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REVIEW ARTICLE

HEALTH-PROMOTING EFFECTS OF BIOACTIVE COMPOUNDS IN BLACKCURRANT (*RIBES NIGRUM L.*) BERRIES

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ABSTRACT
Blackcurrant (BC) is a well-known and appreciated berry fruit in our country and Poland is the largest BC producer among European Union countries and the second, after Russia, producer in the world. Due to the short shelf life of BC, its consumption in fresh form is relatively low , therefore the berries are processed into juices, jams, jellies, and freeze-dried products or alcoholic beverages. The high nutritional value of BC berries result from high content of bioactive compounds (among others, vitamin C, anthocyanins, pectins, organic acids, as well as polyunsaturated fatty acids contained in seeds of the fruit). Anthocyanins (ANTs) create the largest group among all polyphenolic compounds contained in BC. The results from different studies confirm that ANTs are important in attenuation oxidative stress parameters in the organism, and therefore can reduce the risk of certain non-communicable chronic diseases. Consumption of unprocessed and processed blackcurrants (i.e. juices and products containing fruit extracts) may support the nutrition therapy of cardiovascular diseases, certain eye diseases and may normalize the lipid profile of the blood plasma. Additionally, the beneficial profile of unsaturated fatty acids from BC seeds supports the therapy of autoimmune diseases. This article is attempts to summarize the results of the studies on the anti-inflammatory, immunomodulatory, anti-tumor and anti-microbial effects of BC bioactive compounds including the mechanisms of their action depending on the form of the fruit (e.g. juice, whole fruit extract, dried pomace, or seed oil). The article also highlights the potential use of BC in production of functional food, important in the dietary prevention of non-communicable chronic diseases resulting from increased oxidative stress in the organism.

Key words: *anthocyanins, chemoprevention, blackcurrant, inflammation, oxidative stress, polyphenols*

STRESZCZENIE
Czarna porzeczka jest znanym i cenionym owocem jagodowym, a Polska jest największym producentem tych owoców wśród krajów Unii Europejskiej i drugim po Rosji. Ze względu na nietrwałość owoców, ich spożycie w postaci świeżej jest stosunkowo niskie. Z tego też względu owoce czarnej porzeczki najczęściej przeznaczane są do produkcji soków, dżemów, galaretek oraz liofilizatów, suszów oraz napojów alkoholowych. Cenne, z żywieniowego punktu widzenia, właściwości owoców czarnej porzeczki wynikają z wysokiej zawartości związków o charakterze bioaktywnym (w tym m.in. witaminy C, antocyjanów, pektyn i kwasów organicznych oraz wielonienasyconych kwasów tłuszczowych zawartych w pestkach owoców). Antocyjany, z kolei, stanowią największy udział spośród wszystkich związków polifenolowych zawartych w tych owocach. Wyniki badań potwierdzają, że związki te są istotne w przeciwdziałaniu skutkom stresu oksydacyjnego w organizmie, a zatem mogą zmniejszać ryzyko niektórych przewlekłych chorób niezakaźnych. Spożywanie czarnej porzeczki w formie surowej oraz przetworzonej, tj. soków oraz produktów zawierających ekstrakt z owoców może wspomagać dietoterapię chorób układu krążenia, niektórych chorób oczu oraz normalizować profil lipidowy osocza krwi. Dodatkowo, korzystny skład nienasyconych kwasów tłuszczowych zawartych w oleju z nasion owoców może wspomagać terapię chorób o podłożu autoimmunologicznym. Celem niniejszej pracy było podsumowanie badań dotyczących przeciwzapalnego, immunomodulującego, przeciwnowotworowego i przeciwdrobnoustrojowego wpływu związków bioaktywnych zawartych w czarnej porzeczce z uwzględnieniem mechanizmów ich działania w zależności od postaci owocu (tj. sok, suplementy zawierające ekstrakt z owoców, suszone wytłoki lub olej z pestek). Ponadto, w pracy zwrócono uwagę na potencjalne możliwości wykorzystania owoców czarnej porzeczki w produkcji żywności funkcjonalnej, mającej znaczenie w dietoprofilaktyce przewlekłych chorób niezakaźnych wynikających z nasilonego stresu oksydacyjnego w organizmie.

Słowa kluczowe: *antocyjany, chemoprewencja, czarna porzeczka, polifenole, stan zapalny, stres oksydacyjny*

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INTRODUCTION

An optimal fruit intake is one of the factors in prevention of non-communicable chronic diseases. Fruit are one of most important group of food products that provides a substantial amount of bioactive compounds, therefore they should be a part of well-balanced diet. The results from recent research clearly report that regular optimal fruit consumption contributes to the lower risk of cardiovascular diseases, as well as a possible protective effect in decrease the risk of colon cancer, pancreatic diseases, mental health, attention and mood were also reported by other authors [5, 27, 68].

Considering the bioactive compounds present in fruits, the greater attention should be paid to blackcurrant (*Ribes nigrum L.*) (BC), a well-known and appreciated berry fruit in Poland. The long history of cultivation of BC in contributed to the greater popularity of these fruits in our country (both in home gardens and in horticulture production). A report of Main Statistic Office in Poland [26] estimated that the blackcurrant crops in recent years (2015-2019) obtained from orchards fluctuated and reached 130 800 tons in 2016 and decreased to 92 200 tons in 2019 [Fig. 1]. Poland is the leading BC berries producer in European Union countries and the second (after Russia) in the world.

(as a waste of juice processing) are also used to produce a natural colorants or are a source of bioactive ingredients for production of many functional food products [6, 8, 24, 64].

The aim of this study was to summarize the research on the anti-inflammatory, immunomodulatory, antitumor and antimicrobial effects of bioactive compounds contained in blackcurrant, including the mechanisms of their action. In addition, the study highlights the potential use of BC fruit in the production of functional food of importance in the diet and prevention of chronic non-communicable diseases.

NUTRITIONAL VALUE OF BC BERRIES

The BC berries are a rich source of many bioactive and flavour compounds, like soluble sugars, vitamins, minerals, polyphenols, polyunsaturated fatty acids (PUFA), organic acids, vitamins (C, E), soluble and insoluble dietary fiber and tannins [35, 69]. *Nour* et al. [49] showed, based on the analysis of the eight BC cultivars (‘*Abanos*’, ‘*Blackdown*’, ‘*Bogatar*’, ‘*Deea*’, ‘*Record*’, ‘*Ronix*’, ‘*Tenah*’ and ‘*Tinker*’) harvested in Romania, that the content of ascorbic acid in BC fruits ranged from 161.6±3.3 to 284.5±1.5 mg/100 of fresh weight (f.w.). In contrast, *Rachtan-Janicka* et al. [55] reported a lower values of vitamin C (ranging from

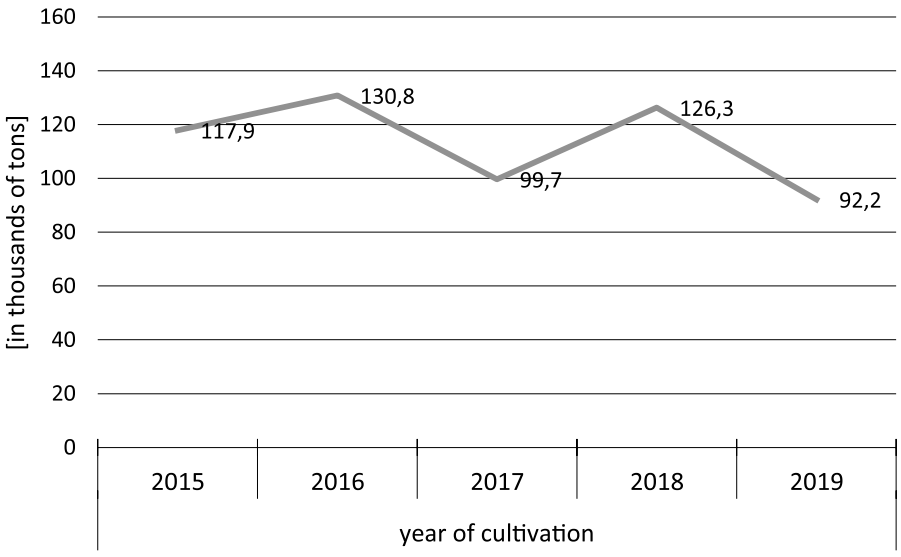

Figure 1. The harvest of blackcurrant fruit in horticulture in 2015-2019 [26]

A blackcurrant berries are known for their characteristic slightly tart and sour taste [6]. A black-colored berries contain a high amount of anthocyanins (ANTs) [49]. Due to the rich source of sugars, pectin, and volatile compounds, BC berries are processed in a wide range of products (like juices, fruit nectars, jams, jellies, alcoholic and non-alcoholic beverages, and dry fruits) [17, 63]. Moreover, the fruit residues

122.4±6.3 to155.9±3.2 mg/100 f.w.) in 3 cultivars (‘*Adler*’, ‘*Tiben*’ and ‘*Titania*’) harvested in Poland. Blackcurrant fruits also contain a substantial amounts of minerals, especially potassium, magnesium and iron (ranging from: 251.1- 305.0; 45.7 – 65.9 and 1.13 – 1.77 mg/100 f.w., respectively). What is more interesting, BC seeds are rich in polyunsaturated fatty acids. As reported by *Šavikin* et al [59], linoleic acid (n-6) (39.6-

46.9%) was the most predominant essential fatty acid, following by γ-linolenic (n-6) (10.7-18.5%), α-linolenic (n-3) (12.9-16.9%), stearidonic (n-3) (2.40-4.45%), and oleic (n-9) (9.86-13.39% w/w). Furthermore, the n-3/n-6 essential fatty acids ratio amongst all analyzed blackcurrant cultivars was ranged from 0.27 to 0.36.

In particular, an important health-promoting properties of BC result from the presence of polyphenolic compounds, that are plant secondary metabolites characterized by strong antioxidant activities. The most important polyphenols present in BC berries are flavonoids and anthocyanins. Environmental factors (such as, latitude, weather conditions and soil quality) significantly determine the quality of berries and as well as the level of bioactive metabolites in fruits [71]. The another key factor affecting the level of bioactive plant compounds is a cultivar variation. The analysis of phenolic compounds in BC berries of different cultivars originated from Scotland (UK), Lithuania, Latvia, Finland and Poland [67] revealed that level of total phenolics ranged from 598 to 2798 mg/100 g, (dry weight, d.w.) and sum of ANTs varied from 532 to 2630 mg/100 g (d.w.). Also, *Rachtan-Janicka* et al. [55] showed the differences in the content of antioxidants compounds in BC fruits harvested in Poland, reporting a high level of phenolics (166-198 mg/100 g f.w.) and anthocyanins (146-179 mg/100 g f.w.) in BC berries with the highest level of these compounds observed in '*Titania*' cultivar.

HEALTH BENEFITS OF BLACKCURRANT ANTHOCYANINS

Anthocyanins (ANTs) are found mainly in blackcurrant fruit skins, but in small amount they also present in leaves [65] and constitute nearly 90% of total polyphenolic compounds present in BC [17]. In addition, 3-O-rutinoside and 3-O-glucoside of cyanidin and delphinidin are the major ANTs [Fig. 2] [15], however they are known from its low bioavailability. The results from the study of *Röhrig* et al. [58] with 5 healthy volunteers (aged 24-32 years) showed that the a single ingestion of 1.5 g BC extract (dissolved in a volume of 200 mL) containing delphinidin-3-rutinoside and cyanidin-3-rutinoside (at a dose of 190±7 mg and 172±8, respectively) showed a highest plasma and urine ANTs concentration after two hours of ingestion, but the recoveries of both tested bioactive compounds in urine samples were 0.04±0.01% (for delphinidin-3-O-rutinoside) and 0.048±0.016% (for cyanidin-3-O-rutinoside). The ANTs exert in organism a direct effects through the intestinal absorption or they may influence indirectly *via* microbiota environment

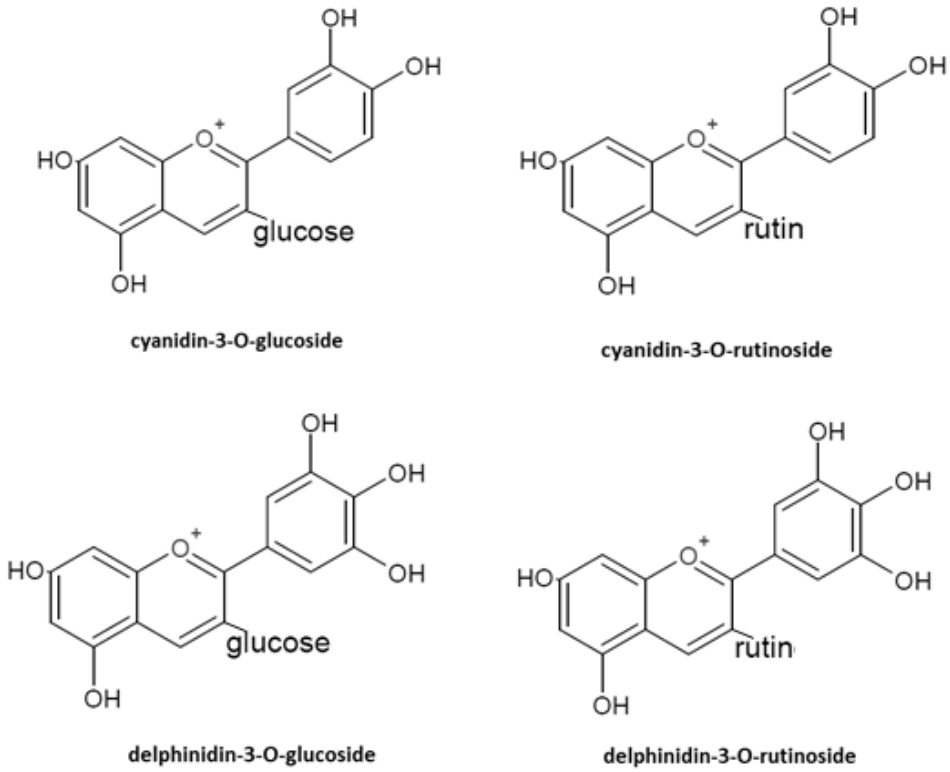

Figure 2. The chemical structure of the main anthocyanins in blackcurrant

[22]. It is known that human microbiota participate in enzymatic bioconversion of bioactive compounds and contributes to its higher antioxidative potential [66]. Despite of its low plasma concentration, ANTs reveal a significant health-promoting properties.

Oxidative stress (OS) is a state of accumulation of reactive oxygen species (ROS) and is observed as a result of an imbalance between pro-oxidative and antioxidant processes. It occurs as a result of excessive production or insufficient degradation of ROS. The biological consequences of oxidative stress include changes in gene expression, inhibition of proliferation, disruption in intercellular communication, that lead to tissue damage and organs dysfunction [31].

The findings from *in vivo* [11], *in vitro* [18] and clinical [13, 25] studies demonstrate a protective role of polyphenols in exerting anti-oxidative effects that decrease the risk of certain non-communicable chronic diseases (chronic inflammatory diseases, some type of cancers, atherosclerosis, type 2 diabetes, neurodegenerative diseases) [61]. Consumption a raw and processed blackcurrants (*i.e.* juices and products with fruit extract), may support the nutrition therapy of cardiovascular diseases, eye diseases and normalize the lipid profile of the blood plasma and obesity) [33, 38, 39, 57]. Additionally, the beneficial composition of unsaturated fatty acids present in fruit seed may support the treatment of autoimmune diseases [14].

ANTI-INFLAMMATORY EFFECTS OF BLACKCURRANT

Inflammation is a complex reaction of organism caused by exogenous (pathogens, allergens, irritants or toxic substances) and endogenous (signals coming from stressed cells, damaged tissues or extracellular matrix breakdown) factors [46]. The mediators of inflammatory pathway (pro-inflammatory cytokines and chemokines, bioactive amines, eicosanoids and bradykinin) can make functional changes in many tissues. The acute phase of inflammation can manifest as swelling, pain, redness, heat or tissue malfunction. When an inflammatory response is triggered by an infection, an immune response is induced by engaging a specific immune cells (like monocytes/macrophages and neutrophils). [45]. This process is mediated by enzyme (cyclooxygenase 2, COX-2) that transforms arachidonic acid to pro-inflammatory prostaglandins (eicosanoids) [56].

Anti-inflammatory potential of BC polyphenols has been widely investigated during last years. A promising results regarded BC treatment come from experiments with obesity-associated inflammation [34]. Diet-induced obesity in animal models is a well-known causative agent of moderate systemic inflammation. *Lee* et al. [38] investigated the effects of BC extract (BCE) in a mice model of obesity-induced nonalcoholic steatohepatitis. The authors reported that feeding mice (for 24 weeks) with 6% of whole blackcurrant extract with high-fat/high-sucrose (HFHS) diet caused an inhibition of pro-inflammatory M1 macrophages infiltration in the liver and down-regulated the hepatic *Tlr4* (toll-like receptor) gene expression when compared with feeding with HFHS diet alone. Moreover, BCE significantly reduced liver weights and triacylglycerol accumulation in liver. The similar results were also obtained by *Nanashima* et al. [48] in the study with ovariectomized (OVX) *Sprague-Dawley* female rats. A 3-month dietary experiment, during which the rats were fed with AIN-93M feed with 3% of BCE (as commercially available supplement contained 38g/100g BCE) revealed a lower gene expression of pro-inflammatory cytokines (*Tnfa*, *Il6* and *Ilb*) in OVX rats compared to control fed group. The improvement in cytokines genes expression were also accompanied by a favorable histological indices in liver. The NAFLD (nonalcoholic fatty liver disease) activity score was markedly decreased in rats fed with BCE compared with control OVX group. In turn, ANTs-rich BC extract ("Currantex 30"; 10 μg/mL total anthocyanins) has been shown to inhibit inflammation response in lungs, eosinophilia, and CCL2 chemokine release in a mouse model of ovoalbumin-induced airway system inflammation. Moreover, the flow-cytometry analysis revealed a significant decrease in total infiltrating immune cells in lungs of mice exposed to inflammation and simultaneously treated with BC extract [62].

In vitro studies with ANTs also confirm a significant impact in alleviation of chronic inflammation through the inhibition of NF-κB signaling. *Ferrari* et al. [23] reported a protective role of cyanidin-3-O-glucoside in an acute *in vitro* model of inflammation. The colon cancer epithelial (Caco-2) cells were exposed to TNFα to induce the mediators of inflammation, and pre-treatment the cells with cyanidin-3-O-glucoside (20-40 μM for 24 hours) reversed the changes induced by TNFα. There was also observed an inhibition of acute inflammation *via* down-regulation of IL-6 mRNA and COX-2 protein expression in colon cells after the exposure to cyanidin-3-O-glucoside. In addition, ANT treatment was also able to induce the expression of Nrf2 transcription factor in order induce the anti-oxidative mechanisms. The similar results were also reported by *Olejnik* et al. [50]. The gastrointestinal digested a freeze-dried BC powder (1mg/mL) down-regulated (by 54%) IL-8 and COX-2 (by 17%) expression in Caco-2 cells. Moreover, there were also observed anti-inflammatory effects in RAW264.7 macrophages. A down-regulation of IL1α (by 76%), IL-1β (by 91%), IL-6 (by 61%), TNFα and

COX-2 expression in LPS-stimulated macrophages in response to BC fruit extract was also noted.

The results from other *in vitro* studies have been proposed a positive effects of BC polyphenolic compounds in alleviation of lung inflammation and risk of allergic asthma. *Hurst* et al. [28], using a human alveolar epithelial (A549) cells, investigated the effects of BC ANTs and proanthocyanidins on eotaxin-3 (CCL26). This chemokine is highly expressed upon stimulation by IL-4 and IL-13 derived from activated Th2 cells, what is frequently observed in asthma patients. What is more, the beneficial effect seemed to be more pronounced for BC proanthocyanidins than for ANTs.

Of the large body of research regarding the anti-inflammatory effects of BC, there are still little evidence from clinical and epidemiological studies, and existing findings bring contradictory results. A randomized, double-blind, placebo-controlled cross-over ANTHONIA study [37] with 30 healthy female students (aged 24.6±1.2 years) did not reveal any significant changes in inflammatory markers (TNF-α, monocyte chemoattractant protein-1 (MCP-1), high-sensitive C-reactive peptide (hs-CRP), IL-2, IL-6, IL-8, and IL-10) after a 14-day intervention with anthocyanin-rich juice or smoothie (contained 840±10 or 983±37 mg/mL ANTs in a volume of 330 mL/d, respectively). In contrast, *Aboonabi* and *Aboonabi* [1] reported that a 4-week dietary intervention (with 320 mg/day of ANTs extracted from wild Norwegian bilberries and blackcurrant) in patients with metabolic syndrome showed significant anti-inflammatory effects. ANTs supplementation caused a down-regulation of Nf-κB-depended pro-inflammatory cytokines (TNF-α, IL-1α and IL-6), as well as COX-2 gene expression in blood cells. Moreover, the another paper from the same study of *Aboonabi* et al. [2] revealed significant reduction of serum hs-CRP following ANTs dietary intervention. The effects of BC ANTs on selected NF-κB-related chemokines were reported in the study conducted by *Karlsen* et al [30]. A group of 118 healthy men and women (aged 40-74 years) were treated with Medox® capsules (300 mg/d for 3 weeks; n=59) with purified ANTs from bilberry and blackcurrant or were subjected to placebo (n=59). There was observed a significant reduction in plasma pro-inflammatory IL-8 (CXCL8) cytokine, RANTES (CCL5) and INFα (NF-κB activation inducer) chemokines.

The subclinical endothelium inflammation is a pivotal factor increasing the risk of atherosclerosis. The pro-oxidant state in endothelium contributes to the modification of monocytes into foam cells (macrophages that absorb oxLDL, oxidized low-density lipoproteins), that release the reactive oxygen species and stimulate the cell adhesion molecules. The study of *Khan* et al. [32] performed among 64 healthy adults who consumed a drink (250 ml, 4 times a day for 6 weeks) with lower or higher content of BC juice (6.4% BC juice with 4 mg/100 ml total anthocyanins or 20% BC juice with 14.3 mg/100 ml total anthocyanins, respectively) compared to placebo drink. The volunteers, who have consumed BC drink with higher anthocyanins level revealed a decreased flow-mediated dilation (FMD%), a parameter related to endothelial reactivity, and lower plasma F2-isoprostanes level (a marker of oxidative stress), compared to placebo group.

ANTI-CANCER PROPERTIES OF BLACKCURRANT

The chemopreventive activity of BC bioactive compounds extensively studied by different authors in *in vitro* and *in vivo* experiments are related to multiple mechanisms [19, 53]. The ANTs possess the ability to arrest a cell cycle and induce apoptosis [44, 47], reduce the risk of DNA damage, and exert an anti-mutagenic action [70].

Diaconeasa et al. [20] studied the anti-proliferative effects (using MTT assay) of ANT extract obtained from BC juice on murine melanoma (B16F10), ovarian carcinoma (A2780) and cervical cancer (HeLa) cell lines. The glycosylated ANTs from BC revealed a dose-dependent reduction in cell proliferation with IC_{50} values of 224 μg/mL, 259.8 μg/mL and 281 μg/mL (in case of B16F10, A2780, and HeLa cells, respectively). It is commonly known that chronic oxidative stress and inflammation cause a higher risk of tumorigenesis. Moreover, the imbalance in redox state in the cells (oxidative environment) stimulates transcription of antioxidative enzymes. This process is controlled at transcriptional level *via* c-active sequences (antioxidant response elements, ARE) that are regulated mainly by Nrf2 transcriptional factor [12]. Anthocyanins, due to their three hydroxyl groups on B-ring and one on C-ring, can act with antioxidant response elements (ARE) *via* Keap1-Nrf2 signaling pathway and stimulate the expression of antioxidative enzymes (such as glutathione reductase, glutathione peroxidase, glutathione transferase and quinone oxidoreductase) [43]. The promising findings on anti-cancer properties of ANTs were shown by *Li* et al. [42]. The authors tested the combined effects of cisplatin (5μg/mL) and cyanidin-3-O-glucoside (400 μg/mL) on HeLa cells and observed that the combination of two substances inhibits the proliferation more distinctively compared to the administration of cisplatin alone. Cyanidin-3-O-glucoside in combination with chemotherapeutic drug contributed to more increased apoptosis and cell cycle arrest in G1 phase than it was observed following cisplatin treatment. It can be

postulated that flavonoids could enhance the activity of chemotherapy drugs. A similar, promising results of anti-tumor properties of ANTs were reported by *Li* et al. [41], who conducted *in vitro* experiments with human epidermal growth factor receptor 2 (HER-2)-positive breast cancer cell lines (MDA-MB-453, BT474, and MDA-MB-453R, BT474R as trastuzumab-resistant lines). The cells were exposed to cyanidin-3-O-glucoside and peonidin-3-O-glucoside (P3G) at concentration ranged from 0.003 to 50 μM. Both tested ANTs (at 5 μM concentration) downregulated phosphorylation of HER-2, AKT (Ser473) and p44/42 MAPK (mitogen-activated protein kinase) kinases. Furthermore, the 24- and 48-hour exposure the cells to cyanidin-3-O-glucoside (5 μM) and peonidin-3-O-glucoside (5 μM) resulted in increased apoptosis. The overexpression of the HER2 gene is responsible for a poor prognosis of recovery form breast cancer, as well as increased risk of an invasive stage and tumor metastasis. The beneficial antiproliferative effect of BC extract was investigated by *Olsson* et al. [51] who showed a concentration-dependent (0.025-0.5%) reduction in cell proliferation of human colon cancer HT29 line. What is more, due to high concentration of vitamin C in BC extract, a synergistic anti-proliferative effect of both vitamin C and ANTs can be postulated.

Studies conducted in animal models with experimentally-induced tumors have also provided a convincing evidence of chemopreventive effect of BC extract [9, 25]. In the study conducted by *Bishayee* et al. [10], male Sprague-Dawley rats fed with diet supplemented with BC skin extract (BCSE; 100 or 500 mg/kg of body weight) were subjected to dietylnitrosoamine (DENA)-induced hepatocellular carcinoma. The macroscopic observations revealed a significantly lower incidence of liver nodules in rats feeding with diet with higher BCSE content compared to DENA exposed animals. The liver specimens from rats in DENA group demonstrated a significantly higher value of proliferative nuclear antigen(PCNA)-positive cells score (a marker of increased cell proliferation) than animals treated with BCSE (at 500 mg/kg bw). At the same time, a higher liver apoptotic index in the liver was observed in animals fed with feed with higher content of BCSE *versus* DENA control group.

ANTI-MICROBIAL ACTIVITIES OF BC ANTHOCYANINS

The antimicrobial activities of dietary plant bioactive compounds against pathogenic bacteria have been extensively reviewed by many authors [3, 16, 54]. Regarding the polysaccharides present in blackcurrant seeds, *Lengsfeld* et al. [40] in *in vitro* study observed a lower adhesion rate of *Helicobacter pylori* to gastric mucosa. The anti-adhesive properties of raw polysaccharides from BC seeds result from the presence of acidic high-molecular weight galactans, which are able to bind to *H. pylori* receptors, and thus can inhibit their ability to implementation in gastric epithelium. In turn, *Kranz* et al. [36] showed an efficient antimicrobial activity of BC extract in suppression of oral *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans, Actinomyces naeslundii, Fusobacterium nucleatum* bacteria colonization compared with 0.2% chlorhexidine, an antimicrobial agent commonly used in mouthwashes. It is postulated that a natural compounds from BC might be a promising tool in the prevention of *periodontitis.* The findings from a 6-week *in vivo* experiment (with Sprague Dawley male rats) [52] proved that BC anthocyanins can modify the intestinal bacterial microbiota. The BC extract (Currantex; 32% w/w of total ANTs content) added to the rats feed (at a dose of 40 g/kg) caused a higher relative abundance of *Bacteroides-Prevotella-Porphyromonas* group and *Lactobacillus* spp., and lower *Bifidobacterium* spp. *Clostridium perfringens.*

POSSIBILITIES OF A PRACTICAL USE OF PHENOLIC COMPOUNDS FROM THE WASTEBLACKCURRANT PRODUCTS

The processing of BC fruit offers an enormous possibility of reusing the BC waste products as valuable functional additives in food. *Baranowski* et al. [8] noticed that residue after BC juice production may be a nutritive additive to “ice” fruit tea or jellies. BC pomace is a rich source of phenolic compounds, especially anthocyanins (up to 49% w/w) from fruit skins [29], soluble (28-30% w/w) and insoluble (47% w/w) dietary fiber [4], essential fatty acids and phytosterols [21] present in seeds.

The cereal-based products are a very popular food products that are enriched with BC to increase organolepic and nutrition value. *Gagneten* et al. [24] reported that the fortification of gluten-free cookies with of 3.75% of BC extract from by-product after juice production caused an 62% increase in total phenolic content without any negative organoleptic changes. The same, *Shmidt* et al. [60] proposed to enrich a savoury crackers in BC pomace. The flour replacement even up to 30% can still make an acceptable product for consumers.

The effects of consumption dietary extract from BC pomace rich in antioxidants was also studied in *in vivo* models [29]. The experiment conducted on 20 New Zealand white rabbits confirmed that feed supplementation with 1.5% of BC pure extract from pomace reduced insulin resistant markers,plasma

triglyceride and total cholesterol concentration in animals maintained on a high-fat diet.

CONCLUSIONS

Based on the research conducted in recent years, BC fruits demonstrate a significant therapeutic potential. The anti-inflammatory, anti-oxidative and anti-cancer properties of BC have been confirmed in many studies. There is still a little evidence coming from human clinical and randomized controlled studies about the beneficial effects of BC polyphenolic compounds, therefore the extrapolation the results obtained from *in vitro* and *in vivo* models to humans should be made with caution. Undoubtedly, blackcurrant fruits consumed at optimal amounts as fresh or used as by-products after juice production additives or supplements exert the anti-oxidative effects and therefore can be used in prophylaxis of many non-communicable chronic diseases, as well as can be used as therapeutic factor to diminish chronic inflammation. A further research perspectives on BC compounds should concern on the mechanisms of anti-cancer activity and the possibility of designing preparations containing blackcurrant to support cancer therapy.

Conflict of interest

The author declares no conflict of interest.

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REFERENCES

1. *Aboonabi A., Aboonabi A.*: Anthocyanins reduce inflammation and improve glucose and lipid metabolism associated with inhibiting nuclear factor-kappaB activation and increasing PPAR-γ gene expression in metabolic syndrome subjects. Free Radic Biol Med 2020;150:30-39 doi: 10.1016/j.freeradbiomed.2020.02.004.
2. *Aboonabi A., Meyer R.R., Gaiz A., Singh I.*: Anthocyanins in berries exhibited anti-atherogenicity and antiplatelet activities in a metabolic syndrome population. Nutr Res 2020;76:82-93 doi: 10.1016/j.nutres.2020.02.011.
3. *Abouzeed Y.M., Zgheel F., Elfahem A.A., Almagarhe M.S., Dhawi A., Elbaz A., Hiblu M.A., Kammon A., Ahmed M.O.*: Identification of phenolic compounds, antibacterial and antioxidant activities of raisin extracts. Open Vet J 2018;8(4):479-484 doi: 10.4314/ovj.v8i4.20.
4. *Alba K., Macnaughtan W., Laws A.P., Foster T.J., Campbell G.M., Kontogiorgos V.*: Fractionation and characterisation of dietary fibre from blackcurrant pomace. Food Hydrocoll 2018;81:398-408 doi:10.1016/j.foodhyd.2018.03.023.
5. *Angelino D., Godos J., Ghelfi F., Tieri M., Titta L., Lafranconi A., Marventano S., Alonzo E., Gambera A., Sciacca S., Buscemi S., Ray S., Galvano F., Del Rio D., Grosso G.:* Fruit and vegetable consumption and health outcomes: An umbrella review of observational studies. Int J Food Sci Nutr 2019; 70(6): 652-667 doi: 10.1080/09637486.2019.1571021.
6. *Archaina D., Leiva G., Salvatori D., Schebor C.*: Physical properties of spray-dried powders from blackcurrant juice and extracts obtained from the waste of juice processing. Food Sci Technol Int 2017;24(1):78-86 doi: 10.1177/1082013217729601.
7. *Archaina D., Sosa N., Rivero R., Schebor C.*: Freeze-dried candies from blackcurrant (Ribes nigrum L.) and yoghurt. Physicochemical and sensorial characterization. LWT 2019;100: 444-449. doi: 10.1016/j.lwt.2018.10.049.
8. *Baranowski K., Baca E., Salamon A., Michalowska D., Meller D., Karas M.:* Możliwości odzyskiwania i praktycznego wykorzystania związków fenolowych z produktów odpadowych: z wytłoków z czarnej porzeczki i aronii oraz z chmielin [Possibilities of retrieving and making a practical use of phenolic compounds from the waste products: blackcurrant and chokeberry pomace and spent hops]. Żywność Nauka Technologia Jakość 2009;4(65): 100 – 109 (in Polish).
9. *Bishayee A, Háznagy-Radnai E, Mbimba T, Sipos P, Morazzoni P, Darvesh AS, Bhatia D, Hohmann J.*: Anthocyanin-rich black currant extract suppresses the growth of human hepatocellular carcinoma cells. Nat Prod Commun 2010;5(10):1613-8.
10. *Bishayee A, Mbimba T, Thoppil RJ, Háznagy-Radnai E, Sipos P, Darvesh AS, Folkesson HG, Hohmann J.*: Anthocyanin-rich black currant (Ribes nigrum L.) extract affords chemoprevention against diethylnitrosamine-induced hepatocellular carcinogenesis in rats. J Nutr Biochem 2011;22(11):1035-46 doi: 10.1016/j.jnutbio.2010.09.001.
11. *Bishayee A, Thoppil RJ, Mandal A, Darvesh AS, Ohanyan V, Meszaros JG, Háznagy-Radnai E, Hohmann J, Bhatia D.*: Black currant phytoconstituents exert chemoprevention of diethylnitrosamine-initiated hepatocarcinogenesis by suppression of the inflammatory response. Mol Carcinog 2013;52(4):304-317 doi: 10.1002/mc.21860.
12. *Biswas M, Chan JY.:* Role of Nrf1 in antioxidant response element-mediated gene expression and beyond. Toxicol Appl Pharmacol 2010; 244(1):16-20 doi: 10.1016/j.taap.2009.07.034.
13. *Braakhuis AJ, Hopkins WG, Lowe TE.*: Effects of dietary antioxidants on training and performance in female runners. Eur J Sport Sci 2014;14(2):160-168 doi: 10.1080/17461391.2013.785597.
14. *Cameron M, Gagnier JJ, Little CV, Parsons TJ, Blümle A, Chrubasik S.*: Evidence of effectiveness of herbal medicinal products in the treatment of arthritis. Part 2: Rheumatoid arthritis. Phytother Res 2009;23(12):1647-1662 doi: 10.1002/ptr.3006.

15. *Cao L., Park Y., Lee S., Kim D.O.:* Extraction, Identification, and Health Benefits of Anthocyanins in Blackcurrants (Ribes nigrum L.). Appl Sci 2021;11(4), 1863 doi: 10.3390/app11041863.
16. *Cesoniene L, Jasutiene I, Sarkinas A.*: Phenolics and anthocyanins in berries of European cranberry and their antimicrobial activity. Medicina (Kaunas) 2009;45(12):992-9
17. *Cortez R.E., Gonzalez de Mejia E.*: Blackcurrants (Ribes nigrum): A Review on chemistry, processing, and health benefits. J food Sci 2019;84(9), 2387-2401 doi: 10.1111/1750-3841.14781.
18. *Desjardins J., Tanabe S., Bergeron C., Gafner S., Grenier D.*: Anthocyanin-rich black currant extract and cyanidin-3-O-glucoside have cytoprotective and anti-inflammatory properties. J Med Food 2012;15(12):1045-1050 doi: 10.1089/jmf.2011.0316.
19. *Dharmawansa K.V.S., Hoskin D.W., Rupasinghe H.P.V.*: Chemopreventive Effect of Dietary Anthocyanins against Gastrointestinal Cancers: A Review of Recent Advances and Perspectives. Int J Mol Sci. 2020;21(18):6555 doi:10.3390/ijms21186555.
20. *Diaconeasa Z., Leopold L., Rugină D., Ayvaz H., Socaciu C.*: Antiproliferative and antioxidant properties of anthocyanin rich extracts from blueberry and blackcurrant juice. Int J Mol Sci 2015;16(2):2352-2365 doi:10.3390/ijms16022352.
21. *Dobson G., Shrestha M., Hilz H., Karjalainen R., McDougall G., Stewart D.*: Lipophilic components in black currant seed and pomace extracts. Eur J Lipid Sci Tech 2012;114(5), 575-582. doi: 10.1002/ejlt.201100313.
22. *Fernandes I., Faria A., Calhau C., de Freitas V., Mateus, N.*: Bioavailability of anthocyanins and derivatives. J Func Foods 2014; 7, 54-66. doi: 10.1016/j.jff.2013.05.010.
23. *Ferrari D, Speciale A, Cristani M, Fratantonio D, Molonia MS, Ranaldi G, Saija A, Cimino F.*: Cyanidin-3-O-glucoside inhibits NF-kB signalling in intestinal epithelial cells exposed to TNF-α and exerts protective effects via Nrf2 pathway activation. Toxicol Lett 2016;264:51-58 doi: 10.1016/j.toxlet.2016.10.014.
24. *Gagneten M., Archaina D.A., Salas M.P., Leiva G.E., Salvatori D.M., Schebor C.*: Gluten-free cookies added with fibre and bioactive compounds from blackcurrant residue. Food Sci Technol Int 2021;56(4),1734-1740 doi: 10.1111/ijfs.14798.
25. *Gopalan A., Reuben S.C., Ahmed S., Darvesh A.S., Hohmann J., Bishayee A.*: The health benefits of blackcurrants. Food Funct 2012;3(8):795-809 doi: 10.1039/C2FO30058C.
26. GUS, Production of agricultural and horticultural crops in 2019, Statistics Poland, Warsaw 2020
27. *Guzek D., Głąbska D., Groele B., Gutkowska K.*: Fruit and Vegetable Dietary Patterns and Mental Health in Women: A Systematic Review. Nutr Rev 2021 doi: 10.1093/nutrit/nuab007
28. *Hurst S.M., McGhie T.K., Cooney J.M., Jensen D.J., Gould E.M., Lyall K.A., Hurst R.D.*: Blackcurrant proanthocyanidins augment IFN-gamma-induced suppression of IL-4 stimulated CCL26 secretion in alveolar epithelial cells. Mol Nutr Food Res 2010; 54 Suppl 2:S159-170 doi: 10.1002/mnfr.200900297.
29. *Jurgoński A., Juśkiewicz J., Zduńczyk Z., Matusevicius P., Kołodziejczyk K.*: Polyphenol-rich extract from blackcurrant pomace attenuates the intestinal tract and serum lipid changes induced by a high-fat diet in rabbits. Eur J Nutr 2014;53(8):1603-1613 doi: 10.1007/s00394-014-0665-4.
30. *Karlsen A., Retterstøl L., Laake P., Paur I., Bøhn S.K., Sandvik L., Blomhoff R.*: Anthocyanins inhibit nuclear factor-kappaB activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults. J Nutr 2007;137(8):1951-1954 doi: 10.1093/jn/137.8.1951.
31. *Karolkiewicz J.*: Wpływ stresu oksydacyjnego na strukturę i funkcję komórek oraz konsekwencje wynikające z uszkodzeń wolnorodnikowych - związek z procesami starzenia [Effects of oxidative stress and free-radicalmediated damage on cell structure and function - connection to aging processes]. Gerontol Pol 2011;19(2):59-67 (in Polish).
32. *Khan F., Ray S., Craigie A.M., Kennedy G., Hill A., Barton K.L., Broughton J., Belch J.J.*: Lowering of oxidative stress improves endothelial function in healthy subjects with habitually low intake of fruit and vegetables: a randomized controlled trial of antioxidant- and polyphenol-rich blackcurrant juice. Free Radic Biol Med 2014;72:232-237 doi: 10.1016/j.freeradbiomed.2014.04.006.
33. *Khoo H.E., Ng H.S., Yap W.S., Goh H.J.H., Yim H.S.*: Nutrients for Prevention of Macular Degeneration and Eye-Related Diseases. Antioxidants (Basel) 2019;8(4):85 doi: 10.3390/antiox8040085.
34. *Kim B., Lee S.G., Park Y.K., Ku C.S., Pham T.X., Wegner C.J., Yang Y., Koo S.I., Chun O.K., Lee J.Y.:* Blueberry, blackberry, and blackcurrant differentially affect plasma lipids and pro-inflammatory markers in diet-induced obesity mice. Nutr Res Pract 2016;10(5):494-500 doi: 10.4162/nrp.2016.10.5.494.
35. *Kowalski R, Gonzalez de Mejia E.*: Phenolic composition, antioxidant capacity and physical characterization of ten blackcurrant (Ribes nigrum) cultivars, their juices, and the inhibition of type 2 diabetes and inflammation biochemical markers. Food Chem 2021;359:129889 doi: 10.1016/j.foodchem.2021.129889.
36. *Kranz S., Guellmar A., Olschowsky P., Tonndorf-Martini S., Heyder M., Pfister W., Reise M., Sigusch B.:* Antimicrobial Effect of Natural Berry Juices on Common Oral Pathogenic Bacteria. Antibiotics (Basel). 2020; 24;9(9):533 doi: 10.3390/antibiotics9090533.
37. *Kuntz S., Kunz C., Herrmann J., Borsch C.H., Abel G., Fröhling B., Dietrich H., Rudloff S.*: Anthocyanins from fruit juices improve the antioxidant status of healthy young female volunteers without affecting anti-inflammatory parameters: results from the randomised, double-blind, placebo-controlled, cross-over ANTHONIA (ANTHOcyanins in Nutrition Investigation Alliance) study. Br J Nutr 2014;28;112(6):925-936 doi: 10.1017/S0007114514001482.

38. *Lee Y., Pham T.X., Bae M., Hu S., O'Neill E., Chun O.K., Han M.J., Koo S.I., Park Y.K., Lee J.Y.:* Blackcurrant (Ribes nigrum) Prevents Obesity-Induced Nonalcoholic Steatohepatitis in Mice. Obesity (Silver Spring). 2019;27(1):112-120 doi: 10.1002/oby.22353.
39. *Lee Y.M., Yoon Y., Yoon H., Park H.M., Song S., Yeum K.J.:* Dietary Anthocyanins against Obesity and Inflammation. Nutrients. 2017;9(10):1089 doi: 10.3390/nu9101089.
40. *Lengsfeld C., Deters A., Faller G., Hensel A.:* High molecular weight polysaccharides from black currant seeds inhibit adhesion of Helicobacter pylori to human gastric mucosa. Planta Med 2004;70(7):620-626 doi: 10.1055/s-2004-827184.
41. *Li X., Xu J., Tang X., Liu Y., Yu X., Wang Z., Liu W.:* Anthocyanins inhibit trastuzumab-resistant breast cancer in vitro and in vivo. Mol Med Rep. 2016;13(5):4007-4013 doi: 10.3892/mmr.2016.4990.
42. *Li X., Zhao J., Yan T., Mu J., Lin Y., Chen J., Deng H., Meng X.*: Cyanidin-3-O-glucoside and cisplatin inhibit proliferation and downregulate the PI3K/AKT/mTOR pathway in cervical cancer cells. J Food Sci 2021;86(6):2700-2712 doi: 10.1111/1750-3841.15740.
43. *Lin B.W., Gong C.C., Song H.F., Cui Y.Y.*: Effects of anthocyanins on the prevention and treatment of cancer. Br J Pharmacol 2017;74(11):1226-1243 doi: 10.1111/bph.13627.
44. *Liu B., Li Z.:* Black Currant (Ribes nigrum L.) Extract Induces Apoptosis of MKN-45 and TE-1 Cells Through MAPK- and PI3K/Akt-Mediated Mitochondrial Pathways. J Med Food 2016;19(4):365-373 doi: 10.1089/jmf.2015.3521.
45. *Medzhitov R.:* Inflammation 2010: new adventures of an old flame. Cell 2010;140(6):771-776 doi: 10.1016/j.cell.2010.03.006.
46. *Medzhitov R.:* Origin and physiological roles of inflammation. Nature 2008; 24;454(7203):428-435 doi: 10.1038/nature07201.
47. *Nanashima N., Horie K., Chiba M., Nakano M., Maeda H., Nakamura T.*: Anthocyanin rich blackcurrant extract inhibits proliferation of the MCF10A healthy human breast epithelial cell line through induction of G0/G1 arrest and apoptosis. Mol Med Rep 2017;16(5):6134-6141 doi: 10.3892/mmr.2017.7391.
48. *Nanashima N., Horie K., Yamanouchi K., Tomisawa T., Kitajima M., Oey I., Maeda H.*: Blackcurrant (Ribes nigrum) Extract Prevents Dyslipidemia and Hepatic Steatosis in Ovariectomized Rats. Nutrients 2020;12(5):1541 doi: 10.3390/nu12051541.
49. *Nour V., Trandafir I., Ionica M.E.*: Ascorbic acid, anthocyanins, organic acids and mineral content of some black and red currant cultivars. Fruits 2011;66(5), 353-362 doi: 10.1051/fruits/2011049.
50. *Olejnik A., Kowalska K., Olkowicz M., Juzwa W., Dembczyński R., Schmidt M.:* A Gastrointestinally Digested Ribes nigrum L. Fruit Extract Inhibits Inflammatory Response in a Co-culture Model of Intestinal Caco-2 Cells and RAW264.7 Macrophages. J Agric Food Chem 2016;64(41):7710-7721 doi: 10.1021/acs.jafc.6b02776.
51. *Olsson M.E., Gustavsson K.E., Andersson S., Nilsson A., Duan R.D.*: Inhibition of cancer cell proliferation in vitro by fruit and berry extracts and correlations with antioxidant levels. J Agric Food Chem 2004;52(24):7264-7271 doi: 10.1021/jf030479p.
52. *Paturi G., Butts C.A., Monro J.A., Hedderley D.:* Effects of Blackcurrant and Dietary Fibers on Large Intestinal Health Biomarkers in Rats. Plant Foods Hum Nutr. 2018;73(1):54-60 doi: 10.1007/s11130-018-0652-7.
53. *Putta S., Yarla N.S., Peluso I., Tiwari D.K., Reddy G.V., Giri P.V., Kumar N., Malla R., Rachel V., Bramhachari P.V., Reddy D.R.S., Bade R., Mannarapu M., Barreto G.E., Lu D.Y., Tarasov V.V., Chubarev V.N., Ribeiro F.F., Scotti L., Scotti M.T., Kamal M.A., Ashraf G.M., Aliev G., Perry G., Sarker S.D., Rao C.V., Bishayee A.*: Anthocyanins: Multi-Target Agents for Prevention and Therapy of Chronic Diseases. Curr Pharm Des 2017;23(41):6321-6346 doi: 10.2174/1381612823666170519151801.
54. *Puupponen-Pimiä R., Nohynek L., Alakomi H.L., Oksman-Caldentey K.M.*: The action of berry phenolics against human intestinal pathogens. Biofactors 2005;23(4):243-251 doi: 10.1002/biof.5520230410.
55. *Rachtan-Janicka J., Ponder, A., Hallmann, E.*: The Effect of Organic and Conventional Cultivations on Antioxidants Content in Blackcurrant (Ribes nigrum L.) Species. Appl Sci 2021;11(11), 5113 10.3390/app11115113.
56. *Rajakariar R., Yaqoob M.M., Gilroy D.W.*: COX-2 in inflammation and resolution. Mol Interv 2006;6(4):199-207 doi: 10.1124/mi.6.4.6.
57. *Reis J.F., Monteiro V.V., de Souza Gomes R., do Carmo M.M., da Costa G.V., Ribera P.C., Monteiro M.C.:* Action mechanism and cardiovascular effect of anthocyanins: a systematic review of animal and human studies. J Transl Med 2016;14(1):315 doi: 10.1186/s12967-016-1076-5.
58. *Röhrig T., Kirsch V., Schipp D., Galan J., Richling E.*: Absrorption of Anthocyanin Rutinosides after consumption of a Blackcurrant (Ribes nigrum L.) Extract. J Agric Food Chem 2019;67, 6792-6797 doi: 10.1021/acs.jafc.9b01567.
59. *Šavikin K.P., Đorđević B.S., Ristić M.S., Krivokuća-Đokić D., Pljevljakušić D.S., Vulić T.*: Variation in the fatty-acid content in seeds of various black, red, and white currant varieties. Chem Biodivers 2013;10(1):157-165 doi: 10.1002/cbdv.201200223.
60. *Schmidt C., Geweke I., Struck S., Zahn S., Rohm H.:* Blackcurrant pomace from juice processing as partial flour substitute in savoury crackers: dough characteristics and product properties. Int J Food Sci Tech 2018;53(1),237-245 doi:10.1111/ijfs.13639.
61. *Serino A., Salazar G.*: Protective Role of Polyphenols against Vascular Inflammation, Aging and Cardiovascular Disease. Nutrients 2018;28;11(1):53 doi: 10.3390/nu11010053.
62. *Shaw O.M., Nyanhanda T., McGhie T.K., Harper J.L., Hurst R.D.*: Blackcurrant anthocyanins modulate CCL11 secretion and suppress allergic airway

inflammation. Mol Nutr Food Res. 2017;61(9) doi: 10.1002/mnfr.201600868.
63. *Sokół-Łętowska A., Kucharska A.Z., Wińska K., Szumny A., Nawirska-Olszańska A., Mizgier P., Wyspiańska D.*: Composition and antioxidant activity of red fruit liqueurs. Food Chem 2014;157, 533-539 doi: 10.1016/j.foodchem.2014.02.083.
64. *Tańska M., Roszkowska B., Czaplicki S., Borowska E.J., Bojarska J., Dąbrowska A.:* Effect of Fruit Pomace Addition on Shortbread Cookies to Improve Their Physical and Nutritional Values. Plant Foods Hum Nutr 2016;71(3):307-13 10.1007/s11130-016-0561-6.
65. *Teleszko M., Wojdyło A.:* Comparison of phenolic compounds and antioxidant potential between selected edible fruits and their leaves. J Funct Food 2015;14,736-746 10.1016/j.jff.2015.02.041.
66. *Tian .L, Tan Y., Chen G., Wang G., Sun J., Ou S., Chen W., Bai W.*: Metabolism of anthocyanins and consequent effects on the gut microbiota. Crit Rev Food Sci Nutr 2019;59(6):982-991 doi: 10.1080/10408398.2018.1533517.
67. *Tian Y., Laaksonen O., Haikonen H., Vanag A., Ejaz H., Linderborg K., Karhu S., Yang B.:* Compositional diversity among Blackcurrant (Ribes nigrum) cultivars originating from European countries. J. Agric. Food Chem 2019; 67, 5621-5633 doi: 10.1021/acs.jafc.9b00033.
68. *Watson A.W., Okello E.J., Brooker H.J., Lester S., McDougall G.J., Wesnes K.A.*: The impact of blackcurrant juice on attention, mood and brain wave spectral activity in young healthy volunteers. Nutr Neurosci 2019;22(8):596-606. doi: 10.1080/1028415X.2017.1420539.
69. *Woznicki T.L., Sønsteby A., Aaby K., Martinsen B.K., Heide O.M., Wold A.B., Remberg S.F.:* Ascorbate pool, sugars and organic acids in black currant (Ribes nigrum L.) berries are strongly influenced by genotype and post-flowering temperature. J Sci Food Agric 2017;97(4):1302-1309 doi: 10.1002/jsfa.7864.
70. *Yamamoto A., Nakashima K., Kawamorita S., Sugiyama A., Miura M., Kamitai Y., Kato Y.*: Protective effects of raw and cooked blackcurrant extract on DNA damage induced by hydrogen peroxide in human lymphoblastoid cells. Pharm Biol 2014;52(6):782-788 doi: 10.3109/13880209.2013.836721.
71. *Yang B., Zheng J., Laaksonen O., Tahvonen R., Kallio H.*: Effects of latitude and weather conditions on phenolic compounds in currant (Ribes spp.) cultivars. J Agric Food Chem 2013;61(14):3517-3532 doi: 10.1021/jf4000456.

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SHORT COMMUNICATION

COVID-19 AND MUCORMYCOSIS (*BLACK FUNGUS*): AN EPIDEMIC WITHIN THE PANDEMIC

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ABSTRACT

The second wave of the COVID-19 pandemic has affected India significantly with country reporting more than 400,000 cases in the month of May 2021 and health system almost collapsing. This was attributed to the new mutant strain also called as the 'Delta Strain' which led to high surge of cases across the country. As the country was stabilising over this situation, another imminent threat in the form of Covid Associated Mucormycosis (CAM) challenged the already burdened health system of India. Also called as 'Black Fungus,' cases of CAM began to rise rapidly in the last week of May 2021 with multiple states reporting steady rise in the number of cases. Based on the published literature, India contributed to approximately 71% of global cases of CAM from December 2019 to start of April 2021, with majority of the cases occurring during the second wave. The present paper focuses on the epidemic of CAM during the second wave in India highlighting the causes, symptoms and various treatment modalities that have been adopted to cure the disease. Also, spotlight has also been thrown on some other nations where cases of CAM have begun to emerge. Some key recommendations are also mentioned which can prove vital towards disease prevention.

Key words: *COVID-19, SARS-CoV-2 pandemic, mucormycosis, India, diabetes, Black fungus*

INTRODUCTION

The Novel Coronavirus disease (COVID-19, SARS-CoV-2), the deadliest pandemic of the millennium has brought the health system of the world down on its knees with millions of people falling prey to this deadly disease. Be a developing country or a developed country, countries all over the world were forced to impose a lock down as a measure of social distancing to curtail the spread of infection [10]. Wearing a face mask has become a new normal to prevent harmful aerosols from entering our respiratory system and causing the deadly infection [5]. According to the latest statistics from the World Health Organization (WHO), globally there are 176,303,596 confirmed COVID-19 cases and 3,820,026 deaths [19]. Currently, renowned pharmaceutical companies have developed vaccines (using different technologies) to protect people from this deadly disease and the process of vaccination is being carried out on priority basis by each and every nation [3]. However, still there is a large gap between the production of vaccines and population to be vaccinated. Hardly the world had emerged from the first wave of COVID-19, many countries including India experienced the second wave of the pandemic in March 2021, which was more deadlier than the first with social distancing measures failing to reduce rapidly increasing case numbers [1]. This was attributed because of the emerging triple mutant strains of the virus with high surge of cases on daily basis crippling the health system of the country [17]. According to WHO, only B.1.617.2, one of the three strains of the B.1.617, first detected in India, is a Variant of Concern (VOC) with greater

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public health risks currently associated with this strain and it has already been reported in 85 countries globally and continues to be reported in new countries across all WHO regions. This is now labelled as the 'Delta Variant' [19]. This has made the virus more transmissible and immune to vaccines or makes it cause more severe disease. As per latest reports, a new variant called as 'Delta Plus Variant' has been reported in some states in India. This variant, in addition to its increased transmissibility, has got stronger binding to receptors of lung cells and potential reduction in monoclonal antibody response [20]. Moreover, the country witnessed a heavy demand of medical oxygen and post COVID cardiac complications during the second wave owing to the increasing number of cases because of the new variant.

To make the situation worse, India witnessed another epidemic, that of mucormycosis (*Black fungus*) amidst the COVID-19 pandemic during the second wave in May 2021. More than 31,000 cases have already been reported so far across the country and more than 2100 people have died as a result of mucormycosis [8]. Majority of cases have been reported from the state of Maharashtra followed by Rajasthan, Gujarat, Madhya Pradesh, Haryana, Delhi and Punjab. Cases of mucormycosis have more than doubled in India in late 2020 compared to the corresponding months of 2019 establishing a direct correlation between COVID-19 and lethal black fungus viral infection now ravaging in almost each and every state of the country [4].

