk of hazardous chemicals being formed, as is the case in the widely used protein hydrolysates for imparting 'meat flavours' to foodstuffs.

In the manufacture of protein hydrolysates through acid hydrolysis, chlorinated compounds can become generated such as 3-chloro-propanediol, which indeed has been detected in vegetable hydrolysates produced by similar methods [27]. Thus for flavour additives, it is recommended that natural products be used in the form of yeast autolysates. It is certain that in the next few years they will replace such previously and widely used protein hydrolysates obtained from acid hydrolysis.

SPENT YEASTS AS A SOURCE FOR BIOACTIVE COMPOUNDS

The nutritional value and basic components of yeasts may vary somewhat depending on the conditions set for growing and the technologies used in their production. The overall content of nitrogenous compounds in dried yeast counted as protein ranges 45 to 55%, of which 80% is protein nitrogen, 10-12% is nucleic acid nitrogen whereas the rest is made up of glutathione, glucosamine, lecithin, and others [25, 27, 36, 37, 38]. Brewer's and baking yeast are characterised by a high β-glucan content; averaging 7.7% [5]. The cell wall constitutes 15-30% of the dry weight and is a complex high molecular weight structure composed of 50-60% β-glucans and 40% mannanoprotein. Yeast also contains around 6% fat, 7% ash and 32% carbohydrate as well as being a good source of B-group vitamins and minerals such as phosphorus, calcium, magnesium and iron. [4] Brewer's yeast is recognised as being a beneficial dietary ingredient making up healthy and nutritious feed for farmed fish as well as being an immunostimulant [25, 45, 51]. Literature studies investigating the possible uses of dried yeast have mainly focused on baker's yeast, where they are chiefly regarded as a dietary supplement; being a source of B-group vitamins, fibre and protein [42, 51]. The beneficial health effects of dietary yeast seen in domestic animals are principally due to the presence of glucan and mannan [22, 23, 41], along with chitin [8].

Interest in yeasts has also risen from the time that dietary nucleic acids were found to be beneficial to farmed animal health in terms of increased immunity and decreased morbidity rates [3, 18, 38, 39]. Nevertheless in human nutrition, the high consumption levels of yeasts observed may be the cause of certain diseases due to their high nucleic acid content [27, 36, 37, 38]. The borderline dose of nucleic acids that has no impact on uric acid levels in blood is 2 g / day, corresponding to an average consumption of 30-50 g of dried yeast. This intake dose is in fact exceeded many times over that recommended for dietary supplements manufactured with yeast [2].

BETA-GLUCANS FROM SPENT YEAST AS PRO-HEALTH ADDITIVES

Polysaccharides isolated from yeast cell walls consist of linearly linked forms of glucan and mannan between β (1-3) / (1-6) positions, where almost 85% of the β-glucan found in yeast cell walls are made up of β (1-3) linear linkages (ie. around 50% of the cell wall by weight). The remaining 12% β-glucan are branched chains linked by β (1-6) bonds [19]. β-glucans from baker's yeast produce similar health effects to those when β-glucans from cereals or fungi are consumed.

Among the β-glucans identified in *Saccharomyces cerevisiae*, zymozan is one of note which is an insoluble long chain glucose polymer with antibacterial properties that enhances immunity, by *inter alia* activating macrophages and the secretion of cytokines such as IL-1, IL-6 and IL-8. Zymozan also stimulates the release of tumour necrosis factor (TNF-α) and it exhibits antioxidant properties [47]. If present in sufficient amounts, β-glucans enhance the immune system by stimulating skin cells to 'quench' free radicals and provide protection against contamination from the environment as well as delaying the cell aging process [4, 6, 46].

At the Department of Functional Food, Ecological

Food and Commodities from the Warsaw University of Life Sciences (SGGW), β-glucans derived from spent brewer's yeast have been found to possess potent and multi-directional biological activity chiefly associated with improving the blood lipid profile and the ability to mobilise the immune system (ie. immunomodulation), together with having prebiotic and antioxidant actions. As a dietary additive fed to rats, β-glucan favourably affects lymphocytes and macrophage levels in the blood, promotes neutrophil phagocytosis in peripheral blood against *Staphylococcus aureus* and *Candida albicans*, increases lymphocyte blast proliferation and significantly increases interferon gamma (INF-γ) release with moderate TNF-α production. Depending on the physicochemical properties of spent brewer's yeast or of dried yeasts containing β-glucan given in dietary supplements, then lowered blood cholesterol and reduced liver lipid concentrations can be observed coupled with increased amounts of the lactic acid-producing bacteria *Bifidobacterium* and *Lactobacillus* in the intestinal microflora when compared to controls along with limiting growth rates of the unfavourable yeast-like fungi *Candada albicans*. Spent yeasts can adjust impaired blood lipid metabolism resulting from an atherogenic diet, they improve intestinal microflora composition and constitute an effective hypo-cholesterolaemic factor irrespective of β-glucan solubilities, but are dependent on the intake dose [48, 52].

The legal options for using yeast-derived β-glucans as a novel food ingredient come under the Regulation of the European Parliament and Council (EU) 2015/2283 from 25th November 2015 concerning novel foods, amending the Regulation of the European Parliament and of the Council (EU) No 1169/2011 and repealing Regulation (EC) No 258/97 of the European Parliament and Council along with Commission Regulation (EC) No 1852/2001 [34, 35]. Thus accordingly, β-glucan yeasts can be used in dietary supplements, fruit drinks, cereal bars, biscuits, crackers, breakfast cereals, yoghurt, chocolate, soup, protein bars and foodstuffs intended for particular nutritional uses; excepting infant formulae.

Introducing β-glucan into foodstuffs bestows upon them the characteristics of functional foods. A study by *Piotrowska* et al. [26] showed that when β-glucan, obtained post-production of brewer's yeast, is added to yogurt in 0.15 to 0.9% amounts then at 0.3% this does not adversely affect sensory food quality, texture and stability of the liquid product during storage. A dietary intake of 250 g of this product can provide the body with 0.7 g of β-glucan, affording pro-health benefits and meeting the criteria set for functional foods [49]. Appearing on the European market, in tablet form, are dietary supplements containing yeast derived β-glucans from the baker's yeast *S. Cerevisiae* and spent yeasts. These are recommended for preventing diseases of the upper respiratory tract, infections and of being of immunological benefit to patients suffering allergies, together with activating monocytes in cancer patients [40, 43].

SPENT YEAST EXTRACTS AS NATURAL FLAVOURING SUBSTANCES

As a mainly cheap biomass, in the form of spent yeasts obtained from breweries, such yeasts are used for manufacturing yeast extracts and to a lesser extent are made in food processing plants where they are grown in various types of specific substrates. In order to release and digest yeast cell contents, a variety of methods are used; the most common ones being autolysis, hydrolysis and plasmolysis. Autolysis and plasmolysis are used for living cell extraction, whilst acid hydrolysis and aqueous extraction are used for non-living cells [15, 30]. Yeast autolysates are concentrated forms of yeast cell contents and are produced principally from autolysis during which hydrolysis occurs spontaneously by the endogenous enzymes present. These yeast autolysates are universally termed as 'Yeast Extracts' [10, 29].

During autolysis, naturally occurring enzymatic reactions take place, where intracellular enzymes become activated by appropriate conditions such as temperature and reaction duration resulting in partial degradation of cell wall structures. Extraction of valuable intracellular proteins, carbohydrates and vitamins is thus facilitated that maintain their native structure. Yeast extracts can also be obtained through using acids or adding enzymes that degrade cell walls and are then referred to as yeast hydrolysates, whereas yeast plasmolysates are obtained by adding large amounts of salts thereby achieving cell wall collapse by osmotic pressure. Autolysates are the purest products of these types because plasmolysates and acid hydrolysates contain large amounts of salt and sodium that are used during plasmolysis or in neutralising acids used in hydrolysis. Autolysis is frequently supplemented by adding proteolytic enzymes to enhance its effectiveness [17, 28].

Yeast extracts have diverse flavours depending on the methods used for their manufacture and are affected by the interactions between amino acids, nucleotides, carbohydrates and peptides present in the extracts. By controlling the manufacturing process, different flavours such as those of chicken soup, meat, cheese, mushrooms and others can be obtained. Meat flavours in extracts are produced, *inter alia,* by the reaction of 5'-nucleotide glutamic acid and cysteine. The sensory properties of the extracts are strongly affected by processes like thickening and drying, during which *Maillard* compounds are formed which are responsible for giving cooked meat flavours. In addition, regulating

the manufacturing process conditions can also impart different colourings to the yeast extracts, from off-white to brown, which can thereby confer a final colour to the finished food product. The presence of glutathione, *Maillard* reaction products and sulphur-containing amino acids also greatly impact on their high antioxidant properties [21].

COMPOSITION AND NUTRITIONAL VALUE OF YEAST EXTRACTS

The chemical composition of yeast extracts depends on the methods and composition of the medium (yeast slurry) used for yeast culturing. The main component of yeast extracts is hydrolysed protein, wherein the average nitrogen values of protein content in the extracts is 73-75% [27, 33].

The free amino acid content may range to 35-40% of the total protein, that includes 10-15 % di-, tri- and tetra-peptides whose molecular weights are below 600 Daltons (Da). Oligo-peptides of 2000 – 3000 Da molecular weight constitute 40-45% of the total protein, whilst the smallest fraction are the larger oligo-peptides at 2-5%. In those yeast extracts obtained by autolysis, the ratio of free amino acids to the di-, tri-, tetra- and oligo-peptides is relatively constant, but may become altered significantly, if exogenous enzymes are added for degrading cell walls. The ratio of total to free amino acids is not constant because at least 85% leucine, alanine, methionine and phenylalanine are present in their free forms whilst for aspartic acid, glycine, and arginine this only ranges at 14-37%. Such outcomes arise from the characteristics of the degradation process through the activity of proteases and peptidases, which during autolysis sever internal chemical bonds within amino acids, whereas carboxy-peptidases and amino-peptidases break the external amino acid bonds. Yeast autolysis products have high contents of B vitamins and trace elements. Vitamin contents can vary depending on the autolysis process, and, so that high amounts are therefore maintained, methodological parameters become vital such as pH, reaction times and sterilisation conditions. Differences in mineral content likewise depend on how the filtration and separation of yeast extracts are performed from their insoluble cellular components such as glucans or mannano-oligosaccharides. A standard yeast extract of 100 g contains about 3 mg of thiamine, 11.9 mg of riboflavin, 68 mg niacin, 3.1 folic acid and 30 mg calcium pantothenate. The mineral content in 100 g of a standard extract contains about 120 mg of calcium, 200 mg magnesium, 3.3 g of potassium and 5 mg of copper; the sodium content obtained when no salts are added is less than 0.5 g [16, 27].

APPLICATIONS OF YEAST EXTRACTS

The legal use of yeast extracts is covered by the Regulation of the European Parliament and Council Regulation (EC) No 1334/2008 of 16th December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods, where extracts are classified as being natural flavourings [7, 35]. Yeast extracts have a GRAS status (Generally Recognised As Safe), which thereby promotes their likely use as natural additives. Yeast autolysates and extracts are used as additives to intensify the flavour and aroma of foodstuffs, especially those enhancing meat characteristics that contain more than 20% of the amino acids glutamic and aspartic acid, (being flavour enhancers), as well as the nucleotides 5'-GMP and 5'-IMP which act synergistically with these amino acids to impart the 'umami' (savoury) taste to foods. The flavour enhancement afforded by such yeast extract additives, through natural means, in the production of ready-to-eat food has permitted a marked reduction in the use of glutamate and ribonucleotides. These yeast formulations are used in the manufacture of many food products, including conventional and organic food: eg. in soups and sauces for reheating ready-to-eat dinner dishes, meat and mushroom fillings, cold meats, pate, savoury snacks and a range of food concentrates. In dietetic and 'light' foodstuffs, with reduced fat or carbohydrates, yeast extracts improve their sensory qualities [14, 24, 27].

Yeast extracts can also mask sour and bitter tastes, so enhancing food flavour, and simultaneously serving as food colourings and antioxidants. In those foodstuffs rich in 5'- GMP (guanosine monophosphate) and 5'-IMP (inosine monophosphate) sweet and salty flavours may be slightly reinforced but at the same time bitter and sour tastes will become significantly lessened. Improving foodstuff flavour by adding yeast extracts arises from the interaction between different amino acids (including glutamic acid), and when 5'-nucleotides are coupled to peptides and their reaction products. The combined action of these components causes a constant stimulation of taste bud receptors creating a greater sensory potential for such flavouring substances. Glutamate is the most important flavour enhancing substance and its impact threshold lies at a concentration of 100-300 ppm, whereas for 5'-GMP and 5'-IMP this threshold stands respectively at 35 and 120 ppm (concentrations in aqueous solution). Glutamate's impact on flavour may however be 10-15 times greater when used in conjunction with the 5'-nucleotides. By having appropriate control over autolysis *via* regulating temperature, pH, and reaction duration this allows manufacturers to change the sensory profile of the produced extracts, thus enabling flavours such as roast meat, bouillon (broth), meat and even cereal to be achieved [28, 29, 53, 14, 32].

Yeast extracts in the form of pastes are valuable components of both vegetarian and conventional diets, where they are used for toast spreads and as an ingredient in soups and ready-to-eat meals [1]. Using pastes or extracts in powder form for vegetarian diets is entirely justifiable as they contain all the essential amino acids, especially large amounts of lysine, valine and isoleucine along with Group B vitamins. Due to their composition, they can be used in those foodstuffs requiring enrichment of amino acids and B vitamins, for example in vegetarian and cereal products made from flour with low wholemeal content or also in vegetable juices [31]. Studies at the Department of Functional Food, Ecological Food and Commodities from the SGGW conducted by *Podpora* et al. [27] demonstrated that autolysate protein can be obtained from spent yeasts possessing specific functional features ie. with an appropriately designed content of free amino acids and amino acids in the form of polypeptides of specific molecular weight, and at the same time having high contents of essential amino acids. Such autolysates could be useful in manufacturing functional foods and dietary supplements similar to current methods for making enzymatic protein hydrolysates derived from milk protein. The study used *Saccharomyces cerevisiae*, a spent brewing yeast from Polish breweries, with a protein content above 42%. According to the conditions applied for autolysis, the products so obtained can have free amino acids ranging from 11.2% to 77.5%, and peptides are suitably designed to range from molecular weights of 1000 Da to 6000 Da. These types of autolysates can thus extend the range of protein hydrolysates found on the market that are used in food supplements and nutritional additives. They can also be particularly useful in vegetarian diets [27].

CONCLUSIONS

As by-products in the manufacture of beer and wine, spent yeasts are increasingly used not only as animal feed additives but as valued and fairly inexpensive nutrition products from which natural food additives are derived thereof such as: β-glucans and yeast extracts. β-glucans obtained from spent foodstuff yeasts, like β-glucans from oats and barley, have several health-promoting effects and can be widely used in food supplements and functional foods.

β-glucans derived from spent yeasts and baker's yeast exhibit high and multidirectional biological activity which has been demonstrated in laboratory animals; mostly consisting of improved lipid profiles in the blood and liver, stimulating immunomodulation as well as producing prebiotic and antioxidant effects. They are safe to use and possess GRAS status (Generally Recognised As Safe). Indeed, they are used in the United States; having been authorised by the Food and Drug Administration (FDA). In Europe, β-glucan and yeast fall under the Regulation of the European Parliament and Council Regulation (EC) No 1334/2008 [35] and the Regulation of the European Parliament and of the Council (EU) No 2015/2283 [34]. Yeast extracts are valuable and natural flavouring additives that reinforce meat flavours in many foodstuffs and spice mixtures, replacing the commonly used protein hydrolysates obtained by acid hydrolysis. Latest studies indicate that they can also be used in functional foodstuffs and dietary supplements as sources of amino acids and peptides. They can thus fulfil the dual function of being a pro-healthy functional additive and simultaneously being an important flavouring additive. The commonly used additive glutamic acid, and its salts, for giving meat profile flavours to foodstuffs is now increasingly being replaced by yeast extracts and spices due to the unfavourable opinions of consumers regarding the former.

Conflict of interest

The authors declare no conflict of interest.

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REVIEW ARTICLE

CHIA SEEDS (SALVIA HISPANICA): HEALTH PROMOTING PROPERTIES AND THERAPEUTIC APPLICATIONS – A REVIEW

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ABSTRACT

Chia has been known for over 5,500 years. Chia seeds were one of the most important components of the diet of Mayas and Aztecs. The chemical composition and technological properties of chia give the plant a high nutritional potential. Chia is a good source of polyunsaturated fatty acids: *omega*-3 and *omega*-6, soluble dietary fiber. It also contains appreciable amount of proteins and phytochemicals. Nutritional value of chia is the reason why it is used in prophylaxis of several non-infectious diseases such as obesity, hypertension, cardiovascular diseases (CVDs), cancer and diabete**s.** Nutritional and therapeutic aspects of chia are currently being researched by many scientific centres. The aim of this article is to present the nutritional and therapeutic values of chia.

Key words: *chia seeds, Salvia hispanica, health, fatty acids*

STRESZCZENIE

Szałwia hiszpańska jest znana od ponad 5500 lat. Nasiona szałwii hiszpańskiej były jednym z najważniejszych składników pożywienia dla Majów i Azteków. Skład chemiczny oraz właściwości technologiczne szałwii hiszpańskiej sprawiają iż roślina ta posiada duży potencjał żywieniowy. Szałwia hiszpańska jest dobrym źródłem niezbędnych wielonienasyconych kwasów tłuszczowych *omega*-3 i *omega*-6 oraz rozpuszczalnego błonnika. Zawiera także znaczne ilości białka i związków fitochemicznych. Wartość odżywcza szałwii sprawia iż roślina ta wykorzystywana jest wspomagająco w profilaktyce wielu chorób niezakaźnych, takich jak: otyłość, nadciśnienie, choroby sercowo-naczyniowe, a także chorób nowotworowych czy cukrzycy. Aspekty żywieniowe i zdrowotne szałwii hiszpańskiej są obecnie przedmiotem badań w wielu ośrodkach naukowych. Niniejszy artykuł ma na celu przybliżenie czytelnikowi walorów żywieniowych i zdrowotnych szałwii hiszpańskiej.

Słowa kluczowe: *nasiona chia, Salvia hispanica, zdrowie, kwasy tłuszczowe*

INTRODUCTION

Salvia is a genus of about 900 species of green plants, shrubs, subshrubs and bushes of the *Salvia* L. family. Chia (*Salvia hispanica* L.) is a representative of the *Salvia* genus. Among the species of the *Labiatae* family chia is distinguished by both high nutritional and therapeutic potential. *Salvia hispanica* L. is an annual plant growing in an area stretching from western Mexico to northern Guatemala. The optimal development of the plant is guaranteed by the warm climate, high rainfall and temperatures of 15-30 °C [13, 14]. The maximum height of the plant is 1 m. It has opposite leaves, which are 4-8 cm long and 3-6 cm wide [38]. The flowers are purple or white and sized 3-4 mm. They are gathered in whorls on top of shoots. The fruits (schizocarps) contain numerous oval seeds, which are about 2 mm long. The seeds are mottle-coloured with brown, grey, black and white [23, 33, 39]. The word 'chia' derives from the Náhuatl word 'Chian', which means 'oily'. The other part of the name *Salvia hispanica* was given to the plant by *Carl Linnaeus* (1707-1778), who discovered the wild-growing plant in the new world and confused it with a native plant from Spain [16]. However, chia comes from Mexico and it was imported to Spain by *Hernán Cortés* [40].

Chia has a high nutritional potential due to the seed composition. The composition depends on genetic factors and on the effect of the ecosystems where the

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plants were grown [5]. Chia seeds contain 16-26% of protein, 31-34% of fat, 37-45% of carbohydrates in total, 23-35% of total dietary fibre (Table 1). Apart from that, they are a source of minerals (calcium, phosphorus, potassium and magnesium), vitamins (thiamine, riboflavin, niacin, folic acid, ascorbic acid and vitamin A) and antioxidant compounds [23, 33]. The energetic value of chia seeds is 459-495 kcal/100 g [15, 27].

The influence of bioactive compounds in chia seeds is the subject of research conducted in numerous scientific centres. The aim of this article is to present the nutritional and therapeutic values of chia.

Table 1. The chemical composition of chia seeds

Component	Content of nutrients in chia seeds [g/100 g d.w.]				
	Reference [38]	Reference [5]	Reference [44]	Reference [5]	Reference [34]
Protein	16.54	19.6	21.52	16.45-26.03	18.65
Fats	30.47	34.4	21.69	29.98-33.50	33.00
Ash	-	4.6	3.63	-	4.35
Carbohydrates	-	41.4	45.30	-	37.73
Dietary fibre	34.4	23.7	-	-	28.36

'-' no data

NUTRITIONAL PROPERTIES OF CHIA SEEDS

Lipids

Lipids are bioactive substances which the human organism needs to accumulate energy, form structural elements of cell membranes and regulate physiological functions. If there are no enzymatic systems capable of forming double bonds at positions n-3 and n-6, the organism cannot synthesise fatty acids, such as ω-3 *alpha*-linolenic acid and ω-6 *alpha*-linoleic acid. Therefore, it is necessary to provide the organism with a supply of lipids in food. Chia seeds contain 25 - 40% of fat, most of which is in the form of polyunsaturated fatty acids, such as ω-3 *alpha*-linolenic acid and ω-6 *alpha*-linoleic acid [33]. As a result of the processes of desaturation and elongation these acids are converted into long-chain polyenoic acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [18]. In comparison with other vegetable oils chia seed oil is characterised by high content of polyunsaturated fatty acids (Table 2). The therapeutic quality of a diet is affected not only by the amount of *omega*-3 PUFAs consumed but also by their proportion to *omega*-6 acids. The adequate ratio between the supply of ω-6 and ω-3 acids is 4-5:1 [25, 46]. However, this proportion is far from recommended (15-20:1) in the diet of an average European inhabitant due to excessive consumption of ω-6 fatty acids and saturated fatty acids. In chia seed oil the ratio between ω-6 and ω-3 acids is 0.32-0.35 [12, 44]. The high content of ω-3 acids in chia seed oil enables reduction of the share of ω-6 acids in daily food rations. Apart from that, an adequate supply of unsaturated fatty acids reduces the risk of ischaemic heart disease and increases immunity of the organism [32].

Table 2. The composition of fatty acids in chia seed oil

Fatty acids	Content of individual fatty acids [% of total fat content]					
	Ref. [12]	Ref. [3]	Ref. [1]	Ref. [22]	Ref.[15]	Ref.[44]
Palmitic acid 16:0	7.10	9.66	6.30	7.2	6.69	5.85
Stearic acid 18:0	3.24	4.34	3.10	3.8	2.67	2.49
Oleic acid 18:1	10.53	6.84	7.50	15.2	10.55	6.16
ω-6 α-linolenic acid 18:2	20.37	17.65	19.90	19.1	17.36	17.47
ω-3 α-linolenic acid 18:3	59.76	64.08	63.4	64.7	62.02	54.49

Proteins

Proteins, peptides, amino acids being different matrices are necessary cell components enabling normal function of the organism. The content of proteins in chia seeds is 16-26%, most of them being prolamins (538 g/kg of crude protein), followed by glutelins (230 g/kg of crude protein), globulins (70 g/kg of crude protein) and albumins (39 g/kg of crude protein) [5, 38, 39]. Patients suffering from coeliac disease can consume chia seeds because they do not contain gluten proteins [35]. Chia seeds contain more proteins than rice, maize, barley or oats seeds [2]. According to the data of the United States Department of Agriculture [38], chia seeds contain 18 amino

acids, including 7 exogenous amino acids, which are considered to be indispensable. The study by *Olivos-Lugo* et al. [39] revealed that glutamic acid, which is responsible for proper functioning of the brain, is the predominant amino acid in chia seeds.

Table 3. The percentage of polyunsaturated fatty acids [PUFAs] in chia oil vs other vegetable oils

Type of oil	PUFAs (% of total fatty acids)			References
	ω-3	ω-6	Total	
Chia	59.76	20.64	80.40	[12]
Perilla	60.93	14.72	75.85	[12]
Flax	42.90	30.90	73.80	[29]
Wheat germ	2.90	56.60	59.60	[29]
Sunflower	0.50	55.90	56.40	[29]
Pumpkin seed	0.50	47.30	47.80	[29]
Rapeseed	9.80	20.30	30.20	[29]

Table 4. The content of indispensable amino acids in chia seeds

Amino acid [g/100 g]	USDA [38]	Amino acid [mg/kg/day]	WHO data for 2002 [54]	WHO data for 1985 [54]
Arginine[a]	2.14	Histidine	10	8-12
Lysine	0.97	Isoleucine	20	10
Histidine	0.53	Leucine	39	14
Phenylalanine	1.01	Lysine	30	12
Leucine	1.37	Methionine + cysteine	15	13
Methionine	0.59	Phenylalanine + tyrosine	25	14
Valine	0.95	Threonine	15	7.0
Threonine	0.71	Tryptophan	4.0	3.5
Total	8.27	Total	184	93.5

[a] not recognised as indispensable in the report published by the FAO/WHO/UNU [WHO 2007]

Dietary fibre

Dietary fibre is an important component of everyday diet. Optimal consumption of dietary fibre, i.e. 25-30 g/day has positive influence on health. The American Dietetic Association established the preferable ratio between insoluble and soluble dietary fibre fractions at 3:1 [9]. The content of fibre in chia seeds is 23-41%, where the insoluble fraction makes about 85% and the soluble fraction makes about 15% [30, 42]. The content of fibre in chia seeds depends on the region of cultivation and climate. Chia seeds contain about twice as much fibre as bran, 4-5 times more than almonds, soy, quinoa or amaranth [38]. They may play an important role in preventing and treating diseases of the digestive and circulatory systems, diabetes, colorectal cancer, kidney stones, haemorrhoids and metabolic disorders [3, 26].

Vitamins and minerals

Vitamins and minerals are necessary for normal function of the organism. An adequate supply of these elements enables optimal control of the amount of hormones, growth regulators and differentiation of cells and tissues. It also protects the organism from oxidative stress. Chia seeds are a source of B vitamins: thiamine (0.62 mg/100 g), riboflavin (0.17 mg/100 g), niacin (883 mg/100 g) and folic acid (49 mg/100 g) [38]. In comparison with rice and maize seeds chia seeds contain more niacin and comparable amounts of thiamine and riboflavin [7].

Table 5. Content of minerals in chia seeds

Minerals	Content of minerals (mg/100 g)		
	Ref. [38]	Ref. [8]	Ref. [28]
Calcium	631	624	580
Phosphorus	860	799	696
Potassium	407	666	870
Magnesium	335	369	403
Iron	7.7	24.4	10.9
Zinc	4.6	6.9	6.0
Selenium*	55.2	78.0	-

* µg/100 g; – no data

Apart from that, chia seeds are a source of minerals. They contain 6 times more calcium, 11 times more phosphorus and 4 times more potassium than cow's milk [7].

Antioxidants

Oxidation is an important biological process, which is indispensable for the production of energy in the human organism. During metabolism molecular oxygen is reduced to water. When electrons are being transferred, free reactive forms of oxygen are being generated, such as hydrogen peroxide, hydroxyl and peroxide radicals. Free radicals are considered to be the cause of neurological diseases, inflammations, immunodeficiency, ageing, ischaemic heart disease, strokes, *Alzheimer's and Parkinson's* diseases and cancers [21, 41].

The following substances have been detected in chia seeds: tocopherols, sterols (approx. 50% β-sitosterol), and polyphenolic compounds, such as protocatechuic acid, gallic and p-coumaric acids, caffeic acid, chlorogenic acid as well as epicatechin, quercetin, kaempferol, rutin and apigenin (Table 6) [12, 24, 42].

The total content of vitamin E in chia seeds is 238-427 mg/kg and it is comparable to peanut oil (398.6 mg/kg), but it is lower than in linseeds (588.5 mg/kg), sunflower (634.4 mg/kg) or soybean (1,797.6 mg/kg). *Reyes-Caudillo et al.* [42] observed that the content of antioxidants was different, depending on the method of their extraction (Table 7).

Table 6. Content of antioxidants in chia seed extracts (mg/g)

Antioxidant	Reference [42]	Reference [6]	Reference [15]
Polyphenols	0.511-0.881	0.914-0.975	0.641
Chlorogenic acid	0.0459-0.102	0.214-0.235	0.00468
Caffeic acid	0.003-0.0068	0.141-0.156	0.03089
Quercetin	0.15-0.268	0.006	0.17
Kaempferol	0.360-0.509	0.024-0.025	0.00017

Table 7. Content of polyphenols in chia seed extracts (mg/g)[42]

Antioxidant	Crude extract	Hydrolised extract
Polyphenols	0.757-0.881	0.511-0.777

THERAPEUTIC AND DIETETIC PROPERTIES OF CHIA SEEDS

The nutritional properties of chia seeds, such as: high content of polyunsaturated fatty acids, vegetable protein, dietary fibre, vitamins, minerals and bioactive substances result in numerous studies on these seeds in order to prove their therapeutic properties. Hypotensive [52], antineoplastic, laxative and analgesic properties are attributed to chia seeds. They are said to protect the cardiovascular system [2], exhibit anti-inflammatory properties, control lipid metabolism [10, 11, 43], have anti-oxidative properties and increase the performance of athletes [49] (Table 8). A randomized, single-blind trial on 20 adults with type 2 diabetes found significant reduction in systolic blood pressure and C-reactive protein concentration in blood plasma even after ingesting 37g chia seeds added to bread per day for 12 weeks, a double increase of α-linolenic acid and eicosapentaenoic acid in plasma was noted as compared to the control group. Anticoagulant and anti-inflammatory effect of chia seeds may help in preventing strokes and heart attacks in type-II diabetic patients [52]. Increase of unsaturated fatty acids in plasma blood was observed also in the study of postmenopausal healthy women supplemented with 25 g milled chia seeds per day for 7 weeks [24].

Effect of ingesting 50 g chia seeds for 12 weeks was examined on 76 adults. This study found no significant reduction in inflammatory markers, body weight, blood pressure, lipid profile and blood sugar levels [36]. Similar results were obtained in the study conducted with 62 obese women supplemented with 25g whole or 25g milled chia seeds [37]. However, reduction in postprandial glycaemia in healthy subjects was showed in another studies [20, 51, 53].

Effect of dietary intervention in checking metabolic syndromes was evaluated through randomized double-blind trial. This trial conducted on 67 adults found significant reduction of triacylglycerols, C-reactive protein concentrations and insulin resistance in group with chia-based diet [19]. It was observed that ingesting 35 g chia flour for 12 weeks decreased total cholesterol level and increased LDL cholesterol [47]. Although the presence of active ingredients in chia seeds contributes to health benefits, safety and efficiency of this medicinal food or natural product, they need to be validated by scientific protocols, since clinical studies on the safety and efficiency of chia seeds are still limited and those reported have not shown conclusive results [50].

Table 8. The therapeutic properties of chia seeds.

Duration of study	Population under study	Supplementation form	Results	References
12 weeks	26 men and women aged 45-55 years (placebo 7; chia flour 19)	35 g chia flour/day	Decreased body weight in the group consuming chia flour, a greater decrease in obese people, no difference from the placebo group. Reduced total cholesterol and increased LDL cholesterol in the supplemented group.	[47]
6-12 weeks	36 young obese rats	133 g chia seeds/ 1 kg diet or 40 g chia oil/ 1 kg diet	Chia seeds and oil reduced oxidative stress in vivo by improving the antioxidant status and reducing lipid peroxidation in diet-induced obese rats.	[31]
5-6 weeks	Hypercholesterolaemic rabbits	10 g chia oil/1kg diet [CD] or 10 g chia oil + 1g cholesterol/ 1 kg diet [HD-Cd]	Reduced concentration of triacylglycerols and increased content of α-linolenic acid in the serum in HD-cd group. Chia seed oil may have protective effect on blood vessels.	[45]
120 minutes	Randomized double-blind trial, 13 healthy people	50 g bread with 0, 7, 15, 24 g chia seeds added	The blood test showed reduced postprandial glycaemia.	[20]
10 weeks	Randomized double-blind trial, 62 overweight women aged 49-75 years	25 g whole chia seeds /day or 25 g ground chia seeds /day	No influence of [whole/ground] seeds on inflammatory markers, blood pressure, body composition. Increased concentration of α-linolenic and eicosapentaenoic acids in the blood serum of obese women consuming ground seeds vs the control group and the group consuming whole chia seeds.	[37]
2 months	Randomized double-blind trial, 67 men and women aged 20-60 years	4 g of chia seeds mixed with palm, oats and soy powder diluted in 250 mL of water/2 per day + reduction diet	Reduced concentration of triacylglycerols, CRP and insulin resistance in the supplemented group.	[19]
7 weeks	10 women after menopause	25 g ground chia seeds / day	Increased concentration of α-linolenic and eicosapentaenoic acids in the serum of women supplemented with ground chia seeds.	[24]
120 minutes	11 healthy men and women	0, 7, 15, 24 g chia seeds added to bread /day	Postprandial glycaemia significantly reduced in comparison with the control group.	[51]
12 weeks	Single-blind trial, 76 obese people (placebo 37; chia seeds 39)	25 g chia seeds in 250 mL water twice a day	Increased concentration of α-linolenic acid in the serum of the group under study vs placebo. No influence of seeds on inflammatory markers, blood pressure, body composition.	[36]
1 month	18 male Wistar rats	150g chia seeds /kg diet or 50g chia oil /kg diet	No influence on IgE concentration in the serum, body weight and thymus weight.	[17]
1 month	32 male rats	160g whole chia seeds/kg diet [T2] or 160g ground chia seeds/kg diet [T3] or 53.4g chia seed oil/kg diet [T4]	Reduced triglyceride concentration in the serum of T2 rats and increased HDL content in the serum of T3 rats in comparison with the control group. Increased concentration of fatty acids 18: 3n–3, 20: 5n–3 and 22: 6n–3 in the serum of T2-T3 rats in comparison with the control group.	[4]
12 weeks	Randomized single-blind trial; 20 men and women aged 18-75 years with type 2 diabetes	37g ground chia seeds added to bread /day	Reduced systolic blood pressure and CRP concentration, a double increase in the concentration of α-linolenic and eicosapentaenoic acids in the serum of patients supplemented with ground chia seeds in comparison with the control group.	[52]
120 minutes	Randomized, controlled, crossover study 15 healthy adults	25 g ground chia seeds with 50 g glucose or 25 g flax with 50 g glucose or alone 50 g glucose	Postprandial glycaemia significantly reduced in comparison with the control group. Chia significantly reduced the mean ratings of desire to eat, prospective consumption and overall appetite score, when compared with flax.	[53]

CONCLUSIONS

Chia seeds are traditionally consumed in Mexico and south-western United States. In the Europe Union countries the marketing of chia *(Salvia hispanica)* seeds as new food ingredients has been permitted only since 13 October 2009 according to the Regulation No. 258/97 of the European Parliament and Council. Due to the chemical composition of chia seeds, which prove their nutritional potential, if they are added to food, they may improve its nutritional value and a diet with chia seeds may be an element of prevention of civilisation-related-diseases.

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ORIGINAL ARTICLE

DIETARY FIBER SOURCES CONSUMPTION AND OVERWEIGHT AMONG POLISH MALE STUDENTS. A CROSS-SECTIONAL STUDY

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ABSTRACT

Background. There has been an increase in the prevalence of overweight and obesity in adolescents and young adults, especially in men than women. Many adolescents have a sedentary lifestyle and consume more processed, low-fiber foods.