CAUSE OF MUCORMYCOSIS (*BLACK FUNGUS*)

According to the present data, *Rhizopus arrhizus* is the predominating agent causing Covid-associated Mucormycosis (CAM) in India [8]. It can be described as "black fungus," because it makes infected tissues turn black. Most mucormycosis infections are life-threatening, and various risk factors, such as diabetic ketoacidosis and neutropenia are present in most cases which can lead to further complications [15]. A majority of patients of CAM have uncontrolled diabetes. Apart from the fact that blood sugar levels are elevated with steroids, COVID-19 itself can induce damage to pancreatic islet cells. Blood sugar levels may also get elevated because of increased resistance to insulin due to the inflammatory reaction. This suggests that indiscriminate and prolonged use of steroids by doctors treating mild COVID-19 patients is the key factor in the development of mucormycosis. According to the guidelines, steroids are recommended only in moderate to severe cases of COVID-19 when oxygen levels drop [8]. Rhino-orbital-cerebral mucormycosis (ROCM) is the most common form of mucormycosis in patients suffering from diabetes mellitus. The infection develops after inhalation of fungal sporangia spores into the paranasal sinuses [4].

According to an advisory issued by the Indian Council of Medical Research, the following conditions in COVID-19 patients increase the risk of mucormycosis infection [14]:

- People with uncontrolled diabetes
- Weakening of immune system due to excessive use of steroids
- Prolonged Intensive Care Unit (ICU) stay / hospital stay
- Co-morbidities / post organ transplant / cancer
- Patients on Voriconazole therapy (used to treat serious fungal infections)

TWO-FOLD RISE IN MUCORMYCOSIS CASES IN A YEAR

According to reports of a multi-centre study conducted in 16 Indian hospitals involving both CAM and non-Covid mucormycosis (non-CAM), it was found that overall mucormycosis case fatality rate was 45.7 percent at 12 weeks [12]. It was also found that newly detected diabetes mellitus was more frequent during the evaluation of mucormycosis among CAM patients that non-CAM patients suggesting that many COVID infected patients may not have been aware of their diabetic condition when diagnosed with black fungus. India is called as diabetes capital of the world; the indiscriminate and non-judicious use of steroids in patients suffering from diabetes potentially led to the surge of mucormycosis in the country. It was reported that there was 2.1-fold rise in mucormycosis cases during the study period compared with 2019 [12].

CLINICAL FORMS AND SYMPTOMS OF MUCORMYCOSIS

On the basis of the anatomical site of involvement, there are various clinical forms of mucormycosis reported in various case series from India. ROCM is the commonest form (45–74%), followed by cutaneous (10–31%), pulmonary (3–22%), renal (0.5–9%), gastrointestinal (2–8%), and disseminated infections (0.5–9%). Breast, ear, spine, heart and bone infections are other unusual sites of infection reported in the literature from India [13].

Mucormycosis begins to manifest as skin infection in the air pockets located behind our forehead, nose, cheekbones, and in between the eyes and teeth [13]. Then it involves the eyes and lungs can even spread to the brain. It leads to blackening or discoloration over the nose, blurred or double vision, chest pain, breathing difficulties and coughing of blood particularly in case of pulmonary mucormycosis (Figure 1). Patients with disseminated infection in the brain can develop mental

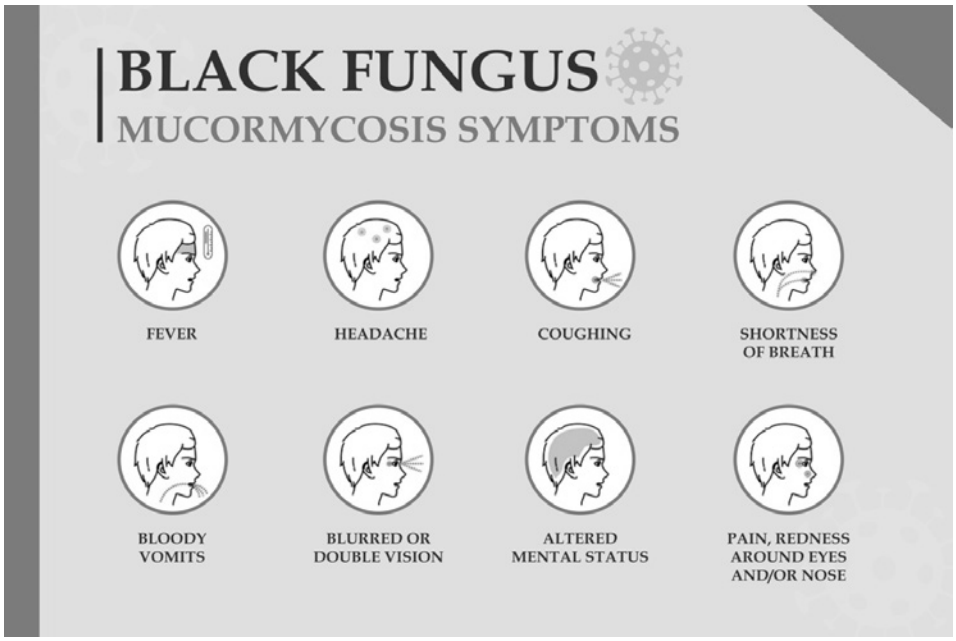

Figure 1. Symptoms of Mucormycosis (Black Fungus)

status changes or coma. According to the advisory issued by the Indian Council of Medical Research, not all cases of blocked nose should be considered as cases of bacterial sinusitis, particularly during/after the treatment of Covid-19 patients. According to medical specialists, patients report to the hospital late considering these symptoms as normal which leads to further complications. The mean duration between diagnosis of COVID-19 and onset of mucormycosis symptoms is approximately 15.6±9.6 days. A delay of even 6 days in seeking treatment doubles the 30 day mortality from 35% to 66% [16].

MANAGEMENT OF MUCORMYCOSIS

The treatment of mucormycosis starts with the early diagnosis and initiation of therapy along with the surgical debridement of the infected tissue, antifungal therapy, and managing the underlying disease. The first-line drug of choice is Amphotericin B (AmB) and later on posaconazole and isavuconazole are prescribed depending on the condition of the patient [6]. Gap in treatment protocol and the financial constraints of patients to afford liposomal AmB are the major drawbacks in managing patients suffering from mucormycosis in India. Moreover, due to the rapid surge of mucormycosis cases in India, there is shortage of AmB.

Different modes of therapy and mortality rates in Indian population are depicted in Figure 2 (it should be duly noted that data shown is based on different studies shown in the main article) [13]. According to existing data, mortality rate was low in patients treated with a combination of AmB and surgical

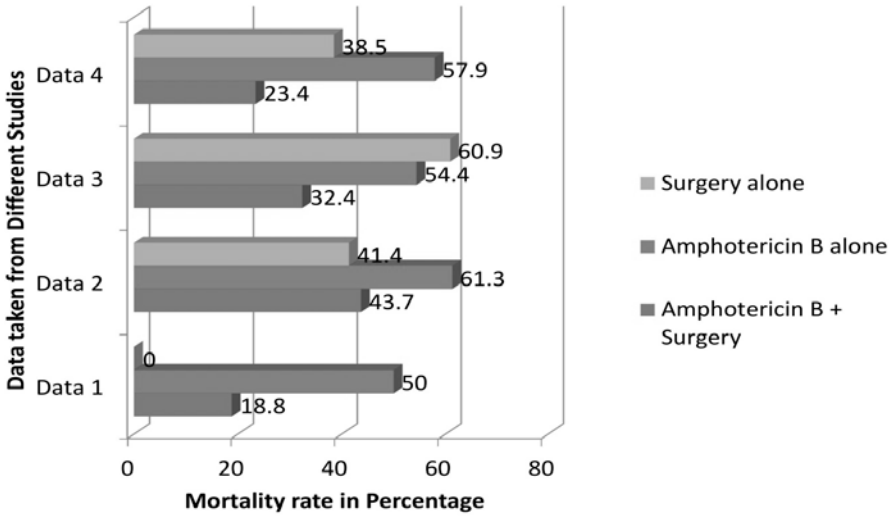

Figure 2. Different modes of therapy and mortality rates in Indian population

debridement of the infected tissue (19–44%) compared with AmB monotherapy (50–61%). These findings are in congruence with the global data [7]. Posaconazole and isavuconazole were used as salvage therapy in the treatment of mucormycosis. A study assessing the safety and efficacy of posaconazole in ROCM patients was conducted in South India. The study findings showed no mortality; there was complete resolution of the disease in 66.6% of the patients and remaining patients reported significant reduction of the disease [9]. The new anti-Mucorales drug, isavuconazole proved to be equally effective as AmB, however, it has been recently introduced in Indian market and its efficacy is still to be assessed in Indian population.

Surgical removal of the infected (necrotic) tissue, debridement, and enucleation of eye (if involved), also proves to be lifesaving. Other clinical interventions like proper cleaning and sterilization of humidifiers and ventilators, utilization of disposable or disinfected personal protective equipment, and proper hygiene maintenance are also equally important in disease prevention [4]. It is also recommended that everyone should follow pristine oral hygiene measures like regular tooth brushing, not using a common holder and disinfecting the toothbrush each time after use by dipping it in some antiseptic mouthwash. One should also dispose the older toothbrush post recovery from COVID-19.

CASES OF MUCORMYCOSIS REPORTED IN THE AMERICAS AND EUROPE

Seven countries in the Region of the Americas have reported to PAHO/WHO or published the detection of 16 CAM cases till 9th June 2021 [11]. These include Brazil, Chile, Honduras, Mexico, Paraguay, Uruguay and the United States of America. Majority of the cases have clinical presentation in the form of ROCM. Diabetes mellitus has been found to be the main underlying disease or co-morbidity in more than 95% of cases apart from other diseases like atypical pneumonia, arterial hypertension etc. Cases have also been reported from other regions like United Kingdom and Italy. Four cases of CAM have also been reported from Netherlands between December 2020 and May 2021 of which three developed the infection during intensive care unit. admission and three lost their lives [2]. The Pan American Health Organization / World Health Organization (PAHO/WHO) has urged Member States to prepare their respective health services to increase clinical suspicion of mucormycosis in patients with COVID-19, especially those with co-morbidities and treatment with corticosteroids and other immunosuppresants.

CASES OF MUCORMYCOSIS IN NEPAL (INDIA'S NEIGHBOUR)

This Himalayan nation and India's immediate neighbour has reported 11 cases of CAM and two people have lost their lives due to this infection [18]. It has been reported that there were only a limited number of mucormycosis cases previously but cases grew rapidly among COVID-19 patients during the second wave of the pandemic which began in early April 2021. There is also shortage of drugs likes AmB and Deoxycholate (used to treat *black fungus* infection) in the Nepali market and now the Government of Nepal is procuring these drugs from international market and WHO.

CONCLUSION AND KEY RECOMMENDATIONS

Following the surge of COVID-19 associated mucormycosis and the Government of India directive, mucormycosis was put under the category of 'notifiable disease' by several states in May 2021. This will provide better insights into the disease burden, population characteristic, risk factors, clinical spectrum and outcomes of these patients. This was also important to check the shortage and black marketing of the drug Amphotericine-B as price of the drug rose exponentially due to the rapid demand. Our findings emphasize the need to be aware of invasive mucormycosis developing in COVID-19 patients, especially while receiving corticosteroids, including patients without (poorly controlled) diabetes mellitus and outside the ICU. An aggressive approach should be employed when invasive mucormycosis is diagnosed, including early surgery and targeted antifungal treatment. Patients receiving oxygen therapy should ensure that the water in the humidifier is clean and is refilled regularly. As fungus can breed on wet surfaces, it should be ensured that there is no leakage of water. Even after recovering from COVID-19, one should not miss any warning signs and symptoms described in the text, as the fungal infection can emerge even weeks or months after recovery. Steroids should be used wisely according to the doctor's advice.

Mass vaccination of the population, sharing health information, building community trust etc. are vital primary health care measures which can protect everyone during COVID-19 (World Health Organization) (Figure 3) [19].

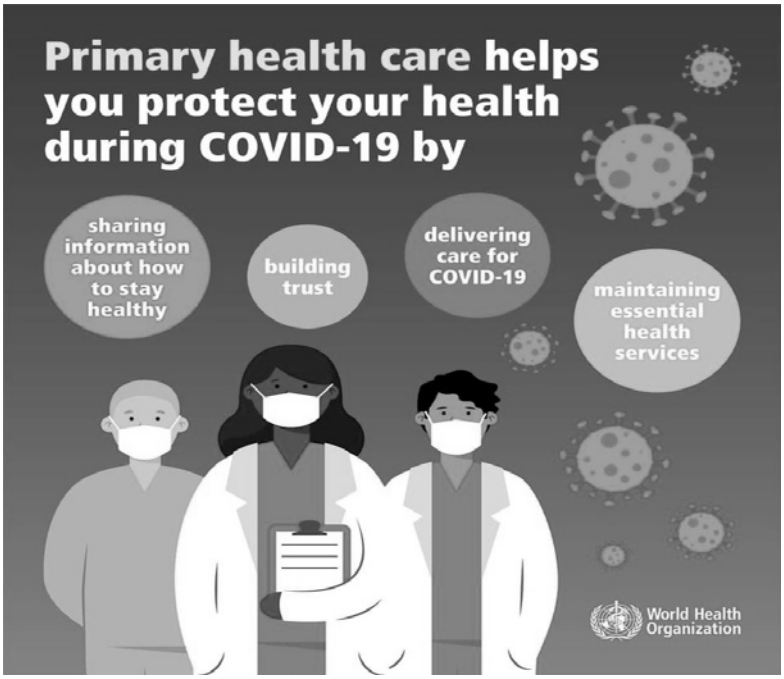

Figure 3. Primary health care measures to protect from COVID-19 according to WHO [19]

REFERENCES

1. *Atreya A.:* Death and dignity in India and Nepal during the second wave of Coronavirus disease 2019. Med Sci Law. 2021:258024211021384. doi: 10.1177/00258024211021384.
2. *Buil J.B., van Zanten A.R.H., Bentvelsen R.G., Rijpstra T.A., Goorhuis B., van der Voort S., Wammes L.J., Janson J.A., Melchers M., Heusinkveld M., Melchers W.J.G., Kuijper E.J., Verweij PE.:* Case series of four secondary mucormycosis infections in COVID-19 patients, the Netherlands, December 2020 to May 2021. Euro Surveill. 2021;26. doi: 10.2807/1560-7917.ES.2021.26.23.2100510.
3. *Carneiro D.C., Sousa J.D., Monteiro-Cunha J.P.:* The COVID-19 vaccine development: A pandemic paradigm. Virus Res. 2021;301:198454. doi: 10.1016/j.virusres.
4. *Christopher N Rocha I., Mehedi Hasan M., Goyal S., Patel T., Jain S., Ghosh A., Denise D Cedeño T.:* COVID-19 and Mucormycosis Syndemic: Double Health Threat to a Collapsing Healthcare System in India. Trop Med Int Health. 2021. doi: 10.1111/tmi.13641.
5. *Forouzandeh P., O'Dowd K., Pillai S.C.:* Face masks and respirators in the fight against the COVID-19 pandemic: An overview of the standards and testing methods. Saf Sci. 2021;133:104995. doi: 10.1016/j.ssci.2020.104995.
6. *Imran M., A S A., Tauseef M., Khan S.A., Hudu S.A., Abida.:* Mucormycosis medications: a patent review. Expert Opin Ther Pat. 2021. doi: 10.1080/13543776.2021.1939308.
7. *Jeong W., Keighley C., Wolfe R., Lee W.L., Slavin M.A., Chen S.C., Kong D.C.M.:* Contemporary management and clinical outcomes of mucormycosis: A systematic review and meta-analysis of case reports. Int J Antimicrob Agents. 2019;53:589-97.
8. Looking beyond the second wave of Covid. Available from: www.epapertribuneindia.com. Accessed on: 12th June 2021.
9. *Manesh A., John A.O., Mathew B., Varghese L., Rupa V., Zachariah A., Varghese G.M.:* Posaconazole: an emerging therapeutic option for invasive rhino-orbito-cerebral mucormycosis. Mycoses. 2016;59:765-772.
10. *Onyeaka H., Anumudu C.K., Al-Sharify Z.T., Egele-Godswill E., Mbaegbu P.:* COVID-19 pandemic: A review of the global lockdown and its far-reaching effects. Sci Prog. 2021;104:368504211019854. doi: 10.1177/00368504211019854.
11. Pan American Health Organization / World Health Organization. Epidemiological Alert: COVID-19 associated Mucormycosis. 11 June 2021, Washington, D.C.: PAHO/WHO; 2021. Available from: www.paho.org. Accessed on: 17th June 2021.
12. *Patel A., Agarwal R., Rudramurthy S.M., Shevkani M., Xess I., Sharma R., Savio J., Sethuraman N., Madan S., Shastri P., Thangaraju D., Marak R., Tadepalli K., Savaj P., Sunavala A., Gupta N., Singhal T., Muthu V., Chakrabarti A., MucoCovi Network3.:* Multicenter Epidemiologic Study of Coronavirus Disease-Associated Mucormycosis, India. Emerg Infect Dis. 2021;27. doi: 10.3201/eid2709.210934.
13. *Prakash H., Chakrabarti A.:* Epidemiology of Mucormycosis in India. Microorganisms. 2021;9:523. doi: 10.3390/microorganisms9030523.
14. Press Information Bureau, Government of India. Available from: https://pib.gov.in/PressReleseDetailm.aspx?PRID=1718501. Accessed on: 15th June 2021.
15. *Sahota R., Gambhir R., Anand S., Dixit A.:* Rhinocerebral Mucormycosis: Report of a Rare Case. Ethiop J Health Sci. 2017;27:85-90.

16. *Sen M., Lahane S., Lahane T.P., Parekh R., Honavar S.G.:* Mucor in a Viral Land: A Tale of Two Pathogens. Indian J Ophthalmol. 2021;69:244-52.
17. Triple-mutant 'Bengal strain' a new worry in second wave. Times of India. Available from: www.timesofindia.com. Accessed on: 14th June 2021.
18. Two dead and 11 suffering from black fungus in Nepal. Available from: https://timesofindia.indiatimes.com/world/south-asia. Accessed on: 18th June 2021.
19. WHO. Coronavirus (COVID-19) Dashboard. Available from: covid19.who.int. Accessed on: 28th June, 2021.
20. 51 Delta plus cases across 12 states, 2 in Punjab, 1 in Haryana. Available from: www.epapertribuneindia.com. Accessed on: 24th June 2021.

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REVIEW ARTICLE

A REVIEW OF APPROVED COVID-19 VACCINES

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ABSTRACT

In the past one and half year elapsed since the identification of the SARS-CoV-2 virus and its genome, an extraordinary effort by the scientific community has led to the development of many vaccine projects. More than ten vaccine candidates throughout the world have been granted approval for emergency use. Existing data suggest that these vaccines have the potential to protect individuals and curb the spread of COVID-19 pandemic. However, long term side-effects and certain unresolved issues associated with vaccine use need to be assessed as the time passes. This study reviews the most recent data of 12 vaccines which have been approved for use and presents information on their doses, composition, mechanism of action, side effects, etc.

Key words: *Covid-19 vaccines, immunization, SARS-CoV-2*

INTRODUCTION

In late December 2019, China reported cases of idiopathic pneumonia in the city of Wuhan. One month later, the causative agent was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 caused the epidemic of coronavirus disease 2019 (COVID-19) in different cities in China, which then spread globally and was later classified as a pandemic by the World Health Organization (WHO) [1, 2].

SARS-CoV-2 belongs to the genus *Betacoronavirus* in the family *Coronaviridae*. SARS-CoV-2 is an enveloped virus that contains a single-stranded positive-sense RNA genome of 29,903 nucleotides in length with 11 open reading frames (ORFs), which encode 27 viral proteins. ORF1a/b is 21,290 nucleotides length and encodes 16 nonstructural proteins (nsp1–nsp16). The last part of the genome is 8613 nucleotides that encodes four structural and six accessory proteins. The structural proteins are the spike (S, virus attachment and major antigenic protein), envelope (E), matrix (M), and nucleocapsid (N) proteins while accessory proteins include ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10 [3, 4].

From a few thousand confirmed COVID-19 cases in January 2020, cases continue to grow globally, as of 17th June 2021, there have been 17,66,93,988 confirmed cases of COVID-19, including 38,30,304 deaths, reported to WHO.

The best way to prevent infection from COVID-19 is to avoid exposure to the virus. The virus spreads mainly from person-to-person through close contact. The basic preventive measures include simple public health measures such as vaccination that are to be followed to reduce the risk of infection with COVID-19. Vaccines greatly reduce the risk of infection by training the immune system to recognize and fight pathogens such as viruses or bacteria. The worldwide endeavor to create a safe and effective COVID-19 vaccine is bearing fruit. More than a dozen vaccines now have been authorized around the globe; many more remain in development.

A COVID-19 vaccine is a vaccine intended to provide acquired immunity against severe acute

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respiratory syndrome coronavirus 2 (SARSCoV2), the virus causing coronavirus disease 2019 (COVID-19).

COMIRNATY (BNT162B2) (PFIZER)

The Pfizer–BioNTech COVID-19 vaccine (INN: tozinameran), sold under the brand name Comirnaty, is an mRNA-based COVID-19 vaccine [5].

Medical use*:* The vaccine is given by intramuscular injection. It is composed of nucleoside-modified mRNA (modRNA) encoding a mutated form of the full-length spike protein of SARS-CoV-2, which is encapsulated in lipid nanoparticles. Vaccination requires two doses given three weeks apart [6, 7].

Composition: In addition to the mRNA molecule, the vaccine contains the following inactive ingredients (excipients): ALC-0315, ((4-hydroxybutyl) azanediyl) bis (hexane-6, 1-diyl) bis (2-hexyldecanoate); ALC-0159, 2-[(polyethylene glycol)-2000]-N, N-ditetradecylacetamide; 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC); Cholesterol; Dibasic sodium phosphate dihydrate; Monobasic potassium phosphate; Potassium chloride; Sodium chloride; Sucrose; Water for injection.

Mechanism of action: Nucleoside-modified messenger RNA (modRNA) encoding the viral spike (S) glycoprotein of SARS-CoV-2; formulated in lipid particles, which enable delivery of RNA into host cells to allow expression of the SARS-CoV-2 S antigen and elicits an immune response to the S antigen, which protects against COVID-19.

Vaccine efficacy: The vaccine showed 95% efficacy at preventing Covid-19 illness [8].

Side effects: The side effect profile of the Pfizer–BioNTech COVID-19 vaccine is similar to that of other adult vaccines. During clinical trials, the side effects deemed very common are (in order of frequency): pain and swelling at the injection site, tiredness, headache, muscle aches, chills, joint pain, and fever. Fever is more common after the second dose [5].

MODERNA COVID-19 VACCINE (mRNA-1273)

The Moderna COVID-19 vaccine, codenamed mRNA-1273, is a COVID-19 vaccine developed by Moderna, the United States National Institute of Allergy and Infectious Diseases (NIAID) and the Biomedical Advanced Research and Development Authority (BARDA) [9].

Medical use**:** It is designed to be administered as two 0.5 mL doses given by intramuscular injection at an interval of four weeks apart [10].

Composition: Nucleoside-modified messenger RNA encoding the SARS-CoV-2 spike glycoprotein (S) stabilized in its prefusion configuration; Lipids [SM-102, Polyethylene glycol (PEG) 2000-dimyristoyl glycerol (DMG), Cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); Tromethamine; Tromethamine hydrochloride; Acetic acid; Sodium acetate; Sucrose [11].

Mode of action: The Moderna COVID-19 Vaccine uses mRNA to provide a blueprint for your cells to build your body's defense against the virus. The nucleoside-modified mRNA in the Moderna COVID-19 Vaccine is formulated in lipid particles, which enable delivery of the nucleoside modified mRNA into host cells to allow expression of the SARSCoV2 Spike antigen. The vaccine elicits an immune response to the Spike antigen, which protects against COVID-19.

Vaccine efficacy: The mRNA-1273 vaccine showed 94.1% efficacy at preventing Covid-19 illness [12].

Side effects: Side effects that have been reported with the Moderna COVID-19 Vaccine include [13]:

- Injection site reactions: pain, tenderness and swelling of the lymph nodes in the same arm of the injection, swelling (hardness), and redness.
- General side effects: fatigue, headache, muscle pain, joint pain, chills, nausea and vomiting, and fever.

ASTRA ZENECA COVID-19 VACCINE (AZD1222); also known as Vaxzevria and Covishield

The Oxford–AstraZeneca COVID-19 Vaccine, codenamed AZD1222, and sold under the brand names Covishield and Vaxzevria among others, is a viral vector vaccine for prevention of COVID-19. Developed by the Oxford University and Astra Zeneca, it is given by intramuscular injection, using as a vector the modified chimpanzee adenovirus ChAdOx1 [14].

Medical uses**:** The Oxford–AstraZeneca COVID-19 vaccine is used to provide protection against infection by the SARS-CoV-2 virus in order to prevent COVID-19 in adults aged 18 years and older. The medicine is administered by two 0.5 ml doses injected intramuscularly into the deltoid muscle (upper arm) four to twelve weeks apart, with the WHO recommending the second is given 8 to 12 weeks after the first for optimum efficacy [15].

Composition**:** The Covishield Vaccine includes the following ingredients: ChAdOx1 nCoV-19 Corona Virus Vaccine (Recombinant) 5 × 10 viral particles (*Recombinant, replication-deficient chimpanzee adenovirus vector encoding the SARS-CoV-2 Spike (S) glycoprotein. Produced in genetically modified human embryonic kidney (HEK) 293 cells); L-histidine; L-histidine hydrochloride monohydrate; Magnesium chloride hexahydrate; Polysorbate 80;

Ethanol; Sucrose; Sodium chloride; Disodium edetate dihydrate (EDTA); Water for injection.

Mode of action: The Oxford–AstraZeneca COVID-19 vaccine is a replication-deficient simian adenovirus vector, containing the full-length. The adenovirus is called replication-deficient because some of its essential genes were deleted and replaced by a gene coding for the spike protein. Following vaccination, the adenovirus vector enters the cells and releases its genes, which are transported to the cell nucleus; thereafter the cell's machinery does the transcription into mRNA and the translation into proteins. The protein of interest is the spike protein, an external protein that enables the SARS-type coronavirus to enter cells through the enzymatic domain of ACE2. Producing it following vaccination will prompt the immune system to attack the coronavirus through antibodies and T-cells if it later infects the body.

Vaccine efficacy: An analysis published on 19 February 2021 showed an efficacy of 76.0% at preventing symptomatic COVID-19 beginning at 22 days following the first dose, increasing to 81.3% when the second dose is given 12 weeks or more after the first [16].

Side effects: The most common side effects in the clinical trials were usually mild or moderate and got better within a few days after vaccination. Vomiting, diarrhea, swelling, redness at the injection site and low levels of blood platelets occurred in less than 1 in 10 people. Enlarged lymph nodes, decreased appetite, dizziness, sleepiness, sweating, abdominal pain, itching and rash occurred in less than 1 in 100 people. In very rare cases (around 1 in 100,000 people) the vaccine can lead to blood clots in combination with low levels of blood platelets [17].

SPUTNIK V (rAd26 and rAd5)

Sputnik V is an adenovirus viral vector vaccine for COVID-19 developed by the Gamaleya Research Institute of Epidemiology and Microbiology in Russia. Also known as Gam-COVID-Vac is a combined vector vaccine, based on rAd type 26 (rAd26) and rAd type 5 (rAd5) - both of which carry the gene for SARS-CoV-2 full-length glycoprotein S (rAd26-S and rAd5-S) [18, 19, 20, 21].

***Medical use*:** It is administered intramuscularly separately with a 21-day interval [20].

Components:

- ***Active ingredient*:** recombinant adenoviral particles of the 26 serotype containing the gene for the protein S of the SARS-CoV-2 virus, in an amount of $(1.0 \pm 0.5) \times 10^{11}$ particles / dose.
- ***Excipients*:** tris (hydroxylmethyl) aminomethane - 1.21 mg, sodium chloride - 2.19 mg, sucrose - 25.0 mg, magnesium chloride - 102.0 μg, EDTA sodium salt dihydrate - 19.0 μg, polysorbate 80 - 250 μg, ethanol 95% - 2.5 μl, water for injection up to 0.5 ml [20].

Mode of action: Sputnik V uses a weakened virus to deliver small parts of a pathogen and stimulate an immune response. It is a vector vaccine based on adenovirus DNA, in which the SARS-CoV-2 coronavirus gene is integrated. Adenovirus is used as a "container" to deliver the coronavirus gene to cells and start synthesizing the new coronavirus›s envelope proteins, "introducing" the immune system to a potential enemy.

***Vaccine efficacy*:** The vaccine efficacy is more than 91% according to the data released [20].

***Side effects*:**

- Short-term general (short-term flu-like syndrome, characterized by chills, fever, arthralgia, myalgia, asthenia, general malaise, headache) and local (soreness at the injection site, hyperemia, swelling) reactions) may develop.
- Less commonly, nausea, dyspepsia, decreased appetite, and sometimes an increase in regional lymph nodes are noted.
- Some patients may develop allergic reactions, a short-term increase in the level of hepatic transaminases, creatinine and creatine phosphokinase in the blood serum [20].

SPUTNIK LIGHT

Sputnik Light is a single dose COVID-19 vaccine developed by the Gamaleya Research Institute of Epidemiology and Microbiology in Russia. It actually consists of the first dose of the Sputnik V vaccine, which is based on the Ad26 vector, and it can be stored at a normal refrigerator temperature of 2–8 °C (36–46^0F) [22, 23, 24].

***Efficacy*:** The single dose Sputnik Light vaccine demonstrated 79.4 per cent efficacy, according to analyzed data taken from 28 days after the injection was administered. An efficacy level of near 80 per cent is higher than that of many two-dose vaccines [25].

***Immunogenicity*:** Sputnik Light can elicit the development of antigen specific IgG antibodies in 96.9% of individuals on the 28th day after vaccination. The Sputnik Light vaccine elicits the development of virus neutralizing antibodies in 91.67% of individuals on the 28th day post immunization. Cellular immune response against the S Protein of SARS-CoV-2 develops in 100% of volunteers on the 10th day. The immunization of individuals with pre-existing immunity against SARS-CoV-2 with Sputnik Light can elicit the increase of the level of antigen specific

IgG antibodies by more than 40x in 100% of subjects 10 days after immunization [26].

***Side effects*:** No serious adverse events were registered after vaccination with Sputnik Light [26, 27].

JANSSEN COVID-19 VACCINE (JNJ-78436735; Ad26.COV2.S)

The Janssen or Johnson & Johnson COVID-19 vaccine is a COVID-19 vaccine that was developed by Janssen Vaccines in Leiden, Netherlands, and its Belgian parent company Janssen Pharmaceuticals, subsidiary of American company [28].

Medical use: It is administered as a single dose [29].

***Components*:** Recombinant, replication-incompetent adenovirus type 26 expressing the SARS-CoV-2 spike protein; Citric acid monohydrate; Trisodium citrate dehydrate; Ethanol; 2-hydroxypropyl-β-cyclodextrin (HBCD); Polysorbate-80; Sodium chloride [30]

Mode of action: A modified type of adenovirus is used that can enter the human cells but don't cause any illness or replicate. After injecting the vaccine, these adenoviruses latch onto proteins of human cells and enter it to release the adenovirus which eventually pushes its own DNA into the nucleus and mRNA containing gene for coronavirus spike protein is made and the spike proteins that are made are recognized by the immune system and antibodies against them are performed.

***Vaccine efficacy*:** The J&J one-dose vaccine was shown to be 66% protective against moderate to severe Covid infections overall from 28 days after injection, though there was variability based on geographic locations. The vaccine was 72% protective in the United States, 66% protective in South America, and 57% protective in South Africa. But the vaccine was shown to be 85% protective against severe disease, with no differences across the eight countries or three regions in the study, nor across age groups among trial participants [30].

Side effects: headache; fever; fatigue; muscle aches; nausea; pain, irritation, redness, and swelling at the site of the injection; Blood clots involving blood vessels in the brain, abdomen, and legs along with low levels of platelets, has occurred in some people who have received the Janssen COVID-19 Vaccine. In people who developed these blood clots and low levels of platelets, symptoms began approximately one to two-weeks following vaccination [31, 32].

COVAXIN (BBV152)

Covaxin (codenamed as BBV152) is an inactivated virus based COVID-19 vaccine developed by Bharat Biotech in collaboration with the Indian Council of Medical Research [33].

Medical use: It is given as an injection into the deltoid muscle of the upper arm. The BHARAT BIOTECH COVID-19 VACCINE (COVAXIN) vaccination series is 2 doses given 4-6 weeks apart [34].

***Components*:** 6μg of whole-virion inactivated SARSCoV-2 antigen (Strain: NIV-2020-770); Aluminum hydroxide gel (250 μg); TLR 7/8 agonist (imidazoquinolinone) 15 μg; 2-phenoxyethanol 2.5 mg; Phosphate buffer saline up to 0.5 ml [34].

Mode of action: COVAXIN induced binding and neutralizing antibody responses and with the inclusion of the Algel-IMDG adjuvant, this is the first inactivated SARS-CoV-2 vaccine that has been reported to induce a Th1-biased response [35].

***Vaccine efficacy*:** Phase 3 results of the COVAXIN, developed by Indian Council of Medical Research (ICMR) in partnership with Bharat Biotech International Limited (BBIL), and has shown an interim vaccine efficacy of 81% in preventing Covid-19 [36]. COVAXIN is found to protect individuals against the Beta (B.1.351) and Delta (B.1.617.2) variants, more commonly referred to as the South African and Indian variants of the Covid-19 virus [37].

Side effects: Injection site pain, swelling, redness, itching, stiffness in the upper arm, weakness in injection arm, body ache, headache, fever, malaise, weakness, rashes, nausea, vomiting were observed after the vaccine was administered. It may cause a severe allergic reaction of which signs are: difficulty in breathing, swelling of the face and throat, a fast heartbeat, rash all over your body, dizziness and weakness [38].

CORONA VAC

CoronaVac, also known as the Sinovac COVID-19 vaccine, is an inactivated virus COVID-19 vaccine developed by the Chinese company Sinovac Biotech [39]. CoronaVac does not need to be frozen and both the final product and the raw material for formulating CoronaVac can be transported refrigerated at 2–8°C (36–46°F), temperatures at which flu vaccines are kept.

Medical use: Two doses should be administered by intramuscular injection in the deltoid region of the upper arm. Vaccine is administered on a 14-28-day schedule [40].

Components: Inactivated SARS-CoV-2 Virus9CZ02 strain); Aluminum hydroxide; Disodium hydrogen phosphate dodecahydrate; Sodium dihydrogen phosphate monohydrate; Sodium chloride [41].

Mode of action: Part of the coronavirus' genetic code is injected into the body, triggering the body to

begin making viral proteins, but not the whole virus, which is enough to train the immune system to attack and hence, immunity is developed.

Vaccine efficacy: A real-world study of millions of people who received Corona Vac, as published by the WHO found the vaccine 67% effective against symptoms, reduced hospitalizations by 85%, intensive care visits by 89%, and deaths by 80% [42].

Side effects: Blood Pressure Increase; Headache; Vaccination site pain; Dizziness; Rash [43].

SINOPHARM BBIBP-CorV COVID-19

Sinopharm BBIBP-CorV, also known as the Sinopharm COVID-19 vaccine, is one of two inactivated virus COVID-19 vaccines developed by Sinopharm›s Beijing Institute of Biological Products, China [44].

Medical use: The WHO recommends the Sinopharm vaccine for people aged 18 years and older, with a gap of 3–4 weeks between the two doses [45].

Components: Sinopharm's SARS-CoV-2 strain (WIV04 strain and Gen Bank number MN996528) was isolated from a patient in the Jinyintan Hospital, Wuhan, China. The virus was cultivated in a qualified Vero cell line for propagation, and the supernatant of the infected cells was inactivated with β-propiolactone (1:4000 vol/vol at 2 to 8^0C) for 48 hours. Following clarification of cell debris and ultrafiltration, the second β-propiolactone inactivation was performed in the same conditions as the first inactivation. The vaccine was adsorbed to 0.5 mg alum and packed into prefilled syringes in 0.5 mL sterile phosphate-buffered saline without preservative, says the WHO [45].

Mode of action: Inactivated vaccines use the killed version of the germ that causes a disease and works by teaching the immune system to make antibodies against the SARS-CoV-2 beta coronavirus.

Vaccine efficacy: In May 2021, peer-reviewed results published in JAMA of Phase III trials in United Arab Emirates and Bahrain showed BBIBP-CorV 78.1% effective against symptomatic cases and 100% against severe cases [46].

Side effects:

- The most common side effects were: headaches; fatigue; injection site reactions
- The WHO identified two serious adverse events that were possibly linked to the vaccine: serious nausea and a rare neurological disorder known as acute disseminated encephalomyelitis. There was also one diagnosis of thrombus (blood clot) in the vaccine group [47].

CONVIDICEA (PakVac, Ad5-nCoV)

Convidicea - Ad5-nCoV, trade-named Convidicea, is a single-dose viral vector vaccine for COVID-19 developed by CanSino Biologics (Tianjin, China) [48]. It conducted its Phase III trials in Argentina, Chile, Mexico, Pakistan, Russia, and Saudi Arabia with 40,000 participants. Convidecia is authorized for use in China, Mexico, Pakistan, Hungary, and Chile. Not authorized by the WHO and European Commission.

Medical use: It is administered as a single dose [49].

Components: Contained replication-defective Ad5 vectors expressing the full-length spike gene based on Wuhan-Hu-1 (GenBank accession number YP_009724390) [49].

Mode of action: The new Ad5 vectored COVID-19 vaccine evaluated in the phase 1 trial is the first to be tested in humans. It uses a weakened common cold virus (adenovirus, which infects human cells readily but is incapable of causing disease) to deliver genetic material that codes for the SARS-CoV-2 spike protein to the cells. These cells then produce the spike protein and travel to the lymph nodes, where the immune system creates antibodies that will recognize that spike protein and fight off the coronavirus.

Vaccine efficacy: In February 2021, data released from an interim analysis of Phase III trials with 30,000 participants and 101 COVID cases showed that globally, the vaccine had an efficacy of 65.7% at preventing moderate cases of COVID-19 and 90.98% efficacy at preventing severe cases [50].

Side effects:

- Injection site adverse reactions included: pain, induration, redness, swelling, itching
- Systemic adverse reactions included: fever, headache, fatigue, vomiting, diarrhea, muscle pain, cough, nausea, etc. [49].

EpiVacCorona

EpiVacCorona is a COVID-19 preventive vaccine developed by the Vector State Research Center of Virology and Biotechnology in Russia. EpiVacCorona is an antigens-based vaccine [51].

***Mode of action*:** The vaccine relies on chemically synthesized peptide antigens of SARS-CoV-2 proteins, conjugated to a carrier protein and adsorbed on an aluminum-containing adjuvant (aluminum hydroxide) [51]. the vaccine does not contain the live virus and forms immunity due to artificially synthesized peptides. EpiVacCorona consists of three synthetic fragments of spike, attached to a carrier protein, which itself is composed of synthetic fragments of the virus nucleocapsid protein, known as N. One peptide is

designed to create antibodies to the spike's receptor-binding domain, the part that hooks onto a human cell protein. The other spike peptides are meant to elicit antibodies that prevent the virus from getting into the cell. The N peptides may generate still other protective responses. VECTOR officials say the vaccine ultimately provides "three lines of defense" [52].

Vaccine efficacy: The efficacy is shown to be 100% according to the study published [53].

RBD-Dimer/ZIFIVAX

ZF2001, trade-named ZIFIVAX, is an adjuvanted protein subunit COVID-19 vaccine developed by Anhui Zhifei Longcom in collaboration with the Institute of Microbiology at the Chinese Academy of Sciences. ZifiVax ZF2001 is a protein subunit vaccine using a dimeric form of the receptor-binding domain (RBD) as the antigen, a harmless piece of the SARS-Cov-2 virus. ZF2001 was first approved for use in Uzbekistan and later China [54].

Medical use: It's a three-dose shot that is spaced out with one month each between shots [55].

LIMITATIONS OF CURRENT VACCINES

Though the vaccines have shown promising results, there are some area which remain unanswered. With such a short duration involved in their development and the novelty of the technologies used, these vaccines will be used with some unanswered issues that only passage of time will allow to elucidate. Issues such as how much protection do these vaccines provide against different variants of Novel Corona Virus? What is the duration of immune response generated? Is there any need for a booster dose after taking recommended doses of a particular vaccine? Can these vaccines be administered to younger children and pregnant women? How much effective are these vaccines against asymptomatic cases i.e. can these vaccines stop transmission of infection from asymptomatic cases? Need to be answered through long term research.

CONCLUSION

Higher efficacy rates were seen with approved vaccines (mRNA based, inactivated and viral vectors vaccines). Pfizer's Comirnaty is the first vaccine to get approval for 12-15-year age group. So far, high reactogenicity was seen with mRNA based vaccines and low with in inactivated vaccines. Those who are administered with viral vectored vaccines and mRNA vaccines need to be observed for thrombotic events and myocarditis respectively.

Conflict of interest
None.

REFERENCES

1. *Zhou P., Yang XL., Wang XG., Hu B., Zhang L., Zhang W.,* et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; 579(7798):270–273. DOI: 10.1038/s41586-020-2012-7
2. *Wu F., Zhao S., Yu B., Chen Y-M., Wang W., Song Z-G., et al.* A new coronavirus associated with human respiratory disease in China. Nature. 2020; 579(7798):265–269. DOI: 10.1038/s41586-020-2008-3
3. *Chan JF-W., Kok K-H., Zhu Z, Chu H., To KK-W., Yuan S., et al.* Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerging Microbes & Infections. 2020; 9(1):221–236. doi: 10.1080/22221751.2020.1719902.
4. *Helmy YA., Fawzy M., Elaswad A., Sobieh A., Kenney SP., Shehata AA.* The COVID-19 Pandemic: A Comprehensive Review of Taxonomy, Genetics, Epidemiology, Diagnosis, Treatment, and Control. *J Clin Med*. 2020;9(4):1225. Published 2020 Apr 24. doi:10.3390/jcm9041225
5. *Dimitrova E.K.* Comirnaty [Internet]. European Medicines Agency. BioNTech Manufacturing GmbH; 2021 Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/comirnaty. (Accessed: 10.07.2021)
6. *Walsh EE., Frenck RW., Falsey AR., Kitchin N., Absalon J., Gurtman A., et al.* Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med 2020; 383:2439-2450. DOI: 10.1056/NEJMoa2027906
7. Information for Healthcare Professionals on Pfizer/BioNTech COVID-19 vaccine [Internet]. GOV.UK. Available from: https://www.gov.uk/government/publications/regulatory-approval-of-pfizer-biontech-vaccine-for-covid-19/information-for-healthcare-professionals-on-pfizerbiontech-covid-19-vaccine. (Accessed: 10.07.2021)
8. *Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med 2020; 383(27):2603–15. doi: 10.1056/NEJMoa2034577.
9. DailyMed - MODERNA COVID-19 VACCINE-cx-024414 injection, suspension [Internet]. U.S. National Library of Medicine. National Institutes of Health. Available from: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=e0651c7a-2fe2-459d-a766-0d59e919f058. (Accessed: 10.07.2021)
10. *Czarska-Thorley D.* COVID-19 Vaccine Moderna [Internet]. European Medicines Agency. 2021. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/covid-19-vaccine-moderna. (Accessed: 10.07.2021)
11. Moderna COVID-19 Vaccine Overview and Safety [Internet]. Centers for Disease Control and Prevention. Centers for Disease Control and Prevention; 2021.

Available from: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/Moderna.html. (Accessed: 10.07.2021)
12. *Baden LR., El Sahly HM., Essink B., Kotloff K., Frey S., Novak R., et al.* Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. New Eng J Med 2021; 384(5):403–16. doi/10.1056/NEJMoa2035389.
13. Moderna COVID-19 Vaccine EUA Fact Sheet for Recipients and ... [Internet]. Moderna COVID-19 Vaccine EUA Fact Sheet for Recipients. 2021 Available from: https://www.fda.gov/media/144638/download . (Accessed: 10.07.2021)
14. Clinical trials [Internet]. Clinical Trials Register. 2021. Available from: https://www.clinicaltrialsregister.eu/ctr-search/trial/2020-001228-32/GB. (Accessed: 10.07.2021)
15. *Smith P.* [Internet]. WHO-2019-nCoV-vaccines-SAGE_recommendation-AZD1222-2021.2-eng.pdf. 2021. Available from: https://apps.who.int/iris/bitstream/handle/10665/340920/WHO-2019-nCoV-vaccines-SAGE_recommendation-AZD1222-2021.2-eng.pdf. (Accessed: 10.07.2021)
16. *Voysey M., Weckx LY., Madhi SA., Clemens SA., Aley PK., Folegatti PM., et al.* [Internet]. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. Lancet. 2021; 397(10277):881-891. doi: 10.1016/S0140-6736(21)00432-3.
17. *Francisco EM.:* AstraZeneca's COVID-19 vaccine: benefits and risks in context [Internet]. European Medicines Agency. 2021. Available from: https://www.ema.europa.eu/en/news/astrazenecas-covid-19-vaccine-benefits-risks-context. (Accessed: 10.07.2021)
18. *Jon Cohen:* Russia's approval of a COVID-19 vaccine is less than meets the press release [Internet]. Science. 2020. Available from: https://www.sciencemag.org/news/2020/08/russia-s-approval-covid-19-vaccine-less-meets-press-release. (Accessed: 10.07.2021)
19. *Callaway E.:* Russia's fast-track coronavirus vaccine draws outrage over safety [Internet]. Nature News. Nature Publishing Group; 2020. Available from: https://www.nature.com/articles/d41586-020-02386-2. (Accessed: 10.07.2021)
20. Gam-COVID-Wack, Satellite V [Internet]. Combined vector vaccine for the prevention of coronavirus infection caused by the SARS-CoV-2 virus (Gam-COVID-Vac). Available from: https://medum.ru/sputnik-v. (Accessed: 10.07.2021)
21. Sputnik V Vaccine [Internet]. Welcome to Precision Vaccinations. Available from: https://www.precisionvaccinations.com/vaccines/sputnik-v-vaccine. (Accessed: 10.07.2021)
22. Sputnik V. General Information [Internet]. Available from: https://sputnikvaccine.com/about-vaccine/. (Accessed: 10.07.2021)
23. An Open Study on the Safety, Tolerability, and Immunogenicity of "Sputnik Light" Vaccine - Full Text View [Internet]. Full Text View - ClinicalTrials.gov. 2021. Available from: https://clinicaltrials.gov/ct2/show/NCT04713488. (Accessed: 10.07.2021)
24. Study to Evaluate Efficacy, Immunogenicity and Safety of the Sputnik-Light - Full Text View [Internet]. Study to Evaluate Efficacy, Immunogenicity and Safety of the Sputnik-Light - Full Text View - ClinicalTrials.gov. Available from: https://clinicaltrials.gov/ct2/show/NCT04741061. (Accessed: 10.07.2021)
25. Mounting evidence suggests Sputnik COVID vaccine is safe and effective [Internet]. mint. Nature. 2021. Available from: nature.com/articles/d41586-021-01813-2. (Accessed: 10.07.2021)
26. An Open Study on the Safety, Tolerability, and Immunogenicity of "Sputnik Light" Vaccine [Internet]. Welcome to Precision Vaccinations. Available from: https://www.precisionvaccinations.com/clinical-trials/open-study-safety-tolerability-and-immunogenicity-sputnik-light-vaccine. (Accessed: 11.07.2021)
27. Desk FPJW. What is Sputnik Light? All you need to know about Russia's single shot COVID-19 vaccine [Internet]. Free Press Journal. Available from: https://www.freepressjournal.in/india/what-is-sputnik-light-all-you-need-to-know-about-russias-single-shot-covid-19-vaccine. (Accessed: 11.07.2021)
28. Janssen COVID-19 Vaccine (Johnson & Johnson) [Internet]. 2021. Available from: https://www.cdc.gov/vaccines/covid-19/info-by-product/janssen/index.html. (Accessed: 11.07.2021)
29. Fact sheet for healthcare providers administering vaccine (vaccination providers). Emergency use authorization (EUA) of The Janssen COVID-19 Vaccine to prevent Coronavirus Disease 2019 (COVID-19). 2021. Available from: https://www.fda.gov/media/146304/download. (Accessed: 11.07.2021)
30. Interim recommendations for the use of the Janssen Ad26.COV2.S (COVID-19) vaccine [Internet]. World Health Organization. World Health Organization. Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-vaccines-SAGE-recommendation-Ad26.COV2.S-2021.1. (Accessed: 11.07.2021)
31. Janssen COVID-19 Vaccine. Fact Sheet for Recipients and Caregivers. Emergency use authorization (EUA) of the Janssen COVID-19 Vaccine to prevent coronavirus disease 2019 (COVID-19) in individuals 18 years of age and older. [Internet]. .Available from: https://www.fda.gov/media/146305/download. (Accessed: 11.07.2021)
32. CDC Recommends Use of Johnson & Johnson's Janssen COVID-19 Vaccine Resume [Internet]. 2021. Available from: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/safety/JJUpdate.html#:~:text=syndrome%20(TTS).-,TTS%20is%20a%20serious%20condition%20that%20involves%20blood%20clots%20with,a%20median%20of%2037%20years. (Accessed: 11.07.2021)
33. Bharat Biotech-Vaccines & Bio-Therapeutics Manufacturer in India. Available from: https://www.bharatbiotech.com/covaxin.html. (Accessed: 11.07.2021)
34. COVAXIN Factsheet-V2_cc.cdr. [Internet]. 2021. Available from: https://www.bharatbiotech.com/

images/covaxin/covaxin-factsheet.pdf. (Accessed: 11.07.2021)
35. *Ganneru B., Jogdand H., Daram VK., Das D., Molugu NR., Prasad SD., et al.* Th1 skewed immune response of whole virion inactivated SARS CoV 2 vaccine and its safety evaluation. iScience. 2021; 24(4):102298. doi: 10.1016/j.isci.2021.102298.
36. *Thiagarajan K.* What do we know about India's Covaxin vaccine? BMJ. 2021;373:n997 doi: 10.1136/bmj.n997.
37. *Sapkal G., Yadav P., Ella R., Abraham P., Patil D., Gupta N., et al.* Neutralization of B.1.1.28 P2 variant with sera of natural SARS-CoV-2 infection and recipients of BBV152 vaccine. Journal of Travel Medicine. 2021. 1-3. doi: 10.1093/jtm/taab077.
38. Fact Sheet for Vaccine Recipients and Caregivers Restricted Use of COVAXIN™ Under Clinical Trial Mode. The Bharat Biotech Covid-19 Vaccine (COVAXIN™) to Prevent Coronavirus Disease 2019 (COVID-19). [Internet]. 2021. Available from: https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/en/biotechver.pdf. (Accessed: 12.07.2021)
39. Posted by Fancy Comma LLC. How Does China's CoronaVac Vaccine for COVID-19 Work? [Internet]. Fancy Comma, LLC. 2021. Available from: https://fancycomma.com/2020/07/22/coronavac-inactivated-covid19-vaccine/ (Accessed: 12.07.2021)
40. SINOVAC BIOTECH LTD. COVID-19 Vaccine (Vero cell), inactivated. [Internet]. 2021. Available from: https://cdn.who.int/media/docs/default-source/immunization/sage/2021/april/4_sage29apr2021_sinovac.pdf?sfvrsn=2d82605d_5(Accessed: 12.07.2021)
41. Qazi A. 20210421-Guidelines-for-Sinovac-6301 [Internet]. 2021. Available from: http://www.hsa.edu.pk/wp-content/uploads/20210421-Guidelines-for-Sinovac-6301.pdf. (Accessed: 12.07.2021)
42. World Health Organization. Evidence Assessment. Sinovac/CoronaVac COVID-19 vaccine. [Internet]. 2021 Available from: https://cdn.who.int/media/docs/default-source/immunization/sage/2021/april/5_sage29apr2021_critical-evidence_sinovac.pdf?sfvrsn=2488098d_5. (Accessed: 12.07.2021)
43. COVID-19 Vaccine (Vero cell), Inactivated (Brief Edition). [Internet]. 2021. Available from: https://www.covidvaccine.gov.hk/pdf/CoronaVac_ENG_PI_brief.pdf. (Accessed: 12.07.2021)
44. COVID-19 Vaccine (Vero Cell), Inactivated (Sinopharm). [Internet]. 2021. Available from: https://www.who.int/docs/default-source/coronaviruse/v.3_21195_sinopharm-vaccine-explainer-24.pdf?sfvrsn=e7507eb4_19&download=true. (Accessed: 12.07.2021)
45. The Sinopharm COVID-19 vaccine: What you need to know [Internet]. World Health Organization. World Health Organization. Available from: https://www.who.int/news-room/feature-stories/detail/the-sinopharm-covid-19-vaccine-what-you-need-to-know. (Accessed: 12.07.2021)
46. Evidence Assessment: Sinopharm/BBIBP COVID-19 vaccine. [Internet]. 2021. Available from: https://cdn.who.int/media/docs/default-source/immunization/sage/2021/april/2_sage29apr2021_critical-evidence_sinopharm.pdf. (Accessed: 12.07.2021)
47. *Saeed BQ., Al-Shahrabi R., Alhaj SS., Alkokhardi ZM., Adrees AO.* Side Effects and Perceptions Following Sinopharm COVID-19 Vaccination. BMJ Yale. 2021 Jul 1. (Preprint) [Internet]. Available from: https://www.medrxiv.org/content/10.1101/2021.06.28.21258847v1. (Accessed: 12.07.2021)
48. Convidicea Vaccine (Ad5-nCoV) Description [Internet]. 2021 Jun 30. Available from: https://www.precisionvaccinations.com/vaccines/convidicea-vaccine. (Accessed: 12.07.2021)
49. *Zhu F-C., Guan X-H., Li Y-H., Huang J-Y., Jiang T., Hou L-H., et al.* Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. The Lancet. 2020;396(10249):479–88. doi: 10.1016/S0140-6736(20)31605-6.
50. CanSino COVID-19 vaccine. Efficacy and effectiveness of the vaccine. [Internet]. 2021 Available from: https://covid-19pharmacovigilance.paho.org/vacuna-de-cansino. (Accessed: 13.07.2021)
51. EpiVacCorona Vaccine [Internet]. Welcome to Precision Vaccinations. Available from: https://www.precisionvaccinations.com/vaccines/epivaccorona-vaccine. (Accessed: 13.07.2021)
52. *Dobrovidova O.* Russia's COVID-19 defense may depend on mystery vaccine from former bioweapons lab-but does it work? [Internet]. Science. 2021. [cited 2021 Jul 13]. Available from: https://www.sciencemag.org/news/2021/04/russia-s-covid-19-defense-may-depend-mystery-vaccine-former-bioweapons-lab-does-it-work. (Accessed: 13.07.2021)
53. International LDS. Watchdog: Russia's EpiVacCorona Vaccine Has '100 Percent Immunological Efficacy' [Internet]. Sputnik International. Sputnik Internationalhttps://cdn2.img.sputniknews.com/i/logo.png; 2021. Available from: https://sputniknews.com/russia/202101191081813036-efficacy-of-russias-epivaccorona-vaccine-is-100-percent-watchdog-says/. (Accessed: 13.07.2021)
54. Zifivax (ZF2001) COVID-19 Vaccine [Internet]. 2021. Available from: https://www.precisionvaccinations.com/vaccines/zifivax-zf2001-covid-19-vaccine. (Accessed: 14.07.2021)
55. *Yang S., Li Y., Dai L., Wang J., He P., Li C., et al.* Safety and immunogenicity of a recombinant tandem-repeat dimeric RBD-based protein subunit vaccine (ZF2001) against COVID-19 in adults: two randomised, double-blind, placebo-controlled, phase 1 and 2 trials. Lancet Infect Dis. 2021:S1473-3099(21)00127-4. doi: 10.1016/S1473-3099(21)00127-4.

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ORIGINAL ARTICLE

OCCURRENCE OF POLYCYCLIC AROMATIC HYDROCARBONS IN HUMAN DIET – EXPOSURE AND RISK ASSESSMENT TO CONSUMER HEALTH*)

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ABSTRACT

Background. Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants, they are also present in food, in which their presence results from environmental pollution and food processing processes. Many compounds from this group, such as benzo(a)pyrene show important toxicity, including genotoxic carcinogenicity. In food heavier PAHs significantly toxic are observed.

Objective. The aim of the study was assessment of consumers exposure to PAHs from the diet of surveyed respondents. The assessment of contaminants content in daily food rations is characterized by less uncertainty factor than the assessment based on data on the contamination of individual foodstuffs and their consumption by humans.

Material and methods. Research material consisted of daily diets obtained from respondents participating in the study. Content of 22 PAHs (fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(c)fluorene, benz(a)anthracene, chrysene, 5-methylchrysene, perylene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(j)fluoranthene, benzo(e) pyrene, benzo(a)pyrene, benzo(ghi)perylene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, dibenzo(a,e)pyrene, dibenzo(a,l)pyrene, dibenzo(a,h)pyrene, dibenzo(a,i)pyrene) in each of diets was tested using liquid chromatography with a fluorescence detector. The samples were purified by saponification, size exclusion chromatography (SEC) and solid phase extraction (SPE).

Results. 52 respondents (n=52) took part in the study. The highest median of PAHs were found for pyrene (1.412 μg/kg), phenantrene (1.276 μg/kg), fluorene (1.151 μg/kg) and fluoranthene (1.087 μg/kg), they were about 10-80 higher than the levels of heavier PAHs. In group of heavy PAHs quantitatively prevailed benzo(e)pyrene (0.109 μg/kg), benzo(b) fluroanthene (0.070 μg/kg), benzo(ghi)perylene (0.065 μg/kg) and perylene (0.059 μg/kg). Generally the median level of contamination with light PAHs was 6.045 μg/kg, while with heavy ones 0.504 μg/kg, in the case of the sum of 4 PAHs regulated in EU law content was 0.301 μg/kg. In the tested samples average 24% of the PAH content was pyrene, light PAHs with a lower toxicity potential accounted for 92% of the content of tested compounds. Sum of 4 regulated PAHs accounted for 58% of content compounds selected by the EU as significant for the assessment of food contamination by PAHs. The composition of the participants' diets was analyzed in terms of determining factors influencing on high levels of PAHs. They were high fat level and presence of smoked or grilled meat and fish products. The mean exposure to benzo(a)pyrene was 0.52 ng/kg b.w. per day, while for the sum of 4 PAHs 3.29 ng/ kg b.w. per day. For light PAHs high exposure was 90.6 ng/kg b.w. per day, while for heavy PAH it was 10.7 ng/kg b.w. per day. Risk assessment was performed by calculating the value of margin of exposure (MoE), which for benzo(a)pyrene and for sum of 4 PAHs were above 25,000 in both considered: mean and high exposure scenario.

Conclusions. Studied diets were a source of exposure to PAHs. Higher levels have been reported for light, less toxic PAH as compared to heavy PAH. In both considered scenarios margin of exposure were >25 000. In case of studied diets no risk for consumer was found.

Key words: *Polycyclic aromatic hydrocarbons, PAHs, benzo(a)pyrene, exposure, margin of exposure, total diet study*

STRESZCZENIE

Wprowadzenie. Wielopierścieniowe węglowodory aromatyczne (WWA) stanowią zanieczyszczenie środowiskowe, występują również w żywności, w której ich obecność wynika z zanieczyszczenia środowiska oraz procesów przetwarzania żywności. Wiele związków z tej grupy wykazuje działalnie toksyczne w tym genotoksyczne kancerogenne, jak np. benzo(a)piren. W żywności obserwuje się cięższe WWA o większym potencjale toksycznym.

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Cel. Celem pracy była ocena narażenia konsumentów na WWA pochodzące z diety badanych respondentów. Ocena zawartości zanieczyszczeń w całodziennych racjach pokarmowych cechuje się mniejszą niepewności niż ocena na podstawie danych o zanieczyszczeniu poszczególnych środków spożywczych i ich spożyciu przez ludzi.
Materiał i metody. Materiał badawczy stanowiły całodzienne diety uzyskane od respondentów biorących udział w badaniu. W każdej z diet badano zawartość 22 WWA (fluoren, fenantren, antracen, fluoranten, piren, benzo(c)fluoren, benz(a)antracen, chryzen, 5-metylochryzen, perylen, benzo(b)fluoranten, benzo(k)fluoranten, benzo(j)fluoranten, benzo(e)piren, benzo(a)piren, benzo(ghi)perylen, indeno(1,2,3-cd)piren, dibenzo(a,h)antracen, dibenzo(a,e)piren, dibenzo(a,l)piren, dibenzo(a,h)piren, dibenzo(a,i)piren), zastosowano technikę chromatografii cieczowej z detektorem fluorescencyjnym. Próbki oczyszczano metodą zmydlania, a następnie techniką chromatografii wykluczenia (SEC) i ekstrakcji do fazy stałej (SPE).
Wyniki. W badaniu udział wzięło 52 respondentów (n=52). Najwyższe wartości mediany występowania WWA w diecie stwierdzono dla pirenu (1,412 μg/kg), fenantrenu (1,276 μg/kg), fluorenu (1,151 μg/kg) i fluorantenu (1,087 μg/kg), były one około 10-80 wyższe niż poziomy zawartości cięższych WWA. W grupie cięższych węglowodorów ilościowo przeważał benzo(e)piren (0,109 μg/kg), benzo(b)fluroanten (0,070 μg/kg), benzo(ghi)perylene (0,065 μg/kg) oraz perylen (0,059 μg/kg). Ogółem poziom mediany zanieczyszczenia lekkimi WWA wynosił 6,045 μg/kg, natomiast ciężkimi 0,504 μg/kg, w przypadku sumy 4 WWA uregulowanych w przepisach UE zawartość wynosiła 0,301 μg/kg. W badanych próbkach przeciętnie 24% zawartości WWA stanowił piren, ogółem lekkie WWA o mniejszym potencjale toksycznym stanowiły 92% zawartości badanych związków. Suma 4 uregulowanych WWA stanowiła 58% zawartości wytypowanych przez UE jako istotne dla oceny zanieczyszczenia żywności. Przeanalizowano skład diet uczestników pod kątem określenia czynników wpływających na wysokie poziomy WWA. Były to wysoka zawartość tłuszczu oraz obecność produktów mięsnych i rybnych wędzonych lub grillowanych. Średnie narażenie na benzo(a)piren wynosiło 0,52 ng/kg m.c./dzień, natomiast na sumę 4 WWA 3,29 ng/kg m.c./dzień. Dla lekkich WWA wysokie narażenie wynosiło 90,6 ng/kg m.c./dzień, natomiast da ciężkich WWA 10,7 ng/kg m.c./dzień. Oceny ryzyka dokonano obliczając wartość marginesu narażenia, który zarówno dla benzo(a)pirenu jak i sumy 4 WWA wynosił powyżej 25 000 dla obu badanych scenariuszy narażenia (średniego i wysokiego).
Wnioski. Badane diety stanowiły źródło narażenia na WWA. Wyższe poziomy odnotowano dla lekkich mniej toksycznych WWA w porównaniu z ciężkimi WWA. W przypadku badanych diet nie stwierdzono ryzyka dla zdrowia konsumentów.

Słowa kluczowe: *wielopierścieniowe węglowodory aromatyczne, WWA, benzo(a)piren, narażenie, margines narażenia, badanie całodziennej diety*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a large group of hydrocarbons composed of at least 2 aromatic rings. These substances are formed in combustion processes and constitute environmental pollution, they are present in water [28], air [27], also are a factors of exposure at certain workplaces [13, 21] and are contained in tobacco smoke [1]. They are also present in food [7, 5, 11, 22]. Higher levels of PAHs are observed in high food with high content of fat, e.g. oils and fats, meat and fish products. These compounds are present also food stuffs subjected to heat (e.g. grilling) or smoke treatment. They show various toxic effects, some light PAHs, e.g. pyrene, are classified as compounds with a low toxic potential, even 1000 times lower than the well-known heavy PAH benzo(*a*) pyrene, which is a carcinogenic factor for humans [22, 10]. The reported value of 1000 results from the use of the toxic equivalent factor (TEF), which assign value of 1 for benzo(a)pyrene. For pyrene, fluorene, fluoranthene and phenanthrene these values are lower and equal 0.001, for chrysene 0.01, for benz(a) anthracene and benzo(*b*)fluoranthene 0.1 [17].

PAHs show little chemical reactivity. In the human body, in the first phase of metabolism, they undergo oxidation on the CYP450 cytochrome to hydroxy- and polyhydroxy hydrocarbons: diols, triols and tetraols. In this process also epoxides can be formed, which characterized by high reactivity and affinity to purine bases [22]. The covalent bond between the epoxide and the DNA base leads to damage (mutation) of the strand, which may cause carcinogenesis. Therefore benzo(*a*)pyrene is classified as a genotoxic carcinogen [22, 15, 18]. The International Agency for Research on Cancer (IARC) classifies this compound in the group of agents with proven carcinogenic effect on humans, i.e. group I [10]. Studies linking toxicity with the structure of the PAH molecule have shown that heavier PAHs are particularly toxic and can create a characteristic spatial structure called 'bay-region'. Epoxides of these compounds show a significant genotoxic and carcinogenic effect [16].

Considering a human exposure it is important to emphasized that PAHs are a group of compounds for which numerous and diverse sources of exposure should be taken into account [10, 20]. In the case of environmental exposure relatively high levels of light PAHs (3-4 ring compounds) are observed in comparison to heavier PAHs with 5 and more rings [22]. For food this difference is less significant due to the both lower levels of light PAHs and higher levels of heavier, more toxic PAHs. These substances are non-volatile, their presence in the environment is related to pollution by dust, especially PM1, PM2.5 and PM10 fractions [27] to which heavier PAHs

show high adhesion. Food contamination by PAHs is favoured by very good fat solubility. Therefore it should be emphasized that from the point of view of risk to human health, food is a particularly important source of exposure. The risk related to the presence of PAHs in food and human environment should be considered taking into account many compounds, two most studied groups are called 16 PAHs according to EPA [29], as well as 15 PAHs according to EU recommendation, usually supplemented with benzo(*c*) fluorene [11, 3]. The PAHs group established by the EPA is universal, these compounds occur both in food and in environment. PAHs from the 16th EU are characteristic of oral exposure.

Over the last few decades many studies have been written on the presence of PAH in food. One of the more comprehensive studies are published by European Food Safety Authority (EFSA) [7, 5] and the former Scientific Committee on Food [22]. The most common approach to exposure assessment is to determine the content of individual PAHs in different foodstuffs. Taking into account available data on the consumption of individual foodstuffs and level of contamination it is possible on this basis to calculate the product of these values which represent exposure of consumers. It is possible to adopt different exposure scenarios with lower and higher levels of contamination or consumption. However this approach is burdened relatively high uncertainty, because in relation to the level of contamination, uncertainty regarding consumption of foodstuffs is much greater. This approach is used in numerous works, including the mentioned EFSA studies.

Another approach which reduce uncertainty related to consumption is the total diet study approach (TDS) [9]. TDS are used to determine the content of many toxic substances including PAHs and various food constituents in reliable diets consumed by consumers. Such diets are much more representative for reliable consumption than calculation based on addition consumption of each foodstuffs. This approach also takes into account increase (or decrease) level of contamination resulting from culinary processing performed by consumers. Determining exposure and then risk to consumer health from TDS studies is more reliable than calculations based on consumption. However a significant limitation of the TDS approach is the much lower availability of consumers' daily diets, mainly related to the difficulty in obtaining appropriate samples. The objective of this study was to assess the exposure and risk to human health related to PAHs presence in diets of consumers, who took part in the study. They prepared samples for testing in line with the rule: "prepare an identical meal for yourself and study organizer". Obtained results were also compared with other similar studies.

MATERIAL AND METHODS

Diets

Diets were accumulated in summer 2015 from 52 study participants. Each participants lived in Warsaw. Each person accumulated a meal from one day. Study participants were asked to prepare meals identically and in identical amounts for themselves and for the purposes of the diet study. All persons were instructed in the preparation of diet samples and their storage. Participants of the study kept 'Test diary' in which they recorded in detail information on food preparation, ingredients used, cooking method and the time of consumption of each meal. Individual meals were packed in separate containers (provided by the test organizer) so that their contents could be verified against information in the diary. Participants reported also the year of birth, sex and body weight on the day of taking the diet.

Handling of samples

The test samples provided by the participants after checking the compliance with the study diary were weighed and then frozen (-18°C ÷ -24°C) until the PAH determinations were made. For the homogenization samples from one participant were combined into 2-4 bulk samples according to the type of food, in particular fat or water content and consistency (high fat and low fat samples were homogenized separately). Homogenization was performed using an UltraTurrax homogenizer until a homogeneous mass was achieved. Before homogenization samples with a high fat content were heated in a water bath at 40°C. The composite samples were weighed and a aliquot portion was taken from each of them to form one laboratory sample representing the participant's entire diet. The weight of the laboratory sample was approximately 50 g.

Reagents and standards

The following reagents were used: ethyl alcohol 96% p.a. (POCh), cyclohexane p.a. (Chempur), ethyl acetate p.a. (Chempur), hexane p.a. (Sigma), dichloromethane p.a. (Baker UltreResi), HPLC acetonitrile (Baker HPLC gradient grade), sodium hydroxide p.a. (Chempur), acetic acid glacial p.a. (Sigma), sodium sulphate anhydrous p.a. (Sigma). Deionized water of HPLC purity was used. BioBeads SX-3 (BioRad) gel was used to purify the sample on a size exclusion chromatography (SEC) column. For solid phase extraction (SPE) purification, 1 g, 6 ml silicagel columns (JT Baker) were used. Following standards were purchased: PAH-Mix 45 from Dr Ehrenstorfer GmbH containing naphthalene, acenafthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(*a*)anthracene, chrysene, perylene, benzo(*b*)fluoranthene, benzo(*k*)

fluoranthene, benzo(*e*)pyrene, benzo(*a*)pyrene, benzo(*ghi*)perylene, indeno(*1,2,3-cd*)pyrene and dibenzo(*a,h*)anthracene each 10 ng/µl in cyclohexane and PAH-Mix 183 from Dr Ehrenstorfer GmbH containing benzo(*c*)fluorine, benz(*a*)anthracene, chrysene, 5-methylchrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*j*)fluoranthene, benzo(*a*) pyrene, benzo(*ghi*)perylene, cyclopenta(*cd*)pyrene, indeno(*1,2,3-cd*)pyrene, dibenzo(*a,h*)anthracene, dibenzo(*a,e*)pyrene, dibenzo(*a,l*)pyrene, dibenzo(*a,h*) pyrene and dibenzo(*a,i*)pyrene each 10 ng/µl in cyclohexane.

Sample preparation

10 g of homogeneous laboratory sample was weighed. Sample was placed in a round bottom flask and then 100 ml of a solution of 1.5 M sodium hydroxide in ethanol was added. The flask fitted with a reflux condenser was heated in a heating bowl to the solution boiling temperature for 2 hours. Then contents of the flask were diluted with 100 ml of 10% acetic acid solution with water and whole mixture was transferred to the separatory funnel. It was extracted with two 50 ml portions of cyclohexane and dried over anhydrous sodium sulfate. The cyclohexane solution was concentrated on rotary evaporator in a water bath at 40°C to a volume of about 5 ml. The resulting concentrated extract was dispensed onto a size exclusion chromatography (SEC) column. Working parameters of the SEC/GPC chromatograph: filling Bio-Beads SX3 gel (in-house packed), sample was dissolved in SEC/GPC solvent cyclohexane:ethyl acetate = 1:1 (v/v) and filled up to 10 ml, 7 ml was injected to loop, initial flow 1 ml/min (during loop washing), then was increased to 2 ml/min. First fraction of 40 ml was discarded and second fraction (proper) of 35 ml was collected. Obtained fraction was concentrated by rotary evaporator in water bath at 40°C. The residue was reconstituted in 1 ml of hexane:dichloromethane = 3:1 (v/v) and applied to previously conditioned SPE column containing 1 g of silicagel, then elute with 8 ml of hexane/dichloromethane (as above). The resulting fraction was concentrated at 40°C under a gentle stream of nitrogen. The residue was reconstituted in 1 ml of acetonitrile.

Determination of PAH content

Standard laboratory equipment and laboratory glassware were used. SEC/GPC purification was performed using a 500 mm x 15 mm (internal diameter) Omnifit glass column (Sigma) and Waters 501 chromatographic pump equipped with the injection valve with a loop of 8 ml, fraction collector valve and electronic controller (of our own design). For separation and measure PAHs content high performance liquid chromatograph was used. HPLC Waters Alliance 2695 was equipped with column Agilent PAH Pursuit 250 mm x 4.6 mm column, 5 µm, integrated with a 10 mm x 4.6 guard column as well as equipped with column oven, automatic sample dispenser equipped and Waters 2475 fluorescence detector.

The determination was performed using a high-performance liquid chromatograph equipped with a column dedicated to the determination of PAHs and a fluorescence detector. A sample volume of 50 µl was injected. Temperature of chromatographic column was set at 30°C. Gradient elution program: solvent A: acetonitrile, solvent B: water; flow 1 ml/min; A: 0 min 50% (vol.), 20 min 50%, 50 min 90%, 75 min 90%, 80 min 100% to 110 min. Frequency of excitation (ex) and emission (em) for individual compounds for fluorescence detector (ex [nm] / em [nm]) and retention times [min]: FLN 270/323 R_t=29,3; PHE 252/370 R_t=32,6; ANT 252/402 R_t=35,5; FLT 280/460 R_t=38,6; PYR 270/375 R_t=40,5; BcFl 309/357 R_t=41,1; BaA 286/387 R_t=46,9; CHR 266/408 R_t=48,0; 5MCh 266/382 R_t=49,8; BeP 286/387 R_t=52,0; BjF 250/510 R_t=51,6; BbF 298/433 R_t=52,7; PER 406/439 R_t=52,8; BkF 298/433 R_t=54,7; BaP 380/406 R_t=56,9; DBahA 286/397 R_t=61,1; DBalP 313/446 R_t=61,4; BghiP 286/408 R_t=64,2; IcdP 300/502 R_t=66,3; DBaeP 386/397 R_t=71,6; DBaiP 292/434 R_t=96,0; DBahP 307/451 R_t=105,3. Each sample was injected twice. Calibration covers 6 points injected twice.

Validation parameters

Determination of PAHs in total diets was performed with in-house validated method. Laboratory is covered by a quality management system compliant with the PN-EN ISO/IEC 17025:2018. Table 1 presents the most important validation parameters for individual compounds, such as limit of detection (LOD), limit of quantification (LOQ), recovery, repeatability, working range, slope of the calibration curve and expanded measurement uncertainty. Validation experiment based on testing 6 samples at each of the three validation levels.

RESULTS AND DISCUSSION

In case of polycyclic aromatic hydrocarbons it is important to analyze the presence of specific groups of compounds, which is distinguished taking into account number of rings, toxicity and source of exposure. One of the most frequently used distinction is division into light PAHs, which include among other 3 and 4 rings and heavy, which include 5 and 6 rings compounds. Light hydrocarbons are observed at higher concentration levels in food and are also present in air and water. For heavier compounds, lower levels are recorded. The heaviest dibenzopyrenes are

Table 1. Validation parameters for the method of determination of polycyclic aromatic hydrocarbons in diets. Abbreviations used in the text are also listed

PAH	Abbreviation	LOD	LOQ	Recovery	Repeatability RSD_r	Expanded uncertainty U_c	Working range	Slope of calibration curve
		µg/kg	µg/kg	%	%	µg/kg	µg/kg	
fluorene	FLN	0.030	0.050	84.2	11.7	18.0	0.05-5.00	$7.02 \cdot 10^6$
phenanthrene	PHE	0.050	0.100	93.1	12.5	20.1	0.10-5.00	$2.23 \cdot 10^6$
anthracene	ANT	0.030	0.050	96.1	12.2	19.0	0.05-5.00	$7.15 \cdot 10^6$
fluoranthene	FLT	0.030	0.050	89.0	13.0	21.1	0.05-5.00	$1.32 \cdot 10^6$
pyrene	PYR	0.050	0.100	98.0	6.6	12.8	0.10-10.00	$3.51 \cdot 10^6$
benzo(*c*)fluorene	BcFl	0.020	0.040	98.5	6.4	11.3	0.04-5.00	$9.65 \cdot 10^6$
benz(*a*)anthracene	BaA	0.010	0.020	99.1	6.3	11.0	0.02-5.00	$7.71 \cdot 10^6$
chrysene	CHR	0.020	0.040	101.2	6.5	11.4	0.04-5.00	$5.51 \cdot 10^6$
5-methylchrysene	5MCh	0.010	0.020	94.8	8.9	15.1	0.02-5.00	$4.04 \cdot 10^6$
perylene	PER	0.020	0.040	74.1	13.2	18.8	0.04-5.00	$1.83 \cdot 10^7$
benzo(*b*)fluoranthene	BbF	0.010	0.020	91.2	8.3	14.5	0.02-5.00	$3.04 \cdot 10^6$
benzo(*k*)fluoranthene	BkF	0.004	0.005	89.4	8.1	14.0	0.005-5.00	$1.58 \cdot 10^7$
benzo(*j*)fluoranthene	BjF	0.060	0.120	103.7	9.1	15.0	0.12-5.00	$6.49 \cdot 10^4$
benzo(*e*)pyrene	BeP	0.010	0.020	90.3	8.9	14.6	0.02-5.00	$1.67 \cdot 10^6$
benzo(*a*)pyrene	BaP	0.005	0.010	83.8	9.4	16.1	0.01-5.00	$4.45 \cdot 10^6$
benzo(*ghi*)perylene	BghiP	0.010	0.020	70.9	12.6	21.3	0.02-5.00	$2.78 \cdot 10^6$
indeno(*1,2,3-cd*)pyrene	IcdP	0.010	0.020	83.4	11.0	17.7	0.02-5.00	$7.09 \cdot 10^5$
dibenzo(*a,h*)anthracene	DBahA	0.020	0.040	91.6	9.1	14.9	0.04-5.00	$5.33 \cdot 10^6$
dibenzo(*a,e*)pyrene	DBaeP	0.020	0.040	71.7	16.8	27.0	0.04-5.00	$4.36 \cdot 10^6$
dibenzo(*a,l*)pyrene	DBalP	0.020	0.040	68.6	17.2	28.0	0.04-5.00	$3.94 \cdot 10^6$
dibenzo(*a,h*)pyrene	DBahP	0.020	0.040	79.4	15.0	24.8	0.04-5.00	$3.47 \cdot 10^7$
dibenzo(*a,i*)pyrene	DBaiP	0.020	0.040	55.9	17.6	36.5	0.04-5.00	$5.63 \cdot 10^6$

found in a few samples. In food, as in other sources of exposure, lower levels of heavier PAHs are observed but decrease in their content compared to light PAHs is relatively smaller than, for example in the air. Another group of compounds often distinguished due to the provisions of EU law [4] is the sum of 4 PAHs, which include benz(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*a*)pyrene and chrysene. Data in this field has shown that they represent about 60% of the content of 4 or more rings PAH in food [7]. Benzo(*a*)pyrene is distinguished among PAHs due to its significant toxicity as well as for comparison with results many previous studies. Additionally, in the case of the list of PAHs established by EC recommendation [3], models with 2 and 8 compounds were also used for risk assessment [7]. In this study PAHs were classified into groups listed in Table 2.

This study involved 52 adult respondents (n=52), including 22 men and 30 women. Since no differences in PAH metabolism are observed between men and women, results were analyzed in relation to the entire study population. For obtained results, which were contents of individual PAHs in the daily diet, quantile parameters were calculated, such as 10th percentile (P10), 25th percentile (P25), median (Me), 75th percentile (P75), 90th percentile (P90), number and rate of results above the limit of quantification (LOQ). These values are presented in Table 3 and in the box plot 1. Statistical calculations for estimation of PAH levels was carried out takin into account *medium bound* approach [6], i.e. values below the limit of quantification (LOQ) were assigned a value of the limit of detection (LOD), while values below the limit of detection (LOD) were assigned a value of zero. For assessment of high consumer exposure value of P95 of contamination is typically used, but due to the limited number of respondents in this study, a more meaningful P90 value with greater statistical certainty was used for this purpose. In the case of the presence of most chemical contaminants of food, including PAHs, normal distribution is not observed, therefore estimators such as mean and standard deviation were not used as non-representative.

Table 2. Classification PAHs into groups used in this study

PAHs	Nap Ace Acy	FLU PHE ANT FLN PYR	BcFl	BaA	CHR	5MCh	PER BeP	BbF BaP	BkF BghiP IcdP DBahA	BjF DBaeP DBalP DBahP DBaiP	CPcdP
22 PAHs		+	+	+	+	+	+	+	+	+	
Light PAHs		+	+	+	+	+					
Heavy PAHs							+	+	+	+	
Sum of 4 PAHs				+	+			+			
PAH2 EC [7]				+	+						
PAH4 EC [7]				+	+			+			
PAH8 EC [7]				+	+			+	+		
15 PAH EC				+	+	+		+	+	+	+
16 PAH EC/JECFA			+	+	+	+		+	+	+	+
16 US EPA	+	+		+	+			+	+		

Table 3. PAH content in the total diet. Table shows number of samples above the limit of quantification and quantile parameters such as: 10th percentile (P10), 25th percentile (P25), median (Me), 75th percentile (P75), 90th percentile (P90). For purpose of statistical calculations *medium bound* approach [6] was adopted

PAH	No. of rings	Results above LOQ		PAHs content in total diet				
		No.	Rate	P10	P25	median	P75	P90
			%	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
FLN	3	52	100	0.578	0.712	1.151	1.39	2.574
PHE	3	52	100	1.123	1.217	1.276	1.654	2.284
ANT	3	52	100	0.138	0.152	0.273	0.322	0.388
FLT	4	52	100	0.704	0.951	1.087	1.527	1.663
PYR	4	52	100	0.956	1.171	1.412	1.976	2.223
BcFl	4	52	100	0.063	0.069	0.096	0.120	0.240
BaA	4	52	100	0.036	0.047	0.062	0.110	0.140
CHR	4	52	100	0.071	0.085	0.117	0.171	0.197
5MCh	4	30	58	< 0.020	< 0.020	0.027	0.038	0.046
PER	5	43	83	< 0.040	0.048	0.059	0.157	0.387
BbF	5	52	100	0.024	0.029	0.070	0.118	0.124
BkF	5	52	100	0.008	0.010	0.017	0.031	0.051
BjF	5	0	0	< 0.120	< 0.120	< 0.120	< 0.120	< 0.120
BeP	5	52	100	0.080	0.084	0.109	0.150	0.204
BaP	5	52	100	0.014	0.016	0.032	0.066	0.122
BghiP	6	52	100	0.023	0.036	0.065	0.082	0.221
IcdP	6	35	67	< 0.020	< 0.020	0.029	0.054	0.126
DBahA	5	0	0	< 0.040	< 0.040	< 0.040	< 0.040	< 0.040
DBaeP	6	0	0	< 0.040	< 0.040	< 0.040	< 0.040	< 0.040
DBalP	6	0	0	< 0.040	< 0.040	< 0.040	< 0.040	< 0.040
DBahP	6	0	0	< 0.040	< 0.040	< 0.040	< 0.040	< 0.040
DBaiP	6	0	0	< 0.040	< 0.040	< 0.040	< 0.040	< 0.040
Light PAHs	3-4			4.171	4.890	6.045	6.89	9.31
Heavy PAHs	5-6			0.221	0.240	0.504	0.794	1.100
Sum of 4 PAHs	4-5			0.151	0.228	0.301	0.469	0.520

For most of tested PAHs results above the limit of quantification were observed in 100% or about 100% of cases. Similarly authors of the study [23], who examining content of PAHs from the EPA list in typical diet ingredients (bread, cookies, tea, coffee, oils, chocolate, spices and fish) also found PAHs levels above the limit of quantification in all samples. A significantly lower number of results above the quantification limit was found for 5-methylchrysene. In case of 6 for indeno(1,2,3-cd)pyrene and benzo(*k*)fluoranthene are comparable. It should be noted that in study cited above, the average values are several times higher than the median value. As there are no details regarding the composition of diets, the reason of difference may be related to the type of diet. In this study, the assumption was that participants consume a freely composed balanced diet, but with the use of various ingredients contributing PAHs to their diet.

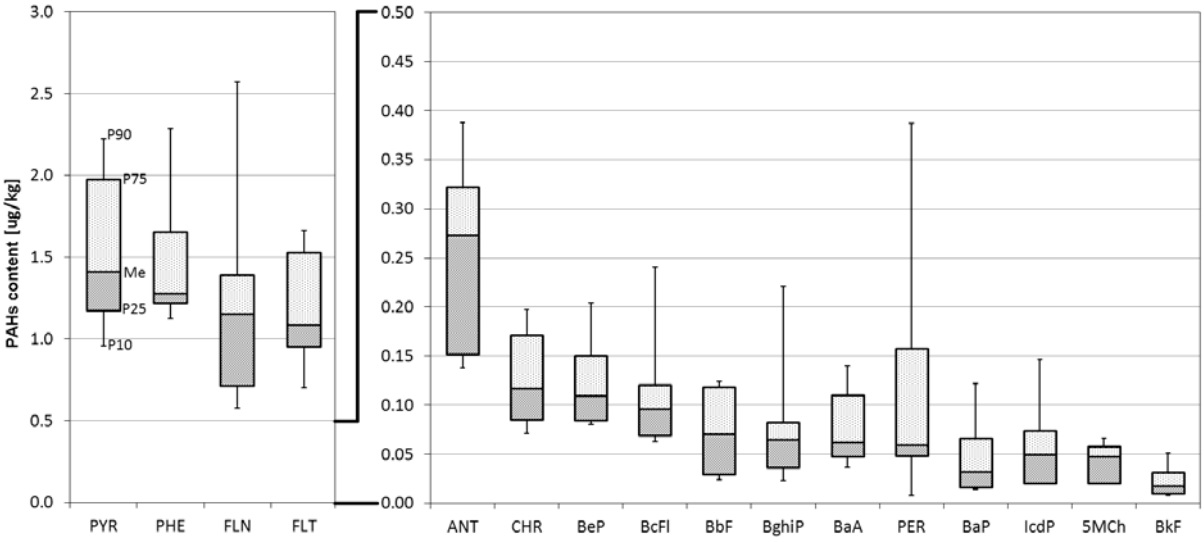

Figure 1. The prevalence of PAHs in studied diets – box diagram represents P25-median-P75 ranges, P10 and P90 values are plotted on the lines to the bars

heavier PAHs: benzo(*j*)fluoranthene, dibenzo(*a,h*) anthracene, dibenzo(*a,e*)pyrene, dibenzo(*a,l*) pyrene, dibenzo(*a,h*)pyrene and dibenzo(*a,i*)pyrene these compounds were not found in tested samples. These compounds are also rarely observed in monitoring of food samples. The highest values of median and P90 occurrence were found for pyrene, phenanthrene, fluorene and fluoranthene, respectively they were about 10-80 higher than the levels of the heavier PAHs. The lowest levels were observed for benzo(*k*)fluoranthene, indeno(*1,2,3-cd*)pyrene and benzo(*a*)pyrene. In group of light PAHs the lowest levels were found for 5-methylchrysene and benz(*a*) anthracene. In case of group of heavier hydrocarbons benzo(*e*)pyrene and benzo(*b*)fluroanthene were predominant. In a comparable study by *Polachowa* et al. [20] the highest median levels were observed for phenanthrene (0.858-0.861 ng/g), fluoranthene (0.191-0.192 ng/g) and pyrene (0.137-0.169 ng/g). These values are significantly lower than those recorded in this work. In the case of heavier PAHs, the author [20] obtained a median in range of 0.006-0.017 ng/g, excluding dibenzopyrene which were not found. Also in this case, results in this study indicates a higher contamination of the diet, ca. 2 times higher in relation to benzo(*a*)pyrene and 3-4 times higher for benzo(*b*)fluoranthene and benzo(*ghi*)perylene, values

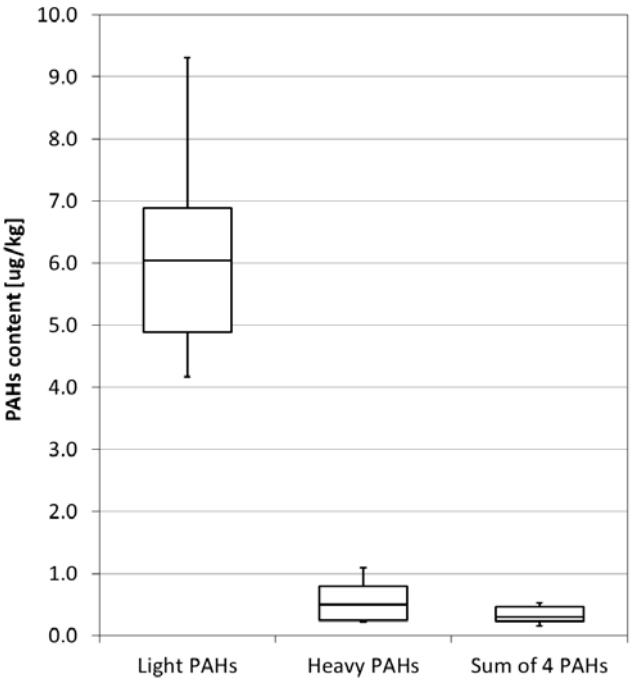

Figure 2. Occurrence of light PAHs, heavy PAHs and the sum of 4 PAHs (benz(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*a*)pyrene and chrysene) in the studied diets – box plot represents the P25-median-P75 ranges, using the lines at the bars P10 and P90 values are shown

The results for the discussed individual PAHs and their groups are presented in the Table 3 and in Figure 2. The median and P90 levels content of light PAHs was respectively 12 and 8 times higher than the content of heavier PAHs observed in the samples.

Taking into account obtained results it is possible to estimate contribution of individual PAHs in the diet, relevant data are presented in Figure 3. In tested samples at the median level pyrene, which did not have a significant toxic effect, contributed ca. 24% of total PAHs. In total light PAHs with a lower toxic potential constituted as much as 92% of all PAHs. EU legislation [4] with regard to food laid down requirements for benzo(*a*)pyrene and sum of 4 PAHs, the justification for establishing requirements for the sum of these compounds was the search for a model of food contamination marker by PAHs, which should represent a reasonably small number of compounds [5]. The level of the sum of 4 PAHs was in the range of 0.228-0.469 µg/kg (P25-P75), median 0.301 µg/kg, which corresponded to 58.4% of the content of all PAHs from EU list and is similar to the previous observations in the mentioned above EFSA report. A significant rate was also found for 4-rings benzo(*c*)fluorene (19%), the remaining compounds constitute 22.6%. Di-benzopyrenes, dibenzo(*a,h*)anthracene and also benzo(*j*)fluoranthene were not observed. Analyzing in detail the content of PAH sit should be stated that level of chrysene and benz(*a*)anthracene accounted for 64% of the total of 4 compounds, similar to the study on diets tested in France. In French study content of these two compounds was 59-70% [25].

PAHs. Greater dispersions were observed at higher concentrations.

An important element of the research was the comparative analysis of the diets of individual participants. For this purpose, two groups of participants were distinguished, whose diets contained the highest levels of PAHs and the lowest levels of PAHs. For each group, diets from 10 participants were qualified – with the lowest and highest levels of PAHs, respectively.

Diets with higher levels of PAHs contained mainly products with a high level of fat or ingredients contributing PAHs to the diet: grilled products, smoked meat and fish products, cocoa-derived products and marine algae (chlorella). In this case higher level of PAHs can results from fat pyrolysis leads to the formation of PAHs, contamination from smoke (smoked meat) and from drying processes (cocoa beans, marine algae) or from environmental pollution (marine algae). Observations made are consistent with data on the presence of PAHs in individual foodstuffs [20, 25, 8, 14, 19]. Lower levels of PAHs were observed in diets containing lunch meat dishes, in particular fried and cooked, composite products with processed meat (e.g. pizza, baked beans), legumes (excluding green beans),

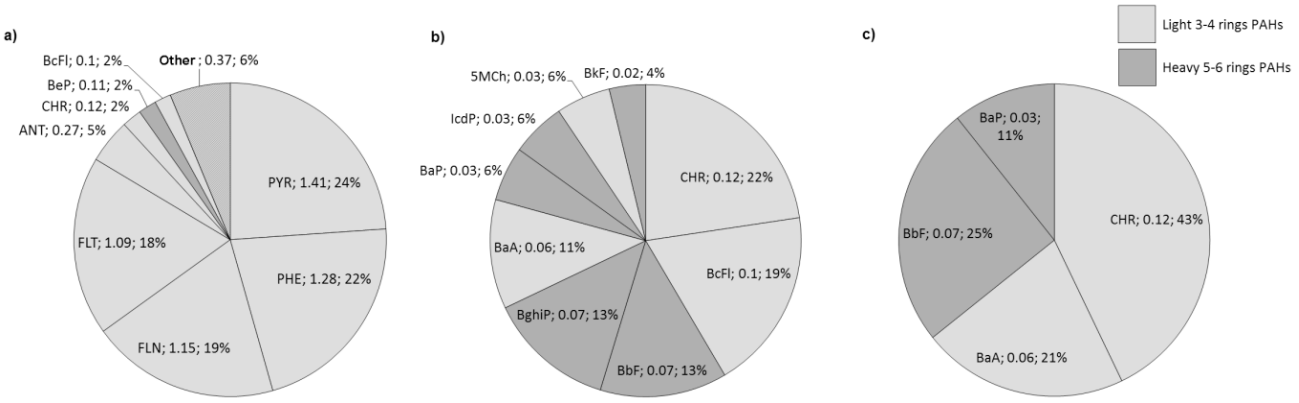

Figure 3. Contribution of individual PAHs in tested diets (level of median): a) in relation to the sum of all tested compounds, b) in relation to 15 PAHs from the EU list (cyclopenta(*cd*)pyrene was not covered), c) in relation to the sum of 4 regulated PAHs in EU regulations [4].

Occurrence of light 3-4 rings PAHs is related to 5-6 rings compounds. Relevant correlation was found between the presence these PAHs groups, regression line and measurement points are shown in Figure 4. Square of the correlation coefficient was determined, which is 0.96 and indicates a significant relationship between the presence of both light and heavy

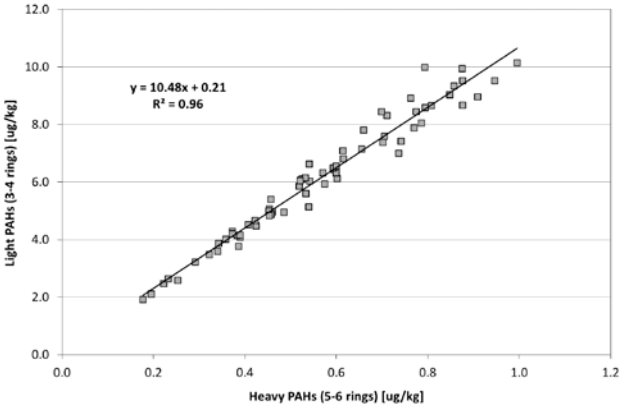

Figure 4. Correlation between presence of light PAHs and heavy PAHs in the studied diets.

nuts and all products with a low fat content, such as vegetables, fruit, salads, as well as potatoes, pasta, rice, dairy products, bread, cookies and confectionery (excluding chocolate). It confirms the key importance of dietary composition for PAH exposure.

Another objective of this research was to determine exposure of consumers to dietary PAHs and to assess the risk to consumer health. Exposure was calculated as the product of the PAH content in diets and consumption taking into account weight of diets provided by the participants. Daily exposure values obtained in this way have been expressed in ng/person. For comparison with the toxicometric parameters and results of other studies exposures were converted ng/kg b.w. per day taking into account corresponding body weight of participants, which were reported by respondents in study diary. Body weight of participants (adults) ranged from 48 kg to 105 kg, the median was 69.7 kg, the mean was 70.0 kg, standard deviation was 13.8 kg. Table 4 presents the quantile values of the exposure of studied population. There was shown exposure to all tested PAHs, light 3-4 rings PAHs, heavy PAHs 5-6 rings, sum of 4 PAHs and benzo(*a*)pyrene.

corresponding to 0.52 ng/kg b.w. per day. In EFSA scientific opinion mean value was ca. 8 times higher: 235 ng/person, while the value expressed in relations to body weight was comparable and amounted to 3.9 ng/kg b.w. per day. On the basis of the collected data exposure of consumers was also assessed in the higher risk group, which corresponding to the high intake of PAHs in studied diets, which is represented by P90. For light PAHs exposure was 90.6 ng/kg b.w. per day, while for heavy PAH it was 10.7 ng/kg b.w. per day. It was 8.5 times lower than the exposure to light PAH similarly to the case of average exposure. The high exposure to sum of 4 PAHs was 5.06 ng/kg b.w. per day, while for benzo(*a*)pyrene it was 1.19 ng/kg b.w. per day. These values were also lower in comparison to the results of exposure assessment published by EFSA [7], which gives 34.5 ng/kg b.w. per day for sum of 4 PAHs and 6.5 ng/kg b.w. per day for benzo(*a*)pyrene. It should be noted that in both this study and EFSA assessment results for high exposure are compared, although EFSA assessment for this purpose uses intake level of P97.5 and average contamination, while in this study risk assessment is based on reliability intake and P90 level of contamination. Table 5 summarizes the results of estimated exposure and exposure values from works of other authors [7, 25, 8, 14, 12, 24, 26].

Table 4. Exposure of participants (n=52) to PAHs in studied diets. Exposure to the sum of all tested PAHs, light PAHs, heavy PAHs, benzo(*a*)pyrene and sum of 4 PAHs (benz(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene and benzo(*a*)pyrene)

Quantile	Exposure to PAHs in diets									
	Light PAHs		Heavy PAHs		Total PAHs		Benzo(*a*)pyrene		Sum of 4 PAHs	
	ng/ person	ng/kg b.w.	ng/ person	ng/kg b.w.	ng/ person	ng/kg b.w.	ng/ person	ng/kg b.w.	ng/ person	ng/kg b.w.
P10	2880	40.6	153	2.2	3033	42.8	9.7	0.14	104.3	1.47
P25	3376	47.6	166	2.3	3542	50.0	11.0	0.16	157.4	2.22
Median	4173	58.9	348	4.9	4521	63.8	22.1	0.31	207.8	2.96
P75	4757	67.1	548	7.7	5305	74.8	45.5	0.64	323.8	4.57
P90	6427	90.6	760	10.7	7187	101.4	84.2	1.19	359.0	5.06
Mean	4237	59.8	390	5.5	4627	65.3	36.7	0.52	233.4	3.29
RSD	32.9%		29.7%		33.8%		32.0%		34.1%	

In most cases (excluding benzo(*a*)pyrene) the median exposure was close to the mean. Mean exposure to heavy PAHs was 5.5 ng/kg b.w. per day, which was 10.9 times lower than exposure to light PAHs. Exposure estimated for sum of 4 PAHs was on average 3.29 ng/kg b.w. per day, which corresponds to exposure 233.4 ng/person per day. Compared to EFSA risk assessment [7] daily exposure to sum of 4 PAHs was estimated at 1729 ng/day, which corresponding to 28.8 ng/ kg b.w. per day (EFSA's opinion based on a body weight of 60 kg) and it was significantly higher than estimated in this study. For benzo(*a*)pyrene the mean exposure was 36.7 ng/person per day, which

Calculated exposure shows even 80-fold differences between certain estimates (compare [25] and [26]). These differences result primarily from the adopted methodologies of exposure estimation. Another important element is also the handling of results below the quantification limit, in this paper the medium bound approach discussed earlier was adopted. Other approaches are often used to increase (upper bound) or decrease (lower bound) mean values and other estimators. There are also different approaches to methods of collecting diets or determining its contamination based on the contribution of individual contaminated ingredients.

Table 5. Comparison of the estimated exposure in this study and studies of other authors

Study	Scope of PAHs	Exposure type	Exposure	Exposure unit
this study	22 PAHs[a]	average (Me of contamination)	63.8	ng/kg b.w./day
		high (P90 of contamination)	101.4	ng/kg b.w./day
	light PAHs[b]	average (Me of contamination)	58.9	ng/kg b.w./day
		high (P90 of contamination)	90.6	ng/kg b.w./day
	heavy PAHs[c]	average (Me of contamination)	4.9	ng/kg b.w./day
		high (P90 of contamination)	10.7	ng/kg b.w./day
	Sum of 4 PAHs[d]	average (Me of contamination)	2.96	ng/kg b.w./day
		high (P90 of contamination)	5.06	ng/kg b.w./day
	BaP	average (Me of contamination)	0.31	ng/kg b.w./day
		high (P90 of contamination)	1.19	ng/kg b.w./day
EFSA, 2008 [7]	PAH8[e]	average (mean of contamination, Me of consumption)	28.8	ng/kg b.w./day
		average (mean of contamination, P97,5 of consumption certain products)	51.3	ng/kg b.w./day
	PAH4[d] = Sum of 4 PAHs	average (mean of contamination, Me of consumption)	19.5	ng/kg b.w./day
		average (mean of contamination, P97,5 of consumption certain products)	34.5	ng/kg b.w./day
	PAH2[f]	average (mean of contamination, Me of consumption)	10.7	ng/kg b.w./day
		average (mean of contamination, P97,5 of consumption certain products)	18.0	ng/kg b.w./day
	BaP	average (mean of contamination, Me of consumption)	3.9	ng/kg b.w./day
		average (mean of contamination, P97,5 of consumption certain products)	6.5	ng/kg b.w./day
Netherland TDS, 1998 [24]	15 PAH[g]	average for individual PAHs	1.1-22.5	µg/person/day
French TDS, 2013 [25]	PAH8[e]	average	2.281	ng/kg b.w./day
		high (P95)	4.454	ng/kg b.w./day
	PAH4[d] = Sum of 4 PAHs	average	1.478	ng/kg b.w./day
		high (P95)	2.998	ng/kg b.w./day
	BaP	average	0.191	ng/kg b.w./day
		high (P95)	0.350	ng/kg b.w./day
Catalonian TDS, 2012 [14]	16 EPA[h]	average	59.2	µg/person/day
Catalonian TDS, 2003 [8]	16 EPA[h]	average	6.72	µg/person/day
Italian TDS, 1994 [12]	16 EPA[h]	average	3.0	µg/person/day
	carcinogenic PAH[i]	average	1.4	µg/person/day
Dutch, 1990 [26]	16EPA[h]	low	5	µg/person/day
	16EPA[h]	high	17	µg/person/day
	carcinogenic PAH[i]	average	11	µg/person/day

TDS – total diet study, Me – median, (a) FLN, PHE, ANT, FLT, PYR, BcFl, BaA, CHR, 5MCh, PER, BeP, BbF, BaP, BkF, BghiP, IcdP, DBahA, BjF, DBaeP, DBalP, DBahP, DBaiP; (b) FLN, PHE, ANT, FLT, PYR, BcFl, BaA, CHR, 5MCh; (c) PER, BeP, BbF, BaP, BkF, BghiP, IcdP, DBahA, BjF, DBaeP, DBalP, DBahP, DBaiP; (d) BaA, CHR, BbF, BaP; (e) BaA, CHR, BbF, BaP, BkF, BghiP, IcdP, DBahA; (f) BaA, CHR; (g) BaA, CHR, 5MCh, BbF, BaP, BkF, BghiP, IcdP, DBahA, BjF, DBaeP, DBalP, DBahP, DBaiP, CPcdP; (h) NAP, ACE, ACY, FLN, PHE, ANT, FLT, PYR, BaA, CHR, BbF, BaP, BkF, BghiP, IcdP, DBahA; (i) BaA, BbF, BaP, BkF, BghiP, IcdP, DBahA.

Undoubtedly total diet study (in this work, also in [8, 12, 14, 25,]) is characterized by less uncertainty than the attempt to estimate contamination based on single diet components [7, 24]. The results obtained in this study are comparable or slightly higher than those presented in [7, 8, 12, 26]. However, they are much higher than in the study [25] concerning French diets and are significantly lower than Spanish study [14], which was performed by the same team as previous study described by [8]. Differences may also arise from a varied diet. Tested national diets are characterized by higher consumption of smoked products and general meat products in contrast to lower consumption of seafood.

In this work risk assessment was performed on the basis of margins of exposure (MoE), which were calculated for sum of 4 PAHs and for benzo(*a*)pyrene. In opinion of EFSA [7], based on the work of *Culp* et al. [2] toxicometric parameters were determined, such as $BMDL_{10}$ for benzo(*a*)pyrene for the genotoxic carcinogenic effect observed in rats which is 0.07 mg/kg b.w. and for the total of 4 PAHs which is 0.34 mg/kg b.w. Margin of exposure is calculated by division of critical toxicometric parameter (dose-response type) and consumer exposure. Indicated value of MoE informs how much less consumer is exposed in relation to dose causing toxic effect with certain probability. Calculated margins of exposure for consumers participating in study in comparison to results of EFSA assessment are presented in Table 6.

It is assumed that risk to consumer health not to be significant for genotoxic carcinogens when MoE value is greater than 10,000 and sometimes even greater than 25,000 in case of conservative approach. For purposes of this risk assessment MoE value of 10,000 has been taken as critical. Calculated margins of exposure in case of benzo(*a*)pyrene and in case of 4 PAHs based on results obtained of this study are much higher than results presented by EFSA [7]. Also for benzo(*a*)pyrene and 4 PAHs in both exposure scenarios (average or high exposure) critical value, as well as conservative value (25,000) is not exceeded.

CONCLUSIONS

The studied diets were a source of exposure consumers to PAHs. Higher levels have been reported for the light, less toxic PAHs compared to the more toxic 5-6 ring PAHs. Greater exposure of consumers to tested compounds is associated with the consumption of products with a high fat content including fried, grilled and smoked meat, as well as smoked fish. Low exposure was resulted from the high proportion of fruit, vegetables and cereal-based products in diets. Exposure of surveyed consumers was comparable to exposure estimated by EFSA for EU consumers. Risk assessment shows that margins of exposure (MoE) are not exceeded for benzo(*a*) pyrene and 4 PAHs in case of both medium and high exposure scenarios.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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REFERENCES

1. *Aquilina, N.J., Delgado-Saborit, J.M., Meddings, C., Baker, S., Harrison, R.M., Jacob III, P., Wilson, M., Yu, L., Duan, M. & Benowitz, N.L.*: Environmental

Table 6**.** Comparison of risk assessment for consumer health based on the exposure determined in this study and results of EFSA assessment

Study	PAHs	Exposure scenario	Exposure [ng/kg b.w.]	$BMDL_{10}$ [mg/kg b.w.]	MoE
this study	BaP	average	0.31	0.07	> 25,000
		high	1.19	0.07	> 25,000
	Sum of 4 PAHs	average	2.96	0.34	> 25,000
		high	5.06	0.34	> 25,000
EFSA, 2008 [7]	BaP	average	3.9	0.07	17,950
		high	6.5	0.07	10,750
	Sum of 4 PAHs	average	19.5	0.34	17,450
		high	34.5	0.34	9,850

$BMDL_{10}$ – benchmark dose low level limit at 10%, MoE – margin of exposure, 4 PAHs: benz(*a*)anthracene, benzo(*b*) fluoranthene, benzo(*a*)pyrene, chrysene

and biological monitoring of exposures to PAHs and ETS in the general population. Environment International 2010;36(7):763-771.
2. *Culp, S.J., Gaylor, D.W., Sheldon, W.G., Goldstein, L.S. and Beland, F.A.*: A comparison of the tumours induced by coal tar and benzo[*a*]pyrene in a 2-year bioassay. Carcinogenesis 1998;19:117124.
3. EC. Commission Recommendation of 4 February 2005 on the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods. Off J EU L 34, 8.2.2005, p. 43-45.
4. EC. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J UE L 364, 20.12.2006, p. 5-24, consolidated version 14/10/2020.
5. EFSA. A Report from the Unit of Data Collection and Exposure on a Request from the European Commission: Findings of the EFSA Data Collection on Polycyclic Aromatic Hydrocarbons in Food. EFSA, 2008.
6. EFSA. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010; 8(3):96 pp.
7. EFSA. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. The EFSA Journal 2008;724:1-114.
8. *Falcó G., Domingo J.L., Llobet J.M., Teixidó A., Casas C., Müller L.*: Polycyclic Aromatic Hydrocarbons in Foods: Human Exposure through the Diet in Catalonia, Spain. Journal of Food Protection 2003;66(12):2325-2331.
9. *Gerald G. Moy G.G., Vannoort R.W.*: Total Diet Studies, Springer, New York 2013.
10. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 92: Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures. World Health Organization, International Agency for Research on Cancer. France 2010.
11. JECFA. A Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives: Chapter 3.6 Polycyclic aromatic hydrocarbons. In: Evaluation of Certain Food Contaminants. Geneve 2006.
12. *Lodovici M., Dolara P., Casalini C., Ciappellano S., Testolin G.*: Polycyclic aromatic hydrocarbons contamination in the Italian diet. Food Add Cont 1995;12(5):703-713.
13. *Marczynski, B., Raulf-Heimsoth, M., Käfferlein, H.U., Förster, K., Spickenheuer, A., Pesch, B., Breuer, D., Angerer, J., Welge, P., Bramer, R., Hahn, J.-U., Mensing, T. & Brüning, T.*: Risk assessment of workers exposed to fumes of bitumen: genotoxic effects and urinary metabolites of naphthalene, phenanthrene, and pyrene. Abstracts of the 44th Congress of the European Societies of Toxicology. Toxicology Letters 2007;172 (Supplement 1), S172-S173.
14. *Martorell I., Nieto A., Nadal M., Perelló G., Marcé R.M., Domingo J.L.*: Human exposure to polycyclic aromatic hydrocarbons (PAHs) using data from a duplicate diet study in Catalonia, Spain. Food Chem Toxicol 2012;50(11):4103-8, doi: 10.1016/j.fct.2012.08.011.
15. *Mensing T., Marczynski B., Engelhardt B., Wilhelm M., Preuss R., Kappler M., Angerer J., Käfferlein H.U., Scherenberg M., Seidel A., Brüning T.*: DNA adduct formation of benzo[*a*]pyrene in white blood cells of workers exposed to polycyclic aromatic hydrocarbons. Int. J. Hyg. Environ.-Health 2005;208:173-178.
16. *Munoz B., Albores A.*: Chapter 6. DNA Damage Caused by Polycyclic Aromatic Hydrocarbons: Mechanisms and Markers. In: Selected Topics in DNA Repair (pp.125-144), InTech, USA 2011.
17. *Nisbet I.C., LaGoy P.K.:* Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). Regul Toxicol Pharmacol 1992;16(3):290-300.
18. *Pavanello S., Pulliero A., Saia B.O., Clonfero E.:* Determinants of anti-benzo[*a*]pyrene diol epoxide–DNA adduct formation in lymphomonocytes of the general population. Mutation Research 2006;611:54-63.
19. *Phillips D.H.*: Polycyclic aromatic hydrocarbons in the diet. Mutation Research 1999, 443, 139-147.
20. *Polachova A. Gramblicka T., Parizeka O., Sram R.J., Stupaka M, Hajslova J., Pulkrabova J.*: Estimation of human exposure to polycyclic aromatic hydrocarbons (PAHs) based on the dietary and outdoor atmospheric monitoring in the Czech Republic. Environmental Research 2020;182:108977, doi: 10.1016/j.envres.2019.108977.
21. *Preuss, R., Roßbach, B., Wilhelm, M., Brüning, T. & Angerer, J.*: External and internal exposure to polycyclic aromatic hydrocarbons (PAH) among workers in the production of fire-proof materials – Proposal of a biological monitoring guidance value. International Journal of Hygiene and Environmental Health, 2006;209(6):575-580.
22. SCF. Scientific Committee on Food: Opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food (SCF/CS/CNTM/PAH/29 Final), Brussels 2002.
23. *Singh L., Agarwal T.*: PAHs in Indian diet: Assessing a cancer risk. Chemosphere, 2018;202:366-376, doi: 10.1016/j.chemosphere.2018.03.100.
24. *Vaessen H.A.M.G., Jekel A.A., Wilbers A.A.M.M.*: Dietary intake of polycyclic aromatic hydrocarbons. Toxicological & Environmental Chemistry 1998;16:281-294.
25. *Veyrand B. Sirot V., Durand S., Pollono C., Marchand P., Dervilly-Pinel G., Tard A., Leblanc J-C., Le Bizec B.*: Human dietary exposure to polycyclic aromatic hydrocarbons: Results of the second French Total Diet Study. Environment International 2013;54:11-17, doi: 10.1016/j.envint.2012.12.011
26. *Vos R.H., Dokkum W., Schouten A., Jong-Berkhout P.*: Polycyclic aromatic hydrocarbons in Dutch total diet samples (1984-1986). Food Chem Tox 1990;28(4):263-268, doi: 10.1016/0278-6915(90)90038-O.