Objective. The aim of this study was to assess the frequency of fiber intake and its selected dietary source consumption in relation to the overweight among Polish male students.

Material and Methods. This cross-sectional study involved 1,233 male students aged 13.0-24.9 years from northern, eastern and central Poland. The respondents completed a self-administered Block Screening Questionnaire for Fruit/Vegetable/Fiber Intake and measurements of their body mass and height were performed. The overweight and obesity prevalence was assessed using international standards.

Results. The most frequently consumed foods by students included: white bread and potatoes, fruit and fruit or vegetable juices. The odds of overweight (including obesity) were lower from 28% (OR=0.72; 95%CI:0.56-0.93) to 31% (OR=0.69; 95%CI:0.50-0.95) with a daily consumption of white bread compared to non-daily consumption of white bread. Consumption ≥4 times/week of prepared vegetables (cooked, preserved or marinated) was associated with 51% lower odds of overweight (OR=0.49; 95%CI:0.27-0.97) compared to consumption less than 4 times/week of these foods. The odds of overweight for the level of fiber intake was insignificant.

Conclusions. This study provides surprising insights regarding high-fiber and low-fiber food consumption and overweight in Polish male students. A lower odds of overweight was associated with a higher frequency consumption of relatively low in fiber foods as white bread and cooked, preserved or marinated vegetables. Most of the students consumed fiber at an unacceptable level, so a beneficial impact of high-fiber foods on overweight prevalence was not shown.

Key words: *adolescent, male, obesity, dietary fiber, food frequency*

STRESZCZENIE

Wprowadzenie. Obserwuje się wzrost częstości występowania nadwagi i otyłości u młodzieży i młodych osób dorosłych, zwłaszcza u mężczyzn niż kobiet. Wielu nastolatków prowadzi siedzący tryb życia i spożywa wysoko przetworzoną, ubogą w błonnik żywność.

Cel. Celem badań była ocena częstotliwości spożycia błonnika i wybranych, pokarmowych źródeł błonnika w relacji do występowania nadwagi u Polskich uczniów i studentów płci męskiej.

Materiał i metody. Badania przekrojowe obejmowały 1 233 uczniów i studentów płci męskiej w wieku 13,0-24,9 lat z północnej, wschodniej i centralnej Polski. Respondenci wypełnili samozwrotny kwestionariusz Block (Screening Questionnaire for Fruit/Vegetable/Fiber Intake). Wykonano pomiary masy i wysokości ciała u każdego z badanych. Oceny występowania nadwagi i otyłości dokonano z wykorzystaniem międzynarodowych standardów.

Wyniki. Do najczęściej spożywanych przez respondentów należały: biały chleb, ziemniaki, owoce, owocowe lub warzywne soki. Iloraz szans występowania nadwagi (w tym otyłości) był niższy o 28% (OR=0,72; 95%CI:0,56-0,93) do 31% (OR=0,69; 95%CI:0,50-0,95) u osób codziennie spożywających białe pieczywo w porównaniu z osobami, które nie spożywały codziennie białego pieczywa. Spożycie 4 razy w tygodniu lub częściej przetworzonych warzyw (gotowanych, konserwowanych lub marynowanych) było związane z 51% niższym ilorazem szans występowania nadwagi (OR=0,49; 95%CI:0,27-0,97) w porównaniu z rzadszym spożyciem tej żywności. Iloraz szans występowania nadwagi dla poziomu spożycia błonnika nie był istotny.

Wnioski. Badanie dostarcza zaskakujących spostrzeżeń dotyczących spożycia żywności o wysokiej i niskiej zawartości błonnika i występowania nadwagi u Polskich uczniów i studentów płci męskiej. Niski iloraz szans występowania nadwagi był związany z częstszym spożyciem żywności o stosunkowo niskiej zawartości błonnika, takiej jak białe pieczywo

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i gotowane, konserwowane lub marynowane warzywa. Większość badanych uczniów i studentów spożywała błonnik na niewystarczającym poziomie, zatem nie odnotowano korzystnego wpływu spożycia żywności wysoko błonnikowej na występowanie nadwagi.

Słowa kluczowe: *młodzież, mężczyźni, otyłość, błonnik pokarmowy, częstotliwość spożycia*

INTRODUCTION

Obesity has been recently classified as a disease of civilization because of the high percentage of people with excessive body fat in developing and industrialized countries. According to the WHO [34] in 2008, 35% of the world's adult population was overweight and 11% was obese. This problem also affects Poland. In 2009, 37% and 16% of adult Poles were overweight and obese, respectively [15]. Over 13 years (1996-2009), the percentage of adult overweight and obese men increased by 26% and 7%, respectively, while in women it increased by 18% and 1%, respectively [15]. There has been a disturbing increase in the prevalence of overweight and obesity in adolescents and young adults, especially those above 20 years of age [5, 15]. Overweight and obesity in childhood and adolescence increase the risk of physical morbidity and premature mortality in adulthood [34]. Obesity has been shown to increase the risk of many chronic diseases, such as cardiovascular disease, type-2 diabetes, certain types of cancer, osteoarthritis, sleep apnea and asthma [18, 23, 34].

The direct cause of obesity is a positive energy balance, maintained for a longer period of time. It is favored by an increase in food consumption over the current energy expenditure and low physical activity. Many overweight or obese adolescents have a sedentary lifestyle and consume more processed foods which are high in fat or sugar, such as chips and sweets, soft drinks, which are available in vending machines in schools and universities [14, 22].

There is convincing evidence that the factors which decrease the risk of obesity include a high dietary fiber intake from wholegrain cereals, fruit, vegetables and legumes [35]. In Poland, in 2009 compared with 2000, daily intake of fiber in households decreased by 14% (from 29.5g/d to 25.4g/d) [15]. The main dietary fiber sources in the Polish diet are: cereal products (41.5%) vegetables (26.4%) and potatoes (11.8%), whereas the lowest dietary fiber sources are legumes (1.8%) [15]. Both Polish and international literature report a lower intake of vegetables and fruit in male than female students [16, 25] and adolescents [23] with respect to the energy value of the diet. Many studies have found that most school and university male students have fiber intake below the recommended level [5, 11, 28, 31, 32]. Changes in student eating habits are often the result of environmental and social changes associated with education. A large number of lessons and the need to take additional paid work may contribute to the dietary deficiencies of young people [33].

More nutritional studies are concentrated on females than males. To the best of our knowledge, there is no up-to-date information regarding dietary fiber source consumption and obesity among male students aged 13.0-24.9 years. A large-scale study related to nutrition, physical activity, health and obesity was carried out on the Polish population in 2000 [29]. After Poland entered the European Union (in 2004) and underwent political, social and economic transformation, an increase in the consumption of processed, low-fiber foods and sedentary lifestyle in Polish society was observed [23]. The aim of the study was to assess the frequency of fiber intake and its selected dietary sources consumption in relation to the overweight among Polish male students aged 13.0-24.9 years.

MATERIAL AND METHODS

Ethical considerations

The study was approved by the Bioethics Committee of the Regional Medical Chamber in Olsztyn on 27 June 2001 (resolution no. 49/2001) and by the Bioethics Committee of the Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn on 17 June 2010 (resolution no. 20/2010). All participants gave their informed and voluntary consent to take part in the study and the obtained information was confidential and used only for scientific purposes.

Study design and sample collection

This cross-sectional study was conducted in 2010-2013 and was a part of a large study (2008-2014) concerning socioeconomic status, food consumption and body weight. The present study involved 1,233 participants aged 13.0-24.9 years, including 830 school male students aged 13.0-18.9 years and 403 university male students aged 19.0-24.9 years. The main inclusion and exclusion criteria of the sample collection, the participation rate of the students and the study design are shown in Figure 1. A quota sampling method was used to obtain similar numbers of respondents by age groups. Respondents were recruited in primary and secondary schools (school students) as well as at the University of Warmia and Mazury in Olsztyn, located in the north-eastern part of Poland (university students). Classes were chosen from the selected primary and secondary schools while students originated from the selected university student groups. The characteristics of the sample, divided into four age groups, are shown in Table 1.

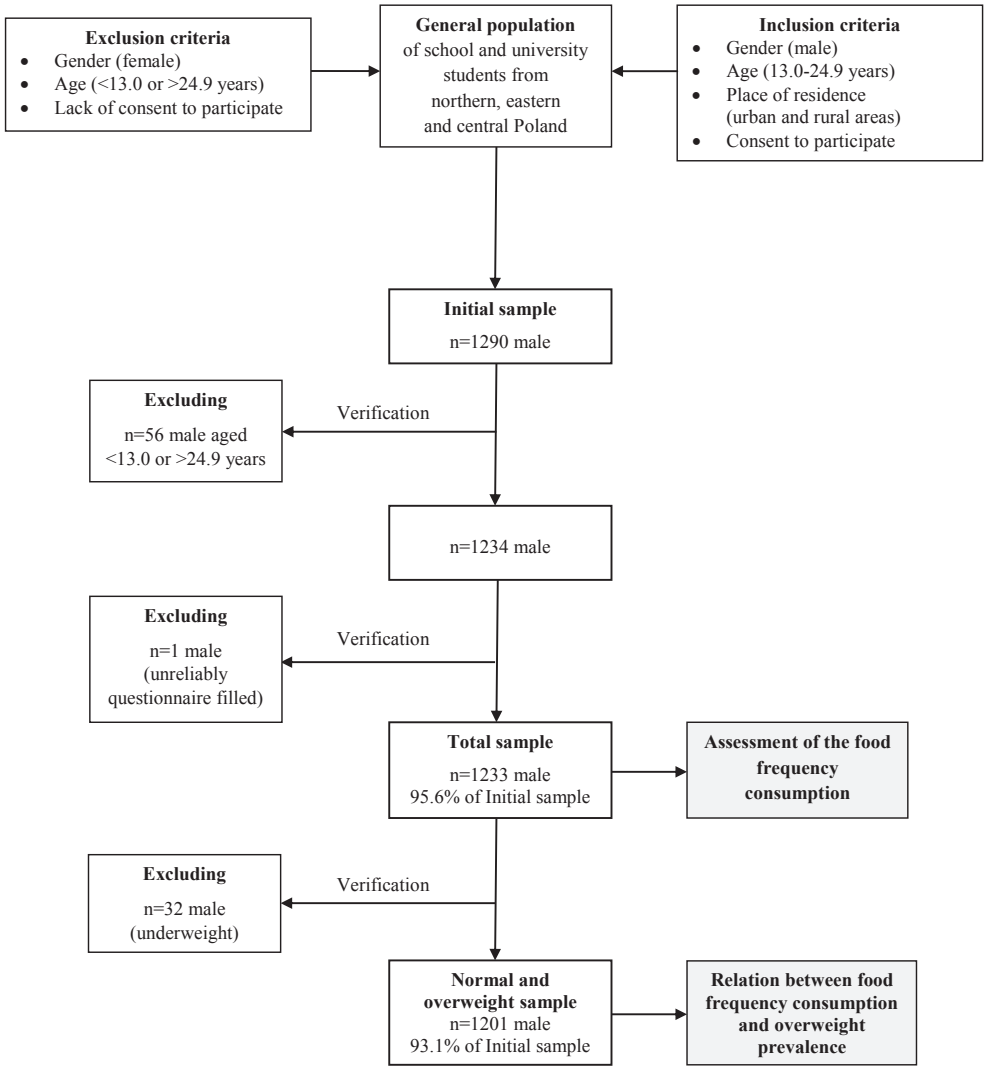

Figure 1. Flow chart of sample collection and study design

Table 1. Sample characteristics by age groups of male students

Category	Total	Age group (years)				p
		13.0-15.9	16.0-18.9	19.0-21.9	22.0-24.9	
Number of subject	1233	483	347	233	170	
Percentage of total sample	100	39.2	28.1	18.9	13.8	
Age (years)*	17.5±3.2 (13.0÷24.6)	14.4±0.7 (13.0÷15.9)	17.3±0.6 (16.0÷18.9)	20.5±0.7 (19.0÷21.9)	23.0±0.8 (22.0÷24.6)	<0.0001
BMI (kg/m^2)*	22.6±3.4 (15.4÷38.5)	21.2^{abc}±3.2 (15.4÷32.8)	22.4^{ade}±2.7 (16.8÷37.7)	23.8^{bdf}±3.4 (17.0÷34.3)	24.9^{cef}±3.5 (18.0÷38.5)	<0.0001
Underweight (%)	2.6	3.3	2.0	3.4	0.6	
Normal weight (%)	68.7	69.4^{ab}	77.2^{acd}	65.7^{ce}	53.5^{bde}	<0.0001
Overweight (%)	24.0	23.0^{a}	18.7^{b}	24.0^{c}	37.6^{abc}	
Obesity (%)	4.7	4.3^{abc}	2.0^{ade}	6.9^{bd}	8.2^{ce}	
Total fiber (points)*	17.5±5.1 (2÷36)	18.6^{ab}±5.7 (2÷36)	18.5^{cd}±4.5 (5÷31)	15.3^{ac}±4.2 (4÷25)	15.5^{bd}±4.5 (5÷31)	<0.0001
Fiber intake <20 points (%)	67.1	58.8^{ab}	61.2^{cd}	81.7^{ac}	81.1^{bd}	
Fiber intake 20-29 points (%)	31.6	38.5^{ab}	38.2^{cd}	18.3^{ac}	18.3^{bd}	<0.0001
Fiber intake ≥30 points (%)	1.3	2.7^{ab}	0.6^{a}	0^{b}	0.6	

*expressed as mean and standard deviation (levels of significance were assessed by the ANOVA test);
Scale of fiber intake: less than once per week (0 points), about one time per week (1 point), 2-3 times per week (2 points), 4-6 times per week (3 points), daily (4 points);
Total fiber intake was expressed on a scale from 0 to 36 points;
() in the brackets indicated minimum-maximum range;
(%) - percentage of the sample or sub-sample (levels of significance were assessed by Pearson Chi^2 test with Yates' correction);
a-a, f-f - statistically significant differences in pairs between age groups (levels of significance were assessed by Tukey's test), p≤0.05.

Food consumption frequency and fiber intake

Information on the consumption of selected dietary fiber sources was obtained by the food frequency method using a self-administered validated Block questionnaire (Block Screening Questionnaire for Fruit/Vegetable/Fiber Intake – BSQFVF) [30] after modification and adjustment to a typical Polish diet and language [10]. The BSQFVF is the most recent screener includes an updated plant food list (nine items) containing dietary fiber: fruit or vegetable juices, fruit (without juices), green salad, potatoes, beans, prepared vegetables (e.g. cooked, preserved or marinated, excluding beans), high-fiber or bran cereal, wholegrain bread and white bread (including French or Italian bread, biscuits and muffins). The food items included in these screening tool are a subset of those found in the 100-item Health Habits and History Questionnaire [30]. The frequency of consumption was expressed in five categories, which were assigned points as follows: less than once per week (0 points), about once per week (1 point), 2-3 times per week (2 points), 4-6 times per week (3 points), daily (4 points). For each person, the points were added and expressed on a scale from 0 to 36 points. Based on the sum of points, fiber intake was considered in three original categories: <20 points – 'Your diet is probably low in important nutrients. You should find ways to increase the fruits and vegetables and other fiber rich foods you eat every day', 20-29 points – 'You should include more fruits, vegetables and whole grains' and ≥30 points – 'You're doing very well! This is a desirable score' [30]. Only 1.3% of the total sample had a fiber intake at the level ≥30 points. For this reason, for further analysis, the scoring categories of fiber intake were re-categorized and two categories were created: <20 points – as an unacceptable level of fiber intake and ≥20 points – as an acceptable, but inadequate level of fiber intake after combining two categories. The cut-offs for the frequency of fiber intake and its dietary source consumption were found arbitrarily, based on the distribution of these variables for the total sample [10]. In view of a similar distribution of fiber intake in students aged 13.0-15.9 years and 16.0-18.9 years, as well as those aged 19.0-21.9 years and 22.0-24.9 years (Table 1), two age groups (13.0-18.9 years and 19.0-24.9 years) were created for further analysis.

Body weight status

Weight (to the nearest 0.1kg) and height (to the nearest 0.5cm) were measured and the body mass index (BMI, kg/m^2) was calculated. During the measurements, participants were dressed in light sportswear. According to international standards developed by Cole et al. [7-8], the BMI of adolescents was converted to the corresponding adults BMI values. The BMI values of both adults and adolescents were then interpreted as follows: underweight (BMI<18.5 kg/m^2), normal weight (18.5≤BMI<25kg/m^2), overweight (25≤BMI<30kg/m^2) and obesity (BMI≥30kg/m^2; Table 1) [36].

Confounders

Respondents were asked about four single factors of their socioeconomic status (SES). Numerical values were assigned to each response category, as follows (in brackets):

I. place of residence: village (1), town ≤20,000-100,000 inhabitants (2), city >100,000 inhabitants (3);
II. paternal education: elementary (1), secondary (2), high (3);
III. maternal education: elementary (1), secondary (2), high (3);
IV. economic situation (self-declared): below average (1), average (2), above average (3).

The SES index was calculated as the sum of the values assigned to the individual response categories to each SES factor. The SES index values were converted into logarithms and then the tertiles of the SES were created to identify respondents with low, average and high SES index [20].

Statistical analysis

The means and standard deviation were calculated for BMI and fiber intake expressed as a sum of points. BMI and fiber intake were logarithmized. An analysis of variance (ANOVA) was used to assess the equality of variance and compare the mean values of BMI and fiber intake between age groups [1]. A post-hoc analysis was conducted using Tukey's honest significance test. The percentage distribution of participants in a total sample and age groups were compared by Pearson Chi^2 test with Yates' correction as necessary. The frequency of fiber intake and its selected dietary sources were expressed as independent dichotomous variables. The association between the frequency of fiber intake and its selected dietary sources and the prevalence of overweight (including obesity) as a categorical dependent variable was evaluated. A logistic regression analysis was performed. The reference group was students with normal weight. Students with underweight were excluded from the logistic regression analysis. Two models were created: Model 1 – crude model and Model 2 – age and SES index adjusted model (other confounders were not investigated). The odds ratio (OR) and 95% confidence interval (95% CI) were calculated. The significance of the odds ratio was assessed by Wald's statistics [1]. A p-value <0.05 was considered as statistically significant. The statistical analysis was performed using STATISTICA statistical software (version 10.0 PL; StatSoft Inc., USA, Tulsa; StatSoft Polska, Kraków).

RESULTS

Body weight status

Most subjects were normal weight (68.7% of the total sample), 24.0% of the total sample were overweight, 4.7% were obese and 2.6% of the total sample were underweight (Table 1). There was a statistically significant increase in mean BMI with age ($p<0.0001$). The highest percentage of overweight and obesity was observed in the students aged 22.0-24.9 years and was 37.6% and 8.2%, respectively (Table 1). Detailed distributions of BMI values for the students in age groups are shown in Figure 2.

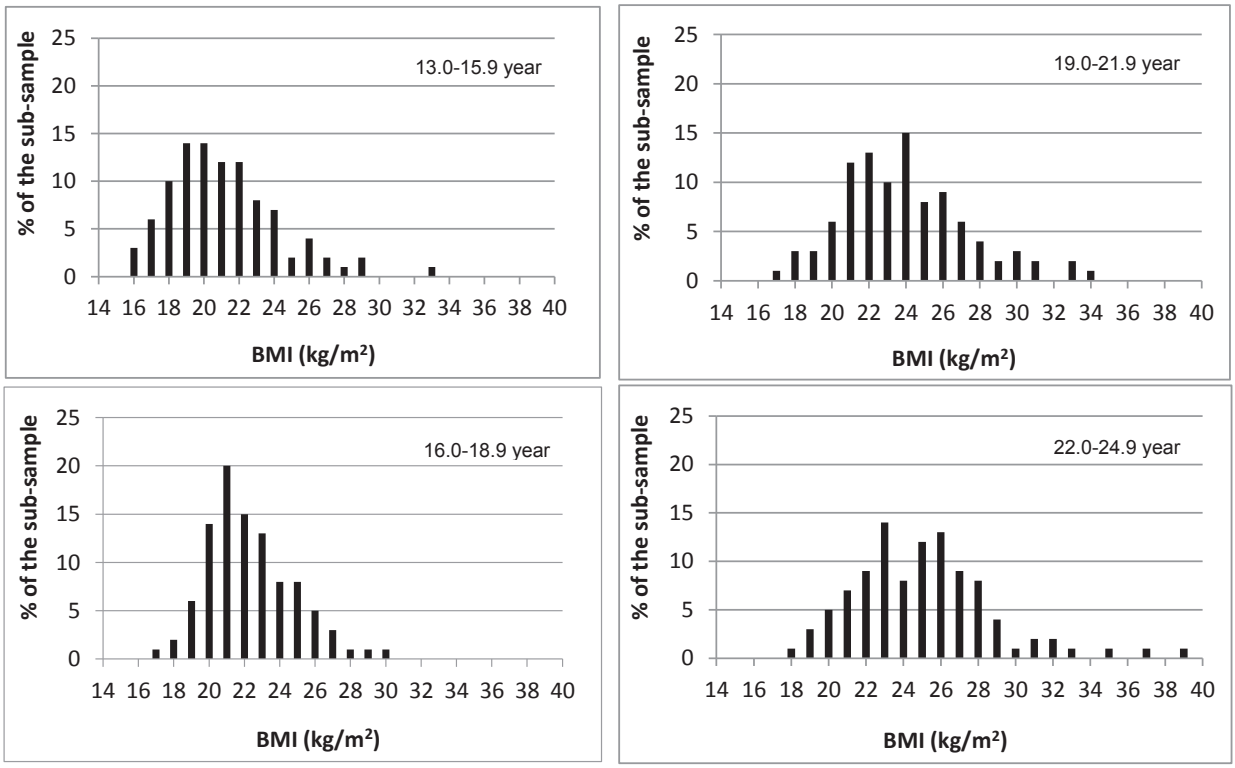

Figure 2. Percentage distributions of BMI values in the age groups of male students

Food consumption frequency and fiber intake

The mean frequency intake of dietary fiber by the students was 17.5 points. Most of the students (67.1%) had fiber intake at an unacceptable level (below 20 points). Fiber intake at the level 20-29 points was found in 31.6% of the students. Only 1.3% of the students had fiber intake at the level of 30 or more points (Table 1). There were significant differences in the consumption of dietary fiber depending on the age ($p<0.0001$). A significantly higher percentage of students aged 13.0-15.9 years (38.5%) and 16.0-18.9 years (38.2%) ate fiber at the level 20-29 points than students aged 19.0-21.9 years and 22.0-24.9 years (18.3%; $p<0.0001$; Table 1). The detailed distributions of dietary fiber intake for students aged 13.0-18.9 years and 19.0-24.9 years are shown in Figure 3.

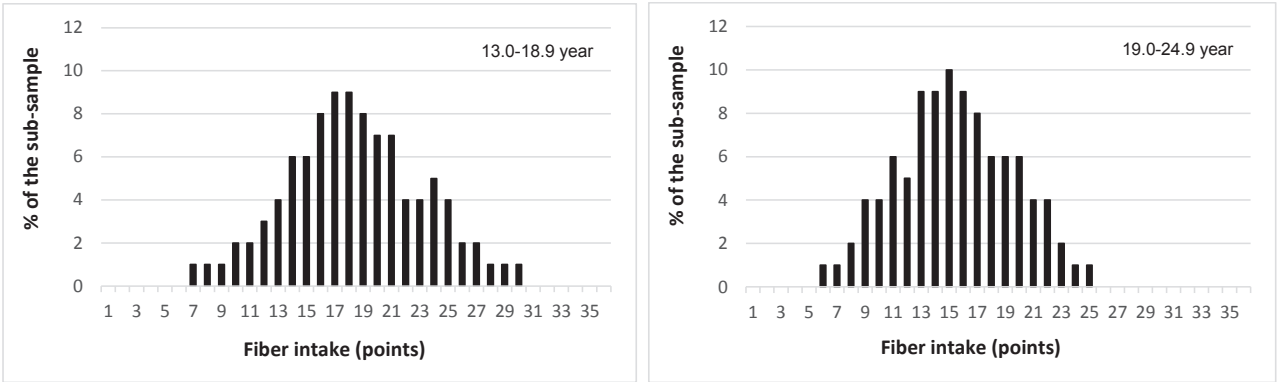

Figure 3. Percentage distributions of BMI values in the age groups of male students

Compared to university students, school students consumed more significantly selected dietary fiber sources, except wholegrain bread (Table 2, Table 3), beans and white bread (Table 3). The most frequently-consumed foods by the school and university students were: white bread, potatoes, fruit and fruit or vegetable juices, but the least frequently consumed were: beans, wholegrain bread, high-fiber or bran cereal and prepared vegetables (Table 2, Table 3). School students ate significantly more fiber than university students (18.5 points vs 15.4 points; p<0.0001; Table 2). Acceptable (but inadequate) levels of fiber intake were 40.4% and 18.4% for school and university students, respectively (Table 3).

Table 2. Fiber intake from selected dietary sources among male students in relation to age (expressed as mean and standard deviation)

Dietary fiber sources	Fiber intake (points)			p
	13.0-24.9 years	13.0-18.9 years	19.0-24.9 years	
Number of subject	1233	830	403	
White bread	3.1±1.2	3.1±1.2	3.2±1.2	NS
Potatoes	2.7±1.2	2.9±1.0	2.2±1.3	<0.0001
Fruit	2.3±1.2	2.5±1.2	2.0±1.2	<0.0001
Fruit or vegetable juices	2.3±1.3	2.5±1.3	1.9±1.2	<0.0001
Green salad	1.9±1.2	2.0±1.2	1.7±1.1	<0.0001
Prepared vegetables	1.7±1.2	1.7±1.2	1.5±1.0	<0.0001
High-fiber or bran cereal	1.5±1.3	1.7±1.3	1.1±1.2	<0.0001
Wholegrain bread	1.3±1.3	1.3±1.3	1.2±1.3	NS
Beans	0.8±1.0	0.8±1.0	0.7±1.0	NS
Total fiber	17.5±5.1	18.5±5.2	15.4±4.3	<0.0001

Scale of food frequency and fiber intake: less than once per week (0 points),
about one time per week (1 point), 2-3 times per week (2 points), 4-6 times per week (3 points), daily (4 points);
Total fiber intake was expressed on a scale from 0 to 36 points;
Levels of significance were assessed by the ANOVA test;
NS – no statistically significant differences.

Dietary fiber sources and overweight

Two out of nine selected dietary fiber sources showed a significant association with the overweight, including obesity, in a logistic regression analysis (Table 4). These included: white bread and prepared vegetables. Daily intake of white bread was associated with lower odds of overweight, including obesity by 28% for the total sample (OR=0.72; 95%CI: 0.56-0.93; p=0.012) to 31% for school students (OR=0.69; 95%CI: 0.50-0.95; p=0.024) compared with non-daily consumption of white bread (Table 4). For university male students, the consumption 4 times or more per week of prepared vegetables (cooked, preserved or marinated) was associated with 51% (OR=0.49; 95%CI: 0.27-0.97; p=0.025) lower odds of overweight, including obesity compared with consumption less than 4 times per week of prepared vegetables (Table 4). There was no reported significant association between the level of fiber intake and the overweight, including obesity among Polish male students (Table 4).

DISCUSSION

The study found an increase in the overweight and obesity prevalence, but a decrease in the frequency intake of fiber and its dietary sources along with male student age. The most frequently-consumed foods among male students were white bread, potatoes, fruit and fruit or vegetable juices and the least-frequently consumed were: beans, wholegrain bread, high-fiber or bran cereal and vegetables. A higher frequency consumption of relatively low in fiber foods as white bread and cooked, preserved or marinated vegetables was associated with a lower likelihood of overweight and obesity in male students. Most of the students consumed fiber at an unacceptable level, so there was no significant effect of the level of fiber intake on overweight prevalence.

The study showed a statistically significant increase of the overweight (by 14.6% points) and obesity prevalence (by 3.9% points) in older than younger students (13.0-15.9 vs. 22.0-24.9 years). This confirmed the results of nationwide research, which found that overweight, including obesity, was increasing by about 15.5% points in males from 13-18 to 19-29 years [29]. In contrast, a decrease of the overweight and obesity prevalence with age was observed by 8.4% points from 11 to 15 years in boys from Czech Republic [24], and by 1% points from 10-13 years to 14-17 years in boys from Lithuania [26].

Table 3. Frequency consumption of selected dietary fiber sources among male students in relation to age

Dietary fiber sources	Frequency of consumption	% of the sample			p
		13.0-24.9 years	13.0-18.9 years	19.0-24.9 years	
	Number of subject	1233	830	403	
White bread	<1 time/week	4.6	4.6	4.7	<0.05
	1 time/week	7.6	7.6	7.7	
	2-3 times/week	13.9	14.2	13.2	
	4-6 times/week	19.1	21.3[d]	14.4[d]	
	daily	54.8	52.3[e]	60.0[e]	
Potatoes	<1 time/week	6.0	2.2[a]	13.9[a]	<0.0001
	1 time/week	9.2	7.0[b]	13.6[b]	
	2-3 times/week	25.1	22.4[c]	30.5[c]	
	4-6 times/week	30.2	34.0[d]	22.3[d]	
	daily	29.6	34.5[e]	19.6[e]	
Fruit	<1 time/week	7.4	4.8[a]	12.7[a]	<0.0001
	1 time/week	15.7	12.8[b]	21.8[b]	
	2-3 times/week	33.4	32.5	35.2	
	4-6 times/week	21.5	23.3[d]	17.9[d]	
	daily	22.0	26.6	12.4[e]	
Fruit or vegetable juices	<1 time/week	9.1	8.0	11.4	<0.0001
	1 time/week	18.3	14.6[b]	26.1[b]	
	2-3 times/week	30.3	29.4	32.3	
	4-6 times/week	19.4	20.4	17.4	
	daily	22.9	27.7[e]	12.9[e]	
Green salad	<1 time/week	14.0	12.8[a]	16.6[a]	<0.0001
	1 time/week	23.7	21.6[b]	28.0[b]	
	2-3 times/week	32.1	30.8	34.7	
	4-6 times/week	17.5	18.9	14.6	
	daily	12.7	15.9[e]	6.0[e]	
Prepared vegetables	<1 time/week	18.1	17.0[a]	20.3[a]	<0.0001
	1 time/week	27.7	26.6	29.8	
	2-3 times/week	33.4	31.7	37.0	
	4-6 times/week	12.6	14.3[d]	8.9[d]	
	daily	8.3	10.4[e]	4.0[e]	
High-fiber or bran cereal	<1 time/week	29.9	24.0[a]	42.2[a]	<0.0001
	1 time/week	25.3	24.0	28.0	
	2-3 times/week	23.4	26.4[c]	17.4[c]	
	4-6 times/week	10.5	13.1[d]	5.0[d]	
	daily	10.9	12.5[e]	7.4[e]	
Wholegrain bread	<1 time/week	37.8	35.5	42.4	NS
	1 time/week	24.4	25.7	21.8	
	2-3 times/week	19.5	20.0	18.6	
	4-6 times/week	9.1	10.1	6.9	
	daily	9.2	8.7	10.2	
Beans	<1 time/week	51.1	49.5	54.3	<0.05
	1 time/week	30.9	31.7	29.3	
	2-3 times/week	11.8	13.3[c]	8.7[c]	
	4-6 times/week	3.2	3.0	3.7	
	daily	3.0	2.5	4.0	
Fiber intake	<20 points	66.8	59.6[a]	81.6[a]	<0.0001
	≥20 points	33.2	40.4[b]	18.4[b]	

a-a, e-e – statistically significant differences in pairs (between age groups), p≤0.05;
Levels of significance were assessed by *Pearson Chi*^{2} test with *Yates'* correction;
NS – no statistically significant differences.

Table 4. The odds ratio (OR) and 95% confidence interval (95% CI) of overweight, including obesity, in male students in relation to the frequency consumption of selected dietary fiber sources and fiber intake

Dietary fiber sources (frequency of consumption)	OR (95% CI)								
	13.0-24.9 years			13.0-18.9 years			19.0-24.9 years		
	Normal weight (N=847)	Overweight, including obesity (N=354)		Normal weight (N=603)	Overweight, including obesity (N=204)		Normal weight (N=244)	Overweight, including obesity (N=150)	
		Model 1	Model 2		Model 1	Model 2		Model 1	Model 2
White bread daily Ref. <daily	1.00	0.76* (0.60-0.98)	0.72* (0.56-0.93)	1.00	0.69* (0.50-0.95)	0.69* (0.50-0.95)	1.00	0.78 (0.52-1.19)	0.68 (0.44-1.04)
Potatoes daily Ref. <daily	1.00	0.78 (0.59-1.03)	0.84 (0.63-1.11)	1.00	0.82 (0.59-1.16)	0.81 (0.58-1.14)	1.00	1.07 (0.63-1.80)	1.14 (0.67-1.94)
Fruit daily Ref. <daily	1.00	0.71* (0.52-0.98)	0.79 (0.58-1.10)	1.00	0.80 (0.55-1.15)	0.75 (0.52-1.10)	1.00	1.26 (0.67-2.40)	1.30 (0.68-2.49)
Fruit or vegetable juices ≥4-6 times/week Ref. <4-6 times/week	1.00	0.73* (0.56-0.94)	0.79 (0.61-1.03)	1.00	0.84 (0.61-1.15)	0.82 (0.59-1.13)	1.00	1.39 (0.88-2.18)	1.34 (0.85-2.12)
Green salad ≥4-6 times/week Ref. <4-6 times/week	1.00	0.89 (0.68-1.17)	0.97 (0.73-1.28)	1.00	0.98 (0.67-1.43)	0.96 (0.68-1.35)	1.00	1.03 (0.62-1.70)	1.06 (0.63-1.78)
Prepared vegetables ≥4-6 times/week Ref. <4-6 times/week	1.00	0.90 (0.66-1.22)	0.98 (0.70-1.38)	1.00	1.30 (0.91-1.86)	1.28 (0.89-1.83)	1.00	0.45* (0.24-0.86)	0.49* (0.27-0.97)
High-fiber/bran cereal ≥4-6 times/week Ref. <4-6 times/week	1.00	0.78 (0.57-1.07)	0.86 (0.63-1.18)	1.00	0.90 (0.62-1.30)	0.89 (0.62-1.29)	1.00	1.26 (0.67-2.40)	1.13 (0.59-2.17)
Wholegrain bread ≥4-6 times/week Ref. <4-6 times/week	1.00	1.08 (0.78-1.48)	1.09 (0.79-1.51)	1.00	1.06 (0.71-1.58)	1.06 (0.71-1.60)	1.00	0.84 (0.49-1.45)	0.89 (0.51-1.55)
Beans ≥2-3 times/week Ref. <2-3 times/week	1.00	1.06 (0.76-1.47)	1.09 (0.79-1.51)	1.00	1.22 (0.82-1.82)	1.22 (0.82-1.82)	1.00	1.18 (0.68-2.06)	1.08 (0.61-1.89)
Fiber intake ≥20 points Ref. <20 points	1.00	0.86 (0.66-1.12)	0.96 (0.73-1.27)	1.00	1.12 (0.81-1.55)	1.10 (0.79-1.53)	1.00	1.46 (0.84-2.55)	1.48 (0.85-2.59)

Model 1 – crude model;
Model 2 –age and SES index adjusted model;
Normal weight (18.5≤BMI<25), overweight incl. obesity (BMI≥25), *≤0.05

In the present study, the most frequently-consumed foods by the students were: white bread, potatoes, fruit and fruit or vegetable juices. The most frequently-consumed foods among male students aged 20-30 years from Italy were: bread and cereals, fresh fruit and raw vegetables [2]. As in our study, an insufficient intake of wholegrain bread and vegetables was also found when assessing the eating habits of university male students from Wroclaw [17] and school male students from Spain [9]. Inadequate consumption of dietary fiber sources, such as whole grain cereals, may result from a lack of availability in canteens or school shops, a long distance to shops (rural communities) or from low incomes [6]. Our study showed that significantly more school students compared to university students often consumed selected dietary fiber sources and ate fiber at a higher level. Differences in the food and fiber intake depending on age are likely a result of changes in dietary habits related to living environment, such as family (school students) or independent life (university students) [2, 31].