27. WHO. Chapter 5.9 PAHs Air Quality Guidelines. In: WHO air quality guidelines for Europe, 2nd edition, 2000.
28. WHO. Polynuclear aromatic hydrocarbons in Drinking-water. Background document for preparation of WHO Guidelines for Drinking-water quality. Geneva, World Health Organization (WHO/SDE/WSH/03.04/59) 2003.
29. *Zelinkova Z, Wenzl T.*: The Occurrence of 16 EPA PAHs in Food – A Review. Polycyclic Aromatic Compounds, 2015;35:2-4, 248-284.

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ORIGINAL ARTICLE

MONITORING SURVEY OF NITRATE CONTENT IN BEETROOT, RADISH AND CABBAGE IN POLAND

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ABSTRACT

Background. Nitrates, compounds commonly occurring in nature, are present for example in vegetables, where they accumulate and become their contaminants. It is estimated that approximately 70-90% of nitrates intake comes from vegetables, which are therefore the main source of human exposure to these compounds through dietary intake. The European Union legislation limits nitrates as contaminants to three leafy vegetables, i.e. lettuce, spinach and rucola. The EU Member States are obliged to monitor nitrate levels in vegetables which may contain significant levels of these compounds.

Objective. To present the results of monitoring surveys on nitrate levels in radish and beetroot as well as in cabbage carried out in Poland between 2012 and 2019.

Material and methods. A total of 966 vegetable samples were tested. Chemical analyses were carried out in accredited laboratories of the State Sanitary Inspection. Analyses were performed by spectrophotometric methods using nitrate reduction on cadmium columns or by HPLC.

Results. The median nitrate content in beetroot was 1,260.0 mg NO_3^-/kg, whilst at the 95th percentile level - 3,222.2 mg NO_3^-/kg. The levels of nitrates in beetroot preserves were lower: 1,030.3 mg NO_3^-/kg (median) and 2337,2 mg NO_3^-/kg (95th percentile). The median content of nitrates in radish and cabbage was 1,337.0 mg NO_3^-/kg and 369,0 mg NO_3^-/kg respectively, while at the 95th percentile the content of these compounds was found to be 3,381.5 mg NO_3^-/kg and 1545,8 mg NO_3^-/kg, respectively.

Conclusions. The nitrate content in radish and cabbage does not pose a risk to the health of consumers, whilst the consumption of beetroot containing significant amounts of the above mentioned compounds may result in exceeding the ADI especially for young children.

Key words: *monitoring, nitrate content in vegetables, beetroot, radish, cabbage*

STRESZCZENIE

Wprowadzenie. Azotany (V), związki powszechnie występujące w przyrodzie, są obecne m.in. w warzywach, w których się kumulują i stanowią ich zanieczyszczenie. Szacuje się, że około 70 – 90% spożywanych azotanów (V) pochodzi właśnie z warzyw, które tym samym są głównym źródłem narażenia człowieka na te związki przy pobraniu wraz z dietą. W ustawodawstwie Unii Europejskiej limity azotanów (V) jako zanieczyszczeń dotyczą trzech warzyw liściastych tj. sałaty, szpinaku i rukoli. Państwa należące do UE mają obowiązek monitorowania poziomów azotanów (V) w warzywach mogących zawierać znaczne ilości tych związków.

Cel badań. Celem pracy jest przedstawienie wyników badań monitoringowych w zakresie zawartości azotanów (V) w burakach, rzodkiewce i kapuście w Polsce latach 2012-2019.

Materiał i metody. Przebadano łącznie 966 próbek buraków, rzodkiewki i kapusty. Wykonawcami badań były akredytowane laboratoria Państwowej Inspekcji Sanitarnej. Analizy wykonywano metodą spektrofotometryczną z użyciem kolumn kadmowych lub metodą HPLC.

Wyniki. Mediana zawartości azotanów (V) w burakach wynosiła 1260,0 mg NO_3^-/kg, natomiast zawartość tych związków na poziomie 95 percentyla - 3222,2 mg NO_3^-/kg. Poziomy azotanów (V) w przetworach z buraków były niższe i wynosiły odpowiednio 1030,3 mg NO_3^-/kg (mediana) oraz 2337,2 mg NO_3^-/kg (95 percentyl). Mediana zawartości azotanów (V) w rzodkiewkach oraz kapuście wynosiła 1337,0 mg NO_3^-/kg oraz 369,0 mg NO_3^-/kg odpowiednio, natomiast na poziomie 95 percentyla stwierdzono zawartość tych związków na poziomie 3381,5 mg NO_3^-/kg oraz 1545,8 mg NO_3^-/kg.

Wnioski. Zawartość azotanów (V) w rzodkiewce i kapuście nie stanowi zagrożenia dla zdrowia konsumentów, natomiast spożycie buraków zawierających znaczne ilości ww. związków może skutkować przekroczeniem ADI zwłaszcza dla małych dzieci.

Słowa kluczowe: *monitoring, zawartość azotanów (V) w warzywach, buraki, rzodkiewka, kapusta*

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INTRODUCTION

Nitrates are naturally occurring chemical compounds and a component of soil and water. They come from the natural decomposition of organic substances, such as plant and animal proteins and animal excretions. This decomposition takes place under the influence of microorganisms and the ammonium ion formed is oxidised to, for example, nitrate. The natural content of nitrates in soil and water, which is approximately 10 mg/l, may be increased as a result of artificial nitrogen fertilisers used in agricultural production, the introduction of excrement from animal breeding into the soil and the release of urban waste into the environment (municipal sewage), industrial waste (fuel refineries) and transport waste (nitrogen oxides from motor vehicles). Nitrates are transferred from soil and water to plants, including vegetables, which can contain tens to thousands of mg/kg of these compounds. The amount of nitrates in vegetables depends on the season in which the vegetables are grown, soil moisture, access to light, temperature, use of fertilisers, and storage of crops.

In 2008, the European Food Safety Authority (EFSA) assessed the risk of consuming vegetables containing nitrates [5]. Vegetables contain more nitrates than other foods and are the main source of human exposure to these compounds through dietary intake. It is estimated that around 70-90% of nitrate intake comes from vegetables. Nitrates are present in vegetables in varying amounts, but when assessing consumer intake of nitrates, it is not just the quantity of vegetables consumed that is important, but also their type. Green leafy vegetables such as spinach, lettuce and rucola contain the highest levels of nitrates, while vegetables whose edible parts are the storage organs of the plant, e.g. carrots, potatoes, leeks, onions, tomatoes, cucumbers, peas and beans, have relatively low levels of these compounds. Technological processes such as washing, cooking, grilling and deep frying have a statistically significant effect on reducing the nitrate content in vegetables.

Nitrates are compounds of low toxicity. However, the compounds formed as a result of nitrate reduction and the products of nitrate metabolism, i.e. nitrites, nitrogen oxides and N-nitroso compounds, have a negative impact on health. Harmful effects of nitrites (and indirectly of nitrates, which are reduced to nitrites) on the body include oxidation of haemoglobin to methaemoglobin, disruption of the processes of digestion of carbohydrates and fats, impaired utilisation of B vitamins, or reduced levels of vitamin A and carotenoides. Nitrites and indirectly nitrates are precursors of N-nitroso compounds with proven carcinogenic effects. Nitrosation reactions can occur both in foodstuffs and in the human body. The stomach's acidic environment promotes the nitrosation reaction. Infants and babies are particularly vulnerable to the harmful effects of nitrites and indirectly of nitrates, as they have no mechanism developed for converting the methaemoglobin formed by the action of nitrites into oxyhaemoglobin.

It should be underlined that EFSA concluded in its opinion of 2008 that estimated human exposure to nitrate in vegetables does not pose a significant risk to consumer health. The benefits of eating vegetables outweigh the risks associated with the intake of nitrates contained in these vegetables.

Nitrates present in food may be both a contaminant (resulting from agricultural practices and from industrial and automotive sectors) and be intentionally added as additives (preservatives) in the manufacturing of certain foodstuffs. In 2017, as part of the re-evaluation programme on the safety of food additives, EFSA issued an opinion on the safety of using nitrates as additives (E 251 sodium nitrate and E 252 potassium nitrate) [6]. For nitrate, the current acceptable daily intake (ADI) of 3.7 mg NO_3^-/kg body weight/day was maintained, corresponding to 222 mg NO_3^-/day for an adult of 60 kg.

The limits for nitrate as a contaminant in food are set by Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (as amended by Regulation (EU) No 1258/2011) [1, 3]. In this document, the maximum levels for nitrate are set for vegetables that accumulate significant amounts of this contaminant, i.e. spinach, lettuce and rucola. The latter vegetable accumulates extremely high levels of nitrate, which is why the limit for this compound is as high as 7000 mg NO_3^-/kg. In addition, according to the aforementioned Regulation, nitrates are limited in processed cereal-based foods and baby foods for infants and young children.

It should be noted that the requirements for nitrate limits in vegetables, which existed in national legislation before Poland's accession to the European Union, covered a much wider range of vegetables. In the Regulation of the Polish Minister of Health of 13 January 2003 [7], vegetables were divided into six groups according to the degree of nitrate accumulation. In addition to lettuce and spinach, the legislation in force at the time also included: radish, beetroot, turnip, kohlrabi, dill, cabbage, chives, kale, green beans, carrot, parsley, garlic, cucumber, cauliflower, leek, celery, broccoli, tomato, potato, onion, pepper, rhubarb, beans (dry beans). The seventh group consisted of vegetables and potatoes for infants and young children. The acceptable level of nitrates in the above vegetables ranged from 200 to 4,500 mg NO_3^-/kg.

Article 9 of Commission Regulation (EC) No 1881/2006 [1] obliges EU Member States to monitor nitrate levels in vegetables which may contain significant levels, in particular green leafy vegetables. The results of these studies should be regularly reported to EFSA.

MATERIAL AND METHODS

Monitoring surveys to determine nitrate levels in radish and beetroot (2012-2013) as well as in cabbage (2014-2019) were carried out in Poland between 2012 and 2019. These vegetables are widely eaten by the Polish consumer. 966 samples were tested.

Sampling and chemical analyses were carried out by employees of the State Sanitary Inspection nationwide. Samples for testing the content of nitrate as a contaminant were taken according to the procedure described in Commission Regulation (EC) No 1882/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of the levels of nitrates in certain foodstuffs [2]. Domestic and imported vegetables were sampled for testing, in the case of imported vegetables, no more than 20% of the samples taken. Imported vegetables were taken when available in the market.

Laboratory-validated and accredited analytical methods were used for the determinations, meeting the requirements of the performance criteria set out in Commission Regulation (EC) No 1882/2006 [2]. Analyses of the nitrate content of the vegetable samples were mostly performed by spectrophotometric methods using nitrate reduction on cadmium columns. In addition, some laboratories of sanitary and epidemiological stations determined nitrates by HPLC.

This paper addresses the results of monitoring studies of nitrate content in selected vegetables (radish, beetroot, cabbage) carried out in Poland over the period 2012-2019. The results obtained will provide a basis for assessing the extent of nitrate intake with vegetables by Polish consumers. It should be noted that in 2010 National Institute of Public Health – National Institute of Hygiene estimated the intake of nitrates through the diet in Poland [4]. The intake of these compounds with vegetables in Poland was more than half lower than that presented in the EFSA opinion of 2008 for the average EU consumer. The reason for the lower than average intake of nitrates with vegetables in Poland may be the lower consumption of vegetables in our country (about half of the value adopted for the EU in 2008) and a different profile of vegetable consumption – the Polish diet is dominated by potatoes, cabbage, beetroot, carrots and tomatoes.

RESULT AND DISCUSSION

Nitrate content in beetroot

The nitrate content in the beetroot is shown in Table 1. In 2012-2013, the nitrate content in 175 samples of fresh and frozen beetroot and 50 samples of preserved beetroot was tested. Beetroot preserves included products such as boiled beetroot, pasteurised beetroot, sliced beetroot in vinegar marinade, grated beetroot (with vinegar and spices) and pickled beetroot.

Table 1. Nitrate content in beetroot (tested in 2012-2013)

Nitrate content [mg NO_3^- /kg]		
	Fresh and frozen beetroot	Beetroot preserves
Number of samples	175	50
Mean	1,420.1	1,151.0
Median	1,260.0	1,030.3
95th percentile	3,222.2	2,337.2
Maximum value	7,330.0	3,066.0

The median nitrate content in beetroot was 1,260.0 mg NO_3^-/kg, while that in beetroot preserves was lower at 1,030.3 mg NO_3^-/kg. A similar correlation between nitrate content in beetroot and beetroot preserves can be observed at the 95th percentile level – nitrate content in beetroot was 3,222.2 mg NO_3^-/kg and in beetroot preserves it was almost 1,000 mg/kg lower – the calculated value was 2,337.2 mg NO_3^-/kg. The fact that the nitrate contents found in beetroot preserves are lower than in fresh beetroot can be explained by the fact that the application of technological processes such as washing, peeling, cooking affects the reduction of nitrate content in vegetables [5].

Assessing the median value of nitrate content in beetroot, it should be stated that both values, i.e. for both fresh beetroot and beetroot preserves, are lower than the maximum permissible level of nitrate specified for beetroot in the Regulation of the Minister of Health of 2003 [7]. The nitrate limit for beetroot specified in the above-mentioned Regulation was 1,500 mg NO_3^-/kg.

The EFSA opinion of 2017 [6] concluded that root and tuberous vegetables were the main source of nitrates in the diet of infants and young children. For this reason, it is of concern that beetroot samples at the 95th percentile level had 2.5 times higher nitrate content than the median, and the maximum value was almost six times higher than the median. Therefore, the possibility cannot be excluded that a beetroot dish containing very high levels of nitrates may be served to young children (under 3 years of age), which may lead to the ADI for these compounds being exceeded. For

example, the ingestion of 50 g of beetroot with a nitrate content of 3,000 mg NO_3^-/kg by a child weighing 12 kg will more than triple the ADI for these compounds (the ingestion of nitrate with beetroot in this case would be 337.8% of the ADI). It is also important to remember that nitrates are also consumed with other components of the diet, such as other vegetables, meat products or water.

On the basis of the results presented herein, it can be concluded that beetroot is a vegetable with a high nitrate accumulation and its consumption may cause the ADI for this compound to be exceeded. It is therefore important to keep nitrate levels in beetroot as low as possible. Setting legal limits on the content of these compounds in beetroot would allow these vegetables to be included in official food control plans. Regular testing of even a small number of samples of beetroot by the official food control authorities, and the consequent threat of withdrawal of products from the market if the nitrate content is exceeded, would draw the attention of manufacturers to the issue of these compounds in beetroot. This could result in particular efforts being made to apply the principles of good agricultural practice when growing this vegetable in order to keep nitrate levels as low as possible.

Nitrate content in radish

The nitrate content in the radish is shown in Table 2. The nitrate content in 149 radish samples was tested over the period 2012-2013.

Table 2. Nitrate content in radish (tested in 2012-2013)

Nitrate content [mg NO_3^- /kg]	
Number of samples	149
Mean	1,531.6
Median	1,337.0
95th percentile	3,381.5
Maximum value	9,921.0

The median content of nitrate in radish was 1,337.0 mg NO_3^-/kg, while at the 95th percentile level the content of these compounds was found to be 3,381.5 mg NO_3^-/kg. In the Regulation of the Minister of Health of 2003 [7], radish was in the same group as beetroot and therefore the limit for nitrate in radish was 1,500 mg NO_3^-/kg, similar to that for beetroot. The median content of nitrates calculated for the radish samples included herein is below the limit of these compounds set for this vegetable in the aforementioned Regulation of the Minister of Health.

The nitrate content at the 95th percentile level is 2.5 times higher than the median value. However, the radish is one of the vegetables consumed in small quantities, mainly in spring in the form of spring vegetables. It is also not a vegetable that forms a significant part of a young children's diet – it is mainly eaten as an addition to sandwiches or salads. It can therefore be concluded that the nitrate content of radish does not pose a health risk to consumers, including young children, despite the fact that this is a vegetable with a high nitrate accumulation rate.

Nitrate content in cabbage

The nitrate content in cabbage is shown in Table 3. The nitrate content in 592 cabbage samples was tested over the period 2014-2019.

Table 3. Nitrate content in cabbage (tested in 2014-2019)

Nitrate content [mg NO_3^- / kg]	
Number of samples	592
Mean	538.7
Median	369.0
95th percentile	1,545.8
Maximum value	3,400.1

The median content of nitrate in cabbage was 369.0 mg NO_3^-/kg, while at the 95th percentile level the content of these compounds was found to be 1,545.8 mg NO_3^-/kg. The limit for nitrate in cabbage provided for in the Regulation of the Polish Minister of Health of 2003 [7] was 750 mg NO_3^-/kg, so it is a vegetable that accumulates less of these compounds than beetroot or radish. However, cabbage is a popular vegetable in Poland, consumed frequently (especially in autumn and winter), and may therefore be a significant source of nitrates in the Polish diet. The median value of nitrate content in cabbage is almost half of the permissible content of these compounds set for this vegetable in the Regulation of the Minister of Health of 2003 [7]. The nitrate content at the 95th percentile level is 4 times higher than the median value.

The consumption of 200 g of cabbage containing 1,500 mg NO_3^-/kg (a value close to the 95th percentile level) by a 60 kg adult will result in an excess of the ADI (135% of the ADI), while the consumption of cabbage with an average nitrate content – the median value – results in an intake of these compounds of 33.2% of the ADI. This shows that the nitrate content in cabbage does not pose a risk to consumer health, despite the fact that the vegetable is an important part of the Polish diet.

SUMMARY

According to the EFSA assessment, vegetables and vegetable-based products are the main source

of nitrate intake from the diet [5]. The total dietary intake of nitrates in Poland did not exceed the ADI for these compounds for adults weighing 60 kg [4]. However, for children, who are of lower body weight than adults, the consumption of vegetables with high nitrate content, such as beetroot, may cause the ADI to be exceeded. Although the benefits of eating vegetables outweigh the risks associated with the intake of nitrate contained in these vegetables [5], by properly designing the diet (e.g. ensuring a variety of vegetables consumed), one can have an impact on minimising the risk of exceeding the ADI for nitrate.

CONCLUSIONS

Beetroot, radish and to a lesser extent cabbage are vegetables that accumulate significant amounts of nitrates.

The median content of nitrates in all the above vegetables was lower than the permissible content of these compounds set for individual vegetables in the Regulation of the Polish Minister of Health of 2003. This shows that, in general, good agricultural practice is applied in vegetable cultivation, which prevents excessive accumulation of nitrates in the above vegetables.

Particular attention should be paid to the nitrate content in the beetroot. The consumption of this vegetable containing significant amounts of the above mentioned compounds may result in exceeding the ADI especially for young children. It is advisable to consider setting a limit on the nitrate content in this vegetable, and this would allow these vegetables to be included in official food control plans.

The nitrate content in radish and cabbage does not pose a risk to the health of consumers.

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Conflict of interest

The Authors declare no conflict of interest.

REFERENCES

1. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal EU L 364 of 20 December 2006, p. 5.
2. Commission Regulation (EC) No 1882/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of the levels of nitrates in certain foodstuffs. Official Journal EU L 364 of 20 December 2006, p. 25.
3. Commission Regulation (EU) No 1258/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuffs. Official Journal EU L 320/15 of 03 December 2011.
4. *Gajda-Wyrębek J., Jarecka J., Kuźma K., Beresińska M., Karłowski K.*: Zawartość azotanów (V) w sałacie i szpinaku. [Content of nitrates in lettuce and spinach]. In: *Karłowski K., Rybińska K., Postupolski J.* (ed.): Ocena narażenia konsumentów na chemiczne i mikrobiologiczne zanieczyszczenia żywności – programy realizowane w latach 2004-2008. [Assessment of consumer exposure to chemical and microbiological food contaminants – 2004-2008 programmes]. National Institute of Public Health – National Institute of Hygiene, Warsaw 2010, 85-98, ISBN 03-89379-18-X.
5. Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission to perform a scientific risk assessment on nitrate in vegetables. The EFSA Journal 2008;689:1-79.
6. Re-evaluation of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives. The EFSA Journal 2017;15(6):4787.
7. Rozporządzenie Ministra Zdrowia z dnia 13 stycznia 2003 roku w sprawie maksymalnych poziomów zanieczyszczeń chemicznych i biologicznych, które mogą znajdować się w żywności, składnikach żywności, dozwolonych substancjach dodatkowych, substancjach pomagających w przetwarzaniu albo na powierzchni żywności. Dz. U. No 37 of 4 March 2003, item 326 (in Polish).

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ORIGINAL ARTICLE

ASSESSMENT OF CAFFEINE INTAKE WITH FOOD BY POLISH FEMALES AND MALES

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ABSTRACT

Background. Caffeine is the most widespread psychoactive substance in the world. With long-term consumption of caffeinated beverages, there is a high probability of overtaking on caffeine.

Objective. The aim of the study was to estimate the consumption of caffeine in the daily caffeine intake of Polish consumers, determine the caffeinated products in the intake of this substance.

Materials and methods. The survey was completed by 433 respondents living in Poland. The research tool was the electronic questionnaire, which consisted of: a) questions about personal data and measurement anthropometric and the level of physical activity and smoking; b) questions regarding the portion size and frequency of consumption of coffee, tea, cocoa, chocolate, energy drinks and colacarbonated beverages.

Results. The main sources of caffeine in the respondents' diet include: coffee (Me 43.64 mg/d) and tea (Me 37.60 mg/d). Approximately 20% of respondents exceeded the threshold of daily caffeine intake (safety level for children and adolescents up to 3 mg/kg b.w, for adults up to 5.7 mg/kg b.w), considered safe.

Conclusions. Respondents who have crossed the safe dose of caffeine intake, should limit the consumption of products being its main source (coffee).

Key words: *caffeine, caffeine intake with food, Poland, women, men*

STRESZCZENIE

Wprowadzenie. Kofeina jest najbardziej rozpowszechnioną substancją psychoaktywną na świecie. Przy długotrwałym spożywaniu napojów kofeinowych istnieje duże prawdopodobieństwo wystąpienia nadmiaru kofeiny.

Cel. Celem pracy było oszacowanie spożycia kofeiny w dziennym spożyciu kofeiny przez polskich konsumentów, określenie udziału produktów zawierających kofeinę w spożyciu tej substancji.

Materiały i metody. W badaniu wzięło udział 433 respondentów mieszkających w Polsce. Narzędziem badawczym był elektroniczny kwestionariusz ankiety, który składał się z: a) pytań dotyczących danych osobowych i pomiarów antropometrycznych oraz poziomu aktywności fizycznej i palenia tytoniu; b) pytań dotyczących wielkości porcji i częstości spożycia kawy, herbaty, kakao, czekolady, napojów energetycznych i napojów gazowanych.

Wyniki. Głównymi źródłami kofeiny w diecie respondentów były: kawa (Me 43,64 mg/d) i herbata (Me 37,60 mg/d). Około 20% respondentów przekroczyło próg dziennego spożycia kofeiny (poziom bezpieczeństwa dla dzieci i młodzieży do 3 mg/kg m.c., dla dorosłych do 5,7 mg/kg m.c.), uznawany za bezpieczny.

Wnioski. Respondenci, którzy przekroczyli bezpieczną dawkę spożycia kofeiny, powinni ograniczyć spożycie produktów będących jej głównym źródłem (kawa).

Słowa kluczowe: *kofeina, spożycie kofeiny z żywnością, Polska, kobiety, mężczyźni*

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INTRODUCTION

Caffeine is the most widespread psychoactive substance in the world. The purine alkaloid is present in coffee beans, tea leaves, cocoa beans, kola nuts (cola vera) and in nearly 60 species of other plants. Caffeine annual consumption is estimated at 120,000 tonnes [17, 30]. In the daily food ration of a Central European resident, the content of this alkaloid ranges between 280-490 mg/day. In Sweden, the consumption of caffeine is greater, resulting from high consumption of coffee. Slightly less caffeine is consumed in the United States, where its average consumption ranges from 193 to 280 mg/day. Polish people consume it relatively little - about 140 mg/day [27]. Caffeine is used as an additive to non-alcoholic beverages in order to achieve a specific taste (eg. in cola). Caffeine is a component of energy drinks and slimming preparations. Through antagonistic influence, caffeine acts as a stimulant, maintains a period of wakefulness, increased mental performance, reduces and delays fatigue. Caffeine is primarily absorbed in the stomach, and, to a lesser extent, in the distal segments of the digestive tract. It is then transported to all tissues, including the brain. With long-term consumption of caffeinated beverages, there is a high probability of overtaking on caffeine, which is characterized by physical and mental disorders [10, 15, 19, 20, 21, 24, 29]. Confirmation of the above conclusions is the opinion issued in 2015 by the European Food Safety Authority, EFSA (European Food Safety Authority) about a safe dose of caffeine intake by various population groups. It was found that the intake of a single dose of caffeine up to 200 mg (about 3 mg/kg body weight of an adult with a body weight of 70 kg) by an adult does not pose a threat to health and safety. In the case of habitual consumption of caffeine from all sources, the acceptable daily intake should not exceed 400 mg for adults (approximately 5.7 mg/kg b.w. per day for 70 kg of an adult), with the exception of pregnant women. The habitual consumption of caffeine by pregnant women should be 200 mg/day without risk to the fetus. EFSA proposes a level of up to 3 mg of caffeine per kilogram of body weight per day (i.e. an individual caffeine level for adults) for regular consumption of caffeine by children and adolescents [5]. A significant increase in the interest of products containing caffeine is associated with western life style, the effect of exposure to the stimulus of advertising. Caffeine-rich products are consumed by a wide range of consumers in all age groups due to their stimulating effect and their high availability. They are very popular among young people, and more and more often among children due to their sweet taste (soft drinks, energy drinks) [4, 6, 16, 26].

The aim of the study was to estimate the consumption of caffeine in the daily food intake of Polish females and males, determine the share of selected products in the intake of this substance.

MATERIALS AND METHODS

The survey was completed by 433 respondents (pupils, students, workers, unemployed, and pensioners) living in Poland and willing to setup the questionnaire. The study was completed by means of a diagnostic survey using the CAWI (*Computer-Assisted Web/Internet Interviewing*) survey technique. A research technique in which the online survey is supervised by a computer system. Questionnaire questions are downloaded from the survey organizer's website and transmitted via the network to any point in which the respondent is located together with a computer connected to the Internet. A person examined in the CAWI system, alone or in the presence of an interviewer, reads the question content from the screen and provides answers that are recorded on the target server. Using a self-administered questionnaire, participants were asked questions regarding their socio- demographic characteristics (including gender, age, education, place of residence, type of professional activity), anthropometric measurements (weight, height), physical activity level (unsystematic physical activity, systematic physical activity, i.e. planned, structured, repeated physical activity for the purpose of maintaining or improving health, lack physical activity) and intake of caffeine-containing beverages (i.e. portion size and frequency of consumption of coffee, tea, cocoa, chocolate, energy drinks and colacarbonated beverages) [11, 13, 18]. For the needs of conducting the analyses, the respondents were divided into groups differentiated in terms of gender (females, males), age (≤18, 19-30, 31-50, ≥51 years), education (primary, vocational, secondary, higher) and place of residence (rural areas, urban areas), as well as declared physical activity (unsystematic, systematic, lack physical activity) and smoking (smoker, non-smoker). Each participant was asked to choose a product with caffeine and the portion that was consumed, and then determine the frequency of consumption of a given product. The following portion sizes were used: a) in the case of coffee, tea, cocoa and chocolate, the respondents entered the size of the portion (eg. 1 teaspoon = 2,5 g coffee beans, grounded, but 2.0 g instant coffee), regardless of the amount of infusion made from it; (b) in the case of beverages: a can with a capacity of 0.33 L or 0.25 L glass bottle with a capacity of 0.2 L, plastic bottles according to the range, and the home measurement - a glass of any indicated capacity from the range of 0.2 L to 0.5 L. The frequency of consumption of products

was determined by the following categories: daily (1 time, 2 times, 3 times, 4 times, 5 times 6 times), several times a week (1 time, 2 times, 3 times, 4 times, 5 times, 6 times), several times a month (once, twice, three times), less often than once a month and never. The study also took into account the type of tea and coffee (eg. tea: leaf/ granulated/express, green/black/red, coffee: ground/granular/instant/decaffeinated/from the coffee machine or mix) and the time of brewing tea (up to 1 minute, 1 minute, 5 minutes). Caffeine content in products was adopted on the basis of data from the literature on the subject and presented in Table 1 [1, 2, 6, 7, 12, 27, 32].

The total daily caffeine intake by respondents was calculated according to the formula:

$$EDI\ (mg/day) = P \cdot F \cdot C \text{ or } EDI\ (mg/kg\ b.w./day) = (P \cdot F \cdot C) / W$$

Where:
EDI – Estimated Daily Intake of caffeine
P – data on the portion size (number of spoons of a loose product or volume of a liquid product expressed in liters);
F – data on the frequency of consumption of portions per day
(multiplicity of intake per day, eg. 3 times per day: 3/1;
per week, eg. 5 times per week: 5/7;
per month, eg. 2 times per month: 2/30);
C – caffeine content in the product (taking into account the type of coffee and tea, brewing time), expressed in mg per teaspoon of loose product, mg per pack of finished product or mg/L);
W – respondent's body weight (kg b.w.).

Example calculations:
eg. for ground coffee, 1 teaspoon = 2.5 g

1.83 g/100 g = 1830 mg/100 g

1830 mg /100 g x 2.5 g = 45.75 mg ground coffee / 1 teaspoon

EDI = [2 teaspoons of ground coffee x 2/1 (two times per day) x 45.75 mg/teaspoon] / 60 kg b.w. = 3.05 mg caffeine from ground coffee per 1 kg b.w.

EDI = (liquid product expressed in ml) = [1 cup of coffee drink mix "2in1" (150 ml) x 1/1 (once per day) x 78 mg/150 ml] / 65 kg b.w. = 1.2 mg caffeine from cup of coffee drink mix "2in1" per 1 kg b.w.

The most frequent drinkers were brewed ground coffee, instant coffee and cappuccino, and among teas, black express tea. The obtained results were summed up and the average daily caffeine intake from all products included in the study was determined. Share of respondents exceeding daily caffeine intake (in %) was assumed by EFSA opinion about safe dose of caffeine in children 3 mg/kg b.w. and in adults 5.7 mg/kg b.w.

Table 1. Caffeine content of selected products [1, 2, 6, 7, 12, 27, 32]

Source	Mean caffeine content
Coffee	
Ground coffee	1.83 g/100 g coffee
Coffee beans (ground before brewing)	2.27 g/100 g coffee
Instant coffee	1.65 g/100 g coffee
Coffee drink mix "2in1"	78 mg/150 ml
Coffee drink mix "3in1"	54 mg/150 ml
Cappuccino chocolate	39.0 mg/150 ml
Cappuccino peanut	44.0 mg/150 ml
Cappuccino creamy	41.0 mg/150 ml
Cappuccino with magnesium	46.0 mg/150 ml
Coffee from the machine	66 mg/150 ml
Espresso coffee	100 mg/60 ml
Decaffeinated coffee	0.1 g/100 g coffee
Decaffeinated instant coffee	0.1 g/100 g coffee
Tea	
Black express tea	14.9 mg/teabag/ 200 ml /15 second 21.8 mg/teabag/ 200 ml /1 minute
Green express tea	22.0 mg/teabag/ 200 ml/1 minute 30.5 mg/teabag/ 200 ml/5 minute
Black leaf tea	33.5 mg teaspoon/ 200 ml/5 minute
Green leaf tea	33.4 mg/teaspoon/ 200 ml/5 minute
Energy drinks	
Energy drinks	80 mg/250 ml
Cocoa/chocolate	
Cocoa/hot chocolate	4-5 mg/150 ml
Milk chocolate	20.8 mg/100 g
Bitter chocolate	66.5 mg/100 g
Cola-type drinks	
Coca-Cola	9.4 mg/100 ml
Pepsi	10.1 mg/100 ml

Statistical analysis

The hypothesis on the normal distribution of the analyzed variables was assessed using the *Shapiro-Wilk* test. *Kruskal-Wallis* ANOVA compared differences in caffeine intake among each group (age, gender, education, place of residence, physical

activity, smoking). In addition, $\chi 2$ was performed to show a relationship between the number of people crossing the safe daily dose of caffeine and gender, age, education, place of residence, physical activity and smoking. All data were performed as mean, standard deviation (SD), median (Me), interquartile range (Q25-Q75) and percentage of participants exceed safe dose of caffeine. Statistical analysis was carried out using statistical program Statistica v.10.0. The level of significance was assumed at $p<0.05$.

RESULTS

Characteristics of respondents

The characteristics of the respondents are presented in Table 2. 433 respondents completed the survey and were included in the analysis. The sample contained approximately equal number of men and women. Most of the respondents were aged 19-30.

Considering the nutritional status, the majority of respondents were characterized by normal BMI. Underweight was found in 9% of respondents. Almost every fifth respondents was overweight, and the BMI indicator for obesity was slightly more than 5% of respondents. Almost 50% of respondents were characterized by secondary education. The highest percentage of respondents were workers. Among all respondents, a similar percentage of people declared a systematic or unsystematic physical activity. 3/4 of respondents are non-smokers. Most respondents inhabited urban areas.

Estimated total caffeine intake and the proportion of products in the consumption of caffeine

Table 3 shows total mean and the estimated relative total daily caffeine intake (mg/day) and the proportion of products in the consumption of caffeine. Total mean daily caffeine intake was 255.75 mg, and estimated relative total daily caffeine intake was 199.72 mg.

The amount of caffeine intake was statistically significantly determined by the type of product consumed ($p<0.0000$). The respondents consumed the most caffeine when they consumed coffee. It accounted for 60.3% of the total intake, amounting to 154.32 mg per day (median 43.64 mg per day). Tea was also an important source of caffeine. Along with it, 70.12 mg of caffeine were supplied (median 37.60 mg), and the contribution to intake was 27.4%.

Assessment of caffeine intake by vs. sex, age, education, place of residence, smoking

The total mean daily caffeine intake per kilogram of body weight was 3.78 mg, and estimated relative total daily caffeine intake was 2.88 mg/kg b.w. Significant differences in relative total daily caffeine intake (mg/kg b.w.) by gender, age, education, and smoking status shown in Table 4. Females (mean: 4.27 mg/kg b.w.; median: 3.35 mg/kg b.w.) consumed more caffeine than males (mean: 3.29 mg/kg b.w.; median: 2.61 mg/kg b.w.) ($p =0.023$).

The highest consumption of caffeine was observed among respondents between 31 and 50 years old (mean: 5.13 mg/kg b.w.; median: 4.62 mg/kg b.w.), and the lowest in adolescents under 18 years old (mean: 2.56 mg/kg b.w.; median: 1.61 mg/kg b.w.) ($p = 0.0001$). The

Table 2. Characteristics of respondents

Variables	n	%
Total subject	433	100
Gender		
females	218	50.3
males	215	49.7
Age (years)		
≤18 (Me 16.6 y.)	58	13.4
19-30 (Me 25.5 y.)	293	67.7
31-50 (Me 41.4 y.)	65	15.0
≥51 (Me 57.9 y.)	17	3.9
Nutritional status by BMI	1	0.2
<16	3	0.7
16 – 16.99	35	8.1
17 – 18.49	287	66.3
18.5 – 24.49	84	19.4
25 – 29.99	15	3.5
30 – 34.99	4	0.9
35 – 39.9	4	0.9
≥ 40		
Education		
primary	65	15.0
vocational	19	4.4
secondary	202	46.7
higher	147	33.9
Type of professional activity	62	14.3
pupils	157	36.3
student	164	37.9
workers	15	3.5
pensioner	35	8.0
unemployed		
Physical activity	175	40.4
unsystematic	183	42.3
systematic	75	17.3
lack		
Smoking		
smoker	99	22.9
non-smoker	334	77.1
Place of residence		
rural areas	90	20.8
urban areas	343	79.2

Table 3. Estimated relative total daily caffeine intake (mg/day) and the proportion of products in the consumption of caffeine (in %)

Caffeine source	Mean ± SD	%	Median	Q25-Q75	p-value
Total caffeine intake	255.75 ± 233.10	100.0	199.72	78.42-370.47	
Coffee	154.32 ± 223.72	60.3	43.64 [a]	0-244.0	
Tea	70.12 ± 86.32	27.4	37.60 [b]	13.43-92.90	
Cocoa/Chocolate	0.65 ± 1.23	0.3	0.30 [c]	0-0.76	<0.0001
Energy drinks	14.31 ± 43.96	5.6	1.33 [c]	0-6.67	
Cola-type drinks	16.50 ± 32.26	6.4	2.78 [c]	0.69-16.79	

p-value - was calculated by analysis of variance *Kruskal-Wallis* ANOVA for $p<0.05$;
a, b, c – statistically significant differences

Table 4. Estimated relative total daily caffeine intake (mg/kg b.w.)

Variables	Mean ± SD	Median	Q25-Q75	p-value
total subject	3.78 ± 3.50	2.88	1.21-5.10	
Gender				
females	4.27 ± 4.02	3.35 [a]	1.36-5.56	0.023
males	3.29 ± 2.80	2.61 [b]	1.06-4.87	
Age (years)				
≤18	2.56 ± 2.38	1.61 [a]	0.65-3.42	
19 - 30	3.74 ± 3.33	2.85	1.24-4.98	0.0001
31 - 50	5.13 ± 4.43	4.62 [b]	2.01-6.65	
≥51	3.68 ± 2.47	3.22	1.39-5.19	
Education				
primary	2.56 ± 2.25	1.49 [a]	0.67-3.11	
vocational	2.74 ± 2.65	1.12	0.29-4.71	0.0002
secondary	3.96 ± 3.57	2.99 [b]	1.46-5.25	
higher	4.23 ± 3.61	3.70 [b]	1.35-5.66	
Place of residence				
rural areas	4.27 ±3.56	3.61	1.33-5.65	0.137
urban areas	3.66 ±3.48	2.78	1.19-4.95	
Physical activity				
unsystematic	3.55 ± 3.16	2.91	1.22-4.95	
systematic	3.76 ± 3.66	2.69	1.06-5.54	0.299
lack	4.39 ± 3.84	3.15	1.38-5.89	
Smoking				
smoker	5.36 ± 4.00	4.59 [a]	2.90-6.68	<0.0001
non-smoker	3.32 ± 3.20	2.32 [b]	1.00-4.71	

p-value - was calculated by analysis of variance *Kruskal-Wallis* ANOVA for $p<0.05$;
a, b – statistically significant differences

daily caffeine intake among respondents with higher (mean: 4.23 mg/kg b.w.; median: 3.70 mg/kg b.w.) and secondary education (mean: 3.96 mg/kg b.w.; median: 2.99 mg/kg b.w.) was significantly higher compared to respondents with primary education (mean: 2.56 mg/kg b.w.; median: 1.49 mg/kg b.w.) ($p = 0.0002$). The total caffeine intake among smokers (mean: 5.36 mg/kg b.w.; median: 4.59 mg/kg b.w.) was significantly higher than non-smokers (mean: 3.32 mg/kg b.w.; median: 2.32 mg/kg b.w.) ($p <0.0001$).

Assessment of intake above (standard/agency) safe criteria

Percentage of respondents exceeding daily caffeine intake is shown in Table 5. Approximately 20% of respondents exceed the daily limit values of caffeine intake. Acceptable daily intake (habitual) of substances set at 5.7 mg/kg b.w., i.e. 400 mg/day, was exceeded in 17.7% of the surveyed males and 22.5% of females. Caffeine consumers below 18 year were assumed by lowest level of caffeine intake according to EFSA opinion (3 mg/kg b.w).

Table 5. Percentage of people exceeding daily caffeine intake (%)

Variables	n	%	p-value
total subject	87	20.1	
Sex			
females	49	22.5 [a]	[a vs. b] 0.259
males	38	17.7 [b]	
Age (years)			[a vs. b] 0.074
≤18	5	8,6 [a]	[a vs. c] 0.001
19-30	57	19.5 [b]	[b vs. c] 0.008
31-50	23	35.4 [c]	[b vs. d] 0.640
≥51	2	11.8 [d]	[a vs. d] 0.934
			[c vs. d] 0.112
Education			[a vs. b] 0.038
primary	6	9.2 [a]	[b vs. c] 0.751
vocational	3	15.8 [b]	[b vs. d] 0.666
secondary	44	21.8 [c]	[c vs. d] 0.866
higher	34	23.1 [d]	[a vs. d] 0.028
Place of residence			
rural areas	22	24.4 [a]	[a vs. b] 0.337
urban areas	65	18.95 [b]	
Physical activity			[a vs. b] 0.201
unsystematic	28	16.0 [a]	[b vs. c] 0.659
systematic	40	21.9 [b]	[a vs. c] <0.0001
lack	19	25.3 [c]	
Smoking			
smoker	34	34.3 [a]	[a vs. b] 0.0001
non-smoker	53	15.9 [b]	

p-value - comparisons with $\chi 2$, $p<0.05$;
a, b, c, d - compare of averages

The respondents between 31 and 50 years old constituted the highest percentage of people who exceeded the dose considered as safe for healthy people. Considering respondents under 18 years old, for whom EFSA proposes a level of normal caffeine intake up to 3 mg/kg/day, the percentage of people who exceed daily intake is 8,6%. The least respondents with primary education exceeded the dose of daily caffeine intake. Significantly more often respondents with lack of physical activity exceeded the daily dose of caffeine (25.3%) than respondents with unsystematic physical activity (16.0%) ($p < 0.0001$). Also, significantly more often smokers (34.3%) exceed the dose of this substance than non-smokers (15.9%) ($p = 0.0001$).

DISCUSSION

The median daily intake of caffeine among the respondents was estimated at 2.88 mg/kg b.w. (199.72 mg/day) and was confirmed by the literature data that the daily caffeine intake in Central Europe was in the range from 3 up to 7 mg/kg b.w. [4, 5]. A similar level of caffeine intake was determined by other authors [31, 32]. In American studies conducted on a representative sample of the population, the average caffeine intake was similar and amounted about 3.01 mg/kg b.w., i.e. 211 mg/day [8]. Similarly, in British studies conducted among women of childbearing age, the caffeine consumption was 173.9 mg/day, i.e. approx. 3.0 mg/kg b.w. [3]. Whereas studies conducted in Austria [22] among adolescents and adults up to 40 years old, showed that the average caffeine intake was at a much higher level, i.e. 5.33 mg/kg b.w. Many studies it was support a hypothesis proved that caffeine intake by women per kilogram body weight was higher than for men. In Japanese studies [31] caffeine intake was 4.9 mg/kg in women, and 4.1 mg/kg b.w. in men. Whereas, in Austrian studies [22], the average caffeine intake in a woman was 5.4 mg/kg b.w./day, and in men 4.8 mg/kg b.w. Similar results were obtained in our these studies. Women consumed on 3.35 mg/kg b.w., and men 2.61 mg/kg b.w., and these differences were statistically significant. Analysing the caffeine intake in terms of age, there a statistically significant relationship was found between the amount of caffeine and age. The highest caffeine intake was consumed by respondents between 31 and 50 years old (4.62 mg/kg b.w.), and the lowest by respondents under 18 years old (1.61 mg/kg b.w.). *Fulgoni et al.* [8] found that 31-50- and 51-70-year-old people consumed approx. 3.05 mg/kg b.w./day, and respondents below 19-year-olds 2,66 mg/kg b.w./day. *Rudolph et al.* [22] recorded higher caffeine consumption with increasing age, where up to 25 years old the daily caffeine intake was 4.7 mg/kg b.w., and above 25 years old was 5.8 mg/kg b.w. A study presented by *Yamada et al.* [31] conducted among the adults of Japanese community and the study of *Tran et al.* [25] conducted among American youth and young adults, as well as American and British studies conducted among young women aged 14-40 and 16-40 respectively 45 years also confirmed the observed trend of increasing the consumption of caffeine with age [3, 28]. Also, in our own studies, it was recorded that after the age of 50, caffeine intake decreased and amounted 3.22 mg/kg b.w. A gradual decrease in caffeine consumption after the age of 50 was also noted by *Knight et al.* [14] (from 2.3 mg to 1.92 mg/kg b.w./day). The lower level of caffeine intake among respondents over 50 years old may result from the decreasing amount of sleep needed with age, and thus the lack of need to regulate the daily rhythm with psychostimulants – the caffeine. Drinking coffee and smoking puts caffeine and nicotine at the top of the list of legally available psychoactive substances. Our research support a significant relationship between smoking and the amount of caffeine consumed. The total caffeine intake among smokers (4.59 mg/kg b.w.) was significantly higher than among non-smokers (2.32 mg/kg b.w.) ($p < 0.0001$). Other researchers

also indicated a similar relationship [3, 28]. Smokers consumed three times more caffeine than nonsmokers (approx. 5.40 mg/kg b.w./day vs. 1.79 mg/kg b.w./day) [3]. In addition, along with increasing the dose of caffeine, the proportion of smokers increased, and decreased non-smokers consuming higher amounts of this substance (more than 200 mg/day, i.e. approx. 2.86 mg/kg b.w./day) [28]. Every fifth of respondents, exceeded the level of acceptable daily caffeine intake set at 5.7 mg/kg b.w./day (i.e. 400 mg/day). More often, this problem affected to females than males (22.5% vs. 17.7%) and respondents between 31 and 50 years old. In turn, lower than in the author's research, exceeding the acceptable daily amount of caffeine intake by adult respondents was noted in the studies presented by *Yamada et al.* [31] in which this dose was exceeded by 15.4% of men and 10.8% of women. However, in the study of *Wetmore et al.* with the level of 200 mg/day, the percentage of people with excessively high intake of this substance was higher (28%) [28]. In contrast, 18% of women of childbearing age living in the United Kingdom exceeded the dose set at 300 mg/day [3]. Knowing that respondents under 18 years old should not consume more than 3 mg/kg b.w./day of caffeine, the percentage of respondents who exceed the safe dose was noted at 32.8%. In the studies of *Santangelo et al.* [23] even more teenagers, almost half (46%) exceeded the upper limits of caffeine intake. Coffee was the richest source of caffeine in the diet of the respondents, and coffee contribution in caffeine intake was approximately 60%. The same percentage of coffee in caffeine intake (60.8%) was obtained by *Rudolph et al.* [22], who examined the adolescents and adults up to 40 years old. In turn, in the studies presented by *Yamada et al.* [31] showed the percentage of caffeine supplied with this product accounted only 47%. Tea was also an important source of caffeine, the contribution was 27.4% of the total daily intake. In the studies of *Yamada et al.* [31] in groups of Japanese and Chinese that tea provided as much as 47% of caffeine. However, the highest share of tea in the collection of caffeine was recorded among the British 53% [9].

CONCLUSIONS

Considering the average caffeine intake along with the diet, as well as the results of the health risk assessment resulting from exceeding the safe dose of caffeine intake among the studied population of Polish people, it is advisable to undertake all activities promoting health-oriented lifestyle and increasing consumer awareness in the field of nutritional knowledge. One of this maybe proper and healthy intake of caffeine as an chemo protective factor of nutrition. This fact was confirmed by the introduction of coffee as a drink that improves the health of the population in the Pyramid of Healthy Eating and Physical Activity by Institute of Food and Nutrition (IŻŻ) in 2016. In turn, among people who exceeded the safe dose of caffeine intake, the consumption of products that are its main source should be limited. It is also important to constantly monitor danger and conduct long-term research, which will significantly extend the knowledge of future generations. In addition, measures should be taken to regulate the descriptions provided by manufacturers on the packaging of products, including those food products in which caffeine is present. Thanks to this knowledge, consumers will be aware of their dietary choices.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Białas, M., Łuczak, H., Przygoński, K.:* Zawartość kofeiny w wybranych napojach kawowych w proszku. Brom. Chem. Toksykol. 2009, 42, 426-430.
2. *Dąbrówka-Molenda, M., Szwedziak, K., Zabłudowska, Ż.*: Analiza zawartości kofeiny w wybranych rodzajach kawy. Post. Tech. Przetw. Spoż. 2019, 2, 68-71.
3. *Derbyshire, E., Abdula, S.*: Habitual caffeine intake in women of childbearing age. J Hum Nutr Diet 2008; 21: 159-164.
4. *Dworzański, W., Opielak, G., Burdan, F.*: Undesirable effects of caffeine. Pol. Merkur. Lek. 2009;27:357-360.
5. EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015. Scientific Opinion on the safety of caffeine. EFSA Journal 2015; 13(5): 4102.
6. EFSAafactasheetsaonacaffeine.ahttp://www.efsa.europa.eu/en/corporate/pub/efsaexplainscaffeine150527
7. *Frankowski , M., Kowalski, A., Ociepa, A.*, et al. Caffeine levels in various caffeine-rich and decaffeinated coffee grades and coffee extracts marketed in Poland. Brom. Chem. Toksykol. 2008, 41, 21-27.
8. *Fulgoni, V.L., Keast, D.R., Lieberman H.R.*: Trends in intake and sources of caffeine in the diets of US adults: 2001–2010. Am J Clin Nutr 2015; 101: 1081–1087. doi:10.3945/ajcn.113.080077.
9. Heatherley, S.V., Mullings, E.L., Tidbury, M.A., et al.: Caffeine consumption among a sample of UK adults. Appetite 2006; 47: 257-259, doi:10.1016/j.appet.2006.07.031.
10. Higdon J.V., Frei B.: Coffee and Health: A Review of Recent Human Research. Crit Rev Food Scien Nutr 2006; 46(2): 101-123.
11. https://www.linkedin.com/pulse/methodologies-compared-advantages-disadvantages-data-via-mancini [cit. 16. 7. 2018].
12. *Jarosz, M., Wierzejska, R., Mojska, H.* et al. Caffeine content in foodstuffs. Brom. Chem. Toksykol. 2009;43:776-781.
13. *Knapik J.J., Austin K. G., McGraw S. M., Leahy G.D., Lieberman H.R.*: Caffeine consumption among active

duty United States Air Force personnel. Food Chem Toxicol 2017; 105: 377-386.
14. *Knight, C.A., Knight, I., Mitchell, D.C.*, et al.: Beverage caffeine intake in US consumers and subpopulations of interest: estimates from the Share of Intake Panel survey. Food Chem Toxicol 2004; 42:1923-1930, doi:10.1016/j.fct.2004.05.002.
15. *Kohler, M., Pavy, A., van Den Heuvel, C.*: The effects of chewing versus caffeine on alertness, cognitive performance and cardiac autonomic activity during sleep deprivation. J Sleep Res 2006;15:358-368.
16. *Mackus M., Van de Loo A.J.A.E., Benson S., Scholey A., Verster J.C.*: Consumption of caffeinated beverages and the awareness of their caffeine content among Dutch students. Appetite 2016;103:353-357.
17. *Mandel, H.G.*: Update on caffeine consumption, disposition and action. Food Chem Toxicol 2002;40:1231-1234.
18. *Mitchell D.C., Knight C.A., Hockenberry J., Teplansky R., Hartman T.J.*: Beverage caffeine intakes in the U.S. Food Chem Toxicol 2014 63:136-142.
19. *Nawrot P., Jordan S., Eastwood J., Rotstein J., Hugenholtz A., Feeley M.*: Effects of caffeine on human health. Food Addit Contam 2003;20(1):1-30.
20. Ozsungur, S., Brenner, D., El-Sohemy, A.: Fourteen well described caffeine withdrawal symptoms factor into three clusters. Psychopharmacology 2009;201:541-548.
21. *Pelczyńska M., Bogdański P.*: Prozdrowotne właściwości kawy. Forum Zaburzeń Metabol. 2019;10(3):112–120.
22. *Rudolph, E., Faerbinger, A., Koenig, J.*: Caffeine intake from all sources in adolescents and young adults in Austria. Eur J Clin Nutr 2014; 68: 793-798, doi:10.1038/ejcn.2014.50.
23. *Santangelo, B., Lapolla, R., Rutigliano, I.*, et al.: Nearly half of the adolescents in an Italian school based study exceeded the recommended upper limits for daily caffeine consumption. Acta Paediatr 2018;107:1055-1059, doi:10.1111/apa.14277.
24. *Suteerapataranon, S., Butsoongnern, J., Punturat, P.*, at al.: Caffeine in Chiang Rai tea infusions: Effects of tea variety, type, leaf form, and infusion conditions. Food Chem 2009; 114: 1335-1338.
25. *Tran, N.L., Barraj, L.M., Bi, X.,* et al.: Trends and patterns of caffeine consumption among US teenagers and young adults, NHANES 2003-2012. Food Chem Toxicol 2016;94:227-242, doi:10.1016/j.fct.2016.06.007.
26. *Wanat, G., Woźniak-Holecka,* J.: Assessment of consumption of caffeine-containing products among university students and secondary school pupils. Probl. Hig. Epidemiol. 2011;92:695-699.
27. *Wierzejska R.*: Caffeine – common ingredient in a diet and its influence on human health. Rocz Panstw Zakl Hig 2012;63:141-147.
28. *Wetmore, C.M., Ichikawa, L., LaCroix, A.Z.*, et al.: Association between caffeine intake and bone mass among young women: potential effect modification by depot medroxyprogesterone acetate use. Osteoporosis Int 2008;19:519-527, doi:10.1007/s00198- 007-0473-2.
29. *Wikoff, D., Welsh, B.T., Henderson, R.*, et al.: Systematic review of the potential adverse effects of caffeine consumption in healthy adults, pregnant women, adolescents, and children. Food Chem Toxicol 2017;109:585-648, doi:10.1016/j.fct.2017.04.002.
30. *Wolde T.*: Effects of caffeine on health and nutrition. A review. Food Sci Qual Manage 2014;30:1-9.
31. *Yamada, M., Sasaki, S., Murakami, K.*, et al.: Estimation of caffeine intake in Japanese adults using 16 d weighed diet records based on a food composition database newly developed for Japanese populations. Public Health Nutr 2010;13:663-672.
32. *Żary-Sikorska E.,Czok, H.*: Estimation the intake of caffeine with the daily food ration in selected population group. In: Designed food Part III. *Walczycka, M., Jaworska, G., Duda-Chodak, A., Tarko T.* (eds.), Publisher: Oddział Małopolski Polskiego Towarzystwa Technologów Żywności, Kraków, Poland, 2011, pp. 59-74, ISBN: 978-83-932389-2-7.