In this study, a higher frequency consumption of relatively low in fiber foods was associated with a lower likelihood of overweight, including obesity by 28-31% for daily consumption of white bread to 51% for 4-6 times or more per week consumption of cooked, preserved or marinated vegetables. Students probably consume typical, especially for Polish male dishes based on white bread (sandwiches), cooked vegetables (soups) and vegetable additives (e.g. pickles), which have the largest share of the supply of fiber in their diet. Similarly, in the NHANES study [4], conducted with the participation of children and adolescents aged 2-18 years, it was observed that the main sources of fiber in their diet were foods that were low in fiber, but were frequently consumed, such as French fries, pizza, white bread or potatoes. The lack of beneficial impact of the frequent consumption of high-fiber foods, such as beans or wholegrain bread, on lowering the overweight prevalence was indeed surprising. Since this effect probably resulted from the low frequency and quantity of high-fiber food consumption, non-typical for a Polish traditional dish, the adopted cut-off did not cause significant differences between students who ate them either often or less often.

There was no significant association between the level of fiber intake and the overweight and obesity prevalence in male students. Infrequent consumption of high-fiber foods caused most of the students to have an unacceptable level of fiber intake, which made it impossible to confirm the beneficial role of dietary fiber in reducing the risk of overweight and obesity. In turn, a lower fiber intake than the recommended level increased the risk of overweight in adolescent school male students from Sao Paulo almost three times [11]. A significantly lower fiber intake in obese compared with underweight was found in male adolescents aged 11-14 years from Italy [31]. To the contrary, a significantly higher intake of fiber in overweight or obese students than in the students with BMI less than 25 kg/m^2 was found by *Frąckiewicz* et al. [13] in university students from Warsaw, Najomi and Najamabadi [21] in university male students from Iran and Turan et al. [32] in male adolescents aged 12-17 years from Turkey. A positive correlation between fiber intake and BMI could result from the generally higher total food consumption by students with excess body mass [13].

Study strengths and limitations

The major limitation of the study is a lack of quantitative data regarding fiber intake. We collected data concerning the frequency of food consumption and then estimated the fiber intake (expressed in points). However, the fiber intake scores (in points) estimated from Block's screening questionnaire were compared with multiple-day dietary records and large validation studies [3, 27] have shown good correlations with fiber intake (grams/day). Secondly, the limitation of the study is a lack of adjustment of the overweight and obesity incidence for energy intake and other nutritional aspects. However, quantitative food consumption does not always reflect the real and usual intake of energy and nutrients, because of the 'day-to-day' variability of food consumption [12]. It has been well-documented that overweight respondents often overestimate the consumption of prohealthy foods, such as fish or vegetables, and underestimate the consumption of unhealthy foods such as fast foods or soft drinks [13]. Thirdly, the limitation of the study is 3-years period of data collection (autumn 2010 to spring 2013). Within this period no changes in trends of food consumption in Poland were observed so summarizing data was justified [15]. Next, the impact of seasonality was limited because of all data were collected in the same seasons (from autumn to spring). Fourthly, the sample was not randomly-selected, but our results on the overweight prevalence in the students are compatible with the results from a national survey [29]. The mean net enrollment rate is relatively high for Polish male aged 13-24 years and is about 70% [15], so it strengthens our results and allows generalizations to be made.

The strength of the study is the measuring of weight and height using a large-scale sample (over 1,200 respondents). Moreover, the international BMI classification was used to assess the overweight in students. An interesting area of the study was to show the frequency consumption of dietary fiber sources and the prevalence of overweight across a wide range of male students aged 13.0-24.9 years instead of comparing female and male students.

CONCLUSIONS

This study provides surprising insights regarding high-fiber and low-fiber food consumption and overweight in Polish male students. A lower likelihood of overweight was associated with a higher frequency consumption of relatively low in fiber foods as white bread and cooked, preserved or marinated vegetables. Most of the students consumed fiber at an unacceptable level, so a beneficial impact of high-fiber foods on overweight was not shown. This highlights the need for more frequent consumption of high-fiber foods and increases fiber intake among Polish male students.

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Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

ASSESSMENT OF DIFFERENCES IN NUTRIENTS CONSUMPTION IN WOMEN DIAGNOSED WITH OSTEOPOROSIS AS COMPARED TO A HEALTHY CONTROL GROUP

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ABSTRACT

Background. Osteoporosis is a condition that has been much more frequent for women, which is related to menopause and to their lower bone mineral density (BMD). Inappropriate diet is among the development factors of the disease.

Objective. To assess differences in consumption of particular nutrients among women with and without osteoporosis diagnosed.

Material and Methods. The study was conducted in 2013 in a group of 100 women aged 51-70, using a questionnaire, including a 24-hour recall related to a participant's nutrients consumptions.

Results. Women suffering from osteoporosis were found to consume significantly lower amounts of fat (by 16%) and energy (by 13%), as well as vitamins: A (by 16%), E (by 20%), B_6 (by 20%), niacin (by 16%) and C (by 19%). Differences in the consumption of minerals have been observed in the cases of calcium, phosphorus, sodium and potassium. The women with osteoporosis were fund to consume lower amounts of those elements, by 14%, 13%, 21% and 19% respectively. On the average, participants of the study in both groups consumed amounts of calcium at a half of the recommended level, and substantially exceeded the recommended values of phosphorus, as well as displaying an inappropriate calcium to phosphorus ratio (0.5:1). As little as 8% of the participants with osteoporosis declared a considerable change in their diet, with increased consumption of dairy products.

Conclusion. The observed nutrition deficiencies in osteoporosis patients may be conducive to a worsened condition, and may lead to an onset of the disease in participants from the control group.

Key words: *consumption, nutrients, osteoporosis, women*

STRESZCZENIE

Wprowadzenie. Osteoporoza to choroba znacznie częściej występująca wśród kobiet, co związane jest z menopauzą i mniejszą szczytową masą kostną. Nieprawidłowy sposób żywienia jest jednym z czynników warunkujących powstawanie choroby.

Cel badań. Oszacowanie różnic w spożyciu składników pokarmowych wśród kobiet z osteoporozą i bez jej zdiagnozowania.

Materiał i metody. Badanie zostało przeprowadzone w 2013 roku, wśród 100 kobiet, w wieku 51-70 lat metodą ankietową, w tym metodą 24-godzinnego wywiadu żywieniowego.

Wyniki. Kobiety z osteoporozą spożywały istotnie mniejsze ilości tłuszczu (o 16%) oraz energii (o 13%), a także witamin: A (o 16%), E (o 20%), B_6 (o 20%), PP (o 16%), C (o 19%). Różnice w spożyciu składników mineralnych zaobserwowano w przypadku wapnia, fosforu, sodu oraz potasu. Kobiety z osteoporozą spożywały mniejsze ilości tych związków odpowiednio o 14%, 13%, 21% i 19%. Osoby badane z obu grup spożywały średnio 2-krotnie zbyt małe ilości wapnia, a znacząco zbyt duże ilości fosforu oraz charakteryzowały się nieprawidłowym stosunkiem wapnia do fosforu (0,5:1). Zaledwie 8% badanych z osteoporozą deklarowało zmianę sposobu żywienia po zdiagnozowaniu schorzenia zwiększając w diecie ilość produktów mlecznych.

Wniosek. Odnotowane nieprawidłowości spożycia mogą w przypadku osób z osteoporozą sprzyjać pogłębianiu schorzenia, a u osób z grupy kontrolnej stać się jego przyczyną.

Słowa kluczowe: *spożycie, składniki pokarmowe, osteoporoza, kobiety*

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INTRODUCTION

The World Health Organisation has defined osteoporosis as a systematic disease of the skeleton, characterised by low bone density levels, and by deficient micro-structure of the bone tissue, leading to increased bone fragility and to occurrence fractures [9].

While osteoporosis is found in both men and women, the latter display a considerably higher risk of osteoporosis-related fractures [7, 29]. The probability of injury with women of Caucasian race aged 50+ amounts to 32% for spinal fractures, 16–17.5% for proximal femoral fractures and 15% for fractures of the forearm. In contrast, the likelihood of proximal femoral fracture in male patients of similar age only amounts to 6% [3]. Other studies indicate that in the 50+ age group a fracture is reported in 30% of women and 8% of men. With a 50-year old woman a life risk of a fracture is 40%, while the corresponding ratio for a man is only 13% [26].

The risk factors for osteoporosis-related fractures include age, gender and ethnic group (osteoporosis is more frequent in people with a fair complexion), but also others, such as the hormonal status, genetic factors and family conditions, as well as specifically a thin body build (underweight), numerous earlier fractures and an insufficient level of physical activity [2, 4, 9, 16, 29, 31]. The risk of osteoporosis may also be heightened by tobacco smoking and by excessive alcohol consumption. Nutritional factors represent an important component in preventive treatment of osteoporosis. Appropriate intake of calcium, vitamin D, phosphorus, sodium and proteins may well decrease the risk of osteoporosis [2, 9, 11, 16, 31].

It has been observed that a certain number of women at perimenopausal age are afraid of a substantial body mass increase and try to reduce it spontaneously, to prevent themselves from becoming overweight or obese. The actions of these women are often in disagreement with rationalistic nutrition principles. Inappropriate dietary habits in the menopausal period may lead to deficits of many important nutrients in that period, may potentially accelerate the aging processes and raise the risk of many diseases, including osteoporosis [2, 19]. The study aimed to assess the differences in consumption of nutritional components in osteoporosis patients against a reference group without osteoporosis, based on purpose-built nutrition interviews.

MATERIAL AND METHODS

The study was conducted in winter/spring of 2013, using a purpose-built questionnaire, in 50 women patients of an osteoporosis treatment centre in Warsaw, diagnosed with osteoporosis, and a control group of 50 women from the Masovian district. All the participants were at age of 51-70 years. Participants in the study gave signed consent.

The questionnaire contained two parts. The first part (demographic summary) comprised the questions related to the participant's age, place of dwelling, level of education, body height and weight, physical activity, use of tobacco and to any changes that took place in the participant's nutrition after osteoporosis was diagnosed. Based on the data on body height and weight, the Body Mass Index (BMI) was calculated for each of the participants [20]. The second part of the questionnaire registered all the meals, dishes, products and drinks (in domestic or weight-based measures) that were consumed by the participant. Consumption was assessed using the method of a 24-hour recall. 24-hours interview was conducted to determine the nutrition irregularities and the diet during that day was typical for each of the subject To specify actual portions of food consumed, a photographic album of products and dishes was used [24]. Using a special 'Żywienie' (*Nutrition*) program, based on food composition tables [15], consumption of energy was assessed for the obtained data, as well as of main nutrients, selected vitamins and minerals. The values obtained were subsequently adjusted for technological and plate losses [27]. The results for intake of energy and major nutrients were presented in the context of current recommended values for age, level of physical activity and proper body mass of participants or in the case of vitamins and minerals were compared to EAR/AI values [10]. Intake of vitamin D from the diet was not estimated in the presented study. Skin synthesis is evolutionarily source of vitamin D and 90% of vitamin D in the body is the endogenous origin [10].

A *Chi*-square test was used for statistical development of the data in order to compare the sample distributions**.** Normality of the distribution was checked using the *Shapiro-Wilk* test. Parametric variables failing to meet the assumptions necessary for the ANOVA test were subject to the U *Mann-Whitney* test using the Statistica ver.10 software. The significance level of α=0,05 was assumed for all calculations.

RESULTS

The study participants in the osteoporosis group and the reference group did not display significant differences in terms of age, place of dwelling, physical activity levels and frequency of tobacco smoking (Table 1). The Body Mass Index (BMI) of participants was within the bracket of 20.4 – 30.9. Among the women with osteoporosis, appropriate body mass was represented by 68% of the group, corresponding to 35% in the control group (statistically significant difference). The remaining participants were overweight. None of the participants was underweight. Among the osteoporosis patients, 80% of participants declared secondary or higher education, corresponding to only 54% in the reference group (statistically significant difference). Education was not found to affect the levels of participants' physical activity.

The majority of the osteoporosis group (80%) declared that they did not change, or only slightly changed,

nutritional habits after being diagnosed with the disease. As little as 8% of the participants from that group hale significantly changed their diet, declaring increased share of dairy products. 12% of the group's respondents were unable to assess the change in their nutritional habits.

Table 1. Characteristics of the study population

Factor	Total n=100	Osteoporotic group n=50	Control group n=50	p***
Age (years)	60±6.0* 51-70**	60±6.0 51-70	61±6.1 51-70	ns
Place of dwelling				
village	24%	8 (16%)	16 (32%)	ns
town	25%	9 (18%)	16 (32%)	
city	51%	33 (66%)	18 (36%)	
Level of education				
primary	7%	1 (2%)	6 (12%)	0.010****
vocational	26%	9 (18%)	17 (34%)	
collage	45%	27 (54%)	18 (36%)	
high	22%	13 (26%)	9 (18%)	
BMI (kg/m^2)	24.7±2.0* 20.4-30.9**	24.7±1.7 20.4-27.8	25.4±2.1 21.1-30.9	0.001****
18,5-24,9	52%	34 (68%)	18 (35%)	
>25	48%	16 (32%)	32 (65%)	
Physical activity				
sedentary	65%	32 (64%)	33 (66%)	ns
moderate	32%	15 (30%)	17 (34%)	
high	3%	3 (6%)	0 (0%)	
Smoking status				
never	46%	27 (54%)	19 (38%)	ns
ex-smoker	30%	13 (26%)	17 (34%)	
current	24%	10 (20%)	14 (28%)	

* - mean ± standard deviation, ** - range, *** - p-value for *Chi*-square test (for age and BMI p-value is the result of U-*Mann-Whitney* test), **** - differences statistically significant ($p \leq 0.05$); ns - values are not statistically different ($p > 0.05$).

Among the participants, important differences were found in terms of consumption of fat and overall energy (Table 2). The women from the control group consumed considerably more fat (by 19%), which influenced a higher overall energy intake (by 16%). No substantial differences were registered in the consumption of proteins, carbohydrates, cholesterol and fibre between the groups, although the patients suffering from osteoporosis tended to consume lower amounts of these components – by 10% (proteins, cholesterol) to 20% (fibre).

In the area of selected vitamins, considerable differences of consumption were displayed among the groups studied (Table 3). Participants in the reference group consumed substantially more of vitamin A (by 19%), vitamin E (by 26%), vitamin B_6 (by 25%), niacin (by 18%) and vitamin C (by 24%), compared to the participants with diagnosed osteoporosis. No differences were observed in the consumption of vitamins B_1 and B_2.

In terms of selected minerals, no difference was observed in the consumption of magnesium and iron. Calcium consumption was deficient in both groups, although it was significantly higher in the reference group (by 17%). Other minerals were also consumed in considerably higher amounts by the controls, including a higher intake of phosphorus (by 15%), sodium (by 27%) and potassium (by 24%).

DISCUSSION

The menopausal period, i.e. the age bracket of approximately 40-50, causes a range of changes in a woman's body. First of all, starting before the menopause, the oestrogens production decreases. The lowered concentration of oestrogens, hormone that stimulate ossification (bone building) and inhibit bone resorption, leads to weaker absorption of calcium in

the digestive tract which causes bone mass loss in perimenopausal and postmenopausal women [19, 31]. Calcium absorption after the 50th year of life remains stable for the following period of approximately 25 years. After the 75. year of life the absorption of calcium falls significantly, by an average of approx. 30% [17]. Moreover, with age, the cutaneous synthesis of vitamin D decreases, and the creation of its active metabolite in kidneys is retarded. The menopausal process leads to a reduced number of vitamin D receptors in target organs. This phenomenon additionally decreases the calcium absorption from the digestive tract. Therefore, the deficit of vitamin D increases the susceptibility to fractures and osteomalacia and affects the development of osteoporosis. In addition, it causes the weakening of the lower limb muscles, decreases the grip strength and leads to a generally lower functional capacity [4, 5, 19, 31]. As a result of these combined processes, just after the menopause one can observe the fastest decrease in the BMD, i.e. bone loss of between 3% and 5% per annum, even though the process slows down significantly in the 65+ age group [1].

The postmenopausal period is also the age of declining professional activity. That is an indirect factor contributing to the decreasing bone density as a result of lower physical activity levels. Physical fitness and general functional capacity of women decreases, while the risk of falls increases, leading to potential osteoporotic fractures [2, 4, 9].

Table 2. Intake of energy and major nutrients by the test group of women

Ingredient	Recommended intake for the group	Total n=100	Osteoporotic group n=50	Control group n=50	p****
Energy (kcal/day)	1890	1743 ± 271* 920 – 2574** 1707***	1617 ± 241 920 – 2303 1585	1869 ± 250 1274 – 2574 1906	0.001*****
Protein (g/day)	33-58	60.5 ± 14.0 30.2 – 107.4 61.5	57.2 ± 15.0 30.2 – 107.4 56.7	63.8 ± 12.6 34.8 – 85.0 63.1	ns
Fat (g/day)	70	68.9 ± 19.9 21.4 – 120.0 68.6	62.9 ± 19.2 21.4 – 103.5 63.6	74.8 ± 19.8 31.5 – 120.0 72.3	0.003*****
Cholesterol (mg/day)	300	303.5 ± 146.3 72.4 – 862.8 266.9	287.9 ± 151.2 81.3 – 862.8 264.8	319.0 ± 140.8 72.4 – 662.1 285.3	ns
Carbohydrates (g/day)	>130	234.9 ± 43.4 124.0 – 327.6 234.6	218.4 ± 41.1 124.0 – 327.6 222.4	251.3 ± 39.4 176.3 – 326.4 251.1	ns
Dietary fibre (g/day)	23	16.0 ± 4.8 5.5 – 28.1 15.4	14.2 ± 4.5 5.5 – 25.8 13.8	17.7 ± 4.3 8.0 – 28.0 16.6	ns

* - mean ± standard deviation, ** - range, *** - median, **** - p-value for the U-*Mann-Whitney* test,

***** - differences statistically significant ($p<0.05$), ns - values are not statistically different ($p>0.05$).

A person's diet plays an important role in osteoporosis prevention, and calcium consumption is among the most important factors. Appropriate consumption of calcium may decrease the risk of osteoporosis significantly [2, 12, 28]. Calcium is found in almost all food products, but its bioavailability from particular products varies considerably, depending on the consumption levels of proteins, minerals (i.e. phosphorus, magnesium, iron, zinc and nutritional fibre), as well as on the presence of the oxalic acid and phytates, as the latter substances, if consumed excessively, disturb the absorption of calcium, while vitamin D and lactose increase the calcium absorption from food products. The ratio of calcium to phosphorus intake is an important part of a nutritional assessment, as excessive supply of phosphorus with a simultaneous deficit of lactose or of vitamin D may inhibit the calcium absorption in the digestive tract. The Ca:P ratio in the diet of postmenopausal women should be as high as 1.3:1 [5]. Maintaining the equimolar Ca:P proportion, favourable for the body, is difficult due to the very common presence of phosphorus in food products. The phosphorus content tends to be increasing, mainly due to the use of additives that are introduced to food products in the technological process [14].

The reduction of bone density is also facilitated by excessive supply of proteins in the early period of life. With consumption of 1 g of protein, the body

releases 1 mg of calcium in urine [5]. Proteins lead to acidification of human urine, whereas calcium plays an indispensable role as a buffering substance. Additionally, a diet high in sodium is also conducive to the loss of calcium in urine. Consuming 1 g of sodium causes the body to release 26 mg of calcium with urine [18]. Animal origin products include low amounts of calcium (except for milk and dairy products), and it is usually in the form that is not easily available in digestion. Additionally, these products often are rich in phosphorus – processed meats are an example. An excessive intake of alcohol and caffeine is also likely to cause increased excretion of calcium in urine [22].

Some plants and related products, such as grain legumes (soya, broad beans), nuts (sunflower seeds, sesame seeds, hazelnuts) or curly kale, include relatively high content of calcium, both in absolute values (mg of calcium per 100 g of the product) and relative to energy content (per 100 kcal). However, the calcium absorption level from plant products is only between 5 and 15%. That is related to a high ratio of fibre, as well as of oxalates, in some plant products, which considerably limits their value as a source of calcium [23].

Table 3**.** Intake of vitamins and minerals by the test group of women

Ingredient	Recommended intake	Total n=100	Osteoporotic group n=50	Control group n=50	p****
Vitamin A (µg retinol eq./day)	500	757.0 ± 467.0* 178.8– 2438** 610.1***	689.8 ± 407.4 192.9 – 2099.9 596.8	824.2 ± 542.0 178.8 – 2438.0 616.1	0.03*****
Vitamin E (mg α-tokopherol eq./ day)	8	10.6 ± 4.5 3.0 – 31.4 9.8	9.4 ± 4.3 3.0 – 25.9 8.6	11.8 ± 5.3 3.1 – 31.4 10.2	0.02*****
Vitamin B_1 (mg/day)	0.9	0.9 ± 0.3 0.4 – 1.7 0.9	0.9 ± 0.3 0.4– 1.7 0.8	1.0 ± 0.2 0.7 – 1.6 1.0	ns
Vitamin B_2 (mg/day)	0.9	1.3 ± 0.34 0.6 – 2.3 1.31	1.3 ± 0.3 0.6 – 2.2 1.24	1.4 ± 0.3 0.7 – 2.3 1.4	ns
VitaminB_6 (mg/day)	1.3	1.8 ± 0.6 0.7 – 3.4 1.7	1.6 ± 0.6 0.7 – 2.7 1.6	2.0 ± 0.6 1.0 – 3.4 1.9	0.03*****
Vitamin PP (mg/day)	11	14.2 ± 5.7 3.6 – 28.6 13.4	13.0 ± 5.8 3.6 – 25.5 12.8	15.4 ± 5.5 5.3 – 28.6 14.9	0.04*****
Vitamin C (mg/day)	60	45.7 ± 25.6 3.5 – 140.0 43.5	40.8 ± 26.0 3.5 – 98.8 40.3	50.6 ± 25.5 11.0 – 140.0 47.6	0.03*****
Calcium (mg/day)	1000	546.5 ± 198.3 177.0 – 1114.9 516.8	504.4 ± 175.3 177.0 – 1055.2 485.1	588.6 ± 196.6 228.3 – 1115.0 591.5	0.01*****
Phosphorus (mg/day)	580	1123 ± 264 555 – 1878 1120	1047 ± 257 555 – 1878 1055	1200 ± 241 730 – 1710 1190	0.003*****
Ca:P	1:1	0.5:1	0.5:1	0.5:1	ns
Magnesium (mg/day)	265	269.1 ± 70.6 122.2 – 439.2 259.8	244.4 ± 68.4 122.2 – 377.7 230.6	293.8 ± 63.1 189.9 – 439.2 285.9	ns
Sodium (mg/day)	1370	1891 ± 716 687 – 4182 1764	1665 ± 637 687 – 4103 1661	2117 ± 710 820.5 – 4182 2026	0.004*****
Potassium (mg/day)	4700	2899 ± 794 1203 – 4743 2766	2589 ± 723 1203 – 4225 2580	3209 ± 763 1564 – 4743 3069	0.003*****
Iron (mg/day)	6	9.4 ± 2.5 3.4 – 16.4 9.3	8.5 ± 2.2 3.4 – 13.4 8.3	10.2 ± 2.4 5.4 – 16.4 9.8	ns

* - mean ± standard deviation, ** - range, *** - median, **** - p-value for the U-*Mann-Whitney* test,

***** - differences statistically significant ($p<0.05$), ns - values are not statistically different ($p>0.05$).

The most valuable sources of calcium are milk and dairy products. Drinking milk contains an average of 120 mg of calcium in 100 g. A similar level of calcium is found in processed dairy products (yoghurts, kephir). Calcium-rich sources also include cheese spreads and rennet cheeses [15], however, while trying to achieve a calcium-rich diet one should choose products with a possibly high ratio of calcium to energy value. Women with lactose intolerance often limit their consumption of dairy products, thus making their diet calcium-deficient. Lack or a low level of lactose in their diet usually leads to a much poorer use of calcium, by as much as 50%. In case of restricted consumption of dairy products, alternative sources of calcium are recommended. These can for instance include processed foods based on soya, whey, buttermilk and products containing casein hydrolysates [21].

Metabolism of bone tissue also requires a diet with an adequate supply of vitamins, including vitamin A, C and K [30]. Along with a lower level of energy in diet, these vitamins were also restricted in the diet of the osteoporosis group. Participants from the group under study reported mainly – which can be grounds for concern – significantly deficient levels of calcium consumption (relevant difference against the control group), and excessive level of phosphorus consumption, with an erroneous ratio between the two. This, coupled with a diet high in sodium and low in potassium, may contribute to a further aggravation of osteoporosis. Similar erratic consumption patterns have also been reported in studies by other authors conducted in groups of female osteoporosis patients [6, 8, 12, 13, 18, 25, 28], as well as in the control group. The latter finding points to a possible future development of osteoporosis in some participants from the control group, particularly those with a lower or deficient body mass (in the adipose tissue adrenal androgens are converted to oestrogens) [9] and with lower bone density.

CONCLUSIONS

1. 8% of the study participants with diagnosed osteoporosis declared significant changes in their diet, with more dairy products introduced. Women with osteoporosis consumed lower levels of fat and energy, which affected a lower intake of vitamins (A, E, B_6, niacin, C) and minerals, including calcium and potassium.
2. Study participants in both groups (diagnosed with osteoporosis and a control) consumed insufficient amounts of calcium, while their diet was excessively rich in phosphorus. The erratic consumption patterns may lead to a worsened condition in osteoporosis group, and may cause the condition in the control group.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

DIET FOR WOMEN WITH IRRITABLE BOWEL SYNDROME – A PRELIMINARY STUDY

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ABSTRACT

Background. Irritable Bowel Syndrome (IBS) is one of the most frequent digestive system diseases, of various medical signs. It is assumed that proper life style, including appropriate, rational diet is a factor helpful for treating such a disorder.
Objective. The purpose of this paper was to assess the selected dietary habits, and to evaluate the nutritional value of daily food rations for patients with a mixed type of Irritable Bowel Syndrome.
Material and Methods. The questionnaire survey involved a group of 32 women suffering from a mixed type of Irritable Bowel Syndrome (The Rome III Diagnostic Criteria were used to diagnose the disease). The control group was comprised of 32 healthy women. The methods used to assess the diet were divided into quantitative and qualitative ones.
Results. The most frequent dietary mistakes among patients with IBS were associated with snacking sweets (83.0% of the subjects) and fruit (17.0% of the subjects) between the meals. A higher intake of sucrose was found amongst women with IBS, than in the case of the control group (p=0.0169). The analysis of the results demonstrated a significantly higher intake of water (derived from drinks and foods) amongst patients with IBS, than in the case of women of the control group (p=0.0267). An insufficient intake of plant proteins and polyunsaturated fatty acids was recorded in both groups. The supply of protein in general, animal protein, fat in general, saturated fatty acids and sodium, exceeded the recommended norm, both amongst women with IBS and women of the control group.
Conclusions. The obtained examination results showed that there are significant dietary improprieties in the diet of women suffering from IBS. In order to eliminate these mistakes in the future, it seems justified to extend the knowledge on rational nutrition amongst patients with IBS.

Key words: *irritable bowel syndrome, dietary habits, daily food rations, diet*

STRESZCZENIE

Wprowadzenie. Zespół jelita nadwrażliwego (IBS – *Irritable Bowel Syndrome*) jest jedną z najczęstszych chorób przewodu pokarmowego o różnym obrazie klinicznym. Przypuszcza się, że prawidłowy styl życia, w tym odpowiednia, racjonalna dieta jest czynnikiem pomocnym w leczeniu tego schorzenia.
Cel. Celem pracy była ocena wybranych zwyczajów żywieniowych oraz ocena wartości odżywczej dziennych racji pokarmowych pacjentek z mieszaną postacią zespołu jelita nadwrażliwego.
Materiał i metody. Badaniami ankietowymi objęto grupę 32 kobiet chorujących na postać mieszaną zespołu jelita nadwrażliwego (do rozpoznania choroby wykorzystano Kryteria Rzymskie III). Grupę kontrolną stanowiły 32 zdrowe kobiety. Metody stosowane dla oceny sposobu żywienia podzielone były na metody ilościowe i jakościowe.
Wyniki. Najczęściej popełnianymi błędami dietetycznymi wśród pacjentek z IBS było dojadanie między posiłkami: słodyczy (83,0% badanych) i owoców (17,0% badanych). Wśród kobiet z IBS stwierdzono wyższe spożycie sacharozy, niż w grupie kontrolnej. Istotnie niższe spożycie błonnika pokarmowego odnotowano wśród pacjentek z IBS niż w grupie kontrolnej (p=0,0169). Analiza uzyskanych wyników wykazała, że pacjentki z IBS istotnie więcej spożywają wody (pochodzącej z napojów i produktów spożywczych) niż kobiety z grupy kontrolnej (p=0,0267). W obu grupach odnotowano niedostateczne spożycie białka roślinnego i wielonienasyconych kwasów tłuszczowych. Podaż białka ogółem, białka zwierzęcego, tłuszczu ogółem, nasyconych kwasów tłuszczowych i sodu, przekraczało zalecaną normę zarówno wśród kobiet z IBS jak i kobiet grupy badanej.
Wnioski. Uzyskane wyniki badań wykazały, że w sposobie żywienia kobiet z IBS istnieją duże nieprawidłowości. Celem eliminacji tych błędów w przyszłości zasadnym wydaje się poszerzenie wiedzy z zakresu racjonalnego żywienia wśród pacjentów z IBS.

Słowa kluczowe: *zespół jelita nadwrażliwego, zwyczaje żywieniowe, dzienne racje pokarmowe, dieta*

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INTRODUCTION

Irritable Bowel Syndrome (IBS) is a frequent digestive system disease. In the European countries and the United States, the percentage of people suffering from this disease is as high as 20,0%, whereas within the Polish population it amounts to ca. 13,0% [31]. Women suffer from the disease twice as often as men, and IBS develops mainly in the third decade of life [31]. When it comes to Irritable Bowel Syndrome, no anatomic alterations, responsible for the condition, were reported. The greatest importance for the development of IBS is attributed to the intestinal motility disorders, visceral hypersensitivity and disorders of the enteric nervous system [23]. The development of the disease may be affected by multiple factors, i.e. past infection of the digestive tract, disturbances in the gut flora, psychosocial and dietary causes, genetic factors and past surgical procedures within the abdominal cavity, or the abuse of antibiotics or laxative and hormonal medicines [31]. The key symptom of the IBS is chronic or recurring pain and/or discomfort in the abdominal cavity, related to the change in defecation rhythm. IBS can be diagnosed upon collecting the detailed medical history, and conducting the subject examination, often without the need for additional, specialist diagnostic tests. What is useful is the document entitled Roman III Criteria, of 2016, which emphasizes the role of the duration and frequency of pain, and the degree of feces formation [23]. Four manifestations of IBS are distinguished: with constipation, with diarrheas, mixed type and undefined type [23]. The purpose of treatment of patients with IBS is to cause regression or significant mitigation of the unpleasant symptoms, which not only cause the conditions but also substantially decrease the comfort of life. In some sufferers, it is possible to achieve many-months or multiannual remissions, i.e. full regression of symptoms (it does not mean full recovery). The diet of a patient with IBS usually depends on individual responses to food products. Although diet is not the cause of this disease, some foods may intensify its symptoms. Therefore, proper diet is an important element of the therapy against this disease, influencing the quality of life of the patients.

The purpose of this paper was to quantitatively assess the daily food rations (DFR), and to evaluate the selected dietary habits of patients with a mixed type of Irritable Bowel Syndrome.

MATERIAL AND METHODS

The questionnaire survey involved 32 women with a diagnosed mixed type of Irritable Bowel Syndrome (on the basis of The Rome III Diagnostic Criteria) [23]. The average age of the subjects was 31.5 ±9.4. Patients were treated at the Gastroenterology Dispensary of the University Hospital in Bialystok, and the Gastroenterology Dispensary of the Independent Public Health Care Institution, Jedrzej Sniadecki Joint Voivodeship Hospital in Bialystok. The study was conducted upon obtaining authorization no. R-I-002/496/2013, of the Bioethics Committee of the Medical University of Bialystok.

The assessment of the nutritional value and the analysis of the dietary habits of women suffering from IBS were conducted during autumn and winter seasons in the years 2015/2016.

The control group was comprised of 32 healthy women from Podlaskie voivodeship, who volunteered to participate in the study. Their average age was 32.9±8.2. Both the test group subjects and the control group subjects understood the aim and the nature of the study, agreed for its conditions and expressed their knowing consent, in writing, prior to their engagement in the study.

The assessment of the nutritional status was carried out on the basis of quantitative specification of the anthropometric features: body mass (kg); body height (cm) waist-hip ratio (WHR) (cm) and waist circumference (measured at the half of the distance between the lower rib cage and the iliac crest); body mass index (BMI) (kg/m^2), ideal body weight (BMI 18.5 – 24.9 kg/m^2), overweight (BMI 25.0 – 24=9.9 kg/m^2), and obesity (BMI ≥30.0 kg/m^2) [14].

In order to evaluate the dietary habits, a survey questionnaire, designed at the Department of Dietetics and Clinical Nutrition, Medical University of Bialystok, was used. The questionnaire included questions concerning the socio-demographic situation of the subjects, and questions concerning the analysis of selected dietary habits: number of the consumed meals, their regularity, snacking between the meals, kinds of the consumed snacks, and eating frequency of selected groups of food products. When it comes to questions concerning the eating frequency of selected food products, the patients had 7 categories to choose from, as follows: from "I don't eat this at all" to "I eat this every day". Suitable ranks were ascribed to each category, as follows; I don't eat this at all – 1; I eat this less often than once a month – 2; I eat this 1-2 times a month – 3; I eat this once a week – 4; I eat this 2 – 3 times a week – 5; I eat this 4 – 6 times a week; I eat this every day – 7. Then, the median of ranks (Me) of the eating frequency of the examined products, and the interquartile range (IQR). The calculations of the arithmetic mean, standard deviation (SD) and percentage, were also used for computation.

The volume of the consumed portions of products and dishes was estimated using cooking weights and measures, and on the basis of the "Album of photographs of food products and dishes" [34]. In order to assess the caloric and nutritional value of daily food rations (DFR), a Diet 5.0 computer program, including the database of the National Food and Nutrition Institute, Warsaw (NFNI) was used, which takes into account the nutrient losses upon culinary

processing. The use of dietary supplements, such as vitamin-mineral preparations, and adding kitchen salt while preparing dishes, were not taken into consideration upon the dietary assessment. The caloric value of an average daily food ration was calculated together with the water content and the following nutrients: protein in general, animal and plant protein, carbohydrates in general, fiber, fats in general, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), cholesterol, sucrose, lactose and sodium. It was assumed that SFAs, MUFAs, PUFAs should provide 7%; 15%; 8% of the daily caloric requirement, respectively. The energy and nutrient values obtained this way were then referred to the present dietary norms – the norm settled at the level of the Estimated Average Requirement for the group (EAR) or Adequate Intake (AI) for women with low Physical Activity Level (PAL 1.4) [13]. The women being tested were ascribed to the aforementioned group on the basis of the collected information on their living conditions and work [13]. The values amounting to 100±10% of the recommended norm were considered appropriate.

The statistical evaluation of the obtained results was carried out with the use of STATISTICA 12.0 program, of StatSoft company. Upon statistical elaboration of the obtained results, U *Mann Whitney* and χ^2 tests were used, along with t-test for two independent samples with *Yates's* correction for continuity, u-test for two structure indicators, calculating also the r *Pearson's* r correlation, adopting the values for which $p<0.05$ as significant.

RESULTS

The studies involved 64 women (32 people suffering from the mixed type of Irritable Bowel Syndrome and 32 healthy subjects), during the autumn-winter season of the years 2015/2016. The group characteristics was shown in Table 1.

Table 1. Sample characteristics

Charakteristics	IBS patients (n) = 32	Controls (n)=32	p value
Age (years)	31.5±9.4	32.9±8.2	0.5481
Body height (cm)	170.2±8.3	170.1±9.0	0.9289
Body weight (kg)	69.5±15.0	71.3±12.0	0.5956
BMI (kg/m^2)	23.9±3.3	24.5±3.2	0.4575
WHR (waist - hip ratio)	0.8±0.08	0.8±0.05	0.2778
Waist circumference (cm)	84.5±12.9	87.8±10.3	0.2758
Marital status: married unmarried	 20 (62.0%) 12 (38.0%)	 24 (75.0%) 8 (25.0%)	0.4975
Education: primary secondary higher	 1 (3.0%) 11 (34.0%) 20 (63.0%)	 3 (9.0%) 9 (28.0%) 20 (63.0%)	0.2680

Values are given as mean±standard deviation or n (%)

The compared groups did not indicate significant differences in terms of anthropometric and demographic traits. Most subjects were married women with appropriate body mass (average BMI of the test group subjects 23.9±3.3 kg/m^2, control group subjects 24.5±3.2 kg/m^2), having a university degree.

Table 2 presents the number and type of the consumed meals, drinks and products that are most often eaten between the meals. When it comes to the assessment of the selected dietary habits, no differences of statistical importance were noted within the compared groups. It was established that a 4-meal dietary plan was prevailing in both of the examined groups (69.0% of subjects in the group with IBS, 72.0% in the control group). Women with IBS most often included breakfast, dinner and supper in their menus (>75,0% of subjects). Similarly, women of the control group most often consumed breakfast (91.0%), dinner (87.0%) and supper (84.0%). Up to 75.0% subjects with IBS and 81.0% of subjects in the control group declared that they eat snacks between the meals. The food products most often consumed by patients with IBS were sweets – 83.0% and fruit – 17.0% of subjects. Whereas, when it comes to snacking between the meals, patients of the control group most often chose sweets (58.0%), fruit (27.0%) and sandwiches (11.0%).

Table 2. Assessment of chosen eating habits among studied subjects

Studied feature	IBS patients (n=32)	Controls (n=32)	p value
Number of meals: ≤3 ≥4	 10 (31%) 22 (69%)	 9 (28%) 23 (72%)	0.4066
Type of meals: breakfast mid-morning meal lunch afternoon tea dinner additional eating between meals	 24 (75%) 20 (62%) 24 (75%) 10 (31%) 25 (78%) 24 (75%)	 29 (91%) 18 (56%) 28 (87%) 11 (34%) 27 (84%) 26 (81%)	 0.1203 0.5027 0.0878 0.8102 0.9175 0.7072
Type of additional snack: sweets fast food sandwiches fruit	n=24 20 (83%) 0 (0%) 0 (0%) 4 (17%)	n=26 15 (58%) 1 (4%) 3 (11%) 7 (27%)	0.1166

In this paper, the customary intake frequency of selected food products was also evaluated (Table 3).

It was established that the test group patients with IBS consumed corn flakes significantly more often (median – 1-2 times a month, R=2.5), in comparison to women of the control group (no consumption, R=10, p=0.0012).

Table 3. Consumption of food groups in irritable bowel syndrome patients and their controls groups

Food products groups	IBS patients (n=32)	Controls (n=32)	p value
	Me (IQR)	Me (IQR)	Me (IQR)
Groats	4.0 (3.0;4.0)	3.7 (3.0;4.0)	0.8120
Rice	4.0 (3.0;5.0)	4.0 (3.0;4.0)	0.4463
Pasta	4.2 (4.0;5.0)	4.0 (3.0;5.0)	0.3020
Cornflakes	2.5 (1.0;4.5)	1.0 (1.0;2.0)	**0.0012**
Oatmeal	5.0 (3.0;5.0)	3.0 (1.0;4.5)	0.0628
Wheat bread	4.0 (3.0;6.5)	5.0 (4.0;6.0)	0.5662
Rye bread	4.5 (3.0;5.5)	5.0 (3.0;6.0)	0.0650
Sweet bread	3.3 (2.0;5.0)	3.0 (1.0;4.0)	0.4130
Flour dishes (dumplings, omelets)	3.5 (3.0;4.5)	3.0 (3.0;4.0)	0.4526
Milk	6.0 (5.0;7.0)	6.0 (4.5;7.0)	0.8489
Yogurts, buttermilk	5.0 (4.0;6.0)	5.0(4.0;6.0)	0.5623
Cottage cheese	4.0 (3.0;5.0)	4.0 (3.0;5.5)	0.4581
Cheese	5.0 (4.0;6.0)	5.0 (4.0;5.0)	0.0742
Cheese with mold	3.0 (1.5;3.0)	1.5 (1.0;3.0)	0.1617
Eggs	4.0 (4.0;5.0)	3.0 (3.0;4.0)	0.9116
Lean pork (pork, beef)	5.0 (4.0;5.0)	5.0(5.0;6.0)	0.3699
Fatty pork (pork knuckle)	1.0 (1.0;2.0)	1.0 (1.0;2.5)	0.2778
Beef, veal	2.0 (1.0;3.0)	2.0 (1.5;3.5)	0.7179
Chicken, turkey	5.0 (4.0;6.0)	5.0 (4.0;6.0)	0.5972
Goose, duck	1.0 (1.0;2.0)	2.0 (1.0;2.0)	0.7264
Fish	4.0 (3.0;4.5)	4.0 (4.0;5.0)	0.2541
Seafood	1.5 (1.0;2.0)	1.0 (1.0;2.0)	0.6174
Meats	6.0 (5.5;7.0)	6.0 (5.5;7.0)	0.7757
Frankfurters, luncheon meat, canned	2.5 (1.0;4.0)	3.0 (1.0;4.0)	0.6392
Butter	6.0 (4.0;7.0)	6.0 (4.0;7.0)	0.8564
Cream	3.5 (2.0;5.0)	4.0 (3.0;5.5)	0.1328
Lard	1.0 (1.0;3.0)	2.0 (1.0;3.0)	0.5073
Vegetable Oils	5.0 (5.0;6.0)	5.0 (5.0;6.0)	0.9978
Margarine, vegetable butter	1.0 (1.0;4.0)	3.0 (1.0;5.0)	0.2446
Potatoes	5.0 (4.0;5.0)	5.0 (4.0;6.0)	0.4446
Vegetables	6.0 (5.0;7.0)	6.0 (5.0;7.0)	0.5442
Fruits	6.5 (6.0;7.0)	6.0 (5.0;7.0)	0.5290
Legumes (peas, beans)	2.0 (1.5;3.0)	2.0 (1.0;3.0)	0.5777
Sugar	4.0 (2.0;7.0)	5.0 (4.0;7.0)	0.5098
Jams	3.0 (3.0;5.0)	4.0 (2.0;4.5)	0.4126
Honey	3.0 (3.5;5.0)	3.0 (2.0;4.0)	0.1121
Carbonated drinks	2.0 (1.5;3.0)	3.0 (1.0;4.0)	0.7107
Juices	4.0 (3.0;5.0)	4.0 (3.0;5.0)	0.9306
Sweets	5.0 (4.5;6.0)	5.0 (4.0;6.0)	0.3085
Fast food	3.0 (2.0;4.0)	2.0 (1.5;3.0)	0.1317
Beer	4.0 (3.0;4.0)	5.0 (4.0;6.0)	0.6106
Wine	3.0 (2.0;3.0)	2.0 (1.0;3.0)	**0.0166**
Spirits	2.0 (1.5;3.0)	2.0 (1.0;7.0)	0.6680
Coffee	7.0 (5.0;7.0)	7.0 (5.0;7.0)	0.9510
Tea	7.0 (5.0;7.0)	7.0 (6.0;7.0)	0.0883

Me-median; IQR-interquartile range

It has been revealed that the patients with IBS drank wine substantially more often (1-2 times a month, R=3.0) than subjects of the control group, who drank wine less often than once a month (R=2.0, p=0.0166).

In comparison to women of the control group, women with IBS most often consumed oatmeal (p=0.0628), pastry (p=0.4130), floury dishes (p=0.4526), blue cheeses (p=0.1617), eggs (p=0.9116), seafood (p=0.6174) and fast-food (p=0.1317). On the other hand, in comparison to healthy women, subjects suffering from IBS were characterized by customary low consumption of groats (p=0.8120),

rice (p=0.4463), wheat bread (p=0.5662), rye bread (p=0.0650), duck and goat meat (p=0.7264), sausages and canned food (p=0.6392), cream (p=0.1328), margarines (p=0.2446), jams (p=0.4126) and sodas (p=0.7107).

The caloric content of the evaluated daily food rations, and the nutrient content (basic nutrients and selected minerals) for women with IBS, and women of the control group was presented in Table 4. A higher intake of sucrose was observed among women with IBS, than in the case of control group. A substantially lower intake of dietary fiber was noted among patients with IBS, than in the case of the control group (p=0.0169). The analysis of the results indicated that patients with IBS consumed significantly more water (derived from drinks and food products) than women of the control group (p=0.0267). In both groups, an insufficient intake of plant protein and polyunsaturated fatty acids was noted.

Table 4. Average energy value and content of selected nutrients in daily food rations of women with irritable bowel syndrome and women in the control group

		IBS patients (n=32)			Controls (n=32)			
Energy and nutrients	Unit	Mean ± SD	Norm	% of the norm	Mean ± SD	Norm	% of the norm	P value
Energy	kcal	2003.0±1269.3	1950.0	102.7	1871.2± 666.1	1950.0	95.9	0.6047
Water	ml	1904.6±542.0	2000.0	95.2	1552.1±691.4	2000.0	77.6	0.0267
Total protein	g	74.2 ± 42.5	58.6	126.8	80.0±37.3	58.6	136.1	0.5872
Animal protein	g	48.2±31.9	29.3	164.8	53.6±34.1	29.3	183.2	0.5126
Vegetable protein	g	24.8±14.1	29.3	84.78	25.00±9.00	29.3	85.4	0.9531
Total carbohydrates	g	260.0 ±145.3	282.7	91.9	231.5± 87.1	282.7	81.9	0.3464
Dietary fiber	g	11.3 ± 12.1	25.0	45.2	17.5± 7.8	25.0	70.0	0.0169
Total fats	g	74.0 ± 62.7	65.0	113.8	69.4±44.7	65.0	106.8	0.5194
Saturated fatty acids	g	29.6± 19.8	15.2	194.7	27.8± 17.4	15.2	182.9	0.3070
Monounsaturated fatty acids	g	30.2± 16.0	32.5	92.9	28.4± 13.6	32.5	87.4	0.6295
Polyunsaturated fatty acids	g	14.2± 14.8	17.3	82.1	13.4± 13.5	17.3	77.4	0.8220
Cholesterol	mg	284.4± 160.8	300.0	94.8	277.4± 240.9	300.0	92.5	0.8917
Saccharose	g	37.2±51.4	195.0	19.1	25.7±17.4	195.0	13.2	0.2395
Lactose	g	7.4±10.7	-	-	5.1±7.0	-	-	0.3215
Sodium	mg	3653.7±1762.0	1500.0	243.6	3576.6±2034.1	1500.0	238.4	0.8717

SD – standard deviation

The total supply of protein in general, animal protein, fat in general, saturated fatty acids and sodium exceeded the recommended norm, both among women with IBS and subjects of the control group.

DISCUSSION

The symptoms of IBS develop mainly in the third or fourth decade of life, but they may also concern children and youth, as well as people over their middle-age or even over the age of 80. More and more studies suggest that most patients with IBS are women aged below 45 [10, 30, 35]. *Khademolhosseini* et al. showed that the incidence of IBS was related to age (most cases concerned young people) [17]. By contrast, no connection was observed between the onset of symptoms of IBS and the age of sufferers in China and in England [11, 37]. In this study, the age of women with IBS was 31.5±9.4, whereas in the control group the age was 32.9±8.2.

It was established that most patients are women with appropriate body mass (average BMI of the subjects – 23.9±3.3 kg/m^2, BMI of the subjects of the control group – 24.5±3.2 kg/m^2). 68.8% of women of the test group and 65.6% of women of the control group had appropriate body mass.

Most of the subjects were married people, having a university degree. *Farzaneh* et al. demonstrated that single persons with secondary or lower education, unemployed and those having a lower BMI (<25 kg/m^2) were remarkably more prone to IBS (particularly when it comes to women with BMI <25 kg/m^2, OR=0.94; 95%: 0.89-0.99, p=0.04) [7]. On the other hand, *Chirila* et al. showed that amongst patients with IBS, 49.5% were overweight (25.0-29.9 kg/m^2), and 20.8% were obese (≥30.0 kg/m^2) [3]. In this study, 25.0% of the sufferers were overweight, and 3.1% were obese.

In the presented dietary assessment of people with Irritable Bowel Syndrome, it has been revealed that in both of the tested groups, a 4-meal dietary plan was prevailing. Both women of the test group, and women of the control group most often consumed breakfast, dinner and supper. The rules of a rational diet promote consumption of 4 to 5 meals a day [13].

The caloric value of a daily food ration of the tested women was in line with the recommended norms, and it amounted to 2003.0±1269.3 kcal in the test group and 1871.2±666.1 kcal in the control group (a discrepancy of no statistical importance). The study of the diet of selected groups of Lower Silesian population, similar in age to the group of evaluated patients, demonstrated a tendency to follow diets with low caloric values, enabling to fulfill the norms at a level of 78,4% of the food rations of healthy women (an average of 1322.7 kcal) [12].

The most common mistake made both by the test group patients and the control group patients was snacking between the meals. Most of the tested women, as many as 83.0%, reached for sweets. Women of the control group consumed those products less commonly (58.0%). The analyzed food rations deliver sucrose at a level of 19.1% of the norm for the group of patients with IBS and 13.2% of the norm for the control group. The recommended daily portion of carbohydrates in our country is 130.0 g, and the amount of energy derived from the added sugars, that are being consumed, should not exceed 10.0% of the total caloric intake needs [13]. A positive correlation between the intensification of symptoms and consumption of products rich in sucrose was observed among patients with IBS. These products may cause excessive gas, flatulence, osmotic diarrhea and stomach ache [9].

Women with IBS did not consume fast food dishes and sandwiches, however these products were consumed by healthy individuals (4.0% and 11.0% of the subjects, respectively). More and more researchers point out that inappropriate diet may contribute to the intensification of IBS ailments. It has been shown that a significant proportion of patients with IBS individually remove some of the food products from their diet, namely those that intensify the disease symptoms, such as: milk, apples, pears, onion, cabbage, peanuts, whole-wheat flour products, coffee and chocolate [5, 19, 36].

In this study, it has been indicated that the intake of dietary fiber in the diet was significantly low among women with IBS (45.2% of the norm), and it amounted to 11.3±12.1g, averagely, in comparison to the patients of the control group (70.0% of the norm, average intake 17,5±7,8 g, discrepancies of statistical importance p=0,0169). The consumption of cereal products, rich in dietary fiber, influences the functioning of the digestive tract. The deficiency of fiber in the diet may cause long-lasting disturbances in the regular intestinal responses. Low supply of fiber in the diet may also constitute one of the primary developmental factors of the IBS with constipation. Gradual increase in its level in the diet may improve the motion functions in the intestines. It was observed that many fiber-rich products, such as whole-grain products, vegetables, legume plant seeds, dark rice, whole-meal pastas cause unfavorable symptoms within the digestive tract, in IBS sufferers. Nevertheless, they should not be entirely removed from the diet, instead only those causing adverse effects should be eliminated [9].

In this study, food products containing cereal grain proteins (wheat, rye and barley) were consumed by women of both of the tested groups, at least 1-2 times a month or more often. Those products may intensify the pain among some of the IBS sufferers, who at the same time do not suffer from coeliac disease (68.0% of patients suffered from stomach ache, flatulence, exhaustion) [2]. Polish study demonstrated that the frequency of positive serological tests for coeliac disease in patients with IBS was remarkably higher than in the control group (32.0 *vs* 0.0, $p<0.001$). The histopathological image of the mucous membrane of the duodenum, in all the people who gave consent to duodenoscopy, from the group with a positive titer of the anti-tTG and/or AGAs antibodies (n = 20), was appropriate. The gluten intolerance among people with IBS occurs significantly more often than in the general population, therefore it is justified for them to undergo serological tests for coeliac disease or gluten intolerance. The most common manifestation of the coeliac disease in patients with IBS is the latent from [25].

It is worth noticing that milk and dairy products were consumed in both of the tested groups, with a similar frequency (2-3 times a week and more often). *Okami* et al. pointed out that the female group with IBS consumed less milk and dairy products, in comparison to the control group [22]. In the study performed by *Chirila* et al., it has been shown that consumption of milk substantially contributes to the occurrence of disease symptoms [3]. The same study, involving 193 people with IBS (80 men and 113 women), indicated that the patients consumed processed canned food, meat preserves, legumes, pastry, fruit compotes and herbal teas significantly more often than healthy subjects [3]. The type of consumed food may contribute to the occurrence of disease symptoms through several mechanisms, including allergy or food intolerance. Atkinson pointed to a significant betterment of general sensation and a 26.0% reduction of the disease symptoms ($p<0.001$), following a 12-week elimination diet, based on the study of the titer of characteristic IgG antibodies, in the presence of selected nutrients [1]. *Drisko* et al. demonstrated an

increased concentration of IgG class antibodies, in the presence of selected nutrients, among 20 patients with IBS. Following 6 months of staying on an elimination diet, accompanied by probiotic supplements, a remarkable reduction of disease symptoms in the form of a decrease in defecation frequency and the degree of experienced pain (p=0.05), along with improvement of the quality of life (p=0.0001) were noted [5]. Some food products may alter the composition of the bacterial flora, directly or indirectly influencing the metabolism of bacteria, and thus evoke the IBS symptoms [20]. Clinical tests indicated that patients with IBS have a different composition of the bacterial flora, from healthy individuals [15, 21, 24]. The molecular analysis of the feces samples showed that the *Firmicutes* / *Bacteroidetes* ratio is 2-times higher among Dutch patients with IBS [21]. According to *Kerckhoffs* et al. study, the *Pseudomonas aeruginosa bacillus* are more often found in patients with IBS, than in healthy ones [16].

In this paper, the assessment of the nutritional value of daily food rations revealed that the intake of protein in general among the tested women was high (126.8% of the norm for patients with IBS and 136.1% of the norm – patients of the control group; a discrepancy of no statistical importance; it equaled 74.2 ±42.5 g in the group with IBS and 80.0±37.3 g in the control group). The study conducted by the authors from Wroclaw showed that the intake of protein among healthy women amounted to 111.5% of the recommended norm of intake [12]. The proportion of this nutrient in the twenty-four-hour caloric value structure should amount to 12.0 – 15.0% [13]. The consumption of animal protein in both of the examined groups exceed the recommended norm (164.0% of the norm – patients with IBS and 183.2% of the norm – patients of the control group) and it amounted on average to 48.2±31.9 g in the study group and 53.6±34.1g in the control group. Insufficient intake of plant protein among the tested women (84.8% of the norm) might reflect the rare consumption of legumes (less often than once a month). Those products contain gas producing oligosaccharides, such as: stachyose and raffinose. Humans do not digest these compounds, they are broken down by bacteria in the large intestine. This process is accompanied by extensive release of gases, methane, carbon dioxide and hydrogen. Because of the lowered pain threshold, IBS sufferers suffer more intensely from such ailments as flatulence, gases, stomach aches, caused by the excessive consumption of plants rich in stachyose and/or raffinose [9].

In this study, it has been demonstrated that the intake of fat in general, among all the tested women, slightly exceeded the recommended norms (it amounted to 113.8% of the norm in the test group and 106.8% in the control group) and constituted on average 74.0±62.7g in the study group and 69.4±44.7 g in the control group. In compliance with the guidelines, the percentage of energy, derived from fats in general, should not be higher than 30.0%. The inappropriately balanced diet, along with excessive consumption of fat in general may lead to weight gain [13]. In this study, it has been shown that butter is a popular fat spread on bread (patients of both groups consumed butter 4-6 times a week). In most patients suffering from IBS, the intake of date may cause the occurrence of gastrointestinal diseases (feeling of fullness, flatulence, nausea). Since lipids may hinder small intestinal motor activity and slow down the motility of intestines, they might also cause retention of gases, and subsequently result in flatulence [8]. On the other hand, there is evidence that lipids stimulate the motor activity in the colon through the gastrocolic reflex. Such a reflex is intensified in patients with IBS, and it leads to diarrhea [27]. *Simrén* et al. [32] also revealed that stomach emptying is hindered if fats are present in the duodenal tube tip. Large quantities of fat consumed at once may excessively stimulate intestinal contractions, therefore it is recommended to consume smaller amounts of this component, apportioned evenly during the day [9].

In both of the examined groups, saturated fatty acids were consumed in excessive quantities (over 180.0% of the norm), amounting on average to 29.6±19.8 g among women with IBS and 27.8±17.4g among women of the control group (a discrepancy of no statistical importance). The supply of the polyunsaturated fatty acids in the diet was insufficient, and equaled 82.1% of the recommended norm, among women with IBS, amounting on average to 14.2±14.8 g, whereas among women of the control group it equaled 77.4% of the recommended norm, amounting on average to 13.4±13.5 g (a discrepancy of no statistical importance). Similar results were obtained by researchers, when it comes to the consumption of SFAs and PUFAs among the inhabitants of Lower Silesia [12].

It has been demonstrated that the average cholesterol content (284.4±160.8 mg in the test group and 277.4±240.9 mg in the control group) in the diets was compliant with the obligatory norms [13].

In accordance with the Polish dietary recommendations, the daily supply of carbohydrates should cover 55.0 – 60.0% of the caloric intake needs of the system [13]. Among the tested women of both groups, the content of this element equaled 91.9% of the recommended norm, and amounted on average to 260.0±145.3g in the group of patients with IBS, and 81.9% of the recommended norm, amounted on average to 231.5±87.1g in the control group (a discrepancy of no statistical importance).

Some carbohydrates, such as fructose (fruit sugar) or lactose (milk sugar) may cause gastric disorders, such as: diarrhea, gases, flatulence or stomach cramps [18]. In this study, fruit and milk were consumed by both tested groups, 4-6 times a week. The lactose content in the examined food rations was 7.4±10.7 g in the group of patients with IBS and 5.1±7.0 g in the control group (a discrepancy of no statistical importance). Among patients with IBS, the sensibility threshold when it comes to these sugars is additionally lowered, hence even their tiny amounts may cause irritation of the digestive tract, leading to disorders. Reports have occurred in literature, elaborating on the positive influence of limiting the amounts of the consumed carbohydrates (FODMAP diet) on the mitigation of gastric disorders [26, 28, 29].

In this study, sweets, cakes, candies, crackers were consumed by both groups of women 2 – 3 times a week. Both sorbitol (E420), xylitol (E967) and mannitol (E421) are polyalcohols showing sweetening properties. They are added in pastry production. Among people with IBS, sorbitol might provoke the occurrence of gases, flatulence, osmotic diarrhea and stomach aches [29, 33, 36]. Xylitol and mannitol also reveal properties to accelerate intestinal peristalsis, which may foster the occurrence of diarrheas, following extensive intake of products containing those compounds [9].

In the performed studies, it is worth noticing that the group of women with IBS consumed fast food too often, in comparison to healthy women. Such products typically contain hot, spicy seasoning. The study of *Esmaillzadeh* et al. [6] demonstrated that the intake of spicy dishes rich in pepper, curry, ginger and turmeric is strictly connected with the disease symptoms, especially among women. Women who consumed hot dishes 10 times a week or more often were twice as much prone to IBS than women who had never consumed spicy food (OR=2.03; 95%CI:1.09-3.77, P=0.02) [6]. Processed food products also contain large amounts of sodium. The food rations of the tested patients exceeded twice the level of appropriate sodium intake. The excess of sodium in CRP is considered to be one of the dietary risk factors in terms of arterial hypertension and stroke [38]. The data derived from research conducted for over a decade point to common occurrence of the coronary artery disease and other cardiovascular diseases [4].

The significant increase in the consumption of wine by patients with IBS, compared to healthy women (p=0.0166), indicated in this study, might have a negative influence on the functioning of the digestive tract of IBS sufferers. The increased doses of ethyl alcohol cause the damage of the mucous membranes, inter alia, in the stomach and in the intestines, which emerge as gastrointestinal suppression of the immune system, and intensified penetration by toxic substances. It can also be the cause of an increased intestinal dysbiosis and systemic infection. Upon regular consumption of ethanol, disorders in digestion and absorption might occur, which promotes deficiencies in nutrients [38]. Through irritating the receptors of nerve endings and shortening the time when chime comes into contact with the intestinal wall, alcohol is the cause of diarrheas. It may also cause the occurrence of severe stomach aches and heartburn [28, 36].

The results concerning the water supply in the group of patients with IBS, which amounted on average to 1904.6±542.0 ml (95.2% of the norm) and was remarkably higher (p=0,0267) than in the control group – 1552.1±691.4 ml, are satisfactory. In this study, all women drank coffee and tea on a daily basis. Such products excessively stimulate the intestinal motility through intensified contractility of the small intestine and increased gastrointestinal excretion. The disorders of the proper functioning if the lower section of the digestive tract contribute to the occurrence of stomach aches, diarrheas, flatulence and heartburn. Caffeine or its derivatives also have diuretic properties, resulting in excreting excessive amounts of fluids during the day. People with IBS should take particular care of an appropriate level of fluids in their diet, in order to maintain the proper degree of hydration [9, 26].

CONCLUSIONS

1. The preliminary study suggests that there are significant improprieties when it comes to the dietary habits of women with IBS. Patients suffering from IBS most often consumed sweets and fruit between the meals. They also much more often consumed corn flakes and wine, in comparison to women of the control group.
2. The examined diets of patients with IBS are characterized by a substantially lower intake of dietary fiber and remarkable higher consumption of water than in the case of women of the control group. In both of the tested groups, insufficient intake of plant protein and polyunsaturated fatty acids was reported. The supply of protein in general, fat in general, saturated fatty acids and sodium exceeded the recommended norm, both among women suffering from IBS and women of the control group.
3. In order to eliminate these mistakes in the future, it seems justified to extend the knowledge on rational nutrition amongst patients with IBS.

Conflict of interest
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

THE USE OF FRUIT EXTRACTS FOR PRODUCTION OF APPLE CHIPS WITH ENHANCED ANTIOXIDANT ACTIVITY

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ABSTRACT

Background. Style and pace of life make consumers more willing to reach for snack products. This group of processed food includes, among others, fruit chips. Due to the increasing incidence of diseases associated with the excessive exposure to free radicals foods enriched with antioxidant compounds, eg. polyphenols, can be introduced into the sale.

Objective. The aim of the study was to use the fruit extracts for the production of apple chips with enhanced antioxidant activity.

Material and methods. 'Golden Delicious' variety of apple fruit was used to produce chips. Apple chips were prepared by slicing, soaking in a sugar solution and pre-drying in a microwave oven. Chips were enriched with extracts prepared from fruits of chokeberry, five-flavor berry, Cornelian cherry, woodland hawthorn, goji berry, Japanese quince and cranberry microcarpa. For this purpose, pre-dried apple slices were soaked (5 min) in ethanolic extract of fruits and then dried to achieve a 5% moisture content. Chips were sensory evaluated and their antioxidant activity and total polyphenols content were determined.

Results. All enriched apple chips were characterized by high antioxidant activity and a relatively high value of total polyphenols content. Chips soaked in extracts of five-flavor berry, cranberry and goji berry were characterized by the highest antioxidant potential. Samples obtained by using chokeberry and Cornelian cherry extracts showed the highest content of polyphenols. High sensory attractiveness of enriched chips was also showed. The chips with the addition of five-flavor berry extract were exceptions. Their taste was not acceptable.

Conclusions. Fruit extracts are a valuable material for chips enrichment. Taking into account all the analyzed differentiators, extracts of Japanese quince, goji berry and woodland hawthorn were found to be the best enriching additives. The chips soaked in extract of five-flavor berry, despite their high antioxidant activity, were disqualified due to very low score of sensory evaluation.

Key words: *apple chips, food supplementation, antioxidant compounds, polyphenols*

STRESZCZENIE

Wprowadzenie. Tempo oraz styl życia sprawiają, że konsumenci coraz chętniej spożywają produkty przekąskowe. Do wyrobów takich należą m.in. chipsy owocowe. Ze względu na rosnącą zachorowalność na choroby związane z nadmierną ekspozycją na wolne rodniki celowe wydaje się wprowadzanie do sprzedaży żywności wzbogaconej związkami przeciwutleniającymi, np. polifenolami.

Cel. Celem badań było wykorzystanie ekstraktów owocowych do wytwarzania chipsów jabłkowych o podwyższonej aktywności przeciwutleniającej.

Materiały i metody. Z jabłek odmiany Golden Delicious przygotowano chipsy jabłkowe poprzez pokrojenie na plastry, wysycenie w zalewie cukrowej i wstępne podsuszenie w piecu mikrofalowym. Chipsy jabłkowe wzbogacano ekstraktami etanolowymi z owoców aronii, pigwowca japońskiego, derenia jadalnego, żurawiny drobnoowocowej, głogu dwuszyjkowego, cytryńca chińskiego i kolcowoju chińskiego. W tym celu podsuszone plastry jabłek moczono (5 min) w ekstrakcie etanolowym z owoców. Następnie plastry dosuszono owiewowo do osiągnięcia 5% wilgotności. Uzyskane chipsy oceniano sensorycznie oraz oznaczano ich aktywność przeciwutleniającą i zawartość polifenoli ogółem.

Wyniki. Wszystkie wzbogacone chipsy jabłkowe miały wyższą aktywność antyoksydacyjną i zawartość polifenoli ogółem niż próby kontrolne. Największym potencjałem antyoksydacyjnym charakteryzowały się chipsy z dodatkiem ekstraktu z cytryńca, żurawiny i goji. Najwyższą zawartością polifenoli odznaczały się chipsy z dodatkiem ekstraktu z aronii oraz derenia. Wykazano także wysoką atrakcyjność sensoryczną chipsów z dodatkami ekstraktów owocowych. Wyjątek stanowiły chipsy z dodatkiem cytryńca, których smak nie był akceptowany.

Wnioski. Ekstrakty owocowe stanowią cenny surowiec wzbogacający chipsy owocowe. Wykazano, że najbardziej wartościowymi ekstraktami owocowymi wzbogacającymi chipsy jabłkowe, ze względu na wysoki potencjał antyoksydacyjny i wyniki oceny sensorycznej, były ekstrakty z owoców pigwowca, goi i głogu. Chipsy z dodatkiem ekstraktu z cytryńca, mimo wysokiej aktywności przeciwutleniającej, uzyskały niską ocenę sensoryczną.

Słowa kluczowe: *chipsy jabłkowe, suplementacja żywności, związki antyoksydacyjne, polifenole*

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INTRODUCTION

Reactive oxygen species (ROS), including free radicals, are formed in all living organisms under the influence of many factors. Therefore it is so important that the antioxidant system functions correctly in order to prevent development of any pathological processes caused by free radicals, that may lead to many diseases including cardiovascular diseases and cancer. Antioxidants are compounds that are able to inactivate ROS. They play a significant role in preventing damage caused by free radicals as they can inhibit ROS production and participate in their transformation into inactive derivatives. Antioxidants include tocopherols, carotenoids, vitamin C, and polyphenols, which make up the most important and abundant group. Fruits and vegetables, tea and wine are the main sources of antioxidants [4, 7].

Chips are popular food products commonly consumed around the world. They are predominant part of the "snack foods", generally eaten between meals. Chips might be made by drying, expanding, extruding, baking or frying. These products are more shelf-stable and attractive for consumers in comparison to the unprocessed raw material. Currently, the most popular snack is potato chips. In the snack food market, there are many varieties of chips manufactured using various spices, flavorings and aromatics [8, 13].

Growing consumer demand for functional food, as well as awareness and understanding of dietary recommendations to eat more fruits and vegetables, encourages food manufacturers to develop new products, which are a valuable source of micro- and macronutrients, fiber and polyphenolic compounds [18]. On the other hand style and pace of life and eating habits can lean buyers to choose processed snacks. Therefore, snacks made from fruit and vegetables are gaining popularity, because they are fat-free, low-calorie products with a low salt content [8].

Enrichment of food products with polyphenols is a type of fortification aimed at shaping the product properties desired by consumers. The presence of the bioactive components improves the quality and affects a specific physiological activity of the body. However, the antioxidant activity of the polyphenols, their content, as well as the absorption capacity of the gastrointestinal tract should be noted [12].

The aim of the study was to use the fruit extracts for the production of apple chips with enhanced antioxidant activity.

MATERIAL AND METHODS

Apple fruits (*Malus domestica* Borkh.) 'Golden Delicious' were used to produce chips. Extracts were prepared from chokeberry (*Aronia melanocarpa* (Michx.) Elliott), five-flavor berry (*Schisandra chinensis* (Turcz.) Baill.), Cornelian cherry (*Cornus mas* L.), woodland hawthorn (*Crataegus oxyacantha* L.), goji berry (*Lycium chinense* Mill.), Japanese quince (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach.) and cranberry microcarpa (*Oxycoccus microcarpus* (Turcz. ex Rupr.) Schmalh.). Fruit came from the pomological orchard of University of Agriculture in Krakow, located in Garlica Murowana near Krakow. Part of the fruits was purchased from the producers of organic farming in Malopolska and Podkarpacie areas. Dried goji berries and five-flavor berries were bought in shops distributing organic food.

Extracts preparation

90 ml of ethanol at a concentration of 80% vol. was added to 10 g of fruits. The high shear homogenizer (19 000 rpm; 5 min; Ultra-Turrax T25 Basic, IKA) was used for extraction. The obtained extracts were filtered, adjusted to 100 ml with the solvent and stored at -20°C.