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ORIGINAL ARTICLE

CHANGES OF BODY COMPOSITION AMONG UNIVERSITY STUDENTS DEPENDING ON THE CONSUMPTION FREQUENCY OF SELECTED BAKERY PRODUCTS

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ABSTRACT

Background. Bakery products such as bread, rolls, croissants and others are an important part of eating habits. Recently, their consumption has been associated with an undesirable increase and prevalence of overweight or obesity.

Objective. The aim of the work was to analyze the influence of the consumption frequency of selected types of bread / bakery products on anthropometric parameters in a group of university students.

Material and Methods. The group was composed of 120 volunteers consuming different types of bread / bakery products with different consumption frequencies during the week. The anthropometric parameters were measured by InBody 720. To obtain information on the frequency of consumption we used the questionnaire method.

Results. The results suggest that in most cases it is not the type of product that is decisive, but its quantity consumed and frequency of consumption supported by low daily physical activity, resp. sedentary lifestyle. We found similar results of the influence of the consumption frequency on anthropometric parameters for all types of bread. Low levels of physical activity, basal metabolism and consumption of selected types of bakery products (wheat bread, wheat rolls, sweet pastries and gluten-free variants) can cause an increase in visceral as well as total body fat, weight gain, BMI, at the expense of fat-free mass. Our results showed that the groups of participants who did not consume a certain type of bread at all, rarely or 1 to 3 times a week, showed higher values of the examined parameters (BMI, body weight, body fat percentage, WHR) compared to the group which consumed a particular type of bakery products on average 4 to 7 times a week. The parameter´s values were largely influenced by the levels of physical activity.

Conclusions. Based on the results it is possible to assume that if the bakery products are the part of a balanced diet with regard to the individual energy needs, it should not be the main cause of overweight / obesity in humans.

Key words: *bakery products, pastry, anthropometry, obesity, health, body fat*

INTRODUCTION

Adult feeding patterns are rooted from childhood experiences. Household healthy food availability and accessibility have been positively associated with healthful meal intake in youth. Modelling of healthful dietary patterns by parents and friends may promote healthy eating among children and adolescents [12,29]. As people age, they should implement healthy eating to maintain an ideal body weight, since both overweight and underweight lead to increased morbidity and mortality [25]. Fast food is very popular among young people, as well as fast consumption of large portions of unhealthy foods and high intake of sweetened drinks combined with average physical activity. The potential risk is also posed by night eating, snacking, and alcohol consumption [3,5,15,51].

Quality food high in proteins, vitamins and minerals, but low in cholesterol, saturated fat, and especially trans-fat should be recommended for weight maintenance. People should consume more of the nutrient-dense whole-grain foods, whole-wheat breads, and whole-grain cereals to meet carbohydrate needs and the consumption of refined foods (white bread, pasta, and other refined products) should be limited [25]. Whole-grains are the key components of healthy eating habits. Thanks to their low energy density and satiating effects are responsible for their potential role in weight control [22,37,50]. Current trends in diet suggest a slight decline in the consumption of bread and traditional bakery products due to concerns about the growing body weight of consumers. It is thanks to this belief that bread is cursed and excluded from many dietary patterns and

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replaced by other, relatively harmless and potentially healthier equivalents. Gluten-free eating behaviours have been in most cases perceived to be healthier than gluten-containing ones. However, the nutritional value and quality of gluten-free products is questionable. Consumption of gluten-free bread is usually associated with excessive energy, animal protein and fat intake and reduced intake of dietary fiber, magnesium and folic acid [45,68]. Gluten-free bread provides twice as much fat, mainly saturated fat in comparison to its equivalents with gluten [2,8,17,45]. In many cases, the fear of weight gain is justified, but with good eating habits, diet and principles of rational nutrition, but especially quantity, bread cannot be considered the primary cause of overweight or obesity, especially given the fact that bread consumption is declining worldwide, but the prevalence of obesity is rising [21].

Healthy eating plays a critical role in risk reduction for various disease states and helps to individuals lose excess fat mass. However, healthy eating must be accompanied by physical activity, proper sleep and adequate time for stress reduction [35,40,47,56].

In practice, the most commonly used parameter to determine overweight or obesity is the body mass index. BMI is useful for the initial screening of the general population for classification of excess weight and it is employed as the anthropometric indicator of excess adiposity. It should be used to classify individuals as having overweight (BMI 25 to 29.9 kg/m^2) or obesity (BMI ≥30 kg/m^2), after taking into account age, gender, ethnicity, fluid status, and muscularity [20]. It is an indirect measure for estimation of total body fat mass. Using BMI as the primary screening tool for obesity and overweight is consistent with recommendations and guidelines developed by many societies and task forces [33,46,48]. However, BMI alone cannot identify excess adiposity and establish a diagnosis of overweight or obesity in all instances [52] and it does not provide accurate information on body fat distribution, so it is appropriate to supplement it with other indicators. The most common means of assessing central obesity are waist circumference and waist-to-height ratio [21] estimated the visceral adipose tissue and reflected a dysfunctional ability of adipose tissue in general to store fat with redistribution to intra-abdominal adipose tissue. The predictive value of waist circumference is generally independent of, and stronger than, BMI [20]. Other measurements of adiposity may be considered, too. Bioelectric impedance is commonly used but is dependent on the hydrational state of individuals [53].

The aim of the survey was to find out how frequency consumption of different kinds of bakery products have been able to influence selected risk factors for overweight or obesity among young adult, especially university students.

MATERIALS AND METHODS

Characteristics of the participants

One hundred and twenty volunteers – students studying at university – were included in the research. The average age of them was 23±1 years. The requirement for participation was the consent of individuals with study and measurement conditions. Participants with the present severe disease or with recommended special dietary regimen were excluded prior to the start of the study.

Dietary Assessment

For study purposes questionnaire method was used to obtain information on frequency consumption of different kinds of bakery products. Each participant completed the questionnaire anonymously. We focused on consumption of wheat bread, whole-grain bread, wheat rolls, sweet pastries and gluten-free bakery products and asked how often the participants consume selected types of bread / bakery products. The options were either 4-7 times a week, 1-3 times a week, rarely or never. Accordingly, we divided the study participants into 4 groups. Part of the questionnaire was also to determine the level of physical activity. This part dealt with the form / type of physical activity performed (aerobic or anaerobic), the length of one exercise process in minutes, the number of days practiced per week and the number of months / years devoted to regular exercise. The main purpose of the research was to analyze the relationship (direct or indirect) between the frequency of consumption of individual types of bakery products and selected anthropometric parameters.

Anthropometric measurements

Body composition was diagnosed by multi-frequency bioelectrical impedance analysis measuring the total impedance at frequencies of 1, 5, 50, 100, 500, 1000 kHz. We used InBody 720 (Biospace Co. Ltd., Seoul, Republic of Korea). Every participant was informed with the measurement procedure, the possible risks of measuring in the case of pregnancy or having an artificial pacemaker at the heart were explained. Before the measurement, participants were asked to excrete and refrain from drinking excessive amounts of water and signed an informed written consent for the measurement procedure and also agreed to the processing of personal data. The Lookin'Body 3.0 software was used to process the results. The following body composition parameters were measured: body mass index (BMI, (kg/m^2), waist-to-hip ratio (WHR), physical condition (points), basal metabolic rate (BMR, kcal), fat-free mass (FFM, %), visceral fat area (VFA, cm^2), percentage of body fat (PBF, %), intracellular water (ICW, %), extracellular

water (ECW, %), total body water (TBW, %). Body height was measured in a standing position without shoes on the electronical medical scales Tanita WB-3000.

Statistical analysis

Microsoft Office Excel 2010 (Los Angeles, CA, USA), XLSTAT (Version 2019.3.1) and STATISTICA Cz version 13 (TIBCO Software Inc., Palo Alto, California, USA) were used for statistical analysis. The changes between groups were evaluated using a one-way analysis of variance (ANOVA) followed by *Tukey's* post hoc test. The data were presented as the means ± standard deviation (SD). The level of statistical significance was set as $P < 0.05$. We used also Pearson´s correlation analysis between parameters.

RESULTS AND DISCUSSION

The total number of volunteers who completed the anthropometric measurement was one hundred and twenty. Table 1 shows the baseline characteristics of the group. Variables were presented as mean with standard deviations. The mean values of the evaluated parameters were in the range of reference limits. The differences in the values of anthropometric parameters of individual groups according to the frequency of consumption of different types of bakery products are shown in Table 2 and Table 2a. Table 3 shows correlation between parameters.

The analysis results of the influence of the consumption frequency of white bread showed that those who avoid its consumption had the highest values of basal metabolism, but also of body weight, which was related to high values of visceral fat. Based on this, the highest BMI and WHR values were confirmed in this group of participants. Unexpectedly, we found the lowest proportion of total and visceral body fat in the group with regular consumption (4 to 7 times a week) of white bread. These consumers had the best fitness values, which was reflected in the highest values of fat-free mass and volume status. However, we did not find significant differences between the individual groups ($P > 0.05$).

We found similar results when evaluating the effect of the frequency of consumption of whole-grain bread. Consumers with the most often consumption of whole-grain bread had the best condition based on physical activity. Good physical condition had a positive effect on fat-free mass and volume status, but a negative effect on BMI, as muscle mass had an effect on increasing its values. From the point of view of central obesity, whole-grain bread consumers with rarely consumption had the worst results. We found significant differences only in WHR ($P < 0.05$).

In the case of wheat rolls, the body composition analysis revealed significant differences between the group that consumed this type of bakery products rarely and the group with the most frequent consumption ($P < 0.05$). The highest values of condition and basal metabolism, as well as fat-free mass and volume status were found in the group with the consumption of wheat rolls 4-7 times a week. We found the worst parameter values of fatness and indicators of obesity (VFA, PBF, WHR, BMI) mainly in the group with occasional consumption of wheat rolls and in the group avoiding their consumption.

Analysis of the effect of the consumption frequency of sweet pastry showed that the worst values of obesity indicators were in the group that consumed this type of pastry 1-3 times a week (VFA, PBF, WHR, BMI). Paradoxically, we found the best results of the mentioned parameters among the participants with regular consumption. In the case of VFA and PBF, we found significant differences ($P < 0.05$). These differences were caused by a higher level of physical

Table 1. Baseline characteristics of body composition in study group

Parameters	Mean	± SD	Max	Min	Mod	Med
Age, years	23	1	26	20	23	23
Height, cm	169.1	8.7	200.0	150.8	168.0	168.8
Weight, kg	65.0	13.3	115.1	46.1	64.7	61.7
Body mass index, kg/m^2	22.6	3.2	31.9	17.0	22.8	22.1
Waist-to-hip ratio	0.86	0.05	1.04	0.78	0.83	0.85
Condition	76	7	108	59	72	75
Basal metabolic rate, kcal	1418	247	2456	1019	ND	1353
Fat-free mass, %	74.6	6.7	91.4	58.3	ND	74.8
Visceral fat area, cm^2	67.8	22.8	140.6	24.9	76.5	65.1
Percentage of body fat, %	25.4	6.7	41.6	8.5	29.6	25.2
Intracellular water, %	62.1	0.5	63.4	61.0	62.2	62.1
Extracellular water, %	37.9	0.5	39.0	36.6	37.8	37.9
Total body water, %	54.6	5.0	67.2	42.7	ND	54.8

Table 2. Body composition according to frequency consumption of different kind of bakery products

Parameters	Wheat bread				Whole-grain bread			
	no	rarely	1-3 times a week	4-7 times a week	no	rarely	1-3 times a week	4-7 times a week
Weight (kg)	67.0	65.3	62.5	63.2	62.9	70.4	64.3	66.2
Body mass index (BMI, kg/m^2)	23.1	22.6	22.0	22.4	22.3	22.9	22.6	23.0
Waist-to-hip ratio (WHR)	0.87	0.87	0.86	0.85	0.85^a	0.90^b	0.86^a	0.85
Condition (points)	77	75	74	78	76.2	75.0	74.6	78.1
Basal metabolic rate (BMR, kcal)	1452	1415	1363	1442	1383	1514	1399	1448
Fat-free mass (FFM, %)	74.6	73.6	74.1	77.8	74.3	74.9	74.3	75.3
Visceral fat area (VFA, cm^2)	69.9	69.7	67.1	55.9	64.7	75.7	67.5	67.8
Percentage of body fat (PBF, %)	25.4	26.5	25.9	22.2	25.7	25.1	25.7	24.7
Intracellular water (ICW, %)	34.0	33.4	33.7	35.5	33.9	34.1	33.8	34.2
Extracellular water (ECW, %)	20.7	20.4	20.6	21.6	20.6	20.7	20.6	20.9
Total body water (TBW, %)	54.7	53.9	54.3	57.1	54.4	54.9	54.4	55.2
	Wheat rolls				Sweet pastry			
	no	rarely	1-3 times a week	4-7 times a week	no	rarely	1-3 times a week	4-7 times a week
Weight (kg)	65.9	65.6	61.5	60.3	62.6	64.3	68.1	62.3
Body mass index (BMI, kg/m^2)	22.9	22.8	21.6	20.7	21.9^a	22.1	23.7^b	21.4
Waist-to-hip ratio (WHR)	0.86	0.87	0.84	0.82	0.86	0.86	0.86	0.84
Condition (points)	76.0	74.5	76.2	76.4	75.5	74.8	76.4	78.2
Basal metabolic rate (BMR, kcal)	1428	1401	1388	1439	1393	1408	1448	1426
Fat-free mass (FFM, %)	74.3	72.6	76.1	81.3	75.4	75.0	73.1	77.7
Visceral fat area (VFA, cm^2)	69.5	74.2^a	59.0	44.2^b	63.2	66.9^a	74.1^a	56.1^b
Percentage of body fat (PBF, %)	25.7	27.4	23.9	18.7	24.6	25.0^a	26.9^a	22.3^b
Intracellular water (ICW, %)	33.9	33.0	34.6	37.0	34.3	34.1	33.3	35.4
Extracellular water (ECW, %)	20.6	20.2^a	21.1	22.7^b	20.9	20.8	20.2	21.5
Total body water (TBW, %)	54.4	53.2^a	55.8	59.7^b	55.3	55.0	53.5	57.0

^{ab} – different symbols in a line mean significant differences in average values; the level of statistical significance was set at $P < 0.05$

Table 2a. Body composition according to frequency consumption of gluten-free bakery products

Parameters	Gluten-free bakery products			
	no	rarely	1-3 times a week	4-7 times a week
Weight (kg)	65.6	63.7	72.8[a]	50.7[b]
Body mass index (BMI, kg/m^2)	22.7	22.3	25.0[a]	19.0[b]
Waist-to-hip ratio (WHR)	0.86	0.86	0.90	0.82
Condition (points)	76.5	74.9	70.8	70.4
Basal metabolic rate (BMR, kcal)	1435	1388	1445	1175
Fat-free mass (FFM, %)	75.0	74.4	67.6	73.3
Visceral fat area (VFA, cm^2)	66.9[a]	68.4	94.2[b]	55.2[a]
Percentage of body fat (PBF, %)	25.0	25.6	32.4	26.7
Intracellular water (ICW, %)	34.2	33.8	30.6	33.1
Extracellular water (ECW, %)	20.8	20.8	18.8	20.6
Total body water (TBW, %)	55.0	54.5	49.4	53.7

[ab] – different symbols in a line mean significant differences in average values;
the level of statistical significance was set at P <0.05

Table 3. Pearson´s correlation between parameters

Parameters	Height (cm)	Weight (kg)	BMI (kg/ m2)	WHR	Condition (points)	BMR (kcal)	FFM (%)	VFA (cm2)	PBF (%)	ICW (%)	ECW (%)
Weight (kg)	0.739										
BMI (kg/m^2)	0.292	0.854									
WHR	0.415	0.651	0.607								
Condition (points)	0.342	0.420	0.311	-0.200							
Basal metabolic rate (kcal)	0.807	0.907	0.658	0.384	0.688						
Fat-free mass (%)	0.375	0.049	-0.238	-0.438	0.714	0.458					
Visceral fat area (cm^2)	0.232	0.596	0.682	0.847	-0.348	0.211	-0.740				
Percentage of body fat (%)	-0.375	-0.050	0.237	0.437	-0.714	-0.459	-1.000	0.739			
Intracellular water (%)	0.110	0.322	0.379	0.064	0.419	0.396	0.278	-0.109	-0.278		
Extracellular water (%)	-0.110	-0.322	-0.379	-0.064	-0.419	-0.396	-0.278	0.109	0.278	-1.000	
Total body water (%)	0.373	0.050	-0.236	-0.435	0.714	0.458	1.000	-0.737	-1.000	0.265	-0.265

P values by *Pearson's* correlation analysis between parameters; the level of statistical significance was set at *P* <0.05 (bold)

activity, which was reflected in the values of fitness, but also fat-free mass. We did not confirm the hypothesis that with increasing frequency of consumption of pastries, the values of obesity indicators increase.

Gluten-free products are intended for patients with celiac disease. In recent years, however, these products have become part of the diet of healthy people. Our results suggest that more frequent (1 to 3 times per week) consumption of gluten-free bakery products can cause weight gain. This is proved by the highest values of obesity and fat indicators (VFA, PBF, WHR, BMI). However, this group of consumers consisted of people who do not depend on strict gluten-free diet. People with gluten intolerance rely on the daily consumption of a gluten-free diet. This disease is associated with weight loss due to indigestion and poor utilization of nutrients. It is therefore necessary to take into account these possible factors influencing the results of the analysis, given that the group of consumers with the most frequent consumption had the lowest values of body weight, BMI, WHR, VFA ($P < 0.05$). At the same time, these consumers had the worst level of physical activity and the lowest values of basal metabolism.

Based on the results, it can be stated that selected types of bread / bakery products play an important role in the regulation of body composition. The epidemiological model of obesity describes diet as the main causative agent of excess weight and scarce physical activity as the second main driver [7,31]. Our study suggests that low levels of physical activity and basal metabolism combined with intake of selected types of bread, especially wheat rolls, sweet pastries and gluten-free variants, may cause an increase in visceral as well as total body fat, BMI, weight gain, and this at the expense of a fat-free mass (especially muscle tissue). The level of physical activity must be taken into account when evaluating anthropometric parameters. In addition, when assessing the impact of consumption of different types of bread / bakery products on the values of anthropometric parameters and indicators of obesity, it is necessary to take into account not only the frequency of consumption, but also the amount of bakery product consumed. This is where we see the limitations of our survey. The results of our study show that the values of anthropometric parameters were significantly affected by the level of physical activity, because in most cases the best results were achieved by consumers with the most frequent consumption of the observed type of bread / bakery products, but also by the highest fitness values. At the same time, it is necessary to take into account the young and vital age of participants with relatively active metabolism, the absence of serious non-infectious disease and the absence of drug treatment affecting metabolic processes in the body.

Young adulthood (17-35 years) has become synonymous with the development of the wrong lifestyle associated with an increased risk of chronic diseases in later years [42,67]. Healthy dietary patterns are a global priority to reduce non-communicable diseases. In the study of *Imamura* et al. [32], better diets were seen in older adults compared with younger adults, and in women compared with men.

Similar findings were found by the authors *Lee* and *Allen* [41]. Men were overall more likely than young women to engage in negative eating habits. Consumption of ultraproccessed foods is continuously increasing and people with regularly physical activity is decreasing and becomes even less frequent with age [38].

Study in Poland showed that the frequency of whole-grain intake (including whole-grain bread) was not correlated with BMI and other body composition parameters. However, individuals who ate white bread (wheat, rye or wheat-rye bread, toast bread) less than once daily were characterized by lower visceral fat levels [39].

Many epidemiological studies have reported that higher intakes of whole grains are associated with a lower BMI, waist circumference and percent fat mass [14,26,43]. Increased intake of cereal fiber is associated with lower body weight and waist circumference over time [14]. Whole grains vary in their fiber and phytochemical content [11]. Oats and barley are high in the soluble fiber, while whole wheat is high in the insoluble fiber. For the health claims it is very important to know the different nutritional profiles of whole grains [11,14]. *Kostecka* et al. [39] found that consumption of whole-grain products did not affect the analyzed body composition parameters. Lower body fat percentage was observed only in women aged 18-39 who consumed whole-grain products once a day / several times a day.

Since 1975, obesity has almost tripled worldwide. More than 1.9 billion adults aged 18 and over were overweight in 2016, and more than 650 million were obese, meaning that 39% and 13% of adults were overweight or obese. In the same year, more than 340 million children and adolescents aged 5-19 suffered from overweight or obesity. In 2019, 38 million children under the age of 5 were overweight or obese [63].

BMI is associated with risk of comorbidities secondary to excess body fat [18,30] and there is a large body of evidence correlating higher BMI with cardiometabolic disease such as diabetes [1,57,58,61] and cardiovascular disease [54,66]. Dysfunction of adipose tissue plays a significant role in the genesis of metabolic disorders [4,23,27,59]. Decreasing body weight by 10% improves risk factors for chronic diseases [49].

BMI is limited for estimating body fat percentage and distribution. In addition to fat mass, the weight measurement incorporates lean mass, bone mass and fluid status. All of these body components contribute to weight independent of fat mass. To determine the degree to which the BMI value is indicative of excess adiposity, muscularity, volume status, sarcopenia, loss of muscle mass and other factors must be considering [10,16,24]. Age is one of the factor that may alter the functional significance of BMI at different ages. Adults tend to loose fat free mass and increase fat mass with increasing age [19]. Oedema can also affect the significance of BMI [64].

Waist circumference should be measured in all patients when evaluating for adiposity related disease risk. In many populations, a WC cut-off point of ≥94 cm in men and ≥80 cm in women should be considered at risk and consistent with abdominal obesity [20]. *Van Dijk* et al. [60] found that both BMI and waist circumference were correlated with all cardiovascular disease risk factors. High waist circumference values, when the BMI is between 25 and 34.9 kg/m^2, are associated with an increased risk of type 2 diabetes, dyslipidemia, hypertension and cardiovascular disease. Over time, changes in waist circumference may indicate an increase or decrease in abdominal fat. Increase in abdominal fat is associated with an increased risk of heart disease [44]. Visceral fat has been associated with a greater cardiometabolic risk as compared with BMI [34].

Energy expenditure is an important component of maintaining a healthy weight. The guidelines for physical activity recommend at least 150 minutes of moderate physical activity or 75 minutes of vigorous physical activity per week, as well as muscle-strengthening activities at least twice per week for health benefits. Many factors affect obesity development, but one of the most important is an appropriate caloric intake and balance between energy intake and expenditure [25,62].

A meta-analysis performed between 1980 and 2017 showed changes in physical activity from adolescence to young adulthood and showed a 13 to 17% decrease in physical activity with age [9]. The study of *Tcymbal* et al. [55] showed that young people aged 18-29 had low levels of physical activity. While only 18.9% did not meet WHO recommendations, in terms of total amount of physical activity and sedentary behavior they were closer to the oldest age group than to the 30-44-year-old group. The most active age group were people between 30 and 44 years old.

The study by *Kerkadi* et al. [36] revealed that physical inactivity, a sedentary lifestyle, and an unhealthy diet are factors that can increase weight and general or abdominal obesity.

The issue of obesity is still highly topical at present, also due to the fact that its prevalence is pandemic. There are many reasons for the emergence and maintenance of obesity, and attention should be paid not only to genetic predispositions, but especially to the lifestyle of an individual, specifically to its two components – diet and physical activity. If there is a regular positive energy balance, either due to excessive energy intake (of course in the form of food) or insufficient energy expenditure, the emergence of overweight first, and later with the uneven trend, obesity is more than obvious. Blaming any food commodity for this condition is irrational. In the case of our intervention bakery products it should be added that while some types of pastries are considered healthier, especially in terms of fiber and other bioactive ingredients, we must not forget the fact that in the case of healthy food, not only its quality but also the quantity of intake is important. Replacing rafined bread with wholemeal bread can lead to a qualitative improvement in diet, but if the amount and time of consumption does not change, there may be an unfavorable trend in the development of body weight and other body composition parameters. Therefore, while adhering to the principles of rational diet focused on quality and quantity in diet, in combination with adequate physical activity, we will ensure not only optimal body weight, but also optimal overall body composition and active and full life.

CONCLUSIONS

A healthy diet, combined with moderate and regular physical activity, is a prerequisite for maintaining good health. Energy balance is an important part of preventing overweight or obesity. Therefore, it is important to pay attention to the correct selection of bakery products, their amount consumed and frequency of consumption. In our study, we observed among university students that groups of participants who did not consume a certain type of bread at all, rarely or 1 to 3 times a week, showed higher values of the examined parameters (BMI, body weight, body fat percentage, WHR) compared to a group that consumed a specific type of bread on average 4 to 7 times a week. The obtained values of the measured parameters of individual groups were largely influenced by the levels of physical activity and basal metabolism. Based on the findings we can conclude that while adhering to certain principles of healthy diet bakery products (within the prevalence of overweight and obesity) should not pose health risks to their consumers.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Abdullah, A., Peeters, A., de Courten, M., Stoelwinder, J.:* The magnitude of association between overweight and obesity and the risk of diabetes: a meta-analysis of prospective cohort studies. Diabetes Res Clin Pract. 2010;89(3):309-319. doi: 10.1016/j.diabres.2010.04.012
2. *Abenavoli, L., Delibasic, M., Peta, V., Turkulov, V., Lorenzo, A.D., Medić-Stojanoska, M.*: Nutritional profile of adult patients with celiac disease. European Review for Medical and Pharmacological Sciences 2015;19(22):4285-4292.
3. *Andersen, G.S., Stunkard, A.J., Sørensen, T.I., Petersen, L., Heitmann, B.L.:* Night eating and weight change in middle-aged men and women. Int J Obes Relat Metab Disord. 2004;28(10):1338-1343. doi: 10.1038/sj.ijo.0802731
4. *Bays, H.E.:* Adiposopathy is "sick fat" a cardiovascular disease? J Am Coll Cardiol. 2011;57(25):2461-2473. doi: 10.1016/j.jacc.2011.02.038
5. *Berkey, C.S., Rockett, H.R., Field, A.E., Gillman, M.W., Colditz, G.A.:* Sugar-added beverages and adolescent weight change. Obes Res. 2004;12(5):778-788. doi: 10.1038/oby.2004.94
6. *Bouchi, R., Asakawa, M., Ohara, N. et al.:* Indirect measure of visceral adiposity 'A Body Shape Index' (ABSI) is associated with arterial stiffness in patients with type 2 diabetes. BMJ Open Diabetes Res Care 2016;4(1):e000188. doi: 10.1136/bmjdrc-2015-000188
7. *Bray, G.A., Kim, K.K., Wilding, J.P.H.:* Obesity: A chronic relapsing progressive disease process. A position statement of the World Obesity Federation. Obes. Rev. 2017;18:715-723. https://doi.org/10.1111/obr.12551
8. *Conte, P., Fadda, C., Piga, A., Collar, C.*: Techno-functional and nutritional performance of commercial breads available in Europe. Food Science and Technology International 2016;22:621-633. doi: 10.1177/1082013216637724
9. *Corder, K., Winpenny, E., Love, R. et al.:* Change in physical activity from adolescence to early adulthood: a systematic review and meta-analysis of longitudinal cohort studies. Br J Sports Med. 2019;53(8):496-503. doi: 10.1136/bjsports-2016-097330
10. *De Lorenzo, A., Bianchi, A., Maroni, P. et al.:* Adiposity rather than BMI determines metabolic risk. Int J Cardiol. 2013;166(1):111-117. doi: 10.1016/j.ijcard.2011.10.006
11. *De Moura, F.F., Lewis, K.D., Falk, M.C.:* Applying the FDA definition of whole grains to the evidence for cardiovascular disease health claims. J Nutr. 2009;139(11):2220S-2226S. doi: 10.3945/jn.109.112383
12. *Decyk-Chęcel, A.:* Children's and adolescents' eating habits. Probl Hig Epidemiol 2017;98(2):103-109.
13. *Drewa, A., Zorena, K.:* Prevention of overweight and obesity in children and adolescents in European countries. Pediatr Endocrinol Diabetes Metab 2017;23(3):152-158 doi:10.18544/PEDM-23.03.0087
14. *Du, H., van der A, D.L., Boshuizen, H.C. et al.:* Dietary fiber and subsequent changes in body weight and waist circumference in European men and women. Am J Clin Nutr. 2010;91(2):329-336. doi: 10.3945/ajcn.2009.28191
15. *Duffey, K.J., Gordon-Larsen, P., Jacobs, D.R.J., Williams, O.D., Popkin, B.M.:* Differential associations of fast food and restaurant food consumption with 3-y change in body mass index: the Coronary Artery Risk Development in Young Adults Study. Am J Clin Nutr. 2007;85(1):201-208. doi: 10.1093/ajcn/85.1.201
16. *Frankenfield, D.C., Rowe, W.A., Cooney, R.N., Smith, J.S., Becker, D.:* Limits of body mass index to detect obesity and predict body composition. Nutrition 2001;17(1):26-30. doi: 10.1016/s0899-9007(00)00471-8
17. *Fry, L., Madden, A.M., Fallaize, R.*: An investigation into the nutritional composition and cost of gluten-free versus regular food products in the UK. Journal of Human Nutrition and Dietetics 2018;31(1):108-120. doi: 10.1111/jhn.12502
18. *Gallagher, D., Heymsfield, S.B., Heo, M., Jebb, S.A., Murgatroyd, P.R., Sakamoto, Y.:* Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index. Am J Clin Nutr. 2000;72(3):694-701. doi: 10.1093/ajcn/72.3.694
19. *Gallagher, D., Visser, M., Sepulveda, D., Pierson, R.N., Harris, T., Heymsfield, S.B.:* How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? Am J Epidemiol. 1996;143(3):228-239. doi: 10.1093/oxfordjournals.aje.a008733
20. *Garvey, W.T., Mechanick, J.I., Brett, E.M., Garber, A.J., Hurley, D.L., Jastreboff, A.M., Nadolsky, K., Pessah-Pollack, R., Plodkowski, R.:* Reviewers of the AACE/ACE Obesity Clinical Practice Guidelines. American Association Of Clinical Endocrinologists And American College Of Endocrinology Comprehensive Clinical Practice Guidelines For Medical Care Of Patients With Obesity. Endocr Pract. 2016;22 Suppl 3:1-203. doi: 10.4158/EP161365.GL
21. *Gažarová, M., Lenártová, P.:* Zmeny telesnej kompozície vplyvom konzumácie celozrnných pekárskych výrobkov. [Changes in body composition due to consumption of whole grain bakery products]. Nitra: Slovak University of Agriculture, 2020. 101 s. ISBN 978-80-552-2283-7. In Slovak
22. *Giacco, R., Della Pepa, G., Luongo, D., Riccardi, G.:* Whole grain intake in relation to body weight: From epidemiological evidence to clinical trials. Nutr

Metab Cardiovasc Dis 2011;21:901-908. doi: 10.1016/j.numecd.2011.07.003
23. *Giorgino, F.:* Adipose tissue function and dysfunction: organ cross-talk and metabolic risk. Am J Physiol Endocrinol Metab. 2009;297(5):E975-E976. doi: 10.1152/ajpendo.00488.2009
24. *Gómez-Ambrosi, J., Silva, C., Galofré, J.C. et al.:* Body mass index classification misses subjects with increased cardiometabolic risk factors related to elevated adiposity. Int J Obes. 2012;36(2):286-294. doi: 10.1038/ijo.2011.100
25. *Gonzalez-Campoy, J.M., St Jeor, S.T., Castorino, K., Ebrahim, A. et al.:* Clinical practice guidelines for healthy eating for the prevention and treatment of metabolic and endocrine diseases in adults: cosponsored by the American Association of Clinical Endocrinologists/the American College of Endocrinology and the Obesity Society. Endocr Pract. 2013;19 Suppl 3:1-82. doi: 10.4158/EP13155.GL
26. *Good, C.K., Holschuh, N., Albertson, A.M., Eldridge, A.L.:* Whole grain consumption and body mass index in adult women: an analysis of NHANES 1999-2000 and the USDA pyramid servings database. J Am Coll Nutr. 2008;27(1):80-87. doi: 10.1080/07315724.2008.10719678
27. *Goossens, G.H.:* The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. Physiol Behav. 2008;94(2):206-218. doi: 10.1016/j.physbeh.2007.10.010
28. *Guthold, R., Stevens, G.A., Riley, L.M. et al.:* Worldwide trends in insufficient physical activity from 2001 to 2016: a pooled analysis of 358 population-based surveys with 19 million participants. Lancet Glob Health 2018;6(10):e1077-e1086. doi: 10.1016/S2214-109X(18)30357-7
29. *Hanson, N.I., Neumark-Sztainer, D., Eisenberg, M.E., Story, M., Wall, M.:* Associations between parental report of the home food environment and adolescent intakes of fruits, vegetables and dairy foods. Public Health Nutr. 2005;8(1):77-85. doi: 10.1079/phn2005661
30. *Heymsfield, S.B., Peterson, C.M., Thomas, D.M. et al.:* Scaling of adult body weight to height across sex and race/ethnic groups: relevance to BMI. Am J Clin Nutr. 2014;100(6):1455-1461. https://doi.org/10.3945/ajcn.114.088831
31. *Huang, H., Yan, Z., Chen, Y., Liu, F.:* A social contagious model of the obesity epidemic. Sci Rep. 2016;28(6):37961. doi: 10.1038/srep37961
32. *Imamura, F., Micha, R., Khatibzadeh, S. et al.:* Dietary quality among men and women in 187 countries in 1990 and 2010: a systematic assessment. The Lancet Global Health 2015;3(3):e132-e142. doi: 10.1016/S2214-109X(14)70381-X
33. *Jensen, M.D., Ryan, D.H., Apovian,, C.M. et al.:* 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. Circulation 2014;129(25 Suppl 2):S102-38. doi: 10.1161/01.cir.0000437739.71477.ee.
34. *Kang, S.H., Cho, K.H., Park, J.W., Yoon, K.W., Do, J.Y.:* Association of visceral fat area with chronic kidney disease and metabolic syndrome risk in the general population: analysis using multi-frequency bioimpedance. Kidney Blood Press Res. 2015;40(3):223-30. doi: 10.1159/000368498.
35. *Katano, S., Nakamura, Y., Nakamura, A. et al.:* Relationship between sleep duration and clustering of metabolic syndrome diagnostic components. Diabetes Metab Syndr Obes. 2011;4:119-125. doi: 10.2147/DMSO.S16147
36. *Kerkadi, A., Sadig, A. H., Bawadi, H. et al.:* The Relationship between Lifestyle Factors and Obesity Indices among Adolescents in Qatar. Int J Environ Res Public Health 2019;16(22):4428. doi: https://doi.org/10.3390/ijerph16224428
37. *Kirwan, J.P., Malin, S.K., Scelsi, A.R. et al.:* A Whole-Grain Diet Reduces Cardiovascular Risk Factors in Overweight and Obese Adults: A Randomized Controlled In Trial. J Nutr. 2016;146(11):2244-2251. doi:10.3945/jn.116.230508
38. *Kolotkin, R.L., Andersen, J.R.:* A systematic review of reviews: Exploring the relationship between obesity, weight loss and health-related quality of life. Clin. Obes. 2017;7:273-289. https://doi.org/10.1111/cob.12203
39. *Kostecka, M., Bojanowska, M., Kostecka, J., Ciołek, A.:* An analysis of dietary patterns and body composition parameters in the Polish population. Rocz Panstw Zakl Hig. 2021;72(1):55-66. doi: 10.32394/rpzh.2021.0152
40. *Lahey, R., Khan, S.S.:* Trends in Obesity and Risk of Cardiovascular Disease. Curr Epidemiol Rep 2018;5(3):243-251. doi:10.1007/s40471-018-0160-1
41. *Lee, J., Allen, J.:* Gender Differences in Healthy and Unhealthy Food Consumption and Its Relationship with Depression in Young Adulthood. Community Mental Health Journal 2020. doi: 10.1007/s10597-020-00672-x
42. *Liu, K., Daviglus, M.L., Loria, C.M. et al.:* Healthy lifestyle through young adulthood and the presence of low cardiovascular disease risk profile in middle age: the coronary artery risk development in (Young) adults (CARDIA) study. Circulation 2012;125(8):996-1004. doi: 10.1161/CIRCULATIONAHA.111.060681
43. *McKeown, N.M., Yoshida, M., Shea, M.K. et al.:* Whole-grain intake and cereal fiber are associated with lower abdominal adiposity in older adults. J Nutr. 2009;139(10):1950-1955. doi: 10.3945/jn.108.103762
44. *Messiah, S.:* Body Composition. In: Gellman M.D. (eds) Encyclopedia of Behavioral Medicine. Springer, Cham [online], 2020. ISBN 978-3-030-39903-0. doi: https://doi.org/10.1007/978-3-030-39903-0_728
45. *Miranda, J., Lasa, A., Bustamante, M.A., Churruca, I., Simon, E.*: Nutritional Differences Between a Gluten-free Diet and a Diet Containing Equivalent Products with Gluten. Plant Foods for Human Nutrition 2014;69:182-187. doi: 10.1007/s11130-014-0410-4
46. *Moyer, V.A., U.S. Preventive Services Task Force:* Screening for and management of obesity in adults: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2012;157(5):373-8. doi: 10.7326/0003-4819-157-5-201209040-00475
47. *Nakata, A.:* Investigating the associations between work hours, sleep status, and self-reported health among full-

time employees. Int J Public Health 2012;57:403-411. doi: 10.1007/s00038-011-0242-z
48. *National Institute for Health and Care Excellence*: Identification, assessment and management of overweight and obesity in children, young people and adults. (Clinical Guideline 189). Available at: http://www.nice.org.uk/guidance/cg189. NICE: National Institute for Health and Care Excellence. 2014.
49. *National Institutes of Health:* Clinical Guidelines on the indentification, evaluation, and treatment of overweight and obesity in adults. The evidence report. Obes Res. 1998;6 Suppl 2:51S-209S.
50. *Pol, K., Christensen, R., Bartels, E.M. et al.:* Whole grain and body weight changes in apparently healthy adults: a systematic review and meta-analysis of randomized controlled studies. Am J Clin Nutr. 2013;98(4):872-884. doi:10.3945/ajcn.113.064659
51. *Rolls, B.J., Roe, L.S., Meengs, J.S.:* Larger portion sizes lead to a sustained increase in energy intake over 2 days. J Am Diet Assoc. 2006;106(4):543-549. doi: 10.1016/j.jada.2006.01.014
52. *Romero-Corral, A., Somers, V.K., Sierra-Johnson, J. et al.:* Accuracy of body mass index in diagnosing obesity in the adult general population. Int J Obes. 2008;32(6):959-966. doi: 10.1038/ijo.2008.11
53. *Seger, J.C., Horn, D.B., Westman, E.C. et al.:* Obesity Algorithm, presented by the American Society of Bariatric Physicians. Available at: www.obesityalgorithm.org. Obesity Medicine Association. 2015. Accessed May 13, 2021.
54. *Strazzullo, P., D'Elia, L., Cairella, G., Garbagnati, F., Cappuccio, F.P., Scalfi, L.:* Excess body weight and incidence of stroke: meta-analysis of prospective studies with 2 million participants. Stroke. 2010;41(5):e418-26. doi: 10.1161/STROKEAHA.109.576967
55. *Tcymbal, A., Andreasyan, D., Whiting, S. et al.:* Prevalence of Physical Inactivity and Sedentary Behavior Among Adults in Armenia. Frontiers Public Health 2020;8:157. doi: https://doi.org/10.3389/fpubh.2020.00157
56. *The GBD 2015 Obesity Collaborators:* Health Effects of Overweight and Obesity in 195 Countries over 25 Years. N Engl J Med 2017;377(1):13-27. doi: 10.1056/NEJMoa1614362
57. *Tobias, D.K., Pan, A., Jackson, C.L. et al.:* Body-mass index and mortality among adults with incident type 2 diabetes. N Engl J Med. 2014;370(3):233-244. doi: 10.1056/NEJMoa1304501
58. *Twig, G., Afek, A., Derazne, E. et al.:* Diabetes risk among overweight and obese metabolically healthy young adults. Diabetes Care. 2014;37(11):2989-2995. doi: 10.2337/dc14-0869
59. *van de Woestijne, A.P., Monajemi, H., Kalkhoven, E., Visseren, F.L.:* Adipose tissue dysfunction and hypertriglyceridemia: mechanisms and management. Obes Rev. 2011;12(10):829-840. doi: 10.1111/j.1467-789X.2011.00900.x
60. *van Dijk, S.B., Takken, T., Prinsen, E.C., Wittink, H.:* Different anthropometric adiposity measures and their association with cardiovascular disease risk factors: a meta-analysis. Neth Heart J. 2012;20(5):208-218. doi: 10.1007/s12471-011-0237-7
61. *Vazquez, G., Duval, S., Jacobs, D.R., Silventoinen, K.:* Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: a meta-analysis. Epidemiol Rev. 2007;29:115-128. doi: 10.1093/epirev/mxm008
62. *WHO:* Global recommendations on physical activity for health. Geneva 2010. Available https://apps.who.int/iris/bitstream/handle/10665/44399/9789241599979_eng.pdf?sequence=1 (Accessed 10.05.2021)
63. *WHO:* Obesity and overweight. Available https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (Accessed 13.05.2021).
64. *Woodruff, B.A., Duffield, A.:* Anthropometric assessment of nutritional status in adolescent populations in humanitarian emergencies. Eur J Clin Nutr. 2002;56(11):1108-1118. doi: 10.1038/sj.ejcn.1601456
65. *World Health Organization Expert Committee:* Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser. 1995;854:1-452.
66. *Wormser, D., Kaptoge, S., Di Angelantonio, E. et al.:* Emerging Risk Factors Collaboration. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. Lancet 2011;377(9771):1085- 1095. doi: 10.1016/S0140-6736(11)60105-0
67. *Zheng, Y., Manson, J. E., Yuan, Ch. et al.:* Associations of Weight Gain From Early to Middle Adulthood With Major Health Outcomes Later in Life. JAMA 2017;318(3):255-269. doi:10.1001/jama.2017.7092
68. *Zuccotti, G., Fabiano, V., Dilillo, D., Picca, M., Cravidi, C., Brambilla, P.*: Intakes of nutrients in Italian children with celiac disease and the role of commercially available gluten-free products. Journal of Human Nutrition and Dietetics 2013;26(5):436-444. doi: 10.1111/jhn.12026

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ORIGINAL ARTICLE

OBESITY, SMOKING STATUS AND THEIR RELATIONSHIPS IN SELECTED POPULATION GROUPS

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ABSTRACT

Background. Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health. Obesity is associated with many chronic diseases, including cardiovascular disease and diabetes, and recently the role of overweight and obesity in lung disease has received new interest. Chronic obstructive lung disease is the third-leading cause of death globally, and both obesity and diet appear to play roles in its pathophysiology. Cross-sectional studies have demonstrated an inverse association between obesity and the prevalence of chronic obstructive pulmonary disease (COPD).

Objective. This study aims to evaluate the relationship between smoking, lipid profile and obesity (body composition changes) in a selected groups of population (30 non-smokers, 30 smokers and 60 COPD patients).

Material and Methods. We evaluated fat mass, fat free mass, body mass index and lipid profile in a group of 120 randomly selected probands (60 COPD patients; 30 smokers without COPD; 30 non-smokers without COPD) to identify possible negative relationships of smoking to body composition. To the measurement of fat mass (FM) and fat free mass (FFM) was used a device Bodystat Quadscan 4000 (Bodystat Ltd, British Isles). The device works by using four-frequency bioelectrical impedance analysis. Laboratory parameters as total cholesterol (T-C), high-density cholesterol (HDL-C), low-density cholesterol (LDL-C) and triacylglycerols (TG) were investigated by automated clinical chemistry analyzer LISA 200th. The measured values were statistically processed and evaluated in a statistical program STATISTICA Cz. Version 7.1. (Kruskall-Wallis test).

Results. A comparison of the mean fat mass we found statistically highly significant differences between the group of COPD patients and non-smokers ($P < 0.001$) and insignificant differences ($P \geq 0.05$) between the other groups of our experiment. A comparison of the mean fat mass values of all three groups of the experiment shows a steady increase in fat from non-smokers (17.66 ± 10.04 kg) to COPD patients with the highest mean value (25.08 ± 10.14 kg). In the group of COPD patients we recorded the lowest average value of FFM (51.76 ± 13.84 kg), in group of smokers the middle (56.06 ± 10.76 kg) and in non-smokers the highest average value of FFM (59.91 ± 9.90 kg) at relatively the same body weight in the groups. Based on calculated body mass index (BMI), we found in group of COPD patients overweight in 15 cases (25%), obesity in 7 patients (11.67%), severe obesity in 14 patients (23.3%) and morbid obesity in 2 patients (3.33%); in the group of smokers overweight in 16 cases (53.33%), obesity in 5 cases (16.6%) and severe obesity in 1 case (3.33%); in non-smokers we recording overweight in 14 cases (46.67%), obesity in 5 cases (16.67%) and severe obesity in 2 cases (6.67%). In the lipid profile of the monitored groups of probands, we observed statistically significant differences only for LDL cholesterol (LDL-C). There was a statistically significant difference ($P < 0.001$) between the group with COPD and smokers, as well as between the group of smokers and non-smokers ($P < 0.05$).

Conclusions. In the vast majority of patients with COPD, the lung damage that leads to COPD is caused by long-term cigarette smoking. The presence and absence of risk factors such as smoking, inappropriate lipid profile and obesity (amount of fat mass) in selected population groups were observed. Additional studies to explore both the quantitative and qualitative changes in body composition with disease process of COPD are required.

Key words: *obesity, cigarette smoking, non-smokers, smokers, COPD patients; lipid profile*

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INTRODUCTION

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health. In 2016, more than 1.9 billion adults, 18 years and older, were overweight. Of these over 650 million were obese. The worldwide prevalence of obesity nearly tripled between 1975 and 2016 [31]. The proportion of obesity among the adult Slovak population was in 2016 for both men and women 25.4 % [30]. The global increase in the prevalence and incidence of obesity has called serious attention to this issue as a major public health concern. Obesity is associated with many chronic diseases, including cardiovascular disease and diabetes, and recently the role of overweight and obesity in lung disease has received new interest [15].

Chronic obstructive pulmonary disease (COPD) is another condition that is associated with severe morbidity and mortality globally [8, 21, 22, 24]. Chronic obstructive lung disease is the third-leading cause of death globally, and both obesity and diet appear to play roles in its pathophysiology [13, 15]. COPD is characterized by airflow obstruction, and is the umbrella term for two conditions: chronic bronchitis and emphysema, both of which are related to similar etiology and may coexist. Lung function, which is readily measured by spirometry, is the defining feature of COPD [21]. Cigarette smoking is the most commonly encountered and readily identifiable risk factor for COPD [7].

Cigarette smoke contains ≈4,700 chemical constituents and can increase production of endogenous reactive oxygen species in target cell populations [27, 28]. However, it is not clear which quantitative factors related to smoking influence the prognosis of COPD patients [1].

Cross-sectional studies have demonstrated an inverse association between obesity and the prevalence of COPD [11]. It is possible that improper lung function increases the risk of developing obesity. This may be due to the following three causes: (1) COPD patients' difficulty breathing while exercising often results in lower levels of physical activity and thus fewer calories burned in exercise, (2) a common side effect of long-term glucocorticosteroid medications is weight gain and (3) due to being hypoxemic both at rest and with exercise, COPD patients are unable to utilize oxygen for the breakdown of fatty acids through beta oxidation [11, 20]. Although the association between obesity and COPD is increasingly recognized, the mechanisms involved and the nature of the relationship are still unclear. One reason for this uncertainty is that studies looking at COPD typically have included current smokers or those with a history of smoking [5]. Since smoking is the number one risk factor for the development of COPD [10], including smokers in the studies makes it more difficult to investigate whether obesity is playing an independent role in the development of COPD, aside from the smoking, and whether obesity is associated with a dose-response relationship [12].

COPD is considered as a systemic disease according to much concomitant comorbidity in patients. These comorbidities significantly impact on patient outcomes. Evidence for this approach has been provided by strong associations with increased rates especially with cardiovascular diseases, metabolic syndrome, anaemia, musculoskeletal disease and pulmonary malignancies. A number of studies have shown a high connectivity between COPD and cardiovascular morbidity and mortality and pulmonary embolism. Hypercholesterolemia probably is hugely responsible for those events [33].

The metabolic defects that ensue in obesity include increased levels of free fatty acids resulting from insulin resistance, increased LDL-cholesterol, VLDL and triglycerides and decrease in HDL-cholesterol. It is most likely that presentation of increased free fatty acids to liver as a function of obesity is primarily responsible for over production of VLDL and this is probably the key to increased LDL via the sequence: VLDL → intermediate density lipoprotein (IDL) → LDL [32].

Inflammation may also play a role in the obesity-COPD association. Obese individuals have elevated levels of a variety of inflammatory markers, including TNF-a, IL-6, and adipose resident macrophages, which results in an increased level of inflammation both locally and systemically [9].

This study aims to evaluate the relationship between smoking, lipid profile and obesity (body composition changes in COPD) in a selected groups of population (30 non-smokers, 30 smokers and 60 COPD patients).

MATERIALS 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.

We evaluated fat mass (FM); fat free mass (FFM); body mass index (BMI) and lipid profile in a group of 120 randomly selected probands (60 COPD patients – the cause of their COPD diagnosis was long-term

cigarette smoking; 30 smokers without COPD; 30 non-smokers without COPD) to identify possible negative relationships of smoking to body composition (especially in relation to fat mass).

The research was approved by the ethics committee, approval number 4/071220/2020; Study Protocol Title: Long-term strategic research of prevention, intervention and mechanisms of obesity and its comorbidities. 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.

To the body weight measure of probands we used a BRUTUS Tanita digital personal scale (Tanita Corporation, Tokyo, Japan). Body weight was determined in underwear (digital scale, accuracy of measurement: 0.1 kg). To the body height measure of probands we used an ultrasonic height measuring unit BODYSON (Ultrasound Height Measuring Unit MZ10020) (ADE GmbH & Co., Hamburg, Germany). The measuring range is 500 - 2500 mm with a division of 5 mm. The meter is characterized by high accuracy, can be checked (spirit level) and its operation is simple. Body mass index (BMI) was calculated from body weight and body height of probands.

To the measurement of fat mass (FM) and fat free mass (FFM) was used a device Bodystat Quadscan 4000 (Bodystat Ltd, British Isles). The device works by using four-frequency bioelectrical impedance analysis (5; 50; 100 and 200 kHz). Regression equations are then derived which relate impedance to fat free mass (FFM) or total body water (TBW) measured by independent techniques. In our work, we adhered to the standard conditions of measurement: the probands were placed on the examination bed in a supine position with their lower limbs outstretched. The upper limbs are placed loosely next to the body, they must not touch the body. Two sensing electrodes with input and output cable are placed on the right hand and right foot, which ensure the supply of electrical current into the body and its feedback detection at the exit from the body and connection to the device software. The probe must not have a pacemaker or insulin pump or any electronic devices. The skin of the limbs must be at normal temperature and dry. Before starting the measurement itself, the personal data of the proband are first entered into the device: sex, age, weight, height, waist and hip circumference, current energy expenditure. This is followed by a custom measurement that takes a few seconds. Subsequently, the software of the device evaluates the given parameters.

Blood from probands was collected during hospitalization or outpatient examination. Laboratory parameters as total cholesterol (T-C), high-density cholesterol (HDL-C), low-density cholesterol (LDL-C) and triacylglycerols (TG) were investigated by automated clinical chemistry analyzer LISA 200$^{th.}$ 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. To the statistically evaluate of our experiment we used the *Kruskall-Wallis* test.

RESULTS AND DISCUSSION

Based on the clinical stage of the disease according to GOLD (stage I. – IV.), were COPD patients (n=60) in the following percentage: stage I. – 26.67%; stage II. – 71.67%; stage III. – 0%; stage IV. - 1.66%. The group of COPD patients consisted of 12 women and 48 men; the group of smokers consisted of 18 women and 12 men and the group of non-smokers consisted of 17 women and 13 men.

From the obtained individual values, we calculated the basic statistical characteristics of probands (Table 1).

Based on calculated body mass index (BMI), we found in group of COPD patients (n=60) cachexia in 1 case (1.67%), underweight in 4 cases (6.67%), normal BMI in 17 cases (28.33%), overweight in 15 cases (25%), obesity in 7 patients (11.67%), severe obesity in 14 patients (23.3%) and morbid obesity in 2 patients (3.33%). In the group of smokers (n=30), we found underweight in 1 case (3.33%), normal BMI in 9 cases (30%), overweight in 16 cases (53.33%), obesity in 5 cases (16.6%) and severe obesity in 1 case (3.33%). No cachexia, underweight or morbid obesity were reported in this group. In the group of non-smokers (n=30) we recording normal BMI in 8 cases (26.67%), overweight in 14 cases (46.67%), obesity in 5 cases (16.67%) and severe obesity in 2 cases (6.67%). In this group were reported no cachexia, underweight or morbid obesity. The results correlate with the incidence of obesity in Slovak population. The results of our experiment showed that COPD patients have a lower body weight and a slightly higher BMI value compared to probands from the general population without COPD.

Because body mass index (BMI) does not take into account fat mass, we measured body composition using BIA in the monitored probands.

Table 1. Basic characteristics of probands (n = 120)

Characteristic	COPD Patients (n=60)		Smokers (n=30)		Non-smokers (n=30)		P - Value
	mean± SD	min. – max.	mean ± SD	min. – max.	mean± SD	min. – max.	
Age (yrs)	69.25 ± 9.90	49 - 87	46.53 ± 9.22	26 - 59	52 ± 6.51	32 - 63	-
Body weight (kg)	76.73 ± 20.23	38.6 – 136.8	77.83 ± 12.76	53.2 – 101.7	77.57 ± 16.34	50 – 117.5	$P \geq 0.05$
FM (kg)	25.08 ± 10.14	10.3 – 57.1	21.77 ± 9.06	7.5 - 45	17.66 ± 10.04	6.5 – 50.7	$P < 0.001$
FFM (kg)	51.76 ± 13.84	22.1 – 81.5	56.06 ± 10.76	38.8 – 75.4	59.91 ± 9.90	43.5 – 80.2	$P < 0.05$
BMI ($kg.m^{-2}$)	28.5 ± 7.05	15.3 – 46.8	26.89 ± 4.07	20 – 36.8	27.29 ± 4.42	18.6 - 37	$P \geq 0.05$
T-C ($mmol.L^{-1}$)	4.69 ± 1.09	2.9 – 8.73	5.01 ± 0.94	3.23 – 6.85	5.33 ± 1.04	2.93 – 7.18	$P < 0.05$
HDL-C ($mmol.L^{-1}$)	1.43 ± 0.55	0.53 – 2.74	1.58 ± 0.35	1.05 – 2.53	1.89 ± 0.27	1.23 – 2.47	$P < 0.001$
LDL-C ($mmol.L^{-1}$)	2.77 ± 1.05	0.62 – 6.29	3.57 ± 0.69	2.38 – 4.67	2.83 ± 1.02	0.93 – 4.53	$P < 0.001$
TG ($mmol.L^{-1}$)	1.23 ± 0.53	0.3 – 2.52	1.34 ± 1.6	0.42 – 9.45	1.34 ± 0.85	0.44 – 4.52	$P \geq 0.05$

Data are expressed as mean ± standard deviation (SD), min. – max.; FM (fat mass); FFM (fat free mass); BMI (body mass index); T-C (total cholesterol); LDL-C (low density cholesterol); HDL-C (high density cholesterol); TG (triacylglycerols)

Bioelectrical impedance analysis (BIA) is a method for estimating body composition, in particular body fat and muscle mass, where a weak electric current flows through the body and the voltage is measured in order to calculate impedance (resistance) of the body. Most body water is stored in muscle. Therefore, if a person is more muscular there is a high chance that the person will also have more body water, which leads to lower impedance. BIA actually determines the electrical impedance, or opposition to the flow of an electric current through body tissues which can then be used to estimate total body water (TBW), which can be used to estimate fat-free body mass and, by difference with body weight, body fat [19].