Preparation of apple chips supplemented with fruit extracts [19]

Apples were washed, thoroughly dried with paper towel and sliced (with peel and apple core) into slices of 3.5 mm thickness. Then the slices were soaked for 1 minute in a saturating mixture containing sucrose (20%), apple juice concentrate (5%), citric acid (0.25%) and SO_2 (0.12%). After draining off the apple slices were treated with microwave (a microwave generator Mars Express, 300 W, 5 min) and subsequently pre-dried in an air-oven (90°C, 2 h). In order to enrich chips with the antioxidant compounds, they were soaked (5 min) in the fruit ethanolic extract. At the end the slices were dried to achieve a 5% moisture content.

Evaluation of the antioxidant activity [20]

The sample of crushed chips was extracted (10 g of chips + 90 ml of methanol) using a high shear homogenizer (19 000 rpm for 5 min). The obtained solutions were filtered and adjusted to a 100 ml by the solvent used.

The antioxidant activity was determined by using the active radical cation ABTS (2,2'-azino-*bis* (3-ethylbenzothiazoline-6-sulphonic acid), Sigma). ABTS radical was generated by chemical reaction between 7 mM aqueous solution of diammonium salt of 2,2'-azino-*bis* (3-ethylbenzthiazoline-6-sulfonate) and 4.9 mM potassium persulfate solution ($K_2S_2O_8$). The solution was kept overnight in the dark at ambient temperature, to terminate the reaction and to stabilize ABTS cation. Just before the analysis, the concentrated solution of ABTS was diluted with phosphate buffer saline (PBS) at pH 7.4, to obtain absorbance value of final solution A = 0.70 ± 0.02 ($ABTS_{0,7}$) measured

with a spectrophotometer (Beckman DU 650) at a wavelength of 734 nm. 100 μL of the appropriate diluted samples were added to 1 mL $ABTS_{0,7}$ and the absorbance was measured 6 minutes after mixing. The antioxidant capacity of the samples was calculated using a standard curve performed on solutions of synthetic vitamin E (Trolox - 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma) and expressed in mg of Trolox/100 mL.

Determination of total polyphenol content [20]

The sample of crushed chips was extracted (10 g of chips + 90 ml of methanol) using a high shear homogenizer (19 000 rpm for 5 min). The obtained solutions were filtered and adjusted to a 100 ml by the solvent used.

Total polyphenol content was determined by the modified *Folin-Ciocalteu* method. 45 mL of double-distilled water was added to 5 mL of appropriately diluted chips' extract or standard (catechin). Then 5 ml of such solution was mixed with 0.25 mL of *Folin-Ciocalteu* reagent (water dissolved at 1:1 v/v, Sigma) and 0.5 mL of 7% Na_2CO_3 (POCh) were added. Samples were incubated for 30 minutes in the dark, before measuring the absorbance on a spectrophotometer at the 760 nm (spectrophotometer Beckman DU 650, against methanol as a blank). The results of total polyphenolic content were obtained based on calibration curve and were expressed as mg of (+) catechin per 100 mL of beverage.

Sensory evaluation of apple chips [15]

Evaluation was carried out by the panel comprising 20 qualified and tested for their sensory sensitivity people. They assessed five basic quality factors (flavor, crispness, color, shape and odor). For the sensory evaluation the 5-point scale with following weight factors: the flavor (0.3); crispness (0.25); color (0.15); shape (0.2) and odor (0.1).

Statistical analysis

There were a minimum of three repetitions of the analysis and the results are shown as the arithmetic mean with standard deviation (± SD). Statistical analysis was performed using InStat v. 3.01 (GraphPad Softwere Inc., USA). A single-factor analysis of variance (ANOVA) with post hoc *Tukey's* test was applied to determine the significance of differences between means. The *Kolmogorov-Smirnov* test was carried out to assess the normality of distribution.

RESULTS AND DISCUSSION

All samples with the addition of fruit extracts showed high antioxidant activity. Chips soaked in the extract of five-flavor berries, small cranberries and goji berries were characterized by the biggest antioxidant potential. These values were higher by 77.68 and 67%, respectively, when compared to the control sample. Among the investigated apple chips, those enriched with an extract from chokeberry showed the lowest activity (Table. 1).

Table 1. Antioxidant activity, total polyphenols content and sensory evaluation of chips enriched with fruit extracts (mean ± SD)

Enrichment	Antioxidant activity [mg Trolox/100 g]	Total polyphenol content [mg catechin/100 g]	Sensory evaluation [points]
Control sample	1041 ± 42 ^{a}	357 ± 5 ^{a}	4.13 ± 0.7 ^{a}
Chokeberry	1504 ± 49 ^{b}	464 ± 18 ^{b}	4.08 ± 0.6 ^{a}
Five-flavor berry	1851 ± 102 ^{c}	371 ± 21 ^{a}	3.54 ± 0.5 ^{b}
Cornelian cherry	1631 ± 26 ^{b,c}	451 ± 28 ^{b,c}	3.98 ± 0.6 ^{a}
Woodland hawthorn	1590 ± 42 ^{b}	421 ± 1 ^{c}	4.14 ± 0.5 ^{a}
Goji berry	1744 ± 32 ^{c}	374 ± 36 ^{a,c}	4.18 ± 0.3 ^{a}
Japanese quince	1695 ± 20 ^{b,c}	395 ± 7 ^{a,c}	4.13 ± 0.7 ^{a}
Cranberry	1757 ± 20 ^{c}	387 ± 7 ^{a,c}	4.05 ± 0.5 ^{a}

The same letter, within the analyzed parameter (column) indicate no statistical significance at $p < 0.05$

Fortification has contributed to the increase in concentration of polyphenol compounds in the examined chips. The highest content of polyphenols was showed by samples soaked in chokeberry extract (about 30% higher than the control sample) and Cornelian cherry extract (28% higher), while the addition of the goji and five-flavor berry extracts had the smallest impact on total polyphenol content (only 5% higher compared to the control sample). Antioxidant activity has not always been correlated with the polyphenol content, due to the fact that the antioxidant activity is also influenced by other components of the fruit. Very high antioxidant activity of five-flavor berry extract was a consequence of the presence of many antioxidant ingredients. Lignans, catechins, anthocyanins and vitamins (C and E) have the greatest impact on the antioxidant activity of these fruits [17]. The basic groups of cranberries polyphenols are the anthocyanins (cyanidin, delphinidin and malvidin), flavones, procyanidins,

flavonols (quercetin, myricetin) and hydroxycinnamic acid derivatives [5]. The chemical composition of goji berries includes a number of antioxidants, which is the cause of high ability to scavenge the free radicals. The orange color of berries comes from carotenoids (0.03-0.5% of dry matter) [2]. However, these fruits are also rich in quercetin and p-coumaric acid [1]. The antioxidant activity of chips supplemented with Japanese quince extract was about 62% higher than the control sample, while total polyphenol content in both samples were at a comparable level (Table 1). High amount of vitamin C (150 mg/100 g) and polyphenols that exhibit synergistic effects with the ascorbate, especially flavonols and procyanidins, affect the antioxidant activity of the Japanese quince [6, 16]. Chips containing the extract of Cornelian cherry were characterized, as in the case of Japanese quince, by 62% higher antiradical capacity than the control sample, but they contained only 28% more polyphenols. Cornelian cherry fruits are classified as a raw materials rich in flavonoids and anthocyanins [21, 22]. Woodland hawthorn contains more than 40 phenolic compounds, especially procyanidins, glycosides of flavonols, anthocyanins and phenolic acids [11]. As a result the antioxidant activity and the polyphenol content of chips with the addition of hawthorn extract increased by 51% and 20%, respectively, in relation to chips without enrichment. Chokeberry fruits are considered as one of the richest sources of polyphenols. Total polyphenol content of chokeberry exceed more than three times concentrations of polyphenolic compounds in other berries, such as cranberries and blackcurrants [14]. Its antioxidant activity is caused by extremely high levels of anthocyanins. However, these compounds are sensitive to high temperatures and oxygen [3]. This may explain why the chips with the addition of chokeberry extract are not characterized by the high antioxidant activity.

It should be noted that the method with the ABTS radical used to measure the antioxidant activity, permits determination both hydrophilic (vitamin C, flavonoids, anthocyanins, phenolic acids) and hydrophobic antioxidants (carotenoids, vitamin E). Accordingly, the high antioxidant activity was not always associated with the polyphenol content [10]. Furthermore, cinnamic acid derivatives are much more effective antioxidants than benzoic acid derivatives which explains the high antioxidant activity of the chips soaked in cranberry extract. Glycosylated derivatives of flavonoids have much lower antioxidant activity compared to their aglycones. This dependence can be observed in chips with the extract of five-flavor berry. It contains lignans mainly in the aglycone forms, thereby causing a high antioxidant activity [9].

Sensory analysis of chips enriched with various fruit extracts showed that almost all products received similar amount of points (3.98-4.18). Only the chips enriched with an extract of five-flavor berry have been scored lower (3.54 pts.), mainly because of the sour-bitter taste. However, among the members of the sensory panel were people who really enjoyed that flavor. All samples soaked in fruit extracts were characterized by a lower sweetness and higher acidity compared to the sample without additives. Noteworthy is also the color of enriched chips, especially with chokeberry extract. Compared to control, they have a pink color, and a sensory panel showed large dispersion in the evaluation of this feature. According to some members, color was good and desirable, while the others evaluated it as not characteristic for apple chips, decreasing the note.

CONCLUSIONS

Fruit extracts are a valuable material for fruit chips enrichment, due to their high antioxidant activity and phenolic content. However, in order to consumer approval, the final products must also be organoleptically attractive. For this reason, the best supplements for apple chips were extracts of Japanese quince fruit, goji berry and woodland hawthorn. The sample soaked in extract of five-flavor berry, despite the high antioxidant activity, was disqualified due to the very low sensory evaluation of these chips.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

BEVERAGE OSMOLALITY AS A MARKER FOR MAINTAINING APPROPRIATE BODY HYDRATION

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ABSTRACT

Background. Osmolalities can be useful markers for determining whether given beverages are suited for maintaining an adequate hydration of the body. Losing 2% of body water relative to body mass reduces the efficiency of body function when undertaking physical effort by around 20%. Deficiencies in water intakes approaching 5-8% of body mass, double the impairment to the body's physical and mental functioning, whereas at a level of 10% the body becomes incapable of performing any sort of physical effort. For such reasons the body's hydration status is vital to its functioning.

Objectives. To asses osmolalities as measured in various types of commercially available mineral waters and non-alcoholic beverages containing different amounts of extracts.

Materials and Methods. Test materials were commercially available mineral waters (of low, medium and high mineral content) along with juices, nectars and drinks that are isotonic, energising and those described as being 'light' and sparkling. Osmolality was measured by the 800CL Osmometer instrument from TridentMed whilst the RL-type refractometer was used for determining extract values.

Results. Isotonic drinks were found to have the same osmotic pressures as bodily fluids at 275 – 295 mOsm/kg water. The osmotic pressure in mineral waters depended on the extent of mineralisation and ranged from 13 mOsm / kg water (low mineral content) to 119 mOsm/kg water (high mineral content). Low osmolalities were also found in 'light' drinks (from 29.3 to 34 mOsm/kg water). Juices, nectars, energising drinks and colas typically have high sugar contents and have high osmolalities ranging 492 – 784 mOsm / kg water. Statistical analysis demonstrated significant associations ($p < 0.05$) between osmolalities and extract content in beverages as well as between osmolalities and mineral content in mineral waters. Upon factor analysis, it was possible to group the tested drinks according to similar osmolalities and extract content.

Conclusions. Osmolalities measured in beverages are a marker that permits drinks to be classified into groups according to their tonicity and their ability to ensure that the body is properly hydrated; this becoming vital in cases when the body requires rapid body fluid replenishment.

Key words: *body hydration, osmolality, extracts, non-alcoholic drinks/beverages.*

STRESZCZENIE

Wprowadzenie. Wartość osmolalności napojów może być wykorzystana jako wskaźnik ich przydatności do właściwego nawadniania organizmu. Utrata wody w wysokości 2% w stosunku do masy ciała obniża wydolność fizyczną o około 20%. Niedobór wody sięgający 5-8% masy ciała powoduje dalsze zaburzenia wydolności fizycznej i psychicznej, a przy stracie wody do 10% masy ciała człowiek jest niezdolny do wykonywania jakiegokolwiek wysiłku fizycznego. Dlatego też kontrola stanu nawodnienia organizmu jest bardzo istotna.

Cel. Celem badań była ocena zmierzonej osmolalności różnych wód mineralnych (nisko, średnio i wysoko zmineralizowanych) oraz soków, nektarów, napojów izotonicznych, energetyzujących, „light" i gazowanych zawierających różne zawartości ekstraktu ogółem (°Bx).

Materiał i metody. Materiał do badań stanowiły komercyjnie dostępne wody mineralne (nisko, średnio i wysoko zmineralizowane) oraz soki, nektary, napoje izotoniczne, energetyzujące i „light" oraz gazowane o różnej zawartości ekstraktu. Osmolalność oznaczono przy wykorzystaniu osmometru 800 CL firmy Trident Med., natomiast zawartość cukrów mierzono refraktometrem typu RL. Wyniki poddano analizie statystycznej wykorzystując program Statistica v. 12.

Wyniki. Stwierdzono, że napoje izotoniczne charakteryzowały się typowym dla płynów ustrojowych ciśnieniem osmolalnym (275-295 mOsm/kg H_2O). Ciśnienie osmolalne wód mineralnych zależało od stopnia ich mineralizacji i wahało się od 13 mOsm/kg H_2O (nisko zmineralizowane) do 119 mOsm/kg H_2O (wysoko zmineralizowane). Niską osmolalność wykazywały także napoje typu „light" (od 29,3 do 34 mOsm/kg H_2O). Soki, nektary, napoje energetyzujące oraz napoje typu „cola" charakteryzujące się wysokim udziałem cukrów wykazywały wysoką osmolalność wynoszącą od 492 do 784 mOsm/kg H_2O. Analiza statystyczna wyników wykazała, że istnieje istotna zależność ($p < 0,05$) pomiędzy osmolalnością

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a zawartością ekstraktu ogółem w napojach, a w wodach mineralnych pomiędzy osmolalnością a zawartością składników mineralnych. Analiza czynnikowa pozwoliła na pogrupowanie badanych napojów produkowanych przemysłowo wg podobnej osmolalności i zawartości ekstraktu ogółem na grupy o podobnej osmolalności i zawartości ekstraktu ogółem.
Wnioski.. Osmolalności zmierzone w napojach są markerami, które pozwalają klasyfikować napoje według grup w zależności od ich toniczności i ich zdolności do zapewnienia, że organizm jest odpowiednio nawodniony. Staje się to niezbędne w przypadkach, gdy organizm wymaga szybkiego uzupełnienia płynu ustrojowego.

Słowa kluczowe*: osmolalność, ekstrakt, napoje bezalkoholowe*

INTRODUCTION

The osmolalities of beverages can be used as markers of their usefulness for achieving appropriate hydration of the body. Whenever fluid intakes become limited or if an uncompensated large water loss occurs, then firstly plasma volume becomes reduced and sodium concentration rises, as does the osmolality. If such water deficiencies are not replenished, water moves out of cells into the extracellular space in order that osmotic equilibrium be maintained. This results in intracellular dehydration, which physiologically manifests itself by thirst, impaired salivation, dry mouth, irritability, insomnia, skin redness, loss of appetite, physical weakness, and abnormal motor coordination. Urinary volume is also decreased along with the excretion of waste metabolic products (urea, creatinine and others), thereby causing body toxicity. When fluid intakes are too low, sweating is reduced, which at high ambient temperatures can cause body overheating. A 2-3% water loss to the body mass lowers physical efficiency by about 20%, whilst water deficiencies of up to 5-8% of body mass results in further deterioration of physical and mental function, and if such water losses are 10% of body mass, a person becomes incapable of exercising any physical effort whatsoever [5]. Therefore, control over the body's hydration is very important. Two factors that predominantly lead to premature exhaustion, are linked to the body's depletion of carbohydrate stores and the loss of water and electrolytes through sweating. Drinking sports drinks, whose main aims are to prevent dehydration and provide energy and electrolytes, can thereby increase the body's functional efficiency [15].

The market for non-alcoholic beverages is always changing in the assortment and intended use of its products, that is conditioned by the expectations and needs of various consumer groups. Drinks are sought for that can quickly quench thirst but simultaneously also supplement body fluids that are lost during intense physical exertion or because of high ambient temperatures; these are isotonic fluids, ie. fluids with an osmotic pressure similar to that of body fluids where the osmolality ranges 275 to 295 mOsm/kg of water. Such drinks are especially recommended to those performing high levels of physical activity, including athletes [12]. They shore-up water deficiencies and restore the body's electrolyte balance.

The different types of energy drinks constitute a significant part of the beverage market and are chiefly intended for people performing high levels of mental activity. Such drinks increase reaction to stimuli? rates and bodily function, enhance mental concentration, counteract fatigue and accelerate metabolism. The non-alcoholic drinks market also consist of fruit juices and nectars which, together with energy drinks, belong to the group of hypertonic beverages with an osmolality higher than 295 mOsm/kg of water. These contain varying amounts of sugars, organic acids, vitamins and minerals and they are absorbed more slowly than water where they contain far more solid material than bodily fluids. Their main task is to supply the body with energy after physical activity. Hypertonic drinks are not recommended to athletes because they reduce the rate of water absorption and may increase the risk of suffering from diarrhoea and gastrointestinal discomfort. Nevertheless they can be drunk in limited quantities after exercise for renewing glycogen stores [12, 15].

Various types of mineral water and beverages known as 'light' have an osmolality of less than 275 mOsm/kg of water and belong to the group of hypotonic drinks. They are especially recommended whenever the so-called hypo-osmotic dehydration occurs, consisting of an excessive loss of excreted water and minerals relative to intake. Hypotonic drinks are rapidly absorbed into the body, sometimes even faster than water. Compared to the other drinks, they contain less sugar, quickly hydrate the body and quench thirst. When also compared to isotonic and hypertonic drinks, they are however unable to rapidly improve the body's water-electrolyte balance due to their lower electrolyte concentration [2]. Hypotonic drinks and mineral water are recommended for those doing moderate amounts of work and not too intensive nor prolonged exercise, which does not generate high levels of sweating [9].

Knowing the osmolality of any given beverage enables appropriate choices to be made for any given situation arising; for example when body fluids need to be quickly replenished after any losses have been incurred. Whilst the content and types of carbohydrate are labelled on the drink's packaging, osmolalities are not provided. In this study, we have therefore measured osmolalities in commercially available mineral waters and non-alcoholic drinks of varying extract content.

MATERIALS AND METHODS

Study materials for testing

These were commercially available mineral waters and other non-alcoholic beverages. Their characteristics are shown in Table 1.

Test methods

Immediately after being opened, samples of the above were taken for measurements of osmolality and total extract content; each being performed in triplicate.

Measuring osmolality

The osmolality of beverages were measured, in triplicate on the OS-3000 osmometer. Samples were degassed then cooled until the moment of crystallization thereby forming a biphasic system; liquid and ice crystals. The heat of crystallization maximally raised the system temperature to its freezing point from which the osmolality of the sample was determined from the freezing point depression; with the results being displayed on the osmometer and calculated as mOsm per kg water.

Measurement of total extract

A refractometric method was used by means of a RL type Refractometer and performed at 20 ºC.

Statistical analysis

All results were analysed using the STATISTICA v. 12 computer program, where standard deviations were calculated along with one-way analysis of variance (ANOVA) and factor analysis.

Table 1. Characteristics of commercially available mineral waters and other non-alcoholic beverages used in the study; as taken from product labels.

Mineral waters of low mineralisation	A	High CO_2 saturation; total mineral content 311.5 mg/l
	B	High CO_2 saturation; total mineral content 285.8 mg/l
	C	High CO_2 saturation; total mineral content 322.2 mg/l
	D	CO_2 unsaturated; total mineral content 420.0 mg/l
Mineral waters of medium mineralisation	A	CO_2 unsaturated; total mineral content 714.0 mg/l
	B	High CO_2 saturation; total mineral content 508.6 mg/l
	C	CO_2 unsaturated; total mineral content 942.9 mg/l
	D	CO_2 unsaturated; total mineral content 946.51 mg/l
Mineral waters of high mineralisation	A	CO_2 unsaturated; total mineral content 5525.3 mg/l
	B	CO_2 medium saturated ; total mineral content 1890.7 mg/l
	C	CO_2 unsaturated; total mineral content 1547.5 mg/l
	D	CO_2 unsaturated; total mineral content 2087.8 mg/l
Isotonic drinks	A	Lemon flavoured; total mineral content 62.1 mg/100 ml; carbohydrate content 6.7%
	B	Orange flavoured; total mineral content 64.4 mg/100 ml; carbohydrate content 5.6%
	C	Lemon flavoured; total mineral content 120 mg/100 ml; carbohydrate content 5.7%
	D	Citrus fruit flavoured; total mineral content 141.6 mg/100 ml; carbohydrate content 5.7%
Energising drinks	A	Containing taurine (0.4%), caffeine (0.03%), inositol, vitamins (niacin, pantothenic acid , B6, B12); carbohydrate content 11%
	B	Containing: caffeine, guarana extract (0.02%); carbohydrate content 11%
	C	Containing: taurine (0.4%), caffeine (0.03%), vitamins (niacin, pantothenic acid , B6, B12), inositol, glucuronolactone; carbohydrate content 11.3%
	D	Containing: taurine (0.4%), glucuronolactone (0.2%), caffeine (0.03%), inositol (0.02%), vitamins (niacin, pantothenic acid , B6, B12); carbohydrate content 11.3%
Juices & nectars	A	Blackcurrant flavoured nectar with blackcurrant juice concentrate (25%)
	B	Orange flavoured juice with orange juice concentrate
	C	Multivitamin juice from concentrates and purees (20%) from: apples 11.9%, oranges 5.5%, grapes, pineapples, lemons, apricots, bananas, guava, mango, peaches, grapefruits and limes
	D	Orange flavoured juice with orange juice concentrate
'Light' drinks	A	Pepsi 'light'; sweeteners: aspartame, acesulfame K
	B	Coca-Cola 'light'; sweeteners: cyclamates, acesulfame K, aspartame
	C	Grapefruit-orange flavoured drinks containing juice concentrates of: grapefruit (20%), oranges (5%) and sweeteners - saccharin sodium, sucralose and acesulfame K
	D	Pineapple-grapefruit flavoured drinks containing juice concentrates of: pineapple(17%), grapefruit (8%), and sweeteners - saccharin sodium, sucralose and acesulfame K.
Other sparking drinks	A	Pepsi, containing sugar (11%), carbon dioxide, acid (phosphoric acid) and caffeine.
	B	Coca-Cola, containing sugar (10.6%), carbon dioxide, phosphoric acid and caffeine.
	C	Sprite, containing sugar (10.6%) glucose-fructose syrup, carbon dioxide, citric acid, sodium citrate and malic acid.
	D	Orangeade, containing sugar (8.6%) and/or glucose/fructose syrup, carbon dioxide, citric acid and black carrot concentrate.

RESULTS

The osmolalities of mineral waters, along with the mineral compositions as provided by the manufacturers are shown on Table 2. These osmolalities varied according to the extent of mineralisation, ranging from 20 mOsm/kg (low-mineralised water) to 119 mOsm/kg of water (highly mineralised water), with the statistics confirming this as shown in Table 3.

Table 2. Osmolalities of tested mineral waters.

Mineral water type		Osmolality [mOsm/kg water]		Total mineral content (from product labels)
		X	**SD**	
Low mineralisation	A	28	1.53	311.5
	B	20	1.53	285.8
	C	28	0.58	322.2
	D	20	0.58	420.0
Medium mineralisation	A	75	1.53	714.0
	B	44	2.00	508.6
	C	54	1.00	942.9
	D	58	0.58	946.9
High mineralisation	A	119	1.53	5525.3
	B	75	0.58	1890.7
	C	69	1.73	1547.5
	D	88	0.51	2087.8

X – Mean; SD – Standard deviation

Table 3. Mineral waters divided into homogenous groupings according to osmolality.

Mineral water type	Osmolality [mOsm/kg water]	Homogenous grouping		
		1	2	3
Low mineralisation	24.00	****		
Medium mineralisation	57.75		****	
High mineralisation	87.75			****

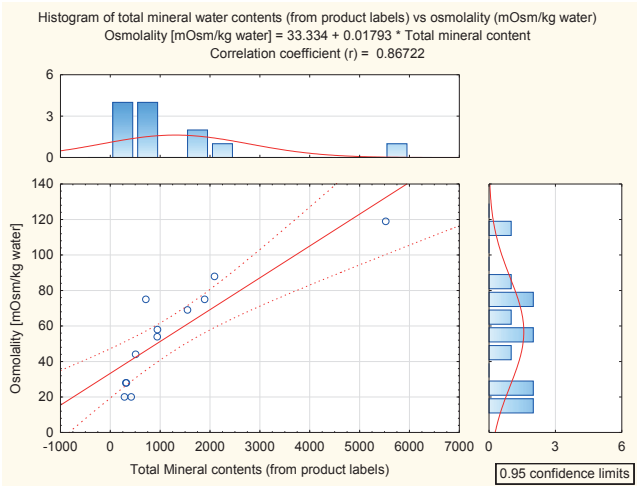

Figure 1. The association between osmolality and total mineral content in mineral waters

Mineral water osmolality significantly rose as the mineral content increased ($p < 0.05$). The total mineral content of the mineral waters was highly correlated ($r = 0.86$) with their osmolality (Figure 1).

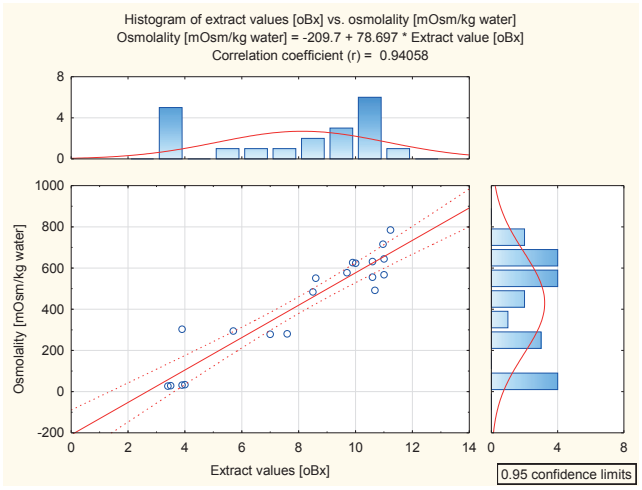

Figure 2. The association between extract values and osmolality in tested beverages

Table 4. Osmolalities and extract contents in non-alcoholic beverages

Type of beverage		Osmolality [mOsm/kg water]		Extract values [°Bx]	
		X	SD	X	SD
Isotonic	A	279	1.73	7	0.06
	B	304	2.52	5.6	0.08
	C	294	7.57	5.7	0.05
	D	281	3.61	7.6	0.09
Energising	A	567	1.53	11	0.12
	B	484	5.03	8.5	0.05
	C	645	5.03	11	0.05
	D	784	6.03	11.23	0.05
Juices & nectars	A	624	1	10	0.04
	B	577	2.52	9.7	0.09
	C	626	0	9.9	0.05
	D	716	1.53	10.97	0.05
'Light' drinks	A	34	1.73	4.00	0.05
	B	29	4.73	3.50	0.09
	C	28	1.53	3.40	0.12
	D	32	1.53	3.90	0.05
Other sparkling drinks	A	492	3.21	10.68	0.05
	B	632	1.53	10.6	0.1
	C	556	2.31	10.6	0.05
	D	551	4.16	8.6	0.05

The measured extract content values and osmolalities of the various groups of commercially available drinks groups are shown in Table 4, with extract values ranging from 5.6 (isotonic drinks) to 11.23 (energy drinks), where these values are characteristic for the beverage type. Large variations were observed in the extract contents of the drink groupings. Isotonic

drinks had fairly low extract contents ranging from 3.90 to 7.60 ºBx and had normal osmolalities (excepting B), that fell within the range found in body fluids (275-295 mOsm/kg water). Energy drinks, juices, nectars and sparkling beverages showed high osmolalities, ranging from 484 to 784 mOsm/kg water for energy drinks, from 577 to 716 mOsm/kg water for juices and nectars and from 492 to 632 mOsm/kg water for other sparkling beverages. The osmolalities of 'Light' drinks were approximately those of the medium-mineralised mineral waters. Statistics confirmed the variability of osmolalities in the drink groupings tested and Table 5 presents the distribution of osmolalities according to their homogeneous groupings. The first group were hypertonic drinks (colas, energy drinks, juices and nectars), the second were light drinks and thirdly the isotonic drinks. Extract content values significantly increased with rising osmolalities, ($p<0.05$), with the correlation coefficient being 0.94, thereby indicating a strong association between the extract contents values and the beverage osmolality (Figure 2).

Table 5. Non-alcoholic beverages divided into homogenous groupings according to osmolality

Type of beverage	Osmolality [mOsm/kg water]	Homogenous groupings		
		1	2	3
'Light'	30.83		****	
Isotonic	289.50			****
Other sparkling drinks	557.75	****		
Energising drinks	620.17	****		
Juices & nectars	635.92	****		

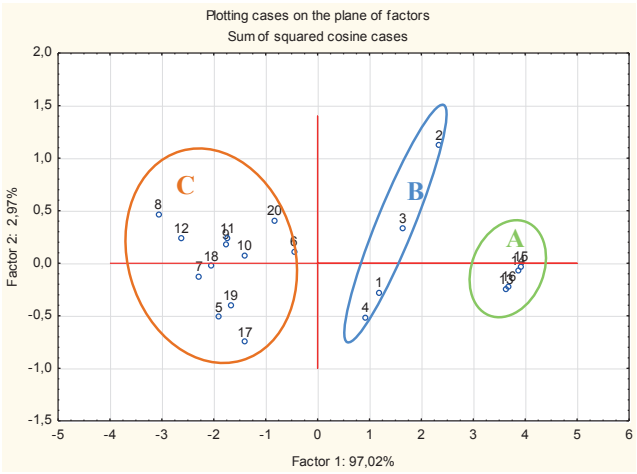

Figure 3. Biplot factor analysis grouping the non-alcoholic beverages according to similar osmolalities and extract content values

Factor analyses allowed the commercially available non-alcoholic drinks to be grouped into three of similar osmolality and total extract content (as shown in Figure 3); ie. light beverages classified as hypotonic drinks (A), isotonic drinks and energy drinks (B) and other carbonated drinks, juices and nectars (C) with high osmolalities characteristic of hypertonic beverages.

DISCUSSION

There is a great variety of commercially available drinks that are recommended for quenching thirst as well as making up any shortfalls in bodily fluids. However, osmolalities are not provided and thus a given drink's ability to replenish water and minerals, which are lost after different types of exercise, is unknown. For this reason our study has compared osmolalities of various commercially available drinks, such as mineral waters, isotonic drinks, energising drinks, 'light' beverages, other sparkling drinks and juices and nectars. Results show that three groups of beverages of similar tonicity can be distinguished as follows: hypotonic drinks, (which include mineral water and light beverages), isotonic drinks and hypertonic drinks (like energising drinks) and 'light' and sparkling beverages. In order that the body's water and electrolyte balance be restored, drinking isotonic fluids is vital as they contain similar amounts of osmotically active substances [19].

A study by *Saat* et al. (2002) investigated body mass fluctuations during intense physical activity causing sweating, and demonstrated that in 80% cases these can be minimised through drinking isotonic beverages containing carbohydrates and selected electrolytes. In commercially available isotonic drinks, apart from water, there are carbohydrates present (mainly glucose, fructose and sucrose) at around 6g/100 ml, sodium, potassium, (sometimes calcium or magnesium) and B group vitamins. In addition to the aforementioned carbohydrates, intensive sweeteners can be added. It is suggested that the extent of intestinal water absorption after drinking isotonic beverages is lower compared to hypotonic beverages, insomuch the latter provides an additional amount of electrolytes for restoring proper electrolyte balance in the body after intensive exercise [11, 15].

There is evidence to show that hypotonic drinks exhibit a higher intestinal tolerance compared to hypertonic drinks [1, 8, 16]. Our study showed that for hypotonic drinks, including mineral waters, their osmolalities depend on the extent of general mineralisation and the ratios between the various mineral components. The extent of mineralisation of the mineral waters is quantitatively expressed as their mineral content. According to these values the mineral waters were divided into three groups: low-mineralised water containing 1 l less than 500 mg of dissolved minerals, medium-mineralised water containing 1 l of 500-1,500 mg of dissolved minerals,

and highly mineralised water containing 1 l of above 1500 mg dissolved mineral components [17]. 'Light' drinks were included in the hypotonic group. Their low tonicity, similar to medium-level mineral waters, resulted from replacing sugars by intense sweeteners; being mainly aspartame and acesulfame K.

The osmolalities of juices, nectars, energy drinks and colas are significantly influenced by the total extract content. *Mettler* et al. [15] reported that the osmolality of beverages rises with total carbohydrate content which highly depends on the proportions of monosaccharides, disaccharides and polysaccharides, together with the levels of organic acids, vitamins and minerals. The literature indicates that sugars' levels in beverages, their calorific value and osmotic activity affect the rates of gastric emptying and intestinal absorption [3, 7, 8, 13, 14]. Juices, nectars, energising drinks and colas are hypertonic fluids, whose consumption reduces the water absorption rates and cause fluid to pass from the plasma and intra-cellular fluid into the intestine, thus hindering proper hydration of the body. Drinking hypertonic drinks during physical exercise increases both the feelings of gastrointestinal discomfort and the risk of diarrhoea.

In contrast when hypertonic drinks are consumed at resting conditions, for example during breaks in exercise, then this does not usually cause any gastrointestinal discomfort. If a drink is to be consumed during exercise, where the risk of gastrointestinal complaints is higher than at rest, then the most appropriate osmolalities are those ranging 200-250 mmol/kg [15]. *Gisolfi* et al. [10] reported that drinking beverages containing 6% carbohydrates is beneficial; in such amounts there is no significant difference in the rates of gastric emptying relative to water. A study by *Burke* [8] aiming to maintain adequate hydration of the body, recommended consuming such drinks both before and during exercise, and in the shortest time immediately after physical activity. This study recommended drinking 4-8% carbohydrate-containing drinks and electrolytes after 60 minutes of physical activity. Nevertheless, drinks of high osmolality are not a cause for concern if achieving rapid hydration is not the main reason for their consumption. To reduce osmolality and increase water absorption, such drinks can be diluted with either water or other hypotonic fluids.

CONCLUSIONS

1. Commercially available mineral waters and non-alcoholic beverages demonstrated large variations in osmolality depending on their mineral content that of the total extract values.
2. Drinks with the highest osmolalities coupled with extract content values were found in energising drinks, other sparkling drinks, juices and nectars; these osmolalities ranging from about 557 to about 636 mOsm/kg of water, thus allowing them to be classified as hypertonic drinks.
3. In comparison, those drinks having the lowest osmolalities and total extract content were mineral waters and light beverages, (ranging from around 24 to around 88 mOsm/kg water), and were thus classified as being hypotonic drinks.
4. The osmolalities of the isotonic drinks tested, were similar to those of body fluids (ie. ranging from 275 to 295 mOsm/kg water).

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

ASSOCIATION BETWEEN BLOOD CHOLINESTERASE ACTIVITY, ORGANOPHOSPHATE PESTICIDE RESIDUES ON HANDS, AND HEALTH EFFECTS AMONG CHILI FARMERS IN UBON RATCHATHANI PROVINCE, NORTHEASTERN THAILAND

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ABSTRACT

Background. Use of pesticides has been documented to lead to several adverse health effects. Farmers are likely to be exposed to pesticides through dermal exposure as a result of mixing, loading, and spraying. Organophosphate pesticides (OPs) are widely used in most of the agricultural areas throughout Thailand. OPs are cholinesterase inhibitors and blood cholinesterase activity is used as a biomarker of OP effects.

Objective. This study aims to determine the association between blood cholinesterase activity and organophosphate pesticide residues on chili farmer's hands and their adverse health effects.

Materials and Methods. Ninety chili farmers directly involved with pesticide applications (e.g. mixing, loading, spraying) were recruited and were interviewed face to face. Both enzymes, erythrocyte acetylcholinesterase (AChE) and plasma cholinesterase (PChE), were tested with the EQM Test-mate Cholinesterase Test System (Model 400). Hand wipe samples were used for collecting residues on both hands and OP residues for chlorpyrifos and profenofos were quantified using gas chromatography equipped with a flame photometric detector (GC-FPD).

Results. The average activity (±SD) of AChE and PChE was 2.73 (±0.88) and 1.58 (±0.56) U/mL, respectively. About 80.0% of the participants had detectable OP residues on hands. The median residues of chlorpyrifos and profenofos were found to be 0.02 and 0.03 mg/kg/two hands, respectively. Half of participants reported having some acute health symptoms within 48 hours after applying pesticides. When adjusted for gender, number of years working in chili farming, and frequency of pesticide use, AChE activity (Adjusted OR = 0.03, 95%CI: 0.01-0.13) and detected OP residues on hands (Adjusted OR = 0.15, 95%CI: 0.02-0.95) were significantly associated with having health effects, but no significant association was found in PChE activity (Adjusted OR = 2.09, 95%CI: 0.63-6.99).

Conclusions. This study suggests that regular monitoring for blood cholinesterase and effective interventions to reduce pesticide exposure to prevent health effects should be provided to chili farmers.

Keywords: *cholinesterase activity, organophosphate pesticide residues, pesticide exposure, health effects*

INTRODUCTION

Thailand is one of the world's largest exporters of agricultural commodities. About 12.09 million Thai people work in the agricultural sector which leads to farming being the top occupation in Thailand [21]. Thailand has been promoting pesticide usage to increase yields and improve the quality of crops. In 2010-2015, around 147,746 tons of pesticides were annually imported to serve the agricultural sector, valued at 600 million USD per year [25]. However, the current use of pesticides among Thai farmers seems to be less effective due to their extensive and inappropriate use which causes environmental contamination and health problems [38]. In 2014, there were 7,954 Thai people afflicted with pesticide poisoning, or 12.25 per 100,000 population; 32.06% were farmers. The major cause of poisoning was organophosphates (OPs) and carbamates (CAs) [3].

Organophosphate pesticides (OPs) are widely used in agriculture throughout Thailand [3]. OPs are cholinesterase inhibitors and high dose exposure to OPs can cause acute effects such as gastrointestinal upset, sweating, tearing, urination problems, bronchial spasms, muscle twitching, muscle weakness, bradycardia, and coma [6, 13, 16, 29]. For chronic exposure at low to moderately high doses, poisoning symptoms include headache, dizziness, nausea, vomiting, abdominal pain, blurred vision, and chest tightness [3, 6, 13, 14, 15,16,

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29, 30, 31, 35, 41]. Moreover, there is evidence linking OP exposure to reproductive effects, non-Hodgkin's lymphoma, and cancer [13].

Thai farmers are exposed to pesticides *via* multiple routes i.e., inhalation, dermal absorption, and unintentional ingestion [36]. For most pesticide handling situations, dermal is most likely to be the given exposure [7]. OP residues on hands can be represented as indicators of exposure [4], and blood cholinesterase enzymes can be used as a biomarker of exposure effects particularly to OPs and CAs [27]. Both enzymes, erythrocyte acetylcholinesterase (AChE) and plasma cholinesterase (PChE) should be measured, as these results will have different value. The AChE activity measure is advantageous to evaluate chronic exposure of OPs and CAs, while the PChE measure is worthwhile in detecting early acute effects of OPs and CAs poisoning [13, 27, 29].

Few studies have investigated the association between cholinesterase (ChE) activity, pesticide exposure, and adverse health effects [5, 14]. Therefore, this study aims to determine the potential association between blood cholinesterase activity, organophosphate pesticide residues on hands, and adverse health effects in chili farmers.

MATERIALS AND METHODS

Study area and subjects

This study was a cross-sectional descriptive study located at Hua Ruea Subdistrict, Mueang District, Ubon Ratchathani Province, Thailand. This area was chosen because of the large number of farmers and agricultural area. It covers a total area of 7,978.9 acre. Over 84% of the total area is under cultivation and the main crops year round include rice, chili, and vegetables. With a population of 9,075 residing in 2,632 households, most of the population is farmers [12].

A total of 90 chili farmers living in Hua Ruea Subdistrict were enrolled. To be eligible for the study, the farmers had to be ≥18 years of age, settled in this area at least 1 year, directly involved with all steps of pesticide application (e.g., mixing, loading, spraying), and no communication problems. Those who had health problems e.g., alcoholism, liver failure, cardiovascular disease, malnutrition, drug addiction, and taking anti-malarial drugs, were excluded.

Data collection

Data collection was done in April 2015 (the high chili growing season). Face-to-face interviews were completed with all participants. Each participant was questioned about demographic characteristics, work characteristics, types and frequency of pesticide use, and acute symptoms related to pesticide exposure.

Cholinesterase measurement

Blood samples (20 µL) were taken from a cleaned fingertip of each participant in capillary tubes during the period of pesticide application by nurses. Blood enzymes erythrocyte acetylcholinesterase (AChE) and plasma cholinesterase (PChE) were tested with the Test-mate ChE Cholinesterase Test System (Model 400) [9, 23, 28, 31, 41], a field spectrophotometric analyzer based on the Ellman method [8]. The results were expressed as units per milliliter (U/mL).

Pesticide residue measurement

Hand wipe sampling method

The hand wipe sampling method was modified from *Geno* et al. [11] and *Taneepanichskul* et al. [36, 37]. Hand wipes were performed using sterilized and chemical free gauze pads (size: 4´4 inches, 8 ply) wetted with a 40% solution of isopropyl alcohol, 10 mL per pad. Two gauze pads were used for wiping pesticide residues on both hands of each participant. Then the wipes were wrapped in laboratory aluminum foil and placed in zip-lock plastic bags. All hand wipe samples were stored in cold boxes with ice packs, shipped to the laboratory, and refrigerated at -20°C until extraction within 7 days and analyzed afterward by gas chromatography.

Extractions of organophosphate pesticides in wipe samples

An extraction method of OP pesticides was adapted from *Farahat* et al. [10] and *Lapparat* et al. [17] to measure pesticide residues on farmer's hands. First, wipe samples were put into a 250-mL flask with 40 mL of ethyl acetate, then agitated *via* a mechanical shaker for 10 min at 150 rpm. Wipe samples were transferred into a second 250-mL flask with 40 mL of ethyl acetate and shaken with a mechanical shaker for 5 min at 150 rpm. The solvent from both flasks were combined and then evaporated by using air pumps until the volume was less than 1.0 mL. The residue was dissolved in 1.0 mL of acetone (pesticide grade). The solution was transferred to a 1.5-ml microcentrifuge tube. After centrifugation for 10 min at 10,000 rpm, only the liquid phase was transferred to a sample vial. Finally, the volume was adjusted with acetone (pesticide grade) to 1.0 mL.

Gas chromatography analysis

Wipe samples were analyzed for chlorpyrifos and profenofos, which were extensively used in this area [24, 36, 37], using an Agilent 7890A gas chromatography (GC) equipped with a flame photometric detector (FPD). The GC run conditions were [17]: HP-5 capillary column (HP-5, 30 m ´ 0.32 mm id, 0.25-µm film thickness) coated with 5% phenyl methyl siloxane. Nitrogen used as carrier gas was set to a flow rate at 2 mL/min, while makeup gas was at 45 mL/min. Air and hydrogen used as detector gas was regulated at 100 and 75 ml/min, respectively. Initially, 1.0 µL of sample was injected into the GC on splitless mode.

The initial temperature of injector and detector was 230 ˚C and 250 ˚C, respectively. The initial condition of the oven was set at 100 ˚C for 2 min, and then it was programmed to increase at 10 ˚C/min to 220 ˚C. The total run time was 24 min. The chromatogram in Figure 1 demonstrates the retention time of chlorpyrifos and profenofos at 9.903 and 11.540 mins, respectively.

Quality control

A calibration curve for quantification was performed using a series of standard solutions at nine concentration levels ranging from 0.001-10.000 µg/mL. The correlation coefficient (r^2) of chlorpyrifos and profenofos was 0.99951 and 0.99931, respectively. For analytical control, the standard solutions were confirmed in every 10 sample measurements presented in the range of linearity. The limit of detection (LOD) was 0.01 mg/kg for chlorpyrifos and 0.02 mg/kg for profenofos. The limit of quantitation (LOQ) for chlorpyrifos and profenofos was 0.02 and 0.05 mg/kg, respectively. The mean recovery of extractions for profenofos was 94.8%, which was in an acceptable range of 80-120% following the Association of Official Agricultural Chemists (AOAC) recommendations [2]. The mean recovery for chlorpyrifos was 64.9%, which was lower than the acceptable range and was a limitation of this study.

Data analysis

Descriptive statistics were used to describe information regarding demographic characteristics, types and frequency of pesticide use, and prevalence of symptoms related to pesticide exposure. *Kolmogorov-Smirnov* tests were used to test distributions for continuous variables. The associations between ChE activity and symptoms related to pesticide exposure were investigated by using point biserial correlation. The relationship between detected OP residues and symptoms were evaluated by using a *Chi*-square test and *Fisher's* exact test. Binary logistic regression analysis was performed to determine potential associations between ChE activity, detected OP residues on hands, and health effects related to pesticide exposure. In our logistic regression analyses, the dependent variable was having health effects (0 = no, 1 = yes), that was defined as "no" when participants reported having none of acute symptom related to pesticide exposure; it was defined as "yes" when participants reported having at least 1 symptom. The independent variables were AChE and PChE activity (continuous) as well as detected OP residues on hands (0 = no, 1 = yes). Odds ratios (OR) and 95% confidence intervals (95%CI) were derived from the logistic regression models. All analyses were conducted with the SPSS statistical software package version 16.0. The significance level was set at 0.05 and 0.01.

For statistical analysis, if the results of OP residues were reported as zero or below the LOD, they were substituted with the LOD [22]. The detected OP residues on hands were defined as "yes" if wipe samples found chlorpyrifos or profenofos or both residues higher than the LOD, otherwise if the residues were lower than the LOD they were considered as "no".

For interpretation of ChE results, the ChE values were classified by using mean values for cut-off points into 2 levels such as abnormal and normal level [23, 28, 31]. If the value was equal to or less than 2.73 U/mL for AChE, and 1.58 U/mL for PChE, it was considered "abnormal level". It was assumed that participants could possibly have pesticide poisoning. If the value of AChE and PChE was more than 2.73 and 1.58 U/mL respectively, it indicated "normal level".

Ethical consideration

This study was approved by the Ethic Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University (Certified code no. 078/2558). All participants signed the written consent before participated in the study.

RESULTS

Demographic characteristics

Demographic characteristics of the participants are summarized in Table 1. Over half of the participants were males (53.3%). The participant's age was in the range of 29 to 83 years. The mean (±SD) age was 49.6 (±10.4) years. The majority of participants (73.6%) had graduated primary school. About 23.3% of participants reported having some chronic diseases, e.g., peptic ulcer, and hypertension. Only 36.7% of participants were drinkers and 20.0% were smokers. All participants worked on chili farms for an average (±SD) of 18.2 (±9.6) years and most of them (74.4%) had owned chili farms of approximately 0.4 to 0.6 acres in size. Also, 62.2% of participants grew other crops during the chili growing season, e.g., spring onions, coriander, and long beans. Over 70.0% of participants had another family members working in chili farming. All participants joined in pesticide application i.e., mixing or loading (83.3%), and spraying (93.3%). Approximately 47.8% of participants applied pesticides twice monthly.

Pesticides used in chili farming

A variety of pesticides were used in chili farming. All of the participants used insecticides, 91.1% used herbicides, and 61.1% used fungicides. The most common insecticides used were avermectins (90.0%), followed by organophosphates, i.e., chlorpyrifos (35.6%), profenofos (33.3%), dimethoate (1.1%), as well as carbamates i.e., methomyl (20.0%), and are detailed in Table 2. Out of 82 participants using herbicides, paraquat (91.5%) was the most often used. Among 55 participants using fungicides, propineb (94.5%) was the most common used. However some participants could not remember the name of pesticides used, so the percent of pesticide use in Table 2 may be underestimated.

Table 1. Demographic characteristics of 90 chili farmers

Characteristics	No. of chili farmers	Percent (%)
Gender		
Male	48	53.3
Female	42	46.7
Age (year)		
Mean ± SD (Min-Max)	49.56 ± 10.36 (29.00-83.00)	
Education level		
Primary education	68	75.6
Secondary education	12	13.3
High school education	7	7.8
Bachelor's degree or higher	3	3.3
Had any chronic disease		
No	69	76.7
Yes	21	23.3
Alcohol consumption		
No	57	63.3
Yes	33	36.7
Smoking habit		
No	72	80.0
Yes	18	20.0
Number of years working in chili farming		
Mean ± SD (Min-Max)	18.16 ± 9.56 (1.00-42.00)	
Chili farm size (acres)		
0.4 - 0.8	67	74.4
0.9 - 1.6	13	14.4
>1.6	10	11.1
Growing other crops during chili growing season		
No	34	37.8
Yes	56	62.2
Frequency of pesticide use per month		
1	22	24.4
2	43	47.8
3	8	8.9
4	17	18.9

Table 2. List of insecticides used in chili farming reported by 90 chili farmers

Chemical class	Common name (Active ingredients)	Trade name	No. of response	%
Botanical, Macrocyclic Lactone	Avermectins	Abamectin, Avermectins	81	90.0
Organophosphate	Chlorpyrifos	Podium, Chlorpyrifos	32	35.6
	Profenofos	Selecron	30	33.3
	Dimethoate	Bazooka	1	1.1
Carbamate	Methomyl	Lannate	18	20.0
Neonicotinoids	Imidacloprid	Provado	31	34.4
Pyrethroid	Cypermethrin	Cypermethrin	8	8.9
	Chlorpyrifos+Cypermethrin	Lampard	3	3.3

Table 3. Cholinesterase activity of the 90 chili farmers

Biomarker	Mean ± SD (U/mL)	Range (U/mL)	Abnormal*		Normal**	
			n	%	n	%
AChE	2.73 ± 0.88	1.20 - 7.17	45	50.0	45	50.0
PChE	1.58 ± 0.56	0.47 - 3.11	46	51.1	44	48.9

* Abnormal level was considered if the value was ≤ 2.73 U/mL for AChE and ≤ 1.58 U/mL for PChE

** Normal level was considered if the value was > 2.73 U/mL for AChE and > 1.58 U/mL for PChE

Table 4. Percentage of positive wipe samples and OP residues (n=90)

Pesticides	Detection frequency [a] (%)	Residues (mg/ kg/ two hands)				
		Range	Median	Mean	SE	SD
OPs	72 (80.0%)	<LOD - 3.41	0.05	0.13	0.04	0.38
Chlorpyrifos	61 (67.8%)	<LOD - 0.96	0.02	0.04	0.01	0.11
Profenofos	58 (64.4%)	<LOD - 3.34	0.03	0.09	0.04	0.36

[a] Detection frequency = Number of wipe samples with detected OP residues higher than the limit of detection (LOD)
LOD = 0.01 mg/kg for chlorpyrifos, and 0.02 mg/kg for profenofos
SE = Standard error of mean, SD = Standard deviation

Cholinesterase activity

The average activity was 2.73 U/mL (±0.88 U/mL) for AChE and 1.58 U/mL (±0.56 U/mL) for PChE. The prevalence of abnormal AChE levels in farmers in this study was 50.0% equal to that of normal levels. For PChE, the prevalence of abnormal levels was 51.1% which was slightly greater than the normal level of 48.9% (Table 3).

Organophosphate pesticide residues on chili farmers' hands

About 80.0% of 90 wipe samples were found to have OP residues, in which 52.2% were found to have both chlorpyrifos and profenofos, 27.8% were found to have either chlorpyrifos or profenofos, and the remaining 20.0% had no residues. As shown in Table 4, 67.8% of the samples were detected with chlorpyrifos, while 64.4% were found to have profenofos. The median residues of chlorpyrifos and profenofos were found to be 0.02 and 0.03 mg/kg/two hands, respectively.

Acute health symptoms related to pesticide exposure

Half of the participants reported having some health symptoms during 48 hours after applying pesticides, in which 27.8% of participants reported 1-3 symptoms and 22.2% of participants reported more than 3 symptoms. The prevalence of acute health symptoms related to pesticide exposure is shown in Table 5. The top three health symptoms were reported to be headache (31.1%), dizziness (27.8%), and fatigue or weakness (22.2%). The main gastrointestinal symptom commonly reported was nausea or vomiting (15.6%). The respiratory symptom most often reported was cough (14.4%). Itching or burning (13.3%) was the most often skin symptom reported.

Table 5. Prevalence of acute symptoms related to pesticide exposure and its associations with ChE activity and detected OP residues on hands

Symptoms	No. of response	AChE*		PChE†		Chlorpyrifos**		Profenofos**	
		r_{pb}	p	r_{pb}	p	c^2	p	c^2	p
Respiratory									
Cough	13 (14.4%)	-0.22	0.04*	0.05	0.68	0.58[a]	0.54	1.03[a]	0.37
Sore throat, dry throat	10 (11.1%)	-0.17	0.12	0.04	0.70	2.54[a]	0.16	0.15[a]	0.75
Difficulty in breathing	6 (6.7%)	-0.19	0.08	0.01	0.95	0.00[a]	1.00	1.00[a]	0.42
Chest pain	5 (5.6%)	-0.13	0.22	0.11	0.29	0.15[a]	1.00	4.56[a]	0.05
Skin									
Itching, burning	12 (13.3%)	-0.28	<0.01**	0.09	0.39	0.33[a]	0.75	3.14[a]	0.11
Rash	8 (8.9%)	-0.23	0.03*	0.18	0.09	0.11[a]	1.00	2.78[a]	0.13
Muscle									
Numbness	4 (4.4%)	-0.04	0.72	0.06	0.58	0.61[a]	0.59	2.84[a]	0.13
Cramp, pain	4 (4.4%)	-0.09	0.41	-0.10	0.36	0.10[a]	1.00	0.38[a]	0.61
Muscle weakness	3 (3.3%)	-0.12	0.25	-0.01	0.90	0.00[a]	1.00	0.01[a]	1.00
Central nervous system									
Headache	28 (31.1%)	-0.46	<0.01**	0.14	0.19	0.93	0.47	0.95	0.33
Dizziness	25 (27.8%)	-0.48	<0.01**	-0.04	0.73	0.23[a]	0.80	2.34	0.15
Fatigue, weakness	20 (22.2%)	-0.36	<0.01**	0.24	0.02*	0.06	1.00	0.22	0.64
Blurred vision	9 (10.0%)	-0.12	0.25	0.14	0.20	0.01[a]	1.00	1.75[a]	0.27
Gastrointestinal system									
Nausea, vomiting	14 (15.6%)	-0.29	<0.01**	-0.01	0.91	0.86[a]	0.54	5.97[a]	0.02*
Abdominal pain	9 (10.0%)	-0.12	0.26	-0.09	0.41	0.68[a]	0.46	7.78[a]	0.01*
Diarrhea	2 (2.2%)	-0.04	0.71	0.16	0.15	0.97[a]	0.56	1.13[a]	0.54
Others									
Excessive sweating	7 (7.8%)	-0.18	0.10	0.15	0.15	0.05[a]	1.00	0.18[a]	0.70
Excessive salivation	3 (3.3%)	-0.14	0.19	0.12	0.27	0.00[a]	1.00	0.01[a]	1.00
Lacrimation	2 (2.2%)	0.00	0.99	-0.02	0.84	0.30[a]	1.00	3.71[a]	0.12
Brittle nails, nail loss	2 (2.2%)	-0.02	0.82	-0.05	0.62	4.30[a]	0.10	3.71[a]	0.12

* Point biserial correlation analysis, r_{pb} = point biserial correlation coefficient
** *Chi*-square test, [a] Fisher's exact test, * Significant at p <0.05, ** Significant at p <0.01

Table 6. Associations between ChE activity, detected OP residues and having health effects related to pesticide exposure by binary logistic regression analysis

Variables	Crude			Adjusted†		
	OR	95%CI	*p-value*	OR	95%CI	*p-value*
AChE	0.13	0.05-0.32	<0.01**	0.03	0.01-0.13	<0.01**
PChE	1.13	0.54-2.39	074	2.09	0.63-6.99	0.23
Detected OP residues‡	0.57	0.20-1.64	0.30	0.15	0.02-0.95	0.04*

* Adjusted for gender, number of years working in chili farming, frequency of pesticide use

** Detected OP residues on hands (0= no, 1 = yes)

OR = odds ratios, 95% CI = 95% confidence interval

* Significant at P <0.05, ** P <0.01

Associations of ChE activity and detected OP residues to health symptoms related to pesticide exposure

The associations of ChE activity and detected OPs on hands to symptoms related to pesticide exposure are shown in Table 5. AChE activity had moderately inverse associations with dizziness (point biserial correlation coefficient, r_{pb} = -0.48, p <0.01) and headache (r_{pb} = -0.46, p <0.01). It also showed weakly inverse associations with fatigue or weakness (r_{pb} = -0.36, p <0.01), cough (r_{pb} = -0.22, p=0.04), skin itching or burning (r_{pb} = -0.28, p <0.01), skin rashes (r_{pb} = -0.23, p=0.03), and nausea or vomiting (r_{pb} = -0.29, p <0.01). PChE activity had a weakly positive association with only fatigue or weakness (r_{pb} = 0.24, p=0.02). Furthermore, detected profenofos residues were significantly related to nausea or vomiting and abdominal pain (Fisher's exact test, p=0.02 and 0.01, respectively), while detected chlorpyrifos residues did not show significant association with any symptoms.

Associations between ChE activity, detected OP residues on hands, and having health effects related to pesticide exposure

The results of binary logistic regression analysis are presented in Table 6. Increased activity of AChE was significantly associated with decreased odds of having health effects related to pesticide exposure (Crude OR = 0.13, 95%CI: 0.05-0.32), and its association was still in the same direction after adjusting for gender, number of years working in chili farming, and frequency of pesticide use (Adjusted OR = 0.03, 95%CI: 0.01-0.13). No statistically significant relationship was observed between PChE activity and having health effects whether adjusted for the confounding factors or not. Moreover, detected OP residues on hands were statistically significantly associated with having health effects (Adjusted OR = 0.15, 95%CI: 0.02-0.95) when adjusted for gender, number of years working in chili farming, and frequency of pesticide use.

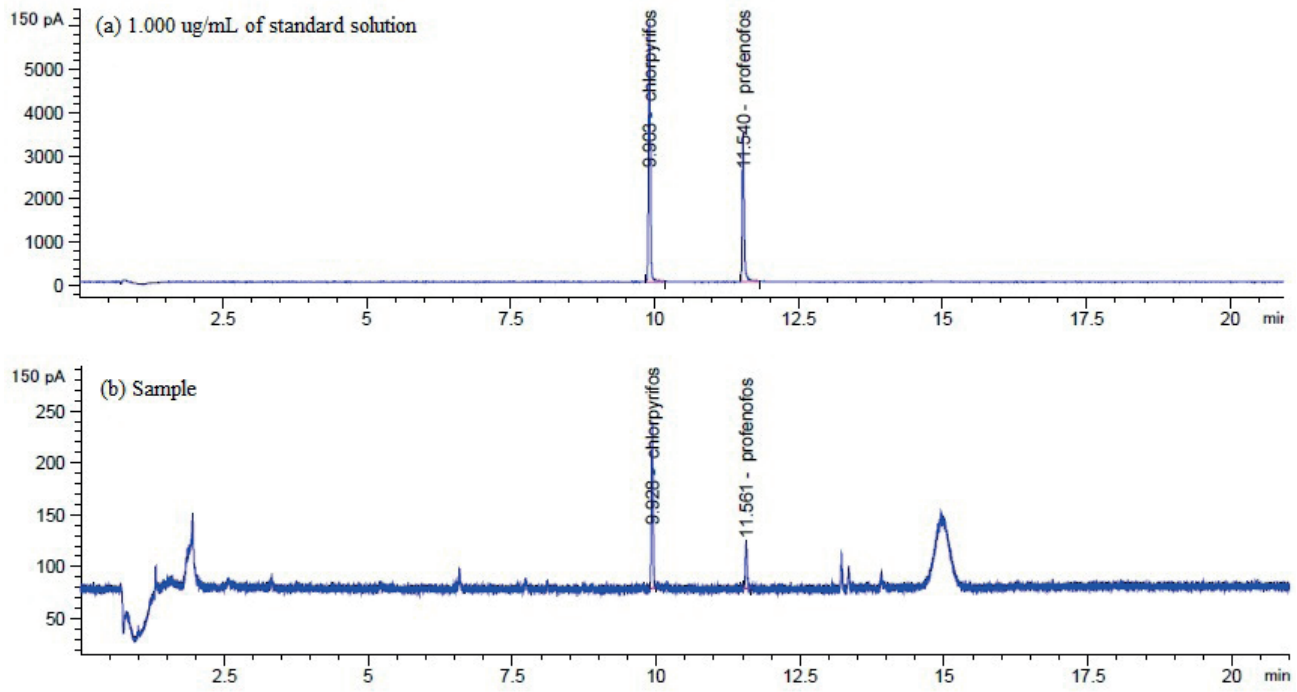

Figure 1. Chromatograms and retention time of chlorpyrifos and profenofos

DISCUSSION

Chili farmers in this study were directly involved in pesticide applications such as mixing or loading (83.3%), and spraying (93.3%). Three quarters of them applied pesticides twice a month or more. Various kinds of pesticides were used in chili farming such as insecticides (100.0%), herbicides (91.1%), and fungicides (61.1%). OPs and CAs, which were ChE inhibiting insecticides, were most commonly used. This study revealed that the average AChE activity of chili farmers (2.73 ±0.88 U/mL) was lower than previous studies, which reported that AChE activity was 3.31 ±0.56 U/mL for elderly people living in the agricultural area in Ubon Ratchathani province [23]; 2.92 ±0.60 U/mL for rice farmers in Chinart province, Central Thailand [28]; and 4.17 ±0.82 U/mL for Kenya agricultural workers [26]. Conversely, the average AChE activity of chili farmers was higher than that of rice farmers in Nakhon Nayok province, Central Thailand (2.63 ±0.55 U/mL) [41] and cacao farmers in Southwestern Nigeria (2.63 ± SE: 0.08 U/mL) [32]. This could be the result of the differences in crop types, agricultural tasks, types of pesticides used, pesticide exposure levels, and personal characteristics such as age, gender, genetic and therapeutic agents [16]. These factors could cause the variation of AChE activity. Furthermore, this study found that around half of chili farmers had abnormal AChE and abnormal PChE. It could be assumed that chili farmers were more likely to get pesticide poisoning and they should be removed from exposure and/or receive the medical treatment. Depression in ChE activity can cause constant firing of electrical signals across synapses in the nervous systems resulting in poisoning symptoms e.g., muscular twitching, trembling, paralyzed breathing, and convulsions [27].

Four-fifths of the chili farmers had detectable OP residues on their hands, in which over half of them had both chlorpyrifos and profenofos. This is evidence that chili farmers were frequently exposed to pesticides through the dermal route. Additionally, 67.8% of wipe samples had detectable chlorpyrifos residues in the range of 0.01-0.96 mg/kg/two hands and 64.4% of wipe samples had profenofos residues in the detectable range of 0.02-3.34 mg/kg/two hands. The percentage of detectable wipe samples and the detectable range of both residues in our study were higher than those reported in the study of *Taneepanichskul* et al. in 2014 [36], although our study had a limitation on extraction recoveries for chlorpyrifos residues. In contrast, their previous study in 2010 reported chlorpyrifos residues on chili farmer's hands greater than those found in our study [37]. The exposure level of OPs varied by the measure of exposure.

In this study, half of the chili farmers experienced some health symptoms after applying pesticides and the most reported symptoms were headache and dizziness. Our findings were consistent with earlier studies [14, 15, 40, 41]. This study exhibited significantly inverse associations of AChE activity with respiratory, skin, central nervous system, and gastrointestinal symptoms, while PChE activity showed a significantly positive association with only fatigue or weakness. Our findings are consistent with the study of *Von Osten* et.al. [40] that demonstrated a significant relationship of AChE inhibition to respiratory and central nervous system symptoms. On the contrary, *Wilaiwan* et al. [41] showed a significant association between AChE level and dizziness, whereas PChE levels were found to have significant associations with respiratory, central nervous system, eye and gland symptoms. Associations between both biomarkers of exposure effects and health symptoms were altered by the measurement of exposure and symptoms.

In addition, this study examined the association between detected OP residues on hands and health symptoms. There was a significant association between detected profenofos residues and gastrointestinal symptoms (e.g. nausea or vomiting, abdominal pain), and no significant association was found for detected chlopyrifos residues. Both chlorpyrifos and profenofos are moderately hazardous pesticides (Class II) by the WHO recommended classification [42]. They can cause ChE inhibition in humans which is linked to overstimulation in the nervous system which causes health effects [1, 18, 19, 20, 39, 42]. Chlorpyrifos, at low levels can cause headache, dizziness, weakness, and runny nose, at moderate exposure, increased lacrimation, salivation and sweating, nausea, vomiting, abdominal cramps, muscle pain, weakness, or cramps, and at high exposure, unconsciousness, convulsion, respiratory depression and paralysis, as well as possible death [1, 19, 20]. Although profenofos is less likely toxic than chlorpyrifos [42], it can cause similar health symptoms [18, 39]. This study demonstrated a significant association between health symptoms and detected profenofos residues on hands which was used as an indicator of OP exposure; however it seems difficult to explain due to a lack of a comparable study. Only one study suggested no significant association was found between reported health symptoms and the proportion of detectable urinary pesticide metabolites used as an indicator of pesticide exposure [34].

Overall, the binary logistic regression results indicated that increased activity of AChE was significantly associated with decreased odds of having health effects. Raised activity of PChE might be related to increased odds of having health effects, but it failed to achieve statistical significance. Association of AChE and PChE with having health effects are not

likely relative. Also the measurement of AChE and PChE had a weak negative association [33]. However, both AChE and PChE should be measured.

Surprisingly, chili farmers with detected OP residues on hands were significantly less likely to have health effects than those without OP residues when adjusted for gender, number of years working in chili farming, and frequency of pesticide use. Possible explanations could be the variety of pesticides were used in chili farming as mentioned previously, so chili farmers were potentially exposed to multiple pesticides through multiple routes other than the OP residues on hands which was focused on in this study. *Von Osten* et al. [40] mentioned that CAs were more likely related to adverse health effects than OPs. Therefore, further study would be required to assess OP and CA exposure through multiple routes such as inhalation and dermal routes and also to determine the potential association between pesticide exposure, ChE activity, and health effects. Several limitations were considered for this study. The prevalence of health symptoms were from subjective evaluation, so it might have recall bias. The health symptoms examined here have multiple causes and may not be caused solely by pesticide exposure.

CONCLUSIONS

Understanding the associations between ChE activity, OP residues on hands, and health effects related to pesticide exposure may be an advantage to prevent health effects related to pesticide exposure in chili farmers. Regular monitoring of AChE and PChE in addition to effective interventions in regards to reducing pesticide exposure to prevent health effects should be provided to chili farmers.

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Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

BIOCHEMICAL PARAMETERS AS MONITORING MARKERS OF THE INFLAMMATORY REACTION BY PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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ABSTRACT

Background. Chronic obstructive pulmonary disease (COPD) is an airway inflammatory disease caused by inhalation of toxic particles, mainly cigarette smoking, and now is accepted as a disease associated with systemic characteristics.

Objective. The aim of this work was to investigate and compare selected biochemical parameters in patients with and without COPD.

Material and Methods. Observation group consisted of clinically stable patients with COPD (n = 60). The control group was healthy persons from the general population, without COPD, who were divided into two subgroups – smokers (n = 30) and non-smokers (n = 30). Laboratory parameters were investigated by automated clinical chemistry analyzer LISA 200th.

Results. Albumin in our measurements showed an average value of 39.55 g.l^{-1} in the patient population; 38.89 g.l^{-1} in smokers and in non-smokers group 44.65 g.l^{-1}. The average value of pre-albumin in the group of patients was 0.28 ± 0.28 g.l^{-1} and 0.30 ± 0.04 g.l^{-1} in smokers group. The average value of the orosomucoid in patients was about 1.11 ± 0.90 mg.ml^{-1}. In the group of smokers, the mean value of orosomucoid was 0.60 ± 0.13 mg.ml^{-1}. The level of C-reactive protein (CRP) in the patient group reached an average value of 15.31 ± 22.04 mg.l^{-1}, in the group of smokers was 5.18 ± 4.58 mg. l^{-1}. Prognostic inflammatory and nutritional index (PINI) in the group of patients showed a mean value of 4.65 ± 10.77 and 0.026 ± 0.025 in smokers.

Conclusions. The results of this work show, that the values of index PINI in COPD patients are significantly higher than in smokers (P <0.001). This along with other monitored parameters indicative inflammation as well as a catabolic process that occurs in the organism of patients with COPD.

Key words: *COPD, albumin, pre-albumin, C-reactive protein, orosomucoid, PINI index.*

STRESZCZENIE

Wprowadzenie. Przewlekła obturacyjna choroba płuc (POChP) jest chorobą zapalną dróg oddechowych spowodowaną inhalacją toksycznych związków chemicznych, pochodzących głównie z palenia papierosów, która charakteryzowana jest jako choroba ogólnoustrojowa.

Cel. Celem niniejszej pracy było oznaczenie we krwi chorych na POChP wybranych parametrów biochemicznych stanu zapalnego oraz porównanie ich stężenia w grupie zdrowych ludzi bez POChP.

Materiał i metody. Grupa badana składała się z klinicznie stabilnych pacjentów z POChP (n = 60). Grupę kontrolną stanowili zdrowi pacjenci z populacji generalnej, bez POChP, którzy zostali podzieleni na dwie podgrupy – palaczy papierosów (n = 30) i niepalących (n = 30). Parametry biochemiczne oznaczano z wykorzystaniem automatycznego analizatora LISA 200.