The results for the parameter "fat mass" in the monitored groups of probands are follows: we found statistically highly significant differences between the group of COPD patients and non-smokers ($P<0.001$) and insignificant differences ($P\geq0.05$) between the other groups of our experiment. A comparison of the mean fat mass values of all three groups of the experiment shows a steady increase in fat from non-smokers (17.66±10.04 kg) to COPD patients with the highest mean value (25.08±10.14 kg) (Figure 1). This finding may be related to the negative effect of cigarette smoke in smokers and patients as well as to the development of chronic obstructive pulmonary disease in patients, as all three groups had approximately the same average body weight (about 77 kg – Table 1).

Although smokers did not show significant difference in mean body mass index than those who never smoked, they showed more metabolically adverse fat distributions with increasing smoking amounts [17]. This finding suggests that smoking is not beneficial for weight control. Therefore, smoking cessation and avoidance of smoking commencement should be addressed as important public health issues in preventing obesity and related complications.

Among smokers, cigarettes smoked per day were positively associated with central fat accumulation, particularly in women [6]. Substantial evidence shows that cigarette smoking induces multiple pathological effects in adipose tissue, such as differentiation of adipocytes, lipolysis, and secretion properties in adipose tissue [29].

Fat free mass (FFM) is made up of muscle and bone mass of the body. Our measurements show that in the group of COPD patients we recorded the lowest average value of FFM (51.76±13.84 kg), in group of smokers the middle (56.06±10.76 kg) and in non-smokers the highest average value of FFM (59.91±9.90 kg) at relatively the same body weight in the groups (about 77 kg - Table 1).

In patients with COPD, this is related to the gradual loss of muscle mass - which is an unfavorable prognostic indicator of their disease; in smokers lower FFM may indicate a negative effect of cigarette smoke on their metabolism and their overall physical and health status.

Smokers are exposed to the effects of oxidative stress, with its negative effects on the cardiovascular and respiratory systems. Recently, osteoporosis, *diabetes mellitus* and obesity have become increasingly common in patients with COPD. The above, together with the ongoing COPD, contributes to reducing the quality of life. Progressive deterioration of lung function in a smoker can be averted only by immediate cessation of smoking and the exclusion of other risk factors, as well as comprehensive treatment including pharmacological, rehabilitation treatment and nutritional intervention. The treatment of COPD must be complex and effective. The pharmacological part of the treatment should gradually improve the health condition, reduce the complications of the disease and the outbreak of exacerbations. Non-pharmacological treatment - nutritional support aims to improve physical fitness and reduce unwanted loss of muscle mass.

Subsequently, we were interested in the lipid profile in the monitored groups of probands. There was no significant difference in the T-C between the

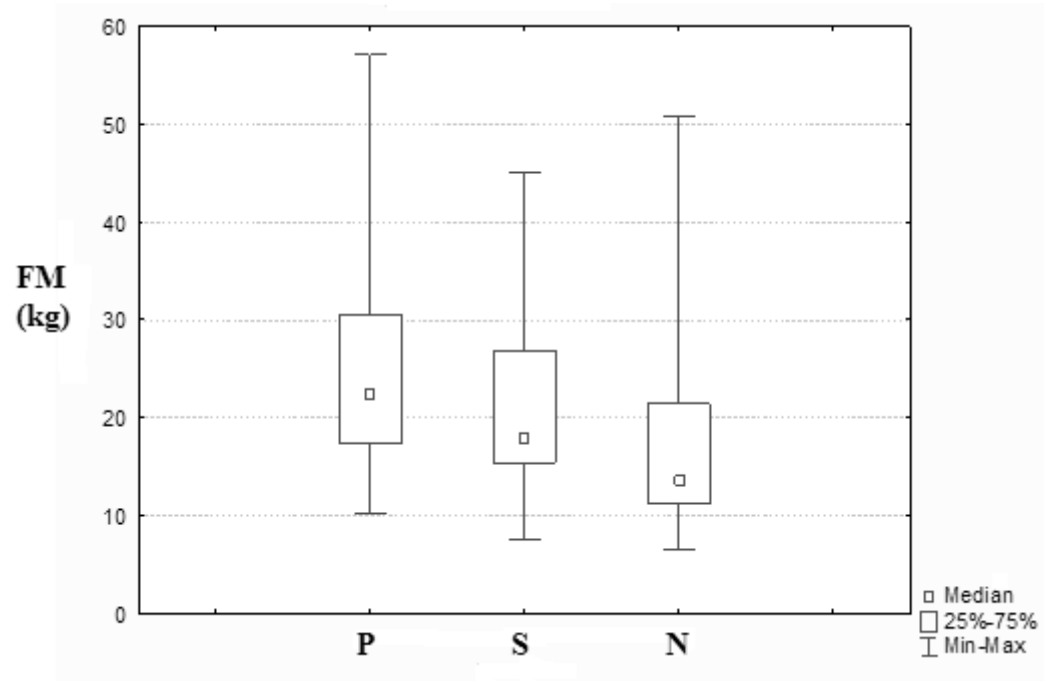

Figure 1. Evaluation of fat mass (FM) by device Bodystat Quadscan 4000 in the monitored groups: COPD patients (P), smokers (S) and non-smokers (N) ($P < 0.001$)

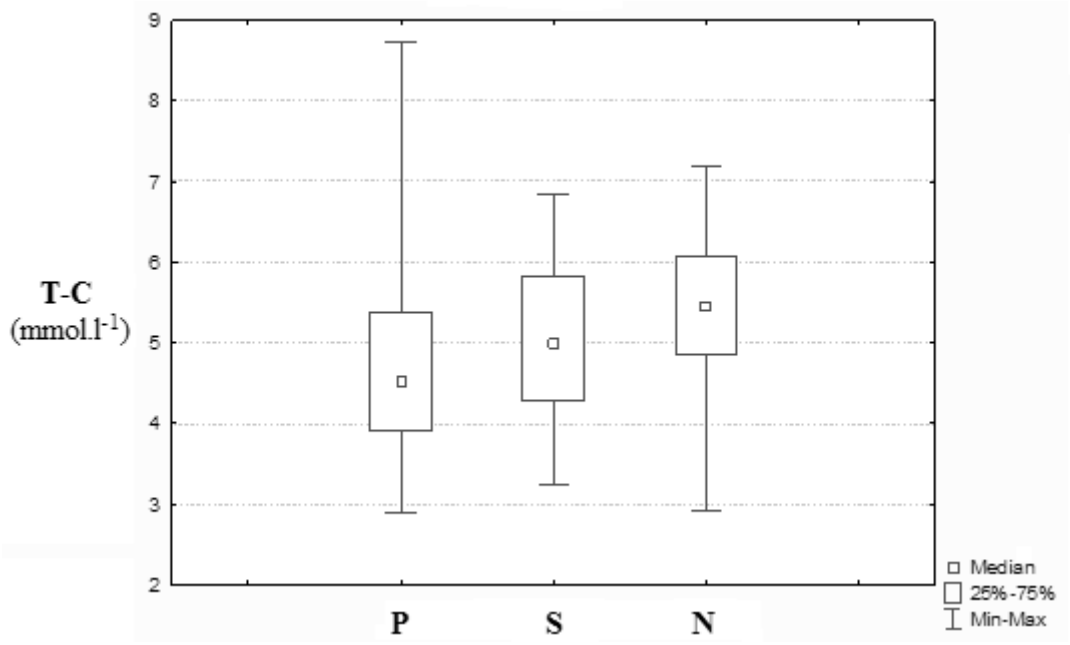

Figure 2. Levels of total cholesterol (T-C) in the monitored groups: COPD patients (P), smokers (S) and non-smokers (N) ($P < 0.05$)

group of patients and non-smokers, and no significant difference ($P \geq 0.05$) was found between the other groups (Figure 2).

Dyslipidemia, a major risk factor for coronary heart disease and metabolic syndrome, is characterized by a cluster of lipid abnormalities such as an elevated level of triglyceride (TG), a reduced level of HDL-C and an increased level of LDL-C. A number of studies have evaluated the relationship between COPD and blood lipid profiles with inconsistent results. While some authors reported reduced serum levels of HDL or increased serum levels of TG in COPD patients [4, 14], others did not observe any significant changes in lipid serum profiles [2].

There was a statistically significant difference ($P<0.001$) in the LDL-C level between the groups of patients and smokers, a statistically significant difference between the group of smokers and non-smokers ($P<0.05$) The mean LDL value in the group of patients was 2.77±1.05 $mmol.l^{-1}$, in the group of smokers 3.57±0.69 $mmol.l^{-1}$ and 2.83±1.02 $mmol.l^{-1}$ in the group non-smokers (Figure 3).

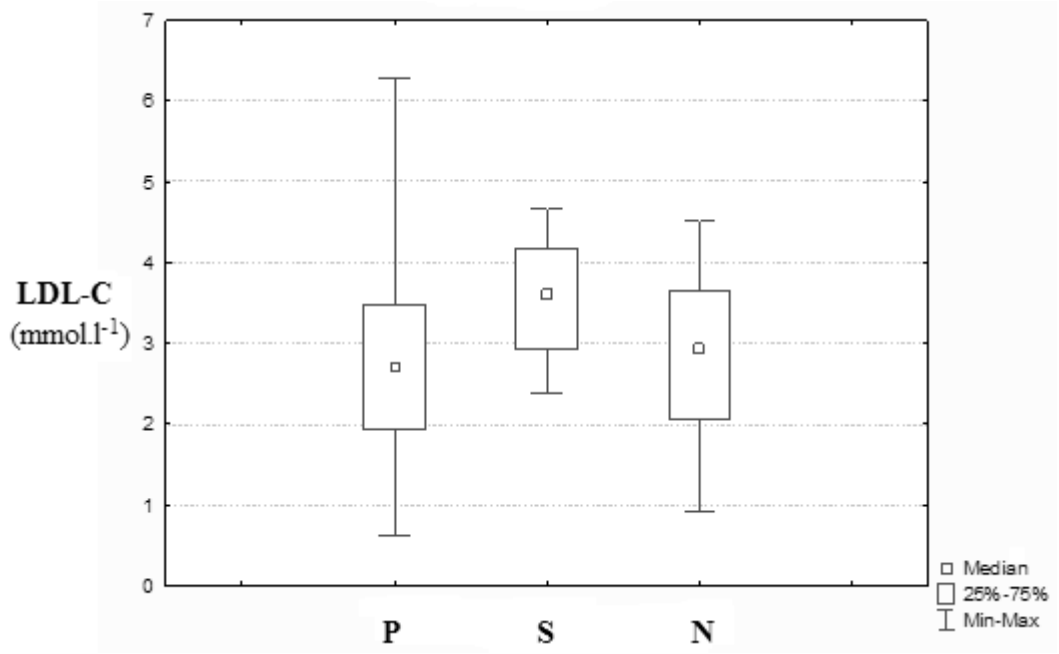

Figure 3. Levels of low density cholesterol (LDL-C) in the monitored groups : COPD patients (P), smokers (S) and non-smokers (N) (P < 0.001)

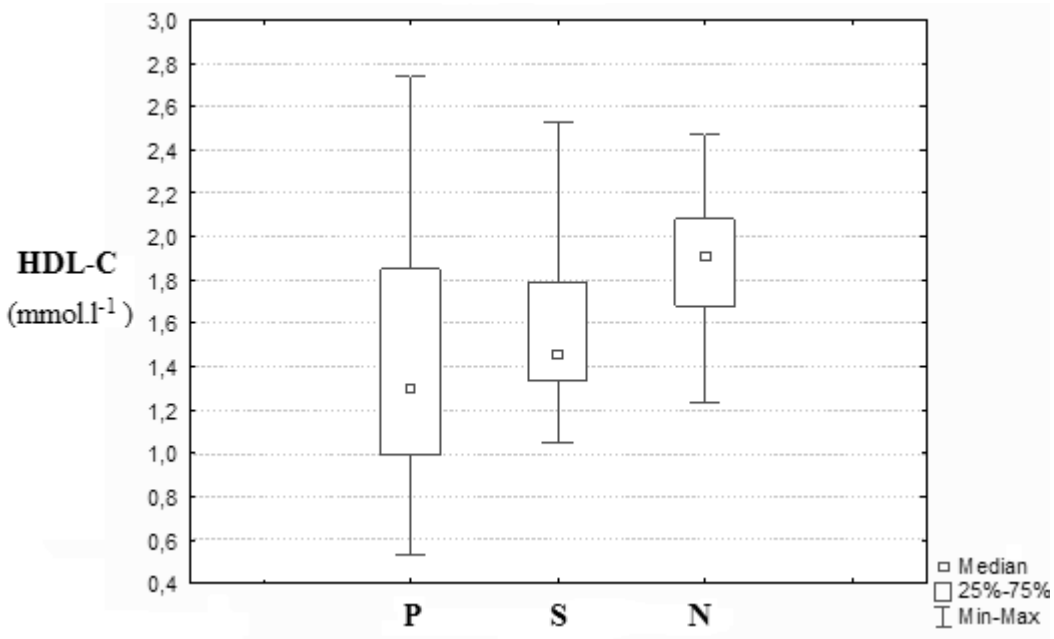

Figure 4. Levels of high density cholesterol (HDL-C) in the monitored groups : COPD patients (P), smokers (S) and non-smokers (N) (P < 0.001)

Hypercholesterolemia in COPD is considered as comorbidity, one of the metabolic syndrome. According to some dates severe COPD is associated with increased levels of HDL-C, which is partially attributable to oral steroid use. HDL-C in this population is not associated with reduced risk of angiographically proven coronary artery disease [25]. We found a statistically highly significant difference (P< 0.001) between the group of patients and smokers, a statistically significant difference between the group of smokers and non-smokers (P < 0.05) (Figure 4).

The prevalence of metabolic syndrome (MS) is high in COPD patients and higher value of triacylglycerols (TG) was the MS component associated with higher risk of five-year mortality in COPD patients [26].

By multiple comparisons of P values, we did not find significant differences between the observed groups in the observed trait (P≥0.05) (Figure 5). The lowest TG values were found in the group of COPD patients; the TG values in the group of smokers and non-smokers were approximately at the same level. This finding indicates the efficacy of treating

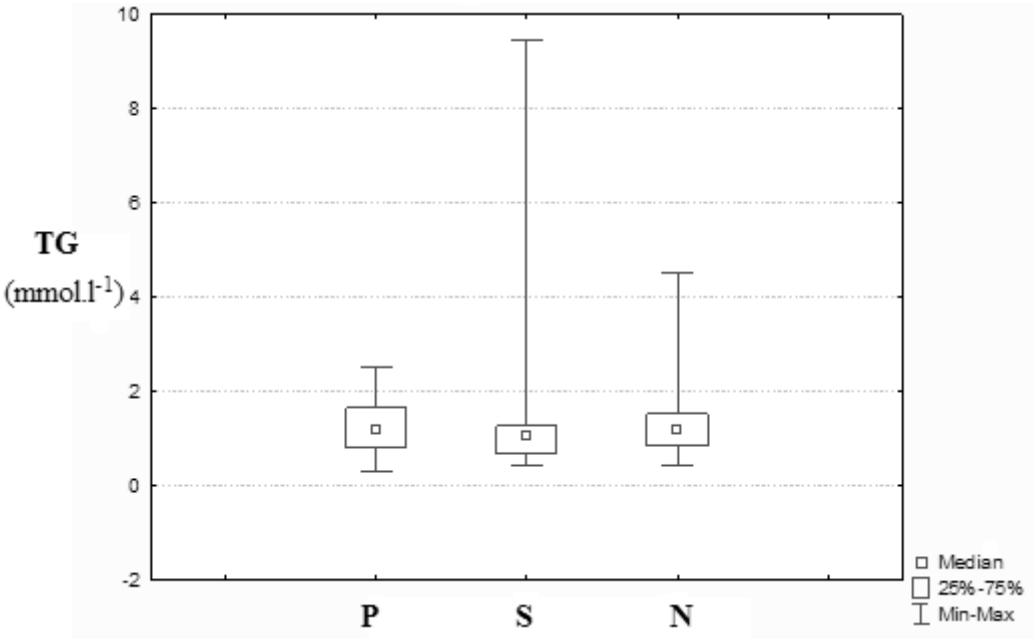

Figure 5. Levels of triacylglycerols (TG) in the monitored groups : COPD patients (P), smokers (S) and non-smokers (N) ($P \geq 0.05$)

dyslipidemia in COPD patients. Patients who were not receiving treatment for dyslipidaemia showed that TG levels were higher in patients with stable COPD than in healthy individuals [34].

In COPD patients, metabolic syndrome did not additionally impact patients' functional outcomes but did impact the prevalence of co-morbidities first of all cardiovascular [3, 16]. According to dates from some studies high level of cholesterol as a part of a metabolic syndrome in COPD patients is associated with more serious and more frequent exacerbations [18, 23].

CONCLUSIONS

In the vast majority of patients with COPD, the lung damage that leads to COPD is caused by long-term cigarette smoking. But there are likely other factors at play in the development of COPD, such as a genetic susceptibility to the disease, because not all smokers develop COPD. There is little data about the combined effects of COPD and obesity.

In our study, we observed the presence and absence of risk factors such as smoking, inappropriate lipid profile and obesity (amount of fat mass) in selected population groups.

In fat mass (FM) we found statistically highly significant differences between the group of COPD patients and non-smokers ($P<0.001$) and insignificant differences ($P\geq0.05$) between the other groups of our experiment. A comparison of the mean FM values of all three groups of the experiment shows a steady increase in fat from non-smokers (17.66±10.04 kg) to COPD patients with the highest mean value (25.08±10.14 kg). Fat free mass (FFM) measurements show that in the group of COPD patients we recorded the lowest average value of FFM (51.76±13.84 kg), in group of smokers the middle (56.06±10.76 kg) and in non-smokers the highest average value of FFM (59.91±9.90 kg) at relatively the same body weight in the groups. In patients with COPD, this is related to the gradual loss of muscle mass - which is an unfavorable prognostic indicator of their disease; in smokers lower FFM may indicate a negative effect of cigarette smoke on their metabolism and their overall physical and health status. In the lipid profile of the monitored groups of probands, we observed statistically significant differences only for LDL cholesterol.

Obesity and COPD are multifactorial diseases and additional studies are required to explore both the quantitative and qualitative changes in body composition with disease process of COPD.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Bai J.W., Chen X., Liu S., Yu L., Xu J.F.*: Smoking cessation affects the natural history of COPD Int J Chron Obstruct Pulmon Dis. 2017; 12: 3323–3328. doi: 10.2147/COPD.S150243.
2. *Basili S, Ferroni P, Vieri M, Cardelli P, Ceci F, Paradiso M, et al.:* Lipoprotein(a) serum levels in patients affected by chronic obstructive pulmonary disease. Atherosclerosis. 1999;147:249–252. doi: 10.1016/S0021-9150(99)00192-6.
3. *Boschetto P., Beghé B., Fabbri L., Ceconi C.:* Link between chronic obstructive pulmonary disease and coronary artery disease: implication for clinical practice. Respirology. 2012;17(3):422–31. http://dx.doi.org/10.1111/j.1440-1843.2011.02118.x.
4. *Can U, Yerlikaya FH, Yosunkaya S.:* Role of oxidative stress and serum lipid levels in stable chronic obstructive pulmonary disease. J Chin Med Assoc. 2015;78:702–708. doi: 10.1016/j.jcma.2015.08.004.
5. *Cecere L.M., Littman A. J., Slatore C. G. et al.*: Obesity and COPD: associated symptoms, health-related quality of life, and medication use. COPD: Journal of Chronic Obstructive Pulmonary Disease, vol. 8, no. 4, pp. 275–284, 2011.
6. *Clair C., Chiolero A., Faeh D., Cornuz J., Marques-Vidal P., Paccaud F., Mooser V, Waeber G., Vollenweider P.:* Dose-dependent positive association between cigarette smoking, abdominal obesity and body fat: cross-selectional data from a population-based survey. BMC Public Health. 2011;11:23. doi: 10.1186/1471-2458-11-23.
7. *Eisner M.D., Anthonisen N., Coultas D., et al.*: An official American Thoracic Society public policy statement: novel risk factors and the global burden of chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2010;182(5):693–718.
8. *Feary J.R., Rodrigues L..C., Smith C.J., Hubbard R.B., Gibson J.E.*: Prevalence of major comorbidities in subjects with COPD and incidence of myocardial infarction and stroke: a comprehensive analysis using data from primary care. Thorax. 2010;65(11):956–962.
9. *Ferrante Jr. A. W.*: Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. Journal of Internal Medicine, vol. 262, no. 4, pp. 408–414, 2007.
10. *Forey B.A., Thornton A. J., Lee P.N.*: Systematic review with meta-analysis of the epidemiological evidence relating smoking to COPD, chronic bronchitis and emphysema. BMC Pulmonary Medicine, vol. 11, no. 1, p. 36, 2011.
11. *Franssen F., O'Donnell D., Goossens G., Blaak E., Schols A.*: Obesity and the lung: obesity and COPD. Thorax, vol. 63, no. 12, pp. 1110–1117, 2008.
12. *Fuller-Thomson E.F., Howden K.E.N., Fuller-Thomson L.R, Agbeyaka S.*: A Strong Graded Relationship between Level of Obesity and COPD: Findings from a National Population-Based Study of Lifelong Nonsmokers. Journal of Obesity, vol. 2018, Article ID 6149263, 9 pages, 2018. https://doi.org/10.1155/2018/6149263
13. *Global Strategy for the Diagnosis, Management and Prevention of COPD*. [Accessed March 9, 2017]. Available from: http://goldcopd.org/wp-content/uploads/2016/12/wms-GOLD-2017-Pocket-Guide.pdf.
14. *Gunay S, Sariaydin M, Acay A.:* New Predictor of Atherosclerosis in Subjects With COPD: Atherogenic Indices. Respir Care. 2016;61:1481–1487. doi: 10.4187/respcare.04796.
15. *Hanson C., Rutten E.P., Wouters E.F.M., Rennard S.*: Influence of diet and obesity on COPD development and outcomes. Int J Chron Obstruct Pulmon Dis 2014;9:723-733. doi: 10.2147/COPD.S50111
16. *Jurczak I., Jurczak K.:* Chronic obstructive pulmonary disease as a risk factor for coronary heart disease. Pol Merkur Lekarski. 2015;38(223):39–41.
17. *Kim J.H., Shim K.W., Yoon Y.S., Lee S.Y., Kim S.S., Oh S.W.:* Cigarette smoking increases abdominal and visceral obesity but not overall fatness: an observational study. PLoS One. 2012;7(9):e45815. doi: 10.1371/journal.pone.0045815.
18. *Küpeli E., Ulubay G., Ulasli S., Sahin T., Erayman Z., Gürsoy A.:* Metabolic Syndrome is associated with increased risk of acute exacerbation of COPD: a preliminary study. Endocr Jour. 2010;38(1):76–82. http://dx.doi.org/10.1007/s12020-010-9351-3 .
19. *Kyle U.G., Bosaeus I., De Lorenzo A.D., Deurenberg P., Elia M., Gómez J.M., Heitmann B.L., Kent-Smith L, Melchior J.C., Pirlich M., Scharfetter H., Schols A.M., Pichard C.:* Bioelectrical impedance analysis-part I: review of principles and methods. Clinical Nutrition. **23** (5): 1226–43. doi:10.1016/j.clnu.2004.06.004. PMID 15380917.
20. *Liu Y., Ma Z., Zhao C. et al.*: HIF-1α and HIF-2α are critically involved in hypoxia-induced lipid accumulation in hepatocytes through reducing PGC-1α-mediated fatty acid β-oxidation. *Toxicology Letters*, vol. 226, no. 2, pp. 117–123, 2014.
21. *Lozano R., Naghavi M., Foreman K., et al.*: Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380 (9859):2095–2128.
22. *Mannino D.M., Thorn D., Swensen A., Holguin F.*: Prevalence and outcomes of diabetes, hypertension and cardiovascular disease in COPD. Eur Respir J. 2008;32(4):962–969.
23. *Mroz R., Lisowski P., Tycinska A., Bierla J., Trzeciak P., Minarowski L., Milewski R., Lisowska A., Boros P., Sobkowicz B., Duszewska A., Chyczewska E., Musial W., MacNee W.:* Anti-inflammatory effects of atorvastatin treatment in chronic obstructive pulmonary disease. Physiol Pharmacol. 2015;66(1):111–28.
24. *Park S.K, Larson J.L.:* Metabolic syndrome and associated factors in people with chronic obstructive pulmonary disease. West J Nurs Res. 2013 Nov 29;
25. *Reed R., Iacono A., DeFilippis A., Eberlein M., Girgis R., Jones S.:* Advanced chronic obstructive pulmonary disease is associated with high levels of high-density lipoprotein cholesterol. Heart Lung Transplant.

2011;30(6):674–8. http://dx.doi.org/10.1016/j.healun.2010.12.010 .
26. *Tanni S., Zamuner A., Schelini K., Coelho L., Caram L., Vale S., Ferrari R., Godoy I., Paiva S., Godoy I.:* Triglycerides are associated with five-year mortality in COPD patients. European Respiratory Journal. 2012. 40:P522.
27. *Van der Toorn M., Smit-de Vries M.P., Slebos D.J., et al.:* Cigarette smoke irreversibly modifies glutathione in airway epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2007;293(5):L1156–L1162.
28. *Van der Toorn M., Rezayat D., Kauffman H.F., et al.*: Lipid-soluble components in cigarette smoke induce mitochondrial production of reactive oxygen species in lung epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2009;297(1):L109–L114.
29. *Wang Z., Wang D., Wang Y.:* Cigarette Smoking and Adipose Tissue: the Emerging Role in Progression of Atherosclerosis. Mediators Inflamm. 2017;2017:3102737. doi: 10.1155/2017/3102737.
30. *WHO:* Nutrition, Physical Activity and Obesity Slovakia. https://www.euro.who.int/__data/assets/pdf_file/0018/243324/Slovakia-WHO-Country-Profile.pdf
31. *WHO*: Obesity and overweight. 2020. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.
32. *Wolf R.N., Grundy S.M.:* Influence of weight reduction on plasma lipoproteins in obese patients. Arteriosclerosis. 1983. 3:160-169.
33. *Zafirova-Ivanovska B., Stojkovikj J., Dokikj D., Anastasova S., Debresliovska A., Zejnel S, Stojkovikj D.:* The Level of Cholesterol in COPD Patients with Severe and Very Severe Stage of the Disease. Open Access Maced J Med Sci. 2016. 15;4(2): 277-282. doi: 10.3889/oamjms.2016.063.
34. *Xuan L., Han F., Gong L., Lv Y., Wan Z., Liu H., Zhang D., Jia Y., Yang S., Ren L., Liu L.:* Association between chronic obstructive pulmonary disease and serum lipid levels: a meta-analysis. Lipid Health Dis. 2018. 21;17(1)263. doi: 10.1186/s12944-018-0904-4.

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ORIGINAL ARTICLE

MEDITERRANEAN DIET ADHERENCE AMONG COSMETOLOGY STUDENTS

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ABSTRACT

Background. The Mediterranean diet (MD) is universally recognized as the healthiest model of nutrition whose beneficial effects help prevent many diet-related diseases.

Objective. The aim of the study was to assess cosmetology students' adherence to the Mediterranean Diet using the Mediterranean Diet Score (MDS).

Material and methods. The study group consisted of 175 cosmetology students of the School of Physiotherapy whose dietary habits were assessed using a validated food frequency questionnaire (FFQ) consisting of 154 food and drink items representative of the diet during the last year. We analyzed data obtained from FFQ and calculated the average number of daily and/or weekly servings from each of the 9 MDS food groups (grains, vegetables, fruits/nuts, milk/dairy products, meat/meat products, legumes, alcohol, olive oil, fish). To obtain more reliable results, we analyzed other products consumed by study participants (sweets, beverages, eggs and potatoes). Study participants were assigned 0, 1 or 2 points for each MDS ingredient. Mediterranean diet adherence was assessed on a 17-point scale. Low adherence to MD was defined at 0-7 points, moderate at 8-10 points, and high at 11-17 points.

Results. The mean MDS was 7.1 ± 2.3. Students who were assigned 11-17 points (n = 20) consumed significantly more vegetables, fruits, nuts, legumes, fish, olive oil and significantly less meat, meat products, milk, dairy, and sweets compared to study participants who were assigned the lowest number of points (n = 78). We reported significant differences in the amounts of consumed vegetables and sweets between participants who received 8-10 points (n = 77) and those who received 11-17 points. The higher MDS was significantly associated with the higher intakes of vegetables and dietary fiber.

Conclusions. The dietary patterns of study group of Polish cosmetology students did not adhere to the MD recommendations.

Key words: *dietary patterns, dietary recommendations, Mediterranean diet, university students*

STRESZCZENIE

Wprowadzenie. Dieta śródziemnomorska (DŚ) to najzdrowszy model żywienia na świecie, który charakteryzuje się korzystnym działaniem w prewencji wielu chorób dietozależnych.

Cel. Ocena zgodności diet studentek kosmetologii z zaleceniami diety śródziemnomorskiej ocenianej wskaźnikiem Mediterranean Diet Score (MDS).

Materiał i metody. Grupę badaną stanowiło 175 studentek kosmetologii z Wyższej Szkoły Fizjoterapii. Ocenę sposobu żywienia przeprowadzono za pomocą walidowanego kwestionariusza częstotliwości spożycia żywności FFQ (ang. food frequency questionnaire), który uwzględniał spożycie 154 produktów w ciągu roku poprzedzającego badanie. Na podstawie FFQ obliczono średnią dzienną i/lub tygodniową zawartość w diecie 9 grup produktów spożywczych: składowych wskaźnika MDS (produkty zbożowe, warzywa, owoce i orzechy, mleko i produkty mleczne, mięso i przetwory mięsne, nasiona roślin strączkowych, alkohol, oliwa, ryby) oraz dodatkowych produktów (słodycze, napoje, jaja i ziemniaki), które nie wchodziły w skład wskaźnika, jednak zostały uwzględnione, ponieważ występowały w dietach badanych i mogły istotnie wpłynąć na wynik badania. Zastosowano trzystopniowy system punktacji, w którym uczestnicy otrzymali 0, 1 lub 2 punkty za każdy składnik. Wynik możliwy do uzyskania na podstawie wskaźnika MDS wynosił 0-17 pkt., przy czym wyższa liczba uzyskanych punktów oznaczała większą zgodność diety z DŚ. Diety studentek, które otrzymały 0-7 punktów określono jako diety o niskiej zgodności z DŚ, 8-10 punktów – o średniej zgodności Z DŚ, a 11-17 punktów – o wysokiej zgodności z DŚ.

Wyniki. Średnia wartość MDS obliczona dla diet studentek kosmetologii wynosiła 7,1 ± 2,3. Diety 20 studentek, które uzyskały wartość wskaźnika MDS w zakresie 11-17 pkt. zawierały istotnie statystycznie więcej warzyw, owoców i orzechów, nasion roślin strączkowych, ryb, oliwy oraz istotnie statystycznie mniej mięsa i jego przetworów, mleka i jego przetworów oraz słodyczy w porównaniu do diet, które uzyskały najmniejszą liczbę punktów (n=78). Dodatkowo występowały istotne różnice między zawartością w diecie warzyw i słodyczy między badanymi, których diety otrzymały

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8-10 pkt. (n=77) w porównaniu do diet, które otrzymały 11-17 pkt. Wraz ze zwiększeniem liczby uzyskanych punktów MDS stwierdzono istotnie większą zawartość warzyw oraz błonnika pokarmowego w dietach badanych studentek.
Wnioski. Diety badanej grupy studentek kosmetologii nie były zgodne z zaleceniami diety śródziemnomorskiej ocenianej wskaźnikiem MDS.

Słowa kluczowe: *zwyczaje żywieniowe, rekomendacje żywieniowe, dieta śródziemnomorska, studenci*

INTRODUCTION

The Mediterranean diet (MD) is universally recognized as the healthiest model of nutrition whose beneficial effects help prevent many diet-related diseases, including cardiovascular disease, type-2 diabetes, cancer, and neurodegenerative disorders. The Mediterranean diet is characterized by a high intake of fruits, vegetables, grains, legumes, olive oil, nuts; moderate consumption of eggs, poultry and dairy, low intake of red meat and red wine consumed with meals [1, 13, 27]. This diet has low glycemic index and a relatively high total fat intake, but is low in saturated fatty acids. The main source of fat intake is olive oil. The diet is rich in dietary fiber, antioxidant compounds and bioactive elements with anti-inflammatory effects. Following MD recommendations may help reduce the risk for chronic diseases [18]. Moreover, MD enhances cognition and academic achievements. It has been shown that greater MD adherence was associated with better learning outcomes [1, 27].

Early adulthood is often associated with lifestyle changes, including eating habits. If students live away from homes their eating patterns often deteriorate [6]. Students' dietary patterns are generally unhealthy and involve skipping meals and consuming a lot of added sugar, saturated fatty acids and alcoholic beverages, which is very often accompanied by a lack of physical activity. Unhealthy eating habits acquired at young age often translate into health problems associated with poor-quality diet in adulthood [27].

We aimed to assess if dietary habits of Polish cosmetology students complied with MD recommendations.

MATERIAL AND METHODS

Study group and methods

The study was conducted in Wroclaw (Poland). The investigation was performer in accordance with the Declaration of Helsinki for Human Research. The research has been approved by bioethical commission of Wroclaw Medical University (No. KB – 519/2019).

The study group consisted of 175 female cosmetology students of the School of Physiotherapy (mean age: 21.3 ± 3.7 years; mean BMI: 21.3 ± 4.2 kg/m^2).

We assessed their dietary habits using a validated food frequency questionnaire (FFQ) consisting of 154 food and drink items representative of the diet during the last 12 months. An average portion size of each product was described, eg. one slice of bread, one glass of milk, one apple, etc. The respondents declared frequency of consumption of each product portion size.

We assessed the frequency of consumption of selected food products on a 9-point scale: never, less than once a month, 1-3 times a month, once a week, 2-4 times a week, 5-6 times a week, once a day, 2-3 times a day, 4-5 times a day, >6 times a day [9]. The collected data was entered into Microsoft Excel v. 2010 spreadsheet.

The declared consumption of food products was converted into grams per day, taking into account the average size of consumed portions. Then, the nutritional value of individual students' dietary patterns was calculated according to the Food and Nutrition Institute in Warsaw [16]. Based on the FFQ, we calculated the daily and/or weekly average intake 9 food products: from the MDS (grains, vegetables, fruits and nuts, milk and dairy products, meat and meat products, legumes, alcohol, olive oil, fish) and, to obtain more reliable results, additional products (sweets, drinks, eggs and potatoes).

We used an updated version of the MDS as outlined by *Stefler* et al. [23] and according to the recommendations on MD indicated by *Sofi* et al. [22]. We defined absolute cut-off values for all MDS components and applied a three-tier scoring system with 0, 1, or 2 points assigned to participants for each component. For food groups typical of the MD (vegetables, fruits and nuts, legumes, grains and fish) we assigned 2 points for the highest intake, 1 point for moderate and 0 points for the lowest intake [23]. For food groups not typical of the MD (meat, milk and dairy products) we assigned 2 points for the lowest intake, 1 point for moderate and 0 points for the highest intake [22]. The first exception was alcohol whose content was assessed on a three-point scale (0-2 points), but we assigned the highest points for the moderate intake. The second exception was olive oil whose content was assessed on a two-point scale (0-1 points). We assigned 1 point for the declared regular use of olive oil, and 0 points if study participants declared otherwise. We used a 17-point MDS with low MD adherence defined at 0-7 points, moderate at 8-10 points, and high at 11-17 points [23]. The scoring system for each food groups is presented in Table 1.

Table 1. Three-tier scoring system with 0, 1 or 2 points assigned to participants for each food groups [22, 23]

Studied parameters	Three-tier scoring system		
	0 points	1 point	2 points
Vegetables [g/day]	<100	100-250	>250
Fruits and nuts [g/day]	<150	150-300	>300
Legumes [g/week]	<70	70-140	>140
Grain products [g/day]	<130	130-195	>195
Fish [g/week]	<100	100-250	>250
Meat and meat products [g/day]	>120	80-120	<80
Milk and dairy products [g/day]	>270	180-270	<180
Alcohol [g/day]	>24	12	12-24
Olive oil	not use olive oil	regular use olive oil	-

We assessed if the average intakes of selected food products adhered to MD recommendations. In addition, we compared the average daily intake of energy and dietary fiber between the study groups and compared our results with dietary habits of Greek students in regard cardiovascular risk status [6].

Statistical analysis was performed with STATISTICA v 12.0 PL (StatSoft Inc., USA). The normality of data distribution was tested with the Lilliefors and Shapiro-Wilk tests, and the homogeneity of variance with the *Levene* test. To compare the three unmatched groups, we used the non-parametric multiple comparison *Kruskal-Wallis* test. The results were considered statistically significant when $p<0.05$.

RESULTS

The mean MDS value for the study participants was 7.1±2.3. After analyzing the collected data, we found low MD adherence in 45% of the study participants (0-7 points), moderate in 44% (8-10 points), and high in 11%. The dietary patterns of female cosmetology students (n = 20) with the highest MDS had statistically significantly higher intake of: vegetables, fruit and nuts, legumes, fish, olive oil and significantly lower intake of meat and its processed products, milk and milk products and sweets compared to diets with the lowest MDS (n = 78). We did not reported differences between grains and alcohol intake.

The dietary patterns of female cosmetology students from the group of moderate MD adherence (n = 77) were characterized by a significantly higher intake of vegetables, legumes and olive oil compared to students whose diets had low MDS (n = 78). We did not reported differences between others food group intake. In the group of female students with

Table 2. Comparison of the mean intake of selected food products, the mean daily energy value and nutrients in the diets of female students who were assigned 0-7, 8-10 and 11-17 MDS

Studied parameters	MDS			*Kruskal-Wallis* test (p)		
	0-7 points	8-10 points	11-17 points	A vs. B	B vs. C	A vs. C
	A (n=78)	B (n=77)	C (n=20)			
Food products/groups of food products						
Vegetables [g/day]	277.42±150.88	355.13±149.41	516.61±215.20	0.0003	0.0348	< 0.0001
Fruits & nuts [g/day]	180.23±114.40	275.98±112.28	361.34±115.82	NS	NS	< 0.0001
Legumes [g/week]	42.77±70.42	137.62±127.15	271.00±281.73	< 0.0001	NS	< 0.0001
Grain products [g/day]	93.53±46.35	110.30±60.56	131.07±77.56	NS	NS	NS
Fish [g/week]	48.06±34.75	76.73±71.42	108.5±86.95	NS	NS	0.0050
Meat & meat products [g/day]	141.02±100.51	113.03±95.42	72.26±85.66	NS	NS	0.0007
Milk & dairy products [g/day]	284.05±148.79	251.35±148.29	198.29±166.75	NS	NS	0.0436
Alcohol [g/day]	4.02±5.05	3.78±4.58	4.01±6.15	NS	NS	NS
Olive oil [g/day]	6.84±5.16	12.15±9.61	12.08±5.04	0.0003	NS	0.0032
Sweets [g/day]	52.17±34.27	52.80±35.53	35.65±32.51	NS	0.0261	0.0305
Beverages [g/day]	252.63±230.15	307.53±320.33	269.38±355.69	NS	NS	NS
Eggs [g/day]	23.16±29.11	22.92±23.43	20.43±16.84	NS	NS	NS
Potatoes [g/day]	66.01±40.97	67.56±47.97	55.17±40.52	NS	NS	NS
Food energy and nutrients						
Food energy [kcal]	1672.55±611.06	1815.78±676.98	1864.36±831.29	NS	NS	NS
Dietary fiber [g]	16.20 ± 6.27	22.83±7.43	30.02±9.49	< 0.0001	0.0318	< 0.0001

A - group with low MD adherence defined at 0-7 points; B - group with moderate MD adherence at 8-10 points; C - group with high MD adherence at 11-17 points;
$p < 0.05$ – statistically significant values.

moderate MD adherence (n = 77) we reported a statistically significantly lower intake of vegetables and significantly higher intake of sweets compared to dietary patterns of study participants with the highest MD adherence (n = 20). We did not reported differences between others food group intake. The average intake of cereals, alcohol, beverages, eggs and potatoes, as well as the energy value did not differ significantly between the studied groups. The above data are presented in Table 2.

DISCUSSION

We reported that the majority of the studied dietary patterns had low or moderate MD adherence. Only 20.0% were assigned the highest number of points. In a study assessing eating habits of the students from the Medical University of Lodz, only 1.2% of women and 0.7% of men reported MD adherence. More than 80.0% of the study participants did not follow any special diet [17]. Similar results were obtained by *Zych* et al. [28] who assessed MD adherence among students from the Medical University of Warsaw. The intake of specific food products in the study group significantly differed from the traditional and modern MD eating principles.

A similar association was observed in a study assessing MD adherence among Greek students in which the majority (73.5%) of study participants had low MD adherence. The authors concluded that a significant proportion of students had abandoned traditional MD in favor of more modern diets [24].

The lower MD adherence has also been reported among youth from other countries of the Mediterranean basin. As shown in the literature, there is a clear trend towards the abandonment of MD recommendations [10], a particularly noticeable behavior among youth, including Poland.

Fruits and vegetables are high in antioxidants, dietary fiber and other bioactive compounds known to possess benefits to human health. In addition, regular fruits and vegetables consumption helps control body weight and prevents obesity.

Whole-grain products contain a significant amount of phytic acid, resistant starch and dietary fiber [20]. They also provide high levels of folic acid, zinc and iron as compared to non-whole grain products [7]. Regular consumption of vegetables, fruits and whole grain products is a practical and effective strategy for reducing the incidence of chronic diseases such as cardiovascular disease, including stroke, certain types of cancer, and type-2 diabetes [19]. Nuts have high nutritional value, because they are a rich source of magnesium, vitamin E, B1, phosphorus, iron, dietary fiber, protein, as well as mono- and polyunsaturated fatty acids. Nuts consumption is an important factor reducing the risk of lifestyle-related diseases [15].

In our study, the intake of vegetables according to MD recommendations differed significantly between the studied groups. However, the intake of fruits and nuts differed significantly only in the groups with low and high MD adherence. Only 11.0% of all study participants followed MD recommendations for daily intake of vegetables and fruits. Similarly, in a study conducted among students from Cyprus the authors reported low intake of vegetables and fruits among the study participants. Only about 30.0% of the studied young adults consumed more than one serving of fruits and vegetables per day [11].

The results of the meta-analysis carried out by *Antonopoulou* et al. [1] reported an insufficient intake of vegetables, fruits and nuts, as well as grain products among Spanish students. The intake of bread in the diets of female students from Warsaw was significantly lower compared to the diets of Greeks in 1978, but similar to the intake of bread in the diets of modern Greeks [28]. The intake of grain products in the diets of cosmetology students was comparable in each group, regardless of the number of points obtained.

Legumes contain high amounts of dietary fiber, protein, many vitamins and minerals, and have a low glycemic index. Consumption of legumes reduces the risk of type-2 diabetes, cardiovascular disease and obesity [26]. We reported significant differences in the intake of legumes between the groups with the lowest and moderate-to-high MD adherence (42.7 ± 70.4 vs. 137.6 ± 127.1 and 271.0 ± 281.7 g/week). However, university students in Cyprus followed MD recommendations for legumes. Almost 50.0% of the study participants consumed more than one serving of legumes per week [11].

Milk and dairy products provide many nutrients to the diet, including complete protein, calcium, phosphorus, magnesium, potassium, zinc, selenium, vitamins A, B2 and B12 [5]. Consumption of fermented dairy products reduces the risk of obesity and type-2 diabetes. The health-promoting effects of dairy products consumption are related to their high content of calcium, some vitamins and protein [20]. The intake of dairy products in the majority of university students in Cyprus complied with MD dietary recommendations week [11].

Our study participants had high intake of dairy, which was inconsistent with MD recommendations. Only the group high MD adherence consumed the recommended servings of dairy products (198.2 ± 166.7 g/day). Authors of the meta-analysis assessing MD adherence in different populations of university students had the same conclusions. They found that the intake of milk and its products in the diets of Spanish students was higher than recommended. In addition, the intake of meat was also higher than recommended and comparable to the results of our study [1]. We

reported significantly higher intake of meat and meat products in the group with low MD adherence compared to the group with high MD adherence (141.0 ± 100.5 vs. 72.2 ± 85.6 g/day). A similar tendency was observed among students from Warsaw whose diets had a significantly higher intake of meat and cheese compared to the Greek population in 1978, which was comparable to the diets of contemporary Greeks living in cities [28]. High consumption of meat, including red and processed meat, is associated with a higher risk of metabolic syndrome and other chronic diseases, including type-2 diabetes. However, the consumption of poultry was inversely correlated with the risk of metabolic syndrome, hypertriglyceridemia and elevated blood pressure. Therefore, health impact of meat consumption depends on its type [14]. According to the MD recommendations, the overall intake of meat, particularly red and processed, should be low [3].

Oily sea fish such as herring, mackerel and salmon are good sources of omega-3 fatty acids and vitamin D. They are rich in minerals and complete protein. The dietary intake of omega-3 polyunsaturated fatty acids (n-3 PUFAs) in fatty sea fish may significantly reduce the risk of cardiovascular disease and have possible anti-inflammatory effects [4, 12]. In our study, students with the highest MD adherence consumed more than twice as many fish as students from with the lowest MD adherence (108.5 ± 86.9 vs. 48.0 ± 34.7 g/week). However, most of the studied participants did not meet the recommended weekly fish intake. In the group of university students from Cyprus, only 28.0% met the weekly intake recommendations for fish, which is at least 2-3 times a week [11].

Monounsaturated fatty acids (MUFAs) in olive oil have many health benefits, e.g., they reduce the risk of type-2 diabetes. In addition, adding olive oil to meals may prevent weight gain by lowering postprandial glucose levels [2]. In our study, the use of olive oil was declared by a significantly greater number of students with moderate and high MD adherence (12.1 ± 9.6 and 12.0 ± 5.0 g/day) compared to students with low MD adherence (6.8 ± 5.1 g / day). The majority of university students from Cyprus consumed high amounts of olive oil [11]. Among Spanish students a significantly higher olive oil intake was recorded in the group with higher MD adherence compared to the group with low MD adherence [8]. However, this association was not confirmed in all groups of Spanish students [1].

About 30.0% of university students from Cyprus consumed sweets several times a day [11], which is in line with similar findings from the studies assessing dietary patterns of Spanish students [1] and American adolescents [21]. In our study, the intake of sweets was significantly higher in the group with low and moderate MD adherence compared to the group with high MD adherence (52.1 ± 34.2; 52.8 ± 35.5 vs. 35.6 ± 32.5 g/day). Sweets and snacks are inversely associated with diet quality. In our study, higher sweets intake was directly associated with lower MD adherence.

When it comes to alcohol, its intake was low and did not differ significantly between the study groups. Similar results were observed in a group of Spanish students. However, the alcohol consumption was higher in the group of students living away from home [1]. It has been reported that moderate alcohol consumption may protect against some chronic diseases [6]. To obtain more reliable study results we assessed dietary intakes of food groups not typical of the traditional MD, such as sweets, drinks, eggs and potatoes. Their high reported intakes may have adversely affected the study results. Some authors included potatoes in the average daily intake of grains [13], and did not assess intakes of additional products, whereas other researchers assessed the intake of additional food products [25].

The dietary patterns of studied Polish cosmetology students differed significantly in the intake of dietary fiber depending on the group. The intake of dietary fiber in the group with the highest MD adherence was 30.0 ± 9.4 g/day, and in the group with the lowest MD adherence only 16.2 ± 6.2 g/day. Insufficient dietary fiber intake was also reported in a study conducted among Greek students - almost 70.0% of men and over 50.0% of women failed to meet the recommended intake of dietary fiber [6], which resulted from the insufficient intake of whole-grain products, vegetables and fruits. Dietary fiber lowers the risk of cardiovascular diseases [6]. In our study, the energy intake of the studied diets was comparable to the results obtained by *Chourdakis* et al. [6] and did not differ significantly between the groups.

CONCLUSION

Dietary patterns of studied cosmetology students of the School of Physiotherapy in Poland did not adhere to the Mediterranean diet recommendations. Changes to these unhealthy eating habits would help improve quality of students' lives today and in the future.

Conflict of interest

The Authors declare no conflict of interest.

REFERENCES

1. *Antonopoulou, M., Mantzorou, M., Serdari, A., Bonotis, K., Vasios, G., Pavlidou, E., Trifonos, C., Vadikolias, K., Petridis, D., Giaginis, C.* Evaluating Mediterranean diet adherence in university student populations: Does this dietary pattern affect students' academic performance and mental health? International Journal of Health

Planning and Management, 2020;35:5–21 doi:10.1002/hpm.2881
2. *Assaf-Balut, C., de la Torre, N.G., DuraÂn, A., Fuentes, M., BordiuÂ, E., del Valle, L., Familiar, C., OrtolaÂ, A., JimeÂnez, I., Herraiz, M.A., Izquierdo, N., Perez, N., Torrejon, M.J., Ortega, M.I., Illana, F.I., Runkle, I., de Miguel, M.P., Montañez, C., Barabash A., Cuesta, M., Rubio, M.A., Calle-Pascual, A.L.* A Mediterranean diet with additional extra virgin olive oil and pistachios reduces the incidence of gestational diabetes mellitus (GDM): A randomized controlled trial: The St. Carlos GDM prevention study. PLoS ONE. 2017;12(10): e0185873 doi.org/10.1371/journal.pone.0185873
3. *Babio, N., Sorli, M., Bulló, M., Basora, J., Ibarrola-Jurado, N., Fernández-Ballart, J., Martínez-González, M.A., Serra-Majem, L., González-Pérez, R., Salas-Salvadó, J.* Association between red meat consumption and metabolic syndrome in a Mediterranean population at high cardiovascular risk: cross-sectional and 1-year follow-up assessment. Nutrition, Metabolism & Cardiovascular Diseases, 2012; 22(3): 200-207 doi:10.1016/j.numecd.2010.06.011
4. *Binia, A., Vargas-Martínez, C., Ancira-Morenoc, M. Gosoniu, L.M., Montoliu, I.,Gámez-Valdez, E., Soria-Contreras, D.C., Angeles-Quezada, A., Gonzalez-Alberto, R., Fernández, S., Martínez-Conde, D., Hernández-Morán, B., Ramírez-Solano, M., Pérez-Ortega, C., Rodríguez-Carmona, Y., Castan, I, Rubio-Aliaga, I., Vadillo-Ortega, F., Márquez-Velasco, R., Bojalil, R., López-Alvarenga, J.C., Valet, P., Kussmann, M., Silva-Zolezzi, I., Tejero, M.E.* Improvement of cardiometabolic markers after fish oil intervention in young Mexican adults and the role of PPARα L162V and PPARγ2 P12A. Journal of Nutritional Biochemistry, 2017;43:98–106 doi.org/10.1016/j.jnutbio.2017.02.002
5. *Cavero-Redondo, I., Alvarez-Bueno, C., Sotos-Prieto, M., Gil, A., Martinez-Vizcaino, V., Ruiz, J.R.* Milk and Dairy Product Consumption and Risk of Mortality: An Overview of Systematic Reviews and Meta-Analyses. Advances in Nutrition, 2019;10:97–104 doi.org/10.1093/advances/nmy128.
6. *Chourdakis, M., Tzellos, T., Pourzitaki, C., Toulis ,K.A., Papazisis, G., Kouvelas, D.* Evaluation of dietary habits and assessment of cardiovascular disease risk factors among Greek university students. Appetite, 2011;57:377–383 doi:10.1016/j.appet.2011.05.314
7. *Christensen, C.B., Mikkelsen, B.E., Toft, U.* The effect of introducing a free breakfast club on eating habits among students at vocational schools. BMC Public Health. 2019;19:369 doi.org/10.1186/s12889-019-6701-9
8. *Cobo-Cuenca, A.I., Garrido-Miguel, M., Soriano-Cano, A., Ferri-Morales, A., Martínez-Vizcaíno, V., Martín-Espinosa, N.M.* Adherence to the Mediterranean Diet and Its Association with Body Composition and Physical Fitness in Spanish University Students. Nutrients, 2019;11:2830 doi:10.3390/nu11112830
9. *Dehghan, M., Ilow, R., Zatońska, K., Szuba, A., Zhang, X., Mente, A., Regulska-Ilow, B.* Development, reproducibility and validity of the food frequency questionnaire in the Poland arm of the Prospective Urban and Rural Epidemiological (PURE) study. Journal of Human Nutrition and Dietetics, 2012;25: 225-232 doi: 10.1111/j.1365-277X.2012.01240.x.
10. *García Cabrera, S., Herrera Fernández, N., Rodríguez Hernández, C., Nissensohn, M., Román-Viñas, B., Serra-Majem y, L.* KIDMED test; prevalence of low adherence to the Mediterranean Diet in children and young; a systematic review. Nutrición Hospitalaria, 2015;32(6): 2390-2399 DOI:10.3305/nh.2015.32.6.9828
11. *Hadjimbei, E., Botsaris, G., Gekas, V., Panayiotou, A.G.* Adherence to the Mediterranean Diet and Lifestyle Characteristics of University Students in Cyprus: A Cross-Sectional Survey. Journal of Nutrition and Metabolism. 2016; Article ID: 2742841 doi.org/10.1155/2016/2742841
12. *Handeland, K., Skotheim, S., Baste, V., Graff I.E., Frøyland, L., Lie, Ø., Kjellevold, M., Markhus, M.W., Stormark, K.M., Øyen, J., Dahl, L.* The effects of fatty fish intake on adolescents' nutritional status and associations with attention performance: results from the FINS-TEENS randomized controlled trial. Nutrition Journal, 2018;17, 30 DOI 10.1186/s12937-018-0328-z
13. *Iaccarino Idelson, P., Scalfi, L., Valerio, G.:* Adherence to the Mediterranean Diet in children and adolescents: A systematic review. Nutrition, Metabolism & Cardiovascular Diseases, 2017;27: 283-299 doi.org/10.1016/j.numecd.2017.01.002
14. *Kim, Y., Je, Y.*: Meat Consumption and Risk of Metabolic Syndrome: Results from the Korean Population and a Meta-Analysis of Observational Studies. Nutrients, 2018;10:390 doi:10.3390/nu10040390
15. *Kulik, K., Waszkiewicz-Robak, B., Biller, E.:* Deklarowana a oznaczona analitycznie zawartość składników odżywczych w różnych rodzajach orzechów [Labelled and analytically determined content of nutrients in various nut types]. Postępy techniki przetwórstwa spożywczego, 2019;2:49-56. (in Polish)
16. *Kunachowicz H., Przygoda B., Nadolna I., Iwanow K.:* Tabele składu i wartości odżywczej. [Food Composition Tables]. Warsaw, PZWL, 2020. (in Polish)
17. *Łaszek M., Nowacka E., Gawron-Skarbek A., Szatko, F.:* Negatywne wzorce zachowań zdrowotnych studentów. Część II. Aktywność ruchowa i nawyki żywieniowe [Negative behavior patterns of students. Part II. Physical activity and eating habits]. Problemy Higieny i Epidemiologii, 2011;92(3):461-465. (in Polish)
18. *Martínez-González, M.A., Hershey, M.S., Zazpe, I., Trichopoulou, A.* Transferability of the Mediterranean Diet to Non-Mediterranean Countries. What Is and What Is Not the Mediterranean Diet. Nutrients, 2017;9:1226 doi:10.3390/nu9111226
19. *Roager, H.M., Vogt, J.K., Kristensen, M., Hansen, L.B.S., Ibrügger, S., Mærkedahl, R.B., Bahl, M.I., Lind, M.V., Nielsen, R.L., Frøkiær, H., Gøbel, R.J., Landberg, R., Ross, A.B., Brix, S., Holck, J., Meyer, A.S., Sparholt, M.H., Christensen, A.F., Carvalho, V., Hartmann, B., Holst, J.J., Rumessen, J.J. Linneberg, A., Sicheritz-Pontén, T., Dalgaard, M.D., Blennow, A., Frandsen, H.L., Villas-Bôas, S., Kristiansen, K., Vestergaard, H., Hansen, T., Ekstrøm, C.T., Ritz, C.,*

Nielsen, H.B., Pedersen, O.B., Gupta, R., Lauritzen, L., Licht, T.R. Whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome: a randomised cross-over trial. Gut, 2019;68: 83–93 doi:10.1136/gutjnl-2017-314786

20. *Schwingshackl, L., Schwedhelm, C., Galbete, C., Hoffmann, G.* Adherence to Mediterranean Diet and Risk of Cancer: An Updated Systematic Review and Meta-Analysis. Nutrients, 2017;9:1063 doi:10.3390/nu9101063
21. *Slining, M.M., Popkin, B.M.* Trends in intakes and sources of solid fats and added sugars among US children and adolescents: 1994-2010. Pediatric Obesity, 2013;8(4): 307–324 doi:10.1111/j.2047-6310.2013.00156.x.
22. *Sofi, F., Macchi, C., Abbate, R., Gensini, G., F., Casini, A.* Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score, Public Health Nutrition, 2013;17(12):2769–2782 doi:10.1017/S1368980013003169
23. *Stefler, D., Malyutina, S., Kubinova, R., Pająk, A., Peasey, A., Pikhart, H., Brunner, E., J., Bobak, M.* Mediterranean diet score and total and cardiovascular mortality in Eastern Europe: the HAPIEE study, European Journal of Nutrition, 2017;56(1):421–429 doi: 10.1007/s00394-015-1092-x
24. *Theodoridis, X., Grammatikopoulou ,M.G., Gkiouras, K., Papadopoulou, S.E., Agorastou, T., Gkika, I., Maraki, M.I., Dardavessis, T., Chourdakis, M.* Food insecurity and Mediterranean diet adherence among Greek university students. Nutrition, Metabolism & Cardiovascular Diseases, 2018;28: 477-485 doi.org/10.1016/j.numecd.2018.02.007
25. *Trichopoulou, A., Orfanos, P., Norat, T., Bueno-de-Mesquita, B., Ocké, M., Peeters, P.H., van der Schouw, Y.T., Boeing, H., Hoffmann, K., Boffetta, P., Nagel, G., Masala, G., Krogh, V., Panico, S., Tumino, R., Vineis, P., Bamia, C., Naska, A., Benetou, V., Ferrari, P., Slimani, N., Pera, G., Martinez-Garcia, C., Navarro, C., Rodriguez-Barranco, M., Dorronsoro, M., Spencer ,E., Key, T., Bingham, S., Khaw, K-T., Kesse, E., Clavel-Chapelon, F., Boutron-Ruault, M-C., Berglund, G., Wirfalt, E., Hallmans, G., Johansson, I., Tjonneland, A., Olsen, A., Overvad, K., Hundborg, H., Riboli, E., Trichopoulos, D.* Modified Mediterranean diet and survival: EPIC-elderly prospective cohort study. BMJ. 2005 doi:10.1136/bmj.38415.644155.8F
26. *Viguiliouk, E., Glenn, A.J.,Nishi, S.K., Chiavaroli, L.,Seider, M.,Khan, T.,Bonaccio, M., Iacoviello, L., Mejia, S.B., Jenkins, D.J.A., Kendall, C.W.C., Kahleov, H., Rahelic, D., Salas-Salvad, J., Sievenpiper, J.L.L.* Associations between Dietary Pulses Alone or with Other Legumes and Cardiometabolic Disease Outcomes: An Umbrella Review and Updated Systematic Review and Meta-analysis of Prospective Cohort Studies. Advances in Nutrition, 2019;10:308–319 doi.org/10.1093/advances/nmz113
27. *Zurita-Ortega, F., San Román-Mata, S., Chacón-Cuberos, R., Castro-Sánchez, M., Muros, J.J.* Adherence to the Mediterranean Diet Is Associated with Physical Activity, Self-Concept and Sociodemographic Factors in University Student. Nutrients, 2018;10(8):966 doi:10.3390/nu10080966
28. *Zych P., Szostak-Węgierek D.:* Ocena zgodności modelu żywienia studentów Warszawskiego Uniwersytetu Medycznego z zasadami diety śródziemnomorskiej [Evaluation of nutritional model compatibility with the principles of the Mediterranean diet in students of the Warsaw Medical University]. Nowa Medycyna, 2013;3:107-110. (in Polish)

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ORIGINAL ARTICLE

EVALUATION OF PRENATAL VITAMIN-MINERAL PREPARATIONS IN THE CONTEXT OF RECOMMENDED DIETARY SUPPLEMENTATION. ARE PREGNANT WOMEN SUPPLIED WITH WHAT THEY SHOULD GET?