Wyniki. Średnie stężenie albumin w grupie chorych wynosiło 39.55 g.l^{-1} ; w grupie zdrowych palących 38.89 g.l^{-1} a w grupie zdrowych niepalących 44.65 g.l^{-1}. Średnie stężenie pre-albuminy w poszczególnych grupach pacjentów i zdrowych palących wynosiło odpowiednio 0.28 ± 0.28 g.l^{-1} oraz 0.30 ± 0.04 g.l^{-1}. Stężenie orosomukoidu w grupie chorych wynosiło 1.11 ± 0.90 mg.ml^{-1}. W grupie palących zdrowych osób średnio 0.60 ± 0.13 mg.ml^{-1}. Stężenie białka C-reaktywnego (CRP) w grupie chorych na POChP wynosiło 15.31 ± 22.04 mg.l^{-1}, a w grupie palących 5.18 ± 4.58 mg. l^{-1}. Prognostyczny wskaźnik odżywienia i stanu zapalnego - PINI *(Prognostic Inflammatory and Nutritional Index)* w grupie pacjentów wynosił średnio 4.65 ± 10.77 a w grupie osób palących 0.026 ± 0.025.

Wnioski: Wyniki niniejszych badań wykazały, że wartości PINI u pacjentów z POChP były statystycznie wyższe (p <0,001) niż w grupie osób palących. Uzyskane wyniki wraz z innymi monitorowanymi parametrami świadczą o stanie zapalnym jak również o procesie katabolicznym, który występuje w organizmie pacjentów z POChP.

Słowa kluczowe: *POChP, albumina, pre-albumina, białko C-reaktywne, orosomukoid, wskaźnik PINI*

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an airway inflammatory disease caused by inhalation of toxic particles, mainly cigarette smoking, and now is accepted as a disease associated with systemic characteristics [16]. COPD can no longer be considered a disease only of the lungs [6]. It is associated with a wide variety of consequences, most notably systemic inflammation. Systemic inflammation is a risk factor for most of the complications that occur in these patients who suffer from cachexia, skeletal muscle abnormalities, hypertension, diabetes, coronary artery disease, cerebrovascular accidents. The origin of systemic inflammation in COPD is unresolved, although several potential mechanisms have been proposed [1].

Albumin is characterized as a negative acute-phase protein and its pool is affected by a number of inflammatory conditions and drugs [21]. Albumin is a serum hepatic protein with a half-life of 14-20 days [2, 4] and the main marker of visceral protein depletion. Supply of albumin in the body is 4-5 $g.kg^{-1}$. In the clinical context of albumin examination and its drop below 25-30 $g.l^{-1}$, it is among nutritional markers. In difficult conditions, the albumin acts as a negative marker and leads to a decline in the recent rise of CRP and other acute phase proteins [30]. Large losses of proteins, primarily albumin, occur in nephrotic syndrome, nephropathy, cirrhosis and chronic bronchitis [29].

Similar to albumin, pre-albumin is also a negative acute phase protein produced by the liver. The half-life of pre-albumin is much shorter (2-3 days) and its total body pool is considerably smaller than albumin [2, 23]. This very short half-life at the same time with high in tryptophan makes a pre-albumin very sensitive indicator of protein deficiency. The determination of pre-albumin to monitor the effectiveness of nutritional support is an appropriate parameter, especially in combination with albumin. Determination of pre-albumin is much more expensive than the determination of albumin, but it is an advantage in that it captures at least 44% of patients at risk of malnutrition even in the period when normal levels of albumin [29]. Pre-albumin is suitable especially for the evaluation of the catabolic state of chronically ill patients [14].

Orosomucoid is the classical acute phase protein exhibiting a 3-4 fold increase during inflammation, and the tissue damage. It is synthesized in the microvascular endothelium. Orosomucoid has a control and dampening effect on the inflammatory cascade, thereby protecting the tissues against damage in inflammation. In patients with proteinuria, orosomucoid predominantly excreted in the urine [19].

Serum level of CRP is increased in COPD patients. It is well known that this inflammatory marker causes a systemic inflammatory process and increases the chance of cardiovascular and cerebrovascular accidents, cachexia and osteoporosis. Therefore, it is recommended to measure the serum level of CRP in COPD patients during their routine clinical visits. These patients should be considered for a more aggressive treatment. Attenuation of systemic inflammation may offer new perspectives in the management of COPD and its comorbidities [12].

PINI index is calculated from four markers, two of which are inflammatory markers (C-reactive protein, and orosomucoid) and albumin, and pre-albumin are nutritional markers. Index PINI is a sensitive and universal means of diagnosing inflammatory diseases and malnutrition at a time subclinical stages of the disease. Healthy adults have PINI values below 1.0. In patients with inflammatory and malnutrition is PINI index progressively increasing [18].

The aim of this study was to investigate selected biochemical parameters in patients with COPD and compare their values with people without COPD. We focused on selected nutritional and inflammatory markers in COPD is typical for a systemic inflammatory response.

MATERIAL AND METHODS

The study was conducted on patients with chronic obstructive pulmonary disease (n = 60) from specialized St. Svorad Hospital Nitra Zobor, Slovakia, who were treated by means of hospitalization or outpatient basis. Observation group consisted of clinically stable patients acute deterioration of the patients was excluded from the reference file. The control group consisted of probands from the general population without COPD, acquired by random selection, who were divided into two subgroups: smokers (n = 30) and non-smokers (n = 30) represented individuals of both sexes. The research was approved by the ethics committee. We received the signed informed consent to be included in the study and carrying out appropriate investigations from all subjects.

The examination of the functional state of the lungs of COPD patients was performed using spirometry and Bodyplethysmographic to confirm the diagnosis and determine the stage of the disease**.** Patients were classified into different groups according to the severity of the disease (Gold I to IV). Lung function was evaluated using spirometer ©2005 ZAN® Meßgeräte, GmbH Germany.

Blood from probands was collected during hospitalization or outpatient examination. Laboratory parameters (albumin, pre-albumin, orosomucoid, C-reactive protein) were investigated by automated

clinical chemistry analyzer LISA 200th. Subsequently, the device calculated from these nutritional and inflammatory markers PINI-index. The device operates at wavelengths from 350-600 nm, in fully automatic mode with 3-stage quality control, automatic control of cuvettes cleanliness, with automatic sample dilution. The device includes software for quality control of the results. The analyzer is working after programming fully automatically.

The measured values were statistically processed and evaluated in a statistical program STATISTICA Cz. version 7.1. The most preferred test for statistical evaluation of our experiment which has a comparative nature is the *Kruskall-Wallis* test.

RESULTS AND DISCUSSION

Measurements of ventilatory capacity are fundamental to the assessment of respiratory health. Spirometric measurement is critical to the diagnosis and management of asthma, COPD, and restrictive lung disease. Respiratory disease is common, and the early effects of cigarette smoking, environmental pollution and occupational exposure demand clinical vigilance and objective measurement [20].

We performed spirometry (measuring of breath) in the group of patients to confirm the diagnosis (COPD), and to determine the disease stage. Table 1 shows average values of the main parameters of spirometry.

Table 1. The main spirometric parameters monitored in patients with COPD (n = 60)

	FEV_1 (l)	**FVC (l)**	**FEV_1/FVC (%)**
$\bar{x}$	1.15	2.14	52.57
± SD	0.63	0.61	16.95
Median	0.94	2.09	51.00
Maximum	3.20	4.11	94.00
Minimum	0.46	1.23	26.00
	% RH FEV_1	**% RH FVC**	**% RH FEV_1/FVC**
$\bar{x}$	40.03	57.93	70.50
± SD	20.63	15.02	22.84
Median	34.50	56.00	67.50
Maximum	99.00	106.00	125.00
Minimum	16	37.00	34.00

FEV_1 – forced expiratory volume in 1 second; FVC – forced volume vital capacity; FEV_1/FVC – ratio FEV_1 and FVC (Tiffeneau-Pinelli index); % RH – percentage of the reference values

If FEV_1 < 80%, it is considered a sign of significantly reduced lung function, in the studied group of patients, the mean FEV_1 was 1.15 ± 0.63 l (equivalent to 40.03 ± 20.63%), which signified that patients had significantly reduced lung function.

Measurement of FEV_1 is an important and accurate parameter because it is reproducible, as well as the objective indicator of lung function [23, 24]. In combination with measuring the ratio of FEV_1/FVC it differentiates the restriction and obstruction pulmonary diseases and provides accurate diagnosis of chronic obstructive pulmonary disease [25].

If the ratio of FEV_1/FVC is less than 70%, the diagnosis of COPD is confirmed [17].

FEV_1/FVC ratio (*Tiffeneau-Pinelli* index) reached in the studied group of patients with COPD the average value of 52.57 ± 16.95%, which clearly confirmed the diagnosis of COPD patients examined. On the basis of spirometry for confirmatory diagnosis, we also determined the stage of COPD by spirometry GOLD classification (I – IV stage). We found that patients with COPD were in the following percentage representation of disease: I. stage – 26.67%; II. stage – 71.67%; III. stage 0%, and IV. stage 1.66%. The similar observations came also *Singh* et al. [24].

Albumin, pre-albumin and CRP orosomucoid rank among the acute phase proteins. Inflammatory reactions in the body are accompanied by increased production of the proteins. For this reason, we consider monitoring the above parameters as important because with COPD patients the inflammation causes remodeling of the pulmonary parenchyma.

Nonspecific elevation of acute phase proteins in serum and other body fluids is caused by acute tissue injury. Initiating moments are the inflammation, rapidly growing tumors, as well as catabolic processes. Increasing levels of acute phase proteins explain the disintegration of body proteins. The subsequent increase in the supply of amino acids in the liver leads to the increased synthesis of acute phase proteins [5].

Albumin in our measurements showed an average value of 39.55 $g.l^{-1}$ in the patients group, 38.89 $g.l^{-1}$ in smokers group and in nonsmokers group 44.65 $g.l^{-1}$. Graphical representation of albumin levels ($g.l^{-1}$) in observed groups (P – patients; S – smokers; N – nonsmokers) is presented in Figure 1.

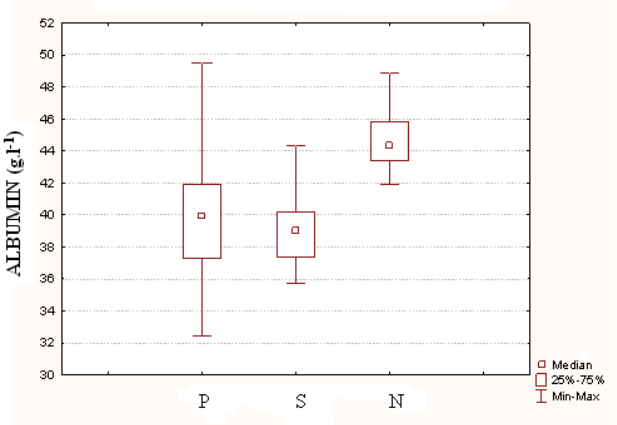

Figure 1. Albumin levels

We found statistically insignificant differences (P≥0.05) between groups of patients and smokers; significant differences (P<0.001) were found between the group of non-smokers and smokers and non-smokers and the group of patients. From the above it is evident that lower levels of albumin were found in groups of smokers and patients, which may reflect the present catabolic processes.

Similar findings also mentioned *Dzúrik* et al. [5] who argued that the concentration of albumin is decreased due to the catabolic degradation, while the permeability of capillaries is increased. Reduced values of albumin and transferrin represent the acute phase dysproteinemia.

The risk of malnutrition or diagnosed malnutrition found in most patients assessed may increase the likelihood of complications during treatment [28].

The pre-albumin level was determined in a group of patients and smokers. In the specified levels of pre-albumin were confirmed statistically highly significant differences (P<0.001) between the group of patients and smokers. The average value of pre-albumin in the group of patients was 0.28 ± 0.28 $g.l^{-1}$ and 0.30 ± 0.04 with smokers $g.l^{-1}$. Pre-albumin values in both groups were within normal ranges from 0.21 to 0.41 $g.l^{-1}$. Figure 2 presents graphs with similar levels of pre-albumin in the groups studied (P – patients; S – smokers).

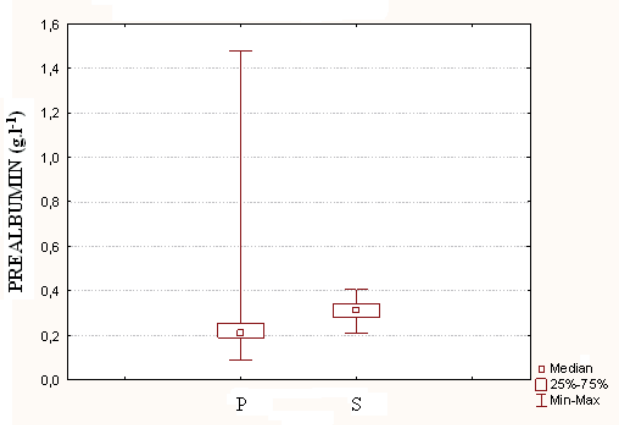

Figure 2. Pre-albumin levels

The level of orosomucoid (ORM) or α-1-acid glycoprotein is increased in inflammatory, cancer and infectious diseases [8]. This plasma protein is widely used as a downstream marker of inflammation and its synthesis is regulated by different proinflammatory cytokines [9, 15]. It is also widely recognized that COPD is an inflammatory disease [7, 11, 26, 27].

The average value of the orosomucoid in patients was about 1.11 ± 0.90 $mg.ml^{-1}$ (median 0.69 $mg.ml^{-1}$; maximum 3.37 $mg.ml^{-1}$, a minimum of 0.45 $mg.ml^{-1}$). In the group of smokers, the mean value of orosomucoid was 0.60 ± 0.13 $mg.ml^{-1}$ (median 0.58 $mg.ml^{-1}$; maximum 1.01 $mg.ml^{-1}$, minimum 0.40 $mg.ml^{-1}$) as it is shown in the Figure 3.

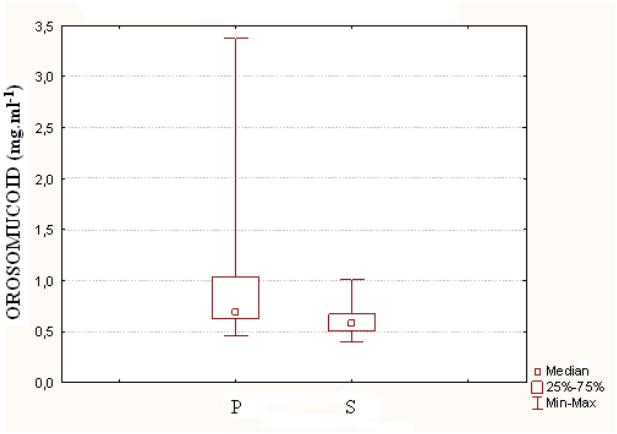

Figure 3. Levels of orosomucoid

By determination of orosomucoid in the blood of smokers and patients, we found higher levels of orosomucoid patients. The results were statistical significant (P <0.001).

The level of C-reactive protein (CRP) in the patient group reached an average value of 15.31 ± 22.04 $mg.l^{-1}$ (median 6.00 $mg.l^{-1}$; maximum 97.30 $mg.l^{-1}$; minimum 0.30 $mg.l^{-1}$), whereas in the group of smokers, the CRP mean value was 5.18 ± 4.58 $mg.l^{-1}$ (median 3.68 $mg.l^{-1}$; maximum 21.22 $mg.l^{-1}$; minimum 0.95 $mg.l^{-1}$). Normal levels of CRP range from 0 to 6.00 $mg.l^{-1}$. Between the groups of patients and smokers the significant difference (P <0.05) was found, as documented in Figure 4. Increases in CRP indicate the presence of inflammation. The amount of CRP level reflects the extent of inflammation, the severity and course of the disease.

We agree with the statement of *Burkhardtová* [3], who claims that in the improvement of health condition, the level of CRP is rapidly decreasing and confirms the success of therapy. It responds much faster than other markers indicating inflammation (e.g. sedimentation, leukocytes), therefore it is a relatively reliable indicator of inflammation.

Recent studies showed that a high level of C-reactive protein in individuals with COPD is associated with low health status in COPD patients [13].

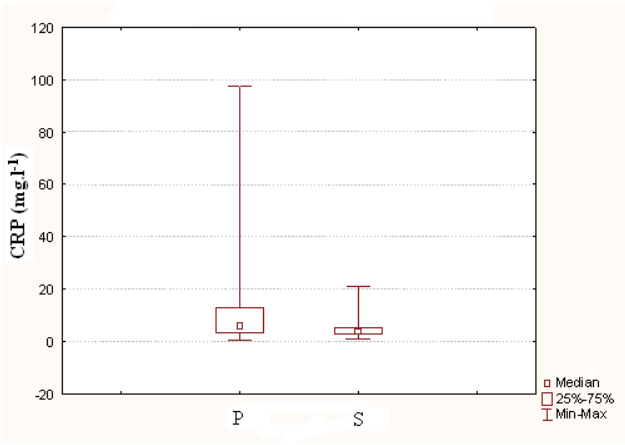

Figure 4. Levels of C-reactive protein (CRP)

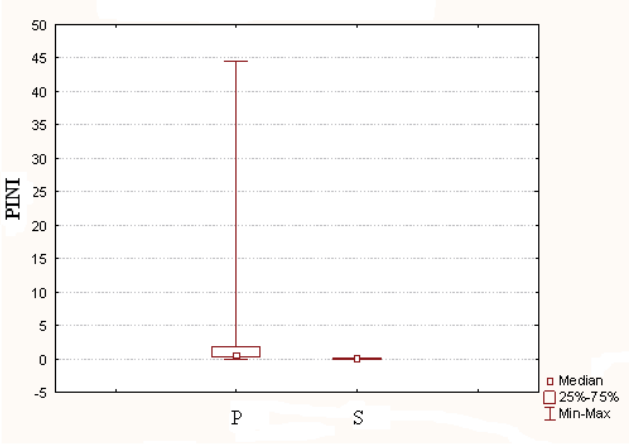

Figure 5. Levels of prognostic inflammatory and nutritional index (PINI)

Prognostic inflammatory and nutritional index - PINI is a sensitive and useful tool for the diagnosis of inflammatory diseases and malnutrition which is already in the subclinical stage of the disease, as proposed by the literary sources [10, 18].

PINI values were compared with values PINI in smokers where we found high statistical evidence values ($P < 0.001$).

PINI in the group of patients showed a mean value of 4.65 ± 10.77 (median 0.44; maximum 44.56; minimum 0.02). For smokers the mean value was of 0.026 ± 0.025 (median 0.016; maximum 0.103; minimum 0.003). From the comparison of PINI values between the two groups we found, that the PINI values with patients were significantly higher. It is an indication of the inflammation and the catabolic process. This process occurs in human patients as part of their disease - COPD. Moreover, we consider PINI as a suitable marker for monitoring the elderly at risk of severe complications that occur in the clinical stage of the disease and as a marker of mortality in seriously sick patients.

CONCLUSIONS

1. The results of this study show that the index values of prognostic inflammatory and nutritional index - PINI in COPD patients are significantly higher than in smokers, which was proved by statistically significant ($P<0.001$) differences between PINI values in observed groups. This observation was accompanied by other inflammation parameters and catabolic processes that occur in the organism of patients with COPD.
2. Each smoker is exposed to oxidative stress, with its negative effects on the cardiovascular and respiratory system. The progressive decline in lung function can only be averted by immediate cessation of smoking and the exclusion of other risk factors. The complex treatment comprising a pharmacological, rehabilitation treatment and nutritional intervention should be initiated.
3. Patients with COPD may be recommended a diet that contains enough energy, rich in amino acids, polyunsaturated fatty acids and antioxidant vitamins (C, E, *beta*-carotene), selenium and glutathione.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

MICROBIOLOGICAL PURITY ASSESSMENT OF COSMETICS USED BY ONE AND SEVERAL PERSONS AND COSMETICS AFTER THEIR EXPIRY DATE

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ABSTRACT

Background. Microbiological purity of cosmetics provides safety of users during their use, prevents physicochemical changes of a preparation, infections and diseases of the skin.

Objective. The aim of this study was to assess the level of microbiological contamination of cosmetics used by one person and by several people and cosmetics after their expiry date in relations to standards for marketed cosmetics, ensuring safety of their use.

Material and Methods. This study was conducted using 55 samples representing 19 types of cosmetics, divided into three groups: used by one person, used by several people and after the expiry date. In cosmetic samples the general numbers of aerobic mesophilic bacteria were determined with the spread plate method on tryptic-soy agar. The presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* were also checked.

Results. The number of aerobic mesophylic bacteria in the tested cosmetics ranged from the level below the method detectability to 1.3×10^7 cfu/g or ml. The presence of *Staphylococcus* spp. was found in 11 (20.0%) tested cosmetic samples and of *P. aeruginosa* in one tested preparation. Yeasts *C. albicans* were not detected, whereas contamination with fungi *Aspergillus* spp. and *Penicillium* spp. ranging from 0.5×10^1 to 1.5×10^1 cfu/g or ml was recorded in four cosmetics. The level of microbiological contamination of cosmetics used by several people was higher than that of cosmetics used by one person. Cosmetics after the expiry date showed the highest microbiological contamination.

Conclusions. The number of users of cosmetic and it expiry date exceeding influenced the level of microbial contamination of preparations.

Key words: *microbiological purity of cosmetics; cosmetics usage; overdue cosmetics; bacteria in cosmetics*

STRESZCZENIE

Wprowadzenie. Czystość mikrobiologiczna kosmetyków zapewnia bezpieczeństwo podczas ich stosowania, zapobiega zmianom fizykochemicznym preparatu oraz infekcjom i chorobom skóry.

Cel badań. Celem pracy była ocena zanieczyszczenia mikrobiologicznego kosmetyków używanych przez jedną i wiele osób oraz kosmetyków przeterminowanych w odniesieniu do norm dla kosmetyków wprowadzonych do obrotu, gwarantujących bezpieczeństwo ich stosowania.

Materiał i metody. W badaniu wykorzystano 55 próbek reprezentujących 19 typów kosmetyków, które podzielono na trzy grupy: używane przez jedną osobę, przez kilka osób oraz przeterminowane W próbkach badanych kosmetyków określano ogólną liczbę tlenowych bakterii mezofilnych metodą posiewu powierzchniowego na podłożu tryptozowo-sojowym. Sprawdzono również obecność *Staphylococcus aureus*, *Pseudomonas aeruginosa* i *Candida albicans*.

Wyniki. W badanych kosmetykach liczba tlenowych bakterii mezofilnych mieściła się w przedziale od poziomu poniżej wykrywalności metody do $1{,}3\times10^7$ j.t.k./g lub ml. *Staphylococcus* spp. wykryto w 11 (20.0%) badanych próbkach, a *P. aeruginosa* w jednej. W żadnym z badanych kosmetyków nie wykryto drożdżaków *C. albicans*, natomiast w czterech stwierdzono zanieczyszczenie pleśniami *Aspergillus* spp. i *Penicillium* spp. wahające się od 0.5×10^1 do 1.5×10^1 j.t.k./g lub ml. Poziom zanieczyszczenia mikrobiologicznego kosmetyków używanych przez kilka osób był wyższy niż używanych przez jedną osobę. Kosmetyki przeterminowane były najbardziej skażone mikrobiologicznie.

Wnioski. Liczba osób używających ten sam kosmetyk oraz przekroczenie jego terminu ważności wpływają istotnie na poziom skażenia mikrobiologicznego preparatu.

Słowa kluczowe: *czystość mikrobiologiczna kosmetyków, użycie kosmetyków, kosmetyki przeterminowane, bakterie w kosmetykach*

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INTRODUCTION

From obtaining raw materials, through the technological process, to the use of the ready product by a consumer, there is a risk of microbiological contamination of a cosmetic products [17]. Avoiding primary contamination, along with the cleanness of production surfaces of devices, production hygiene, microbiological air pollution, personal hygiene of the staff and the quality of the used raw materials, ensure the quality and safety of produced cosmetics [17, 25]. During the use a cosmetic is exposed to secondary contamination, connected with the way of its use by the consumer, storage conditions, the type and size of a packaging, as well as the time of use and the number of users of the given product [17].

According to the guidelines contained in the Ordinance of the Minister of Health of 23 December 2002 [22], meeting both qualitative and quantitative requirements (Table 1) allows for authorization of the cosmetic for use. Quantitative requirements divide cosmetics into two categories. Category I refers to cosmetics for children, cosmetics intended for use in the area of eyes and on mucous membranes, and the other cosmetic products compose category II. Additional tests ale also performed for the presence of *Escherichia coli*, *Salmonella* spp., *Clostridium perfringens* or *Burkholderia cepacia*, which may pose a potential threat to the consumer [10].

Water and materials of animal, plant and mineral origin used in production of cosmetics may cause their contamination with microorganisms from the genus *Bacillus, Clostridium, Pseudomonas, Micrococcus, Flavobacterium* and yeasts [3, 21, 23]. For this reason, cosmetics additionally must contain natural and synthetic preservatives, ensuring their microbiological purity.

The microorganisms often isolated from contaminated cosmetics in tests for microbiological purity of cosmetics are relatively anaerobic species from the genus *Staphylococcus*: *S. aureus, S. epidermidis* and *S. warneri* [6]. The most common cause of skin infections caused by the use of a cosmetic contaminated with *S. aureus* are strains MRSA (methicillin-resistant *Staphylococcus aureus*) [4]. The species *S. aureus*, being an element of natural human microflora, is responsible for purulent skin infections, such as: folliculitis, sycosis, boil, hidradenitis suppurativa and bacterial conjunctivitis. *S. aureus* may cause bullous impetigo in newborn babies (SSSS – staphylococcal scalded skin syndrome) caused by epidermolysine generated by this species. [8].

Both in lotions, soaps, shampoos and cosmetics for eye makeup the genus *Pseudomonas* is mainly represented by *P. aeruginosa* and *P. putida* [20]. *P. aeruginosa* may lead to eye infection, particularly to the ulceration and deep infection of the cornea, which results to its damage [19].

Cosmetics for body rinsing and conditioning and colour cosmetics may contain microorganisms, such as: *Serratia marcescens, Citrobacter freundii*, *P. putida*, *Enterobacter* spp. or *Klebsiella* spp. [20]. *K. pneumoniae*, *E. cloacae*, *C. freundi* and *S. marcescens* may cause pneumonia, urinary tract infections and sepsis [17, 20]. *B. anthracis* is the causing agent of anthrax, and *B. cereus* is responsible for opportunistic food poisoning and eye infections, may be found in cosmetics. *Clostridium tetani* which causes tetanus have also been isolated [8].

Eye shadows and mascaras may be contaminated *Staphylococcus* spp., *P. aeruginosa* and *K. pneumoniae*, also with the microorganisms *Micrococcus* spp., *Corynebacterium* spp., *Acinetobacter* spp., *Moraxella* spp., *Neisseria* spp. [20]. *Micrococcus* spp. are the etiological agent of infections. *Moraxella catarrhalis* and *Moraxella lacunata* are responsible for infections of the respiratory tract as well as ears and eyes [11].

The fungi most frequently isolated from hand creams and lotions are: *C. albicans*, *Aspergillus niger* and *Aspergillus fumigatus* as well as *Penicilium* spp, and mostly yeasts are isolated from mascaras and eye shadows. Not only do fungi lower the quality of cosmetic products, but they can induce infections of the skin and mucous membranes, as well as hair and nails [17].

The aim of this study was to assess the microbiological contamination of cosmetics used by one person and by several people and cosmetics after their expiry date in relation to the standards for marketed cosmetics that ensure the safety of their use. Such studies are very important. The obtained results helped assess the potential health threat for people who use cosmetics both within their expiry date and thereafter. This research also gave the answer to the question whether the use of cosmetics by several people increases the risk of it contamination and increases the threat for its users.

MATERIALS AND METHODS

In quantitative and qualitative tests of microbiological purity of cosmetics, 55 samples of cosmetics were used representing 19 types of cosmetics (Table 2), which were divided into three groups: (1) cosmetics used by one person before the expiry date, (2) cosmetics used by several people before the expiry date and (3) cosmetics used after the expiry date. The same categories of cosmetics were tested in each group of cosmetic preparations. Expiry periods and the number of people used tested cosmetics are presented in Table 2.

Cosmetics used in the study were conditioning products intended to personal hygiene and beautifying cosmetics, including preparations in aqueous and non-aqueous form. Colour cosmetics were both dry cosmetics and sticks and suspensions. Conditioning preparations in which microbiological purity was determined were characterized by oil-water character, as in the case of emulsions, and a high content of water in suspensions, fluids and milks. These were cosmetics for hair washing

and conditioning, peeling, body lotions, face creams, intimate hygiene washes and cleansing milks. The study also involved cosmetics intended for children, which included shower gels and skin conditioning creams. Cosmetics of I category accounted for 49% of the tested cosmetic products, and the other cosmetics 51%.

and the presence of yeasts from the genus *Candida* was evaluated, and then incubation was prolonged to 5 days under the same thermal conditions, to determine the occurrence and numbers of moulds. Identification of the grown fungi in respect of the genus was carried out by the macro-and microscopic assessment of grown colonies based on the mycological atlas [12].

Table 1. Microbiological requirements for cosmetic products [9]

Microbiological indices	Number of microorganisms	
	Cosmetics of category I	Cosmetics of category II
General number of aerobic mesophylic microorganisms (bacteria and moulds)	>100 cfu/g or ml	>1000 cfu/g or ml
Staphylococcus aureus	Absent in 0.1 g/ml	Absent in 0.1 g/ml
Pseudomonas aeruginosa	Absent in 0.1 g/ml	Absent in 0.1 g/ml
Candida albicans	Absent in 0.1 g/ml	Absent in 0.1 g/ml

The spread plate method was used to assess the microbiological quality of cosmetics. Three series of dilutions were performed for each tested cosmetic. Samples with a weight of 1 g or 1 ml were mixed with the neutralizer (buffered solution of sodium chloride with peptone with pH 7.0) in a ratio of 1:10 and a series of decimal dilutions in 0.9% NaCl (Avantor) were made to a level of 10^{-4}. In hydrophobic cosmetics an addition of 0.1% (m/v) polysorbate 80 was applied.

Tryptic Soy Agar (TSA) (Beton Dickinson) was used to determine the total number of aerobic bacteria. Each of the dilutions was inoculated on two Petri plates with TSA, transferring 0.1 ml of prepared dilution and spreading it throughout the agar surface. Cultures were incubated for one day at 37˚C, and then the number of grown colonies were counted on each medium and the number of bacteria was determined, expressed in cfu/g or cfu/ml.

Microbiological quality tests of cosmetics were carried out by introduction of a sample (0.1 g or 0.1 ml) into 200 µl of a neutralizer, thorough mixing and inoculation of the whole volume on the culture media suitable for the tested microorganisms. In the case of hydrophobic cosmetics, an addition of 0.1% (m/v) polysorbate 80 was applied. For positive samples, grown colonies were counted and their number was calculated for 1 g or 1 ml of the tested cosmetic.

To determine the presence of *S. aureus* we used the *Baird–Parker* medium (BTL Sp. z o.o.), and grown Staphylococci (incubation for 24 h at 37˚C) were identified based on catalase formation, coagulase-bound, the so-called clumping factor (CF), and free.

The presence of bacteria *P. aeruginosa* was detected on the cetrimide medium (PYA, Becton Dickinson). Cultures were incubated for 24 hours at 37˚C. The oxidase test was used to confirm the occurrence of *P. aeruginosa*.

Fungi in the tested cosmetics were detected on the Sabouraud medium with dextrose (Becton Dickinson). Cultures were incubated for 24 hours at room temperature

RESULTS

Of the tested cosmetics before the expiry date used by one person, the requirements concerning microbiological purity were met by: peeling, where microbiological contamination was 2×10^2 cfu/ml, and cream for children, where no aerobic mesophylic bacteria were detected (Table 3m). Contamination exceeding the maximal values was also shown in 89.0% of cosmetics used by one person. The highest level of microbiological contamination among cosmetics used by one person, exceeding the standards of microbiological purity, was recorded in hand cream, where the number of aerobic mesophylic microorganisms amounted to 2.8×10^6 cfu/g, and the lowest in eye cleansing milk – 1.5×10^3 cfu/ml (Table 3). Based on the obtained results concerning cosmetics used by several people (Table 3), it was observed that 84.0% of the tested cosmetics underwent contamination exceeding the adopted standards. Of cosmetics of I category, the lowest microbiological contamination with aerobic mesophilic bacteria was detected in cream for children, 1.5×10^3 cfu/g, and the highest in eye cleansing milk – 9.0×10^4 cfu/ml (Table 3). Based on the obtained results, it was stated that the contamination of cosmetics of II category ranged between 5×10^3 and 2.6×10^5 cfu/g or ml. The lowest level of microbiological contamination of cosmetics of II category used by several people was indicated in hair mask, and the highest in fluid (Table 3). No contamination with aerobic mesophylic microorganisms was recorded in intimate hygiene wash or peeling. Contamination of face cream did not exceed 5×10^1 cfu/g (Table 3).

In 55.0% of cosmetics used by several people, a higher microbiological contamination with aerobic mesophilic bacteria was found than in the cosmetics used by one person (Table 3).