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ABSTRACT

Background. The composition of preparations, intended for pregnant women is an important issue of proper dietary supplementation. The range of such products on the market is very wide and their composition is not regulated by law.

Objectives. To evaluate the composition of preparations for pregnant women in the context of 2014 Polish Gynecological Society (PTG) recommendation and the 2020 recommendation of the Polish Society of Gynecologists and Obstetricians (PTGiP).

Materials and methods. A range of preparations was collected in pharmacies and e-pharmacies in 2019. The nutrient content was determined based on the information on the unit packaging or the pharmacy's website. The content of folic acid, vitamin D, iodine, DHA, and iron was assessed.

Results. There were 33 vitamin-mineral preparations (VMPs) on offer on the analyzed market. All preparations contained folic acid, of which 55% contained precisely the dose recommended by the PTG, and 45% of the preparations is compliant in this respect with the recommendation of the PTGiP. Ninety seven percent of VMPs contained vitamin D. Fifty percent of them did not contain this vitamin at the dose recommended by the PTG, and half of the preparations do not meet the PTGiP recommendation either. Ninety seven percent of VMPs contained iodine. Out of them 44% contained a too low dose of iodine, by the PTG standards, but only 9% of preparations do not meet the PTGiP recommendation in this respect. DHA was a component contained in 73% of VMPs. Among them, 33% contained the dose recommended by the PTG for women who eat little fish and 88% of the preparations are in line with the new recommendation PTGiP. Eighty two percent of preparations contained iron, which in the light of the PTGiP recommendation is debatable.

Conclusions. The composition of many VMPs did not reflect experts' recommendations regarding the type and amounts of particular nutrients.

Key words: *dietary supplements, vitamins, minerals, pregnancy, dietary recommendations*

STRESZCZENIE

Wprowadzenie. Skład preparatów, przeznaczonych dla kobiet ciężarnych jest ważną kwestią prawidłowej suplementacji diety. Rynek takich produktów jest bardzo szeroki, a ich skład nie jest regulowany prawnie.

Cel. Ocena preparatów dla kobiet w ciąży, w kontekście rekomendacji Polskiego Towarzystwa Ginekologicznego (PTG) z 2014 r. oraz najnowszych zaleceń Polskiego Towarzystwa Ginekologów i Położników (PTGiP) z 2020 r., dotyczących stosowania witamin i składników mineralnych w czasie ciąży.

Materiał i metody. Asortyment preparatów został zebrany w aptekach, w czterech dzielnicach Warszawy oraz metodą online w sześciu e-aptekach. Zawartość składników odżywczych ustalono na podstawie składu preparatów, zamieszczonego na opakowaniu jednostkowym lub podanego na stronie internetowej apteki. Pod uwagę wzięto składniki, które w diecie kobiet ciężarnych należy uzupełniać tj. kwas foliowy, witaminę D, jod, DHA, a dodatkowo oceniono także zawartość żelaza.

Wyniki. W obrocie handlowym znajdowały się 33 asortymenty preparatów witaminowo-mineralnych. Wszystkie preparaty zawierały kwas foliowy, z czego 55% zawierało dawkę taką, jak zalecało PTG, a 45% preparatów jest zgodne z zaleceniami PTGiP. 97% preparatów zawierało witaminę D, ale 50% z nich dostarczało zbyt małej dawki tego składnika, w stosunku do obu rekomendacji. 97% preparatów zawierało jod, z czego 44% nie dostarczało dawki zalecanej przez PTG, ale tylko 9% nie spełnia w tym zakresie zaleceń PTGiP. DHA zawarty był w 73% preparatów witaminowo-mineralnych. 33% tych preparatów zawierało w dawce dziennej ilość DHA rekomendowaną przez PTG, a 88% zawierało minimalną dawkę ustaloną przez PTGiP. Powszechnym składnikiem preparatów było żelazo (82% preparatów), które w świetle zaleceń PTG wymaga suplementacji tylko u kobiet z ryzykiem niedokrwistości, a w świetle zaleceń PTGiP tylko w przypadkach niedoboru udokumentowanego wynikami badań.

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Wnioski. Skład wielu preparatów witaminowo-mineralnych nie odzwierciedla rekomendacji ekspertów, odnośnie suplementacji poszczególnych składników odżywczych w okresie ciąży.

Słowa kluczowe: *suplementy diety, witaminy, składniki mineralne, ciąża, zalecenia żywieniowe*

INTRODUCTION

During pregnancy, the need for many nutrients increases. For most vitamins, the increased demand, compared to women who are not pregnant, ranges from 6% to 50%, and for minerals from 9% to 50%. This applies to folic acid, iron, and zinc to the greatest extent and much less so to vitamin B12, choline, and selenium. It is worth emphasizing that according to the most recent nutritional standards for the Polish population (2020), there has been no increase in the need for calcium, phosphorus, potassium, vitamin D, vitamin K, and biotin in pregnant women. Other ingredients that are particularly important during pregnancy include docosahexaenoic acid (DHA): *omega*-3 fatty acid, which should be daily taken in the additional amount of 100-200 mg during pregnancy [20].

Research shows that the dietary intake of some nutrients is highly deficient. In pregnant Polish women, the amount of folic acid covers only 27-55% of the demand, for iodine, it is 38-65%, for iron 34-48%, for calcium 60-84%, and in the case of vitamin D, whose deficiency affects the entire population, the diet covers 12-25% of the nutritional requirement of expectant mothers [3, 15, 19, 34, 40]. At the same time, some studies indicate that some vitamins and minerals are consumed in excessive amounts. The intake of vitamin A reaches 156% of the nutritional standard, of vitamin E 140% [6, 40], of vitamin B12 146% [16, 40], and even higher is the intake of phosphorus (166-235%) [3, 16, 40] and manganese (250-300%) [16, 40]. The situation is less clear when it comes to other nutrients. Some studies reveal deficiencies of magnesium (71-87% of the norm) and zinc (77-89% of the norm) [3, 15, 16]; others indicate that their intake covers the demand [40]. Evident deficiencies in the diet of pregnant women appear in DHA, the intake of which, in the light of a few existing studies in Poland, amounts to 60-280 mg daily [4, 12, 35], with recommendations standing at 350-450 mg [20].

Due to the high requirement for individual nutrients, their particular importance for the course of pregnancy, and their limited dietary intake, it is recommended that they be taken in the form of preparations. According to the most recent 2020 recommendation issued by the Polish Society of Gynecologists and Obstetricians (PTGiP), dietary supplementation in pregnant women should include four nutrients - folic acid, vitamin D, DHA and, in pregnant women with no history of thyroid disease, also iodine. Iron, which is widely regarded as a critical nutrient during pregnancy due to new scientific reports on the adverse effects of its excessive intake, should only be taken if hemoglobin or ferritin levels are reduced [42]. The same nutrients were listed in the recommendations of Polish Gynecological Society (PTG) back in 2014, albeit their position was more flexible for iron and admitted its legitimate application, provided that the patient was featuring the risk of developing anemia [43]. Both cited positions specify a daily dose of folic acid, vitamin D, iodine, DHA, and iron to be possibly taken, and as regards other components, the experts agreed that routine supplementation for all pregnant women was not advisable.

Research to-date indicates that dietary supplementation with vitamins and minerals is commonly used in Poland and concerns 79-98% of pregnant women [9, 13, 14, 9, 36, 37]. The same percentage appears in other countries: the US, Canada, and Australia (78%-98% of women) [5]. Most women (74-77%) use VMPs during that period [11, 23, 36]. The market of dietary supplements is currently vast, and their composition is not regulated by law, therefore significant differences may appear.

OBJECTIVES

The aim of the study was to assess the composition of preparations intended for pregnant women as to the type of nutrients contained and their respective doses, in terms of the PTG recommendation [43] and the most recent PTGiP recommendation [42]. The article also discusses the issue of maximum doses of vitamins and minerals in the daily dose of supplements proposed in 2019 in Poland by the Panel on Dietary Supplements (an advisory body to the Chief Sanitary Inspector) [30, 31, 32, 33]. Besides, the study sought to address the discrepancies between the information provided on the labels of dietary supplements, indicating to what extent they cover nutritional requirements and scientifically established nutrition standards.

MATERIAL AND METHODS

An assortment of preparations addressed to pregnant women, intended both for the entire period of pregnancy and for individual trimesters, was collected in pharmacies in four districts of Warsaw (Centrum, Mokotów, Ursynów, and Gocław) between April and May 2019, and sourced online in six pharmacies (e-zikoapteka.pl, doz.pl, cefarm-24.pl, apteline.pl,

i-apteka.pl, e-melissa.pl) between June and November 2019. The analysis did not include preparations used for pregnancy planning and during breastfeeding. The content of vitamins, minerals, and other nutrients in a daily dose of preparation was determined based on the unit packaging or the pharmacy's website. For the doses of 1-2 tablets/capsules a day, the maximum dose was used. The average nutrient content in the analyzed preparations was calculated, considering only the preparations containing specific nutrients.

The doses of components requiring supplementation in all pregnant women, i.e., folic acid, vitamin D, iodine, DHA, and additionally iron, were juxtaposed with the recommendations of the PTG in 2014, and of the PTGiP in 2020 [43, 42].

RESULTS

Characteristics of the preparations

In total, the study covered 33 vitamin-mineral preparations (VMPs) available on the Polish market, six preparations presented as *omega*-3 fatty acids (DHA), four single folic acid preparations, and one preparation containing iron combined with vitamin C. Of the 33 VMPs, only one had the status of an over-the-counter drug (OTC), and the remaining preparations were dietary supplements.

The nutrient that was contained in all VMPs was folic acid. The next most commonly available ingredients were vitamin D and iodine, present in 32 preparations (97%), followed by iron, present in 27 VMPs (82%) (Table 1 and Table 2). Among vitamins, vitamin K was the least frequently used, contained in four preparations (12%), and in the case of minerals, phosphorus (1 preparation – 3%) and molybdenum and chromium (3 preparations – 9%).

In the composition of 24 VMPs (73%) additionally DHA was present, and the composition of 12 (36%) was featuring EPA (Table 2). Apart from vitamins, minerals, and *omega*-3 fatty acids, only few preparations contained also plant ingredients: ginger extract (one preparation), chamomile extract (one preparation); amino acids: arginine and taurine (one preparation), glycine and aspartic acid (one preparation) and soy protein, which is a source of many amino acids (one preparation). In addition, inositol was present in one preparation.

Composition of VMPs vs. recommended dietary supplementation

Folic acid

All commercially available VMPs contained between 400 and 800 μg of folic acid, with an average daily dose of 545.5 μg. Most of them contained the exact dose recommended by PTG, i.e., 400 μg of folic acid (18 preparations – 55%), and the second most numerous group were preparations containing 800 μg (9 preparations – 27%) (Table 1). Single folic acid preparations for pregnant women contained on average 450 μg of this nutrient (ranging between 200 – 800 μg) (Table 3).

Thirty nine percent of VMPs and 50% of single folic acid preparations contained calcium L-methylfolate or (to a lesser extent) glucosamine salt of (6S)-5-methyltetrahydrofolic acid: the forms of the vitamin which are directly active in human body.

Vitamin D

The average content of vitamin D in preparations containing this vitamin (32 preparations – 97%) was 30.4 μg (1216 IU). Only 16 VMPs (50%) contained 50 μg (2000 IU) of vitamin D. At the same time as many as six preparations (19%) contained only 5 μg (200 IU) in a daily dose, and another five preparations (16%) only 10 μg (400 IU) of this vitamin (Table 1).

Iodine

Among preparations containing iodine in their composition (32 preparations – 97%) the average amount of iodine was 175.3 μg. Most of them (17 preparations, i.e. 53%) contained 200 μg of iodine, whereas three preparations (9%) had a dose lower than 150 μg (75 – 140 μg) (Table 2).

Iron

In the group of 27 iron-containing VMPs (82%), the average dose was 28.5 mg, which is close to the requirement for pregnancy (27 mg). The largest number of preparations (17 preparations – 63%) supplied 26-30 mg of this element, while three preparations (11%) contained a large amount of iron – 60 mg of iron in a daily dose (Table 2). A single iron preparation addressed to pregnant women stood out as supplying 32 mg of iron.

DHA

In the preparations, which next to vitamins and minerals also contained DHA (24 preparations – 73%), the latter's dose ranged from 50 to 800 mg, 343 mg on average. Among them, eight preparations (33%) contained at least 600 mg DHA, and 21 preparations (88%) contained at least 200 mg DHA (Table 2). In preparations being the only source of *omega*-3 fatty acids, the daily dose of DHA was higher (on average 441 mg) and ranged from 250 to 600 mg (Table 4).

DISCUSSION

In recent years, hand in hand with the overall market growth for dietary supplements, there has also been an increase in preparations on offer for pregnant women. In 2014, Warsaw pharmacies had 20 VMPs

Table 1. Vitamins contained in vitamin-mineral preparations (quantities in a daily dosage)

Name of the preparation	A (µg)	D (µg)	E (mg)	K (µg)	Thiamin (B1) (mg)	Riboflavin (B2) (mg)	Niacin (mg)	Pantothenic acid (mg)	B6 (mg)	B12 (µg)	Biotin (µg)	Choline (mg)	C (mg)	Folic acid (µg)
Acti vita-miner Prenatal + DHA	500	10	10	0	1.66	2	17.8	10.3	2.2	1.2	150	0	100	400
Centrum Femina DHA	500	12.5	24	0	1.4	1.7	22	6	1.9	2.6	35	0	100	400
Composita Mama DHA	0	50	0	0	0	0	0	0	2.6	4	0	125	0	800
DuphaVit Pregna	0	50	0	0	0	0	0	0	0	0	0	0	0	800
Elevit Pronatal	1081	12.5	15	0	1.55	1.8	19	10	2.6	4	200	0	100	800
Falvit mama	501	5	12	0	1.4	1.6	16	6	1.9	3	0	0	120	400
Femibion Natal 1	0	20	13	0	1.2	1.6	15	6	1.9	3.5	60	0	110	800
Femibion Natal 2 Plus	500	10	24	0	1.5	1.6	20	10	2.2	2.7	100	0	180	600
Feminovit + DHA	0	50	15	0	1.4	1,5	18	6	1.8	2.6	50	0	80	400
Fertil Care	333	10	12	70	5	2	20	6	10	6	150	0	80	400
Folik Mama 1	0	0	0	0	0	0	0	0	0	0	0	0	0	400
Folik Mama 2 /3	0	20	0	0	0	0	0	0	0	0	0	0	0	400
LedeeVit	0	10	10	0	1.4	1.6	18	6	2	2.6	50	0	80	400
LadeeVit Optima	0	50	0	0	0	0	0	0	0	0	0	0	0	400
Mama activ Doppelherz	334	5	13	0	1.2	1.5	15	6	1.9	3.5	60	0	110	600
Mama dha	0	25	0	0	0	0	0	0	0	0	0	0	0	400
Mama DHA Premium +	0	50	0	0	0	0	0	0	0	0	0	0	0	400
Mama Premium activ Doppelherz	0	50	0	0	0	0	0	0	0.7	1.25	0	0	0	400

Omegamed optima DHA z alg	0	50	0	0	0	0	0	0	0	0	0	0	0	800
Omegamed optima Forte DHA z alg	0	50	0	0	0	0	0	0	0	0	0	0	0	800
Pharmaton Matruelle	333	5	15	0	1.4	1.4	18	0	1.9	2.6	30	0	85	600
Pregna Plus	0	50	0	0	0	0	0	0	0	0	0	0	0	600
Pregnal Evital	0	5	12	0	0	0	0	0	1.9	2.6	0	0	0	400
Prenatal classic	0	50	0	0	0	0	0	0	2.6	4	0	125	0	800
Prenatal duo	0	50	11.7	0	0	0	0	0	2.6	4	0	125	0	800
Prenatal uno	0	50	0	0	0	0	0	0	1.25	1	0	125	0	800
Solgar Witaminy i Minerały Prenatalne	300	5	10	0	0.85	1	10	5	1.25	4	150	2	50	400
Symvital Mama	0	50	0	0	0	0	0	0	0	0	0	0	0	400
TRIMiD 1	0	50	0	11.25	0	0	0	0	2.24	0	0	0	0	600
TRIMiD 2	0	50	0	11.25	0.21	0.21	0	0	2.24	0.375	0	0	0	600
TRIMiD 3	0	50	0	11.25	0	0	0	0	0	0	0	0	0	400
Vita-min plus Mama	180	5	12	0	1.1	1.4	16	6	1.4	2.5	50	0	80	400
Vita-miner Prenatal	500	10	10	0	1.66	2	17.8	10.3	2.2	1.2	150	0	100	400
Average vitamin content (min.; max.) in the preparations containing particular vitamins														
	460.2 (180; 1081)	**30.4** (5.0; 50.0)	**13.7** (10.0; 24.0)	**25.9** (11.25; 70.0)	**1.5** (0.21; 5.0)	**1.5** (0.21; 2.0)	**17.3** (10.0; 22.0)	**7.2** (5.0; 10.3)	**2.3** (0.7; 10.0)	**2.8** (0.375; 6.0)	**95** (30.0; 200.0)	**100.4** (2.0; 125.0)	**101.1** (50.0; 180.0)	**545.5** (400.0; 800.0)
Daily requirement of pregnant women for vitamins [1]														
	770	15	10	55	1.4	1.4	18	6.0	1.9	2.6	30	450	85	600

Table 2. Minerals, DHA and EPA contained in vitamin-mineral preparations (quantities in a daily dosage)

Name of the preparation	Iron (mg)	Calcium (mg)	Magnesium (mg)	Iodine (µg)	Zinc (mg)	Manganese (mg)	Molybdenum (µg)	Copper (mg)	Phosphorus (mg)	Selenium (µg)	Chromium (µg)	DHA (mg)	EPA (mg)
Acti vita-miner Prenatal + DHA	28	200	50	150	15	1	0	1	0	20	0	220	44
Centrum Femina DHA	27	140	60	175	11	2	0	0.5	0	30	40	200	0
Composita Mama DHA	27	0	200	220	0	0	0	0	0	0	0	600	120
DuphaVit Pregna	30	0	0	200	0	0	0	0	0	0	0	250	0
Elevit Pronatal	60	125	100	0	7.5	1	0	1	125	0	0	0	0
Falvit mama	20	240	0	200	10	1.5	50	1	0	55	0	0	0
Femibion Natal 1	0	0	0	150	0	0	0	0	0	0	0	0	0
Femibion Natal 2 Plus	28	0	70	150	15	1	0	1	0	0	0	200	0
Feminovit + DHA	27	0	0	150	11	0	50	1	0	60	0	200	43
Fertil Care	17	200	150	140	15	0	0	1	0	50	0	0	0
Folik Mama 1	0	0	0	150	0	0	0	0	0	0	0	300	0
Folik Mama 2	0	0	200	150	0	0	0	0	0	0	0	300	0
LedeeVit	27	0	75	200	10	1	0	1	0	60	0	160	32
LadeeVit Optima	26	0	60	200	0	0	0	0	0	0	0	600	40
Mama activ Doppelherz	15	200	90	100	10	0	0	0	0	0	0	200	44
Mama DHA	0	0	200	200	0	0	0	0	0	0	0	600	34
Mama DHA Premium +	0	0	200	200	0	0	0	0	0	0	0	800	68
Mama Premium activ Doppelherz	26	0	100	200	0	0	0	0	0	0	0	600	130

Omegamed optima DHA z alg	30	0	0	200	0	0	0	0	0	0	0	200	0
Omegamed optima Forte DHA z alg	30	0	0	200	0	0	0	0	0	0	0	600	0
Pharmaton Matruelle	27	0	10	200	11	0	50	1	0	60	30	150	30
Pregna Plus	27	0	0	200	0	0	0	0	0	0	0	50	0
Pregnal Evital	26	0	0	200	0	0	0	0	0	0	0	200	40
Prenatal classic	60	0	0	200	0	0	0	0	0	55	0	0	0
Prenatal duo	60	0	0	200	0	0	0	0	0	55	0	600	70
Prenatal uno	0	0	0	150	0	0	0	0	0	0	0	0	0
Solgar Wit. i minerały prenatalne	14	650	225	75	7.5	0.5	0	1	0	12.5	12.5	0	0
Symvital Mama	27	0	200	200	0	0	0	0	0	0	0	600	0
TRIMiD 1	18	0	0	150	0	0	0	0	0	0	0	200	0
TRIMiD 2	20	0	75	150	0	0	0	0	0	0	0	200	0
TRIMiD 3	30	0	150	200	0	0	0	0	0	0	0	200	0
Vita-min plus Mama	14	100	50	200	10	1	0	1	0	0	0	0	0
Vita-miner Prenatal	28	200	50	150	15	1	0	1	0	20	0	0	0
Average mineral content, minimum and maximum in the preparations containing particular minerals													
Average Minimum Maximum	**28.5** 14 60	**228.3** 100 650	**115.8** 10 225	**175.3** 75 220	**11.4** 7.5 15	**1.1** 0.5 2	**50.0** 50 50	**1.0** 0.5 1.0	**125** 125 125	**43.4** 12.5 60	**55.8** 12.5 40	**343** 50 800	**63** 30 130
Daily requirement of pregnant women for minerals [1]													
	27	1000	360	220	12	2	not established	1	700	60	not established	350 – 450	

Table 3. Single folic acid preparations for pregnant women

Name of the preparation	Folic acid in a daily dose (µg)
Folian Naturell	400
Folian forte Naturell	800
Kwas foliowy DOZ Product	400
Myo Folic	200
Folic acid content	
Average	450
Minimum	200
Maximum	800

Table 4. Single DHA preparations for pregnant women (quantities of components in a daily dosage)

Name of the preparation	DHA (mg)	EPA (mg)	Other *omega*-3 fatty acids (mg)
DHA z alg DOZ Product	250	-	-
Mumomega	300	42	-
Möller′s Baby tran norweski	600	400	200
Omegamed pregna DHA z alg	400	-	-
Pregna 250 DHA	500	-	-
Prenatal DHA	600	70	-
Component content in preparations containing particular components			
Average Minimum Maximum	**441** (250; 600)	**171** (42; 400)	**200** (200; 200)

for the period of pregnancy [38], and this market has now grown by 65% (33 preparations). In 2014, the bulk of preparations (70%) were registered as dietary supplements, and 25% had the status of dietary foods for special medical purposes. Currently, after the legislation on dietetic products was repealed as of 20 July 2016 [24], almost all preparations are dietary supplements.

One of the ingredients recommended for all pregnant women is folic acid (a B vitamin). Research indicates that in pregnancy, unlike in its preceding period, almost all women take folic acid in the form of multi-component preparations (77% out of 83% of women using supplementation) [39]; therefore, the formulation of such preparations is an essential issue for correct supplementation. In light of the PTG recommendation, the routine use of folic acid in a dose of 400 µg a day concerned only the first trimester of pregnancy [43]. On the other hand, according to the latest recommendation of the PTGiP, women who are not at increased risk of fetal neural tube defects should take 400 – 800 µg of folic acid a day in the first trimester of pregnancy and 600 – 800 µg in the following trimesters [42]. All commercially available VMPs contained folic acid, of which more than half contained the exact dose recommended by the PTG. Of the four single folic acid preparations, one provided only 200 µg of this vitamin, which does not correspond with all previous recommendations issued by Scientific Societies. However, the PTGiP has recently modified the dose and the period of intake of folic acid, among others, increasing its amount in the second and third trimester of pregnancy to 600 µg at least [42]. Consequently, 55% of preparations, with their current formulation, will not meet the latest recommendations, and this finding applies to an even greater extent to single folic acid preparations (75%). Simultaneously, it should be emphasized that so far, the routine dose of folic acid has been 400 µg [43], and presumably, therefore most VMPs contain such an amount of this vitamin. It is also evident that the manufacturers of preparations must be allowed some time to possibly change the formulation of their products in the context of the amended recommendations.

In none of the analyzed preparations, either single or multi-component, the dose of folic acid would exceed the upper safe level (UL) of 1000 µg per day, which means that the correct use of such preparations, in combination with the usual dietary intake of folates, should be safe. Nevertheless, as one study showed, more than 33% of the studied population of pregnant women took folic acid from two sources - in the form of a single preparation and a VMP, which resulted in exceeding the UL [36]. At this point, it is important to highlight that in 2019, the Panel on Dietary Supplements (PDS) opined that the daily dose of folic acid in supplements for pregnant women should not exceed 800 µg. Furthermore, it recommended placing a warning on the labels of supplements: *"in pregnant women, use after consultation with a doctor."* [30].

In the last decade, an increasing number of scientific studies have pointed to genetically determined folic acid metabolism problems due to enzyme deficiencies in a significant proportion of the white population [1, 18, 41]. This means that dietary supplementation with folic acid, which requires conversion to 5-methyltetrahydrofolate (5-MTHF) to become bioactive in the body, may prove ineffective in such individuals. An alternative is to use metafolin, i.e., the calcium salt of L-5-methyltetrahydrofolate acid (L-5-MTHF), and glucosamine salt of (6S)-5-methyltetrahydrofolic acid, which are reduced, active forms of folate that directly enters the human bloodstream [1, 7, 27]. These compounds were approved for use in dietary supplements by the Commission

Regulation [8, 9], and were also included in the list of chemical forms of folates in the Polish regulation [25]. Although the PTGiP emphasizes that currently there are no indications for the use of reduced forms of folic acid, among analyzed preparations L-5-MTHF or glucosamine salt of (6S)-5-methyltetrahydrofolic acid were used in almost 40% of VMP and in every second single folic acid preparation, usually in combination with the standard form of folic acid, constituting half of the total folate dose contained in the product.

With respect to vitamin D, according to the PTG recommendation, pregnant women should take 2000 IU (50 μg) of vitamin D daily [43]. New recommendations of PTGiP condition the dosage of this vitamin with the patient's BMI. Women with normal body weight are recommended to take 1500 – 2000 IU (37.5 – 50 μg) of vitamin D, and women with obesity – after consultation with a doctor – a higher dose, even up to 4000 IU (100 μg) [42]. Only half of the VMPs contained the PTG-recommended dose of this vitamin and the same situation occurs with regard to the PTGiP recommendations. Although there has been a significant improvement made in this respect, compared to the market situation analyzed in 2014, when only 15% of preparations had an adequate amount of vitamin D [38], nevertheless most preparations still provide a low dose of this vitamin, even as little as 5-10 μg (200 – 400 IU). As shown by numerous studies, such a daily dose is not able to increase vitamin D concentration in the blood of patients to the level recommended in pregnancy [2, 28, 29]. Given that an even higher dose of vitamin D has been recommended for pregnant women with obesity (as much as 4000 IU - 100 μg), it should be noted that the PDS expressed the position on the daily dose of this vitamin in supplements which should not exceed 2000 IU (50 μg) [31]. Therefore, women with obesity will need to have their vitamin D intake individually determined by a physician, next to the VMP that they may be using.

The iodine dose to be taken by pregnant women in line with the PTG recommendation so far, was 200 μg a day [43]. Considering the most recent PTGiP recommendations, all pregnant women (without a history of thyroid disease) are advised to take a dose of 150 – 200 μg a day [42]. The situation concerning iodine content in preparations for pregnant women has definitely improved over the years. In 2005, it was present only in a few VMPs (43%) [36], while currently, iodine is a component of 97% of VMPs. Fifty three percent of currently analyzed preparations contained its dose, complying with the PTG recommendations (200 μg), but as much as 91% will meet the PTGiP recommendations in this respect (150-200 μg). It should be noted that one VMP did not contain this mineral, but since that preparation has the status of a drug, information about the absence of iodine is included in the package insert for patients. As a result, women using this preparation should be aware that such an essential nutrient should be supplemented separately. In the light of the resolution of the PDS, the maximum level of iodine in supplements for pregnant women should not exceed 200 μg in a daily dose [32], which is in line with the recommendations on dietary supplementation.

Iron is a fairly controversial mineral in the context of dietary supplementation. According to the PTG, if risk factors for anemia appear in pregnancy, women should take 26 – 27 mg of iron [43]. The latest position of the PTGiP emphasizes that iron should only be taken by women with anemia, whereas in the case of women without anemia but with reduced ferritin levels, dietary supplementation with low doses of iron, i.e., up to 30 mg a day, may be allowed from week 16 of pregnancy onward [42]. Therefore, according to the most recent recommendations, iron should not be used by pregnant women on their own, so its presence in most vitamin-mineral preparations (82%) can be problematic. Iron has been in any case present in all VMPs for years; therefore, 78-80% of pregnant women taking such preparations also took iron [11, 36]. Both cited experts' recommendations indicate that low doses of iron (up to 30 mg) should be taken in justified situations, and yet three preparations contained 60 mg of this mineral. The maximum dose of iron in supplements for pregnant women set by the PDS in 2019 is 30 mg, so the composition of some preparations will most likely need to be changed in the future. According to the Panel's resolution, such supplements should also be provided with a warning: *"product for pregnant women, use after consultation with a doctor."* [33]. Although the resolutions of the Panel mentioned above do not have the status of law, it should be assumed that they will be taken into account in the evaluation of dietary supplements carried out by inspection authorities.

A vital nutrient during pregnancy is DHA, and because of its low dietary intake, the need for supplementation has been stressed for years. For women who did not eat much fish, the PTG recommended a DHA intake of at least 600 mg [43]. The PTGiP suggests that all pregnant women should take DHA in a dose of at least 200 mg a day and that higher doses should be considered for women with low fish consumption [42]. In Poland, only 3-19% of pregnant women consume fish according to the recommendations (at least twice a week) [4, 10, 17, 35]; therefore, higher doses of supplementation practically apply to most pregnant women. Although there have been changes in the composition of preparations in terms of DHA content in recent years, despite recommendations existing for years, this

ingredient was not present in 27% of vitamin-mineral preparations, and in some, its dose was less than 200 mg. A study conducted in 2014-2015 found that VMPs contained an average of 130 mg of DHA [35] and currently, the situation is much more favorable. The average DHA content in VMPs was 343 mg, and in preparations containing only *omega*-3 fatty acids 441 mg. Still, it should be remembered that women who do not eat fish should make use of additional DHA preparations next to VMP. According to the recently published study by *Knapik* et al., polyunsaturated fatty acids are supplemented by less than 25% of pregnant women [14], whereas the study by *Wierzejska* et al. shows that 28% of pregnant women take DHA [35].

Concerning the remaining vitamins and minerals, experts from Scientific Societies now state that their routine supplementation is not recommended and should only apply to women for whom it is medically justifiable [42, 43]. However, some experts believe that multivitamin-mineral supplementation is essential, pointing to most components' deficiencies, especially among women with gestational diabetes [16]. A cautious approach to universal supplementation also prevails in the United States, where the supply of these nutrients in the form of preparations is recommended only for mothers at risk of deficiencies thereof [5]. In general, it is felt that dietary supplementation should be tailored to the patient's needs to the maximum extent, which may prove most beneficial and protect the patient against excessive intake of nutrients [5, 16, 21, 43]. Caution of widespread intake of VMPs is further corroborated by studies demonstrating a link between dietary supplementation and an increase in body weight and body fat in newborns [22].

Apart from components that should be taken by all patients, the most frequently used vitamin in analyzed preparations was vitamin B6 (22 preparations), B12 (21 preparations), and magnesium (20 preparations). In the case of eight vitamins, the average content of particular vitamins exceeded pregnant women's requirements by 7-316%. Compared to the recommended intake, the highest doses concerned biotin (316%), even though, according to the nutritional standards, the need for biotin does not increase during pregnancy. High amounts of some nutrients in preparations have been observed for years. Studies conducted in 2005-2007 showed that the amount of some vitamins provided by VMPs covered up to 667% of the requirement for pregnant women, and the dose of minerals up to 227%. High doses in the preparations were recorded mostly for vitamin B6, B12, and biotin, and negligible and practically irrelevant in the pool for magnesium [36]. Also the study by *Hamułka* et al. conducted in 2008 showed that dietary supplements were supplying vitamin C, B2, folic acid, and iron above the norm (120-128%) [11]. In none of the preparations analyzed in the study did the content of vitamins and minerals exceed their UL. Next to the content of vitamins in the daily dose of preparations, there is also a problem of an inappropriate approach to diet supplementation. Studies indicate that 4.7 - 22% of pregnant women take several preparations simultaneously, which results in exceeding the recommended intake 5-fold or even 6-fold [11, 36].

When discussing the composition of the preparations and the extent to which they cover pregnant women's requirements, one cannot fail to mention legal regulations concerning the labelling of dietary supplements. On their packaging, like in all food products, the quantity of vitamins and minerals is expressed as a percentage of the Reference Intake (RI) [26]. At present, RI has been determined for an average adult, so this value does not reflect the recommended intake for pregnant women, whose nutrient requirements increase. A good example is that of folic acid, for which RI stands at 200 μg, and according to the nutrition standards, the requirement for pregnant women is 600 μg. This means that in the case of 400 μg of this vitamin in the daily dose of a supplement (which was generally found in the analyzed preparations), the label indicates the amount covering 200% of RI, while during pregnancy, this only supply 66% of the recommended intake. The second issue is many years' delay in providing an amended regulation of RI, in line with changing nutritional standards based on new scientific data. A good example here is vitamin D, for which RI still stands at 5 μg, while in light of nutrition standards, the amount necessary in the diet of all adults and pregnant women is 15 μg. As it was the case with folic acid, a supplement offering a dose of 5-10 μg of vitamin D (often contained in preparations) is labeled as providing 100-200% of RI, whereas, for the currently determined requirement, it only supplies 33-66%. Pregnant women may not be aware of such differences, and perhaps doctors or pharmacists either. Therefore, the legislation relating to labelling of dietary supplements needs to be revised, following changing nutritional standards, to avoid misinterpretation by patients regarding the amount of nutrients.

CONCLUSIONS

All 33 evaluated VMPs contained folic acid. Fifty five percent of them provide the exact amount of this nutrient according to recommendation PTG (2014), but less than half of preparations meet the new recommendation PTGiP (2020).

Ninety seven percent of VMPs contained vitamin D. Fifty percent of them contain too low dose of this vitamin in relation to both recommendations.

Ninety seven percent of VMPs contained iodine. Out of them 44% did not contain a dose of iodine recommended by PTG, but only 9% of preparations do not meet the PTGiP recommendation.

Seventy three percent of VMPs contained DHA. One-third of them provided the dose recommended by the PTG and 88% of preparations meet the new recommendation PTGiP.

Eighty two percent of VMPs contained iron. Sixty three percent of them contain this mineral in dose compliant with PTG recommendation, but according to the PTGiP recommendation iron should not be taken by all pregnant women on their own accord.

The composition of many VMPs did not reflect experts' recommendations regarding the type and amounts of particular nutrients.

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Conflict of interest

The author declare no conflict of interest.

REFERENCES

1. *Bailey S.W., Ayling J.E.*: The pharmacokinetic advantage of 5-methyltetra-hydrofolate for minimization of the risk for birth defects. Sci Rep 2018; 8:4096. doi:10.1038/s41598-018-22191-2378.
2. *Bartoszewicz Z., Kondracka A., Krasnodębska-Kiljańska M., Niedźwiedzka B., Popow M., Ambroziak U., Bednarczuk T.*: Vitamin D insufficiency in healthy pregnant women living in Warsaw. Ginekol Pol 2013;84:363-367
3. *Bojar I., Owoc A., Humeniuk E., Wierzba W., Fronczak A.*: Inappropriate consumption of vitamins and minerals by pregnant women in Poland. Ann Agric Environ Med 2012; 19(2):263-266
4. *Broś-Konopielko M., Białek A., Oleszczuk-Modzelewska L., Zaleśkiewicz B., Różańska-Walędziak A., Teliga-Czajkowska J., Tokarz A., Czajkowski K.:* Consumption of fish and seafood by pregnant Polish women and the supply of docosahexaenoic acid and eicosapentaenoic acid from these products. Fam Med Prim Care Rev 2017;19(3):191-195
5. *Brown B., Wright C.*: Safety and efficacy of supplements in pregnancy. Nutrition Reviews 2020;78(10):813–826
6. *Bzikowska A., Czerwonogrodzka-Senczyna A., Riahi A., Weker H.*: Nutritional value of daily food rations of overweight and normal weight pregnant women. Rocz Panstw Zakl Hig 2017;68(4):375-379
7. *Cochrane K.M., Mayer C., Devlin A.M., Elango R., Hutcheon J.A., Karakochuk C.*: Is natural (6S)-5-methyltetrahydrofolic acid as effective as synthetic folic acid in increasing serum and red blood cell folate concentrations during pregnancy? A proof-of-concept pilot study. Trials 2020; 21: 380. https://doi.org/10.1186/s13063-020-04320-3
8. Commission regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. Official Journal EU L 314/36; 1.12.2009
9. Commission regulation (EU) 2015/414 of 12 March 2015 amending Directive 2002/46/EC of the European Parliament and of the Council as regards (6S)-5-methyltetrahydrofolic acid, glucosamine salt used in the manufacture of food supplements. Official Journal EU L 68/26; 13.3.2015
10. *Gacek M.*: Some health behaviours and selected health status coefficients in a group of pregnant women. Probl Hig Epidemiol 2010;91(1):48-53
11. *Hamułka J., Wawrzyniak A., Pawłowska R.:* Assessment of vitamins and minerals intake with supplements in pregnant women. Rocz Panstw Zakl Hig 2010;61(3):269-275
12. *Harton A., Choroszewska A., Gajewska D., Myszkowska-Ryciak J.:* Intake of polyunsaturated fatty acids by pregnant women. Probl Hig Epidemiol 2013;94(3):605-609
13. *Kaim I., Penar A., Sochacka-Tatara W., Gałaś A., Jędrychowski W.:* Farmakologiczna suplementacja witaminami i składnikami mineralnymi w okresie ciąży. Badanie epidemiologiczne w Krakowie. Przegl Lek 2004;61:776
14. *Knapik A., Kocot K., Witek A., Jankowski M., Wróblewska-Czech A., Kowalska M., Zejda J.E., Brożek G.:* Dietary supplementation usage by pregnant women in Silesia — population based study. Ginekol Pol 2018;89(9):506-512
15. *Kocyłowski R., Lewicka I., Grzesiak M., Gaj Z., Sobańska A., Poznaniak J., von Kaisenberg C., Suliburska J.*: Assessment of dietary intake and mineral status in pregnant women. Arch Gynecol Obstet 2018;297:1433-1440
16. *Kozłowska A., Jagielska A.M., Okręglicka K.M., Dabrowski F., Kanecki K., Nitsch-Osuch A., Wielgoś M., Bomba-Opon D.:* Dietary vitamin and mineral intakes in a sample of pregnant women with either gestational diabetes or type 1 diabetes mellitus, assessed in comparison with Polish nutritional guidelines. Ginekol Pol 2018; 89(11):581–586
17. Ługowska K., *Kolanowski W.*: Spożycie ryb przez kobiety w ciąży. Żyw Człow Metabol 2017;2:87-97
18. *Moczulska H., Pesz K., Gach A., Borowiec M., Sieroszewski P., Sąsiadek M., Jakubowski L., Wielgoś M.:* MTHFR genetic testing: Recommendations of the Polish Society of Gynecologists and Obstetricians and the Polish Human Genetics. Ginekol Perinatol Prak 2017;2(5):234–238
19. *Myszkowska-Ryciak J., Gurtatowska A., Harton A.:* Nutritional knowledge and selected aspects of

the diet of pregnant women. Probl Hig Epidemiol 2013;94(3):600-604.
20. Normy żywienia dla populacji Polski i ich zastosowanie (red. *Jarosz M., Rychlik E., Stoś K., Charzewska J.*), Narodowy Instytut Zdrowia Publicznego – Państwowy Zakład Higieny, Warszawa, 2020
21. *Oh C., Keats E.C., Bhutta Z.A.*: Vitamin and mineral supplementation during pregnancy on maternal, birth, child health and development outcomes in low- and middle-income countries: A systematic review and meta-analysis. Nutrients 2020;12,491; doi:10.3390/nu12020491
22. *Petry C.J., Ong K.K., Hughes I.A., Dunger D.B.:* Multiple micronutrient supplementation during pregnancy and increased birth weight and skinfold thicknesses in the offspring: The Cambridge Baby Growth Study. Nutrients 2020;12,3466; doi:10.3390/nu12113466
23. *Pieszko M., Ciesielska-Piotrowicz J., Skotnicka M., Małgorzewicz S.:* Behaviour health pregnant women with secondary and higher education – preliminary studies. Pediatr Med Rodz 2017; 3(1):94-102
24. Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control. Official Journal UE L 181/35; 29.6.2013
25. Regulation of the Minister of Health of 24 April 2017 amending the regulation on the composition and labelling of dietary supplements. Dz. U. z dnia 18.05.2017, poz. 979
26. Regulation (EU) No 1169/2011 of the European Parliament and of Council of 25 October 2011 on the provision of food information to consumers. Dz. U. L 304; 22.11.2011
27. *Seremak-Mrozikiewicz A.:* Metafolin – alternative for folate deficiency supplementation in pregnant women. Ginekol Pol 2013;84:641-646
28. *Shakiba M., Iranmanesh M.R.:* Vitamin D requirement in pregnancy to prevent deficiency in neonates: a randomized trial. Singapore Med 2013;54(5):285-288
29. *Skowrońska-Jóźwiak E., Adamczewski Z., Tyszkiewicz A., Krawczyk-Rusiecka K., Lewandowski K., Lewiński A.*: Assessment of adequacy of vitamin D supplementation during pregnancy. Ann Agric Environ Med 2014;21:198-200
30. Uchwała nr 7/2019 Zespołu ds. Suplementów Diety z 11 czerwca 2019 w sprawie wyrażenia opinii dotyczącej maksymalnej dawki kwasu foliowego w zalecanej dziennej porcji w suplementach diety. www.gov.pl/web/gis/zespol-do-spraw-suplementow-diety
31. Uchwała nr 4/2019 Zespołu ds. Suplementów Diety z 22 maja 2019 w sprawie wyrażenia opinii dotyczącej maksymalnej dawki witaminy D w zalecanej dziennej porcji w suplementach diety. www.gov.pl/web/gis/zespol-do-spraw-suplementow-diety
32. Uchwała nr 15/2019 Zespołu ds. Suplementów Diety z 25 października 2019 w sprawie wyrażenia opinii dotyczącej maksymalnej dawki jodu w zalecanej dziennej porcji w suplementach diety. www.gov.pl/web/gis/zespol-do-spraw-suplementow-diety
33. Uchwała nr 20/2019 Zespołu ds. Suplementów Diety z 13 grudnia 2019 w sprawie wyrażenia opinii dotyczącej maksymalnej dawki żelaza w zalecanej dziennej porcji w suplementach diety. www.gov.pl/web/gis/zespol-do-spraw-suplementow-diety
34. *Wierzejska R.:* Consumption of vitamin D and its sources in diet of pregnant women. Żyw Człow Metabol 2018;4:172-180
35. *Wierzejska R., Jarosz M., Wojda B., Siuba-Strzelińska M.:* Dietary intake of DHA during pregnancy: a significant gap between the actual intake and current nutritional recommendations. Rocz Panstw Zakl Hig 2018;69(4):381-386
36. *Wierzejska R., Jarosz M., Siuba-Strzelińska M.:* Diets supplementation of pregnant women - recommendation and practice. Ginekol Położ 2012;1(23):70-78
37. *Wierzejska R., Jarosz M., Sawicki W., Bachanek M., Siuba-Strzelińska M.:* Vitamin D concentration in maternal and umbilical cord blood by season. Int J Environ Res Public Health 2017;14:1121; doi:10.3390/ijerph14101121
38. *Wierzejska R.:* Dose of the vitamin D in prenatal supplements and the current prevention its deficiency at mothers and newborns. Ginekol Położn 2015;3(37):49-53
39. *Wierzejska R., Wojda B.:* Folic acid supplementation in pregnancy and prevention of fetal neural tube defects. Przegl Epidemiol 2020;74(2):362-369
40. *Więch M., Kawiak-Jawor E., Barańska M., Weker H.*: Diets of pregnant women in relations to current recommendations. Bromat Chem Toksykol 2019;2:114-120
41. *Wolski H., Kocięcka M., Mrozikiewicz A.E., Barlik M., Kurzawińska G.:* Coexistence of the 677C>T and 1298A>C MTHFR polymorphisms and its significance in the population of Polish women. Ginekol Pol 2015;86(10):742-747
42. Zespół Ekspertów Polskiego Towarzystwa Ginekologów i Położników. Rekomendacje Polskiego Towarzystwa Ginekologów i Położników dotyczące suplementacji u kobiet ciężarnych. https://www.ptgin.pl/sites/default/files/page-2020/Rekomendacje PTGiP suplementy w ciąży 2020-07-28 bo.pdf
43. Zespół Ekspertów Polskiego Towarzystwa Ginekologicznego. Rekomendacje Polskiego Towarzystwa Ginekologicznego w zakresie stosowania witamin i mikroelementów u kobiet planujących ciążę, ciężarnych i karmiących. Ginekol Pol. 2014; 85(5): 395-399.

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STATEMENT OF THE COMMITTEE OF HUMAN NUTRITION SCIENCE OF THE POLISH ACADEMY OF SCIENCES ON THE USE OF DIETARY SUPPLEMENTS CONTAINING VITAMINS AND MINERALS BY ADULTS*

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ABSTRACT
The use of dietary supplements (supplementation) is the individual enrichment of the diet with ingredients naturally occurring in food. As a rule, dietary supplements should be used periodically. In nutritional practice, there are many indications for dietary supplementation, but the decision to take dietary supplements should be made by consumers wisely and only in justified situations, when there is a risk that the usual diet does not provide vitamins and minerals in an amount adequate to meet dietary recommendations. However, we should remember about the real dangers of taking too large doses of vitamins and minerals. Many people using dietary supplements, especially several types at the same time, may experience undesirable side effects and deterioration of health, and in addition, people taking medicines may seriously disrupt or weaken the effect of the drug, or even lack the therapeutic effect of the drug. The document presents 10 steps and rules for the use of dietary supplements available on the market, which are addressed to the general population.

Key words: *diet, minerals, vitamins, supplements, shortages, nutrition, food, adults*

STRESZCZENIE
Stosowanie suplementów diety (suplementacja) to indywidualne uzupełnianie diety w składniki naturalnie występujące w żywności. Z założenia suplementy diety powinny być przyjmowane okresowo. W praktyce żywieniowej istnieje wiele wskazań do suplementacji, ale decyzja o przyjmowaniu suplementów diety powinna być podejmowana przez konsumentów z rozsądkiem i tylko w uzasadnionych sytuacjach, gdy istnieje ryzyko, że zwyczajowa dieta nie dostarcza witamin i składników mineralnych w ilości odpowiedniej do pokrycia zapotrzebowania organizmu. Należy jednak pamiętać o realnym niebezpieczeństwie wynikającym z przyjmowania zbyt dużych dawek witamin i składników mineralnych. U wielu osób stosujących suplementy diety, zwłaszcza kilka rodzajów jednocześnie, mogą wystąpić niepożądane skutki uboczne i pogorszenie stanu zdrowia, a u osób przyjmujących leki – może dodatkowo dojść do poważnego zakłócenia lub osłabienia działania leku, a nawet braku efektu leczniczego. W dokumencie przedstawiono 10 kroków i zasad korzystania z dostępnych na rynku suplementów diety, które skierowano do populacji generalnej.

Słowa kluczowe: *dieta, składniki mineralne, witaminy, suplementy, niedobory, żywienie, żywność, osoby dorosłe*

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The use of dietary supplements (supplementation) is the individual enrichment of the diet with ingredients naturally occurring in food. As a rule, dietary supplements should be taken periodically [1]. In nutritional practice, there are many indications for supplementation, but the decision to take dietary supplements should be made by consumers wisely and only in justified situations, when there is a risk that a regular diet will not provide vitamins and minerals in an amount adequate to comply with dietary recommendations. Dietary supplements that have been introduced to the Polish market are safe and should not pose a threat to the health and life of consumers, but their improper use may pose a threat. Taking dietary supplements should be consulted with doctor, nutritionist or pharmacist as there is a risk of overdosing or interactions with food, medicines or other supplements. Eating a varied diet based on available food should always be considered as the first step in improving the nutritional status and health.

LEGAL REGULATIONS AND DIETARY SUPPLEMENTS

The Act of Food Safety and Nutrition of 25 August 2006 [10], defines a **dietary supplement** as a **food, supplementing a normal diet**, being a concentrated source of vitamins or minerals or other substances with a nutritional or other physiological effect, single or complex, marketed in dosage form, in the form of: capsules, tablets, dragees and other similar forms, powder sachets, liquid ampoules, dropper bottles and other similar forms of liquids and powders intended for consumption in small, measured unit amounts, **excluding products with the properties of a medicinal product within the meaning of pharmaceutical law**.

The above conditions should be met cumulatively.

The ingredients of dietary supplements can be:

- vitamins: A, D, E, K, B vitamins (B_1, B_2, B_6, B_{12}, niacin, pantothenic acid, folic acid, biotin), vitamin C;
- minerals: calcium, phosphorus, magnesium, iron, zinc, copper, iodine, selenium, fluorine, manganese, sodium, potassium, chlorine (chlorides) and others, e.g. boron, chromium, silicon, molybdenum;
- other substances, e.g. amino acids, fatty acids, dietary fiber, pro and prebiotics, substances of plant origin and others having a potential biological effect on the body.

Dietary supplements are labeled with the following information on the packaging:

- the term “dietary supplement”;
- the name of the category of nutrients or substances characterizing the product or an indication of their properties;
- the portion of the product recommended for consumption during the day;
- a warning about not exceeding the recommended daily dose;
- **a statement that dietary supplements cannot be used as a substitute (replacement) for a varied diet;**
- a statement that dietary supplements should be stored out of reach of small children;
- information on the content of vitamins and minerals;
- the content of vitamins and minerals as a percentage of the recommended daily intake.

The labeling of dietary supplements must not attribute to them the property of preventing, treating, or cure human diseases or suggest such properties. This policy also applies to advertising.