The results of microbiological purity assessment of cosmetics after the expiry date showed the highest

Table 2. Expiry periods and the number of people using tested cosmetics

Cosmetic	Expiry period (after open) [months]	Time after expiry date [months]*	Number of people using cosmetic**
Body lotion	12	1	4
Eye shadow	24	6	2
Fluid	12	4	3
Cream for children	6	1	2
Hand cream	36	3	5
Foot cream	24	5	3
Face cream	6	2	4
Face mask	6	1	2
Hair mask	6	3	3
Eye cleansing milk	12	7	3
Toothpaste	12	1	4
Peeling	12	10	3
Intimate hygiene wash	6	1	2
Mouthwash	12	1	4
Blush	9	8	3
Shampoo	12	2	3
Lipstick	24	11	2
Mascara	6	5	3
Shower gel for children	12	2	2

* - concern tested cosmetics after expiry date
** - concern tested cosmetics used by several people

Table 3. General numbers of aerobic mesophylic bacteria in tested cosmetics

Cosmetic	Cosmetics used by one person, before the expiry date		Cosmetics used by several people, before the expiry date		Cosmetics after the expiry date	
	Bacteria number [cfu×ml^{-1}] or [cfu×g^{-1}]	Standard deviation	Bacteria number [cfu×ml^{-1}] or [cfu×g^{-1}]	Standard deviation	Bacteria number [cfu×ml^{-1}] or [cfu×g^{-1}]	Standard deviation
Body lotion	$1{,}5\times10^{4}$	$7{,}1\times10^{3}$	1×10^{4}	$5{,}7\times10^{3}$	$2{,}4\times10^{6}$	$3{,}8\times10^{5}$
Eye shadow	5×10^{3}	$7{,}1\times10^{3}$	1×10^{4}	-	$1{,}8\times10^{6}$	$2{,}6\times10^{6}$
Fluid	1×10^{4}	-	$2{,}6\times10^{5}$	$3{,}7\times10^{5}$	$1{,}1\times10^{6}$	$7{,}3\times10^{5}$
Cream for children	n.d.*	-	$1{,}5\times10^{3}$	$7{,}1\times10^{2}$	5×10^{4}	$7{,}1\times10^{4}$
Hand cream	$2{,}8\times10^{6}$	$8{,}3\times10^{5}$	$1{,}5\times10^{4}$	$2{,}1\times10^{4}$	$1{,}3\times10^{7}$	$3{,}9\times10^{6}$
Foot cream	1×10^{4}	$1{,}4\times10^{4}$	5×10^{4}	$7{,}1\times10^{4}$	$2{,}8\times10^{5}$	$1{,}5\times10^{5}$
Face cream	2×10^{4}	$1{,}4\times10^{4}$	5×10^{1}	7×10^{1}	$1{,}6\times10^{5}$	$2{,}3\times10^{5}$
Face mask	-**	-	1×10^{5}	$1{,}4\times10^{5}$	-	-
Hair mask	$1{,}3\times10^{4}$	$1{,}8\times10^{4}$	5×10^{3}	$7{,}1\times10^{3}$	$1{,}3\times10^{5}$	$1{,}8\times10^{5}$
Eye cleansing milk	$1{,}5\times10^{3}$	$2{,}1\times10^{3}$	9×10^{4}	$1{,}3\times10^{5}$	$2{,}1\times10^{5}$	$2{,}6\times10^{5}$
Toothpaste	1×10^{4}	$1{,}4\times10^{4}$	$1{,}5\times10^{4}$	$7{,}1\times10^{3}$	6×10^{4}	-
Peeling	2×10^{2}	$2{,}8\times10^{2}$	n.d.	-	$1{,}5\times10^{2}$	$2{,}1\times10^{2}$
Intimate hygiene wash	5×10^{3}	$7{,}1\times10^{3}$	n.d.	-	1×10^{6}	$4{,}8\times10^{5}$
Mouthwash	$4{,}5\times10^{4}$	$2{,}1\times10^{4}$	$1{,}8\times10^{4}$	$2{,}1\times10^{4}$	$1{,}7\times10^{4}$	$2{,}4\times10^{4}$
Blush	2×10^{3}	$2{,}8\times10^{3}$	$1{,}5\times10^{4}$	$2{,}1\times10^{4}$	$3{,}2\times10^{6}$	$2{,}9\times10^{6}$
Shampoo	1×10^{4}	$1{,}4\times10^{4}$	2×10^{4}	$2{,}8\times10^{4}$	$9{,}7\times10^{5}$	$6{,}8\times10^{5}$
Lipstick	1×10^{4}	-	$3{,}5\times10^{3}$	$2{,}1\times10^{3}$	$2{,}2\times10^{4}$	$2{,}4\times10^{4}$
Mascara	1×10^{4}	-	$1{,}5\times10^{4}$	$2{,}2\times10^{4}$	$5{,}5\times10^{3}$	5×10^{4}
Shower gel for children	1×10^{4}	$1{,}4\times10^{4}$	$2{,}1\times10^{4}$	$8{,}9\times10^{4}$	2×10^{3}	$2{,}8\times10^{3}$

* n.d.- not detected
** -- not tested/determined

level of contamination with aerobic mesophylic bacteria of all the tested samples. Of the cosmetics after the expiry date of I category, the highest level of microbiological contamination was found in eye shadow – 1.8×10^6 cfu/g, and the lowest in shower gel for children – 2.0×10^3 cfu/ml (Table 3). The number of aerobic mesophilic microorganisms in the other cosmetics after the expiry date ranged from 1.5×10^2 cfu/g in peeling, which was the only cosmetic after the expiry date that met the requirements of the Ordinance of the Minister of Health of 23 December 2002, and 1.3×10^7 cfu/g in hand cream (Table 3).

The presence of bacteria of the genus *Staphylococcus* was indicated in 11 (20.0%) examined samples of cosmetics. They included 6 (10.9%) cosmetics used by several people and 5 (9.1%) cosmetics after the expiry date. In the above cosmetic products the lowest contamination caused by *Staphylococcus* spp. was detected in the blush after the expiry date and its contamination amounted to 5.0×10 cfu/g (Table 4). The highest contamination by these cocci, which amounted to 1.0×10^2 cfu/g, was found in the eye shadow after the expiry date (Table 4). *S. aureus* occurred only in the lipstick after the expiry date, and other species of this genus *Staphylococcus* were present in the other cosmetic products.

Qualitative studies of microbiological purity for detecting *P. aeruginosa*, show their presence in the intimate hygiene wash used by one person. The studied cosmetic contained 2.1×10^2 cfu/ml (Table 4).

Table 4 - Presence of microorganisms in tested cosmetics

Cosmetic	Cosmetics used by one person, before the expiry date			Cosmetics used by several people, before the expiry date			Cosmetics after the expiry date		
	Number of microorganisms [cfu×ml^{-1}] or [cfu×g^{-1}]			Number of microorganisms [cfu×ml^{-1}] or [cfu×g^{-1}]			Number of microorganisms [cfu×ml^{-1}] or [cfu×g^{-1}]		
	Staphylococcus spp.	*P. aeruginosa*	Fungi	*Staphylococcus* spp.	*P. aeruginosa*	Fungi	*Staphylococcus* spp.	*P. aeruginosa*	Fungi
Body lotion	n.d.*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Eye shadow	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	$1,0\times10^2$ ($1,4\times10^2$)	n.d.	n.d.
Fluid	n.d.	n.d.	n.d.	$1,5\times10^1$ ($2,1\times10^1$)	n.d.	n.d.	n.d.	n.d.	n.d.
Cream for children	n.d.	n.d.	n.d.	n.w.	n.d.	n.d.	$1,5*10^1$ ($0,7\times10^1$)	n.d.	n.d.
Hand cream	n.d.	n.d.	$1,5\times10^1$ ($2,1\times10^1$)	$1,0\times10^1$ ($0,1\times10^1$)	n.d.	n.d.	n.d.	n.d.	n.d.
Foot cream	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Face cream	n.d.	n.d.	n.d.	$1,5\times10^1$ ($2,1\times10^1$)	n.d.	n.d.	n.d.	n.d.	n.d.
Face mask	-	-	-	$6,5\times10^1$ ($6,3\times10^1$)	n.d.	n.d.	-	-	-
Hair mask	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	$1,5\times10^1$ ($0,7\times10^1$)
Eye cleansing milk	n.d.	n.d.	n.d.	$1,5\times10^1$ ($2,1\times10^1$)	n.d.	n.d.	n.d.	n.d.	n.d.
Toothpaste	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	$2,5*10^1$ ($2,1\times10^1$)	n.d.	n.d.
Peeling	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Intimate hygiene wash	n.d.	$2,1\times10^2$ ($5,6\times10^1$)**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mouthwash	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	$0,5\times10^1$ ($0,7\times10^1$)
Blush	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	$0,5\times10^1$ ($0,7\times10^1$)	n.d.	$0,5\times10^1$ ($0,1\times10^1$)
Shampoo	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lipstick	n.d.	n.d.	n.d.	$6,0\times10^1$ ($8,4\times10^1$)	n.d.	n.d.	$2,5\times10^1$ ($0,3\times10^1$)	n.d.	n.d.
Mascara	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Shower gel for children	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

* n.d.- not detected, **- standard deviation

No presence of fungi was detected in cosmetics used by several people. Among the tested cosmetics after the expiry date, contamination with fungi stayed within range from 0.5×10^1 cfu/g or ml in the case of the mouthwash and the blush and 1.5×10^1 cfu/g win the case of the hair mask and the hand cream used by one person (Table 4). The fungi that contaminated the tested cosmetics were *Aspergillus* spp. and *Penicillium* spp. No contamination of cosmetic preparations with the *C. albicans* was recorded.

DISCUSSION

In the cosmetics used in this study no physicochemical changes were found that could indicate microbiological contamination, such as: a change in colour, smell, change in consistence, appearance of sediment or phase separation. Similar study results are reported by *Hugbo* et al. [11], *Abu Shaqra* and *Al-Groom* [1] as well as *Mwambete* and *Simon* [15]. The results differed from the present were shown by Muhammed [14], who noted changes in colour, the appearance of sediment and cloudiness of a cosmetic batch.

The majority of cosmetics tested in the present study which were used by one person before the expiry date did not meet the requirements of the Ordinance of the Minister of Health of 23 December 2002 [22]. Preparations which contained a high percentage of water were characterized by a higher level of microbiological contamination. Similar results were presented by *Campana* et al. [6], who indicated that the microbiological contamination of preparations stays within the range from 1×10^2 to 3×10^4 cfu/ml for cosmetics for personal hygiene. No contamination of oil/water emulsions and toothpastes were found. Similar results were obtained by *Lamikanra* and *Okeke* [13], who noted higher microbiological contamination in water cosmetics.

Onurdağ et al. [18] results differ from the present results of microbiological purity of used colour cosmetics (100% contaminated samples). Only in 5 (6.9%) used make-up cosmetics *Onurdağ* et al. [18] observed contamination with aerobic mesophylic bacteria exceeding the standards of microbiological purity.

In the present study, in the group of cosmetics used by one person, rods of *P. aeruginosa* were isolated in the intimate hygiene wash. *Staphylococcus* have not been observed. In contrast to the results obtained by *Behravan* et al. [5], *Dashen* et al. [7] and *Campana* et al. [6], *Staphylococcus* spp. were the most often isolated potentially pathogenic bacteria in this group of cosmetics.

Varied microflora, specific of each person, at not following the principles of hygiene, is the cause of a higher level of microbiological contamination of cosmetics which are used by several people. Based on the present study, it can be observed that *Staphylococcus* spp., being an element of the human microflora, are the most often isolated from cosmetic preparations used by several people (10.9% of samples). This is confirmed by the results obtained by *Anelich* and *Korsten* [2], who studied 58 samples of cosmetics and proved the presence of bacteria from the genus *Staphylococcus* in 9.0% of samples. The presence of *Pseudomonas* spp. was found in 30.0% of samples, *Enterobacter* spp. - in 17.0%, and the mould *Aspergillus* spp. in 13.0% of samples [2]. In the present study, no *Pseudomonas* spp. were indicated in this group of cosmetics. *Naz* et al. [16], in turn, observed the presence of *S. aureus* in 100% of samples of make-up sponges and brushes, and *P. aeruginosa* and fungi in more than 50% of the tested cosmetics. The most of used waxes were contaminated with *S. aureus* and *P. aeruginosa*, and contamination with fungi was low.

CONCLUSIONS

Based on the obtained results, it was found that the contamination of cosmetics which are past their sell-by date or were used by more than one person is considerably higher than in cosmetics before their expiry date or used by single person. Most often isolated potentially pathogenic microorganisms are *Staphylococcus* spp., which constitute an element of the natural microflora of the human skin.

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Conflict of interest

The authors declare that they have no conflict of interest.

Financial disclosure

The authors have no financial interests related to the material.

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ORIGINAL ARTICLE

IMMUNE RESPONSE CYTOKINES AS POTENTIAL BIOMARKERS FOR DDT INDUCED NEURODEGENERATION

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ABSTRACT

Background: The world incidence rate of Alzheimer's Disease (AD) is 64 cases per 1,000 individuals. Due to the aging population, the prevalence of AD is however increasing and yet, little remains known about the etiology of AD.

Objective: Previous studies suggested relationships between AD, neuro-inflammation and organochloride pesticide exposures, therefore, we aimed to study the association between DDT and possible biomarkers of AD.

Materials and Methods: We explored literature on inflammation, pesticide exposure and biomarkers associated with AD. We measured eligible markers in adult C57BL/6J mice treated with DDT for 4 months (dose=3 mg/kg/day); Hippocampi tissue gene expression was quantified by qPCR. IL-1β expression was compared in test vs. control mice using t-tests. Furthermore, we studied population data to: explore the immunological markers, identify gaps and possible approaches for addressing them.

Results: Average serum levels of IL-1β were significantly higher ($p<0.05$) in the DDT treated mice compared to controls. IL-1β stimulates APP and Aβ41 syntheses, which may be associated with AD pathogenesis. Gaps identified included: (1) Parallel analysis of genetic and environmental risk factors; (2) Definition of toxin-induced neuro-inflammation focusing on microglial physiology. Studies focusing on the physiological effects of DDT, focusing on epigenetic aberrations may aid in the description of the effect of DDT on gene expression; (3) The blood-brain-barrier limits comparisons between peripheral and brain-localized IL-1β and DDT concentrations, suggesting the need for robust measurement schemes. We report that there is still much uncertainty regarding biomarkers associated with AD pathogenesis.

Conclusions: Currently, we cannot confidently report that DDT has a causal role in AD incidence. However, by first quantifying the cytokine concentrations post-exposure to DDT, by measuring the metabolite DDE, we can further explore potential drifts in immune marker concentrations that could provide a platform for future studies.

Key words: *biomarkers, DDT, Alzheimer's disease, pesticide exposure, immune response*

INTRODUCTION

Dementia incidence has increased exponentially worldwide despite extensive efforts to understand the disease's pathogenesis. This work focused on Alzheimer's Disease (AD), which accounts for approximately 60-80% of prevalent dementia cases. The prevalence of AD varies geographically [29]. Studies in Europe found that the age-adjusted prevalence of AD was 4.4 per every 100 persons [21]. A U.S. study conducted in a representative sample of individuals 70 years of age or above reported the AD prevalence as 9.7 per every 100 persons in the population [28]. The burden of dementia is increasing among lower income countries and in higher income countries among groups with lower education and income. In addition, the prevalence is lower among those who receive treatment for cardiovascular risk factors associated with AD [20]. In the US, the prevalence of AD has increased while the incidence has declined significantly [28] which may at least be partially attributed to patients living longer due to improved treatment and patient management. However, the incidence of dementia appears to be

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remain constant in certain populations. The previous was shown in the CHAP study, which looked at the variation in incidence over time among African-American compared to White individuals, and found no change in secular trends over time [32]. There are a number of hypotheses that may explain some part of this trend: development of new diagnostic technology, refinement of disease criteria for diagnosis, and in-depth understanding of on-set mechanism – all of which could result in alternative diagnoses of conditions other than AD which would have been previously diagnosed as AD.

Often, patients who have developed neurodegenerative diseases are diagnosed because they demonstrate symptoms such as losing items, forgetting important dates/events and other daily routines [23]. The routine diagnostic approach is to perform medical exams among "at risk" individuals given that to date there is no laboratory based test that is able to fully explain risk of developing AD, aside for the risk factor gene APOE4 [37]. AD's symptoms are latent to its pathogenesis, potentially rendering intervention of little to no use since once senile plaques are formed in the brain (a hallmark of AD pathogenesis), it becomes difficult to reverse the pathological process [5].

The organochlorine pesticide DDT was banned in the U.S. in 1972, however, the population continues to experience DDT exposure because the compound persists in the environment, has bio-accumulated in the food chain, and is still used in developing countries. In September 2006, the World Health Organization (WHO) declared its support for the indoor use of DDT in African countries where malaria remains a major health problem, citing that benefits of the pesticide outweigh the health and environmental risks [8]. The WHO position is consistent with the Stockholm Convention on POPs, which bans DDT for all uses except for malaria control.

Organochlorine pesticides have been shown to stimulate an innate immune response resulting in increased levels of pro-inflammatory cytokines (e.g. IL-6, INF- γ, TNF- α, IL-1 β, IL-10, and IL-17A) [25]. The immune capabilities of glial cells might play a key role in the regulation and rate of neurodegenerative diseases. Acetylcholinesterase blood levels, as a biomarker of organophosphate pesticides exposure, have been associated with loss of cognitive function. [34]. It has also been shown now that acetylcholinesterase plays a role in the immune response [38], providing a rationale for why it may be of interest to study cytokines as potential biomarkers due to their role in immune responses, driven by Aβ42 and their overall consistency across different ethnic groups. A second study conducted navigated the potential differences in C-Reactive Protein (CRP) levels among Hispanic Whites and Caucasian whites [26]. These results indicate that there is a need to further understand the localized immune phenomena in the brain and to further explore potential biomarkers of outcome of disease.

Population-based studies have assessed whether exposure to organochlorine pesticides is associate with the prevalence of AD. Previous work found that elevated serum DDE levels are associated with increased risks for developing AD [30]. Higher levels of exposure to DDT (the parent to the DDE metabolite) occurred mainly in the work place, particularly amongst farmers. Some occupational studies found that the elderly are more likely to be exposed to AD-associated pesticides [2]. A 5-year follow-up study found that men had a higher risk of AD then women, associated with agricultural occupations, or rural residency versus urban residency.

Overall, given that various studies have associated DDT exposure to increased risk of AD and to a hyper-reactive immune response, our study aimed to address the association between pesticide exposure and AD by quantifying various cytokines that are hallmarks for an innate immune response.

MATERIALS AND METHODS

Methods

To understand the toxin's role in mammals a mouse model was implemented (C57BL/6J, *Mus musculus*). Six DDT treated, six DDE treated and 6 control male mice in the study population; the dose was 3 mg/kg/day for a period of 4 months with Institutional Animal Care and Use Committee (IACUC) approval. Once the dosing period concluded the animals were sacrificed and their hippocampi collected and stored in liquid nitrogen. One DDE treated mouse died during the dosing period and was excluded from the study analysis. The cause was unknown and there seemed to be not particular indication that could have explained the loss.

Sample preparation

Hippocampus tissue samples

Samples were thawed in ice and standard sonication technology was used to homogenize the tissues to ensure maximum homogenization. The samples were sonicated in trizol solution, between every sample the sonicator was run in molecular grade water to clear of potential debris, reducing the chances of cross-contamination among samples.

Once samples were prepared a QUIAGEN RNA Isolation Mini Kit was used to extract approximate 50 μL of RNA from each sample. The standard manufacturer protocol was followed with the exception that the last spin in the collecting tube was done at 1000 G. No secondary collection was required given that the concentration of RNA was enough to conduct all required dilutions for the cDNA synthesis.

RNA concentrations were measured using nanodrop technology. The nanodrop measurement was done at 260/280 wavelength for particle precision.

cDNA synthesis

Standard laboratory protocol was conducted using Thermocycler technology, with 4 recurring programmed steps for optimal temperature conditions for cDNA synthesis from RNA. The total volume of diluted RNA was 10 µL, with an RNA concentration of 0.6 ng/mL. Depending on the initial concentration (found by nanodrop analysis), samples were diluted with de-ionized water to achieve normality among samples.

qPCR preparation/execution/reading methods

Preparing samples for qPCR was initially done in a 96-well plate, followed by transfer in duplicates to a 384-well plate. Each well in the 384-well plate required a total of 10µL of RNA, therefore a total volume of 22.5µL were initially mixed in the 96-well plate. Each well in the 96-well plate had a solution that included 5µL of the primers selected (INF-gamma, IL-1beta, iNOS, IL-10, IL-6, IL-17A as well as house-keeping genes GAPDH, TBP), 5µL of each cDNA sample diluted in water and 12.5µL of SYBR green. The total volume in each well was 22.5µL. The 96-well plate was then spun down at 1000 rpm for 2 minutes. Next, a multi-tip pipette was used to transfer to the 384-well plate 10 µL duplicates of the SYBR Green, primers and cDNA solutions. The 384-well plate was covered in aluminum foil and spun for at 1000 rpm for a minute. The plate was then sealed, the barcode scanned and wells were labeled using Viaa7 manager page.

Viia7 technology (for RT-qPCR) was used to run automated qPCR 384-well plates. The plate was placed in the Viaa7 machine and the protocol was uploaded into the system. The total run time was approximately 5 hours. Once the automated system went to full completion, the data sheet was extracted as an Excel file for analysis. From the melting point graphs provided by the Viia7 system, primers were identified as high/low expression (melting curve should plateau). Duplicate Ct values for each of the samples, were averaged. *Student's* t-test was then applied to each criteria group (DDT, DDE, and Control) and bar plots were plotted by cytokine.

Ct values that were overexpressed were used to identify potential markers that may be translated to having some sort of impact at the population level. A second search of original and review articles was conducted using key words Alzheimer's disease, dementia, risk factors, incidence, burden, immune response and epidemiology. The in-depth review consisted of studies that focused on *in vivo, in vitro*, and population-based studies of associations between environmental exposures and the induction of AD. Sequentially, population studies were the basis for suggesting risk factors associated with increased AD and potential involvement of resident cells in the central nervous system.

RESULTS AND DISCUSSION

AD is primarily of interest in the scientific community not only for its burden to society, but also because its etiology not yet fully understood. The experimental design for the DDT toxicology work was driven by an initial literature search that aimed to explore eligible biomarkers that may play a role in AD pathology. Previous work on has focused on biomarkers of disease with a focus on the most common acute immune response biomarker in systemic circulation. However, CRP can be measured with confidence in the peripheral serum and not in brain tissue. This could be because it is induced in the liver but once it systematically circulates, the BBB may be disrupted by CRP. Exposure to DDT can cause acute immune responses in the peripheral system [41], however we aimed to address the influence of DDT in the central nervous system with a focus on its influence on the hippocampus. Not ruling out the influence of CRP specifically, but keeping in mind pathways associated with its induction. Markers presented in Table 1 were selected for their eligibility to be measured in hippocampal tissue as well as in blood samples.

Table 1. Acute immunity markers and their significance in Alzheimer's Disease (AD) pathways

Biomarker	Reasoning for study selection
IL-6	Stimulates binding to TLR-4 (major mechanism for detection of LPS positive bacteria) and induces the formation of CRP. Aβ42 activates resident microglia which in turn secrete.
TNF- α	Major biomarker for systemic infection.
IL-1 β	Aggravates the immune response by promoting more APP synthesis and by promoting the production of more Aβ proteins by astrocytes. Its overexpression may promote the phosphorylation of TAU leading to NFT formation and/or neuronal death.
IL-10, IL-17A	Synergistically overexpressed by by Aβ.
INF- γ	It is the major activator of macrophage and it induces MHCII (which is found in antigen presenting cells such as macrophage and astrocytes).
C Reactive Protein (CRP)	Most ubiquitous biomarker for innate immunity.

These markers were quantified in DDT, DDE and Control mice by qPCR. From the gene expression data obtained it was clear that CRP was below the LOD, suggesting that a breach in the BBB had not occurred, however needs to be further be quantified by more robust measurements in our future studies. Applying t- test with *Welch's* correction a p-value of 0.0451 was reported for the IL-1β, the only marker overexpressed in the DDT mice when compared to control mice (Figure 1).

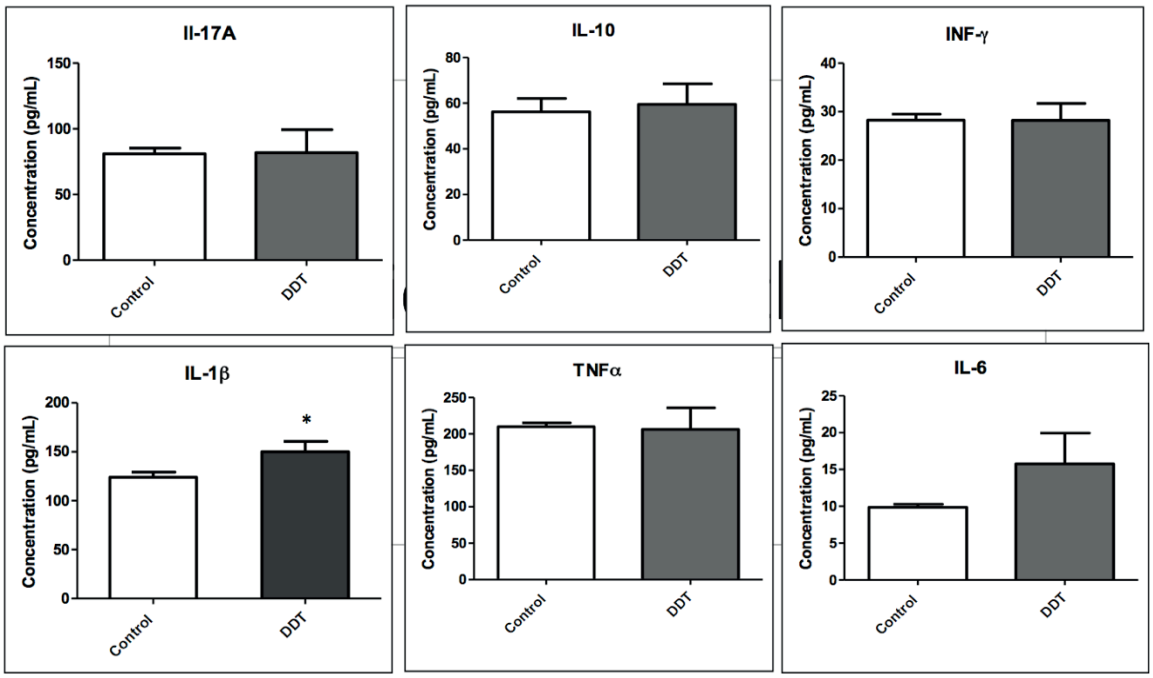

Figure 1. Immune marker concentration data for controls compared to DDT samples

Having reported a significant increase in concentration of IL-1β for the DDT treated mice, we conclude that DDT may in fact influence acute immune responses and that this induction may be treated as a biomarker of response. The reported concentrations were in pg/mL, which is consistent the resident immune cells in the brain having a different role from macrophage cells in the periphery. We therefore would not expect the concentrations to be in the microgram scale. This was an interesting finding, given the role that IL-1β may play in AD onset at an early stage suggesting that gene expression data may provide insight on the effect of DDT in the expression of these markers. IL-1β aggravates astrocytes promoting APP synthesis, the precursor protein associated with increased levels of *beta*-amyloid proteins. If cleavage of Aβ induces Aβ42 oligomers, the progression of AD is almost certain given that Aβ42 is seminal in formation of senile plaques which drive AD. Consequently, Aβ42 may also stimulate IL-1β expression. This suggests an additive mechanism caused by DDT exposure, which was not explored in this particular study, but is a future direction in our work in order to further understand disease pathogenesis. Moving forward, it may be best to fit linear regression and larger samples sizes that ensure a power of 80% or greater. Moreover, IL-1β may promote phosphorylation of TAU proteins, which then can join to one another to form Neuro Fibrillary Tangles (NFTs). That is a better test to neuronal death. Part of the distinction found in AD is the formation of senile plaques which are dependent on the TAU protein expression. Since IL-1β was overexpressed in the DDT treated mice and not the controls, we can further hypothesize that there may be an inductive effect caused by the exposure. To fully understand the outcome, we first must address the source of the DDT, its effects on health and the prevalence of disease related to environmental/occupational exposures, controlling for confounding factors. Unfortunately, not enough secondary data is present that would allow us to analyze this association for effect modification.

Important to note, running qPCR for immune biomarkers requires that the cDNA be more concentrated than many typical protocol indicate. Given that we were exploring potential markers in brain tissue (not usually present in the absence of pathology), the dilution for this particular expression was optimized to 1:3 where usual dilutions factors are taken as 1:10. This particular aspect was important to consider since it may influence the sensitivity and consistency of the measurements for gene expression. We also recognize that we were limited in our samples given that the number of hippocampi collected for the mice in each group was n=6. However, this was an

exploratory study and the data were consistent even in this small sample size. It suggests that after revisiting our study design and moving forward with future studies, we have justification for including a larger sample size, allowing us to observe the effects of aging and sex in our toxicity model.

Understanding that we reported a possible, reliable marker, moving forward we were interested in exploring exposures that may lead to increased rates of AD formation, to identify potentially at risk populations and the distribution of disease found in the literature. A second review was conducted using keywords Alzheimer's disease, dementia, risk factors, incidence, burden, immune response and epidemiology which generated work that was included here. The published literature was reviewed for: age distribution of disease, risk factors, occupational exposures and genetic factors that may be associated with AD.

From the search, it was concluded that AD has been studied in a variety of settings to understand the potential risk factors for the disease as well as to understand its distribution among the population. From the belief that cigarette smoking may play a protective role in the development of Parkinson's disease, many *in vivo* studies [39] aimed to study if this relationship held true for AD. Some cross-sectional studies found that individuals who smoked had a reduced likelihood of developing AD [40]. These studies, however, failed to address the fact that individuals who smoke may be dying at a younger age than those who did, creating a bias towards the null hypothesis, since senile plaque formation rates are higher in the elderly than in the younger population. We can almost certainly conclude that age may be modifying the associations. On the other hand, recent prospective cohort studies found that individuals who smoked were two times as likely to develop AD as their counterparts who didn't smoke [1]. The previous study claims that smoking was not an additional risk factor for those who carried the APOE4 allele. However, as a standalone, the APOE4 allele is a risk factor for developing AD [35]. The APOE gene is located on chromosome 19 and it occurs in 3 common forms: APOE2, APOE3, and APOE4. APOE4 specifically has been studied with interest since at least 40% of AD patients will have at least one copy of the APOE4 allele.

Ethnically, 25% of Caucasian AD patients have at least one allele copy while African patients have a higher occurrence and Oriental AD patients have a lower occurrence of the gene copy when compared to their Caucasian counterparts [19]. Although, APOE4 is neither necessary nor sufficient to cause AD, it is strongly correlated to the disease and therefore it is considered a risk factor for Caucasians; limiting its relevance to one specific subgroup of the population. Moreover, APOE4 has not been associated as risk factor among Hispanics and Blacks whom seem to have higher risk of AD whether or not they possess a copy of the gene.

Another set of population-based studies has addressed whether exposure to organochlorine pesticides increases the risk or AD or not previous work found that elevated serum DDE levels are associated with increased risks for developing AD [30]. Exposure to DDT (the parent to the DDE metabolite) has been reported to occur mainly in the work place, particularly amongst farmers with increased rates of exposures in countries where use of DDT has yet to become banned. Other occupational studies found that the elderly are more likely to be exposed to AD-associated pesticides [2]. This 5 year follow up study found that men had a higher risk of AD then women associated with occupational exposure, having a main job in agriculture, or a rural residency. The occupational exposure association was in analysis with an RR of 2.9, which remained significant once age and education level were adjusted for (RR=2.4, 95% CI: 1.0, 5.6). Although pesticide exposure has been studied as a potential environmental risk factor, there are many other factors to be considered. Another previous review suggests important risk factors to consider [4].

Finally, a longitudinal study conducted reported that mental retardation (RR=2.03, 95% CI: 0.13, 2.66), *Parkinson's* disease (RR=2.56, 95% CI: 0.69, 9.50), Leukemia (RR=3.24, 95% CI: 0.37, 28.46) serve as genetic risk factors. These risk factors for AD have also been correlated in other population studies published in different countries [36]. This same study also found that exposure to pesticides/fertilizers (RR=4.35, 95% CI: 1.05, 17.90), ink dyes (RR=1.45, 95% CI: 0.57, 3.68), paints/varnishes (RR=1.21, 95% CI: 0.46, 3.21), liquid plastics/rubbers (RR=1.01, 95% CI: 0.12, 8.38), glues/adhesives (RR=1.41, 95% CI: 0.49, 4.05), and radiation (RR=3.57, 95% CI: 0.38, 33.38) were environmental/occupational risk factors, implying to genetic and environmental risk factors. Although this particular study reported an increased likelihood of developing the disease based on both genetic and environmental exposures independently, their analysis was not performed for both types of risk factors in parallel. In addition, the confidence intervals were broad, suggesting that the internal and external validity of the data may be confounded.

CONCLUSIONS

In summary, the work presented here suggest that there is value in understanding the role of DDT and its possible association with AD, using the immune system as proxy for the effect. Although we haven't proposed a causal mechanism, the data provided here

may serve as a framework indicative of the need to address the gaps in the literature that could perhaps lead to an improved understanding of AD pathogenesis and progression.

Conflict of interest

The authors declare no conflict of interest.

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IN MEMORIAM: PROFESSOR MARCELLO SPANÓ (1954-2017)

Sorrowful message from the Editors of the journal *Roczniki Panstwowego Zakladu Higieny – Annals of the National Institute of Hygiene.*

In January 2017 we lost Professor Marcello Spanó, Member of the International Scientific Board of our journal. Professor Marcello Spanó held the position of senior scientist in the Laboratory of Toxicology, Unit of Radiation Biology and Human Health, ENEA (Italian National Agency for New Technologies, Energy and Sustainable Economic Development), Casaccia Research Center, Rome, Italy.
Below there is 'In memory' note written by his friends and collleagues.

In memory of Marcello Spanó

Whilst conversating with his research team at ENEA in Rome, Marcello Spanó was hit by cardiac arrest, and died at the hospital without regaining conscience few days later - on January 19, 2017.

The meaningless death of Marcello at an age of 62 is a terrible loss for his family, his research team and collaborators at ENEA in Rome and all his colleagues and friends in a large international network of researchers.

Marcello figured out at an early stage that a laboratory method to measure DNA damage in spermatozoa, the so called Sperm Chromatin Structure Assay (SCSA), had a great potential in research as well as in clinical practice. He established a collaboration with US scientist Professor Don Evenson, who invented this assay and got it to work at his Lab at ENEA in Rome. Subsequently Marcello's Lab has played a major role in numerous epidemiological and experimental studies throughout Europe addressing toxic effects on male reproductive function by chemicals in our environment. Of major importance it turned out that the SCSA parameter called DFI (DNA Fragmentation Index) is an independent predictor of male fertilizing potential and therefore has become an important part of diagnostics in infertility make-up. During the past few years Marcello and his team has worked hard to develop and implement techniques to look into epigenetic changes in the human male genome in spermatozoa. He was brutally taken away just when this work was hoped to provide new important insight into male reproductive function and its susceptibility to environmental chemicals.

However, Marcello was not only a great scientist but also a humanist with profound interest in literature, travels and ancient as well as modern history and politics. His ability to communicate with other people and to create social relationships was remarkable. Perhaps, because he was an excellent listener. In human relations understanding was much more important than judging, the progress and performance of the group more important than that of the individual. Marcello insisted on scientific integrity and high standards and never was tempted to harvest publicity from premature or preliminary research results.

Marcello was a great friend and fan of Scandinavia which he showed, not only by scientific collaboration and by helping colleagues at Lund University with setting up and running SCSA. He also learned to speak and read Swedish by taking lessons at the Swedish Church in Rome. Many of his e-mails and SMSs sent to his Nordic friends were written in – close to perfect – Swedish.

Although research was an important part of his life, he also appreciated his leisure time, which he spent by meeting family, friends, reading, travelling and watercolor painting.

Marcello was a very unique person – both as researcher, colleague and friend. His early death is a tremendous loss to science, colleagues at ENEA and his large international collaborative research network. We will miss Marcello's ever supporting back-up, his optimistic and humoristic approach to life and partnerships, his patience and understanding, a real friend one could always rely on.

Jens Peter Bonde and Aleksander Giwercman

February, 2017

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Abstract. It should contain 250-300 words and consist of the following sections: Background, Objective, Material and methods, Results, Discussion and Conclusions. The abstract of a review should not be divided into sections.

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3. *Shridhar G.*, *Rajendra N.*, *Murigendra H.*, *Shridevi P.*, *Prasad M.*, *Mujeeb M.A.*, *Arun S.*, *Neeraj D.*, *Vikas S.*, *Suneel D.*, *Vijay K.*: Modern diet and its impact on human health. J Nutr Food Sci 2015;5:6 doi:10.4172/2155-9600.1000430.
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Books and chapter in a book:

5. *Riley D.M., Fishbeck P.S.*: History of methylene chloride in consumer products. In: *Salem H.*, *Olajos E.J.* (eds.). Toxicology in Risk Assessment. London, Taylor & Francis, 2000.

Legislative acts:

6. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J EU L 364, 20.12.2006.

Internet source:

7. The Rapid Alert System for Food and Feed (RASFF) Portal. Available https://webgate.ec.europa.eu/rasff-window/portal (accessed 18.10.2010)

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Abbreviations, symbols, units. Generally known and used abbreviations may be left unexplained, others must be explained at the first use in the text. Metric SI units are recommended, however also other generally used units are accepted.

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