DIETARY SUPPLEMENTS AND MEDICINES

A medical claim that states, suggests, or implies that a product or ingredient(s) has properties for treating or preventing disease(s) are proprietary to medicaments.

Dietary supplements, like medicines and medical devices, are in the form of tablets, dragees, capsules, drops, powders, but the differences between these products are fundamental (Table 1). This applies not only to the method of operation, but also to the principles of marketing authorization, intended use, and the possibility of advertising the preparation and placing it on the market. A drug differs from a medical device, among other, mode of action. Medicinal products (medicines) have a pharmacological effect, i.e. they cure or prevent a disease, and medical devices only have a physical and mechanical effect. Thus, the effect of the medical devices is limited compared to the medicines. Diet supplements, on the other hand, are used to enrich the usual diet (based on typically consumed food). **They do not have any healing properties, but they can support the functioning of the body, improving its nutritional status.**

In the light of legal regulations [2, 3, 5, 7, 8, 9], the maximum content of vitamins and minerals in a daily portion of a dietary supplement should be determined taking into account:

- the upper level of safe intakes (UL) for vitamins and minerals based on scientific risk assessment and generally accepted scientific data, taking into account the varying degrees of sensitivity of different groups of consumers;

able 1. The main differences between food supplements and medicines

Differences	Food supplement	Medicines
Intended use	**A food supplement is used to supplement the nutritional value of a regular diet.** It is intended for healthy individuals who do not have an adequate supply of certain ingredients in their diet.	**A medicine is used to treat or prevent diseases.** It is intended for people who are ill or at increased risk of developing the disease.
Safety	**Food supplements satisfy the requirements applying to foodstuffs.** The content of an ingredient in the product may differ from the amount declared on the label by -20% to +50% for vitamins and -20% to +45% for minerals.	**Each medicine is subject to detailed testing of its composition.** Only small, strictly defined differences in the content of a given component resulting from the test method used are permitted.
Placing on the market in Poland	A food supplement is a food item and therefore does not require a marketing authorisation. **The decision to market it is taken by the entrepreneur, who notifies the Chief Sanitary Inspector** (GIS). **In case of any doubt, the Chief Sanitary Inspector may initiate a clarification procedure.**	A medicine, before it can be marketed, **must be approved by the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products** (URPL, WMiPB). The product label shows the marketing authorisation number of the medicines.
Inclusion in a relevant list	Products that have been approved as food supplements are listed in the special Register of Products **www.rejestrzp.gis.gov.pl**	Medicines that have been authorised for marketing in Poland are included in the Official List of Medicinal Products **www.urpl.gov.pl**

Source: https://gis.gov.pl/wp-content/uploads/2020/01/suplementy-diety_A5.pdf

- intake of vitamins and minerals from food and drinking water, including fortified foods;
- recommended intakes of vitamins and minerals for the population, taking into account different groups of consumers.

In Poland, the Team for Diet Supplements, which functions within the Sanitary and Epidemiological Council, as an advisory body to the Chief Sanitary Inspector (GIS), has been entrusted with determining the maximum content of vitamins and minerals in dietary supplements. The Team for Diet Supplements develops opinions in the form of resolutions on the maximum daily amounts of vitamins and minerals in dietary supplements (in the recommended daily dose) intended for adults, with additional guidelines (Table 2).

Additional guidelines of the Team for Diet Supplements regarding the maximum content of vitamins and minerals in dietary supplements [4]:

- **vitamin D** - before use, it is advisable to test the concentration of 25(OH)D in the blood and consult the result of the test with a doctor or pharmacist;
- **vitamin C** - in the labeling of dietary supplements with a high content of vitamin C, it is recommended to include a warning: "Do not use in people with a predisposition to the formation of kidney stones or suffering from kidney stones";
- **vitamin A** - 800 μg in the form of retinol equivalent (retinol and retinyl esters) and 7 mg in the form of **β**-carotene;
- **folic acid** - 800 μg if the supplement is marked as intended for pregnant women; in addition, it is recommended to include a warning: "In pregnant women, use after consulting a doctor";
- **niacin** - 830 mg in the form of nicotinic acid amide or 16 mg in the form of nicotinic acid;
- **pantothenic acid** - 10 mg in the form of pantethine or 200 mg in the other chemical forms, expressed as pantothenic acid;
- **iodine** - 200 μg if the supplement is designated as intended for pregnant and lactating women;
- **iron** - 30 mg if the supplement is marked as intended for pregnant women; in addition, it is recommended to include a warning: "Supplement for pregnant women, use after consulting a doctor";
- **vitamin K** - in the labeling of dietary supplements with a high content of vitamin K, it is recommended to include a warning: "The supplement should not be consumed by people taking anticoagulants containing vitamin K antagonists (eg. warfarin and acenocoumarol)";
- **boron** - 3 mg;
- **chromium** - 200 μg.

THE INTAKE OF FOOD SUPPLEMENTS AND CONSUMER SAFETY

The use of food supplements containing vitamins and minerals may, for some people, improve compliance with dietary recommendations and more fully meet the body's requirements for these nutrients. However, it should be remembered that **taking excessive doses of vitamins and minerals involves a real risk** (Table 2 and 3). For many individuals,

taking food supplements, especially several types of supplements at the same time, undesirable side effects and deterioration of health may occur and, in those taking medicines, the effect of their medicines may additionally be seriously affected or impaired or the therapeutic effect may even be completely absent. For these reasons, food supplements need to be appropriately labelled by the manufacturer, i.e. to include reliable information on contraindications to the use of these preparations, to indicate possible interactions with medicines, food components or components of other food supplements and to recommend that consumers consult their doctor before using them, especially if they are ill or taking medicines.

TEN STEPS AND RULES FOR USING FOOD SUPPLEMENTS

Based on current scientific knowledge and existing legislation, ten steps and rules have been formulated for using food supplements available on the market:

1. Ongoing education of the public concerning the principles of proper nutrition and a well-balanced diet through the consumption of a wide variety of foods.
2. Before using a food supplement, a qualitative and quantitative assessment of the diet should be conducted by a dietician or another professional, taking into account the individual needs of the consumer according to gender, age, physical activity and physiological state (pregnancy, breastfeeding).

Table 2. Recommended daily allowances (RDA) of vitamins and minerals in Poland in relation to the upper safe level of intake (UL) from food, drinking water and food supplements jointly, as well as their maximum daily amounts in food supplements (according to the values provided by the Team for Diet Supplements) [4]

Component	RDA for an adult	UL values for an adult	Maximum daily amount in the recommended daily portion of the supplement
Vitamin A (µg)	700 [1]/ 900 [2]	3000	800
Vitamin D (µg)	15 (600 IU)	100 (4000 IU)	50 (2000 IU) [6]/ 100 (4000 IU) [7]
Vitamin E (mg)	8 [1]/ 10 [2]	300	250
Vitamin K (µg)	55 [1]/ 65 [2]	no data	200
Vitamin C (mg)	75 [1]/ 90 [2]	no data	1000
Thiamine (mg)	1.1 [1]/ 1.3 [2]	no data	100
Riboflavin (mg)	1.1 [1]/ 1.3 [2]	no data	40
Niacin (mg)	14 [1]/ 16 [2]	nicotinic acid – 10 mg nicotinamide – 900 mg	16
Vitamin B_6 (mg)	1.5 [1]/ 1.7 [2]	25	18
Folacin (µg)	400	1000 as folic acid	600 as folic acid
Vitamin B_{12} (µg)	2.4	no data	100
Biotin (µg)	30	no data	not established
Pantothenic acid (mg)	5	no data	10
Calcium (mg)	1000 [3]/ 1200 [4]	2500	not established
Phosphorus (mg)	700	no data	450
Magnesium (mg)	320 [1]/ 420 [2]	250 [5]	400
Iron (mg)	18 [1]/ 10 [2]	no data	20
Zinc (mg)	8 [1]/ 11 [2]	25	15
Copper (mg)	0.9	5	2
Iodine (µg)	150	600	150
Selenium (µg)	55	300	not established
Fluoride (mg)	3 [1]/ 4 [2]	7	3.5
Manganese (mg)	1.8 [1]/ 2.3 [2]	no data	1.8

[1] women; [2] men; [3] for women under 50 and men under 65; [4] for women 50 or over, and men 65 or over; [5] value for the intake of magnesium from food supplements and magnesium added to food (excluding magnesium naturally occurring in products); [6] for the healthy adult population under 75; [7] for the healthy adult population 75 or over

Table 3. The presence of risks related to excessive intake of vitamins or minerals with food supplements in adults [6]

Group	Health risk	Component
A	No evidence of risk to human health at current levels of consumption (from all sources in total)	thiamine (vitamin B_1), riboflavin (vitamin B_2), biotin, vitamin B_{12}, pantothenic acid, vitamin K, chromium (III)
B	The risk of exceeding UL* is low	vitamins: B_6, C, D, E, folic acid, nicotinamide, phosphorus, magnesium, molybdenum, selenium, potassium
C	There is a risk of excessive intake when using supplements	vitamin A, beta-carotene, calcium, copper, iodine, iron, manganese, zinc

* UL (Upper Level) – upper safe level of the component intake with food, drinking water and food supplements

3. If a diet is found to be poorly balanced in relation to the dietary recommendations, making changes to food intake and ensuring the consumption of food enriched with vitamins and minerals.
4. Carrying out medical and biochemical tests on nutritional status to assess health condition and confirm vitamin and mineral deficiencies in the body.
5. Choosing an appropriate food supplement, while eliminating the risk of potential interactions associated with the simultaneous intake of several food supplements or interactions between a food supplement and medicines.
6. Using food supplements only from verified sources.
7. Educating patients on the use of the food supplement to minimize the risk of adverse reactions resulting from excessive intake, i.e. exceeding the upper safe intake level (UL) for vitamins and minerals (including diet, drinking water and food supplements).
8. Seeking follow-up advice from a doctor and a dietician to monitor the effectiveness of the food supplement and, if necessary, to change the type or dose of the food supplement.
9. After periodic dietary supplementation and confirming the elimination of vitamin and mineral deficiencies in the body, discontinuing the intake of the food supplement and following a well-balanced diet.
10. For population groups at higher risk of deficiencies, e.g. children, adolescents, the elderly and pregnant [11] or lactating women, following the recommendations addressed to those groups by the relevant expert panels and, in the case of sick persons, following individual medical advice.

REFERENCES

1. *Brzozowska A., Roszkowski W., Pietruszka B., Kałuża J.:* Witaminy i składniki mineralne jako suplementy diety [Vitamins and minerals as dietary supplements]. Żywność Nauka Technologia Jakość 2005;4(Supl.):5-16 (in Polish).
2. Discussion Paper on the setting of maximum and minimum amounts for vitamins and minerals in foodstuffs, European Commission, 2006. Available https://ec.europa.eu/food/sites/food/files/safety/docs/labelling_nutrition-supplements-discus_paper_amount_vitamins_en.pdf (Accessed 17.05.2021).
3. Dyrektywa 2002/46/WE Parlamentu Europejskiego i Rady z dnia 10 czerwca 2002 r. w sprawie zbliżenia ustawodawstwa państw członkowskich odnoszącego się do suplementów diety [Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements], Dz. U. L 183 z 12.7.2002, 51-57 z późn. zm. (in Polish) [Official Journal of the European Communities L 183, 12.7.2002].
4. *Jarosz M., Rychlik E., Stoś K., Charzewska J.* eds. Normy żywienia dla populacji Polski i ich zastosowanie [Nutrition recommendations for the Polish population and their application]. Warsaw, NIZP-PZH 2020 (in Polish). Available https://www.pzh.gov.pl/wp-content/uploads/2020/12/Normy_zywienia_2020web-1.pdf (Accessed 17.05.2021).
5. Overview on Tolerable Upper Intake Levels as derived by the Scientific Committee on Food (SCF) and the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), Summary of Tolerable Upper Intake Levels, September 2018. Available https://www.efsa.europa.eu/sites/default/files/assets/UL_Summary_tables.pdf (Accessed 17.05.2021).
6. *Richardson D.P.:* Risk management approaches to the setting of maximum levels of vitamins and minerals in food supplements for adults and for children aged 4–10 years. Food Supplements Europe, July 2014. Available https://foodsupplementseurope.org/wp-content/themes/fse-theme/documents/publications-and-guidelines/fseriskmanagement.pdf. (Accessed 17.05.2021).
7. Rozporządzenie Ministra Zdrowia z dnia 9 października 2007 r. w sprawie składu oraz oznakowania suplementów diety [Regulation of the Polish Minister of Health of 9 October 2007 on the composition and labeling of dietary supplements], Dz. U. 2007, 196, 1425, z późn. zm. (in Polish).
8. *Stoś K., Głowala A., Ziółkowska I.:* Normy a suplementacja [Nutrition recommendations and supplementation]. In: *Jarosz M., Rychlik E., Stoś K., Charzewska J.* (eds.) Normy żywienia dla populacji Polski i ich zastosowanie [Nutrition recommendations

for the Polish population and their application]. Warsaw, NIZP-PZH 2020;414-424 (in Polish).
9. Suplementy witamin i składników mineralnych: model zarządzania ryzykiem [Vitamin and mineral supplements: a risk management model] (translation: *Stoś K*. Żyw Człow Metab 2005;32:5-29).
10. Ustawa z dnia 25 sierpnia 2006 r. o bezpieczeństwie żywności i żywienia [The Act of Food Safety and Nutrition of 25 August 2006]. Dz.U. 2006, no 171 item 1225, amendments 2020.2021 (in Polish). Available https://sip.lex.pl/akty-prawne/dzu-dziennik-ustaw/bezpieczenstwo-zywnosci-i-zywienia-17302608 (Accessed 17.05.2021).
11. *Zimmer M., Sieroszewski P., Oszukowski P., Huras H., Fuchs T., Pawlosek A.:* Polish Society of Gynecologists and Obstetricians recommendations on supplementation during pregnancy. Ginekol Pol 2020;91:644-653 doi: 10.5603/GP.2020.0159.

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ORIGINAL ARTICLE

SYSTEMIC IMMUNE-INFLAMMATION INDEX (SII) AND NEUTROPHIL TO LYMPHOCYTE RATIO (NLR) ARE USEFUL MARKERS FOR ASSESSING EFFECTS OF ANTI-INFLAMMATORY DIET IN PATIENTS BEFORE CORONARY ARTERY BYPASS GRAFTING

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ABSTRACT

Background. One of the risk factors responsible for coronary artery disease (CAD) is an inadequate diet that is frequently deficient in anti-inflammatory components, such as polyphenols and omega-3 fatty acids. The neutrophil to lymphocyte ratio (NLR) and the systemic immune-inflammation index (SII) are inflammatory markers that may reflect a diet's anti-inflammatory potential.

Objective. The aim of this study was to evaluate the effects that CAD patients' nutrition patterns have on NLR and SII.

Material and methods. A retrospective study assessed the dietary habits and inflammatory marker levels in patients with advanced CAD before they underwent coronary artery bypass grafting (CABG) (n=101). Patients were divided into subgroups based on their NLR and SII levels.

Results. Subgroups with lower NLR and SII levels had consumed significantly more eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (p=0.02). The group with a lower ratio of omega-6 to omega-3 fatty acids (<4:1) also had lower NLR and SII levels (p=0.007 and p=0.01, respectively). Statistically significant negative correlations were found between EPA and DHA, as well as omega-3 intake, and both NLR and SII values. No statistically significant differences were found between the subgroups with lower and higher NLR and SII values for polyphenol intakes.

Conclusions. Inflammatory markers such as NLR and SII may reflect an anti-inflammatory diet consumed by cardiac patients. A simultaneous assessment of dietary habits and inflammatory parameters is beneficial in the possible prevention of adverse cardiovascular incidents after CABG. There is also a need to establish reference values for SII and NLR.

Keywords: *inflammatory marker, anti-inflammatory diet, omega-3, cardiovascular disease, NLR, SII*

STRESZCZENIE

Wprowadzenie. Jednym z czynników ryzyka choroby wieńcowej jest nieodpowiednia dieta, często uboga w składniki o działaniu przeciwzapalnym, takie jak polifenole i kwasy tłuszczowe omega-3. Stosunek liczby neutrofilów do limfocytów (NLR) oraz wskaźnik ogólnoustrojowej reakcji immunologiczno-zapalnej (SII) są markerami stanu zapalnego, które mogą odzwierciedlać potencjał przeciwzapalny diety.

Cel badań. Celem pracy była ocena wpływu sposobu żywienia pacjentów z chorobą wieńcową na poziom NLR i SII.

Materiał i metody. W retrospektywnym badaniu oceniono sposób odżywiania oraz poziom markerów stanu zapalnego u pacjentów z zaawansowaną chorobą wieńcową przed poddaniem ich zabiegowi pomostowania aortalno-wieńcowego (CABG) (n=101). Pacjentów podzielono na podgrupy w zależności od poziomów NLR i SII.

Wyniki. Podgrupy z niższym poziomem NLR i SII spożywały istotnie więcej kwasu eikozapentaenowego (EPA) i dokozaheksaenowego (DHA) (p=0,02). Grupa z niższym stosunkiem kwasów tłuszczowych omega-6 do omega-3 (<4:1) miała również niższe poziomy NLR i SII (odpowiednio p=0,007 i p=0,01). Stwierdzono istotne statystycznie ujemne korelacje pomiędzy EPA i DHA oraz spożyciem kwasów omega-3 a wartościami NLR i SII. Nie wykazano istotnych statystycznie różnic w spożyciu polifenoli pomiędzy podgrupami o niższych i wyższych wartościach NLR i SII.

Wnioski. Markery zapalne, takie jak NLR i SII, mogą odzwierciedlać dietę przeciwzapalną stosowaną przez pacjentów kardiochirurgicznych. Jednoczesna ocena sposobu żywienia i parametrów stanu zapalnego jest korzystna w ewentualnej

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prewencji niekorzystnych incydentów sercowo-naczyniowych po CABG. Istnieje również potrzeba ustalenia wartości referencyjnych dla SII i NLR.

Słowa kluczowe: *marker stanu zapalnego, dieta przeciwzapalna, omega-3, choroby sercowo-naczyniowe, NLR, SII*

INTRODUCTION

Many dietary compounds have been shown to have beneficial effects on atherosclerotic cardiovascular disease (CVD) risk factors [21]. One of these, polyunsaturated fatty acids (PUFAs), has been reported to improve inflammatory markers and common CVD risk factors [17, 28]. Inflammatory markers are essential in determining which group of patients has a higher risk of developing CVD and a worse prognosis following diagnosis [10, 12]. A traditional and well-known biomarker is C-reactive protein (CRP), formed in the liver in response to pro-inflammatory interleukin-6 (IL-6) [24]. A CRP level above 3.0 mg/dL is associated with a high risk of CVD [20].

The neutrophil to lymphocyte ratio (NLR) is an increasingly used inflammatory biomarker that was originally associated with cancer and now also with CVD [6], and recently as a prognostic marker in COVID-19 patients [22]. Due to its low cost and ease of calculation, it could complement blood morphology and constitute a parameter for routine diagnostics [13]. NLR was also reported to be correlated with a risk of rehospitalisation and higher long-term mortality due to recurrent cardiovascular events in patients after cardiac surgery [12].

The systemic immune-inflammation index (SII) has been recently becoming more popular [29]. The index's score is obtained by multiplying the values of neutrophils and platelets and then dividing by the number of lymphocytes. The SII enables the identification of patients that have a poor prognosis following coronary artery bypass grafting (CABG) [5]. Compared to common risk factors, SII has been found to be more effective in predicting cardiac events in patients with coronary artery disease (CAD) and after surgery [5, 32].

In addition to inflammation, nutrition is also important in the development of CVD [4]. Plant polyphenols, as well as *omega*-3 polyunsaturated fatty acids are dietary components that have primarily antioxidant, anti-inflammatory and anticoagulation properties, thus having beneficial effects on the organism [3, 9, 16]. It is also worth taking heed of the proper ratio of *omega*-6 to *omega*-3 fatty acids – it should be about 4:1. When consumed in excess, *omega*-6 fatty acids exhibit pro-inflammatory properties compared to the anti-inflammatory *omega*-3. An imbalance in the ratio, caused by inadequate dietary consumption, can also affect inflammatory parameters [7].

The aim of this study was to evaluate the nutritional patterns of patients with CAD before their planned CABG, taking particular account of their intake of polyphenols and fatty acids. We analysed also the effects of diet on two inflammatory markers: NLR and SII. We found that the group of patients that had lower NLR and SII levels had a higher dietary intake of plant polyphenols and anti-inflammatory fatty acids. Furthermore, decreased values of these inflammatory markers were observed in patients with lower *omega*-6 to *omega*-3 fatty acid ratios.

MATERIALS AND METHODS

Patients

Initially, the study recruited a total of 110 patients with CAD who were before planned CABG. All CABG procedures were performed at the Department of Cardiac Surgery, Faculty of Cardiology and Cardiac Surgery, Medical University of Lodz (Poland). All the patients enrolled in the study presented with complex coronary lesions including left main disease, multiple-vessel disease or a coronary anatomy that was not amenable to percutaneous coronary intervention (PCI), which was reflected by a SYNTAX Score > 22 points (intermediate and high risk).

The exclusion criteria were: history of intracranial disorders (including stroke), renal dysfunction (eGFR<60 mL/min/1.73 m^2), primary and metastatic brain tumours and a history of head trauma, and major surgery or high level trauma within the previous 6 weeks. An active inflammatory state was detected when CRP >10.0 mg/dl, thrombocytopenia was defined as less than 100 x 10^9 PLT/L, anticoagulation with INR ≥1.5. The dietary criteria excluded persons following an alternative diet (e.g. rigorous low-calorie/vegetarian/elimination diets) and those with a daily calorie intake <1000 kcal or >5000 kcal.

Finally, after applying the above criteria, 101 patients were included in the study. The retrospective analysis used data collected from December 2016 to June 2019. The characteristics of the group (n=101) were: female sex: 5 patients (5.0%) and male sex: 96 patients (95.0%); age: 65.1±8.5 years; BMI (body mass index) 28.2±4.8 kg/m^2; hypertension: 82 (81.2%); dyslipidaemia: 95 (94.1%); peripheral artery disease: 6 (5.9%); EuroSCORE: 5.6±2.0 points. All the patients were on aspirin till the day of their operation. A complete set of all clinical and laboratory data was gathered for the study group.

Laboratory results

To assess the CRP level, a Modular Analytics EVO Cobas 6000 analyser (Roche, Basel, Switzerland) was used to carry out the Tina-quant C-Reactive Protein Gen.3 immunoturbidimetric test. Blood count analysis used a Sysmex XE-2100 analyser (Sysmex Corporation, Kobe, Kansai, Japan). Complete blood morphology results were used to calculate the SII (immune-inflammation index – multiplying the values of neutrophils and platelets and then dividing by the number of lymphocytes) and NLR (neutrophil/lymphocyte ratio) values.

Dietary assessment

A validated 125-item food frequency questionnaire (FFQ) was used to assess the energy intake, as well as the intake of polyphenols and fatty acids [25]. The questionnaire is characterized by satisfactory repeatability and it can be assumed that it is a reliable tool for assessing the diet, as well as the intake of particular food components during the previous year.

Based on the patients' declarations, it was assumed that the frequency of consumption of the products described was constant over the year and the daily intake could be calculated according to this frequency. To estimate the daily intake from the responses, frequency conversion factors presented as times/day were used (numerical values were given in brackets for each frequency).

Respondents indicated how frequently they had consumed products, choosing one of the possible options: never (0 times/day), several times a year (0.02 times/day), once a month (0.03 times/day), 2–3 times a month (0.08 times/day), once a week (0.143 times/day), twice a week (0.286 times/day), 3-4 times a week (0.5 times/day), 5-6 times a week (0.786 times/day), or every day (1 times/day). They also estimated the size of portions by choosing respectively how many glasses, pieces, slices, tablespoons or teaspoons they had consumed. For each food product, weight (expressed in grams) was assigned according to the standard portions used. The collected data was entered into a previously prepared calculation sheet, which contained data on the polyphenol and fatty acid amounts in the analysed products (per serving of the product). The content of polyphenols in the diet was calculated based on the Phenol-Explorer base, which includes information on the content of phenolic compounds (such as total polyphenols, flavonoids, phenolic acids, flavan-3-ols, lignans, and stilbenes) in plant origin products [25]. Dietary fat content (such as saturated, monounsaturated and polyunsaturated fatty acids; cholesterol; *omega*-3 and *omega*- 6 fatty acids; EPA and DHA) was calculated using the Aliant dietary program (Anmarsoft, Poland).

Statistical analysis

A statistical analysis was performed using Statistica 13.1 software (Statsoft, Poland). The normality of the distribution of the analysed variables was assessed using the *Shapiro-Wilk* test. Descriptive statistics were used to characterize the parameters. Mean (±SD) was used to describe normal variables, while the median (Q1-Q3) was used for variables deviating from the normal distribution. *Student's* t-test was used to analyse the significance of differences between groups for variables with normal distribution. Analysis of variables whose distribution differed from the normal was performed using the U *Mann-Whitney* test. The correlation between the studied variables was analysed using *Spearman's* rank correlation coefficient. *Cohen's* kappa coefficient was used to assess the agreement of the parameters under study. The differences in the analysed variables were considered to be statistically significant if the p-value was <0.05.

Ethics

All patients were informed about the detailed purpose of the study and then gave their voluntary written consent to participate. The study was approved by the Local Bioethics Committee (RNN/24/17/KE).

RESULTS

Our study calculated the inflammatory markers of patients before their planned CABG. Table 1 shows the detailed characteristics of the levels of the analysed inflammatory markers (SII and NLR) in the studied group of cardiac surgery patients (n=101).

In the next step, the patients (n=101) were divided into two subgroups based on the median SII value: 1) with lower SII (n=50) and 2) with higher SII (n=51). The cut-off value was the median in all patients, SII=430.2. The distribution of estimated

Table 1. Characteristic of inflammatory parameters (SII and NLR) in patients with advanced CAD (n=101)

Inflammatory marker	Mean	SD	Median	Q1	Q3	Min	Max
SII	502.2	312.0	430.2	342.9	539.8	151.8	2330.2
NLR	2.42	1.08	2.15	1.75	2.81	1.03	8.46

NLR – neutrophil to lymphocyte ratio; SII – systemic immune-inflammation index; SD – standard deviation; Q1– lower quartile; Q3 – upper quartile

daily dietary intake of polyphenols, including the differences between the group with lower and higher SII is presented in Table 2. There were no statistically significant differences in polyphenol intake between groups with higher and lower SII values. There was also no statistically significant correlation between this inflammatory marker and the dietary content of polyphenol compounds.

Table 3 shows the characteristics of the estimated daily dietary intake of fatty acids, taking into account the different value of the SII parameter. Statistically significant differences were found between the groups with higher and lower SII in the intake of EPA and DHA, as well as between the ratio of *omega*-6 to omega-3 fatty acids. The intake of *omega*-3 fatty acids in the studied groups was on the verge of statistical significance. Moreover, a statistically significant correlation was noted between SII and the intake of *omega*-3 fatty acids (r= -0.24, p=0.01), EPA and DHA acids (r= -0.28, p=0.005), as well as for the ratio of *omega*-6 to *omega*-3 fatty acids (r=0.26, p=0.008).

Subsequently, patients (n=101) were divided into two subgroups based on the median NLR value: 1) with lower NLR (n=51) and 2) with higher NLR (n=50). The cut-off value was the median for all patients, NLR=2.15.

Table 4 presents the estimated daily intakes of polyphenols according to the differences between the group with lower and higher NLR values. An analysis of the levels of polyphenol consumption between

Table 2. Comparison of estimated daily dietary intake of polyphenols based on SII in patients with advanced CAD

	Total (n=101)	Lower SII (n=50)	Higher SII (n=51)	p
Polyphenols (mg)	1780 (1460-2319)	1805 (1460-2533)	1666 (1448-2208)	p=0.41
Flavonoids (mg)	855 (645-1135)	866 (581-1164)	846 (656-1080)	p=0.88
Flavan-3-ols (mg)	513 (370-630)	519 (331-670)	491 (389-629)	p=0.99
Phenolic acids (mg)	442 (290-586)	439 (298-715)	449 (271-574)	p=0.38
Lignans (mg)	20.88 (14.46-30.43)	20.32 (13.26-28.22)	23.56 (14.45-33.56)	p=0.22
Stilbenes (mg)	0.14 (0.05-1.07)	0.15 (0.04-0.96)	0.14 (0.05-1.09)	p=0.67

Results are presented as median (Q1-Q3). SII – systemic immune-inflammation index. *Mann-Whitney* U test was used to assess the significance of differences

Table 3. Comparison of estimated daily dietary intake of fatty acids based on SII in patients with advanced CAD

	Total (n=101)	Lower SII (n=50)	Higher SII (n=51)	p
Saturated fatty acids (g)	32.64 (23.99-42.73)	32.32 (25.09-40.97)	33.59 (23.75-44.84)	p=0.77
Monounsaturated fatty acids (g)	44.51 (31.77-56.11)	41.19 (32.33-53.36)	46.50 (30.67-59.31)	p=0.48
Polyunsaturated fatty acids (g)	21.25 (15.09-28.17)	20.88 (18.24-26.54)	21.89 (14.07-29.97)	p=0.86
EPA+DHA (g)	0.72 (0.40-1.12)	0.88 (0.49-1.53)	0.57 (0.40-0.81)	p=0.02
Omega-3 fatty acids (g)	4.13 (3.03-6.13)	4.67 (3.49-6.68)	3.75 (2.58-5.73)	p=0.05
Omega-6 fatty acids (g)	16.52 (12.10-21.17)	16.24 (12.73-20.32)	16.77 (10.90-23.22)	p=0.97
Omega-6/*omega*-3 ratio	3.62 (2.97-5.02)	3.33 (2.36-4.87)	4.07 (3.36-5.29)	p=0.01
Cholesterol (mg)	342.66 (278.95-441.67)	327.83 (279.50-416.75)	349.04 (274.22-474.33)	p=0.75

Results are presented as median (Q1-Q3). SII – systemic immune-inflammation index; EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid. *Mann-Whitney* U test was used to assess the significance of differences.

patients with lower and higher NLR levels showed no statistically significant differences. Furthermore, there was no statistically significant correlation between these variables.

The differences in daily dietary fatty acid intake between groups with lower and higher NLR levels are shown in Table 5. The group with lower and higher NLR showed statistically significant differences in the intake of EPA and DHA acids and the ratio of *omega*-6 to *omega*-3. A statistically significant correlation was found between the NLR parameter and the amount of *omega*-3 fatty acids consumed per day (r= -0.21, p=0.04), DHA and EPA acids (r= -0.22, p=0.03) and also the ratio of *omega*-6 to *omega*-3 fatty acids (r=0.27, p=0.005).

Due to the potential importance of the *omega*-6 to omega-3 fatty acids ratio, an analysis was made of the effect that levels of inflammatory markers (SII and NLR) had on this parameter. Patients (n=101) were divided into two subgroups: 1) lower ratio of omega-6 to *omega*-3 fatty acids (n=51) and 2) higher ratio of *omega*-6 to *omega*-3 fatty acids (n=50). The cut-off value was the median for all patients of the *omega*-6 to *omega*-3 ratio = 4:1.

The group with a lower ratio of *omega*-6 to *omega*-3 exhibited lower levels of SII and NLR (Figure 1).

Table 4. Comparison of estimated daily dietary intake of polyphenols based on NLR in patients with advanced CAD

	Total	Lower NLR (n=51)	Higher NLR (n=50)	p
Polyphenols (mg)	1780 (1460-2319)	1811 (1506-2526)	1674 (1385-2209)	p=0.24
Flavonoids (mg)	855 (645-1135)	883 (632-1164)	850 (654-1081)	p=0.87
Flavan-3-ols (mg)	513 (370-630)	514 (340-671)	501 (398-630)	p=0.92
Phenolic acids (mg)	442 (290-586)	458 (298-715)	416 (271-573)	p=0.11
Lignans (mg)	20.88 (14.46-30.43)	21.33 (17.02-30.74)	19.75 (12.99-30.43)	p=0.43
Stilbenes (mg)	0.14 (0.05-1.07)	0.11 (0.04-0.81)	0.20 (0.05-1.10)	p=0.30

Results are presented as median (Q1-Q3). NLR – neutrophil to lymphocyte ratio. *Mann-Whitney* U test was used to assess the significance of differences.

Table 5. Comparison of estimated daily dietary intake of fatty acids based on NLR in patients with advanced CAD

	Total (n=101)	Lower NLR (n=51)	Higher NLR (n=50)	p
Saturated fatty acids (g)	32.64 (23.99-42.73)	32.32 (24.25-41.39)	33.08 (23.95-43.59)	p=0.88
Monounsaturated fatty acids (g)	44.51 (31.77-56.11)	40.11 (31.77-54.77)	46.32 (31.77-58.37)	p=0.40
Polyunsaturated fatty acids (g)	21.25 (15.09-28.17)	20.10 (16.31-26.54)	22.60 (14.80-29.35)	p=0.55
EPA+DHA (g)	0.72 (0.40-1.12)	0.84 (0.50-1.46)	0.56 (0.39-0.81)	p=0.02
Omega-3 fatty acids (g)	4.13 (3.03-6.13)	4.52 (3.25-6.84)	3.96 (2.60-5.52)	p=0.08
Omega-6 fatty acids (g)	16.52 (12.10-21.17)	15.88 (12.10-20.09)	17.34 (11.66-23.51)	p=0.28
Omega-6/*omega*-3 ratio	3.62 (2.97-5.02)	3.27 (2.32-4.82)	4.14 (3.44-5.27)	p=0.001
Cholesterol (mg)	342.66 (278.95-441.67)	353.92 (283.94-430.01)	330.80 (271.39-443.52)	p=0.45

Results are presented as median (Q1-Q3). NLR – neutrophil to lymphocyte ratio; EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid. *Mann-Whitney* U test was used to assess the significance of differences.

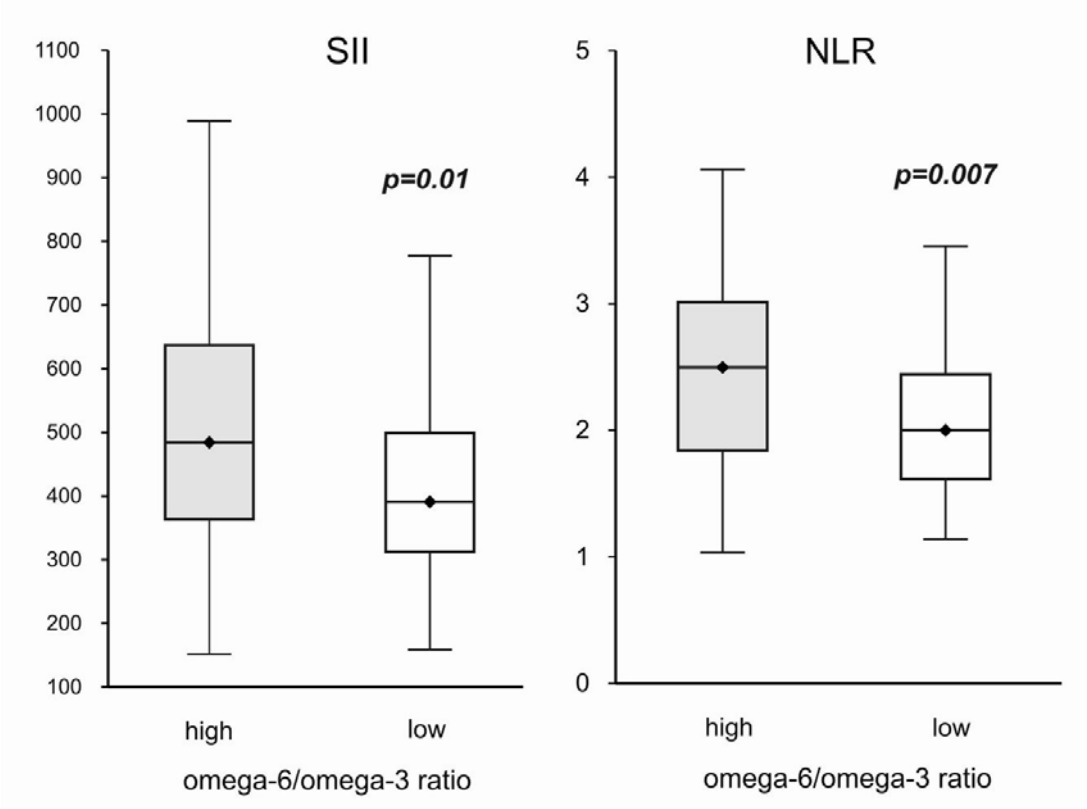

Figure 1. Levels of inflammatory markers (SII and NLR) in the groups with high and low ratio of *omega*-6 to *omega*-3 fatty acids. Results are presented as median (horizontal section), Q1-Q3 (bottom and top of the box), min and max values (whiskers). The *Mann-Whitney* U test was used to assess the significance of differences.

The differences were statistically significant for both SII and NLR values. Additionally, the NLR and SII inflammatory markers, are strongly and statistically significantly correlated with each other ($r=0.79$, $p<0.001$).

Furthermore, the association between the ratio of *omega*-6 to *omega*-3 fatty acids and inflammatory markers was tested using *Cohen's* agreement test. Agreement was confirmed between the analysed variables. A significant association was found in two cases – between SII and the *omega*-6 to *omega*-3 fatty acids ratio: agreement (%) - 61.4, *Cohen Kappa* 0.23 (95% CI 0.03-0.42, $p=0.011$) and between NLR and the *omega*-6 to *omega*-3 fatty acids ratio: agreement (%) - 65.4, *Cohen Kappa* 0.31 (95% CI 0.11-0.50, $p=0.001$). Concordance between the *omega*-6 to *omega*-3 fatty acids ratio and the inflammatory markers was in the poor to fair range, but reached a level of statistical significance.

DISCUSSION

There is an undeniable link between inflammation, diet, and cardiovascular disease. Plant polyphenols and *omega*-3 fatty acids are responsible to a large extent for a diet's cardioprotective effect [9, 16, 33]. With an anti-inflammatory diet, it is possible to reduce NLR values [25], which predict complications after cardiac surgery [31].

The aim of this study was to evaluate the nutrition of patients suffering from CAD, especially with regard to anti-inflammatory polyphenols and *omega*-3 fatty acids. Currently, a number of simple inflammatory markers are available, such as CRP (standard diagnostic marker detected in plasma) as well as SII and NLR (markers easily calculated from blood morphology) [1, 11, 32]. We hypothesized that patients that are following an anti-inflammatory diet may have reduced levels of inflammation markers before CABG.

A comparison of the intake of plant phenolic compounds with diet showed that the group with lower SII values was characterized by having a higher consumption of polyphenols, flavonoids, flavan-3-ols, lower phenolic acids and lignans, and almost the same intake of stilbenes compared to the group with higher SII. Higher intakes of polyphenols, flavonoids, phenolic acids, and lignans, and a lower consumption of stilbenes were observed in the group with lower NLR compared to patients with higher NLR. The daily amount of flavan-3-ols was almost at the same level. However, these differences were not statistically significant.

The results of our previous study regarding CRP and NLR values were similar to those reported in the current study, despite differences in the severity

of CAD. Also, the intake of *omega*-3 fatty acids was not significantly different [25]. Basing on our previous results, we left out the PLR (platelet to lymphocyte ratio) and MPVLR (mean platelet volume to lymphocyte ratio) parameters from the current study. Those markers' values were not related to the dietary intake of polyphenols or *omega*-3 fatty acids [25, 26]. Interestingly, differences in CRP levels were observed between the total group of patients analysed in the current study, and a group of healthy volunteers (median [Q1-Q3] 1.7 [1.1-3.6] vs 1.0 [0.5-1.8]) [27].

Available publications mostly negate an association between the intake of *omega*-3 fatty acids and traditional inflammation markers [15, 30]. No changes have been observed in the levels of inflammatory markers, such as hs-CRP, IL-6, and sICAM, between placebo and *omega*-3 supplementation groups [15]. A meta-analysis by *Vors* et al. has shown no difference in the effect of EPA or DHA supplementation on levels of inflammatory markers (such as CRP, IL-6, or TNF- α) [30]. When analysing the nutritional intake of fatty acids, the group with lower levels of SII and NLR was characterized by lower intakes of saturated, monounsaturated and polyunsaturated fatty acids and *omega*-6 to *omega*-3 fatty acids ratio, while having a higher intake of *omega*-3 fatty acids, EPA and DHA. This observation is difficult to discuss due to the limited literature on this subject [2]. The intake of *omega*-6 fatty acids in our study was practically the same in both subgroups with high and low values of SII, whereas the subgroup with a lower NLR had a lower daily intake of these fatty acids compared to the subgroup with a higher NLR. These differences were statistically significant only for the ratio of *omega*-6 to *omega*- 3, EPA and DHA, and *omega*-3 fatty acids (when assessing the level of SII), therefore it is reasonable to assume that these compounds have the most important impact on the anti-inflammatory effect of patients' diets. This observation supports the findings of previous studies [25] and suggests also that the panel of markers should include a further inflammatory parameter, SII. In the context of the above-cited study describing the lack of effect of *omega*-3 fatty acids consumption on CRP, IL-6 and TNF-α levels [15, 30], our view is that the usefulness of the NLR and SII markers should be given due consideration.

Our study confirmed the role of the *omega*-6 to *omega*-3 fatty acids ratio as a key parameter for assessing a patient's diet. Significant associations were found between SII and the *omega*-6 to *omega*-3 fatty acids ratio, as well as between NLR and the *omega*-6 to *omega*-3 fatty acids ratio. Attention is increasingly being paid, not only to the ratio of *omega*-6 to *omega*-3, but also to the quotient of EPA and arachidonic acid (AA), compared to the ratio of DHA to AA, in assessing possible adverse cardiovascular incidents. It has been reported that patients with one classical risk factor of CVD but a higher EPA to AA ratio were ultimately at a lower risk of CVD [18].

We found that levels of SII and NLR were significantly reduced in the subgroup of patients that had a lower ratio of *omega*-6 to *omega*-3 fatty acids. There are no published studies that estimate the effect of *omega*-3 fatty acids or plant polyphenols on SII levels. However, as our study has demonstrated, SII could be used as a predictor of a diet's anti- inflammatory effect. Interestingly, one recent published study has demonstrated that a modified diet has an effect on NLR in patients after surgery [19]. In our study, the group of patients with a higher *omega*-6 to *omega*-3 ratio had an average NLR of 2.5. In the study by *Sari* et al., the NLR index reflected the severity of CAD in 180 patients after coronary angiography. An NLR level above 2.3 was a prognostic element for CAD [23]. In a study carried out by *Yang* et al. on 5,602 patients with CAD, the cut-off value of the SII index was 694.3 x 10^9/L. It has been shown that a lower SII value was associated with a reduced risk of a non-fatal stroke or infarction, hospitalization due to heart failure or cardiac death [32].

Due to the lack of guidelines regarding standards for inflammatory markers, such as NLR and SII, *Fest* et al. [8] has proposed reference values for these parameters in the Rotterdam Study. That prospective cohort study included 8,711 individuals with a minimum age of 45 years, and the data was collected from 2002 to 2014. After calculating SII and NLR values based on blood count, the differences between levels were also considered according to age and gender. As presented in the results, the mean NLR and SII values calculated for patients participating in our study were 2.42 for NLR and 502.2 for SII. Whereas the reference values proposed by *Fest* et al. [8] for the 65–75 age group (the average age of patients in our study was 65.1±8.5 years) were mean values of 1.82 for NLR and 455.0 for SII. This definitely shows that in our study both inflammatory markers were above the presented reference levels. Due to their simplicity and accessibility, these markers should be used as practical prognostic indicators to identify patients who are at high cardiac risk. Using SII and NLR for monitoring the anti-inflammatory effects of diet is a novel approach but is fully justified, in our opinion, in the light of recent findings. The our study's results support the proposal of *Artiach* et al., who suggested a need for further studies using novel methods that would more precisely identify the specific dietary predictors that influence inflammation in patients with CAD [2]. Our results are also in line with the developing trend of diet personalization [6, 14] and may enhance the panel of examinations allowing the better matching of

diet to patient phenotype. In addition, a diet-mediated reduction of subthreshold clinical status in patients before CABG may contribute to an improved final outcome of surgical intervention [5, 12].

CONCLUSIONS

We conclude that there exists a need to assess a patient's nutritional habits, especially among cardiac patients. The level of NLR and SII can play an important role in patient supervision, reflecting the patient's diet while at the same time being an integral diagnostic element. The above-mentioned inflammatory markers can be an important tool in monitoring compliance with an anti-inflammatory diet. There is a need for precise reference values to be established for the NLR and SII indices in order to be able to correctly relate patient results to commonly accepted standards. The combined evaluation of nutritional and inflammatory marker levels can serve as a simple means of assessing the risk of adverse cardiovascular events following CABG. Early intervention, including modification of the diet, can significantly improve the health condition of patients. Non-pharmacological approaches, such as diet modifications, should be an auxiliary but integral part of the treatment of cardiac patients. We suggest also the consideration of a model in which a multidisciplinary team that includes a cardiologist/cardiac surgeon, a dietitian, and a laboratory diagnostician, could work together to provide the most effective patient care.

Conflict of interest

The Authors declare no conflict of interest.

REFERENCES

1. *Afari ME, Bhat T.* Neutrophil to lymphocyte ratio (NLR) and cardiovascular diseases: an update. Expert Rev Cardiovasc Ther 2016; 14: 573-577 DOI: 10.1586/14779072.2016.1154788
2. *Artiach G, Sarajlic P, Back M.* Inflammation and its resolution in coronary artery disease: a tightrope walk between omega-6 and omega-3 polyunsaturated fatty acids. Kardiol Pol 2020; 78: 93-95 DOI: 10.33963/KP.15202
3. *Bijak M, Sut A, Kosiorek A, Saluk-Bijak J, Golanski J.* Dual Anticoagulant/Antiplatelet Activity of Polyphenolic Grape Seeds Extract. Nutrients 2019; 11: DOI: 10.3390/nu11010093
4. *Boden S, Wennberg M, Van Guelpen B, Johansson I, Lindahl B, Andersson J, Shivappa N, Hebert JR, Nilsson LM.* Dietary inflammatory index and risk of first myocardial infarction; a prospective population-based study. Nutr J 2017; 16: 21 DOI: 10.1186/s12937-017-0243-8
5. *Dey S, Kashav R, Kohli JK, Magoon R, ItiShri, Walian A, Grover V.* Systemic Immune-Inflammation Index Predicts Poor Outcome After Elective Off-Pump CABG: A Retrospective, Single-Center Study. J Cardiothorac Vasc Anesth 2020: DOI: 10.1053/j.jvca.2020.09.092
6. *Di Renzo L, Cinelli G, Dri M, Gualtieri P, Attina A, Leggeri C, Cenname G, Esposito E, Pujia A, Chiricolo G, Salimei C, De Lorenzo A.* Mediterranean Personalized Diet Combined with Physical Activity Therapy for the Prevention of Cardiovascular Diseases in Italian Women. Nutrients 2020; 12: DOI: 10.3390/nu12113456
7. *DiNicolantonio JJ, O'Keefe JH.* Importance of maintaining a low omega-6/omega-3 ratio for reducing inflammation. Open Heart 2018; 5: e000946 DOI: 10.1136/openhrt-2018-000946
8. *Fest J, Ruiter R, Ikram MA, Voortman T, van Eijck CHJ, Stricker BH.* Reference values for white blood-cell-based inflammatory markers in the Rotterdam Study: a population-based prospective cohort study. Sci Rep 2018; 8: 10566 DOI: 10.1038/s41598-018-28646-w
9. *Golanski J, Szymanska P, Rozalski M.* Effects of Omega-3 Polyunsaturated Fatty Acids and Their Metabolites on Haemostasis-Current Perspectives in Cardiovascular Disease. Int J Mol Sci 2021; 22: DOI: 10.3390/ijms22052394
10. *Golia E, Limongelli G, Natale F, Fimiani F, Maddaloni V, Pariggiano I, Bianchi R, Crisci M, D'Acierno L, Giordano R, Di Palma G, Conte M, Golino P, Russo MG, Calabro R, Calabro P.* Inflammation and cardiovascular disease: from pathogenesis to therapeutic target. Curr Atheroscler Rep 2014; 16: 435 DOI: 10.1007/s11883-014-0435-z
11. *Gurbuz O, Kumtepe G, Ozkan H, Karal IH, Velioglu Y, Ercan A, Yuksel A, Ener S.* Predictive Value of Neutrophil-Lymphocyte Ratio for Long-Term Cardiovascular Event Following Coronary Artery Bypass Grafting. Braz J Cardiovasc Surg 2020; 35: 274-284 DOI: 10.21470/1678-9741-2018-0362
12. *Haran C, Gimpel D, Clark H, McCormack DJ.* Preoperative Neutrophil and Lymphocyte Ratio as a Predictor of Mortality and Morbidity After Cardiac Surgery. Heart Lung Circ 2021; 30: 414-418 DOI: 10.1016/j.hlc.2020.05.115
13. *Haybar H, Pezeshki SMS, Saki N.* Evaluation of complete blood count parameters in cardiovascular diseases: An early indicator of prognosis? Exp Mol Pathol 2019; 110: 104267 DOI: 10.1016/j.yexmp.2019.104267
14. *Livingstone KM, Celis-Morales C, Navas-Carretero S, San-Cristobal R, Forster H, Woolhead C, O'Donovan CB, Moschonis G, Manios Y, Traczyk I, Gundersen TE, Drevon CA, Marsaux CFM, Fallaize R, Macready AL, Daniel H, Saris WHM, Lovegrove JA, Gibney M, Gibney ER, Walsh M, Brennan L, Martinez JA, Mathers JC.* Characteristics of participants who benefit most from personalised nutrition: findings from the pan-European Food4Me randomised controlled trial. Br J Nutr 2020; 123: 1396-1405 DOI: 10.1017/S0007114520000653
15. *Mackay I, Ford I, Thies F, Fielding S, Bachoo P, Brittenden J.* Effect of Omega-3 fatty acid

supplementation on markers of platelet and endothelial function in patients with peripheral arterial disease. Atherosclerosis 2012; 221: 514-520 DOI: 10.1016/j.atherosclerosis.2011.12.041
16. *Massaro M, Scoditti E, Carluccio MA, De Caterina R*. Nutraceuticals and prevention of atherosclerosis: focus on omega-3 polyunsaturated fatty acids and Mediterranean diet polyphenols. Cardiovasc Ther 2010; 28: e13-19 DOI: 10.1111/j.1755-5922.2010.00211.x
17. *Mazidi M, Mikhailidis DP, Sattar N, Toth PP, Judd S, Blaha MJ, Hernandez AV, Penson PE, Banach M, International Lipid Expert P, Lipid, Blood Pressure Meta-analysis Collaboration G*. Association of types of dietary fats and all-cause and cause-specific mortality: A prospective cohort study and meta-analysis of prospective studies with 1,164,029 participants. Clin Nutr 2020; 39: 3677-3686 DOI: 10.1016/j.clnu.2020.03.028
18. *Nelson JR, Raskin S*. The eicosapentaenoic acid:arachidonic acid ratio and its clinical utility in cardiovascular disease. Postgrad Med 2019; 131: 268-277 DOI: 10.1080/00325481.2019.1607414
19. *Ortiz-Reyes LA, Chang Y, Quraishi SA, Yu L, Kaafarani H, de Moya M, King DR, Fagenholz P, Velmahos G, Yeh DD*. Early Enteral Nutrition Adequacy Mitigates the Neutrophil-Lymphocyte Ratio Improving Clinical Outcomes in Critically Ill Surgical Patients. Nutr Clin Pract 2019; 34: 148-155 DOI: 10.1002/ncp.10177
20. *Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC, Jr., Taubert K, Tracy RP, Vinicor F, Centers for Disease C, Prevention, American Heart A*. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003; 107: 499-511 DOI: 10.1161/01.cir.0000052939.59093.45
21. *Penson PE, Banach M*. Natural compounds as anti-atherogenic agents: Clinical evidence for improved cardiovascular outcomes. Atherosclerosis 2021; 316: 58-65 DOI: 10.1016/j.atherosclerosis.2020.11.015
22. *Pimentel GD, Dela Vega MCM, Laviano A*. High neutrophil to lymphocyte ratio as a prognostic marker in COVID-19 patients. Clin Nutr ESPEN 2020; 40: 101-102 DOI: 10.1016/j.clnesp.2020.08.004
23. *Sari I, Sunbul M, Mammadov C, Durmus E, Bozbay M, Kivrak T, Gerin F*. Relation of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio with coronary artery disease severity in patients undergoing coronary angiography. Kardiol Pol 2015; 73: 1310-1316 DOI: 10.5603/KP.a2015.0098
24. *Shrivastava AK, Singh HV, Raizada A, Singh SK*. C-reactive protein, inflammation and coronary heart disease. The Egyptian Heart Journal 2015; 67: 89-97
25. *Sut A, Chizynski K, Rozalski M, Golanski J*. Dietary intake of omega fatty acids and polyphenols and its relationship with the levels of inflammatory markers in men with chronic coronary syndrome after percutaneous coronary intervention. Kardiol Pol 2020; 78: 117-123 DOI: 10.33963/kp.15078
26. *Sut A, Pytel M, Zadrozny M, Golanski J, Rozalski M*. Polyphenol-rich diet is associated with decreased level of inflammatory biomarkers in breast cancer patients. Rocz Panstw Zakl Hig 2019; 70: 177-184
27. *Szymanska P, Boncler M, Golanski J*. Cellular and biochemical predictors of high platelet reactivity assessed by Multiple Electrode Aggregometry (MEA) in healthy individuals. Diagn Lab 2021; in press:
28. *Tamtaji OR, Milajerdi A, Reiner Z, Dadgostar E, Amirani E, Asemi Z, Mirsafaei L, Mansournia MA, Dana PM, Sadoughi F, Hallajzadeh J*. Effects of flaxseed oil supplementation on biomarkers of inflammation and oxidative stress in patients with metabolic syndrome and related disorders: A systematic review and meta-analysis of randomized controlled trials. Clin Nutr ESPEN 2020; 40: 27-33 DOI: 10.1016/j.clnesp.2020.09.017
29. *Ustundag Y*. Relationship between CRP, systemic immune-inflammation index and routine hemogram related inflammatory markers in low grade inflammation. International Journal of Medical Biochemistry 2018: DOI: 10.14744/ijmb.2017.08108
30. *Vors C, Allaire J, Mejia SB, Khan TA, Sievenpiper JL, Lamarche B*. Comparing the Effects of Docosahexaenoic and Eicosapentaenoic Acids on Inflammation Markers Using Pairwise and Network Meta-Analyses of Randomized Controlled Trials. Adv Nutr 2021; 12: 128-140 DOI: 10.1093/advances/nmaa086
31. *Weedle RC, Da Costa M, Veerasingam D, Soo AWS*. The use of neutrophil lymphocyte ratio to predict complications post cardiac surgery. Ann Transl Med 2019; 7: 778 DOI: 10.21037/atm.2019.11.17
32. *Yang YL, Wu CH, Hsu PF, Chen SC, Huang SS, Chan WL, Lin SJ, Chou CY, Chen JW, Pan JP, Charng MJ, Chen YH, Wu TC, Lu TM, Huang PH, Cheng HM, Huang CC, Sung SH, Lin YJ, Leu HB*. Systemic immune-inflammation index (SII) predicted clinical outcome in patients with coronary artery disease. Eur J Clin Invest 2020; 50: e13230 DOI: 10.1111/eci.13230
33. *Zampelas A, Magriplis E*. Dietary patterns and risk of cardiovascular diseases: A review of the evidence. Proceedings of the Nutrition Society 2020; 79: 68-75

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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.

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